ABSTRACT

Title of Document: DEVELOPMENT OF OKARA POWDER AS A GLUTEN FREE ALTERNATIVE TO ALL PURPOSE FLOUR FOR VALUE ADDED USE IN BAKED GOODS

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Soymilk and tofu production yields large quantities of agrowaste (okara). Okara is high in fiber and protein making it a potential nutritious food ingredient. The shelf life of only a day makes it difficult to work with in large scale operations. To increase the value of okara, the dried product was incorporated with other ingredients to create a gluten-free flour (AF). Analysis of biscuits and cookies made with AF yielded poor product height, spread and texture. However, pancakes and muffins made with AF were more successful. AF was not significantly different ($p < 0.05$) from the control in the muffin product in regards to springiness, hardness and height. Sensory evaluation of the muffins found AF muffins out-performing a commercial gluten free flour, and was not significantly different from the control in flavor and texture, with no distinguishable beany flavor. These studies demonstrate that AF produced from okara can be used to increase the value of the agrowaste.

DEVELOPMENT OF OKARA POWDER AS A GLUTEN FREE ALTERNATIVE TO ALL PURPOSE FLOUR FOR VALUE ADDED USE IN BAKED GOODS

By

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Chapter 1: Introduction

Okara, the pulpy by-product of the soy milk and tofu processing industry, is a potentially nutritious product that is high in fiber, protein, carbohydrates, vitamins, minerals, and fat. Besides its application as an animal feed, okara is used directly in food dishes such as soups and salads, or fermented into products such as tempeh in Asian countries known to consume more soy products. America and other Western countries have not utilized okara for foods as it is generally considered a waste product with strong beany flavor, making it unacceptable to U.S. consumers. In America, okara is largely an agrowaste and is either used in animal feeds or sent to landfills for disposal, which creates an ecological problem due to the increase in sales of the soymilk and tofu industry in the U.S. Presently, small scale production of okara is observed in home cooking where fresh soymilk is produced in household kitchen. Direct incorporation of freshly produced okara into food dishes appears to increase due in part to popular online recipes. However, no industrial product made of soy okara is currently available commercially. A possible use for okara is in baked goods as it has a large amount of fiber and protein. Even greater value can be added to okara if it can be properly introduced into gluten-free formulations, a market that is not only growing (as the incidence of celiac disease is increasing), but also lacks a successful gluten-free all purpose flour. Therefore, a gluten-free all purpose flour formulated with okara as the main ingredient should offer a value-added application for this agrowaste while increasing the nutritional value of gluten-free baked goods.

Chapter 2: Literature Review

2.1 Soybeans: Typical uses and varieties

Soybeans, a legume in the *Leguminosae* family, subfamily *Papilionoideae* and genus *Glucine*, L (Liu 1997), are grown world-wide for several uses ranging from food sources (soy sauce, soymilk, tofu) to biofeuls (Liu, 1995; Liu 1997; Hill et al., 2006) Asian countries lead the market in using soy for food consumption (Liu, 1997; Setchell, 1998). However, the United States is currently the largest soybean producer in the world accounting for about 38% of production in 2007 (IBIS World). In the United States, most of the soybeans are used for production of oils or for animal feeds (Liu, 1997; Xie, 2008). In general, major differences in soybeans are their protein and oil content, the chemical composition of the protein and oils, and the appearance of the seeds themselves (Liu, 1995). This has led to two major groups of soybeans: food soybeans and oil soybeans (Liu, 1997).

2.2: Soymilk processing

In the United States, a popular use for soybeans is the production of soymilk. Several methods exist to make soymilk. Which method is used depends on the manufacturer and the desired product. Soymilk is an extraction of the aqueous portion of the soybean slurry after soybeans have been allowed to soak in water and then ground (Riaz 2006). Figure 1.1 shows the general process for soymilk manufacturing. An important processing step is heating of the soymilk to deactivate enzymes inherent in the soybean such as lipoxygenase and trypsin inhibitors (Liu, 1997) that not only cause oxidative rancidity (and therefore off flavors characterized as beany-ness in the final soymilk), but can also inhibit absorption of nutrients (i.e. trypsin). Differences in soymilk production include: soaking water temperature (hot or cold); separation of the pulp (okara) from the soymilk pre or post cooking; blanching the un-ground beans before grinding; adding chemicals to the blanching or grinding step; grinding size; and the number of times the okara is washed (Cai, 1997: Riaz 2006). For example, the Illinois method of soymilk production blanches the whole beans prior to grinding to inactivate the enzymes before they have an opportunity to be released from the whole bean into the soymilk (Lui, 1997). The combination of heat and sodium bicarbonate inactivates the lipooxygenase enzyme (LOX) which is mainly responsible for oxidation in soymilk (Riaz 2006).

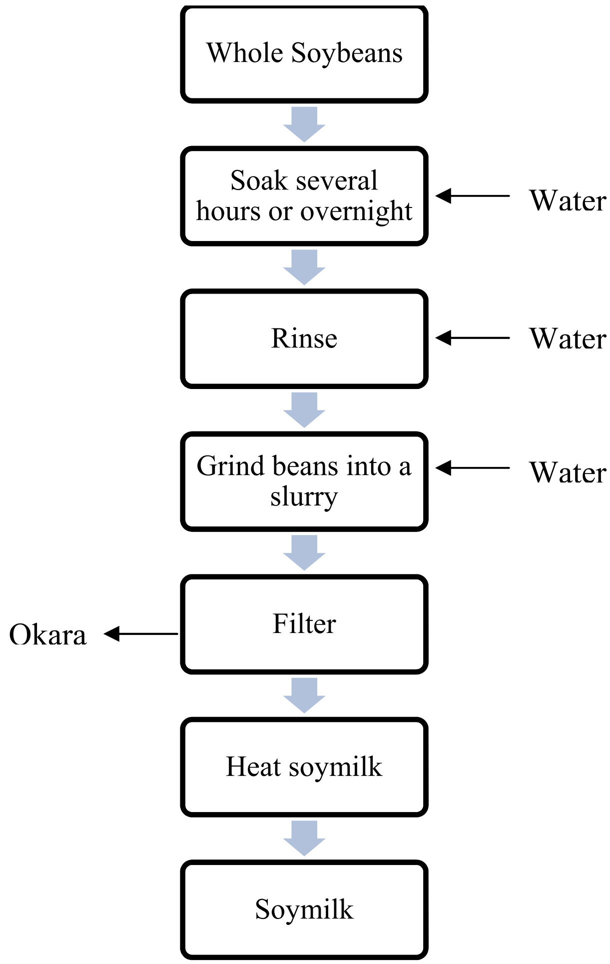

Figure 2.1 Diagram of the Chinese method for soymilk production: adapted from Liu, 1997

2.2.1 Soymilk processing: soymilk industry in United States

In the United States, soymilk production is a $482.8 million industry. Along with snack bars and meat substitute, soymilk represents 90% of soy food sales in 2008 (Mintel, 2008). Between 2003 and 2008, the soymilk market segment has grown 61% (Mintel, 2008), indicating a fast-growing market with increasing quantities of okara as a natural by-product of this growing industry.

2.2.2 Soymilk processing: okara production

Soymilk and tofu production generates large quantities of okara. Liu (1997) found that for every pound of soybean used to make soymilk, 1.1pounds of okara is produced, whereas Riaz (2006) stated that 1.4 – 1.8 kg of okara is produced for every kilogram of soybeans used for soymilk processing. Yoshi and others (1997) reported that $8x10^5$ tons of okara was produced in Japan annually.

2.3: Okara Composition

Soybeans are high in protein, fat, and fiber. After soymilk extraction, much of that fat, fiber, and protein remains in okara (O'Toole, 1999; Riaz, 2006). Several studies of the macro-nutrient contents of okara have been published and a summary of these findings is found in table 1.1. On a dry basis, Liu stated that okara contains 25.4-28.4% protein, 9.3-10.9% fat, 40.2-43.6% insoluble fiber, 12.6-14.6% soluble fiber and 3.8-5.3% soluble carbohydrates. This is consistent with the findings from other researchers (Table 2.1). Moisture content of the raw okara is variable depending on soymilk processing and the efficiency with which soymilk is extracted from the wet pulp (Table 2.1). Ranges in the reported findings from several authors illustrate the variability in okara, not only in nutritional composition but also in its moisture content. More research studies on okara have focused on the nutritional properties of it, such as antioxidant activity (Jimenez-Escrig et al., 2009). In this case, bioactive peptides were extracted from okara protein isolates that had undergone hydrolysis for proper protein sequence analysis. Simulated digestion of okara protein hydrolysates had significant reduction power and radical scavenging activity.

Okara contains high amounts of dietary fiber. It is recommended that adults consume 21-40g of fiber per day (Maeos-Aparici) as soluble dietary fiber has been linked to: prebiotic activity, reduction of cholesterol levels, anti-inflammatory and anti-carcinogenic effects in the gastro-intestinal tract, and increased fecal bulk. Insoluble fiber can be used to control diarrhea and constipation along with being a possible treatment for irritable bowel (Mateos-Aparicio et al., 2010).

Moisture sorption isotherm of freeze-dried okara showed that in its dry state, okara exhibits "typical sigmoide shape II isotherm" (Garcia et al., 2000) and is similar to commercial soy protein isolates found on the market. Evaluation of the adsorption/desorption hysteresis loops in the dried okara showed small amounts of hysteris comparable with other starch foods, having the maximum deviations at an a_w of around 0.7 (Garcia et al., 2000; Al-Muhstaseb et al., 2002). Knowledge of dried okara isotherms can aide in proper storage of the product (Oyelade et al., 2008). The okara exhibited hysteresis along all water activities, yet was not as pronounced as it would be for a high starch food, which is in agreement with the make-up of okara having large amounts of fiber and lesser quantities of starch (Garcia et al., 2000). At an a_w between 0.6 and 0.7 at both room temperature and elevated temperature, absorption increased dramatically, suggesting that dried okara when maintained in storage should be kept at lower humidity levels to maintain low levels of moisture.

Table 2.1 Summary of proximate analysis of okara based on dry weight unless specified

Protein (%)	Fat (%)	Fiber (%)	Carbohydrate (%)	Moisture (%)	Reference
28	9.3	44.58**	5.42	-	Khare et al., 1995
25.4 – 28.4	9.3-10.9	52.8 – 58.2	3.8 – 5.3	-	Liu, 1997
36.6	14.1	11.8	34.6***	-	Garcia et al., 2000
25	10-15	-	-	70-80*	Lescano et al., 2005
37.5	20	31.1	-	68.91*	Surel and Couplet, 2005
29	11	60	-	75-80*	Wachiraphansakul and Devahastin, 2005
24.0	15.2	42.8	5.1	8.9	Matsumoto et al.,2007
24.5-37.5	9.3-22.3	14.5-55.4	-	-	Jimenez-Escrig et al., 2008
31.7	14.7	33.6	-	8.4	Katayama and Wilson, 2008
28.52	9.84	55.48	2.56	-	Redondo-Cuenca et al., 2008
3.6	1.4	9.2	-	85*	Zhu et al., 2008
34.7	23.2	-	37.9***	-	Vishwanathan et al., 2011

*Moisture of raw material prior to drying
**Value obtained by subtracting “free carbohydrates” from “total carbohydrate”
***Carbohydrate by difference

2.3.1 Okara composition: Isoflavones

Okara contains the isoflavones that are also found in whole soybeans and other soybean products. The three main isoflavones in soy are: daidzein, genistein, and glycitein. These can occur in four different chemical forms: aglycons, glucosides, acetylglucosides and malonylglucosides (Wang, 1994; Coward, 1998; Mathias, 2006). Isoflavones, a class of phytoestrogens, can act as antioxidants and are credited with many of anti-carcinigenic effects of soy based foods (Wang and Murphy, 1994; Coward, 1998; Setchell, 1998; Mathia et al., 2004). Sourel and Couplet identified two of the isoflavones (daidzein and genistein) in three different forms (glucosides, malonylglucosides and acetylglucosides) in okara. Jimenez-Escrig reported that okara contains isoflavones at a concentration of 0.14g/100g of dry matter. As previously stated, isoflavones can act as antioxidants, but as phytoestrogens, they can act as estrogen agonists or antagonists, bind to the estrogen receptor which is thought to lead to lower incidences of breast cancer (Setchell, 1998). However the exact mechanism by which soy isoflavones act remains to be fully elucidated (Setchell, 1998; Coward et al., 1997; Mathias et al., 2004).

2.4: Current uses of okara

There are several different uses for okara due to its nutritionally rich composition. These uses range from extraction processes for macronutrients to an array of food uses.

2.4.1 Current uses of okara: protein extraction

Protein can be added to a wide variety of products and can help the physical and nutritional properties of several products. Many commercially available foods are supplemented with protein such as: Healthy Choice® Chicken Tortilla Soup, Dannon®

DanActive Immunity Yogurt, Bolthouse Farms® Perfectly Protein Chai Tea Vanilla, Kashi ™ TLC Chewy Granola Bars Dark Chocolate Cherry. Ma and others (1997) isolated proteins from okara via isoelectric precipitation of an alkaline extract and studied the physicochemical and functional properties of the okara protein isolate (OPI) as compared to a commercial soy protein isolate (Supro 610). Their objective was to validate the use of OPI as a food ingredient. OPIs had good amino acid profiles, which were comparable to those of the commercial product and were comparable to FAO/WHO standards and the protein had good digestibility comparable to the control (Supro 610). The Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the United Nations University (UNU) have a joint consultation where protein standards for proper health are evaluated and set (WHO, 2007). Dietary requirements for protein are set so that the minimum intake satisfies metabolic demands, and must therefore contain adequate amounts of “nutritionally indispensible amino acids” (WHO, 2002). OPI was reported to have an amino acid profile comparable to these standards. Cysteine, methionine, and tyrosine were low in OPI and solubility was worse than that of Supro 610 at both alkaline and acidic pH. Emulsifying properties of OPI were equivalent to those of Supro610, but lacked in emulsion stability. Fat binding capacity of OPI was significantly greater than that of the Supro 610 and no significant difference was found between the two samples for water holding capacity. Additionally, OPI had better foaming properties. It was concluded that in all areas except for solubility, OPI performed as well as, if not better than, the commercial soy protein isolate, Supro 610.

To improve the poor solubility of OPI, Ma and Chan (1999) employed acid deamidation. Due to the intense heat treatment in soymilk processing, the proteins in okara denature, accounting for its poor solubility. As in the previous study, Supro 610 was used as a reference protein. OPI was treated with three different levels of HCl (0.01N, 0.1N, 0.3N) and then tested for functional and nutritional properties. Solubility improved with higher levels of deamidation (higher concentrations of HCl) especially in acidic and alkaline conditions. Water holding capacity decreased with acid modification whereas fat binding capacity was un-altered with the acid treatment. The emulsification activity index increased with increases in HCl concentrations, while there was no significant change in the emulsion stability index. Foaming ability increased with greater modification, however, foaming stability greatly decreased with higher concentration of HCl. All of the essential amino acids were present in OPI and they either met, or exceeded, FAO/WHO/UNU (1995) patterns. However, protein digestibility decreased as modification level increased. The 0.1N HCl alteration yielded the best overall product, thereby strengthening the possibility of using okara as a functional food ingredient.

Chan and Ma also modified okara proteins using trypsin (1999). The purpose was again to improve the solubility of OPI by using an enzymatic process. The same commercial soy protein isolate was used as a reference and trypsin was used as the hydrolysis enzyme. The solubility of OPI increased over all pH levels as the degree of hydrolysis (DH) increased. Fat absorption ability decreased as DH increased, whereas the water holding capacity increased as DH increased. Emulsification activity increased in OPIs as DH increased, however, the emulsification stability decreased as trypsin digestion increased. Foamability decreased considerably with the addition of trypsin. However, as hydrolysis increased, so did foamability. Foam stability was negatively affected with degree of hydrolysis; therefore, those with the highest degree of hydrolysis had the lowest foam stability. As with their previous study, the amino acid composition of OPIs met or exceeded the essential amino acid pattern for FAO/WHO/UNU. Digestibility of OPI decreased somewhat with the increase in hydrolysis, but were still relatively high (above 74%) across all hydrolysis degrees. These findings support the use of okara protein as an additive in food products.

2.4.2 Current uses of okara: fiber extraction

Extraction of the fiber from okara can be used in food products for fiber supplementation. Products that are currently supplemented with fiber include: Atkins ™ Morning Start Breakfast Bars, Fiber One® products (bread, yogurt, cereal, pancake and muffin mix, breakfast and snack bars), and Metamucil® products. A study conducted by Redondo-Cuenca and others (2008) analyzed the fiber content from okara to assess its potential as a food additive. Okara fiber had very little excess flavor and no color, indicating that it could be a viable ingredient in foods. Analysis via the AOAC Method revealed okara having 55.48g/100g dry matter of fiber versus 24.36g/100g dry matter for soybean seeds. Additionally, the ratio of insoluble fiber to soluble fiber (IF/SF) is 11 in okara versus 6 for soybean seeds. The high yield of okara makes it a good starting material for fiber supplementation.

The health benefits of using okara fiber was studied by Jimenez-Escrig and others (2008). Healthy rats were fed a diet rich in dietary fiber from okara. They evaluated the effect of this diet on biochemical parameters, mineral balance, prebiotic effect, and antioxidant status. Okara was freeze dried and de-fatted prior to being added into the diet (up to 10% okara fiber). After four weeks, it was found that the defatted, dietary fiber rich diet did not negatively affect the health of the rats, suggesting that the okara diet was safe for consumption. Additionally, it lowered weight gain when fed in the same quantities as the control diet and increased food efficiency. There was also a significant decrease in total serum cholesterol levels, along with a suppression of an increase in total serum cholesterol levels, an increase in antioxidant status and higher apparent calcium absorption and retention. This study indicates that okara fiber can have a promising future as a fiber supplement in food items.

Matsumoto and others (2007) fed mice an okara-supplemented diet to investigate if okara could help reduce obesity. After a 10-week period, mice on the okara-supplemented diet at levels of 20% and 40%, had a body weight 15% lower than those of the control mice with the same volume of food. Mice on the 20% and 40% okara supplemented diet also had 40% lower weight gain than he control mice, indicating okara may have weight loss effects, increasing the overall value of okara as a food ingredient.

Dietary fiber was extracted from okara employing three different extraction techniques (Surel and Couplet, 2005). Analysis of the effect of the fiber on gastrointestinal function was analyzed, using water-holding capacity (WHC) as the study parameter. Purification of the fiber included oven drying the okara, followed by soxhlet fat extraction and proteolysis (protease P-5380). This technique yielded 80.5% total dietary fiber (TDF). A secondary method began the purification process by allowing the wet okara to undergo proteolysis, followed by soxhlet fat extraction generating a TDF yield of 75%. A final method studied had the wet okara undergoing proteolysis followed by lipolysis yielding only 50% TDF. The WHC of the okara fibers were on par with the commercial fiber products available. The second method of fiber extraction had the best WHC. Isoflavone content was also studied. The researchers concluded that extracted fibers should not be dried at high temperatures as this can lead to a change in the isoflavones as well as alter the microstructure of the fibers and decrease overall WHC. The extracted fiber from these methods contained some residual protein and fat, making it difficult to use the okara as a pure fiber product. However, good WHC translates to possible uses in food products that can tolerate small quantities of fat or protein.

Mateos-Aparicio et al. (2010) intended to increase the functionality of the dietary fiber from okara through use of high hydrostatic pressure and temperature. At a pressure of 400MPa and 60°C, soluble dietary fiber (SDF) content in the okara fiber increased. As previously stated, soluble fiber has beneficial health effects when consumed in appropriate quantities. Increasing the amount of SDF increases the value of using okara fiber in food supplementation.

2.4.3 Current uses of okara: lipid extraction

Depending on the soybean varietal used, okara can have large quantities of fat making it a possible material for lipid extraction. Quitain and others (2005) extracted oil from okara using supercritical carbon dioxide. Oil yields were 3.09g per 100g of dried okara. This is a yield of about 25% (assuming total available oil for extraction from dried okara is 12g/100g). Yields increase to 4.54g/100g and 7.81g/100g sample (64% recovery) if the supercritical carbon dioxide has 5 mol% EtOH and 10 mol% EtOH (respectively) added as entrainer. This high yield makes extraction of oil components from okara a viable process. Additionally, the isoflavones, genistein and daidzein, were co-extracted along with the oils. This adds value to the oil recovery process as these isoflavones are credited with positive health effects such as antioxidant ability, anti-inflammatory properties, anti-mutagenic ability and more (Quitan, 2005).

2.4.4 Current uses of okara: foods

Soybeans are a staple in Asian cuisine and have been cultivated for thousands of years in China, Japan and neighboring countries (Lager, 1945: Liu, 2006). This long history with soybeans has allowed Asian countries to become more innovative with okara, which is highly perishable having a shelf life of only a day. Unless okara is frozen or dried, its fatty composition, high moisture content and nutritive components (Waliszewski 2002; Lescano, 2005) give rise to a rancid scent making its disposal unpleasant. In light of this, many Asian countries have found a variety of ways to make use of okara, which is very nutritious, in many food items such as soups, salads, baked goods and fermented food products such as tempeh (Liu, 1997; O'Toole, 1999; Riaz, 2006). It is also used as an ingredient in animal feeds. Tempeh is commonly made by pressing okara into cakes and allowing it to ferment (Liu 1997). Table 2.2 summarizes use of okara in bioprocessing and fermentation reactions for food, medicine, and other products. However, as fermentation reactions still leave behind a considerable amount of waste (Sanghvi, 2009), utilization of okara for fermentation and bioprocessing reactions does not make complete use of okara.

Whereas Asian countries have found many ways to use okara, most Western countries consider it an agrowaste with very little value (Waliszewski et al, 2002; Riaz, 2006) with the most common application the United States being animal feed (Liu, 1997; O'Toole, 1999). Soybeans are a modern crop in the United States and large-scale farming of these legumes did not begin until the early 1900's (Liu, 2006), causing Americans to fall behind in how to make use of the by-product of a growing industry.

Soy is still working its way into the mainstream food supply and many Americans are not yet accustomed to soy's flavor and have a strong dislike to it (Rimal, 2008), making it difficult for companies to use okara which can have a strong beany flavor. Attempts to use soy in food formulations have found that beany flavor is the major sensory characteristic that makes their products unacceptable (Xie, 2008; Riaz, 2008), and overall flavor of products decreases with increased amounts of soy product (Genta, 2002). Katayama and Wilson (2008) produced a soy based snack food and found that beany aroma and flavor was the major problem in developing a soy-based food. Xie and others

(2008) developed a puffed okara/rice cake where they supplemented rice with a dried okara product. Here, beany flavor and aroma was not a significant factor, and consumers liked best the rice cakes containing 70% okara. This confirms that okara can be used in a food product, but only when the characteristic beany flavor is undetectable. Waliszewski and others (2002) created a tortilla supplemented with okara in an attempt to increase the protein content in tortillas and found the maximum acceptable supplementation of okara to be only 10%. This highlights that there is a need for a food product that is high in okara and acceptable to consumers. In Rimal's study, he stated that if soy food products are to become popular, they have to have more flavor and appeal to a large base of consumers.

A consumer base open to soy products is the vegan and vegetarian market (Rimal, 2008). The high protein content in soy makes it an attractive food source for individuals who lack a good protein source in their diets, and has led to some individuals making soymilk at home. This has led to an increase in online blogs (www.okaramountain.blospot.com) where individuals share recipes and ways to use okara. The home cook can easily make use of small quantities of okara by freezing the leftover pulp until later use, thereby keeping it fresh, whereas large-scale production lines find storage of the raw okara a problem. Distribution and storage of a highly perishable item make it an undesirable product to use as an ingredient in food formulations.

Table 2.2 Summary of okara as a substrate for bioprocessing reactions

Purpose	Product	Organism	Remarks	Ref.
Medicinal	Aldose reductase (AR) inhibitor	*Aspergillus sp.*	8-hydroxydaidzein identified as AR inhibitor; likely daidzein is a precursor	Fujita et al., 2004
	Lingzhi	*Ganoderma lucidum*	May need adjustment of C/N for best results	Hsieh and Yang, 2004
Traditional food	Meitauza	*Bacillus subtilis*	Improve antioxidant activity and therefore enhance value of okara and Meitauza	Zhu et al. 2008
Fatty acids	DHA and EHA	Thraustochytrid strains	Thraustochytrid strains able to use okara as substrate for DHA production, but with low yields	Fan et al., 2001
Antioxidant	Antioxidative peptides	Proteases	Hydrolysis of okara protein yielded di- and tri- peptides with antioxidant capabilities	Yokomizo, 2002
	Citric acid	*Aspergillius niger sp. Aspergillus terreus sp.*	Four fold increase of citric acid yield when pre-fermented by *Aspergillus terreus* (5.1g citric acid/100g dry solid)	Khare et al.1995
Agricultural Uses	Lipopeptide antibiotic (surfactin)	*Bacillus subtillis sp. (recombinant)*	Yields as high as 2g/kg wet weight, 4-5 times more than liquid medium	Ohno et al., 1995
	Lipopeptide antibiotic (iturin A: plant anitbiotic)	*Bacillus subtillis sp.*	Decreases fermentation time ; increases in yield from 6-10 fold depending on study	Ohno et al., 1996; Mizumoto et al., 2006
	Okaramines	*Aspergillus aculeatus*	Insecticide found to be effective against silkworms	Hayashi et al., 1999

2.5: Drying okara

As previously mentioned, okara can have high quantities of moisture after the soymilk has been extracted (Table 2.1). Many researchers have studied ways to dry this material for ease of storage and transportation.

2.5.1 Drying okara: Jet spouted bed dryer

Wachiraphansakul and Devahastin (2005) studied the effects of inlet air velocity, inlet air temperature, bed height and heating duration on the drying kinetics of okara with a jet spouted bed dryer. They also studied quality parameters of the okara after it was dried; specifically the urease index (a measure of anti-nutritional factors, mainly trypsin inhibitor), percent change in protein content and color. They used a stainless steel jet spouted bed dryer and dried okara to about 13% moisture. Higher inlet air velocity led to a higher rate of drying at all inlet air temperatures. A lower bed height resulted in a higher drying rate at all levels of inlet air velocity and air temperature. Higher inlet air temperature led to a faster rate of drying, especially at higher inlet air velocity. Constant drying ended after a period of 5-10 minutes and in all drying conditions, the change in protein content was not significant. All of the drying conditions used had acceptable levels of urease index (between 0.045-0.092) and the lowest value was found when all the variables were at their optimum drying conditions. Failure to heat okara to adequate temperatures allows the trypsin inhibitor to be present, leading to okara having anti-nutritional factors.

Color changes were minimal for all color values based on a CIE L*a*b* color scale. The L* value, which indicates lightness (L* of 100 = white, L* of 0 = black), did not have any significant change unless compared at the two extremes of drying conditions. The b* value (-b* = blue, +b* = yellow), changed in the positive direction, meaning the dried okara was more yellow than the raw okara. Inlet air temperature and initial bed height were the two variables that contributed the most to yellowness in the dried product. The a* value (-a* = green, +a* = red), had the most significant, change and all the parameters had a significant effect on the redness of the okara.

2.5.2 Drying okara: drying effects

Surel (2005) found that oven drying okara altered the fiber structure and decreased its water holding capacity. It was hypothesized that oven drying reduces the okara fiber's ability to rehydrate. There was no significant decrease in the isoflavone content in the okara after heat treatment; however, Surel mentioned that other studies have shown that high temperatures have a negative effect on isoflavone content. The higher the temperature, the more the isoflavones change and decrease in quantity. However, since there is still debate going on as to the beneficial properties of isoflavones (Coward et al, 1998; Mathias et al, 2006), retaining okara's other nutrients such as fiber and protein, are more pertinent parameters to retain.

2.6 Celiac disease

Celiac disease can be an adverse effect of the consumption of gluten derived from wheat products, specifically the gliadin fraction of wheat protein, and is characterized by an inflammation of the mucosa lining the small intestine (Gallagher et al., 2003; Moore et al., 2004). Fractions from other grains can illicit similar responses (secalins from rye, hordeins from barley and avidins from oats) and can cause malabsorption of many nutrients including iron, folic acid, calcium and fat soluble vitamins (Gallagher et al., 2004; Moore et al., 2004). Gluten is the main structural protein in baked goods, making it difficult to formulate gluten free bakery products for individuals suffering from celiac disease (Moore et al., 2003; McGee, 2004; Gallagher et al., 2004). Many gluten free products are formulated with only starch to help mimic the texture and feel of bread, which leads to gluten free diets low in fiber (Moore et al., 2003; Gallagher et al., 2004). As well as nutritional problems, many gluten free products lack favorable flavor and texture, as gluten provides elasticity to baked goods (Hartwig, 1983; Moore et al., 2003, Gallagher et al., 2004). Therefore, when formulating gluten free products, it is important to not only maintain textural qualities similar to the gluten containing counterparts, nutritional factors and flavor must also be considered.

Chapter 3: Objectives

The ultimate goal of this project was to identify a value-added application for okara in baked goods that make use of its nutritional properties. In order to achieve the goal, this project will:

- Formulate a gluten-free flour and characterize its properties
- Use the gluten-free flour in a variety of baked goods (dough- and batter-based) and assess the textural properties of the products
- Conduct sensory analysis of the formulated product for consumer acceptance

Chapter 4: Materials and Methods

4.1: Preparation of a gluten free flour

4.1.1 Formulation of flour

A novel gluten free baking flour was formulated using okara powder (6% moisture w/w, SunOpta, Alexandria, Minnesota), white rice flour (Bob's Red Mill®, Milwaukie, Oregon), tapioca starch (Ener-G®, Seattle, Washington), inulin (Metamucil®, Cincinnati, Ohio), calcium carbonate (Now Foods®, Bloomingdale, Illinois), cream of tartar (McCormick®, Sparks, MD), and xanthan gum (Bob's Red Mill®, Milwaukie, Oregon). This gluten-free all purpose flour, aka Alpha Formulation (AF) was compared to regular all purpose flour (control) obtained from a generic line at a local grocery store (Safeway®, Kensington, MD) along with a commercially available gluten-free all purpose flour (CGF).

4.1.2 Testing parameters of flour

All color measurements on the flour samples were conducted using the HunterLab ColorFlex Colorimeter (Reston, Virginia) on the L*a*b* scale. Water activity was determined using the AW Lab Set H (Novasina, Lachen, Switzerland). The density of the samples was measured by weighing 10 mL of each respective flour and then converting to the measurement of g/mL. Water holding capacity (WHC) of flours was determined by adding 1 g of the flour sample to 10mL of deonized water in a centrifuge tube. Centrifuge tubes were weighed before centrifugation. Samples were vortexed for 30 seconds at high speed and then sat for 5 min prior to centrifuging at 3000 rpm for 30 min at 4°C. After centrifuging, the supernatant was decanted, tubes were re-weighed, and WHC was found by the equation:

Equation 1: $$WHC = \frac{(m_{tube\ ac*} - m_{empty\ tube}) - (m_{flour})}{m_{flour}}$$

m = mass in g
*ac = after centrifugation

Analysis of the AF flour's nutritional profile was conducted at rtechlabs™ (Arden Hills, Minnesota). Proximate analysis (ash, moisture, and protein content via Kjeldahl reaction), fat content (cholesterol and fatty acid profile), carbohydrate (total dietary fiber, sugar profile via HPLC, total carbohydrate), total calories, vitamin A, vitamin C, calcium, iron and sodium content was determined.

4.2 Utilization of AF in dough products

For the purpose of assessing the functionality of the alpha formulation in dough products, standard recipes and baking procedures of biscuits and cookies were employed and AF was substituted for regular all purpose flour. These two flours were also compared against CGF. The physical properties (height, weight, spread, texture, color) of the products were analyzed and compared. All ingredients used in the products were acquired from a local supermarket (Safeway®, Kensington, MD).

4.2.1 Biscuit formulation

Biscuit doughs (Deen, 2008) were prepared by using the biscuit method by blending all dry ingredients together (2 cups flour, 1 teaspoon sugar, 1 tablespoon baking powder, 1 teaspoon salt) and then cutting in the butter before adding the liquid (3/4 cup milk). The dough was mixed until there were no dry particles of flour and the liquid had been fully incorporated into the dry ingredients. All biscuit doughs were rolled to ½" thickness, cut with a 2" diameter circular cutter and baked in a 350° F oven.

4.2.2 Biscuit characterization

Weight of uncooked biscuit dough was measured and hardness of the dough was determined using a TA-XT2i Texture Analyzer (Texture Technologies, Robbinsville, New Jersey). Samples of the uncooked dough were placed into a cylindrical test cell . An aeration plunger was used to remove random pockets of air that occur naturally in the dough when placing it in the cell. A flattening plunger was then employed to even out the top surface of the sample prior to using a 5mm aluminum probe. Hardness of the dough was determined using the biscuit hardness test from the texture analyzer program. The probe was inserted to a depth of 20mm with a test speed of 3mm/sec. The biscuits were baked in convention oven at 350°F for 20-25 min and cooled. Upon reaching room temperature, the spread and weight of the biscuits were measured. The average height of the samples was measured by stacking six biscuits. The color of the top and the bottom of the biscuits was measured using the HunterLab ColorFlex Colorimeter. The hardness of the biscuits was determined with the TA-XT2i Texture Analyzer equipped with a 50 kg load cell and the knife blade attachment on a 3-point bend rig. The blade attachment penetrated the sample to 50% of the average height. The maximum force achieved was marked as hardness of the biscuit. The moisture loss during baking was assessed by calculating the difference between pre-bake and post-bake weights.

4.2.3 Snickerdoodle formulation

Snicerkdoodles (Gand, 2010) were made via the creaming method where sugar (2 cups) and butter (1 cup) were beaten on a mixer with a paddle attachment until light and fluffy. The wet ingredients were then add (2 eggs, 1 tablespoon light corn syrup, 2 ½ teaspoons vanilla extract) and beaten until well incorporated. Lastly, the dry ingredients (3 ½ cups flour, 1 tablespoon baking powder, 2 teaspoons baking soda, ¼ teaspoon salt, ¼ teaspoon cinnamon) were added and mixed until just combined. Cookie dough was then scooped onto a baking sheet using a #50 scoop (5/8 oz) and the dough balls were slightly flattened to a thickness of about 1cm. Cookies were baked in a 350°F for 9-11 min.

4.2.4 Snickerdoodle characterization

The weight, height and texture of the dough were measured prior to baking. Dough hardness was measured in the same fashion as the biscuit dough. After the products were cooled to room temperature, the weight and diameter of the samples were measured by placing 6 cookies side to side. The height of the cookie was measured by stacking 6 cookies and measuring the total height. The color of the top and bottom of the cookies was determined using the Colorimeter and the hardness and fracturability of the samples was found measured with the TA-XT2i Texture Analyzer equipped with a 5kg load cell. A 3pt bend rig with a 4cm gap was employed along with the knife blade attachment. Cookies were cut using a penetration depth of 20mm and a test speed of 3mm/sec. Maximum force was noted as hardness of the product while distance to break was considered fracturability.

4.3 Utilization of AF in batter products

Different types of batters, namely buttermilk pancake and banana muffin, were prepared to assess the performance of AF against all purpose flour and Bob's Red Mill Gluten Free All Purpose Flour. All batters were prepared in the conventional manner following typical home formulations with standard measuring equipment. Ingredients were purchased at a local supermarket (Kensington, MD).

4.3.1 Buttermilk pancake formulation

Buttermilk pancake (Stewart, 2001) batter was made by first mixing all the dry ingredients (2 cups flour, 2 teaspoons baking powder, 1 teaspoon baking soda, ½ teaspoon salt and 1 tablespoon sugar) in a bowl. Two eggs were whisked together with 3 cups buttermilk, and 3 tablespoons of melted butter were mixed into the wet ingredients. Finally, the wet ingredients were added to the dry ingredients and mixed together until just combined. Pancakes were cooked on a medium high temperature (ca.145°C) teflon skillet lubricated with unsalted butter, for about 2 minutes per side.

4.3.2 Buttermilk pancake characterization

The batter thickness was analyzed by depositing 30 mL of batter into a 50 mL graduated conical bottom centrifuge tube (Fisher Scientific, Suwanee, GA) using the TA-XT2i Texture Analyzer (10 mm persplex probe; 1 mm/sec pre-test speed; 1 mm/sec test speed; 10 mm/sec post test speed; probe distance of 10 mm; 1. 0g trigger force; 400 points per second). The batter weight per pancake (1.125 fluid oz) was measured and the after cooling, the average spread of the pancake was determined by placing four pancakes side by side and measuring their average diameter. The height and weight of the products was measured along with color (HunterLab ColorFlex Colorimeter) after cooking. The softness of pancakes was determined using the TA-XT2i texture analyzer with a 25 mm Perspex probe using 40% strain, 1 mm/sec pre-test and post-test speed, with a 5 g trigger force taking 400 pps.

4.3.3 Banana muffin formulation

Banana muffins (De Laurentiis, 2010) were formulated via the muffin method. All dry ingredients (3 cups flour, 1 teaspoon baking soda, 1 teaspoon salt, ½ teaspoon baking powder, ½ teaspoon ground cinnamon, ½ teaspoon ground nutmeg) were mixed together in a bowl, In a separate container, all the wet ingredients (2 cups sugar, 1 cup vegetable oil, 3 eggs, 1 teaspoon vanilla, 4 ripe mashed bananas) were mixed together. The wet ingredients were then added to the dry ingredients and mixed until just combined. All muffins were baked in conventional muffin pans lined with muffin liners sprayed with non-stick cooking spray at 325°F in a conventional oven for 20-25 min, then cooled for 5 min prior to cooling on a rack.

4.3.4 Banana muffin characterization

The batter was tested for overall thickness in the same manner as the pancake batter. The average weight of the batter was recorded (1.687 fl. oz). The height and weight of the muffins were measured upon reaching room temperature. The color measurements of the tops and the inside were conducted using the HunterLab ColorFlex Colorimeter. The hardness and springiness of muffins was measured using the TA-XT2i Texture Analyzer (2 cm x 2 cm x 3 cm sample, 25 mm texture probe). The maximum force (hardness) was attained at 25% compression of the product height and applied for 30 s following the equation:

Equation 2: $$Springiness = \left(\frac{F(t=30s)}{F_{max}}\right) \times 100$$

Where:
F = force
t = time

4.4 Scanning Electron Microscopy (SEM)

Scanning electron micrographs (SEM) was employed to see the properties of the three flours studied along with the final structure of the muffin products. SEM was run at the University of Maryland NanoCenter at its NispLab using the Hitachi SU-70 (Tokyo, Japan). Flours were placed directly upon stainless steel coupons equipped with an adhesive tape. Flours were photographed at varying magnifications. Baked muffins were allowed to cool to room temperature prior to being crumbled by hand and frozen. Muffin crumbs were freeze dried prior to running SEM. Muffin samples were placed on stainless steel coupons equipped with adhesive tape and then gold plated. Muffins were observed under varying magnifications to assess protein structure and starch interactions.

4.5 Sensory analysis

Consent from the University of Maryland Institutional Review Board was obtained to run sensory on human subjects. To assess overall product acceptance of the banana muffin, a 9-point hedonic test was employed. Volunteers (n = 68) for sensory analysis were college aged students enrolled at the University of Maryland ranging in age from 18-34. Panelists were informed of possible allergens and to not participate if they had any sensitivities to the ingredients used in the sample preparation. Criteria for banana muffins were assessed on a likeness scale ranging from “dislike extremely” to “like extremely” with increments between the two extremes, along with a 5 point scale rating the intensity of beany flavor (Appendix 1). Panelists were presented with water and saltine crackers for palate cleansing. Samples were presented to the volunteers one at a time along with a corresponding sensory sheet. After assessment of one of the samples, tasters were then given instructions to cleanse their palates and then they were given a new sample. Banana muffins were prepared as previously described, however they were baked in standard miniature muffin tins for ease of sample preparation and distribution. Results obtained from the sensory analysis were analyzed comparing overall acceptance of the control against CGF muffins and AF muffins.

4.6 Statistical analysis

Results from all of the tests were collected and calculated as means ± SEM from quintuplet sampling. Analysis of variance (ANOVA) was run to determine variance of the means with Pr > F at 0.05, followed by Tukeys test using SAS 9.2 software (Cary, North Carolina) with a $p < 0.05$ to assess significance within all of the sample means. Sensory data underwent additional ANOVA with interactions, followed again by Tukeys test as previously mentioned.

4.7 Photography

All visual photography of products was taken using a Canon Rebel X DSLR camera using an 18 – 35mm Canon lens. Additional lighting for the products was supplied by spotlight lamps. Products were photographed on 1cm square graph paper for ease of comparison.

Chapter 5: Results and Discussion

5.1 Formulation of AF

Gluten is an important component of wheat flour and is credited with the elastic, cohesive structure found in bakery goods (Gallagher et al, 2004). To create an optimal composition for a gluten free flour, the positive aspects of gluten had to be mimicked, which means the composition of okara had to be taken into account prior to optimization of the product. Wheat derived all purpose flour contains an average of 10 – 13.5% protein along with 76.3% carbohydrates with 2.7% dietary fiber (McGee, 2004; USDA Nutrient Database, 2010). As previously noted, the protein content of okara can vary from 25-37% with high contents of fiber (40.2 -43.6% insoluble fiber, 12.6 – 14.6% soluble fiber). To decrease these high levels of fiber and protein, rice flour and tapioca starch were employed. This served the dual purpose of increasing the starch content of the flour and decreasing the overall protein content to levels more similar to all purpose flour. This also serves the purpose of increasing the starch content of okara and decreasing its fiber content. Okara has a high water holding capacity (Table 5.2), in part due to its high protein content. Adding inulin, a soluble dietary fiber (Niness, 1999), decreases the overall viscosity of the flour formulation and to increases the humectancy of the final baked products (Niness, 1999). Additionally, as gluten free products diets tend to lack good sources of fiber (Gallagher et al., 2004), additions of inulin, along with okara's already high fiber content, make the alpha formulation a possible alternative for celiacs who may be lacking a good source of fiber in their diet.

To impart the cohesiveness and elasticity indicative of gluten, xanthan gum was employed. Gums and hydrocolloids are commonly used in gluten free baked products to help mirror the crumb structure and air pockets created in part by interactions between gluten (protein) and starch (Gallagher et al., 2004). Additions of 1% have been shown to increase the volume of baked goods (Miller and Hoesney, 1993; Gallagher et al., 2004). Xanthan is used in the formulation to aid in the cohesive properties of the batter and help generate a more uniform crumb structure (Miller and Hoseny, 1993; García-Ochoa, 2000). Calcium carbonate, along with cream of tartar, was added to the formulation to aid in leavening. As okara tends to be dense when wet, extra leavening in the flour itself will aid the products made with AF in gaining more volume and in turn, apparent fluffyness.

Table 5.1: Flour formulation criteria

	Nutritional Attributes					Physical Attributes			
Ingredient	Protein	Starch	Fiber	Fat	Minerals	Texture	Leavening	Crust	Extensibility
Okara	X		X	X	X	X			
Rice Flour	X	X				X		X	
Tapioca		X				X			
Inulin			X						X
CaCO3					X		X		
C.O.							X		
Tartar									
Xanthan						X			

Table represents formulation of AF and contributions from each specific component

5.2 Flour properties

The alpha formulation was significantly different than the control AP flour and CGF in density, water activity and color measurements. The density of the alpha formulation is less than both the control and CGF, indicating that for the same amount of volume, less flour is needed. This can be beneficial from a shipping perspective as less dense materials are cheaper to ship. Additionally, it helps decrease overall costs for a product as less flour is needed by weight for the same amount of product. The high protein content and high fiber content in okara increases its water holding capacity (WHC) as seen in table 5.2, which leads to the alpha formulation having a higher WHC, which then ultimately leads to products that are more viscous with harder textures. The WHC of the other flours studied are significantly lower than those of the okara (7.459 ± 0.095, not reported) and the alpha formulation, which correlates to products made from those flours having softer textures.

The water activity of the alpha formulation falls between the control and CGF, indicating that its shelf life and microbial stability is similar to those products already on the market. Using a CIE L*a*b* color scale, it was found that the alpha formulation is lighter in color ($L = 92.26 \pm 0.05$; where 100 indicates pure white) than the control flour and CGF. The lightness of the flour consistently leads to products that are lighter in color than the control. However, lightness of a product can often be controlled by length of cooking time allowing products to brown more in the oven or on the stove. Additionally, the okara flour tended more towards the green color in the a* value ($a = -0.72 \pm 0.02$; negative a* values indicate green colors) whereas the control and the CGF both had a* values in the positive direction indicating more redness. Although these values are different, it is important to note that none of the a* values vary widely from 0, which makes the differences so small that they are hard to perceive with the naked eye. The b* value for the alpha formulation (10.07 ± 0.17) falls within the b* values for the control and CGF. The b* value indicates blueness (-b*) and yellowness (b*). All of the flours have positive b* values, which are associated with a more yellow color, and the alpha formulation falls between the two commercially available flours.

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b* values, which are associated with a more yellow color, and the alpha formulation falls between the two commercially available flours.

Table 5.2: Flour properties

Parameters	Control	CGF	AF
Density (g/ml)	0.73 ± 0.04[a]	0.76 ± 0.03[a]	0.50 ± 0.12[b]
a_w	0.434 ± 0.001[c]	0.547 ± 0.006[a]	0.488 ± 0.002[b]
Color			
L*	90.58 ± 0.41[b]	87.83 ± 0.24[c]	92.26 ±0.05[a]
a*	0.26 ± 0.03[b]	0.70 ± 0.05[a]	-0.72 ± 0.02[c]
b*	8.45 ± 0.09[c]	15.89 ± 0.13[a]	10.07 ± 0.17[b]
Water Holding Capacity ($g\ H_2O$/g flour)	0.825 ± 0.053[c]	1.054 ± 0.004[b]	2.693 ± 0.033[a]

Values with the same superscript along the same row are not found to be significantly different using Tukey's test, n = 3, p>0.05

5.2.1 Nutritional properties of flour

The AF was sent to a commercial laboratory (rtechlabs®) for nutritional analysis. Proximate analysis of all three flours gave very different results. As expected with the control flour, it had a protein content around 10%, as most all purpose flours have a protein content ranging from 10-12% (McGee, 2004). Both AF and CGF had similar protein contents and had almost 9% protein. Products with 6.25g of soy protein or more may qualify for a health claim as stated by 21CFR101.82. The ingredients in AF that donate significant protein are both the okara and the rice flour and although it may not qualify as a high soy protein food, a claim stating the amount of soy protein provided per serving would be appropriate for the package (21CFR101.82). Moisture content for the control was close to 12% while AF had a lower moisture content (9.42%). This is likely one reason that many of the products made with AF were harder as they needed to compensate for lower starting moisture contents. Fat content of the control flour was very low, being less than 1% per 100g of product. As okara is a derivative of soy, and soybeans can have high fat contents based on variety (Liu et al., 1995), it is expected to find AF having a high fat content for flours (3.77%). The CGF, which is composed of garbanzo bean flour, potato starch, tapioca flour, white sorghum flour, and fava bean flour, also had a high fat content (2.94%). Raw garbanzo beans have 6.04g of fat per 100g (USDA Nutrient Database, 2010), and the same company that produces CGF also has a garbanzo bean flour, and it states its fat content to be 2.00g/30g serving, correlating to 6.66g/100g, which is close to the reported amount by the USDA. As garbanzo bean flour is the first ingredient listed in the ingredient list on the commercial flour, it is likely that most of the fat can be attributed to it. Sorghum flour also contains a significant amount of fat containing 3.29g/100g (USDA Nutrient Database, 2010). These two ingredients that are used in the CGF formulation lead to the fact that CGF contains more fat than the control.

The overall carbohydrate content of the control flour was found to be quite high (76.31%), however this is expected as all purpose four comes mainly from the endosperm of the wheat kernel and therefore would have a high carbohydrate content (Charley and Weaver, 1982). AF was found to have a similar carbohydrate content, yet less than the control (71.53%) and more than CGF (64.70%). The most drastic differences among the flours was the total dietary fiber content, where AF was found to have almost 8 times more fiber than the control and almost 2.5 times more fiber than CGF. As many individuals suffering from celiac disease tend to have diets deficient in fiber (Moore et al., 2003; Gallagher et al., 2004), the fiber content of AF makes it a valuable product to use in gluten free baked goods. High fiber foods have been approved by the FDA to carry a health claim if the food is a grain, fruit or vegetable and if the product is low in fat (21CFR101.76). Low fat foods must contain less than 3g of fat per serving (21CFR101.62) and the reference serving size for flour products is 30g (21CFR101.12) making the amount of fat per serving of AF to be 2.63g. Additionally, to be considered a high fiber product, AF would require 20% or more the dietary reference intake for fiber per serving (21CFR101.54). AF contains 6.43g of dietary fiber per serving and the DRI for fiber ranges from 21g-38g per day depending on age and gender (USDA NAL Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acid, 2002). This would make AF a high fiber food for many individuals thereby making AF eligible to hold a health claim for fiber.

Moreover, AF has other benefits that make it valuable for consumers, such as omega-3 fatty acids (0.29%), polyunsaturated fatty acids (2.13%), and monounsaturated fatty acids (0.79%). Omega 3 polyunsaturated fatty acids (PUFA) have been linked to increased health effects such as decreased risk of cardiovascular disease, anti-inflammatory effects, and proper brain development (Ruxton et al., 2004). A closer look at the fatty acid profile of AF shows 51.2% of the fatty acids being linoleic acid, an essential fatty acid needed for proper health and a precursor to arachidonic acid, along with 7.9% linolenic acid, another essential fatty acid and a precursor to both docosahexanoic acid (DHA) and eicosapentaenoic acid (EPA) (Ruxton et al., 2004). Both of these fatty acids are essential in a balanced diet as the human body is unable to synthesize either of these (Ruxton et al., 2004). Other fatty acids found in AF include palmitic acid (13.5%), stearic acid (4.0%), and oleic acid (21.6%).

Finally, AF contains 1850mg of calcium and 3.85mg of iron per 100g. Calcium is a necessary mineral for proper bone health and increased bone density, and is especially valuable for individuals at risk for osteoporosis (21CFR101.72). Depending on and individual's and and gender, calcium intake dietary reference intakes can vary from 1000mg – 1300mg. With AF having 555mg of calcium per serving, it can be considered "high in calcium" or "an excellent source of calcium" (21CFR101.54). Iron is a vital mineral in the human body and is needed for a variety of different functions such as oxygen transfer, DNA synthesis and electron transport (Lieu et al., 2001). A lack of iron in the diet can lead to anemia in extreme cases and in less extreme cases fatigue, poor athletic performance, impaired ability to maintain body temperature in a cold environment and more (Craig, 1994). Vegetarians are thought to be at risk for iron deficiency as iron is better absorbed from animal sources (Craig, 1994). Unfortunately, both soy protein and calcium inhibit iron absorption in food (Craig, 1994). Therefore, although it seems like an asset to for AF to have naturally occurring iron, it is not as valuable as it seems due to the high amount of calcium present in AF along with the inherent property of being a soy product, both of which work against iron absorption, especially from plant sources (Craig, 1994).

Table 5.3 Proximate analysis of flours per 100g

	Control*	CGF**	AF
Moisture (g)	11.92	N/A	9.42
Total Calories	364	294.12	355
Protein (g)	10.33	8.82	8.77
Total Fat (g)	0.98	2.94	3.77
Carbohydrate, by difference (g)	76.31	64.70	71.53
Fiber, total dietary (g)	2.7	8.82	21.43
Sugars (g)	0.27	2.94	~1.2
Total Ash (g)	0.47	N/A	6.51

*Values from USDA Agricultural Research Service, 2010
**Values from CGF nutritional label calculated to represent 100g

Table 5.4 Micronutrient Composition of AF

Description	Value per 100g db*
Calcium	1850mg
Iron	3.85mg
Sodium	112mg
Vitamin A (Retinol), by HPLC	<40 IU
Vitamin C	<1.0mg

*db – Dry basis

Table 5.5 Composition of sugar in AF

Sugars, by HPLC	% in Sample
Fructose	0.2
Glucose	<0.1
Lactose	<0.1
Maltose	<0.1
Sucrose	1.0

Table 5.6 Fat composition of AF sample

Fat Composition	% in Sample
Total Fat (g)	3.77
cis-monounsaturated fat	0.79
Cis – polyunsaturated fat	2.13
Omega 3 fat	0.29
Saturated fat	0.68
Fatty Acid Composition (% of Total Fat)	
Linoleic (C18:2)	51.2
Oleic (C18:1)	21.6
Palmitic (C16:0)	13.5
Linolenic (C18:3)	7.9
Stearic (C18:0)	4.0

5.3 Dough formulations

To assess the ability of the alpha formulation to function well in baked goods, different products were created and tested on several attributes. Baked goods that fall in the dough category are those that are dryer and can be rolled and molded into different shapes prior to baking and includes products such as biscuits and cookies along with breads (McGee, 2004). For the purpose of this study, biscuits and cookies were evaluated using the control all purpose flour, CGF, and AF.

5.3.1 Biscuits

When compared against the control and CGF, the alpha formulation biscuits were significantly shorter indicating poor rise while baking. The pre bake weight (44.54 g ± 1.12) was significantly less than that of both the control and CGF, however, this can be expected due to the alpha formulation being less dense than either the control or CGF. It is interesting to note that although the alpha formulation has both more protein and fiber

(Table 5.7) it lost significantly more moisture during baking (6.37 g ± 0.12). This may indicate poor network formation between the starch and protein, allowing moisture to escape the product during baking. This would correlate well with the product height after baking, as the alpha formulation was significantly shorter (2.18 cm ± 0.13) than either the control or CGF.

The success of many baked goods, especially breads and biscuits, is due to gluten's ability to form a network that can encase gasses during baking, lending to product height and volume (Gallagher et al., 2004). A poor protein/starch network would be unable to expand and grow in the oven as the gasses would escape. This is also in line with the height and moisture loss of the CGF biscuits which also had poor height after baking (3.08 cm ± 0.08) and a large amount of moisture loss (6.0 g ± 0.15). Biscuits in America are characterized by crusty outsides and either fluffy or tender interiors (McGee, 2004). As such, spread of the dough is not considered as important of a quality characteristic as much as height is, with tall biscuits with smooth, even sides being considered fluffier and more desirable (Charley and Weaver, 1998). Regardless, spread was measured in all of the biscuits and it was found that the alpha formulation and the control were not significantly different. Color of the biscuits (both tops and bottoms) was measured using CIE L*a*b* and it was found that alpha formulation biscuits were lighter (L* = 73.25 ± 0.95) than both the control and CGF. This is likely due to the alpha formulation being lighter in color to the other two flours, thereby imparting more lightness to the alpha biscuits. However, lightness is variable and dependent on baking time (longer baking times allow for more browning reactions to occur), and if the product contains any additional coating such as sugar or egg whites to aid in browning. Another possible reason for lighter tops is that increases in moisture content increase Maillard browning reactions (Martins et al., 2001), and as the alpha formulation lost the most moisture during baking, it would have the least amount of Maillard browning.

The bottom of the biscuit in the alpha formulation was significantly darker ($L^* = 45.37 \pm 3.16$) than either the control or CGF. This could potentially indicate that the alpha formulation is able to conduct heat better allowing for more darkening against the metal baking sheet. One possible explanation for this is that okara is higher in fat compared to other flours (Table 5.3). A higher fat content can reach higher temperatures and allow for increases in the Maillard browning reactions (Martins et al., 2001), leading to the bottom of the biscuits being darker. The values for a* in the top crust of the biscuits was not significantly different for any of the products and they all tended towards the red values. Although the a* values for the bottom of the biscuits were significantly different, they tended towards the red spectrum and were not perceptively different. The b* value for color was not found to be significantly different for any of the biscuits.

As evidenced by the higher water holding capacity of the alpha formulation, the uncooked biscuit dough for the alpha formulation was found to be significantly harder than either the control or the CGF. This hardness carried through in the baked product and the alpha formulation exhibited the greatest amount of hardness after baking. A hard biscuit is not desirable for consumers (McGee, 2004), and as such, the alpha formulation for use in a biscuit would not be recommended.

5.3.2 Snickedoodles

Snickerdoodles, a sugar cookie with no particulate matter within the batter, (Gand, 2010), was made with the alpha formulation flour and compared to cookies prepared with the control AP flour and CGF. Height after baking was found to be significantly different in the alpha formulation being taller (1.59cm ± 0.04) than both the control and CGF, which correlates with the alpha formulation having less spread after baking (6.21cm ± 0.17). Like the biscuit, this is due in part to the alpha formulations higher WHC. Mass of the cookies prior to baking and post baking was not significantly different throughout all three flour varieties. This falls in line with the moisture loss not being significantly different either. However, it is important to note that the alpha formulation yielded a product with a greater mass than either of the other two cookies. This may indicate an interaction between the alpha formulation and the egg protein in the cookie formulation causing a tight water holding network that keeps water and gases trapped in the product instead of releasing them into the environment (Stadelman, 1999; McGee, 2004). This correlates with the evidence from the biscuits which do not contain egg protein and therefore lost more moisture when baking.

Cookie dough texture prior to baking was found to be significantly harder (279.164 ± 7.024 g) than either the control or CGF, while CGF was found to be softer (41.592 ± 1.942g) than the control (63.89 ± 1.582g). The low WHC of CGF along with its lack of gluten formation contributes not only to its softness in dough texture, but also to its significantly greater spread (9.35cm ± 0.33). The hardness of the AF can be attributed to its higher WHC, which is in line with the results found form the biscuit dough. This hardness mirrored to the final baked product in hardness, being significantly harder (3613.599g ± 448.063) than either the control or CGF. Fracturability of the baked snickerdoodle (measured as the distance to break the cookie) was found. The control and CGF had similar fracturability (1.635g ± 0.308 and 1.185g ± 0.204 respectively) whereas AF had less fracturability (2.367 g ± 0.192) requiring more distance to break. This indicates that the cookie made with AF had a stronger protein and starch network with less water available for spreading that in turn led to a thicker, denser cookie with less snap and fracturability.

The color of the top of AF snickerdoodles had a larger L* value (L* = 59.64 ± 2.87) indicating more lightness. This is expected, as AF is lighter in color (Table 5.4) than the control and CGF. For a bakery product, where darkness and crust color is often dependent on bake time, lightness is a variable that can be controlled. As opposed to the biscuit where the L* value was significantly darker in the AF biscuit, the L* value for the bottom of the cookies was lighter (L* = 46.44 ± 1.32) than either the control or CGF, being found significantly different ($p < 0.05$).

A likely cause for this is the bake time for the cookies versus the biscuits (9-11 min vs. 20-25 min respectively). The longer bake time for the biscuits allows for more browning to occur especially on the bottoms of the products that have direct contact with a heated surface. The a* values for both the tops and the bottoms of the cookies were significantly different for all of the flour varieties tested, however they were all within a similar range tending towards the red (positive a* values between about 13 and 18) and being imperceptibly different to the naked eye. The b* value indicating blueness or yellowness was not significantly different in the bottoms of the cookies, however for the tops of the cookies, the control was significantly different than both CGF and AF tending more towards the blue range (b* = 33.11 ± 0.92). Regardless, all b* values were in the yellow range. Similarities in color for all dough products, indicates that AF would be as suitable gluten free alternative for all purpose flour in dough products in regards to color. However, due to its high WHC, the textures of all dough products along with its physical dimensions was not successful. Therefore it is suggested that AF undergo further development before utilization of AF in dough bakery products.

Table 5.7: Dough product characteristics

	Control	CGF	AF
Biscuit			
Height (cm)	4.06 ± 0.36 [a]	3.08 ± 0.08 [b]	2.18 ± 0.13 [c]
Weight (g)			
Pre bake	47.80 ± 1.51 [a]	46.77 ± 2.07 [ab]	44.54 ± 1.12 [b]
Post bake	43.06 ± 2.55 [a]	40.77 ± 1.94 [ab]	38.17 ± 1.09 [b]
Difference	4.74 ± 1.48 [b]	6.0 ± 0.15 [ab]	6.37 ± 0.12 [a]
Spread (cm)	5.48 ± 0.33 [b]	6.38 ± 0.08 [a]	5.16 ± 0.09 [b]
Color (top)			
L*	68.66 ± 2.67 [b]	68.19 ± 1.42 [b]	73.25 ± 0.95 [a]
a*	14.26 ± 1.22 [a]	9.39 ± 0.96 [a]	9.79 ± 0.45 [a]
b*	32.28 ± 5.79 [b]	35.49 ± 1.18 [a]	35.47 ± 1.04 [a]
Color (bottom)			
L*	54.59 ± 3.06 [a]	50.85 ± 1.13 [a]	45.37 ± 3.16 [b]
a*	15.64 ± 1.29 [b]	16.39 ± 0.73 [ab]	17.72 ± 0.65 [a]
b*	35.34 ± 1.13 [a]	35.34 ± 1.52 [a]	32.75 ± 2.16 [a]
Texture			
Dough hardness (g)	107.751 ± 5.61 [b]	95.058 ± 2.841 [b]	288.867 ± 14.291 [a]
Hardness (kg)	1.309 ± 0.11 [b]	1.37 ± 0.147 [b]	5.715 ± 0.25 [a]
Snickerdoodle			
Height (cm)	1.10 ± 0.08 [b]	0.98 ± 0.05 [c]	1.59 ± 0.04 [a]
Weight (g)			
Pre bake	21.00 ± 0.49 [a]	21.58 ± 0.48 [a]	21.44 ± 0.41 [a]
Post bake	17.10 ± 2.37 [a]	18.82 ± 0.48 [a]	19.20 ± 0.33 [a]
Difference	3.91 ± 2.18 [a]	2.76 ± 0.07 [a]	2.24 ± 0.08 [a]
Spread (cm)	8.06 ± 0.17 [b]	9.35 ± 0.33 [a]	6.21 ± 0.17 [c]
Color (top)			
L*	48.94 ± 0.99 [c]	54.43 ± 1.52 [b]	59.64 ± 2.87 [a]
a*	16.51 ± 0.30 [a]	14.80 ± 0.80 [b]	13.67 ± 0.24 [c]
b*	33.11 ± 0.92 [b]	36.23 ± 1.01 [a]	35.93 ± 0.70 [a]
Color (bottom)			
L*	42.57 ± 1.94 [b]	42.48 ± 1.28 [b]	46.44 ± 1.32 [a]
a*	17.28 ± 0.15 [b]	17.73 ± 0.35 [a]	16.42 ± 0.25 [c]
b*	31.97 ± 1.28 [a]	32.71 ± 0.57 [a]	31.26 ± 0.90 [a]
Texture			
Dough hardness (g)	63.890 ± 1.582 [b]	41.592 ± 1.942 [c]	279.164 ± 7.024 [a]
Hardness (g)	2896.528 ± 291.410 [b]	1755.578 ± 358.360 [c]	3613.599 ± 448.060 [a]
Fracturability (mm)	1.635 ± 0.308 [b]	1.185 ± 0.204 [b]	2.367 ± 0.192 [a]

Average ± SD n = 5; Values with the same superscript along the same row are not significantly different using Tukey's test, p>0.05

5.4 Batter products

As opposed to a dough, a batter is characterized by its high water content and fluid formulation (McGee, 2004). Batters rely less on gluten for structure and instead depend more heavily upon starch for proper crumb structure as larger quantities of liquid decrease the overall proportions of gluten and therefore hydration of starch granules becomes the primary form of structure (McGee, 2004). AF was used to make both pancakes and banana muffins to assess possible uses of AF in batters for gluten free bakery products.

5.4.1 Buttermilk pancake

After pancakes reached room temperature, dimensional data (height, weight, spread) was taken. The control and AF were not found to be significantly different in regards to height ($p < 0.05$) with a measurements close to 1 cm. Height is an important quality characteristic as tall pancakes are often associated with fluffiness as taller pancakes will have more trapped air which leads to taller pancakes (Siegeleman et al., 2003). A possible explanation for the successful height of the AF pancakes is its WHC, which allows the pancakes to retain moisture and volume when cooking. As the pancakes contain eggs in the ingredients, a desirable interaction between the egg protein and the AF may be occurring that increases its textural properties and its ability to trap air. Egg protein has the ability to trap air as evidenced in several egg foam uses from angel food cake to meringue (McGee, 2004), as such the egg protein in the batter may aide AF in creating a structure capable of withholding gases while cooking.

However, as the AF pancakes had the least amount of spread (7.1 cm ± 0.24), a likely reason for AF's height is that the batter did not spread well when poured on the pan to cook, which yields a product with more vertical height as there is less horizontal movement. Poor horizontal movement is in line with the high WHC of the AF flour, which generates a very thick batter. The data from AF is consistent with the CGF pancakes that had a thinner batter and therefore the least amount of height and the greatest spread. The mass of the pancakes pre cooking was significantly different between all of the batters (Table 5.8) with CGF having the lowest mass and AF having the greatest mass, which can be attributed to the high WHC of AF even though AF has the least dense flour. As batters have higher quantities of liquid, AF has a greater opportunity to gain mass from water as it holds the most water per gram of flour. Post cooking weight of the control and AF was not found to be significantly different ($p < 0.05$) and CGF was found to have the lowest mass of all the flours. Moisture loss for the pancakes was the greatest in AF (8.66 g ± 3.66) with the control having the least amount of moisture loss.

A large volume of lost moisture indicates a poor protein starch network, with water and gas able to escape and poor fiber hydration (Sangnark and Noomhorm, 2004). As mentioned earlier, batters as opposed to doughs, depend more on starch for structure. As such, large quantities of moisture being lost can indicate that the starches and the fiber inherent in the AF batter are not absorbing enough moisture and retaining it throughout the cooking process. It is likely that due to the method of mixing for pancakes, where the dry components and wet components are kept apart until the end, negatively influenced the WHC of the AF flour. The wet portion of the pancake batter contains egg proteins, sugar, and butter, all which may interfere with the ability of the starches and fiber in AF to properly absorb liquid and remain hydrated during the cooking process, and instead, the pancake lost large quantities of moisture while cooking. Pancakes made with the control flour have the addition of gluten to help form a structure and trap air, yielding the tallest, fluffiest pancakes (McGee, 2004). Pancakes made with CGF, were less viscous than the other two batters (Table 5.8) and therefore the batter spread more thinly on the pan when poured. This increased surface area, decreased vertical height, and allowed for more surface area for evaporation of liquid.

Pre cooking batter texture was significantly different for each of the batters with CGF having the softest batter (3.268 g ± 0.088) and AF having the hardest batter (21.472 g ± 0.441) again likely due to the differences in WHC. Post cooking texture was measured and AF had a significantly harder texture (282.008 g ± 77.901) than either the control or CGF. Regardless of the hard texture of the AF pancakes, it is important to note that they also had the greatest variability in the samples as evidenced by its standard deviation (45.12g), indicating that some of the pancakes were not consistent in their textures. This may be linked to uneven cooking in the product and areas where the pancake did not spread as well causing some parts of the product being thicker and softer and other parts being thinner and harder. Uneven texture is indicative of a batter that does not spread well when poured, which may indicate a competitive nature of the fiber to bind water, decreasing available water for protein and starch interactions, resulting in a poor structure matrix (Tudorica et al., 2002; Siegelman et al., 2003). This may make the AF undesirable at this point for continued use in pancake batters. Thinning of the batter with milk or buttermilk would generate a more fluid product that would have more even texture after baking. Additions of extra dairy would mostly be up to the end user or the producer. Future formulations are required to decrease viscosity and thereby yield pancakes with more flow, spread and even texture. However, as CGF was not as successful as AF in achieving height, AF is the more desirable gluten free flour for pancakes from the perspective of fluffiness.

5.4.2 Banana muffins

The height of the banana muffins made with AF (5.32 cm ± 0.19) was not found to be significantly different from the control ($p > 0.05$, table 5.8) and had the maximum height of all three flour varieties tested. A possible explanation for this height is an interaction of the AF with the egg protein in the batter, allowing the muffin to form a strong crumb structure in the oven, trap gases during baking thereby increasing height, and maintain its structure after cooling. Another possibility is the amount of fiber within the AF combined with a long bake time in the oven allows the fiber in AF a greater opportunity to bind with, swell and retain water (Sangnark and Noomhorm, 2003), contributing to a sought-after crumb structure that can add overall height in the finished product. This is further supported by moisture loss and final product mass in the AF muffin, having a similar moisture loss (5.55 g ± 2.58) and baked product weight (57.01 g ± 1.64) as the control and CGF, with all of the products having similar masses before baking. Additionally, as muffins are contained within a rigid structure with only one surface open to the oven (the top), it is likely that less water is able to escape from the muffin. Less evaporation from the muffin top translates to a more moist product with better textural characteristics, increased product mass, and better product height.

Analysis of the color of the muffin tops yielded L* values that were not significantly different for all of the flour varieties tested ($p > 0.05$, n=5), with AF having an L* value between the control and CGF (Table 5.8). The values for a* in the muffin tops found that AF had significantly more redness (a* = 15.59 ± 1.01) than either the control or CGF. However, it was not so different as to cause a perceptible color difference to the naked eye. The b* value in the muffin tops was not significantly different between AF and the control. Internal crumb color was also measured using the L*a*b* color scale and it was found that in both a* and b* color values, none of the products were significantly different. The L* value, which indicates lightness, was only significantly different for CGF, which is expected as the CGF flour is darker than either the control and AF. The control flour was found to be between CGF and AF in regards to lightness and AF had the lightest crumb color (L* = 56.21 ± 1.62). The texture of the raw muffin batter was found for each of the flours tested. All of the batters had significantly different textures ($p < 0.05$, n=5) and AF had the hardest batter tested (Table 5.8), which can be attributed to its high WHC. The control and AF muffin were not found to be significantly different in hardness or springiness ($p > 0.05$). In both of these criteria, CGF was found to be softer in batter texture and less springy than either the control or AF.

The success of the AF muffin can be attributed to the high WHC of the AF flour. This allows large quantities of moisture to be absorbed in the batter. Additionally, as mentioned earlier, as the muffin only has one surface through which moisture can escape, the WHC of AF works in favor of obtaining a moist product with good textural qualities. The muffin also contains egg protein which can interact with the AF flour to generate a strong crumb structure, consistent with results from the cookie. A strong crumb structure allows for more springiness in the muffin as springiness is measured as compression force over a set amount of time (Eq. 2). Due to these factors, it can be stated with some certainty that AF is not only a good alternative to all purpose flour in gluten free formulations, but it out performs CGF which is currently available on the market in several supermarkets.

Table 5.8: Batter products

	Control	CGF	Okara alpha
Pancakes			
Height (cm)	0.98 ± 0.13 [a]	0.48 ± 0.08 [b]	0.90 ± 0.19 [a]
Spread (cm)	8.2 ± 0.2 [b]	9.5 ± 0.1 [a]	7.1 ± 0.2 [c]
Weight			
Pre bake (g)	32.39 ± 1.09 [b]	28.03 ± 0.79 [c]	36.62 ± 0.95 [a]
Post bake (g)	28.13 ± 0.86 [a]	21.74 ± 1.24 [b]	27.96 ± 3.89 [a]
Difference (g)	4.25 ± 1.72 [b]	6.29 ± 1.29 [ab]	8.66 ± 3.66 [a]
Color			
L*	46.24 ± 6.87 [b]	62.34 ± 3.51 [a]	55.71 ± 5.10 [a]
a*	14.26 ± 1.22 [a]	8.81 ± 2.52 [b]	11.95 ± 2.05 [ab]
b*	32.28 ± 5.79 [a]	37.45 ± 0.91 [a]	32.18 ± 4.21 [a]
Texture			
Raw batter (g)	5.645 ± 0.827 [b]	3.268 ± 0.088 [c]	21.472 ± 0.441 [a]
Hardness (g)	210.280 ± 12.836 [a]	226.519 ± 35.248 [a]	282.008 ± 77.901 [a]
Muffins			
Height (cm)	4.42 ± 0.14 [ab]	4.26 ± 0.09 [b]	5.32 ± 0.19 [a]
Weight			
Pre bake (g)	62.52 ± 2.68[a]	61.47 ± 0.52[a]	62.56 ± 1.70[a]
Post bake (g)	57.49 ± 2.51[a]	56.06 ± 0.61[a]	57.01 ± 1.64[a]
Difference (g)	5.03 ± 4.84 [a]	5.41 ± 0.98 [a]	5.55 ± 2.58 [a]
Color (crust)			
L*	48.81 ± 1.59 [a]	45.58 ± 0.71[b]	46.83 ± 2.19 [ab]
a*	13.95 ± 0.57 [b]	13.33 ± 0.35[b]	15.59 ± 1.01 [a]
b*	29.63 ± 1.18 [a]	27.27 ± 0.20 [b]	30.33 ± 1.35 [a]
Color (crumb)			
L*	51. 44 ± 2.56 [a]	45.78 ± 3.68 [b]	56.21 ± 1.62 [a]
a*	7.63 ± 0.52 [a]	6.94 ± 0.33 [a]	7.03 ± 0.90 [a]
b*	24.03 ± 0.69 [a]	22.76 ± 1.09 [a]	23.15 ± 2.29 [a]
Texture			
Raw batter (g)	10.564 ± 0.417 [b]	6.074 ± 1.348 [c]	26.858 ± 3.327 [a]
Hardness (g)	239.73 ± 21.77 [a]	138.86 ± 18.50 [b]	236.29 ± 45.12 [a]
Springiness (g)[φ]	63.19 ± 0.92 [a]	59.49 ± 0.93 [b]	64.01 ± 0.59 [a]

Average ± SD, n = 5; Values with the same superscript along the same row are not significantly different using Tukey's test, p>0.05

[φ]Values closer to 100 indicate more springiness

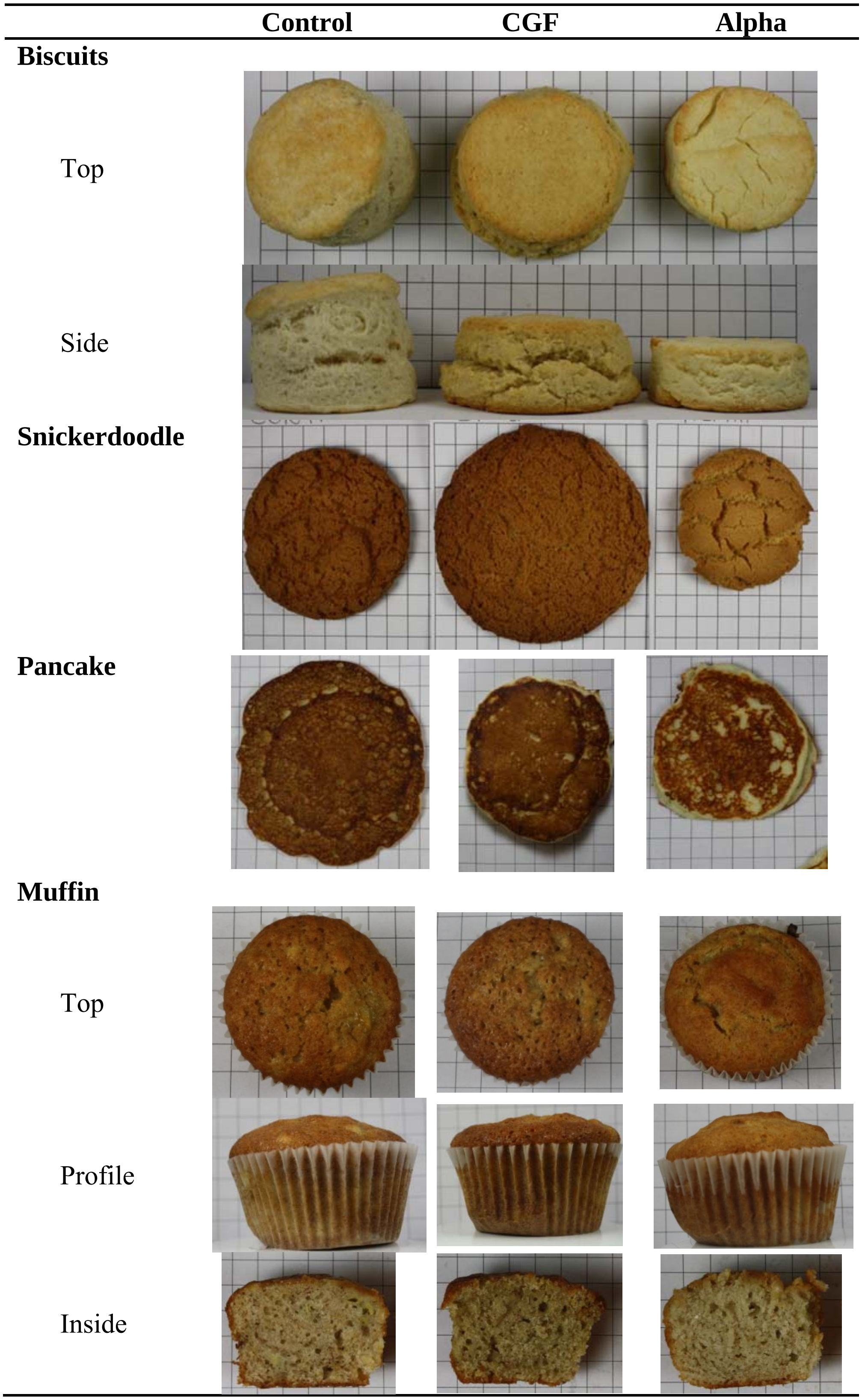

Fig. 5.1 Visual of products from different angles. Grid paper represents a 1cm x 1cm square

<u>5.5 SEM</u>

Figure 5.2 shows scanning electron micrographs of all the three flours under 300X magnification. Examination of the particles reveals that the control, A, contains rough, uneven flour particles along with smaller smooth granules. The AF micrograph shows almost all of the particles being of uneven texture and size with very few smooth, round granules, while the CGF micrograph contains mostly smooth, large granules. Rough surfaces may indicate small capillary areas that can easily absorb water and retain moisture in final products. This would correlate well with the high WHC of AF (Table 5.2), as the flour contains almost exclusively rough particles. The smooth granules of CGF may indicate a particle that shields itself from moisture absorption. This falls in line with the lower WHC of CGF than AF (Table 5.2).

A combination of rough and smooth particles in the control flour allows the control to absorb and retain water while at the same time, not be too aggressive in water binding. Further inspection of the flours shows that the particle size of AF tends to be larger than those in the control as can be seen by areas of AF being dominated by very large particles. Sangnark et al. (2004) reported that dietary fibers of larger sizes to bind water more efficiently than fibers with smaller sizes. This difference in particle size could be a reason for AF having such a high WHC. It is likely that by simply decreasing particle size, WHC can be decreased in AF and lead to more successful products, such as biscuits, cookies, or pancakes, which seemed to be adversely affected by the WHC of AF.

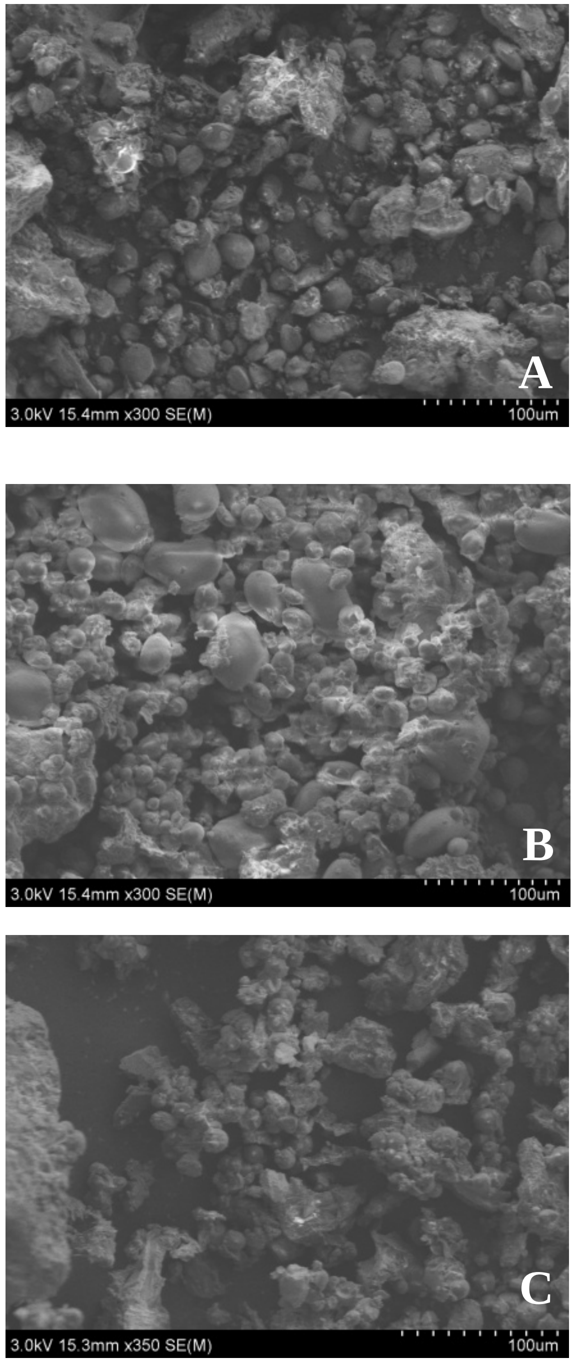

Figure 5.2: Scanning electron micrograph of different flours: control (A), CGF (B) and AF (C)

Due to the banana muffins having the most desirable textural properties, they were chosen for scanning electron microscopy in order to better examine reasons for the desirable structure. Magnification at a level of 600X revealed similar surface structures throughout the three flours tested. However, closer examination of AF shows a more open structure as evidenced by increased areas or ruptures on the surface of the structure, similar to a flaky sort of structure. A more open structure correlates with more air and thereby increased rise yielding a product with better height. The CGF micrograph has fewer of these ruptures indicating a more dense, closed structure that correlates with a product with decreased height and rise. Comparison of these images with data collected from the muffin characterization (Table 5.6) supports these findings. Additionally, when the CGF and AF micrograph images are compared to those from the control, it is evident that AF and the control have more similar textures having increased open areas and more flakiness. These images further the understanding of how the AF flour interacts with other ingredients generating a structure that is open and similar to the structure attained with the control flour.

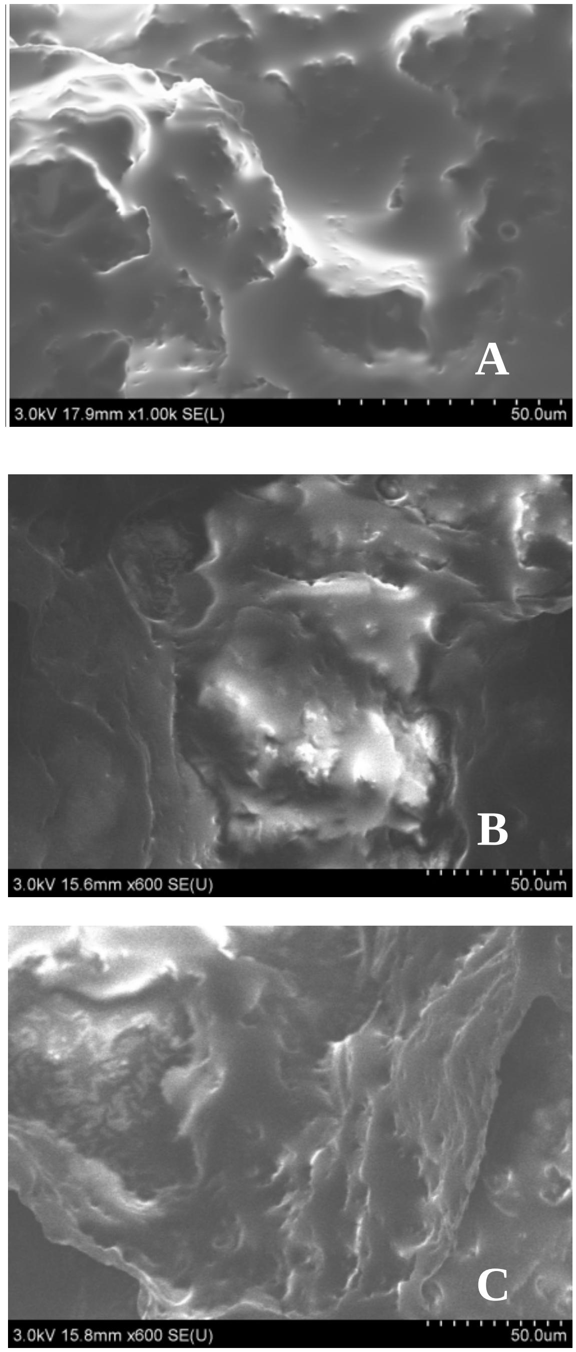

Figure 5.3: Scanning electron micrograph of banana muffins made with control (A), CGF (B) and AF (C)

5.6 Sensory analysis

Sensory testing on banana muffins on eight different attributes is presented in Table 5.7. There were 68 participants overall and almost all participants answered every question. There were very minimal instances where a volunteer did not fill in one of their answers on the sensory sheet. These data points were omitted in statistical analysis in the SAS program by indicating those few instances with having a 'missing value'. Overall, it was found that the control was liked the most overall with 83% (54 of 65 responses) of panelists responding that they like the product and 63% (41 of 65 responses) would buy the product. Out of a possible score of 9 (indicating "like extremely"), the control consistently scored at 6 or above, representing a muffin that is well liked.

On the basis of beany flavor, it was found that most individuals did not find any beany flavor (a score of 5 indicates no beany flavor). The CGF scored well in color and appearance, but did poorly in textural attributes along with flavor attributes. This translated into only 23% (15 of 65 panelists) of the panelists liking the product overall and only 14% (9 or 65 panelists) would purchase the product. This correlates with the score that CGF received for overall flavor gaining only a 4.2 ± 1.9, indicating a dislike of the overall product. Comments from the sensory panel showed agreements among most panelists of a dislike of the flavor of CGF. Additionally, CGF got the lowest score in beany flavor (3.6 ± 1.6) which correlates with more beany flavor, as a 1 would indicate very strong beany flavor. The muffins made with AF received scores similar to those of the control and none of the scores were found to be significantly different ($P < 0.05$)

except for intent to buy, and received the best score for overall appearance ranking close to a 7 (6.9 ± 1.8) on the 9 point scale. This shows that although it did not do as well on overall flavor as the control (6.1 ± 2.0 versus 6.8 ± 1.6 respectively), it did well in the overall liking of the product with 70% (45 of 65 responses) of panelists responding that they like the product and 42% (27 of 65 responses) would buy the product. Due to the control and AF not having any significant difference on all attributes tested, it can be concluded that the panelists liked the two muffins equally, making AF a good gluten free substitute.

Furthermore, although AF contains soy the sensory data showed that there was very little to no beany flavor in the product, receiving close to a 6 (4.5 ± 1.5) indicating no beany flavor and not being significantly different to the control which received a score of 4.9 ± 1.2. This data is of special significance as it reveals that the panelists were unable to classify what "beany flavor" was (Appendix B) since there were still panelists that ranked the control flour, which has no soy in it, to having some amount of beany flavor, and many panelists commented on not knowing what soy tasted like. However, as the beany flavor for AF and the control were not significantly different, it represents a well liked product with very little beany flavor.

Previous attempts to use okara in food products stated that beany flavor was a major detriment to the products and made using okara unacceptable for consumer acceptance (Genta et al., 2002, Katamaya and Wilson, 2008). With AF containing little to no beany flavor, it would be a suitable replacement for regular all purpose flour for individuals suffering from a gluten intolerance in regards to sensory perception and flavor. Moreover, the data clearly identifies AF as the superior gluten free flour in regards to flavor and texture. With a large sample size of 68 individuals, it can be said with certainty that AF can compete with the commercially available gluten free flour in batter formulations, especially muffins.

Interactions of the flours with age and gender showed few areas with significant interactions (Table 5.10, 5.11, 5.12). This indicates that overall, regardless of age or gender, we can expect to see the same preferences as in Table 5.9 for a full population, which is a full compilation of preferences without taking into considerations interactions between ages and genders. However, looking at just gender and age as stand-alone factors, it is seen that differences do occur in different age groups of crumb color, top appearance and flavor (data not shown, $p > 0.05$). Gender differences include the attributes of springiness, grain texture, moistness of muffin, overall flavor, overall likeness of the product and intent to buy, with women scoring less on all of the mentioned attributes (data not shown, $p > 0.05$). However, this data lumps together all of the scores for all of the muffin varieties suggesting that women are more likely to score products in the lower extreme pulling all of the scores down which then would appear as a significant difference between men and women. Possible future research could focus on examining reasons for females scoring all of the muffins lower.

Table 5.9 Sensory responses for 9-point hedonic test for overall acceptance

Attribute	Control	CGF	AF
Crumb color	6.9 ± 1.3 [a]	6.1 ± 1.7 [b]	6.8 ± 1.6 [a]
Muffin top texture	6.6 ± 1.5 [a]	5.2 ± 1.9 [b]	6.5 ± 1.8 [a]
Overall appearance	6.6 ± 1.5 [a]	5.1 ± 1.8 [b]	6.9 ± 1.8 [a]
Springiness	6.0 ± 1.6 [a]	4.6 ± 2.0 [b]	6.2 ± 1.8 [a]
Crumb grain	6.1 ± 1.8 [a]	4.3 ± 2.0 [b]	6.0 ± 1.9 [a]
Muffin moistness	6.1 ± 2.1 [a]	4.8 ± 1.9 [b]	6.1 ± 2.1 [a]
Overall flavor	6.8 ± 1.6 [a]	4.2 ± 1.9 [b]	6.1 ± 2.0 [a]
Beany Flavor*	4.8 ± 1.4 [a]	3.5 ± 1.6 [b]	4.5 ± 1.5 [a]
Like product**	83% [a]	23% [b]	70% [a]
Would buy product**	63% [a]	14% [c]	42% [b]

Average ± SD, n = 68; Same letter across the same row indicates no significant difference $P > 0.05$
*Based on a 6pt scale
**Based on Yes (1) or No (0) response

Table 5.10 Sensory analysis of banana muffins by flour type, age, and gender (M = male, F = female)

	Control‡						CGF§						AF†					
	17-18$^{\alpha}$		19-21		22-34		17-18		19-21		22-34		17-18		19-21		22-34	
Attribute	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Crumb color	6.7 ± 1.0^{a}	7.4 ± 1.3^{a}	6.7 ± 1.2^{a}	6.7 ± 1.3^{a}	7.6 ± 0.9^{a}	6.5 ± 1.5^{a}	5.8 ± 1.9^{a}	7.1 ± 1.4^{a}	5.3 ± 1.6^{a}	5.8 ± 1.5^{b}	6.6 ± 2.1^{a}	6.0 ± 2.1^{a}	7.4 ± 1.1^{a}	6.9 ± 1.6^{c}	6.8 ± 1.9^{a}	6.5 ± 1.5^{a}	7.0 ± 2.3^{a}	6.7 ± 0.8^{a}
Muffin top texture	6.3 ± 1.5^{a}	7.2 ± 1.2^{b}	6.5 ± 1.1^{a}	6.2 ± 1.6^{a}	7.8 ± 1.3^{a}	5.8 ± 2.4^{c}	5.3 ± 1.8^{a}	6.0 ± 2.0^{a}	4.8 ± 1.2^{d}	4.6 ± 1.6^{e}	6.4 ± 1.8^{a}	4.0 ± 2.3^{f}	6.4 ± 1.5^{a}	6.9 ± 2.1^{g}	6.9 ± 2.2^{a}	5.8 ± 1.6^{a}	7.0 ± 1.2^{a}	6.3 ± 1.0^{a}
Overall appearance	5.7 ± 1.0^{a}	7.0 ± 1.7^{h}	6.1 ± 1.2^{a}	6.8 ± 1.3^{a}	7.2 ± 1.5^{a}	5.7 ± 2.0^{a}	4.5 ± 0.9^{g}	6.1 ± 1.9^{a}	5.1 ± 1.2^{f}	4.4 ± 1.8^{e}	6.6 ± 2.1^{a}	4.7 ± 2.5^{a}	7.6 ± 1.1^{d}	6.9 ± 2.1^{c}	6.8 ± 1.9^{b}	6.5 ± 1.8^{b}	7.2 ± 1.9	6.7 ± 1.2
Springiness	5.6 ± 1.9^{a}	6.7 ± 1.1^{b}	5.4 ± 1.2^{a}	5.1 ± 1.6^{a}	7.2 ± 1.5^{f}	6.7 ± 1.9^{a}	4.9 ± 1.1^{a}	4.7 ± 1.8^{e}	5.2 ± 2.1^{a}	4.0 ± 2.2^{c}	5.6 ± 2.1^{a}	3.0 ± 1.4^{d}	6.9 ± 1.0^{g}	6.2 ± 1.5^{h}	6.1 ± 2.1^{a}	5.7 ± 1.9^{a}	7.0 ± 1.9^{i}	5.2 ± 2.3^{a}
Crumb grain	6.3 ± 1.7^{l}	6.7 ± 1.6^{k}	6.3 ± 1.6^{i}	4.9 ± 1.7^{a}	7.2 ± 2.2^{a}	5.7 ± 2.0^{j}	4.5 ± 1.1^{a}	4.5 ± 1.9^{h}	5.5 ± 1.3^{a}	3.7 ± 2.1^{f}	4.2 ± 2.4^{a}	2.5 ± 1.4^{g}	6.8 ± 1.3^{e}	6.0 ± 1.7^{d}	5.8 ± 1.9^{b}	5.6 ± 2.1^{a}	7.0 ± 2.0^{c}	4.7 ± 2.1^{a}
Muffin moistness	6.8 ± 2.6^{h}	6.2 ± 2.0^{i}	6.5 ± 1.7^{f}	4.8 ± 2.0^{a}	6.8 ± 2.2^{a}	6.8 ± 1.6^{g}	4.1 ± 1.6^{a}	5.4 ± 2.2^{a}	4.9 ± 1.2^{a}	4.6 ± 1.8^{a}	5.6 ± 1.1^{a}	2.8 ± 1.3^{e}	6.9 ± 1.1^{d}	6.1 ± 1.9^{c}	6.2 ± 2.7^{a}	5.6 ± 2.0^{a}	7.8 ± 1.3^{b}	4.8 ± 1.9^{a}
Overall flavor	7.1 ± 1.3	7.7 ± 0.9	6.5 ± 1.7	6.0 ± 1.5	7.6 ± 1.3	5.2 ± 2.0	4.4 ± 1.9	5.0 ± 1.9	5.2 ± 1.3	3.4 ± 1.7	4.0 ± 2.8	2.8 ± 1.6	6.7 ± 0.9	6.2 ± 2.0	6.0 ± 2.0	5.6 ± 1.9	8.2 ± 0.8	4.5 ± 2.4
Beany Flavor*	4.3 ± 1.3^{a}	5.2 ± 1.0^{e}	4.3 ± 1.1^{a}	4.8 ± 1.2^{d}	5.8 ± 0.5^{a}	5.0 ± 1.2^{a}	3.3 ± 1.4^{a}	3.8 ± 1.8^{a}	4.0 ± 1.3^{a}	3.0 ± 1.2^{c}	4.3 ± 2.9^{a}	3.2 ± 2.3	4.8 ± 1.7^{a}	4.5 ± 1.3^{a}	4.2 ± 0.8^{a}	4.2 ± 1.7^{a}	6.0 ± 0.0^{b}	4.2 ± 2.0^{a}
Like product (%)**	89^{a}	95^{m}	75^{j}	65^{k}	100^{l}	33^{a}	25^{i}	35^{a}	17^{f}	16^{g}	40^{h}	0^{a}	90^{d}	72^{e}	82^{b}	50^{a}	100^{c}	33^{a}
Would buy (%)**	67^{a}	80^{h}	50^{a}	41^{a}	100^{g}	33^{a}	16^{e}	25^{f}	8^{b}	5^{c}	40^{a}	0^{d}	50^{a}	50^{a}	46^{a}	25^{a}	80^{a}	17^{a}

Average ± SD; Different letters across the same row indicate a significant interaction between age, gender, and flour type using ANOVA and Tukeys $p < 0.05$

*Based on a 6pt scale with 6 indicating no beanyness

**Based on Yes (1) or No (0) response

‡ M (17-18) n = 9, F (17-18) n = 20; M (19-21) n = 12, F (19-21) n = 17; M (22-34) n = 5, F (22-34) n = 6

§ M (17-18) n = 8, F (17-18) n = 20; M (19-21) n = 12, F (19-21) n = 19; M (22-34) n = 5, F (22-34) n = 6

† M (17-18) n = 10, F (17-18) n = 18; M (19-21) n = 12, F (19-21) n = 16; M (22-34) n = 5, F (22-34) n = 6

Table 5.11 Sensory analysis of banana muffins by flour type and gender (M = male, F = female)

	Control‡		CGF§		AF†	
Attribute	**M**	**F**	**M**	**F**	**M**	**F**
Crumb color	6.8 ± 1.1^{b}	6.9 ± 1.3^{b}	5.7 ± 1.8^{c}	6.4 ± 1.6^{b}	7.0 ± 1.7^{a}	6.7 ± 1.4^{b}
Muffin top texture	6.7 ± 1.3^{a}	6.6 ± 1.6^{a}	5.2 ± 1.6^{b}	5.2 ± 2.0^{b}	6.7 ± 1.8^{a}	6.4 ± 1.8^{a}
Overall appearance	6.2 ± 1.3^{b}	6.7 ± 1.6^{a}	5.2 ± 1.5^{b}	5.2 ± 2.0^{b}	7.2 ± 1.6^{a}	6.7 ± 1.8^{a}
Springiness	5.8 ± 1.6^{a}	6.0 ± 1.6^{a}	5.2 ± 1.7^{b}	4.2 ± 2.0^{b}	6.6 ± 1.7^{a}	5.8 ± 1.8^{a}
Crumb grain	6.5 ± 1.7^{a}	5.8 ± 1.8^{a}	4.9 ± 1.5^{b}	3.9 ± 2.0^{b}	6.4 ± 1.7^{a}	5.7 ± 1.9^{a}
Muffin moistness	6.7 ± 1.7^{a}	5.7 ± 2.1^{a}	4.8 ± 1.4^{c}	4.7 ± 2.1^{b}	6.7 ± 2.0^{a}	5.7 ± 2.0^{a}
Overall flavor	6.7 ± 2.2^{a}	6.7 ± 1.7^{a}	4.7 ± 1.9^{b}	4.0 ± 2.0^{b}	6.7 ± 1.7^{a}	4.3 ± 1.6^{b}
Beany Flavor*	4.6 ± 1.2^{a}	5.0 ± 1.1^{a}	3.8 ± 1.6^{b}	3.4 ± 1.6^{b}	4.7 ± 1.3^{a}	5.7 ± 2.0^{b}
Like product (%) **	85^{a}	74^{a}	24^{b}	22^{b}	85^{a}	58^{b}
Buy product (%) **	65^{a}	58^{a}	16^{b}	13^{b}	52^{a}	35^{b}

Average ± SD, Different letters across the same row indicate a significant interaction between gender, and flour type using ANOVA and Tukeys $p < 0.05$
*Based on a 6pt scale with 6 indicating no beanyness
**Based on Yes (1) or No (0) response
‡ Male n = 26 , Female n = 43
§ Male n = 25, Female n = 45
† Male n = 27, Female n = 40

Table 5.12 Sensory of banana muffin by flour type and age

	Control‡			CGF§			AF†		
Attribute	**17-18**	**19-21**	**22-34**	**17-18**	**19-21**	**22-34**	**17-18**	**19-21**	**22-34**
Crumb color	7.1 ± 1.2 [a]	6.7 ± 1.2 [a]	7.1 ± 1.3 [a]	6.7 ± 1.7 [a]	5.6 ± 1.6 [b]	6.5 ± 2.0 [a]	7.1 ± 1.4 [a]	6.6 ± 1.6 [a]	6.9 ± 1.6 [a]
Muffin top texture	6.9 ± 1.4 [a]	6.3 ± 1.4	6.6 ± 2.1	5.8 ± 2.0 [b]	4.7 ± 1.5 [b]	5.3 ± 2.3 [b]	6.8 ± 1.9 [a]	6.3 ± 1.9 [a]	6.7 ± 1.1 [a]
Overall appearance	6.6 ± 1.6 [b]	6.5 ± 1.3 [b]	6.6 ± 1.8 [a]	5.6 ± 1.8	4.7 ± 1.6 [c]	5.7 ± 2.4	7.2 ± 1.8 [a]	6.6 ± 1.8 [a]	6.9 ± 1.5 [a]
Springiness	6.3 ± 1.5 [a]	5.2 ± 1.4 [a]	6.9 ± 1.6 [a]	4.8 ± 1.6 [c]	4.5 ± 2.2 [c]	4.4 ± 2.2 [c]	6.4 ± 1.4 [a]	5.9 ± 2.0 [a]	6.0 ± 2.2 [b]
Crumb grain	6.6 ± 1.6 [a]	5.5 ± 1.8 [a]	6.5 ± 2.1 [a]	4.5 ± 1.7 [b]	4.4 ± 2.0 [b]	3.3 ± 2.0 [b]	6.3 ± 1.7 [a]	5.7 ± 2.0 [a]	5.7 ± 2.3 [a]
Muffin moistness	6.4 ± 2.2 [a]	5.5 ± 2.0 [a]	6.7 ± 1.8 [a]	5.0 ± 2.1 [b]	4.7 ± 1.6 [b]	4.2 ± 1.9 [b]	6.4 ± 1.7 [a]	5.8 ± 2.3 [a]	6.2 ± 2.2 [a]
Overall flavor	7.5 ± 1.0 [a]	6.2 ± 1.6 [a]	6.5 ± 2.1 [ab]	4.8 ± 1.9 [c]	4.1 ± 1.8 [c]	3.5 ± 2.2 [c]	6.4 ± 1.7 [a]	5.8 ± 1.9 [b]	6.2 ± 2.6 [a]
Beany Flavor*	4.9 ± 1.2 [a]	4.6 ± 1.1 [a]	5.3 ± 1.0 [a]	3.7 ± 1.6 [a]	3.4 ± 1.3 [b]	4.0 ± 2.4 [a]	4.6 ± 1.4 [a]	4.2 ± 1.4 [a]	4.9 ± 1.7 [a]
Like product (%) **	93 [a]	74 [a]	78 [a]	32 [b]	17 [b]	20 [b]	79	63 [a]	50 [a]
Would buy product (%) **	76[a]	48 [a]	78 [a]	21 [b]	7 [b]	20 [b]	50 [a]	33 [a]	52 [a]

Average ± SD, Different letters across the same row indicate a significant interaction between age and flour type using ANOVA and Tukeys $p < 0.05$
*Based on a 6pt scale with 6 indicating no beanyness
**Based on Yes (1) or No (0) response
‡ 17-18 n = 29, 19-21 n = 29, 22-34 n = 11
§ 17-18 n = 28, 19-21 n = 31, 22-34 n = 11
† 17-18 n = 28, 19-21 n = 28, 22-34 n = 11

Chapter 6: Conclusion

A gluten-free flour was formulated using the pulpy by-product of soy milk processing (okara). AF was analyzed for nutritional constituents and it was found to be high in protein, dietary fiber, calcium, and omega 3 fatty acids. The gluten free flour was assessed for performance by formulating a variety of products using AF, mainly falling under the heading of doughs (dry) or batters (wet). AF did not perform well in dough products such as biscuits and muffins where gluten formulation plays the biggest role in regards to texture, mouthfeel, and product volume/height. This poor performance was attributed to lack of gluten in the product along with AF having large amounts of fiber that interfered with proper protein starch interactions. This indicates that more formulation is required before using AF as a substitute for wheat flour in dough products. A possible solution is lowering the WHC of AF by decreasing fiber size and thereby decreasing overall WHC. Batter products (pancakes and muffins) performed well when formulated with the AF. Batters have higher moisture to flour ratios and depend more on starch for structure and texture. This worked in favor of AF in batter products resulting in pancakes and muffins with good overall volume and texture. SEM images supported these findings. Finally, sensory testing of consumer acceptance confirmed the desirable flavor, texture and appearance of AF muffins. Out of a large sample size of 68 volunteers, AF consistently out-performed CGF in texture and flavor and was comparable in taste, texture and appearance to the control muffin. Preferable flavor, texture, and objective measurements along with increased nutritional benefits indicates that AF is possibly a suitable gluten free alternative to wheat flour for individuals suffering from celiac disease. Additionally, AF is comparable to a commercial gluten-free flour in batter formulations suggesting a novel marketable ingredient for formulation of a variety of gluten free products.

Appendix A

Sensory analysis sheet for panelists:

Product Code: ___________

Age:___________ Gender: Male Female

Instructions:

If you have any food allergies (soy, egg, wheat) please do not participate in the survey.

Participation in this survey is voluntary. If you do not wish to participate, let the survey leader know by folding the survey in half.

Eat a saltine cracker and drink some water to cleanse your palate before and after tasting each product.

Read through all of the categories before tasting the product and then rank the product on the attributes below. (circle your choice)

Please leave any extra comments you may have about the product in the comments section only.

Circle the choice that best describes your preference in the following categories:

Crumb Color

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neutral	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Muffin top texture

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neutral	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Overall muffin appearance

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neutral	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Springiness of muffin when biting

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neutral	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Crumb grain in the mouth

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neutral	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Moistness of muffin

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neutral	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Overall flavor of the muffin

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neutral	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Beany Flavor

Very Strong	Strong	Moderate	Light	Very Light	None

Overall, do you like the product? YES NO

Would you buy this product? YES NO

Comments:

Appendix B

Compilation of comments from sensory analysis. Numbers in parenthesis indicate number of comments, in addition to the stated comment, that was the same or similar.

Control:

- I like it! (7)
- Very dry. Too grainy (8)
- Do not typically like soy products, but enjoyed it a lot
- Muffin had dark appearance
- Color looks like its banana flavored, appearance is flat.
- Not soft enough (gluteny)
- I don't know what soy tastes like (5)
- Don't eat muffins often (3)
- Long brown stringy things are weird looking
- Odd texture, poor flavor, need more banana and less "oaty" taste
- Very real banana flavor that is slightly masked in other muffins
- The muffin was a little too spongey
- The top of the muffin was kind of sticky (probably due to a bag storage). I couldn't detect the beany flavor, and I consume primarily vegan products – It was great!
- Kind of dense
- I don't eat muffins often, so I probably wouldn't buy this product. As far as muffins go, I liked it a lot!

CGF:

- Seemed to fall apart a little too easy (4)
- Really bad overall taste, could not be paid to eat this/tastes weird (8)
- Too flat
- I do not like banana flavored products (3)
- I don't exactly know what soy tastes like, but this has an off flavor I figured was soy. It doesn't taste right or banana-y (1)
- Don't know what soy tastes like (4)
- Texture seems inconsistent
- Extremely dry, darkish comes off sort of grayish, hard to swallow (3)
- Was okay
- This muffin had a more unique flavor, it was a little dry, but tasted more healthy
- Too much moisture on the top of the crumb (4)
- Too strong beany flavor, don't taste banana (1)
- Perhaps it was cooked too long?

AF:

- Best muffin (1)
- Was a little burnt on the bottom, but didn't effect taste too much. Very good!
- I have no idea what soy tastes like…
- Tasty (3)
- Little chewy, but still great texture
- More natural flavor and look

- I don’t like banana muffins very much (3)
- Best texture out of the three, worst taste…not enough flavor? Seemed bland. (1)
- The beany flavor was much more prevalent in this muffin, but I liked its consistency and moistness
- It was a little hard for my liking (1)
- It was ok, but I wouldn’t buy it
- Not sure what beany flavor tastes like (3)
- Looks moist but tastes dry, better flavor than other muffins, but still not good overall
- Well rounded (1)
- Very nice looking, but too much spice in taste
- Top was a little dry, tasted like it had artificial sweetener in it
- Flavor does not appeal to me
- Very dry

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