CHEMICAL AND FUNCTIONAL PROPERTIES OF BROSIUM ALICASTRUM SEED POWDER (MAYA NUT, RAMÓN NUT)

Chad Thomas Carter
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CHEMICAL AND FUNCTIONAL PROPERTIES OF BROSIMUM ALICASTRUM SEED POWDER (MAYA NUT, RAMÓN NUT)

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Food, Nutrition and Culinary Science

by
Chad Thomas Carter
May 2015

Accepted by:
Dr. Margaret Condrasky, Committee Co-Chair
Dr. Julie Northcutt, Committee Co-Chair
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ABSTRACT

*Brosimum alicastrum* is a member of the Moraceae family of edible botanicals. The fruit from this tree contains an edible seed that may be dried or roasted, ground into powder and incorporated into baked goods. Although researchers have claimed that *B. alicastrum* seed powders have high nutritional value, there is very little published evidence to support these claims. Moreover, little is known about the functional properties of *B. alicastrum* seed powders in traditional baked goods. Thus, the purpose of this research was to determine the nutritional composition of *B. alicastrum* seed powder and the feasibility of incorporating this seed powder into a sugar snap cookie formulation.

A series of experiments were conducted to determine baseline nutritional composition data for *B. alicastrum* seeds from two different locations, one in Guatemala and the other in Nicaragua. This was done in order to assess any differences in the composition of the seed based on harvest location. This research also serves to determine if there is a change in nutritional composition when the seeds are roasted to a specific temperature.

Nutritional differences were found in *B. alicastrum* seed powder as an effect of the country in which the seeds were harvested (Guatemala or Nicaragua), and as an effect of roasting the seeds. Seeds from Nicaragua were found to have a more favorable nutritional profile than seeds from Guatemala. Roasting generally increased the relative concentrations of nutrients, with the exception of minerals, which remained constant, and amino acids, which decreased.
Unroasted and roasted Nicaraguan *B. alicastrum* seed powder was incorporated into a sugar snap cookie formulation in equal quantities with white wheat flour. These cookies were analyzed for their nutritional composition, and compared to control cookies baked with 100% all-purpose white wheat flour. Additions of *B. alicastrum* seed powder resulted in decreases in protein and carbohydrates, when compared to control cookies. Cookies baked with *B. alicastrum* seed powder had higher levels of fat and ash when compared to control cookies.

Experiments were conducted to determine the technical feasibility of incorporating *B. alicastrum* seed powder into a standardized laboratory sugar snap cookie formulation. This included determining if and how changes in harvest location, roasting, and level of incorporation affected the technical characteristics of the final product. As *B. alicastrum* seed powder replacement levels increased, cookies became darker, redder, and more yellow when compared to control cookies baked with 100% all-purpose white wheat flour. Hardness and toughness values decreased as replacement levels of *B. alicastrum* seed powder increased, while brittleness remained unchanged. There was no effect of county of harvest or roasting on texture values. Cookie spread factor also decreased as replacement levels of *B. alicastrum* seed powder increased. Country of origin or roast had no effect on spread factor values.

It can be concluded that *B. alicastrum* seed powders are a viable indigenous replacement to wheat flour in this sugar snap cookie formulation. The small differences in nutritional and functional properties are offset by the fact that *B. alicastrum* seeds are an indigenous food source in Central America and one that does not have to be imported.
ACKNOWLEDGMENTS

This project was a collaborative undertaking and many people assisted along the way. I would like to first thank my wife, Jessica Boynton. This endeavor would not have been possible without her support. I would like to thank my advisors Dr. Margaret Condrasky and Dr. Julie Northcutt for all of their valuable assistance, guidance, and support. I would also like to thank committee member Dr. Ronald Thomas for his guidance and input on the project. Special thanks go to Erika Vohman, of the Maya Nut Institute, for providing information, literature, and expertise, along with samples for analysis.

There were many individuals who assisted with technical aspects of the project. I would like to thank Charlie Mustard for his expert assistance in roasting the B. alicastrum seeds and Dr. Brian Kiepper for assistance with particle size determinations. Thank you to Mark Floerke for assistance with cookie baking methodology and to Dr. James Rieke for assistance with statistics and randomization.

There were also many individuals who either directly or indirectly supported me throughout this project. Thank you to Marie Hegler and Lindsay Koeper for putting me up, to Samantha King Cekic for help with research and in the lab, and to the many members of my Creative Inquiry group who researched B. alicastrum seed powder and worked on incorporating it into food products. Thanks to Chris Colthrope for assistance with references and Maciel González for assistance with translations.

A final thank you goes to the Clemson Food Science Department students, faculty, and staff for always being approachable, helpful, supportive, and kind.
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I. CHEMICAL AND FUNCTIONAL PROPERTIES OF *BROSIMUM ALICASTRUM* SEED POWDER (MAYA NUT, RAMÓN NUT)

**Introduction**

In 1975, the National Academy of Science listed *Brosimum alicastrum* as “an underexploited tropical plant with promising economic value”, and called for the seeds of this tree to be studied for their nutritional composition (National Academy of Sciences, 1975). Several years later, Peters and Pardo-Tejeda (1982) noted that since this designation, little work had been done concerning the utilization of *B. alicastrum* seeds as a food source. Only a few peer reviewed studies have been published regarding the use of *B. alicastrum* as a human food ingredient (Thompson, 1930; Roys, 1931; Pardo-Tejeda & Muñoz, 1981; Puleston, 1982; Gillespie, Bocanegra-Ferguson, & Jimenez-Osornio, 2004; Yates & Ramírez-Sosa, 2004), and no comprehensive study has been performed on the nutritional characteristics of the seeds from this plant. In the countries where *B. alicastrum* primarily grows (Southern Mexico to Bolivia), this apparent lack of interest may be due to government subsidized corn and feeding programs, or the stigma held by rural families that *B. alicastrum* is a famine food (Peters & Pardo-Tejeda, 1982). There is a small but growing demand for *B. alicastrum* seed powder in the U.S., which is typically sold under the designations of Maya Nut, Ramón Nut or Ramón Seed. Under these names, it has been added to a limited number of commercially available products.

The *B. alicastrum* tree has several common names associated with the regions where it grows in Central America. In Mexico, the tree has over 50 common names including: Ramón, Ojite, Ojoche, Capomo, Jushte, Ash, and Ox (Puleston, 1968; Peters
& Pardo-Tejeda, 1982). Puelston (1968) lists over 50 names for the tree based on the region where it grows. It is known as Ramón, Masico, Iximché, and Ujuxte in Guatemala, Ojushte in El Salvador, Barúu in Venezuela, and Guaimaro in Colombia and Cuba. Maya Nut is the common name used in the United States and was coined by The Maya Nut Institute (Maya Nut Institute, 2014). For the purposes of this thesis, the Latin name Brosimum alicastrum, and the names Maya Nut, Ramón Nut or Ujushte, which are commonly found in the literature, will be used.

**Species and Range**

*B. alicastrum* is part of the Moraceae family of botanicals and is related to the fig, mulberry, breadfruit, and jackfruit (Puleston, 1968). This evergreen tree takes approximately twenty years to reach maturity, reaching heights of 30 to 35 meters, and a trunk diameter of one meter or greater. The tree has bright green leaves, gray bark, and whitish-yellow wood (Puleston, 1968). Populations of the tree are dioecious, monoecious or hermaphroditic, and do not change over time (Puleston, 1968; Sanchez Garduno, 2005).

Although the primary growing region for *B. alicastrum* ranges from southern Mexico to Bolivia, the tree can also be found in the Caribbean, Cuba, Trinidad, and Jamaica. The tree has been introduced to Haiti, Hawaii, Puerto Rico, Kenya and Uganda (Puleston, 1968; Vohman, 2014). Peters and Pardo-Tejeda (1982) reported that the tree is dominant in Mexico, and is widely distributed throughout that country. It grows at elevations between 50 and 2000 meters above sea level, primarily in hot, humid subtropical forests, although the tree is drought tolerant (Peters & Pardo-Tejeda, 1982).
Interviews conducted by Gillespie et al. (2004) found that established trees do not need irrigation or fertilization which supports the observation of drought resistance.

Fruiting times vary by region. Fruiting in Tikal, Guatemala occurs twice a year, once in February, and again in August (Puleston, 1968). Peters (1983) reported that fruiting in Veracruz, Mexico which has similar conditions to Tikal, only occurs once a year. He suggested that different fruiting times may be due to the occurrence of three different varieties of the tree in Tikal (Ramon Blanco, Ramon Amarillo, Ramon Colorado) (Puleston, 1968; Peters, 1983). This is corroborated by The Maya Nut Institute, which reported that in Chiapas, Mexico and in El Salvador, different trees fruit during different seasons, and the fruits, leaves, and tree architecture are slightly different (Vohman, 2014). In Mexico, the trees flower between November and February, and fruits ripen from March to June (Pardo-Tejeda & Muñoz, 1981). However, because trees at Tikal have different fruiting characteristics than trees found elsewhere, it is possible that the Mayans selectively bred these trees to fruit abundantly as a source of food (Peters, 1983). A list of specific flowering and fruiting times in Mexico can be found in Pardo-Tejeda and Muñoz (1981), and a list of flowering and fruiting times throughout its range can be found on the Maya Nut Institute website digital library (Maya Nut Institute, 2014).

*B. alicastrum* fruits are 1.5 to 2.5 cm in diameter, weigh approximately 4.5 g., and contain one seed comprising 3.0 g of this weight, about 67%. Fruits are yellowish green before maturity, and upon maturity, some remain yellowish green, while others turn orange, yellow, or red. (Puleston, 1968; Vohman, 2014).
**B. alicastrum** Seed Proximate Composition, Calories, and Total Sugars

Little information has been published in peer reviewed journals on the nutritional composition of the *B. alicastrum* seed. After an extensive review of literature, the author of the present study found that much of the nutritional information on *B. alicastrum* has been cited and recited from work that was done by a few independent labs for Central American government organizations from 1950 to the mid 1970’s (Coelho, Bramblett, Quick, & Bramblett, 1976; Pardo-Tejeda & Muñoz, 1981; Peters & Pardo-Tejeda, 1982; Ortiz, Azañón, Melgar, & Elias, 1995). Other sources provide averages of data that have been manipulated, to some degree, with no clear indication of how the original data were collected and presented. Peters and Pardo-Tejeda (1982) provided averaged data for nutrient values and amino acid content of *B. alicastrum* seeds. The data they presented were from unpublished laboratory analysis, and these authors did not provide any information about the methods that were used to generate the data.

Table 1 summarizes a representation of the previous work on the proximate composition of dried *B. alicastrum* seeds. The table is comprised of data that has been generated recently by independent labs or has been mentioned in several articles in the literature (Coelho et al., 1976; Pardo-Tejeda & Muñoz, 1981; Flaster, 2007; Salguero, A.I.A. & Bressani, R., 2013). When necessary, the data was converted to a dry weight basis for comparison. Limited data are available on the amino acid profile, as well as the vitamin and mineral content of *B. alicastrum* seeds.
The Institute for Nutrition in Central America and Panama-Interdepartmental Committee on Nutrition for National Development (INCAP-ICNND) published a nutrition analysis on the *B. alicastrum* seed in 1961, as part of a Table of Food Composition in Latin America. This data was cited by Coelho et al. (1976), as well as Pardo-Tejeda and Munoz (1981). The analysis was performed on dried *B. alicastrum* seeds from Yucatán, Mexico. Seeds were found to have a moisture content of 6.05% with 363 calories per 100 grams of seed.

Original values were converted to dry weight basis; protein, fat, carbohydrates, ash, and crude fiber were 12.13%, 1.70%, 81.48%, 4.68%, and 6.60% respectively.

Pardo-Tejeda and Munoz (1981) provided a review of chemical analyses performed on all parts of the *B. alicastrum* tree from 1950 to 1977. The National Institute of Child Protection in Mexico (INPI) provided Pardo-Tejeda and Munoz (1981) with nutritional composition data, from an unpublished analysis, for their publication. The analysis was performed on dried seeds from Veracruz, Mexico. Seeds were found to have a moisture content of 12.17%. On a dry weight basis, protein, fat, carbohydrate, ash, and crude fiber were 10.22%, 2.02%, 83.55%, 4.21% and 8.9%, respectively.

Zamorano University in Honduras analyzed the nutritional composition of *B. alicastrum* seeds from Honduras. The analysis report was received as a personal communication (Vohman, 2012). Based on their findings, seeds were found to have a moisture content of 7.72% with 281 calories per 100 grams of
seed. On a dry weight basis the protein, fat, carbohydrates, ash, dietary fiber, and sugars were 8.80%, 1.37%, 85.89%, 3.96%, 21.71%, and 14.93% respectively.

A study by Salguero and Bressani (2013) on the glycemic index of _B. alicastrum_ seed was published in the Journal of the University of the Valley of Guatemala. It is unclear if this journal is peer reviewed or what the rejection rate is for publications, as that is not reported. The article was based on Salguero’s (2010) thesis, and her methods were included. The seed obtained for cooking was harvested from Suchitepéquez, Guatemala and received dried. The seed was cooked for one hour, at 98°C, in water and 0.5% calcium hydroxide. The seed was then dehydrated to 10.64% moisture. Cooked seed nutritional composition on a dry weight basis for protein, fat, carbohydrate, ash, dietary fiber, and crude fiber was 11.48%, 1.51%, 79.83%, 3.08%, 4.91%, and 4.10% respectively.

Roasted seed was purchased from Petén, Guatemala. The roasted _B. alicastrum_ seed moisture found to be 8.33%. Nutritional composition for the roasted seed on a dry weight basis for protein, fat, carbohydrates, ash, dietary fiber, and crude fiber was 11.48%, 0.75%, 79.42%, 4.06%, 14.04%, and 4.30% respectively (A. I. A. Salguero, 2010; Salguero, A.I.A. & Bressani, R., 2013).

T. Flaster (2007), Executive Director of Botanical Liaison, LLC., produced a Generally Recognized as Safe (GRAS) Self-Affirmation Report entitled ‘Ramón Seed (Brosimum alicastrum sw.) and Ramón Seed-Derived Ingredients for use in Traditional Foods’. This report contained a complete analysis of roasted _B. alicastrum_ seed performed by Silliker Laboratories,
Chicago, IL, and included amino acids, sugars, fatty acids, vitamins and minerals.

This report also provided a good general overview of the *B. alicastrum* tree and seed. Roasted seeds were harvested in Guatemala and were found to have a moisture content of 10.95% with 346 calories per 100 grams. On a dry weight basis protein, fat, carbohydrates, ash, sugars, dietary fiber, and total sugars were 10.42%, 0.49%, 85.57%, 3.53%, 21.27%, and 10.50% respectively (Flaster, 2007).

Based on these previously reported findings, data on the proximate composition of *B. alicastrum* seed is unsubstantiated, as little information on analytical methods exists. Furthermore, proximate composition data varies significantly. Therefore, additional information that relates chemical composition, methodology, seed-source, and seed treatment is needed.

**Historical Uses of *B. alicastrum* as a Food Source**

Traditionally, *B. alicastrum* fruit pulp, which has been described as having the flavor of tart apricots, was eaten raw or used to make marmalades (Roys, 1931). Thompson (1930) suggested the fruit was nutritious, could be eaten raw, or the seeds could be ground to make tortillas while the leaves could be used as animal fodder. *B. alicastrum* seed has been reported to be a valuable source of nutrition during years where other agriculture crops failed. For example, it was widely used during World War II when corn was scarce. The leaves provide a source of food for livestock in Cuba, Guatemala, Mexico and Jamaica, and the fruits are fed to cows, horses and pigs (Yates & Ramírez-Sosa, 2004).
Previous researchers have proposed that *B. alicastrum* was cultivated and used by the ancient Mayan Civilization from 300 to 900 AD. *B. alicastrum* trees are prevalent near Mayan ruins, suggesting that ancient Mayans cultivated these trees (Puleston, 1968). Puelston (1968) reported a significant positive correlation (R=0.86) between Maya settlements and *B. alicastrum* trees, and concluded that these trees were likely cultivated by the Mayans and used as a subsistence food. Puelston (1982) later suggested that the *B. alicastrum* tree was the reason ancient Maya Civilizations were able to sustain their populations and thrive. He postulated that during the fruiting season (7 to 10 weeks), a woman and 2 to 3 children, working approximately 1.5 hours a day, could collect enough *B. alicastrum* seeds to provide food for an average family for a year. Puleston (1982) also noted, with women and children responsible for family subsistence, the men were free to build monuments that stand to this day. These findings are controversial, as Lambert and Anson (1982) argued that the trees were prevalent at Mayan ruins because those ruins provide favorable soil conditions for natural habitat growth. Peters (1983) argued that this prevalence of *B. alicastrum* trees may be due to bats, which are key seed dispersers and choose habitats in Maya ruins, combined with limestone rich soils which the trees prefer.

**Contemporary Cultural Knowledge of *B. alicastrum***

Yates and Ramirez-Sosa (2004) attempted to determine whether adolescents in El Salvador had knowledge of the *B. alicastrum* (Ujushte) seed and its use as food. This was a follow up to a study that found most adults had knowledge of or had consumed *B. alicastrum* seed. These authors speculated that because *B. alicastrum* (Ujushte) was widely used during World War II, in place of corn, that the seed may suffer a stigma of
being a food of the poor, and that children from higher income families may have no knowledge of it. They found that only 38.4% (N=177) of seventh to ninth grade students in their study had heard of *B. alicastrum* seed, and 30.5% (N=177) had eaten *B. alicastrum* seed. However, these results were not correlated with self-reported socioeconomic status (Yates & Ramírez-Sosa, 2004).

Interviews of 18 Maya natives in Yucatán, Mexico conducted by Gillespie et al. (2004) found that all of these individuals had *B. alicastrum* trees planted in their home gardens, and reported the best use of this tree was as a forage crop for animals. Using this tree as forage was thought to help boost the family’s income. Older individuals in these interviews had knowledge of past use of the seed as food, and one individual recalled using it when locusts destroyed the maize crop (Gillespie et al., 2004). Puleston recounted a story of a corn shortage in 1967 in Guatemala, which prompted the locals to go out and collect *B. alicastrum* seeds (Puleston, 1968).

**Current and Potential Uses for *B. alicastrum* as a Food Source**

When *B. alicastrum* seeds are dried or roasted, and ground into a powder, this seed powder can be incorporated into baked goods such as breads, cakes and cookies (Flaster, 2007). The roasted and ground seed powder has flavors of coffee and chocolate which intensify with a darker (longer) roast. Where fresh seeds can be obtained, they are boiled in ash or lime (calcium hydroxide), cleaned, ground, and used to make tortillas. This process is similar to corn kernel processing (Yates & Ramirez-Sosa, 2004). The seeds can be roasted, ground, and brewed to produce a drink similar to coffee. The seeds can also be ground and mixed with milk and sugar for another type of beverage. The
fresh seeds, which have a flavor similar to potatoes, can also be boiled and mashed in the same way as potatoes (Peters & Pardo-Tejeda, 1982; Puleston, 1968).

A generally recognized as safe (GRAS) self-affirmation report, prepared for the United States Food and Drug Administration by Flaster (2007) gives the following uses for *B. alicastrum* seed powder:

“Ramón seed and powder derived from the Ramón seed are intended for use as ingredients in traditional foods including baked goods and baking mixes, beverages and beverage bases, breakfast cereals, grain products and pastas, gravies and sauces, and milk products as a source of protein” (p.4).

**Commercial Availability of *B. alicastrum* Seed, Seed Powder, and Products**

Currently, there are few commercially available products that utilize *B. alicastrum* seed as an ingredient, outside the regions where it is produced. *B. alicastrum* seed powder (labeled Ramón) is used in Mary’s Gone Crackers gluten-free Chocolate Chip and Double Chocolate Cookies (Mary's Gone Crackers, 2014). Guayakí produces a coffee substitute named Java Mate that is comprised of yerba mate and roasted *B. alicastrum* (Ramón) seeds (Guayaki Brand Yerba Mate, 2014). TJ Enterprise sells *B. alicastrum* seed as a drink, under the trade name Maya Mojo, as a Rainforest blend similar to tea, or a dark roast to be used as a coffee substitute. TJ Enterprises also sells *B. alicastrum* meal as Maya Breadnut Nutmeal/Flour and suggests this product be used in baking (TJ Enterprises, 2014). TeeChia produces two Super Seeds Cereals, Blueberry Date and Cranberry Apple, both of which contain *B. alicastrum* (Ramón) seed powder and are listed as certified gluten-free (TeeChia, 2014). Teccino includes *B. alicastrum* (Ramón)
seed in its herbal coffee alternatives. The seed is used in six of their products, each with a different flavor profile including French Roast, Maya Chai, Chocolate, French Vanilla, Chocolate Raspberry, and Dandelion Dark Roast Herbal Coffee. The latter is also listed as gluten-free (Teccino, 2014). Harmonic Arts Botanical Dispensary sells roasted $B. alicastrum$ (Ramón) seed in whole or powdered form. They also include it in an energy drink called Kick Start (Harmonic Arts, 2014). MontaVida includes $B. alicastrum$ (Ramón) seed in its coffee blends (MontaVida, 2014). The Denver Brewing Company produced a Maya Nut Brown Ale using roasted and ground $B. alicastrum$ (Maya Nut) seeds. This beer was a special release and may no longer be available from the brewer (Denver Beer Company, 2014). The Maya Nut Institute sells roasted $B. alicastrum$ (Maya Nut) powder through their website (Maya Nut Institute, 2014).

**The Maya Nut Institute and Healthy Kids Healthy Forests**

The Maya Nut Institute began as the Equilibrium Fund in 2001. Their mission is “to find balance between people, food and forests by teaching rural communities about the value of Maya Nut for food, fodder, ecosystem services, and income” (Maya Nut Institute, 2014). Since 2002, the institute has trained over 20,000 women from over 1,200 communities, primarily in Guatemala, Nicaragua, El Salvador, Mexico, and Honduras. These women are taught recipes, nutrition, processing, and marketing of Maya Nut. The Maya Nut Institute lists among its accomplishments as having “demonstrated lasting impacts on: rainforest conservation, reforestation, health and nutrition, food security; women’s income, self-esteem and status; maternal health and infant birth weights” (Maya Nut Institute, 2014).
As a part of their mission, the Maya Nut Institute has developed a program entitled Healthy Kids Healthy Forests (HKHF). The HKHF program seeks to provide school children in Central America with a healthy lunch that includes a Maya Nut-based cookie and a Maya Nut-based drink (Maya Nut Institute, 2014). The Maya Nut drink is generally milk or water with roasted ground Maya Nut suspended thoroughly; however, they are developing a drink that combines a number of cereal crops and soy for an enhanced nutrition profile (Vohman, E. 2014). In 2008, the HKHF program began providing Maya Nut-based school lunches for over 8000 children in Guatemala. This program has expanded to El Salvador, Nicaragua, Honduras and Mexico (Maya Nut Institute, 2014).

**B. alicastrum as an Animal Food Source**

The *B. alicastrum* tree serves as a food supply for mammals and birds including trogons, parrots, toucans, and squirrels (Peters, 1989). Bats of the genus *Artibeus* are known to collect and consume the fruits of the tree. When returning to their roosts, they drop the seed, thereby promoting seed dispersion. The bats can disperse seeds up to five kilometers from the source tree (Peters, 1983). From April to June, when *B. alicastrum* fruits are available, it is a major part of bat diets (Vazquez-Yanes, Orozco, François, & Trejo, 1975).

In Tikal, Guatemala, *B. alicastrum* trees are a major food source for both spider (Ateles geoffroyi) and howler (Alouatta villosa pigra) monkeys, who consume the fruit, seeds, and leaves (Coelho et al., 1976). Red howler monkeys (*Alouatta seniculus*) in Venezuela were found to prefer *B. alicastrum* leaves in two of the three years the
monkeys were studied. In 1999, the leaves made up 57% of the foliage diet of the red howler monkeys, but decreased to 22% in 2001 (Lopez, Terborgh, & Ceballos, 2005).

**B. alicastrum Use as Livestock Feed and Forage**

Puelston (1968) noted that the leaves of *B. alicastrum* tree were used to feed mules. “In the jungled heart of the Penten the tree is essential for the maintenance of mules used in the chicle industry. Few trees in the world have leaves which are edible in this way, and its rapid recuperative powers make it doubly valuable” (p. 86). Peters and Pardo-Tejeda (1982) reported that experimental *B. alicastrum* plantations were being cultivated for cattle forage in Yucatán and Veracruz, Mexico. These plantations were expected to produce double the amount of forage of traditional pastures. In addition, this forage is available year round (Peters & Pardo-Tejeda, 1982).

Several livestock nutrition studies have been conducted to determine the nutritional value of *B. alicastrum* seed and fodder for different types of livestock. A 1978 study replaced sorghum with *B. alicastrum* seed in chicken and pig diets. No differences were found for feed conversion ratio in chickens at *B. alicastrum* seed replacement levels of 0, 50 and 100 percent. While there was no difference in feed conversion ratio with a *B. alicastrum* seed replacement level of 30% in pig feed, there was a significant decrease in weight gain when *B. alicastrum* seed was replaced at 60% (Lozano, Shimada, & Avila, 1978). This decrease in weight gain was likely due to the protein level in the reformulated diet, as low levels of protein may cause retardation of growth.

A 1977 study found that eight month old lambs voluntarily consumed *B. alicastrum* forage over other forage types and that the digestibility of *B. alicastrum*
forage was no different from the other forage types offered (Yerena, Ferreiro, Elliott, Godoy, & Preston, 1977). A study on tree fodder preference by cattle found that heifers preferred *B. alicastrum* leaf fodder over four other tree fodder types. An increase of *B. alicastrum* fodder intake coincided with a decrease in grass intake (Sandoval-Castro, Lizarraga-Sanchez, & Solorio-Sanchez, 2005). In a study conducted with sheep, *B. alicastrum* fodder was replaced with *Lysiloma latisiliquum* (False Tamarind). As *L. latisiliquum* was increased in the diet, dry matter, organic matter and crude protein intake decreased along with digestibility and nitrogen balance (Castro-González, Alayón-Gamboa, Ayala-Burgos, & Ramírez-Avilés, 2008). In a 2012 study, sheep were offered feed blocks supplemented with foliage from 4 different tree species, *Acacia cochliacantha*, *Brosimum alicastrum*, *Guazuma ulmifolia*, and *Leucaena lanceolata*. Only one other fodder, *L. lanceolata*, was considered better than *B. alicastrum*, although both were considered “highly palatable to livestock” (Martínez-Martínez et al., 2012). These data suggest that *B. alicastrum* fodder is a valuable source of animal feed for livestock and poultry.

**Productivity**

A three year study performed by Puleston (1982) found that *B. alicastrum* trees in the forest of Tikal produced an average of 1,763 kilograms of seed per hectare, per year. These trees were not cultivated, and therefore were in competition with other plants. This production figure could be increased if trees are planted with uniform spacing to accommodate up to 125 trees per hectare. At this ideal spacing configuration, Puleston estimated production at 5,680 to 9,090 kg of *B. alicastrum* seeds per hectare (Puleston,
Studies of seed production obtained from individual trees have varying results. In a previous study, Puelston (1968) reports a harvest in April at Tikal, Guatemala of 37.6 kg of seed from one tree, and cites other studies that estimate 60 kg of seed harvested from a large tree (Gonzalez, 1939) and 75 kg harvested from a medium sized tree in humid regions (Martinez, 1936; Puleston, 1968). Measurements in Veracruz, Mexico put productivity at 65 kg per tree per year, but the author cautioned that these production figures were subject to a number of variables that could change from year to year (Peters, 1983). It was estimated that in Veracruz, Mexico, collection and processing seed from established trees could produce 80,000 metric tonnes of dry seed a year (Pardo-Tejeda & Sanchez Munoz, 1980; Peters & Pardo-Tejeda, 1982). Peters and Pardo-Tejeda (1982) speculated that the limiting factor in B. alicastrum collection is the market. No well-developed market for B. alicastrum seed exists, and therefore, the price of seeds is too low to encourage harvesting (Peters & Pardo-Tejeda, 1982). Pardo-Tejeda and Sanchez-Munoz (1980) described the need for systems of distribution and harvesting, as well as a need for increased interest in order to make B. alicastrum seed a viable alternative in livestock feeding programs.

**Harvesting and Processing**

Over ninety percent of fallen B. alicastrum seeds can be harvested without a detrimental effect to population regeneration (Peters, 1989). Seeds are harvested from the forest floor and allowed to dry in the sun for 10-30 days. They can also be partially dried in the field, and then sent to processing facilities for further drying. Once the seeds are fully dried (to 12% moisture), they are cleaned to remove shell fragments and other
foreign material. Seeds can then be shipped in this “raw” dried form. These dried seeds can be ground into a powder, or can be roasted and then ground (Flaster, 2007). *B. alicastrum* seed and seed powder is commercially available in the following forms: unroasted, light roasted, and dark roasted.

A drying experiment described by Pardo-Tejeda and Sanchez-Munoz (1981) found that by using a dryer in a coffee processing plant, 2000 kg of seed could be dried below 12% moisture in 8 hours. The authors suggested that seeds could be processed in coffee facilities because these facilities are only used during coffee harvest and are unused for the other half of the year.

**Technical Feasibility of Incorporation of *B. alicastrum* Seed into Food Products**

It has been established that *B. alicastrum* has been used as a conventional food source, and has recently been incorporated into a number of products that are currently available. However, a review of the literature provides no information on the technical aspects of incorporating *B. alicastrum* seed powder into food products. Furthermore, whereas there is an abundance of research on incorporating seed powders/flours such as pumpkin, amaranth, and legumes, into baked goods, there is little information regarding the incorporation of tree seed (from fruit) powders/flours into baked goods. A cookie formulation is a simple, yet effective, starting point to assess the technical feasibility of incorporating *B. alicastrum* seed powder into a baked product. According to Chavan et al. (1993) cookies are a good candidate for replacement of other flour types for several reasons: “1.) they are widely consumed, 2.) exhibit good eating qualities, 3.) are
acceptable to varied selections of the population, 4.) possess relatively long shelf life” (p. 212).

**Cookie Definition**

Cookie, as it is known in North America, is derived from the Dutch word Koekje. In Britain and other countries, it is known as a biscuit. The term biscuit refers to a variety of products that include cookies, crackers, hard biscuits (sweet and semi-sweet), and wafers. These products can be traced back to the middle ages when twice cooked breads, *panis biscocuts*, were made for sea voyages using flour, salt, and water. In North America these were called pilot biscuits or hardtack (Manley, 2011). Manley (2011) attributes the success of biscuits to four factors: “1.) their relatively long shelf life, 2.) their great convenience as food products, 3.) the human liking and weakness for sugar and chocolate, 4.) their relatively good value for money” (p. 3). This thesis focuses on a particular variety of cookie, the sugar snap, which is baked from short dough, characterized by a high fat content. Wheat flour is the main component of short dough, with large percentages of sugar and fat. The dough is referred to as short, because it does not form a gluten network (Chevallier, Colonna, Della Valle, & Lourdin, 2000; Manley, 2011). Short dough cookies are predominant in the North American market and the term cookie will be used rather than biscuit for the purposes of this thesis (Manley, 2011).

**Wheat Production**

Historically the cultivation of wheat can be traced back to between 10,000 and 8,000 B.C. in the Tigris and Euphrates river valley of Mesopotamia (Atwell, 2001). Today, wheat is one of the most important cereal products in the world. In the United
States, wheat is the primary cereal grain grown for human consumption. In 2012, the worldwide production of wheat was 671,496,872 metric tonnes (MT), and was the fourth most produced commodity in the world, with a production value of $79,285,035 USD. Sugar, maize, and rice, in that order, are the only crops that have greater worldwide production quantities. Production of wheat in the Americas (North, Central, South) in 2012 was 108,954,670 MT, roughly 16 percent of worldwide production, and the fifth most produced commodity in that region with sugar cane, maize, soybeans, and milk being the top four products. In Central America, wheat is the ninth most produced commodity at 3,276,824 MT, which represents 0.5 percent of worldwide production and 3 percent of production in the Americas. In 2011, the top two imports for Central America were maize at 12,296,490 MT, and wheat at 5,509,404 MT (FAOSTAT, 2014).

**Wheat Used in Baking**

Most wheat comes from two species, *Triticum aestivum*, which is used in breads, cakes, cookies and a variety of other baked goods, and *Triticum durum*, a hard yellow wheat, which is primarily used in pasta and pizza dough (Atwell, 2001; Pareyt & Delcour, 2008). *T. aestivum* can be divided into several other sub categories, but the primary division is between soft and hard wheat, which describes how easily the wheat can be milled. Hard wheat, which requires more force to be crushed, is used almost exclusively for bread production. Soft wheat has a variety of uses which includes cookie production (Atwell, 2001; Pareyt & Delcour, 2008). Wheat can also be classified by the season in which it grows, spring or winter, as well as by color, red or white, due to the presence or absence of pigment (Atwell, 2001).
The primary function of wheat flour in baked goods is to provide bulk and structure (Lai & Lin, 2006). Flour provides the matrix for other ingredients, which act to make the cookie tough or tender (Pareyt & Delcour, 2008). Cookies are comprised of soft wheat flour, sugar and fat in varying proportions, and may include chemical leaveners, yeast, syrups, salt, or emulsifiers (Chevallier et al., 2000; Pareyt & Delcour, 2008). Cookies are typically produced from soft wheat flour, because the development of gluten is undesirable in these products. The high fat and sugar content of the cookie contributes to plasticity and cohesiveness without forming a gluten network. Fat in the formulation also acts to coat the flour particles to limit their absorption of moisture. High ash in flour is correlated with high pentosan content, which absorbs excess moisture (Lai & Lin, 2006). Cookies may also contain additions of fruits, nuts, chocolate, seeds, or may be comprised of meals, grits, flours, starches or powders of non-wheat ingredients (Manley, 2011). Sugar snap cookies vary in the quantities of flour, sugar and fat, but are generally comprised of 47.5 to 54 percent flour, 33.3 to 42 percent sugar and 9.4 to 18 percent fat (Wade, 1988).

Replacement of Wheat Flour in Cookies

Extensive research has been conducted on replacing white wheat flour with different types of non-wheat cereals, seeds, starches, and other ingredients. Commonly, these replacements are made either to enhance the nutritional aspects of the cookie, or to utilize an ingredient that is indigenous to a certain location. This thesis reviews typical flour replacement cookie research. It is not intended to be comprehensive, but rather to serve as an illustration of the various types of ingredients or categories that have been
studied. These studies utilize a variety of cookie baking methodologies and analytical
techniques, such that direct comparisons between studies may not be appropriate.
Furthermore, these studies represent a wide variety of geographical locations, and thus,
sensory panel ratings of products may not be broadly applicable to all regions or cultures.
Studies are presented in reverse chronological order. Control cookies, unless otherwise
noted, are those comprised of 100% white wheat flour. Significance as discussed in this
review is at the 95% confidence level (P < 0.05).

Chickpea

Yamsaengsung, Berghofer, and Schoenlechner (2012) measured the effects of
chickpea (*Cicer arietinum*) flour replacements on cookies made with white wheat flour,
whole wheat flour, amaranth flour and buckwheat flour at replacement levels of 0, 20, 40,
60, 80, and 100 percent. These authors measured color, spread ratio, and hardness as it
related to cookie quality and consumer acceptance. They also conducted a sensory panel
to determine consumer perception of color, texture, taste, and overall impression.
Chickpea flour, amaranth flour and buckwheat flour were all milled to a particle size of
less than 250 microns. White wheat flour and whole wheat flour were purchased at a
particle size less than 250 microns. Cookie ingredients were not based on a standard
formulation. Dough was mixed and rested in a climate chamber at 20°C and 50% relative
humidity for 30 minutes, rolled to a thickness of 3 mm, cut with a 5.0 cm cutter, and
baked at 175°C for 10 minutes.

As chickpea flour replacement was increased in cookies made with white wheat
flour, the lightness (L*) values significantly decreased, while cookies made with whole
wheat and amaranth had an increase in L* values. For buckwheat flour cookies there was no significant difference in L* values at any level of chickpea flour replacement. Values for redness (a*) significantly increased with higher levels of chickpea flour replacement, for cookies baked using white wheat flour and buckwheat flour. There were significant differences for redness values in cookies baked with chickpea replacement to whole wheat flour and amaranth flour, but these differences were not linear with respect to replacement levels of chickpea flour. Yellowness (b*) values increased significantly at each level of chickpea replacement for all flour replacements.

Chickpea flour replacements to white wheat flour did not substantially change spread factor values. Cookie diameter and thus spread factor are standard measurements for evaluating the quality of soft wheat flour; a larger diameter or spread factor is usually a measure of better quality cookie flour. Soft wheat flour generally produces cookies with the ideal spread ratio (Kaldy, Rubenthaler, Kereliuk, Berhow, & Vandercook, 1991). Cookies baked with 100 percent chickpea flour had the same spread ratio as control cookies (100% white wheat). The largest spread ratio was found for cookies baked with a 20% chickpea flour replacement. When chickpea flour replaced whole wheat flour, the control cookies (100% whole wheat) had the largest spread ratio, but this was not significantly different from cookies baked with 100% chickpea flour. When chickpea flour was used to replace whole wheat flour at 20, 40, and 60%, all cookies had similar spread ratios but were significantly different from controls and 100% chickpea cookies. The addition of chickpea flour to amaranth flour cookies caused significant decreases in spread ratio as replacement levels increased. When chickpea flour replaced buckwheat
flour, spread ratios were similar for control (100% buckwheat) and 20% chickpea replacements. Cookies baked at replacement levels higher than 20% had significantly lower spread ratios from controls, but remained similar to each other (Yamsaengsung et al., 2012).

Hardness was measured on all of the cookie replacement levels (Yamsaengsung et al., 2012). Hardness was measured as the force, in newtons, to penetrate a cookie with a cylindrical probe. Cookies baked with 100% white wheat flour had the lowest overall hardness values (1.65±0.5N) among all the flour types used for control cookies baked with 100% of their respective flours. Chickpea flour cookies at the 100% level had hardness values (2.40±0.9N) between 100% buckwheat (1.76±0.3N) cookies, and 100% amaranth cookies (3.09±0.7N). As chickpea flour replacement levels increased in the white wheat flour cookies, cookie hardness increased significantly compared to the controls. Increased replacements of chickpea flour did not affect hardness substantially beyond the 20% replacement. The replacement of chickpea flour for whole wheat flour decreased the hardness of cookies; however, this change was not significant until replacement reached the 60% level. When amaranth flour was replaced by chickpea flour, hardness decreased up to the 60% replacement level, but then increased at the 80% and 100% replacement levels. Hardness increased with chickpea flour addition to buckwheat flour cookies, although these changes only became significant at the 80% replacement level.

A sensory panel of 25 participants determined color, texture, taste, and overall impression attributes of cookies baked with chickpea flour replacements. Cookies were
evaluated using a 10 cm line scale, with 10 being the most positive rating. The highest color rating within each group, in decreasing order between groups were: white flour 20%/chickpea flour 80% (7.29±2.1), buckwheat flour 80%/chickpea flour 20% (7.14±2.1), amaranth flour 20%/chickpea flour 80% (6.83±2.1) whole wheat flour 60%/chickpea flour 40% (6.25±2.8). The highest taste (flavor) ratings within each group, in decreasing order between groups were: whole wheat flour 60%/chickpea flour 40% (7.01±2.6), white wheat flour 80%/chickpea flour 20% (5.70±2.3), amaranth flour 40%/chickpea flour 60% (4.93±2.6), and buckwheat flour 60%/chickpea flour 40% (4.59±1.6). The highest texture rating within each group, in decreasing order between groups were: white wheat flour 100% (7.06±2.3), whole wheat flour 60%/chickpea flour 40% (6.14±2.4), buckwheat flour 100% (5.39±3.4), amaranth flour 40%/chickpea flour 60% (5.34±2.4). The highest overall rating within each group, in decreasing order between groups were: whole wheat flour 60%/chickpea flour 40% (6.32±1.8), white wheat flour 80%/chickpea flour 20% (5.86±2.3), amaranth flour 40%/chickpea flour 60% (5.66±2.7), buckwheat flour 40%/chickpea flour 60% (4.67±2.4) (Yamsaengsung et al., 2012).

**Flaxseed**

Khouryieh and Aramondi (2012) studied the effects of incorporating flaxseed flour into cookies at replacement levels of 6, 12 and 18 percent. These authors measured moisture content, water activity, color, spread ratio, and cookie hardness. They also utilized a sensory panel to determine appearance, color, texture, flavor and overall acceptability. Cookies were prepared according to American Association of Cereal
Chemists (AACC) official method 10-54 with modifications (AACC 10-54, 2000).

Dough was rolled to a thickness of 7mm, cut with a 6cm diameter cutter and baked in a conventional oven at 204°C for 8 minutes.

Moisture content and water activity of the cookies decreased as the amount of flaxseed was increased in the cookie formulation. There was no difference in the moisture and water activity between cookies made with 12% and 18% flaxseed replacement. At each increase of flaxseed flour, from controls to 18% replacement, there was a significant difference in color. L* values and hue angle decreased with flaxseed additions while chroma, a* and b* values increased as flaxseed replacement increased.

Cookie height decreased significantly at each flaxseed replacement level while width and spread ratio significantly increased, with the 18% flaxseed cookies having the largest width and spread ratio. Cookie hardness was measured using a three point bend test. Hardness, measured as the amount of force, in grams, to snap cookies, significantly increased with each level of flaxseed replacement. Cookies baked at the 18% replacement level required the greatest force to snap (2723±383g).

A sensory panel of 103 participants evaluated cookies for appearance, color, texture, flavor and overall acceptability using a 9 point hedonic scale, with 9 being the most favorable rating. The cookies baked with a 6% flaxseed replacement were rated the highest (most acceptable) on all sensory attributes except texture, although these differences were not significant when compared to control cookies. The cookies made with 18% flaxseed flour replacement received unfavorable scores on all sensory attributes. Cookies baked at a 12% flaxseed replacement level were rated significantly
lower than the controls regarding texture and overall acceptability (Khouryieh & Aramouni, 2012). Based on these data, flaxseed replacement in cookies is acceptable up to the 12% replacement level, but does not enhance consumer perception over controls.

**Pulses (Legumes)**

Zucco, Borsuk, and Arntfield (2011) tested cookies made with flours derived from pulses, including navy bean, pinto bean, and green lentil. Pulse flour replacement levels were 25, 50, 75 and 100 percent. The researchers evaluated both fine and coarse milled flours. The authors measured cookie weight, spread ratio, hardness, and color. Cookies were prepared using AACC method 10-50.05 with some modifications (AACC 10.50.05, 2010). Dough was rolled to a thickness of 0.3cm, cut with a 6.35cm diameter cutter, and baked in a convection oven at 182°C for 5.5 minutes.

The weight of cookies increased with all pulse flours tested, with complete replacement (100%) having a significantly higher weight than partial replacements. Partial replacements were significantly heavier than the control but not different from one another. When coarse and fine milled pulse flours were compared, there was only a cookie weight difference with pinto bean flour, where coarse flour produced a heavier cookie than fine milled flour. Cookies substituted with pulse flours were significantly thicker than the controls, with the exception of green lentil flour. Coarse milled green lentil flour decreased cookie thickness when incorporated at 100%, while fine milled green lentil flour increased cookie thickness as the level of substitution increased. There were significant differences in widths of pulse flour cookies compared to controls. Cookies from fine milled pulse flours had smaller widths than the controls, while cookies
from coarse milled pulse flours had a larger width than the controls. There was no significant difference in width when the replacement level of fine pulse flours was increased; however, as the replacement level of coarse pulse flours increased, the cookie width also significantly increased. Zucco et al. (2011) hypothesized that the decrease in spread was due to increased protein found in the pulse flour as compared to wheat flour. Decreased spread could also be caused by higher levels of damaged starch, as seen in lower widths of cookies made with fine milled pulse flour. These fine milled flour cookies could have a higher level of damaged starch when compared to coarse milled pulse flours (Zucco et al., 2011).

Cookie hardness was measured in newtons using a three point bend test. Hardness, as peak force to snap a cookie, was found to be significantly greater in cookies made with lentil and pinto bean flour, when compared to the control, but was not significantly different for cookies made with navy bean flour. Replacements made with fine milled flours had an effect on hardness. As substitution levels increased, cookie hardness also increased. Increasing the level of coarse milled flours did not give this effect (Zucco et al., 2011).

Color was measured to determine a whiteness value. The control cookies had a higher whiteness value ($L^*$) than cookies baked with pulse flours. Among cookies baked with pulse flours, pinto bean flour had the highest whiteness values. As substitution levels of pinto bean flour increased, whiteness levels decreased up to the 75% level, and 75% substitution was not different from complete substitution. Coarse flours had higher
whiteness values than fine flours at the 25% and 50% replacement levels (Zucco, et al., 2011).

**Amaranth**

Sindhuja, Sudha, and Rahim (2005) incorporated amaranth flour into a sugar snap cookie formulation at replacement levels of 0, 5, 10, 15, 20, 25, 30, and 35 percent. These authors measured weight, spread ratio, and hardness. They also utilized a six member sensory panel to evaluate the attributes of surface color, surface cracking, texture, mouthfeel, and flavor. An overall quality score was calculated from sensory data. Amaranth seeds were milled to 340 microns (60 mesh). Cookies were produced using AACC method 10-50 D (AACC 10-50D, 1975), rolled to a thickness of 7.5 mm, cut with a 7.5 cm diameter cutter and baked at 205°C for 13 minutes.

Cookie weight was highest for cookies baked at the 25% amaranth flour replacement level (19.90±0.13g). This was significantly different from cookies baked with 20, 30 and 35% replacements (19.72±0.23g, 19.70±0.18g, 19.42±0.15g), which were all of similar weights. The lightest cookie was formulated with 10% amaranth flour (18.90±0.21g) followed by the control cookie (19.46±0.11g). The differences between the 10% amaranth flour replacement and the control were significant. As the replacement level of amaranth flour was increased, cookie width significantly decreased. The relationship between replacement level and thickness was not linear. The greatest thickness was found in cookies at 10% replacement (12.2±0.08mm) with 20% replacement cookies (12.1±0.09mm) being similar. Thickness decreased at 25% to 35% replacement with control cookies (10.9±0.08mm) having the smallest thickness. When
spread ratio was calculated, the control cookies (7.82±0.03) had a significantly larger spread ratio, followed by cookies baked with 10% replacement of amaranth flour (6.69±0.03). Cookies formulated with 20-35% amaranth flour replacement showed no significant difference in spread ratio, although the group was significantly different from both the control and the 10% cookies. Cookie hardness was measured as force to snap in kilograms, using a three point bend test. There was no significant difference between control (4.94±0.19kg), 10% (5.0±0.28kg) and 20% (4.89±0.21kg) amaranth replacement cookies on hardness measured as force to break (kg). There was a reduction in hardness at the higher levels of amaranth flour replacement, with cookies at the 35% replacement level having a significantly lower hardness value (3.03±0.32kg).

A six member sensory panel evaluated the attributes of surface color, surface cracking, texture, mouthfeel, and flavor. Each characteristic was rated on a 10 point scale and all categories were added together to give an overall quality score. Cookies baked with 25% amaranth were rated the highest for all attributes with an overall quality score of 43.2. This score was significantly different from all other overall quality scores except for 30% replacement cookies which received an overall quality score of 42.9. Control cookies scored the lowest in terms of overall quality. The researchers added emulsifiers to the 25% amaranth cookies but not controls, which significantly improved overall quality scores, and produced softer cookies with a higher spread ratio (Sindhuja et al., 2005).

**Black Rice**

Lee et al. (2005) incorporated black rice flour (*Oryza sativa* L. Indica type) into a cookie formulation at replacement levels of 5, 10, 15 and 20 percent. These authors
measured cookie moisture, dough moisture, pH, spread factor, and color. A sensory panel was also utilized to evaluate flavor, savory taste, color, and texture. Black rice was milled to approximately 420 microns (40 mesh). Cookies were baked according to AACC official method 10-50D. Cookie dough was allowed to rest in a refrigerator for 30 minutes before being rolled to a thickness of 0.4cm, cut with a 4.26cm diameter cutter and baked in a convection oven at 180°C for 12 minutes.

Cookie moisture significantly decreased in cookies baked with 5% (1.66±0.05%) and 20% (1.87±0.38%) black rice flour as compared to the controls (2.32±0.13%). Cookies at 10% (2.40±0.12%) and 15% (2.26±0.17%) black rice flour replacement were not significantly different, with respect to moisture content, from controls. Dough moisture remained consistent at all replacement levels (16.16±0.09-16.56±0.12%). A significant decrease in pH was measured, as compared to controls (5.88±0.01), for cookies baked with 5% black rice flour (5.56±0.03). Cookies baked with 10% black rice flour (5.85±0.03) did not have a significantly different pH from controls. There were significant increases in pH for cookies baked with 15% (5.97±0.01) and 20% (6.09±0.02) black rice flour replacements.

Cookies baked with 15% black rice flour replacement (11.50±0.05) had the highest spread factor, which was significantly different from all other replacement levels. Cookies baked at 5% (11.11±0.07), 10% (11.23±0.15), and 20% (11.24±0.06) replacements did not have significantly different spread factors from one another. Control cookies had the lowest spread factor (10.28±0.06) which was significantly different from all replacement levels. Lightness (L*) and yellowness (b*) values significantly decreased
with each replacement level of black rice flour while redness ($a^*$) values significantly increased. There was no significant difference in hardness of the cookies, as measured in force to fracture (grams).

A sensory panel of 10 trained panelists evaluated the cookies on flavor, savory taste, color and texture using a 9 point hedonic scale. All of these sensory characteristics significantly increased as black rice flour replacements increased. Cookies baked with 10% replacement of black rice flour received a significantly higher score for flavor (Lee, et al., 2005)

**Pumpkin Seed**  
Giami, Achinewhu, and Ibaakee (2005) evaluated cookies formulated with defatted fluted pumpkin seed flour (*Telfairia occidentalis* Hook) at replacement levels of 0, 5, 10, 15, 20, and 25 percent. These authors measured cookies for weight, spread ratio, hardness, and chemical composition. A sensory panel evaluated cookies for color, texture, flavor, and overall acceptability. Cookies were baked according to the procedure described by McWatters et al. (2003). Dry ingredients and shortening were mixed by hand, and then mixed mechanically for 3.5 minutes after egg was added. The dough was rolled to 0.4 cm thickness, cut with a 5.8 cm diameter cutter and baked at 180°C for 10 minutes.

There was no significant increase in the weight of cookies at the 5% replacement level (12.7±0.4g) when compared to controls (12.5±0.6g). As pumpkin seed flour replacement levels increased, the cookie weight significantly increased. Cookies baked with 30% pumpkin seed flour replacement had the heaviest weights (15.0±0.8g). There
was no significant difference in spread ratio between controls (6.6±0.3) and cookies baked with pumpkin seed flour replacement levels of 5% (6.4±0.2), 10% (5.9±0.4), and 15% (5.8±0.5). At replacement levels of 10% and 15%, the width and thickness were significantly lower than the control or 5% replacement cookies. At pumpkin seed flour replacement levels of 20 and 25%, the cookies, while similar to each other, had a significantly reduced diameter, height, and spread factor from controls and 5%, 10%, and 15% replacements.

Cookie hardness was measured by a compression test as shear stress in newtons. There were no significant difference in hardness between the control cookies (59.4±1.0N) and those formulated with 5% (58.2±0.8N), 10% (56.9±0.6N), and 15% (55.8±0.5N) pumpkin seed flour replacements. At replacement levels of 20% (38.6±0.4N) and 25% (35.4±0.7N) cookies became softer, and differences were significant from controls and 5-15% pumpkin seed flour replacement cookies, but not from one another.

As the percentage of pumpkin seed flour was increased in the cookies, protein, ash, calcium, potassium and phosphorus increased, while moisture, fiber, calories, and iron remained unchanged.

Cookies were evaluated by a 26 member sensory panel using a nine point hedonic scale. There was no significant difference in the sensory characteristics of color, texture, flavor, or overall acceptability between the control cookies and cookie made with 5-15% replacement levels of pumpkin seed flour. These cookies were rated the highest (most favorable). At the 20 and 25% replacement levels there was a significant difference from the 0-15% replacement levels for texture and overall acceptability, however there was no
difference between cookies baked at the 20 and 25% replacement levels. A significant difference for flavor was found at the 20% level (less acceptable), which was significantly different from cookies baked at the 25% replacement level. Perceived color difference only became significant at the 25% replacement level. Panelists reported cookies containing more than 15% pumpkin seed flour to be crumbly, dark, having an aftertaste, and a beany flavor (Giami, et al., 2005).

**Corn and Potato**

Singh, Singh, Sharma, and Saxena (2003) studied the effects of incorporating corn and potato flours at replacement levels of 2, 4 and 6 percent into cookies. These authors measured cookies for spread factor (ratio), color and hardness. Three separate potato cultivars were boiled in water for thirty minutes, shredded and dried in a hot air dryer. The dried potatoes were ground and passed through a 212 micron sieve. Cookies were made using AACC methods (AACC, 1995). Dough was mixed using a pinhead mixer, rolled to 1 cm thickness, cut with a 4.5 cm cutter and baked at 205°C for 12 minutes in a reel oven.

Additions of corn or potato flour increased the spread factor of the cookies, with the cookies containing potato flour having a greater spread than those baked with corn flour. Color measurements of the cookies showed decreasing whiteness (L*) values when corn and potato flours were added, with potato flour cookies having a higher L* value than corn flour cookies. Color values for a* and b* increased with corn and potato flour additions, with greater values found in corn additions. Hardness was measured in grams of force required to shatter a cookie using a blade. Hardness (fracture force) decreased
with the addition of potato and corn flour. When corn and potato flours were replaced at the 6% level, fracture force for control cookies was the highest followed by corn flour replacement cookies, and the lowest fracture force was measured in cookies made with potato flour (Singh et al., 2003).

**Millet and Cowpea**

McWatters, Ouedraogo, Resurreccion, Hung, and Phillips (2003) replaced wheat flours in sugar cookies with White fonio (*Digitaria exilis*), a type of millet, and California blackeye cowpea (*Vigna unguiculata*). Cookies were made by a 50% replacement of wheat with fonio or cowpea. Other cookies were baked with equal quantities (33%) of wheat, fonio, and cowpea, and also with 25% wheat/75% fonio, 25% wheat/50% fonio/25% cowpea. The researchers’ measured cookies without wheat flour, which were comprised of 75% fonio/25% cowpea and 50% fonio/50% cowpea. The authors measured cookies for spread ratio, hardness and color. A sensory panel further evaluated cookies for the characteristics of appearance, color, flavor, texture, and overall acceptability. Cookies were prepared using the method described by McWatters (1978), sheeted to 9.0 mm thickness and cut with a 3.8 cm diameter cutter. Cookies were baked in a 204°C conventional electric oven (McWatters et al., 2003).

The control cookies, containing 100% wheat flour had the highest spread ratio (5.86), followed by the cookies containing 50% wheat/50% fonio (5.21) and 50% wheat/50% cowpea (5.43), which were not different from each other. As the level of wheat flour decreased, the spread ratio also decreased, with the lowest spread ratio measured in the 75% fonio/25% cowpea cookie (4.39).
Hardness was measured in newtons as the maximum peak force to shear a cookie. The hardest cookies consisted of 50% fonio/50% cowpea (720.3N), and the softest were 50% wheat/50% fonio (399.4N) and 25% wheat/75% fonio (421.4). The control cookie (568.4N) was an intermediate in terms of force to shear.

Top color measurement of lightness found the control cookie (74.3) and the 50% wheat/50% cowpea (73.4) cookies to have the highest L* values, and the 75% fonio/25% cowpea (63.2) cookie to have the lowest L* value. There was no significant difference between treatments for redness values (a*). Yellowness (b*) was highest in 50% wheat/50% cowpea cookies (29.6), and lowest in 75% fonio/25% cowpea cookies (19.6).

A sensory panel determined that only the control cookies and the 50% wheat/50% fonio cookies were acceptable from a flavor standpoint, however, there was no significant difference in the panelists perceptions of the overall appearance, color, or texture of the cookies before they tasted them (McWatters et al., 2003).

**Quinoa**

Lorenz and Coulter (1991) evaluated cookies baked with replacements of high and low spread wheat cookie flour with 5, 10, 20, and 30 percent replacements with quinoa flour. These authors measured spread ratio, top grain scores, and color. A sensory panel was utilized to assess appearance, color and texture.

As the percent of quinoa flour was increased in the high spread wheat flour cookie formula, cookie spread and top grain scores decreased. The top color of the cookie became darker (lower L* values) as quinoa percentages were increased. A sensory panel
found flavor improvements in cookies baked with up to 20% quinoa flour, although the cookies became bitter, as detected by panelists, at the 30% replacement level.

When quinoa was incorporated into a cookie baked with low spread wheat flour, there was a slight decrease in cookie spread. The appearance and texture of the cookies, as judged by a sensory panel, were not affected by the replacement with quinoa flour and flavor scores improved up to the 20% level. At 30% quinoa replacement level, the cookies became bitter, as detected by panelists. When 2% lecithin was added to the low spread flour formulation, to improve emulsification, cookie spread increased beyond the low spread flour treatments made without lecithin (Lorenz & Coulter, 1991).

**Summary of Wheat Flour Replacement Research**

When compared to control wheat flour cookies, as non-wheat flour replacement levels increased, cookie thickness increased for cookies made with navy bean, pinto bean, and amaranth flour. The opposite occurred when flaxseed, green lentil, and pumpkin flours were incorporated, which resulted in decreased cookie thickness. As replacement levels of non-wheat flours increased, there was an increase in width for flaxseed and coarse milled pulse flours. There was a decrease in width for amaranth, pumpkin, and fine milled pulse flours.

Spread ratios increased as replacement levels of non-wheat flours increased for flaxseed, black rice, corn and potato flours. Spread ratios decreased for fonio, cowpea and quinoa flours. There was no change in in spread ratios for chickpea flour replacements of white wheat flour.
Hardness increased as replacement levels of non-wheat flours increased for chickpea, flaxseed, green lentil, pinto bean, and cowpea flours. Hardness decreased with replacements of amaranth, pumpkin, corn, potato, and fonio flours. Hardness was unchanged with replacements of pinto bean and black rice flours.

Whiteness ($L^*$) decreased as non-wheat flour replacement levels increased for chickpea, flaxseed, navy bean, pinto bean, green lentil, black rice, corn, potato, fonio, cowpea, and quinoa flours. Redness ($a^*$) values increased as replacement levels increased for chickpea, flaxseed, corn and potato flours. Redness values decreased for black rice flours. Yellowness ($b^*$) values increased as replacement levels increased for chickpea, flaxseed, black rice, corn and potato flours.

Cookie weight increased as non-wheat flour replacement level increased for navy bean, pinto bean, green lentil, pumpkin, fonio, and cowpea flours. Flaxseed flour increases resulted in a decrease in cookie moisture and water activity. Black rice flour increase caused an increase in cookie pH.

These studies show is that it is difficult to predict how flour replacement will affect width, spread, hardness, and color, since it varies widely depending on flour replacement type and percent replacement. Furthermore, these studies show that an optimum wheat-replacement for baked goods has not been found that mimics wheat for color, texture, appearance, etc.

**Research Objectives**

The research objectives for this thesis are twofold. First, to provide baseline nutritional composition data for *Brosimum alicastrum* seeds from two different locations,
one in Guatemala and the other in Nicaragua. This is done in order to assess any differences in the composition of the seed based on harvest location. This research will also determine if there is a change in nutritional composition when the seeds are roasted to a specific temperature. This research is not intended to be comprehensive and aims to serve as a baseline comparison so that future studies can be carried out and compared to these results.

The second objective is to determine the technical feasibility of incorporating *Brosimum alicastrum* seed powder into a laboratory sugar snap cookie formulation. This includes determining if and how changes in harvest location, roasting, or level of incorporation affect the technical characteristics of the final product.
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<sup>1</sup>Data reported on a dry weight basis (g/100g) with the exception of moisture and calories. Superscripts 2, 4, 5, and 6 have been converted to dry weight from original data.


<sup>4</sup>Unpublished Laboratory report from Zamorano Food Science Laboratory, Francisco Morazan, Honduras. Received as a personal communication from E.Vohman 4.16.2012.


<sup>7</sup>Yucatán and Veracruz are states in Mexico. HND=Honduras, GTM=Guatemala.

<sup>8</sup>Calculated from original data.
II. EFFECTS OF ROASTING ON THE NUTRITIONAL COMPOSITION OF GUATEMALAN AND NICARAGUAN BROSIMUM ALICASTRUM SEED POWDER (MAYA NUT, RAMÓN NUT).

Abstract

*Brosimum alicastrum* is part of the Moraceae family of botanicals and is related to the fig, mulberry, breadfruit, and jackfruit (Puleston, 1968). *B. alicastrum* fruits contain one seed comprising 3.0 g of the fruit’s total weight, about 67%. When *B. alicastrum* seeds are dried or roasted, and ground into a powder, this powder can be incorporated into baked goods such as breads, cakes and cookies (Flaster, 2007). As *B. alicastrum* can be utilized in various foodstuffs, it is the purpose of this research to determine baseline nutritional composition data for *B. alicastrum* seeds from two locations, Guatemala and Nicaragua, in order to determine whether there are differences in the composition of the seeds based on where they are grown and harvested. This research also intends to determine if there is a change in nutritional composition when the seeds are roasted to a specific temperature and when they are incorporated into a cookie.

Unroasted and roasted *B. alicastrum* seed powder was analyzed for proximate composition, sugars, amino acid profile, vitamins A & C, minerals, and gluten. Unroasted *B. alicastrum* seed powders from Guatemala and Nicaragua were found to be different in their nutritional composition. Roasting affected the chemical composition of both the Nicaraguan and the Guatemalan seed powders, in many cases increasing relative
quantities of nutrient when compared on a dry weight basis. Roasting decreased the total sugars, which changed the percentage of the total solid constituents.

Cookies were baked using either 100% all-purpose white wheat flour (control) or a 50% replacement of either unroasted or roasted seed powder from Nicaragua. Cookies were evaluated for the same nutritional components as the seed powders. Control cookies had significantly higher levels of protein and carbohydrates. Cookies baked with Nicaraguan *B. alicastrum* seed powder replacements had higher levels of fat, ash, and moisture. Cookies baked with *B. alicastrum* seed powder had significantly higher levels of calcium, copper, magnesium, phosphorus, potassium, zinc and sodium. There was little to no change in mineral levels from the unroasted to the roasted seed powder cookies.
Introduction

In 1975, the National Academy of Science listed *Brosimum alicastrum* as “an underexploited tropical plant with promising economic value”, and called for the seeds of this tree to be studied for their nutritional composition (National Academy of Sciences, 1975). Several years later, Peters and Pardo-Tejeda (1982) noted that since this designation, little work had been done concerning the utilization of *B. alicastrum* seeds as a food source. Only a few peer reviewed studies have been published on the use of *B. alicastrum* as a human food ingredient (Thompson, 1930; Roys, 1931; Pardo-Tejeda & Muñoz, 1981; Puleston, 1982; Gillespie, Bocanegra-Ferguson, & Jimenez-Osornio, 2004; Yates & Ramírez-Sosa, 2004), and no comprehensive study has been performed on the nutritional characteristics of the seeds of this plant. In the countries where *B. alicastrum* primarily grows (from southern Mexico to Bolivia), this apparent lack of interest may be due to government subsidized corn and feeding programs, or the stigma held by rural families that *B. alicastrum* is a famine food (Peters & Pardo-Tejeda, 1982). There is a small but growing demand for *B. alicastrum* seed powder in the U.S., which is typically sold under the designations of Maya Nut, Ramón Nut or Ramón Seed. Under these names, it has been added to a limited number of commercially available products.

*B. alicastrum* is part of the Moraceae family of botanicals and is related to the fig, mulberry, breadfruit, and jackfruit (Puleston, 1968). This evergreen tree takes approximately twenty years to reach maturity, reaching heights of 30 to 35 meters, and a diameter of one meter or greater. Populations of the tree are dioecious, monoecious or hermaphroditic, and do not change over time (Puleston, 1968; Sanchez Garduno, 2005).
*B. alicastrum* fruits are 1.5 to 2.5 cm in diameter, weigh approximately 4.5 g., and contain one seed comprising 3.0 g of this weight, about 67%. Fruits are yellowish green before maturity, and upon maturity, some remain yellowish green, while others turn orange, yellow, or red (Puleston, 1968; Vohman, 2014).

Although the primary growing region for *B. alicastrum* ranges from southern Mexico to Bolivia, the tree can also be found in the Caribbean, Cuba, Trinidad, and Jamaica; and has been introduced to Haiti, Hawaii, Puerto Rico, Kenya and Uganda (Puleston, 1968; Vohman, 2014). Peters and Pardo-Tejeda (1982) reported that the tree is dominant in Mexico, and is widely distributed throughout that country. It grows at elevations between 50 and 2000 meters above sea level, primarily in hot, humid subtropical forests, although the tree is drought tolerant (Peters & Pardo-Tejeda, 1982).

When *B. alicastrum* seeds are dried or roasted, and ground into powder, this powder can be incorporated into baked goods such as breads, cakes and cookies (Flaster, 2007). This roasted seed powder has flavors of coffee and chocolate which intensify with a darker (longer) roast. Where fresh seeds can be obtained, they are boiled in ash or lime (calcium hydroxide), cleaned, ground, and used to make tortillas. This process is similar to corn kernel processing (Yates & Ramirez-Sosa, 2004). The seeds can be roasted, ground, and brewed to produce a drink similar to coffee. The seeds can also be ground and mixed with milk and sugar for another type of beverage. The fresh seeds, which have a flavor similar to potatoes, can be boiled and mashed in the same way as potatoes (Puleston, 1968; Peters & Pardo-Tejeda, 1982).
A generally recognized as safe (GRAS) self-affirmation report, prepared for the United States Food and Drug Administration by Flaster (2007) gives the following uses for *B. alicastrum* seed powder:

“Ramón seed and powder derived from the Ramón seed are intended for use as ingredients in traditional foods including baked goods and baking mixes, beverages and beverage bases, breakfast cereals, grain products and pastas, gravies and sauces, and milk products as a source of protein” (p.4).

Little information has been published in peer reviewed journals on the nutritional composition of the *B. alicastrum* seed. After an extensive review of literature, the author of the present study found that much of the nutritional information on *B. alicastrum* has been cited and recited from work that was done by a few independent labs for Central American government organizations from 1950 to the mid 1970’s (Coelho, Bramblett, Quick, & Bramblett, 1976; Pardo-Tejeda & Muñoz, 1981; Peters & Pardo-Tejeda, 1982; Ortiz, Azañón, Melgar, & Elias, 1995). Other sources provide averages of data that have been manipulated, to some degree, with no clear indication of how the original data were collected and presented. Peters and Pardo-Tejeda (1982) provided averaged data for nutrient values and amino acid content of *B. alicastrum* seeds. The data they presented were from unpublished laboratory analysis, and these authors did not provide any information about the methods that were used to generate the data.

Table 1 summarizes a representation of the previous work performed to date on the proximate composition of dried *B. alicastrum* seeds. The table is comprised of
data that has been generated recently by independent labs or has been referenced in several articles in the literature (Coelho et al., 1976; Pardo-Tejeda & Muñoz, 1981; Flaster, 2007; Salguero, A.I.A. & Bressani, R., 2013). When necessary, the data was converted to a dry weight basis for comparison. Limited data are available on the amino acid profile, as well as the vitamin and mineral content of *B. alicastrum*.

Previous research has reported a wide range in data for moisture (6.0 to 12.2%), protein (8.8 to 12.1%), fat (0.5 to 2.0%), carbohydrates (79.4 to 85.9%), ash (3.1 to 4.7%) and crude fiber (4.1 to 8.9%). Dietary fiber was reported to vary from 4.9 to nearly 22%, while total sugar was only reported by two other sources (10.5% or 14.9%). These authors also noted that calories in 100 grams of *B. alicastrum* seed powder ranged from 281 to 363 calories.

Although these data provide an estimate of the proximate composition of *B. alicastrum*, in many cases the methodology is not provided for comparison. Furthermore, proximate composition data varies significantly. Moreover, there is no data available that describe the effects of roasting on the nutritional composition of *B. alicastrum* seeds and seed powders. Therefore, the purpose of this research is to determine baseline nutritional composition data for *B. alicastrum* seeds from two different locations, one in Guatemala and the other in Nicaragua. This is done in order to determine whether there are differences in the composition of the seed based on where they are grown and harvested. This
research also intends to determine if there is a change in nutritional composition when the seeds are roasted to a specific temperature.

**Materials and Methods**

**Sourcing *B. alicastrum* Seeds**

Whole dried *B. alicastrum* seeds were obtained in single lots from two countries, Guatemala and Nicaragua. Guatemalan *B. alicastrum* seeds, purchased as Ramón Nut, (lot # 5403) were obtained from Mountain Rose Herbs (PO Box 50220, Eugene, OR, 97405, www.mountainroseherbs.com). *B. alicastrum* seeds from Guatemala were harvested from the Maya Biosphere Reserve (17°28'46.9"N 89°51'02.0"W). Nicaraguan *B. alicastrum* was purchased from the Maya Nut Institute (PO Box 2371, Crested Butte, CO, 81224, www.mayanutinstitute.org). *B. alicastrum* from Nicaragua was harvested from Volcan San Cristobal-Casita Natural Reserve (12°40'19.61"N 86°57'58.86"W). Coordinates represent a center point for these reserves, not actual harvest locations, which are larger areas. Appendix A shows the harvest locations relative to each other.

**Roasting *B. alicastrum* Seeds**

*B. alicastrum* seeds were roasted in a Diedrich Coffee Roaster, 24kg capacity (Diedrich Manufacturing, Inc., P.O Box 430, Ponderay, ID, 83852 www.diedrichroasters.com). Equal quantities, 2360 grams, of whole Guatemalan and Nicaraguan *B. alicastrum* seeds were roasted separately to a final temperature of 160°C. Roasting began when the temperature in the roaster reached 188°C at full flame. No air adjustment was made to the roaster, which resulted in approximately 20% air flow. Roasting temperature was monitored and recorded at one minute intervals. Adjustments
to the flame during roasting were recorded as actions. The roasting process varied slightly for each sample; however, the final temperature was the same. A detailed roasting protocol for seed from each country can be found in Appendix B.

**Grinding B. alicastrum Seed**

All *B. alicastrum* seed was ground to conform to the standards for flour set by the United States Code of Federal Regulations 21C.F.R. § 137.105 which states: “not less than 98 percent of the flour passes through a cloth having openings not larger than those of woven wire cloth designated 212μm (No. 70)” (Cereal Flours and Related Products, 2014). This was achieved through a step down process of grinding and sieving *B. alicastrum* seeds until particle size was less than 212μm. The grinding process generated heat and thus, the seeds were frozen prior to grinding to minimize the impact the heat from processing.

Whole *B. alicastrum* seeds were stored in a freezer at 0°C, in a vacuum sealed bag, for a minimum of 12 hours prior to grinding. Immediately after removal from the freezer, the temperature was recorded and the seeds were ground coarsely in a Mazzer Luigi, Super Jolly coffee grinder (Mazzer Luigi s.r.l., Via Mogliansese 113, I - 30030 Gardigiano di Scorze’ - Venezia – Italy, www.mazzer.com). Temperature and weight were recorded after each grinding step, and the ground seed was sieved through a 212μm (#70) screen (Certificate of compliance #70BS8F589291, ASTM specification E11). Any *B. alicastrum* seed powder that did not pass through the sieve was weighed, sealed in a vacuum bag, and placed in a blast freezer, USECO Catr Cook Chill Model # 31-003 (USECO, 869 Seven Oaks Suite 140, Smyrna, TN, 37167, www.useco.com), until the
temperature was 0°C or less. The remaining coarse *B. alicastrum* seed powder was then ground using a WonderMill grain mill (The WonderMill Company, Pocatello, ID, 83201) on the finest setting (pastry). The product of grinding in the WonderMill was sieved through a 212μm (#70) screen, and what remained on the sieve was sealed in a vacuum bag, chilled in the blast freezer, and reground in the WonderMill. This process was repeated until what remained on the sieve was less than 2% of the total weight; this was then added to the final ground *B. alicastrum* seed powder.

**Nutritional Analysis of *B. alicastrum* Seed Powder**

Analysis of ground *B. alicastrum* seed powder and cookies were performed in triplicate for all categories unless otherwise noted. Samples analyzed were unroasted and roasted *B. alicastrum* seed powder from Guatemala and Nicaragua along with cookies made with roasted and unroasted seed powder from Nicaragua. Roasted and unroasted samples were from the same lot from each country.

**Proximate Composition**

**Moisture**

Total moisture of *B. alicastrum* seed powder (unroasted and roasted), and cookies baked with seed powder, was determined using AACC International Method 44-15.02, Moisture - Air Oven Methods with modifications (AACC 44-15.02, 2014). Briefly, this method uses approximately 2 grams of sample, weighed on a Mettler Toledo top loading balance model # PB303, (Mettler-Toledo (Schweiz) GmbH, Im Langacher 44, 8606 Greifensee, Switzerland), in a 5.7cm diameter x 1.6 cm deep round aluminum dish, and dried in a convection oven, (Blue M Stabil Therm Model # OV-490A-2, Blue M Electric
Company, Blue Island, IL, 60406), for 12 hours at 105°C. Sample dishes were transferred to a desiccator containing 4A Molecular Sieve (Delta Adsorbents, 24 Congress Circle West, Roselle, IL, 60172), and allowed to cool for 1 hour. Samples were weighed after cooling, and percent moisture was calculated from the weight difference as loss on drying.

**Protein**

Crude protein was determined in samples by measuring nitrogen content using combustion analysis and multiplying nitrogen levels by 6.25 (conversion factor for 16% nitrogen in protein). Samples were tested by the University of Missouri-Columbia Agricultural Experiment Station Chemical Laboratories using Combustion Analysis, AOAC Official Method 990.03 (2006). Crude protein was calculated using 6.25 x nitrogen value.

**Crude Fat**

Crude fat was determined using the Soxhlet method with an ether solvent. Analyses were performed by the University of Missouri-Columbia Agricultural Experiment Station Chemical Laboratories using ether extraction, AOAC Official method 920.39 A (2012).

**Ash**

Percent ash was determined using combustion analysis where previously dried samples are heated at 550°C in a muffle furnace and cooled in a desiccator. Determination of percent ash was performed by Clemson University Agriculture Services Laboratory using AOAC Method 923.03 (2012).
Carbohydrate

Total carbohydrate was calculated by subtracting crude protein, total fat, moisture, and ash from total weight of the sample (Food Labeling, 2014).

Additional Nutrient Analysis

Fiber

Neutral and Acid Detergent fiber determinations were made by Clemson University Agriculture Services Laboratory using the method described by Goering and Van Soest (1970).

Sugars

The sugar profile of *B. alicastrum* seed powder and cookies was completed by the University of Missouri-Columbia Agricultural Experiment Station Chemical Laboratories using gas liquid chromatography and mass spectrometry (Kakehi & Honda, 1988; Churms, 1982). Sugar profile includes glucose, fructose, sucrose, lactose, and maltose.

Amino Acid Profile

Amino acid analysis was completed by the University of Missouri-Columbia Agricultural Experiment Station Chemical Laboratories using AOAC Official Method 982.30 E (AOAC 982.3 E, 2006). This method used high performance liquid chromatography coupled with ninhydrin post column derivation and quantification using absorbance at 570 nm or 440 nm. Amino acids included; Taurine (Tau), Hydroxyproline (Hyp), Aspartic Acid (Asp), Threonine (Thr), Serine (Ser), Glutamic Acid (Glu), Proline (Pro), Lanthionine (Lan), Glycine (Gly), Alanine (Ala), Cysteine (Cys), Valine (Val), Methionine (Met), Isoleucine (Ile), Leucine (Leu), Tyrosine (Tyr), Phenylalanine (Phe),
Hydroxylysine (Hyl), Ornithine (Orn), Lysine (Lys), Histidine (His), Arginine (Arg), and Tryptophan (Trp).

**Vitamins A and C**

Vitamin A (total carotenoids) and vitamin C (ascorbic acid) determinations were made by The University of Nebraska – Lincoln, Small Molecule Analysis Lab (143 Filley Hall, Lincoln, NE, 68583). Vitamin A was analyzed spectrophotometrically using the methods described by Dere et al. (1998). Vitamin C determination was made using AOAC official method 984.26(AOAC 984.26, 2012).

**Mineral Element Analysis**

Mineral determination was performed by Clemson University Agriculture Services Laboratory using wet ashing with nitric acid and hydrogen peroxide and inductively coupled plasma-mass spectrometry. Minerals that were analyzed include: calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), phosphorous (P), potassium (K), sulfur (S), zinc (Zn), and Ca/P ratio (Jones & Case, 1990; Jones, Wolf, & Mills, 1991; Plank, 1992).

**Gluten**

Gluten tests were performed solely on the unroasted *B. alicastrum*, after it was ground into powder. For each of the three replications, unroasted *B. alicastrum* seed powder from each country was ground and immediately tested for gluten using the EZ Gluten test (test batch ID – EZG130610-32), ELISA Technology Inc., 2501 NW 66th Court, Gainesville, FL 32653, www.ezgluten.com. The test has a threshold of 10 ppm.
This test has been certified by the AOAC as a validated performance test method, certificate number 51101 (AOAC 051101, 2012).

**Cookie Analysis**

Cookies were baked using unroasted and roasted *B. alicastrum* seed powder from Nicaragua, and subjected to all analyses described above, with the exception of gluten analysis. Cookies were prepared from Nicaraguan *B. alicastrum* seed powder to determine if baking would alter the composition sufficiently to result in the need for further analyses. Samples were taken from two replicates of 6 cookies each (12 cookies). All cookies were combined and ground in a food processor. Only cookies baked using seed powder from Nicaragua were used for chemical analysis.

**Cookie Formulation and Baking Methods**

In a separate experiment, cookies were baked using either unroasted or roasted *B. alicastrum* seed powder from Nicaragua, at 50% replacement, by weight, in place of all-purpose white wheat flour. Control cookies were made with 100% all-purpose white wheat flour. These cookies were baked according to The American Association of Cereal Chemists (AACC) International Method 10-5.05, Baking Quality of Cookie Flour, with modifications (AACC 10.50.05, 2014). Dextrose was omitted from the cookie formulation. Cookies were baked in a convection oven at 190°C for 9 minutes.

**Cookie Ingredients**

All ingredients, other than the *B. alicastrum* seeds, were either purchased from a wholesale distributor or in local markets.
All-purpose flour used was Sir Galahad King Arthur Flour, which contained the following ingredients: wheat flour enriched (niacin, reduced iron, thiamine mononitrate, riboflavin, folic acid) and malted barley flour, Lot # 30871PO0121316:17MM, The King Arthur Flour Company, Norwich, VT, 05055.

Sugar used was Dixie Crystals All Natural Pure Cane Sugar Extra Fine Granulated, Lot # S1300-3-12:40, Imperial-Savannah LP, Sugar Land, TX, 16200.

Shortening used was Golden Chef All-Purpose All Vegetable Shortening, that was comprised of partially hydrogenated soybean and cottonseed oil; Lot# 072312DE10105013.24, Stratus Foods LLC, Memphis, TN, 38016.

Baking soda used was Arm and Hammer Pure Baking Soda, containing sodium bicarbonate, Lot # BC303909, Arm and Hammer Division of Church and Dwight Co, Inc., 469 N. Harrison Street, Princeton, NJ, 08543.

Salt used was Diamond Crystal Fine All Natural Sea Salt 100% Pure California Sea Salt, Lot# 30012ADBAA, Cargill Inc., Minneapolis, MN, 55440.

**Statistical Analysis**

The data analysis for this thesis was generated using SAS software, Version 9.3 of the SAS System for Windows Copyright © 2012 SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA. Data were analyzed for statistical significance using the General Linear Model procedure of SAS software (SAS, 2012). Sources of variation in the data were country-of-origin (Guatemalan or Nicaraguan), seed-roasting (roasted or unroasted) and replication. Main effects of the model and their interactions were tested
for statistical significance (P < 0.05) using the residual error. Least square means statement was used to predict population margins for means separation at a significance level of 0.05.

**Results and Discussion**

**Proximate Composition**

With the exception of moisture data, all nutritional data for seed powders are presented and compared on a dry weight basis. Cookie data are presented and compared on an as sampled basis. Control cookies refer to cookies baked with 100% all-purpose white wheat flour. “Unroasted cookies” refers to cookies baked with 50% unroasted Nicaraguan seed powder and 50% all-purpose white wheat flour. “Roasted cookies” refers to cookies baked with 50% roasted Nicaraguan seed powder and 50% all-purpose white wheat flour.

Table 2 shows the proximate composition of the unroasted and roasted *B. alicastrum* seed powder from Guatemala and Nicaragua. The proximate composition for cookies baked with 100% all-purpose white wheat flour (control) and, 50% replacement of all-purpose white wheat flour with unroasted or roasted *B. alicastrum* seed powder from Nicaragua are shown in Table 3. Proximate composition data includes: moisture, protein, fat, carbohydrates, and ash.

**Moisture**

Moisture content was measured after grinding, and after roasting and grinding. Unroasted Nicaraguan seed powder had the highest moisture content (11.75 g/100g), followed by unroasted Guatemalan seed powder (9.72 g/100g), roasted Nicaraguan seed
powder (8.78 g/100g), and the lowest moisture was measured in roasted Guatemalan seed powder (7.32 g/100g).

Previous research has shown that moisture levels of *B. alicastrum* seeds vary significantly between countries-of-origin (Table 1). Moisture levels may be controlled during seed processing and handling by controlled or modified atmospheric storage (low temperature, humidity and oxygen levels); however, these types of control measures are limited in the countries where *B. alicastrum* trees are grown. During the present study, roasting reduced the moisture level in *B. alicastrum* seeds (Table 2). All-purpose white wheat flour was analyzed for moisture, using identical methods, alongside *B. alicastrum* seed powders. All-purpose flour had a total moisture of 9.76% (N=36). This moisture level is consistent with the moisture level found in the unroasted seed powder from Guatemala (9.72%). This seed powder was purchased from a commercial distributor and may have been manipulated to control moisture content.

Cookies baked with *B. alicastrum* seed powder had significantly higher moisture contents than control cookies (5.89 g/100g). Roasted cookies (6.74 g/100g) did not have a significantly different moisture level from unroasted cookies (6.71 g/100g). In the formulation of the cookie dough, moisture levels of the wheat flour and the seed powders were determined before dough mixing. Adjustments were made to the level of water incorporated into the dry ingredients, in order to compensate for any moisture differences in the flour or seed powder. All cookie dough had the same level of moisture prior to baking, which suggests that the proteins in *B. alicastrum* may provide mechanisms for retaining moisture. Additional information on the characteristics of the proteins in *B.*
*B. alicastrum* (structure, size, isoelectric point) would be useful in understanding the higher moisture level found in cookies baked with *B. alicastrum* seed powder.

**Protein**

Protein levels for all four treatments were significantly different when compared on a dry weight basis. Roasted seed powder from Nicaragua (12.14 g/100g) had the highest protein content followed by unroasted seed powder from Nicaragua (11.74 g/100g). Seed powder from Guatemala had lower protein content than that from Nicaragua, with roasted Guatemalan seed powder (10.55 g/100g) having a higher protein level than unroasted Guatemalan seed powder (10.37 g/100g). Although statistically significant, overall protein content in the seed powders varied by 1.77 g/100g, with roasting of Nicaraguan seeds resulting in a 0.4 g/100g difference in protein while roasting of Guatemalan seeds resulted in a 0.18 g/100g difference in protein. Within a country, these differences are of little practical significance and may be reflective of changes in other constituents, such as sugar (Table 4). There was a greater decrease in total sugars from the unroasted to roasted forms from Nicaragua compared to those from Guatemala.

The closest matches for protein content, from the literature, for unroasted and roasted seed powder from Nicaragua was found in unroasted seed from Yucatán, Mexico (12.13 g/100g), cooked seed from Guatemala (11.48 g/100g), and roasted seed from Guatemala as analyzed by Salguero (2013) (11.48 g/100g) (Table 1). Unroasted and roasted seeds from Guatemala had protein levels closer to dried seed from Veracruz. Flaster (2007) found roasted seed from Guatemala to have a protein content of 10.42 g/100g, and the findings of the present study are in agreement with those of Flaster.
Salgero (2013), on the other hand, found slightly higher protein content (11.48 g/100g) in roasted Guatemalan seed.

Control cookies had a significantly higher protein content (7.43 g/100g) than those baked with B. alicastrum seed powder. There was no significant difference in protein content between unroasted (6.52 g/100g) and roasted cookies (6.51 g/100g).

For this research, it was assumed that the nitrogen content of the proteins in B. alicastrum was 16%; a similar assumption was made by Flaster (2007). However, the percent nitrogen may be higher than 16%, as it is in wheat flour proteins. Wheat flour proteins have been found to contain 17.5% nitrogen, based on high levels of glutamic acid and proline which comprise nearly 50% of the amino acids profile of gliadens and glutenins (Atwell, 2001). Additional work is needed regarding the determination of the appropriate conversion factor (Tkachuk, 1969). As cookies were baked using a 50% split between wheat and B. alicastrum it may be more appropriate to use two separate conversion factors. There was only a 0.4 g/100g difference in protein from the roasted to the unroasted Nicaraguan seed powder. At a 50% replacement for wheat flour, this difference was no longer significant.

**Fat**

Roasted seed powder from Guatemala had a significantly higher fat content (1.35 g/100g) than all other treatments. This was followed by unroasted Guatemalan seed powder (0.74 g/100g) which had a significantly higher fat content than either of the Nicaraguan seed powders. There was no significant difference in fat contents of roasted (0.64 g/100g) and unroasted (0.58 g/100g) seed powders from Nicaragua. Fat content was
found at the greatest levels in the roasted Guatemalan seed powder (1.35%). Previous research by Zamorano (2009) found that *B. alicastrum* seeds from Honduras had a similar level of fat at 1.37 g/100g. However, Flaster (2007) and Salguero (2013) found fat levels in roasted Guatemalan seed at 0.49 g/100g and 0.75 g/100g respectively. Other research has reported the range for fat levels in unroasted seeds was 1.37-2.02 g/100g. Cooked seed fat levels were 1.51 g/100g. Relative quantities of crude fat nearly doubled after roasting the Guatemalan seed which had 0.74 g/100g of fat before roasting. This change in fat content was less pronounced with the unroasted Nicaraguan seed (0.58 g/100g), which had a small and insignificant increase in fat after roasting (0.64 g/100g). The fat levels in the Nicaraguan seed more closely resemble the fat levels in the roasted Guatemalan seed measured by Flaster (2007) and Salguero (2013).

While unroasted (16.09 g/100g) and roasted (15.87 g/100g) cookies had a significantly higher fat content than control cookies (15.04 g/100g), there was no significant difference between the unroasted and roasted cookies regarding fat content. While the difference in fat content between *B. alicastrum* and wheat flour cookies was small, it was statistically significant. The added fat content to cookies (as vegetable shortening) was controlled during ingredient scaling and mixing and thus, small differences in fat may not be of practical significance for functionality.

**Carbohydrates**

Carbohydrate content, calculated by difference, was highest for unroasted Guatemalan seed powder (85.12 g/100g) followed by roasted Guatemalan seed powder (83.89 g/100g), unroasted Nicaraguan seed powder (83.61 g/100g) and roasted
Nicaraguan seed powder (82.62 g/100g). Differences were significant between all treatments.

Roasted (68.62 g/100g) and unroasted (68.38 g/100g) cookies had a significantly lower level of carbohydrates than control cookies (70.61 g/100g), but were not significantly different from one another.

As carbohydrates were calculated by difference, higher levels of protein and ash in the roasted Nicaraguan seed powder contributed to lower level of carbohydrates. Unroasted Guatemalan seed powder had the lowest levels of protein and ash which explains the highest level of carbohydrates.

Ash

Ash was at the highest levels in the roasted samples with Nicaraguan seed powder (4.6 g/100g) having higher levels than Guatemalan seed powder (4.2 g/100g). Unroasted seed powder had lower levels of ash with unroasted Nicaraguan seed powder (4.1 g/100g) having higher levels than unroasted Guatemalan seed powder (3.8 g/100g). Analyses of ash gave highly repeatable results with no standard deviation (Table 2). There was a 0.8 g/100g difference in the ash levels from the highest level of ash, in roasted Nicaraguan seed to the lowest level in unroasted Guatemalan seed. Ash levels were higher in the roasted forms than in their unroasted counterparts. This is due to the heat stability of the minerals that make up total ash. At high temperatures during roasting other components are volatilized or burned off while ash remains.

Roasted (2.27 g/100g) and unroasted (2.30 g/100g) cookies did not have significantly different levels of ash, but the levels were significantly higher than control
cookies (1.03 g/100g). There was only a 0.4 g/100g difference in the level of ash between unroasted and roasted seed powders from Nicaragua. This accounts for the small and insignificant difference in the cookies baked with the unroasted and roasted forms of the seed powders. The Nicaraguan B. alicastrum seed powder cookies had an approximately 1.25 g/100g greater ash content than the control cookies, suggesting that B. alicastrum seed powder has a higher overall mineral content than wheat flour.

Additional Nutrient Analysis

Neutral Detergent Fiber

Neutral detergent fiber (NDF) was measured at the highest levels in roasted seed powder from Nicaragua (11.0 g/100g), followed by the unroasted seed powder from Nicaragua (9.8 g/100g). These differences were significant (P<0.05) and significantly different from the roasted (7.5 g/100g) and unroasted (8.1 g/100g) seed powders from Guatemala, which were not significantly different from one another.

Neutral detergent fiber, which is a measure of hemicellulose, cellulose and lignin, accounts for the insoluble fiber fraction in food/feeds. NDF is not a measure of dietary fiber which includes water soluble non-starch polysaccharides (NSP) such as β-glucans, pectins, gums and mucilages (Van Soest, Robertson, & Lewis, 1991). Correlations can be made between detergent fiber results and total dietary fiber; however this is not within the scope of this work (Wolfrum & Lorenz, 2009). There was a significant difference in NDF between roasted Nicaraguan and unroasted Nicaraguan seed powders, which may be attributed to the decrease in total sugars, causing relative quantities of other components to increase. However, there was no significant change in NDF between unroasted and
roasted Guatemalan seed powder, which had a smaller decrease in total sugar. Guatemalan seed powders, overall, had lower levels of NDF than Nicaraguan forms.

Dietary fiber was measured by Zamarano (2009) in unroasted seeds and Flaster (2007) in roasted seeds at 21.71 g/100g and 21.2 g/100g respectively. These measurements are nearly double or more than double the NDF values generated by this analysis. This suggests that B. alicastrum seed powder may therefore have a quantity of NSP not detected by detergent fiber analysis.

When seed powder was made into cookies, no significant difference in NDF was found for roasted (11.8 g/100g), unroasted (10.1 g/100g) and control cookies (9.2 g/100g). This may be due to small sample size and large standard deviations.

**Acid Detergent Fiber**

Acid detergent fiber (ADF) compared on a dry weight basis, was found at the highest levels in roasted Nicaraguan seed powder (8.5 g/100g), which was significantly higher than the levels measured in the unroasted seed powder from Nicaragua (6.5 g/100g). While both forms of Guatemalan seed powder were not significantly different from one another, the roasted seed powder (5.3 g/100g) and unroasted seed powder (4.9 g/100g) had a significantly lower level of ADF than both types from Nicaragua.

Acid detergent fiber (ADF) measures only cellulose and lignin, and the process does not recover the hemicellulose recovered by NDF determination. This fiber is not digestible. ADF measurements are similar to crude fiber measurements, however crude fiber does not account for all of the lignin (Wolfrum & Lorenz, 2009). Increases in fiber after roasting can be attributed to decreased sugar content, which changed the relative
quantities of other constituents. Crude fiber measurements from the literature are between 4.10 and 8.90 g/100g for *B. alicastrum* seeds. Given that ADF may be higher than crude fiber, the ADF quantities from this experiment fall in this range.

There were no significant differences in ADF for roasted (5.2 g/100g), unroasted (3.7 g/100g) and control cookies (3.4 g/100g). This may be due to small sample size and high standard deviations.

**Sugars**

Table 4 shows the sugar profile data for unroasted and roasted *B. alicastrum* seed powders from Guatemala and Nicaragua. Table 5 shows the sugar profile data for cookies baked with 100% all-purpose white wheat flour, or 50% replacement of all-purpose white wheat flour with unroasted or roasted *B. alicastrum* seed powder from Nicaragua.

**Total Sugar**

Unroasted seed powders had the highest levels of total sugar with unroasted Nicaraguan seed powder (7.66 g/100g) having a significantly higher level than unroasted Guatemalan seed powder (4.93 g/100g). Roasted Nicaraguan seed powder (3.98 g/100g) had significantly higher total sugar content than roasted Guatemalan seed powder (2.48 g/100g). There were significant differences for all treatments regarding total sugar content. There was a greater percentage of sugar loss in the roasted Nicaraguan seed than in the roasted Guatemalan seed. This decrease in sugar content may be responsible for the increases in relative quantities of protein, fat, ash, and fiber as discussed above. Sugar loss may be attributed to Maillard reactions during roasting. After roasting there were small reductions in fructose and larger reductions in glucose and maltose; all of these
sugars are reducing sugars and therefore, could be involved in non-enzymatic browning. Nicaraguan seed had a higher level of maltose than Guatemalan seed which may explain the greater reduction in total sugars. Loss of maltose accounted for 2.52 g/100g of the total sugar reduction in Nicaraguan seed after roasting. Sucrose was lost to a lesser extent, possibly because it must first be inverted into glucose and fructose before Maillard reaction can take place (Damodaran, Parkin, & Fennema, 2008).

Unroasted cookies (49.09 g/100g) had significantly higher total sugar content than control (35.49 g/100g) or roasted cookies (32.78 g/100g), which were not significantly different from one another. The total sugar content of the control cookies was comprised solely of sucrose, which was added to the cookie formulation. Cookies baked with unroasted seed powder had the highest sugar content based on the sugar content naturally present in the unroasted Nicaraguan seed powder (7.66 g/100g). According to the USDA Nutrient Database, wheat flour, white, all-purpose, enriched, unbleached (#20581) has 0.27 g/100g of total sugar, which does not add an appreciable amount of total sugar to the total sugar content of the control cookie (U. S. Department of Agriculture, 2014). Cookies baked from roasted Nicaraguan seed powder had lower total sugar than the control cookies, and this may be due to caramelization or Maillard reaction during roasting and subsequent baking.

**Fructose, Glucose, Lactose, Maltose**

Unroasted seed powder from Guatemala had the highest level of fructose (0.85 g/100g). While this level of fructose was not significantly different from unroasted...
Nicaraguan seed powder (0.79 g/100g), it was significantly different than fructose levels in roasted seed powders from Guatemala (0.62 g/100g) and Nicaragua (0.60 g/100g).

Unroasted Nicaraguan seed powder (1.57 g/100g) had significantly higher levels of glucose than all other treatments. Unroasted seed powder from Guatemala (1.26 g/100g) had significantly higher levels of glucose than roasted seed powder from Guatemala (0.59 g/100g) and roasted seed powder from Nicaragua (0.78 g/100g). There was no significant difference in glucose levels between roasted powders from Guatemala and Nicaragua.

Lactose was only found in roasted seed powder from Nicaragua (0.04 g/100g). Lactose was not detected in Guatemalan seed powders or in the unroasted Nicaraguan seed powder.

Maltose was significantly higher in unroasted Nicaraguan seed powder (3.46 g/100g) than in all other treatments. There was no significant difference between the maltose levels in unroasted seed powder from Guatemala (1.15 g/100g) and roasted powder from Nicaragua (0.94 g/100g). Roasted Guatemalan seed powder (0.05 g/100g) had the lowest level of maltose, which was significantly different from all other treatments.

Nicaraguan roasted seed powder had the highest level of sucrose (1.83 g/100g) and was significantly different from all other treatments. There was no significant difference in sucrose levels between unroasted Guatemalan seed powder (1.67 g/100g) and roasted Nicaraguan seed powder (1.62 g/100g). Roasted Guatemalan seed powder
(1.21 g/100g) had the lowest levels of sucrose, which was significantly different from all other treatments.

Unroasted cookies were the only treatment where fructose was measured (0.67 g/100g). Fructose was not detected in roasted cookies or controls. As the sugar in the control cookies was comprised predominantly of added sucrose, no fructose was found in the finished product.

Unroasted cookies (0.80 g/100g) had a significantly higher level of glucose than roasted cookies (0.45 g/100g). Glucose was not detected in the control cookies.

Roasted cookies (1.35 g/100g) had significantly higher maltose content than unroasted cookies (0.09 g/100g). This finding is unexpected as unroasted Nicaraguan seed powder had significantly higher levels of maltose than its unroasted counterpart. Maltose was not detected in control cookies.

Unroasted cookies (47.53 g/100g) had a significantly higher sucrose content than controls (35.49 g/100g) or roasted cookies (30.98 g/100g), which were not significantly different from one another. Unroasted seed powder cookie sugar content was expected to be greater given the high levels of naturally occurring sugars in the unroasted Nicaraguan B. alicastrum seed powder.

**Indispensable Amino Acids**

Table 6 shows the indispensable amino acid data for unroasted and roasted B. alicastrum seed powders from Guatemala and Nicaragua. Table 7 shows the indispensable amino acid data for cookies baked with a 50% replacement of all-purpose white wheat flour with unroasted or roasted B. alicastrum seed powder from Nicaragua.
Indispensable amino acids include histidine, isoleucine, leucine, lysine (as lysine and hydroxylysine), methionine, phenylalanine, threonine, tryptophan and valine.

Overall, the unroasted Nicaraguan seed powder had the highest levels of indispensable amino acids. Lysine was the only exception to this, with unroasted seed powder from Guatemala having the highest lysine content (0.61 g/100g). Roasted seed powder from Nicaragua consistently had the second highest levels of indispensable amino acids, with the exception of lysine (0.34 g/100g). Generally, roasting only slightly decreased indispensable amino acid quantities in the Nicaraguan seed powder. There was no change after roasting for histidine, phenylalanine, and threonine. There were small (0.01-0.05 g/100g) but significant reductions in isoleucine, leucine, methionine, tryptophan and valine. The most pronounced reduction due to roasting Nicaraguan seed was found for lysine (0.16 g/100g reduction). Lysine is readily used in the Maillard reaction as a source of primary amines (Damodaran et al., 2008). Differences across all treatments for each amino acid were small even when they were significant and ranged from 0.01g/100g to 0.11g/100g. Lysine, again, was the exception to this. Unroasted Guatemalan and roasted Nicaraguan seed powders had the greatest difference in lysine content (0.27 g/100g). Even though differences were small, they could account for a large portion of the amino acid present. For example, unroasted Nicaraguan seed powder had 0.05g/100g of methionine and unroasted had 0.03g/100g, a difference of only 0.2g/100g, but nearly half of the total.

Unroasted Guatemalan seed powder had higher levels of most indispensable amino acids than did its roasted counterpart, but primarily lower levels than Nicaraguan
seed powder of both types. The exception again was lysine, which was at its highest levels in unroasted Guatemalan seed powder. Lysine levels in the roasted Guatemalan seed powder were higher than in the roasted Nicaraguan seed powder. The Guatemalan seed powder had a small (0.01g/100g) but significant increase in threonine after roasting. For the Guatemalan seed powder, roasting had no effect on the levels methionine and tryptophan. There was a small (0.01-0.06 g/100g) but significant decrease due to roasting for histidine, isoleucine, leucine, phenylalanine and valine. The largest indispensable amino acid decrease after roasting Guatemalan seed was measure in lysine content (0.14 g/100g loss).

Control cookies had a greater or equal content of indispensable amino acids than cookies made with 50% unroasted B. alicastrum seed powder from Nicaragua. Cookies made with roasted Nicaraguan seed powder had consistently lower indispensable amino acid quantities than control cookies. Control cookies also had the highest level of protein of the three treatments. There was no difference between control cookies and cookies baked with unroasted Nicaraguan seed powder for lysine and tryptophan. There was no significant difference in the indispensable amino acid content between cookies baked with unroasted and roasted seed powder for: histidine, isoleucine, leucine, methionine, tryptophan and valine. There were small but significant reductions due to roasting for lysine and threonine. There was a small increase in phenylalanine from the unroasted seed powder cookies to the roasted. With the exception of leucine (0.14 g/100g difference) and the phenylalanine (0.12 g/100g difference), the differences from highest
to lowest indispensable amino acid content across all three treatments were small (0.01-0.07 g/100g).

**Conditionally Indispensable Amino Acids**

Table 8 shows the conditionally indispensable amino acid data for unroasted and roasted *B. alicastrum* seed powders from Guatemala and Nicaragua. Table 9 shows the conditionally indispensable amino acid data for cookies baked with a 50% replacement of all-purpose white wheat flour with unroasted or roasted *B. alicastrum* seed powder from Nicaragua. Conditionally indispensable amino acids include arginine, cysteine, glycine, proline (proline and hydroxyproline), and tyrosine.

Overall, unroasted seed powder from Nicaragua had the highest levels of conditionally indispensable amino acids. There was no significant difference in the levels of conditionally indispensable amino acids between the unroasted and roasted Nicaraguan powders for cysteine and tyrosine. There were significant reductions, from the unroasted to the roasted powders from Nicaragua for arginine, glycine, and proline (0.02-0.10 g/100g loss).

Unroasted Guatemalan seed powder had the second highest quantities, overall, of conditionally indispensable amino acids, but was relatively close to roasted Nicaraguan seed powder concerning the quantities of these amino acids. Cysteine levels were greater in both types of Nicaraguan seed powder and roasted Nicaraguan seed powder had higher levels of glycine than unroasted Guatemalan powder. There were significant reductions of conditionally indispensable amino acids from the unroasted to the roasted forms of Guatemalan seed powder for arginine, glycine, proline, and tyrosine. These reductions
were small but significant (0.02-0.08 g/100g). There was no significant change in the level of cysteine from the unroasted to roasted Guatemalan seed powders.

Control cookies had significantly higher levels of conditionally indispensable amino acids than either the cookies baked with unroasted or roasted seed powder from Nicaragua. There were no significant differences between cookies baked with unroasted or roasted Nicaraguan seed powder for arginine and cysteine. There was a reduction of conditionally indispensable amino acids from the cookies baked with unroasted seed powder to cookies baked with roasted seed powder for glycine (0.01g/100 g reduction) and proline (0.04 g/100g) reduction. Tyrosine levels increased significantly (0.03 g/100g) from the unroasted to the roasted seed powder cookies. Although significant, the differences between the highest and lowest levels of conditionally indispensable amino acids were small for arginine, cysteine glycine, and tyrosine (0.04-0.07 g/100g). Control cookies had a high level of proline compared cookies baked with roasted Nicaraguan seed powder and the difference was more substantial (0.32 g/100g difference).

**Dispensable Amino Acids**

Table 10 shows the dispensable amino acid data for unroasted and roasted *B. alicastrum* seed powders from Guatemala and Nicaragua. Table 11 shows the dispensable amino acid data for cookies baked with a 50% replacement of all-purpose white wheat flour with unroasted or roasted *B. alicastrum* seed powder from Nicaragua. Dispensable amino acids include alanine, aspartic acid, glutamic acid and serine.

Unroasted Nicaraguan seed powder had the highest overall levels of dispensable amino acids, with the exception of serine, which was higher in roasted Nicaraguan seed
powder. From the unroasted to the roasted forms for the Nicaraguan seed powder; there was no change for alanine, and a decrease in aspartic and glutamic acids. There was a large decrease in aspartic acid due to roasting (0.42 g/100g reduction); there were smaller decreases in the other dispensable amino acids (0.0-0.4 g/100g difference).

Unroasted Guatemalan seed powder had the second highest levels of dispensable amino acids with the exception of serine (0.23 g/100g), which was higher in the roasted (0.32 g/100g) and unroasted (0.28 g/100g) Nicaraguan seed powders. There were significant reductions from the unroasted to the roasted Guatemalan seed powder for alanine, aspartic and glutamic acids. The decrease was largest for aspartic acid (0.25 g/100g), and smaller for the others (0.01-0.03 g/100g). Serine increased from the unroasted to the roasted Guatemalan seed powder (0.03 g/100g increase).

Control cookies had a statistically significant higher level of alanine (0.21 g/100g), glutamic acid (2.38 g/100g), and serine (0.32 g/100g). Cookies baked with unroasted Nicaraguan seed powder had the highest level of aspartic acid (0.49 g/100g). This is the only instance where an individual amino acid was measured at a greater quantity in seed powder cookies than in the control cookies. There were no significant differences between unroasted and roasted seed powder cookies for alanine and serine. There was a significant reduction due to roasting for levels of aspartic acid (0.09 g/100g decrease), and glutamic acid (0.03 g/100g decrease).

**Vitamins and Minerals**

Table 12 shows the vitamin and mineral data for unroasted and roasted *B. alicastrum* seed powders from Guatemala and Nicaragua. Table 13 shows the vitamin
and mineral data for cookies baked with a 50% replacement of all-purpose white wheat flour with unroasted or roasted *B. alicastrum* seed powder from Nicaragua. Vitamin data includes: vitamin A (measured as total carotenoids) and vitamin C (measured as ascorbic acid). Mineral data includes: calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sulfur, zinc, sodium, and chloride.

**Vitamin A (Total Carotenoids)**

The highest level of total carotenoids was measured in the roasted forms of the seed powder with roasted Nicaraguan (1820 μg/100g) having the highest levels of total carotenoids; followed by roasted Guatemalan (1393 μg/100g), unroasted Guatemalan (1188 μg/100g) and unroasted Nicaragua (899 μg/100g). Differences were significant between treatments. Roasting the Guatemalan seed increased the relative quantities of total carotenoids by 205μg/100g, while roasting the Nicaraguan produced a greater increase of 921μg/100g. Data should be confirmed using high performance liquid chromatography (HPLC) analysis.

Carotenoids are unstable and susceptible to heat degradation. Thermal processing, of various types has been shown to reduce the total carotenoid levels in foods (Delgado-Vargas, Jiménez, & Paredes-López, 2000; Lešková et al., 2006). Although loss of carotenoids due to thermal processing is common, there are exceptions. Thermal processing, in the form of blanching, was shown to increase α and β-carotene in cowpea, peanut and pumpkin leaves. Cooking peanut, pumpkin and sweet potato leaves at 98°C in water, increased total carotenoid levels (Mosha, Pace, Adeyeye, Laswai, & Mtebe, 1997). Caramelization, as a result of high drying temperature of spice paprika pepper
pods, increased levels of β-carotene, cis- β-carotene, monoesters, and diesters (Markus, Daood, Kapitany, & Biacs, 1999).

Total carotenoid content was highest for roasted cookies (1460 μg/100g), followed by unroasted cookies (593 μg/100g) and control cookies (61 μg/100g). Differences were significant (P<0.001).

Due to the high levels of vitamin A in unroasted and roasted seed powder it is expected that cookies baked with these seed powders would have higher levels of total carotenoids than controls. These results show that *B. alicastrum* seed powder has higher levels of total carotenoids than the all-purpose white wheat flour used in this research.

**Vitamin C (Ascorbic Acid)**

Unroasted seed powder from Nicaragua had a significantly higher level of ascorbic acid (41mg/100g) than all other treatments, which decreased when it was roasted (23 mg/100g). Unroasted seed powder from Guatemala (16 mg/100g) as well as roasted seed powder from Guatemala (11 mg/100g) had significantly lower ascorbic acid contents than unroasted Nicaraguan seed powder.

Ascorbic acid is thermally liable and will degrade at temperatures higher than 100°C (Van den Broeck, Ludikhuyze, Weemaes, Van Loey, & Hendrickx, 1998). The temperature of *B. alicastrum* seed reached 160°C, before it was forced air cooled. Seeds were heated well beyond the temperature necessary for thermal degradation and a loss in ascorbic acid is expected during roasting. This data should be confirmed using HPLC methods of analysis. The much higher levels of ascorbic acid measured in the unroasted
Nicaraguan seed suggests that environmental conditions were more favorable for ascorbic acid biosynthesis.

Ascorbic acid content was not significantly different (P=0.2672) for unroasted (13±4mg/100g), control (10±4mg/100g), and roasted cookies (10±4mg/100g).

The cookies baked with unroasted seed powder had a slightly, but not significantly higher level of ascorbic acid than controls, or cookies baked with roasted seed. The presence of ascorbic acid in the control cookies is an unexpected result, as it is not clear which ingredient contributed the ascorbic acid. It is possible that 10 mg/100g is the lower threshold for the test. These results should be confirmed using HPLC analysis.

**Calcium**

Unroasted seed powder from Guatemala (206 mg/100g) had the highest levels of calcium and had significantly higher levels than all other treatments. Roasted Guatemalan seed powder (190 mg/100g), and both unroasted (189 mg/100g) and roasted (190 mg/100g) seed powders from Nicaragua did not have significantly different levels of calcium. Roasting did not cause a significant decrease in calcium for the Nicaraguan seed. Roasting did produce a small but significant decrease in calcium for the Guatemalan seed. Roasting of a wild legume seed (*Canavalia cathartica*) at 180°C for 20 minutes caused decreases in calcium in one related experiment, while in another, this roasting had no significant effect on calcium levels. (Seena, Sridhar, & Jung, 2005; Seena, Sridhar, Arun, & Young, 2006).

Calcium was higher in both unroasted and roasted cookies (50 mg/100g), than in control cookies (10 mg/100g). Differences were significant (P<0.0001). This data shows
that *B. alicastrum* has higher levels of calcium than all-purpose white wheat flour used in this experiment.

**Copper**

There was no significant difference between copper levels in roasted seed powders from Nicaragua (1.0 mg/100g) and Guatemala (1.0 mg/100g). These powders had small but significantly higher levels of copper than their unroasted counterparts from Nicaragua (0.9 mg/100g) and Guatemala (0.9 mg/100g) which were not different from one another. Small but statistically significant differences in copper from the unroasted to the roasted forms could be due to a small sample size and small standard deviation. It could also be attributed to the loss of other constituents after roasting which would increase relative quantities of copper. As copper is heat stable, it would not be degraded or lost during heat treatment.

Copper content was highest in unroasted cookies (0.4 mg/100g) followed by roasted cookies (0.3 mg/100g), and control cookies (0.1±0.1mg/100g). Differences were significant (P<0.0052). Comparison between groups, for statistical significance, was not possible due to small sample size and standard deviation. It is likely that the only significant difference is between control cookies and those baked with *B. alicastrum* seed powder.

**Iron**

Unroasted seed powder from Nicaragua (6.1 mg/100g) had the highest levels of iron, although this level was not significantly different from the roasted Nicaraguan seed powder (5.6 mg/100g). Both seed powders from Nicaragua had significantly higher
levels of iron than seed powders from Guatemala; however, iron levels in unroasted (3.6 mg/100g) and roasted (4.0 mg/100g) seed powders from Guatemala were not significantly different from one another. There were no significant changes in iron content due to roasting.

There were no significant differences for iron content in unroasted (3.0 mg/100g), roasted (2.9 mg/100g), and control cookies (2.9 mg/100g). The all-purpose white wheat flour used in this experiment was fortified with iron. When wheat flour quantity was reduced by half and replaced with Nicaraguan *B. alicastrum* seed powder, iron levels remained constant. This result suggests Nicaraguan *B. alicastrum* naturally contains comparable iron levels to enriched white wheat flour.

**Magnesium**

There were no significant differences in magnesium levels between roasted (208 mg/100g) and unroasted (200 mg/100g) Nicaraguan seed powders. There were also no significant differences in magnesium levels between roasted (173 mg/100g) and unroasted (173 mg/100g) Guatemalan seed powders. Nicaraguan seed powders had significantly higher magnesium contents than Guatemalan seed powders.

Magnesium levels were higher in unroasted (60 mg/100g) and roasted (60 mg/100g) cookies, than in control cookies (20 mg/100g). Differences were significant (P<0.0001).

**Manganese**

There was no significant difference in manganese levels for any treatment. Unroasted and roasted seed powders from Nicaragua had equal levels of manganese (0.6
mg/100g). Unroasted and roasted seed powders from Guatemala had equal levels of manganese (0.7 mg/100g).

There were no significant differences between manganese content for unroasted (0.3 mg/100g), roasted (0.3 mg/100g) and control cookies (0.4 mg/100g).

**Phosphorus**

Phosphorus levels were significantly different between countries, with Nicaraguan seed powder having higher levels than Guatemalan. There was no significant difference in phosphorus levels between the unroasted (163 mg/100g) and roasted (171 mg/100g) Nicaraguan seed powders. There was also no significant difference between the unroasted (137 mg/100g) and roasted (147 mg/100g) Guatemalan seed powders.

Phosphorus was higher in unroasted (70 mg/100g) and roasted (70 mg/100g) cookies, than in control cookies (60 mg/100g). Differences were significant (P<0.0001).

**Potassium**

Potassium levels were significantly higher in roasted (1498 mg/100g), and unroasted (1439 mg/100g) Nicaraguan seed powder. There were no significant differences in potassium levels between unroasted seed powder from Nicaragua, roasted seed powder from Guatemala (1399 mg/100g), and unroasted seed powder from Guatemala (1381 mg/100g).

Potassium was higher in unroasted (360 mg/100g) and roasted (360 mg/100g) cookies than in control cookies (60 mg/100g). Differences were significant (P<0.0001).
Sulfur

Roasted (88 mg/100g) and unroasted (91 mg/100g) Nicaraguan seed powders had higher levels of sulfur than Guatemalan roasted (79 mg/100g) and unroasted (78 mg/100g) seed powders. Sulfur levels were not significantly different from unroasted to roasted forms but were significantly different between countries.

Control cookies (77 mg/100g) had higher sulfur content than unroasted (60 mg/100g) and roasted (60 mg/100g) cookies. Differences were significant (P=0.0012).

Zinc

Zinc was measured at a higher level in roasted seed powder from Nicaragua (2.9 mg/100g), with zinc levels in the unroasted Nicaraguan seed powder (2.7 mg/100g) being significantly lower. Seed powders from Guatemala had a significantly lower level of zinc than powders from Nicaragua, although the unroasted (1.6 mg/100g) and roasted (1.6 mg/100g) forms from Guatemala were not different from one another.

Roasted cookies (1.0 mg/100g) had the highest zinc content followed by unroasted (0.9 mg/100g) and controls (0.6 mg/100g). Differences were significant (P<0.001).

Sodium and Chloride

There were no significant differences in sodium levels for any of the treatments. Sodium levels were slightly higher for unroasted Guatemalan seed powder (21.6 mg/100g), followed by unroasted Nicaraguan seed powder (18.1 mg/100g), roasted Guatemalan seed powder (15.2 mg/100g), and roasted Nicaraguan seed powder (11.7 mg/100g). There were also no significant differences in chloride levels for any
treatments. Unroasted seed powder from Guatemala had the highest levels of chloride (41 mg/100g) followed by the roasted form (39 mg/100g). Roasted seed powder (15 mg/100g) and unroasted seed powder (15 mg/100g) from Nicaragua had relatively equal quantities of chloride. Standard deviations for sodium and chloride contents were high, which may have not shown a difference that possibly existed.

Cookies baked with roasted seed powder (371.0 mg/100g) had the highest sodium content followed cookies baked with unroasted seed powder cookies (336.3 mg/100g) and controls (304.7 mg/100g). Differences were significant (P=0.0355). There was no significant difference (P=0.0732) in chloride content for roasted cookies (367 mg/100g), unroasted cookies (313 mg/100g), and controls (293 mg/100g).

**Gluten**

*B. alicastrum* seed powders were tested for gluten because gluten-free foods have gained in popularity in recent years. All gluten tests were negative for unroasted *B. alicastrum* seed powder from Nicaragua and Guatemala. These tests measured gluten level concentrations in *B. alicastrum* seed powder to be under 10 ppm. The U.S. Food and Drug Administration has set the limits for gluten in food to be under 20 ppm, in order to be labeled “gluten-free” (U.S. Food and Drug Administration, 2014). *B. alicastrum* seed and seed powder, in compliance with this definition, is gluten-free.

**Conclusions**

Unroasted *B. alicastrum* seed powders from Guatemala and Nicaragua were found to be different in their nutritional composition. These differences are likely due to environmental conditions, such as soil quality and rainfall, or to genetic variations within the species, and include handling post-harvest. When compared on a dry weight basis,
Nicaraguan seed powder had higher levels of almost all nutrients measured. When unroasted Nicaraguan seed powder was compared to unroasted Guatemalan seed powder, Nicaraguan seeds had higher levels of protein, ash, and fiber, while the opposite was observed for carbohydrate and fat. Unroasted Nicaraguan seed powder had higher total sugar content.

Nearly all of the indispensable amino acids measured, with the exception of lysine, were significantly higher in the unroasted Nicaraguan seed powder when compared to the unroasted Guatemalan seed powder. Lysine was higher in the Guatemalan variety and tryptophan levels were not significantly different. Unroasted Nicaraguan seed powder had significantly higher levels of all conditionally indispensable amino acids measured, when compared to unroasted seed powder from Guatemala. Serine was the only dispensable amino acid measured that was found in significantly higher levels in unroasted Guatemalan seed powder, when compared to unroasted Nicaraguan seed powder.

Roasted Nicaraguan seed powder had higher relative quantities of vitamin A, while unroasted Nicaraguan seed powder had higher vitamin C content. Unroasted seed powder from Guatemala had a significantly higher level of calcium. Unroasted Nicaraguan seed powder had significantly higher levels of iron, magnesium, phosphorus, sulfur, and zinc. There were no significant differences between countries for copper, manganese, potassium, sodium and chloride.

Roasting affected the chemical composition of both the Nicaraguan and the Guatemalan seed powders, in many cases increasing nutrient levels when compared on a
Roasting significantly decreased most of the indispensable amino acids. The only increase was small (0.01 g/100g) but significant for threonine in the Guatemalan seed powder. There was no significant change after roasting Guatemalan seeds for histidine, methionine and tryptophan. There was a significant decrease in roasted Guatemalan seed powder for isoleucine, leucine, lysine, phenylalanine and valine. Roasting Nicaraguan seed significantly decreased isoleucine, leucine, lysine, methionine, tryptophan and valine. Roasted Nicaraguan seed did not differ from its unroasted form for histidine, or phenylalanine. With regard to the conditionally indispensable amino acids, cysteine remained unchanged in roasted Guatemalan seed, while the remaining amino acids measured decreased significantly. Roasting Nicaraguan seed did not change cysteine or tyrosine levels although arginine, glycine, and proline levels significantly decreased.

Roasting significantly increased relative quantities of total carotenoids (vitamin A) in seed powders from both Guatemala and Nicaragua. Vitamin C significantly decreased in roasted Nicaraguan seeds. While there was a vitamin C decrease in roasted Guatemalan seeds, it was not significant. Of all minerals measured in the Guatemalan
seed powder, roasting only affected calcium and copper. Calcium decreased significantly while copper increased significantly. With the exception of copper and zinc, which significantly increased, roasting Nicaraguan seed did not significantly change the mineral content. Roasted Nicaraguan seed powder had the highest mineral content of all the treatments with the exception of calcium, as well as the highest vitamin A content.

Cookies were baked using either 100% all-purpose white wheat flour (control) or with a 50% replacement of either unroasted or roasted seed powder from Nicaragua. Two batches of six cookies each were combined and ground in a food processor for chemical analysis. Results were presented on an as sampled basis. Control cookies were found to have significantly higher levels of protein and carbohydrates. Cookies baked with Nicaraguan *B. alicastrum* seed powder replacements had significantly higher levels of fat and ash. Moisture was also significantly higher in cookies baked with *B. alicastrum* seed powder. There was no difference between cookies baked with the unroasted and roasted seed powders for moisture, protein, fat, carbohydrates, and ash. Although cookies baked with the roasted seed powder had a higher level of fiber, this level was not significantly different from controls or cookies baked with unroasted seed powder. Cookies baked with unroasted seed powder had significantly higher total sugar content while there was no difference between controls and roasted seed powder cookies.

Control cookies had significantly higher levels of nearly all indispensable amino acids. Lysine and tryptophan levels were not significantly lower in unroasted seed powder cookies. Lysine was significantly lower in roasted seed powder cookies although this difference was small. There were also small but significant decreases in
phenylalanine and threonine in cookies baked with unroasted seed powder compared to
cookies baked with roasted seed powder. The differences between controls and the
lowest amino acid level for cookies baked with either unroasted or roasted seed powder
were moderate for leucine and phenylalanine, but were smaller for all other indispensable
amino acids. Control cookies had significantly higher levels of all conditionally
indispensable amino acids measured. Tyrosine significantly increased from the unroasted
to the roasted *B alicastrum* seed powder cookies, although this increase was small. There
were also small but significant decreases from unroasted to roasted seed powder cookies
for glycine and proline. While the differences between amino acid levels in controls and
the lowest amino acid level for cookies baked with either unroasted or roasted seed
powder were large for proline, they were smaller for the remaining conditionally
indispensable amino acids measured.

Cookies baked with roasted Nicaraguan seed powder had the highest level of
vitamin A. Unroasted cookies had the highest level of vitamin C, although this was not
significant. Cookies baked with *B. alicastrum* seed powder had significantly higher levels
of calcium, copper, magnesium, phosphorus, potassium, zinc and sodium. There was
little to no change in mineral levels from the unroasted to the roasted seed powder
cookies. There was no difference between any treatment for iron, manganese, and
chloride. It should be noted that the wheat flour used was fortified with reduced iron,
whereas *B. alicastrum* seed powder is not. Control cookies had a significantly higher
level of sulfur than cookies baked with either unroasted or roasted seed powder.
Nicaraguan seed powder had a more favorable nutrition profile than Guatemalan seed powder. Cookies were baked with Nicaraguan seed powder. Without additional evidence, it is unclear as to whether or not Nicaraguan seed powder has superior nutritional qualities when compared to other growing regions and climates. Chemical composition can also change from year to year based on a number of factors. Cookies baked with *B. alicastrum* seed powder have higher levels of fiber, and ash than cookies baked with 100% white wheat flour. There are decreases in protein as well as indispensable amino acids. While these decreases may be statistically significant, they are small enough to not detrimentally affect the overall protein quality. Cookies baked with *B. alicastrum* seed powder have an increase in overall mineral content when compared to control cookies. Given these considerations, from a nutritional perspective, *B. alicastrum* seed powder is a suitable substitute for all-purpose white wheat flour in cookies.

**Future Research Recommendations**

- Comprehensive study on the chemical composition of *B. alicastrum* seed, using a larger sample size and generating year to year data, in order to establish more accurate chemical composition data.
- Isolation and purification of proteins to study structure and gelation properties.
- Evaluation of anti-nutrients absence or presence in *B. alicastrum* seed powder.
- Evaluation of the digestibility of *B. alicastrum* seed.
- Solubility/particle size determinations for *B. alicastrum* seed powders.
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Table 1: Proximate composition, calories and total sugars\textsuperscript{1} of \textit{Brosimum alicastrum} seeds.

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<th>INPI\textsuperscript{3}</th>
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<tr>
<td>Moisture</td>
<td>6.05</td>
<td>12.17</td>
<td>7.72</td>
<td>10.64</td>
<td>8.33</td>
<td>10.95</td>
</tr>
<tr>
<td>Calories</td>
<td>363</td>
<td>-</td>
<td>281</td>
<td>-</td>
<td>-</td>
<td>346</td>
</tr>
<tr>
<td>Protein</td>
<td>12.13</td>
<td>10.22</td>
<td>8.80</td>
<td>11.48</td>
<td>11.48</td>
<td>10.42</td>
</tr>
<tr>
<td>Fat</td>
<td>1.70</td>
<td>2.02</td>
<td>1.37</td>
<td>1.51</td>
<td>0.75</td>
<td>0.49</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>81.48\textsuperscript{8}</td>
<td>83.55\textsuperscript{8}</td>
<td>85.89</td>
<td>79.83</td>
<td>79.42</td>
<td>85.57</td>
</tr>
<tr>
<td>Ash</td>
<td>4.68</td>
<td>4.21</td>
<td>3.96</td>
<td>3.08</td>
<td>4.06</td>
<td>3.53</td>
</tr>
<tr>
<td>Dietary Fiber</td>
<td>-</td>
<td>-</td>
<td>21.71</td>
<td>4.91</td>
<td>14.04</td>
<td>21.27</td>
</tr>
<tr>
<td>Crude Fiber</td>
<td>6.60</td>
<td>8.90</td>
<td>-</td>
<td>4.10</td>
<td>4.30</td>
<td>-</td>
</tr>
<tr>
<td>Total Sugars</td>
<td>-</td>
<td>-</td>
<td>14.93</td>
<td>-</td>
<td>-</td>
<td>10.50</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Data reported on a dry weight basis (g/100g) with the exception of moisture and calories. Superscripts 2, 4, 5, and 6 have been converted to dry weight from original data.


\textsuperscript{4}Unpublished laboratory report from Zamorano Food Science Laboratory, Francisco Morazan, Honduras. Received as a personal communication from E. Vohman 4.16.2012.


\textsuperscript{7}Yucatán and Veracruz are states in Mexico. HND=Honduras, GTM=Guatemala.

\textsuperscript{8}Calculated from original data.
### Table 2: Proximate composition\(^1\) of unroasted and roasted *Brosimum alicastrum* seed powder from Guatemala and Nicaragua.

<table>
<thead>
<tr>
<th>Proximate</th>
<th>Guatemala</th>
<th>Nicaragua</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unroasted</td>
<td>Roasted</td>
</tr>
<tr>
<td>Moisture</td>
<td>9.72±0.06</td>
<td>7.32±0.09</td>
</tr>
<tr>
<td>Protein(^2)</td>
<td>10.37±0.01</td>
<td>10.55±0.04</td>
</tr>
<tr>
<td>Fat</td>
<td>0.74±0.04</td>
<td>1.35±0.03</td>
</tr>
<tr>
<td>Carbohydrate(^3)</td>
<td>85.12±0.04</td>
<td>83.90±0.07</td>
</tr>
<tr>
<td>Ash(^4)</td>
<td>3.8</td>
<td>4.2</td>
</tr>
<tr>
<td>NDF(^5)</td>
<td>8.1±0.5</td>
<td>7.5±0.2</td>
</tr>
<tr>
<td>ADF(^6)</td>
<td>4.9±0.1</td>
<td>5.3±0.6</td>
</tr>
</tbody>
</table>

\(^{a-d}\) Means ± standard deviation in a row without common superscripts are significantly different at \(P<0.05\). N=3 for all variables except moisture where N=9.

\(^1\)Data reported on a dry weight basis (g/100g), with the exception of moisture data which is reported on an as sampled basis.

\(^2\)Protein = N x 6.25 (16% Nitrogen in Protein)

\(^3\)Calculated By Difference

\(^4\)Standard deviation is 0 for all values. P<0.0001

\(^5\)Neutral Detergent Fiber

\(^6\)Acid Detergent Fiber

### Table 3: Proximate composition\(^1\) of all-purpose wheat flour cookies baked using 50\% replacement of roasted and unroasted *Brosimum alicastrum* seed powder from Nicaragua, compared to control cookies baked with 100\% all-purpose wheat flour.

<table>
<thead>
<tr>
<th>Proximate</th>
<th>Control</th>
<th>Unroasted</th>
<th>Roasted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>5.89±0.17</td>
<td>6.71±0.07</td>
<td>6.74±0.16</td>
</tr>
<tr>
<td>Protein(^2)</td>
<td>7.43±0.04</td>
<td>6.52±0.06</td>
<td>6.51±0.04</td>
</tr>
<tr>
<td>Fat</td>
<td>15.04±0.69</td>
<td>16.09±0.03</td>
<td>15.87±0.13</td>
</tr>
<tr>
<td>Carbohydrate(^2)</td>
<td>70.61±0.67</td>
<td>68.38±0.13</td>
<td>68.62±0.20</td>
</tr>
<tr>
<td>Ash</td>
<td>1.03±0.06</td>
<td>2.30±0.10</td>
<td>2.27±0.06</td>
</tr>
<tr>
<td>NDF(^3)</td>
<td>9.2±1.0</td>
<td>10.1±2.2</td>
<td>11.8±0.4</td>
</tr>
<tr>
<td>ADF(^4)</td>
<td>3.4±1.0</td>
<td>3.7±0.6</td>
<td>5.2±1.4</td>
</tr>
</tbody>
</table>

\(^{a-b}\) Means ± standard deviation in a row without common superscripts are significantly different at \(P<0.05\). N=3 for all variables except moisture where N=9.

\(^1\)Data reported on an as sampled basis (g/100g). A combined sample of twelve cookies, six cookies from two replicates, for each treatment was measured in triplicate.

\(^2\)Calculated by difference

\(^3\)Neutral detergent Fiber

\(^4\)Acid detergent Fiber
Table 4: Sugar profile\(^1\) of unroasted and roasted *Brosimum alicastrum* seed powder from Guatemala and Nicaragua.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Guatemala</th>
<th>nicaragua</th>
<th>Nicaragua</th>
<th>Nicaragua</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unroasted</td>
<td>Roasted</td>
<td>Unroasted</td>
<td>Roasted</td>
</tr>
<tr>
<td>Total Sugars</td>
<td>4.93(^{b})±0.12</td>
<td>2.48(^{d})±0.14</td>
<td>7.66(^{a})±0.14</td>
<td>3.98(^{c})±0.20</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.85(^{a})±0.01</td>
<td>0.62(^{b})±0.19</td>
<td>0.79(^{a})±0.01</td>
<td>0.60(^{b})±0.07</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.26(^{b})±0.02</td>
<td>0.59(^{c})±0.19</td>
<td>1.57(^{a})±0.02</td>
<td>0.78(^{c})±0.07</td>
</tr>
<tr>
<td>Lactose</td>
<td>ND(^2)</td>
<td>ND</td>
<td>ND</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>Maltose</td>
<td>1.15(^{b})±0.05</td>
<td>0.05(^{c})±0.01</td>
<td>3.46(^{a})±0.09</td>
<td>0.94(^{b})±0.06</td>
</tr>
<tr>
<td>Sucrose</td>
<td>1.67(^{b})±0.07</td>
<td>1.21(^{c})±0.03</td>
<td>1.83(^{a})±0.05</td>
<td>1.62(^{b})±0.04</td>
</tr>
</tbody>
</table>

\(^{a-d}\) Means ± standard deviation in a row without common superscripts are significantly different at \(P<0.05\). N=3 for all variables.

\(^1\)Data reported on a dry weight basis (g/100g).

\(^2\)Not Detected.

Table 5: Sugar profile\(^1\) of all-purpose wheat flour cookies baked using 50% replacement of roasted and unroasted *Brosimum alicastrum* seed powder from Nicaragua, compared to control cookies baked with 100% all-purpose wheat flour.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Control</th>
<th>Unroasted</th>
<th>Roasted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Sugars</td>
<td>35.49±0.81(^{b})</td>
<td>49.09±1.28(^{a})</td>
<td>32.78±1.63(^{b})</td>
</tr>
<tr>
<td>Fructose</td>
<td>ND(^2)</td>
<td>0.67±0.04</td>
<td>ND</td>
</tr>
<tr>
<td>Glucose</td>
<td>ND</td>
<td>0.80±0.03(^{a})</td>
<td>0.45±0.02(^{b})</td>
</tr>
<tr>
<td>Lactose</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Maltose</td>
<td>ND</td>
<td>0.09±0.01(^{b})</td>
<td>1.35±0.07(^{a})</td>
</tr>
<tr>
<td>Sucrose</td>
<td>35.49±0.81(^{b})</td>
<td>47.53±1.23(^{a})</td>
<td>30.98±1.56(^{b})</td>
</tr>
</tbody>
</table>

\(^{a-b}\) Means ± standard deviation in a row without common superscripts are significantly different at \(P<0.05\). N=3 for all variables. A combined sample of twelve cookies, six cookies from two replicates, for each treatment was measured in triplicate.

\(^1\)Data reported on an as sampled basis (g/100g).

\(^2\)Not Detected.
Table 6: Indispensable amino acids\(^1\) of unroasted and roasted *Brosimum alicastrum* seed powder from Guatemala and Nicaragua.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Guatemala</th>
<th>Nicaragua</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unroasted</td>
<td>Roasted</td>
<td>Unroasted</td>
<td>Roasted</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.10(^{bc})</td>
<td>0.09(^c)</td>
<td>0.11(^a)</td>
<td>0.11(^{ab})</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.29(^b)</td>
<td>0.23(^c)</td>
<td>0.33(^a)</td>
<td>0.28(^b)</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.39(^c)</td>
<td>0.35(^d)</td>
<td>0.45(^a)</td>
<td>0.43(^b)</td>
</tr>
<tr>
<td>Lysine(^2)</td>
<td>0.61(^a)</td>
<td>0.47(^c)</td>
<td>0.50(^b)</td>
<td>0.34(^d)</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.03(^b)</td>
<td>0.03(^b)</td>
<td>0.05(^a)</td>
<td>0.03(^b)</td>
</tr>
<tr>
<td>Methionine/Cysteine</td>
<td>0.10(^{bc})</td>
<td>0.10(^c)</td>
<td>0.15(^a)</td>
<td>0.12(^b)</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.29(^b)</td>
<td>0.27(^c)</td>
<td>0.33(^a)</td>
<td>0.33(^a)</td>
</tr>
<tr>
<td>Phenylalanine/Tyrosine</td>
<td>0.47(^b)</td>
<td>0.44(^c)</td>
<td>0.53(^a)</td>
<td>0.54(^a)</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.25(^c)</td>
<td>0.26(^b)</td>
<td>0.31(^a)</td>
<td>0.31(^a)</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.09(^a)</td>
<td>0.09(^d)</td>
<td>0.09(^a)</td>
<td>0.08(^b)</td>
</tr>
<tr>
<td>Valine</td>
<td>0.34(^c)</td>
<td>0.29(^d)</td>
<td>0.40(^a)</td>
<td>0.36(^b)</td>
</tr>
</tbody>
</table>

\(^{a-d}\) Means in a row without common superscripts are significantly different at \(P<0.05\). N=3 for all variables. Standard deviation is 0.01 or less for all values.

\(^1\)Data reported on a dry weight basis (g/100g).

\(^2\)Lysine + Hydroxylysine
Table 7: Indispensable amino acids\(^1\) of all-purpose wheat flour cookies baked using 50% replacement of roasted and unroasted *Brosimum alicastrum* seed powder from Nicaragua, compared to control cookies baked with 100% all-purpose wheat flour.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Control</th>
<th>Unroasted</th>
<th>Roasted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>0.15(^a)</td>
<td>0.10(^b)</td>
<td>0.10(^b)</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.26(^a)</td>
<td>0.20(^b)</td>
<td>0.20(^b)</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.49(^a)</td>
<td>0.35(^b)</td>
<td>0.35(^b)</td>
</tr>
<tr>
<td>Lysine(^2)</td>
<td>0.16(^a)</td>
<td>0.16(^a)</td>
<td>0.15(^b)</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.10(^a)</td>
<td>0.05(^b)</td>
<td>0.05(^b)</td>
</tr>
<tr>
<td>Methionine/Cysteine</td>
<td>0.24(^a)</td>
<td>0.15(^b)</td>
<td>0.14(^b)</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.36(^a)</td>
<td>0.24(^c)</td>
<td>0.25(^b)</td>
</tr>
<tr>
<td>Phenylalanine/Tyrosine</td>
<td>0.56(^a)</td>
<td>0.36(^c)</td>
<td>0.40(^b)</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.18(^a)</td>
<td>0.17(^b)</td>
<td>0.16(^c)</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.08(^a)</td>
<td>0.07(^ab)</td>
<td>0.07(^b)</td>
</tr>
<tr>
<td>Valine</td>
<td>0.29(^a)</td>
<td>0.22(^b)</td>
<td>0.23(^b)</td>
</tr>
</tbody>
</table>

\(^{a-c}\) Means in a row without common superscripts are significantly different at \(P<0.05\). Standard deviation is 0.01 or less for all values. \(N=3\) for all variables. A combined sample of twelve cookies, six cookies from two replicates, for each treatment was measured in triplicate.

\(^1\)Data reported on an as sampled basis (g/100g).

\(^2\)Lysine + Hydroxylysine
Table 8: Conditionally indispensable amino acids\(^1\) of unroasted and roasted *Brosimum alicastrum* seed powder from Guatemala and Nicaragua.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Guatemala Unroasted</th>
<th>Guatemala Roasted</th>
<th>Nicaragua Unroasted</th>
<th>Nicaragua Roasted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>0.38(^b)</td>
<td>0.30(^c)</td>
<td>0.39(^a)</td>
<td>0.29(^c)</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.08(^b)</td>
<td>0.07(^b)</td>
<td>0.10(^a)</td>
<td>0.09(^a)</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.32(^c)</td>
<td>0.29(^d)</td>
<td>0.37(^a)</td>
<td>0.35(^b)</td>
</tr>
<tr>
<td>Proline(^2)</td>
<td>0.43(^c)</td>
<td>0.38(^d)</td>
<td>0.49(^a)</td>
<td>0.44(^b)</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.18(^b)</td>
<td>0.16(^c)</td>
<td>0.20(^a)</td>
<td>0.21(^a)</td>
</tr>
</tbody>
</table>

\(a-d\) Means in a row without common superscripts are significantly different at \(P<0.05\). N=3 for all variables. Standard deviation is 0.01 or less for all values.

\(^1\)Data reported on a dry weight basis (g/100g).

\(^2\)Proline + Hydroxyproline

Table 9: Conditionally indispensable amino acids\(^1\) of all-purpose wheat flour cookies baked using 50% replacement of roasted and unroasted *Brosimum alicastrum* seed powder from Nicaragua, compared to control cookies baked with 100% all-purpose wheat flour.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Control</th>
<th>Unroasted</th>
<th>Roasted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>0.26(^a)</td>
<td>0.20(^b)</td>
<td>0.19(^b)</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.14(^a)</td>
<td>0.10(^b)</td>
<td>0.09(^b)</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.25(^a)</td>
<td>0.22(^b)</td>
<td>0.21(^c)</td>
</tr>
<tr>
<td>Proline</td>
<td>0.80(^a)</td>
<td>0.52(^b)</td>
<td>0.48(^c)</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.20(^a)</td>
<td>0.12(^c)</td>
<td>0.15(^b)</td>
</tr>
</tbody>
</table>

\(a-c\) Means + standard deviation in a row without common superscripts are significantly different at \(P<0.05\). Standard deviation is 0.01 or less for all values. N=3 for all variables. A combined sample of twelve cookies, six cookies from two replicates, for each treatment was measured in triplicate.

\(^1\)Data reported on an as sampled basis (g/100g).

\(^2\)Proline + Hydroxyproline
Table 10: Dispensable amino acids\(^1\) of unroasted and roasted *Brosimum alicastrum* seed powder from Guatemala and Nicaragua.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Guatemala</th>
<th>Nicaragua</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unroasted</td>
<td>Roasted</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.25(^b)</td>
<td>0.24(^c)</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>1.25(^b)</td>
<td>1.00(^d)</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>0.56(^c)</td>
<td>0.53(^d)</td>
</tr>
<tr>
<td>Serine</td>
<td>0.23(^d)</td>
<td>0.26(^c)</td>
</tr>
</tbody>
</table>

\(^a-d\) Means in a row without common superscripts are significantly different at \(P\leq 0.05\). N=3 for all variables. Standard deviation is 0.01 or less for all values.

\(^1\)Data reported on a dry weight basis (g/100g).

---

Table 11: Dispensable amino acids\(^1\) of all-purpose wheat flour cookies baked using 50% replacement of roasted and unroasted *Brosimum alicastrum* seed powder from Nicaragua, compared to control cookies baked with 100% all-purpose wheat flour.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Control</th>
<th>Unroasted</th>
<th>Roasted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>0.21(^a)</td>
<td>0.17(^b)</td>
<td>0.17(^b)</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>0.28(^c)</td>
<td>0.49(^a)</td>
<td>0.40(^b)</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>2.38(^a)</td>
<td>1.39(^b)</td>
<td>1.36(^c)</td>
</tr>
<tr>
<td>Serine</td>
<td>0.32(^a)</td>
<td>0.24(^b)</td>
<td>0.23(^b)</td>
</tr>
</tbody>
</table>

\(^a-c\) Means ± standard deviation in a row without common superscripts are significantly different at \(P\leq 0.05\). Standard deviation is 0.01 or less for all values. N=3 for all variables. A combined sample of twelve cookies, six cookies from two replicates, for each treatment was measured in triplicate.

\(^1\)Data reported on an as sampled basis (g/100g).
Table 12: Vitamins and minerals\(^1\) of unroasted and roasted *Brosimum alicastrum* seed powder from Guatemala and Nicaragua.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Guatemala</th>
<th>Nicaragua</th>
<th>Guatemala</th>
<th>Nicaragua</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unroasted</td>
<td>Roasted</td>
<td>Unroasted</td>
<td>Roasted</td>
</tr>
<tr>
<td>Vitamin A(^2)</td>
<td>1188(\pm 77)</td>
<td>1393(\pm 40)</td>
<td>899(\pm 66)</td>
<td>1820(\pm 17)</td>
</tr>
<tr>
<td>Vitamin C(^3)</td>
<td>16(\pm 7)</td>
<td>11(\pm 8)</td>
<td>41(\pm 11)</td>
<td>23(\pm 12)</td>
</tr>
<tr>
<td>Calcium</td>
<td>206(\pm 6)</td>
<td>190(\pm 6)</td>
<td>189(\pm 7)</td>
<td>190(\pm 6)</td>
</tr>
<tr>
<td>Copper</td>
<td>0.9(\pm 0)</td>
<td>1.0(\pm 0.1)</td>
<td>0.9(\pm 0)</td>
<td>1.0(\pm 0)</td>
</tr>
<tr>
<td>Iron</td>
<td>3.6(\pm 0.5)</td>
<td>4.0(\pm 1.1)</td>
<td>6.1(\pm 0.3)</td>
<td>5.6(\pm 0.3)</td>
</tr>
<tr>
<td>Magnesium</td>
<td>173(\pm 13)</td>
<td>173(\pm 0)</td>
<td>200(\pm 6)</td>
<td>208(\pm 0)</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.7(\pm 0.1)</td>
<td>0.7(\pm 0.1)</td>
<td>0.6(\pm 0.1)</td>
<td>0.6(\pm 0.1)</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>137(\pm 6)</td>
<td>147(\pm 6)</td>
<td>163(\pm 6)</td>
<td>171(\pm 6)</td>
</tr>
<tr>
<td>Potassium</td>
<td>1381(\pm 63)</td>
<td>1399(\pm 27)</td>
<td>1439(\pm 52)</td>
<td>1498(\pm 17)</td>
</tr>
<tr>
<td>Sulfur</td>
<td>78(\pm 0)</td>
<td>79(\pm 6)</td>
<td>91(\pm 0)</td>
<td>88(\pm 0)</td>
</tr>
<tr>
<td>Zinc</td>
<td>1.6(\pm 0.1)</td>
<td>1.6(\pm 0.1)</td>
<td>2.7(\pm 0.1)</td>
<td>2.9(\pm 0.2)</td>
</tr>
<tr>
<td>Sodium</td>
<td>21.6(\pm 19.1)</td>
<td>15.2(\pm 11.2)</td>
<td>18.1(\pm 7.0)</td>
<td>11.7(\pm 3.0)</td>
</tr>
<tr>
<td>Chloride</td>
<td>41(\pm 32)</td>
<td>39(\pm 13)</td>
<td>15(\pm 7)</td>
<td>15(\pm 6)</td>
</tr>
</tbody>
</table>

\(^{a-d}\) Means ± standard deviation in a row without common superscripts are significantly different at \(P<0.05\). N=3 for all data except for Vitamin C.

\(^1\)Data reported on a dry weight basis (mg/100g) unless otherwise noted.

\(^2\)Vitamin A as total carotenoids (μg/100g)

\(^3\)Vitamin C as ascorbic acid. N=7 for Guatemala Unroasted, N=8 for Guatemala Roasted, N=4 for Nicaragua Unroasted, N=5 for Nicaragua Roasted
Table 13: Vitamins and minerals\(^1\) of all-purpose wheat flour cookies baked using 50\% replacement of roasted and unroasted *Brosimum alicastrum* seed powder from Nicaragua, compared to control cookies baked with 100\% all-purpose wheat flour.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Control</th>
<th>Unroasted</th>
<th>Roasted</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A(^2)</td>
<td>61±31</td>
<td>593±66</td>
<td>1460±60</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vitamin C(^3)</td>
<td>10±4</td>
<td>13±4</td>
<td>10±4</td>
<td>0.2672</td>
</tr>
<tr>
<td>Calcium</td>
<td>10±0</td>
<td>50±0</td>
<td>50±0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Copper</td>
<td>0.1±0.1</td>
<td>0.4±0.1</td>
<td>0.3±0.1</td>
<td>0.0052</td>
</tr>
<tr>
<td>Iron</td>
<td>2.9±0.1</td>
<td>3.0±0.2</td>
<td>2.9±0.3</td>
<td>0.5629</td>
</tr>
<tr>
<td>Magnesium</td>
<td>20±0</td>
<td>60±0</td>
<td>60±0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.4±0.0</td>
<td>0.3±0.1</td>
<td>0.3±0.1</td>
<td>0.2160</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>60±0</td>
<td>70±0</td>
<td>70±0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Potassium</td>
<td>60±0</td>
<td>360±0</td>
<td>360±0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sulfur</td>
<td>77±6</td>
<td>60±0</td>
<td>60±0</td>
<td>0.0012</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.6±0.1</td>
<td>0.9±0.0</td>
<td>1.1±0.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sodium</td>
<td>304.7±16.0</td>
<td>336.3±13.1</td>
<td>371.0±34.5</td>
<td>0.0355</td>
</tr>
<tr>
<td>Chloride</td>
<td>293±21</td>
<td>313±12</td>
<td>367±29</td>
<td>0.0732</td>
</tr>
</tbody>
</table>

Means ± standard deviation. N=3 for all variables unless otherwise noted. A combined sample of twelve cookies, six cookies from two replicates, for each treatment was measured in triplicate.

\(^1\)Data reported on an as sampled basis (mg/100g) unless otherwise noted.

\(^2\)Vitamin A as total carotenoids (μg/100g).

\(^3\)Vitamin C as ascorbic acid. N=9
III. EFFECTS OF INCORPORATING UNROASTED AND ROASTED 
*BROSIMUM ALICASTRUM* (MAYA NUT, RAMÓN NUT) SEED POWDERS 
INTO SUGAR SNAP COOKIES.

Abstract

Seeds from *Brosimum alicastrum*, a tree from the Moraceae family of botanicals, can be dried or roasted and ground into a powder that can be incorporated into baked goods such as breads, cakes and cookies. The purpose of this research was to determine the functional properties of sugar snap cookies prepared using *B. alicastrum* seed powder.

Cookies were formulated by replacing all-purpose white wheat flour with 0, 25, or 50 percent unroasted or roasted *B. alicastrum* seed powder harvested from Guatemala or Nicaragua. *B. alicastrum* seed powders and cookies were evaluated for moisture, water activity, pH, and color. Additionally, cookies were measured for weight, spread factor, hardness, toughness, and brittleness.

As replacement levels of *B. alicastrum* seed powder were increased in cookie formulations, there was a decrease in pH, lightness (L*), cookie width, spread factor, hardness, and toughness. Similarly, higher levels of *B. alicastrum* seed powder in the cookie formulations produced cookies with higher percent moisture, redness (+a*), and yellowness (+b*). Cookie water activity, weight, and brittleness remained consistent within the 25% and 50% seed replacement treatments. As replacement levels of *B. alicastrum* seed powder increased, cookie widths and spread factors decreased (p<0.05), while cookie thickness remained relatively unchanged (p>0.05). Top colors for cookies became darker (lower L*) as seed powder replacements of *B. alicastrum* increased (p<0.05). *B. alicastrum* cookies also had higher redness and yellowness values when
compared to controls (0% replacement). Overall, *B. alicastrum* seed powder replacements produced a cookie that was softer than an all wheat flour cookie, with similar brittleness.

**Introduction**

The primary function of wheat flour in baked goods, such as sugar snap cookies, is to provide bulk, structure and texture in the form of a matrix that entraps other ingredients (Lai & Lin, 2006; Pareyt & Delcour, 2008). Cookies are comprised of soft wheat flour, sugar and fat in varying proportions, and may include chemical leaveners, yeast, syrups, salt or emulsifiers (Chevallier, Colonna, Della Valle, & Lourdin, 2000; Pareyt & Delcour, 2008). Soft wheat flour is typically used in cookie production because the development of an extensive gluten network is undesirable in these products (Lai & Lin, 2006). The high fat and sugar content of cookies contributes to plasticity and cohesiveness. Fat in the formulation also acts to coat the flour particles to limit their absorption of moisture. Cookies may also contain fruits, nuts, chocolate, seeds, or may be comprised of meals, grits, flours, starches or powders of other grains or seeds (Manley, 2011). Sugar snap cookies vary in quantities of flour, sugar and fat, but are generally comprised of 47.5 to 54 percent wheat flour, 33.3 to 42 percent sugar and 9.4 to 18 percent fat (Wade, 1988).

Extensive research has been conducted on replacing white wheat flour with different types of non-wheat cereals, seeds, starches, and other ingredients. Commonly, these replacements are made either to enhance the nutritional aspects of the cookie, or to utilize an ingredient that is indigenous to a certain location. Most of the research on
utilizing replacements for white wheat flour in cookies focuses on how the replacement affects width, thickness, spread ratio/factor, hardness, and color. Several studies also include a sensory component, where cookies are rated by consumers in the categories of flavor, color, texture, appearance, and overall acceptability. Previous research on replacements for wheat flour in cookies has utilized a variety of baking methodologies and analytical techniques, making direct comparisons difficult (Lorenz & Coulter, 1991; Chavan, Kadam, & Reddy, 1993; McWatters, Ouedraogo, Resurreccion, Hung, & Phillips, 2003; Singh, Singh, Sharma, & Saxena, 2003; Giami, Achinewhu, & Ibaakee, 2005; Lee, Kim, & Kim, 2005; Sindhuja, Sudha, & Rahim, 2005; Zucco, Borsuk, & Arntfield, 2011; Khouryieh & Aramouni, 2012; Yamsaengsung, Berghofer, & Schoenlechner, 2012). These studies represent a wide variety of geographical locations, and thus, sensory panel ratings of products may not be broadly applicable to all regions or cultures. Table 14 shows a summary of selected previous studies that have evaluated wheat flour replacements.

Overall, the aforementioned studies show that when compared to control wheat flour cookies, as non-wheat flour replacement levels increased, thickness increased in cookies made with navy bean, pinto bean, and amaranth flour (Sindhuja et al., 2005; Zucco et al., 2011). The opposite occurred when flaxseed, green lentil, and pumpkin flours were incorporated, causing decreased cookie thickness (Giami et al., 2005; Zucco et al., 2011; Khouryieh & Aramouni, 2012). As replacement levels of non-wheat flours increased, there was an increase in width for flaxseed and coarse milled pulse flours (Zucco et al., 2011; Khouryieh & Aramouni, 2012). There was a decrease in width for
amaranth, pumpkin, and fine milled pulse flours (Giami et al., 2005; Sindhuja et al., 2005; Zucco et al., 2011).

Spread ratios increased as replacement levels of non-wheat flours increased for flaxseed, black rice, corn and potato flours (Lee et al., 2005; Singh et al., 2003; Khouryieh & Aramouni, 2012). Spread ratios decreased for fonio, cowpea, and quinoa flours (Lorenz & Coulter, 1991; McWatters et al., 2003). There was no change in spread ratios for chickpea flour replacements of white wheat flour (Yamsaengsung et al., 2012).

Several studies measured the effects of flour replacement on cookie hardness. Hardness, measured as force to fracture or shear, increased as replacement levels of non-wheat flours increased for chickpea, flaxseed, green lentil, pinto bean, and cowpea flours (McWatters et al., 2003; Zucco et al., 2011; Khouryieh & Aramouni, 2012; Yamsaengsung et al., 2012). Hardness decreased with replacements of amaranth, pumpkin, corn, potato, and fonio flours (McWatters et al., 2003; Singh et al., 2003; Giami et al., 2005; Sindhuja et al., 2005). Hardness was unchanged with replacements of pinto bean and black rice flours (Lee et al., 2005; Zucco et al., 2011).

Previous studies demonstrated that cookie whiteness (L*) decreased as non-wheat flour replacement levels increased for chickpea, flaxseed, navy bean, pinto bean, green lentil, black rice, corn, potato, fonio, cowpea, and quinoa flours (Lorenz & Coulter, 1991; McWatters et al., 2003; Singh et al., 2003; Lee et al., 2005; Zucco et al., 2011; Khouryieh & Aramouni, 2012; Yamsaengsung et al., 2012;). Redness (a*) values increased as replacement levels increased for chickpea, flaxseed, corn and potato flours (Singh et al., 2003; Khouryieh & Aramouni, 2012; Yamsaengsung et al., 2012). Redness values
decreased for black rice flours (Lee et al., 2005). Yellowness ($b^*$) values increased as replacement levels increased for chickpea, flaxseed, black rice, corn and potato flours (Singh et al., 2003; Lee et al., 2005; Khouryieh & Aramouni, 2012; Yamsaengsung et al., 2012).

Studies showed that cookie weight increased as non-wheat flour replacement levels increased for navy bean, pinto bean, green lentil, pumpkin, fonio and cowpea flours (McWatters et al., 2003; Giami et al., 2005; Zucco et al., 2011). Flaxseed flour increase caused a decrease in cookie moisture and water activity (Khouryieh & Aramouni, 2012). Black rice flour increase resulted in an increase in cookie pH (Lee et al., 2005).

Review of data from previous studies demonstrates that it is difficult to predict how flour replacement will affect width, spread, hardness, and color of cookies, since these factors vary widely with the replacement type and percent. Furthermore, these studies show that an optimum wheat-replacement for baked goods has not been found that mimics wheat for color, texture, appearance, and sensory qualities. Another possible replacement for wheat flour in baked products is Brosimum alicastrum seed powder. While *B. alicastrum* seed powder is currently used in baked goods in Central America, there is little or no information available on the effect of these seed powders on the physical characteristics of baked products (moisture, water activity, pH, spread factor, color, texture).

*B. alicastrum* is a member of a family of edible botanicals (Moraceae) which includes the fig, mulberry, breadfruit, and jackfruit (Puleston, 1968). *B. alicastrum* fruits
are 1.5 to 2.5 cm in diameter, weigh approximately 4.5 g., and contain one seed comprising 3.0 g of this weight, about 67%. When *B. alicastrum* seeds are dried or roasted, and ground into a powder, this powder can be incorporated into baked goods such as breads, cakes and cookies (Flaster, 2007). This ground roasted powder has flavors of coffee and chocolate which intensify with a darker (longer) roast. Fresh seeds are typically boiled in ash or lime (calcium hydroxide), cleaned, ground, and used to make tortillas. This process is similar to corn kernel processing (Yates & Ramirez-Sosa, 2004). The seeds can be roasted, ground, and brewed to produce a drink similar to coffee. The seeds can also be ground and mixed with milk and sugar for another type of beverage. The fresh seeds, which have a flavor similar to potatoes, can also be boiled and mashed in the same way as potatoes (Puleston, 1968; Peters & Pardo-Tejeda, 1982).

Previous research demonstrated that *B. alicastrum* has been used as a conventional food source, and it has been incorporated into a number of products that are currently available (Puleston, 1968; Peters & Pardo-Tejeda, 1982; Puleston, 1982; Peters, 1983; Flaster, 2007). However, no information is available on the technical aspects of incorporating *B. alicastrum* seed powder into food products. Furthermore, whereas there is an abundance of research on incorporating seed powders/flours such as pumpkin, amaranth, legumes, and non-cereal grains into baked goods, there is little information regarding the incorporation of tree seed (from fruit) powders/flours into baked goods. A cookie formulation is a simple yet effective starting point to assess the technical feasibility of incorporating *B. alicastrum* seed powder into a baked product.
According to Chavan et al. (1993) cookies are a good candidate for replacement of other flour types for several reasons: “1.) they are widely consumed, 2.) exhibit good eating qualities, 3.) are acceptable to varied selections of the population, 4.) possess relatively long shelf life” (p.212). Thus, the purpose of this research is to determine the effects of incorporating *B. alicastrum* seed powder into a sugar snap cookie formulation on the functional properties of moisture, water activity, pH, spread factor, weight, surface color and texture. This includes determining the impact of changes in harvest location, roasting, or levels of incorporation.

**Materials and Methods**

**Sourcing *B. alicastrum* Seeds**

Whole dried *B. alicastrum* seeds were obtained in single lots from two countries, Guatemala and Nicaragua. Guatemalan *B. alicastrum* seeds, purchased as Ramón Nut, (lot # 5403) were obtained from Mountain Rose Herbs (PO Box 50220, Eugene, OR, 97405, www.mountainroseherbs.com). *B. alicastrum* seeds from Guatemala were harvested from the Maya Biosphere Reserve (17°28′46.9″N 89°51′02.0″W). Nicaraguan *B. alicastrum* was purchased from the Maya Nut Institute (PO Box 2371, Crested Butte, CO, 81224, www.mayanutinstitute.org). *B. alicastrum* from Nicaragua was harvested from Volcan San Cristobal-Casita Natural Reserve (12°40′19.61″N 86°57′58.86″W). Given coordinates represent a center point for these reserves, not actual harvest locations, which are larger areas. Appendix A shows the harvest locations relative to each other.
Roasting *B. alicastrum* Seeds

*B. alicastrum* seeds were roasted in a Diedrich Coffee Roaster, 24 kg capacity (Diedrich Manufacturing, Inc., P.O Box 430, Ponderay, ID, 83852 www.diedrichroasters.com). Equal quantities, 2360 grams, of whole Guatemalan and Nicaraguan *B. alicastrum* seeds were roasted separately to a final temperature of 160°C. Roasting began when the temperature in the roaster reached 188°C at full flame. No air adjustment was made to the roaster, which resulted in approximately 20% air flow. Roasting temperatures were monitored and recorded at one minute intervals. Adjustments to the flame during roasting were recorded as actions. The roasting process varied slightly for each sample; however, the final temperature was the same. A detailed roasting protocol for seed from each country can be found in Appendix B.

Grinding *B. alicastrum* Seed

*B. alicastrum* seed was ground to conform to the standards for flour set by the United States Code of Federal Regulations 21C.F.R. §137.105 which states: “not less than 98 percent of the flour passes through a cloth having openings not larger than those of woven wire cloth designated 212 µm (No. 70)” (Cereal Flours and Related Products, 2014). This was achieved through a step down process of grinding and sieving *B. alicastrum* seeds until particle size was less than 212µm. The grinding process generated heat; thus, the seeds were frozen prior to grinding to minimize the impact the heat from processing.

Approximately 300 grams of whole *B. alicastrum* seed were stored in a freezer at 0°C, in a vacuum sealed bag, for a minimum of 12 hours prior to grinding. Immediately
after removal from the freezer, the temperature was recorded and the seeds were ground coarsely in a Mazzer Luigi, Super Jolly coffee grinder (Mazzer Luigi s.r.l., Via Moglianese 113, I - 30030 Gardigiano di Scorze' - Venezia – Italy, www.mazzer.com). Temperature and weight were recorded after each grinding step, and the ground seed was sieved through a 212 μm (#70) screen (Certificate of compliance #70BS8F589291, ASTM specification E11). Any *B. alicastrum* seed powder that did not pass through the sieve was weighed, sealed in a vacuum bag, and placed in a blast freezer, USECO Catr Cook Chill Model # 31-003 (USECO, 869 Seven Oaks Suite 140, Smyrna, TN, 37167, www.useco.com) until the temperature was 0°C or less. The remaining coarse *B. alicastrum* seed powder was then ground using a WonderMill grain mill (The WonderMill Company, Pocatello, ID, 83201) on the finest setting (pastry). The product of grinding in the WonderMill was sieved through a 212μm (#70) screen, and what remained on the sieve was sealed in a vacuum bag, chilled in the blast freezer, and reground in the WonderMill. This process was repeated until what remained on the sieve was less than 2% of the total weight; this was then added to the final ground *B. alicastrum* seed powder.

**Cookie Formulation and Preparation**

Using AACC International Method 10-5.05, Baking Quality of Cookie Flour (AACC 10.50.05, 2014), cookies were baked using either unroasted or roasted *B. alicastrum* seed powder from either Nicaragua or Guatemala in the quantities discussed below. Cookies were baked using 100% all-purpose white wheat flour (control) or a combination of all-purpose flour and *B. alicastrum* seed powder. Three batches of six
cookies were baked for each replicate and treatments consisted of the following: control, 25% *B. alicastrum* seed powder replacement by weight, or 50% *B. alicastrum* seed powder replacement by weight. Each seed powder replicate was baked with its own set of control cookies so that direct comparisons could be made. Type of flour (unroasted or roasted), country of origin (Guatemala or Nicaragua), and percent replacement were randomized during the baking process. Three replicates were performed for each treatment: unroasted Guatemalan *B. alicastrum* seed powder, roasted Guatemalan *B. alicastrum* seed powder, unroasted Nicaraguan *B. alicastrum* seed powder, and roasted Nicaraguan *B. alicastrum* seed powder.

One day prior to cookie baking, *B. alicastrum* seeds were ground according to the procedure described previously, and a sample was taken for triplicate analysis of moisture, pH, water activity, and color. The remaining *B. alicastrum* seed powder was sealed in a vacuum bag until the next day’s baking experiments. Additionally, in preparation for the baking experiment the following day, a quantity of all-purpose white wheat flour was removed from bulk storage, mixed thoroughly, sampled for analysis, and sealed in a vacuum bag.

Cookie dough was mixed using a KitchenAid® 4 ½ Quart Classic Plus Mixer (Model# KSM75WH, KitchenAid®, Inc., 701 Main Street, St. Joseph, MI 49085, www.kitchenaid.com) divided and placed on a Vollrath Wear-Ever® heavy duty aluminum sheet pan, 45.1 x 37.2 cm, with a 13 gauge (1.83mm) thickness (Vollrath, 1236 N 18th Street, Sheboygan, WI 53082, www.vollrath.com). Dough was then rolled to a uniform thickness using two 7.25 mm thick steel gauge strips (Carbo-Cut, Inc., 3937
Chimney Rock Road, Hendersonville, NC, 28792) with a 25.4 cm long x 7 cm diameter rolling pin (Mario Batali by Fletchers’ mill 10 inch rolling pin, Fletchers’ Mill 1687 New Vineyard Rd, New Vineyard, ME 04956, www.fletchersmill.com) covered with surgical seamless tubular gauze size 3 (Surgitube GL221, Derma Sciences, 214 Carnegie Center Suite 300, Princeton, NJ, 08540). Cookies were cut using a seamless, nylon, 60 mm inside diameter cutter (Fat Daddios, PO Box 30175, Spokane, WA 99223, www.fatdaddios.com).

Cookies were baked in an Electrolux Icon electric convection oven (model # E36DF76EPS, Electrolux, S:t Göransgatan 143, Stadshagen, Stockholm, Sweden, www.electrolux.com). The oven was outfitted with four 16.5 x 14.5 x 1.0 cm ceramic baking stones. Two stones were placed side by side between the top element and the baking rack and two stones were placed side by side between the bottom element and the baking rack. Cookies were baked using the convection bake setting at 190°C for 9 minutes. Oven temperature was recorded at the first thirty seconds of baking, and at 30 second intervals during baking using a HANNA Instruments HI935005N K-Type Waterproof Thermocouple Thermometer (HANNA Instruments®, Woonsocket, RI, www.hannainst.com) with a Comark ATT29 K-Type Oven/Air Temperature Probe (Comark Instruments, 52 Hurricane Way, Norwich, Norfolk, United Kingdom, www.cormarkinstruments.com) located above the geometric center of the baking pan.

**Ingredients**

All ingredients, other than the *B. alicastrum* seeds, were either purchased from a wholesale distributor or in local markets.
All-purpose flour was Sir Galahad King Arthur Flour, which contained the following ingredients: wheat flour enriched (niacin, reduced iron, thiamine mononitrate, riboflavin, folic acid) and malted barley flour, Lot # 30871PO0121316:17MM, The King Arthur Flour Company, Norwich, VT, 05055.

Sugar used was Dixie Crystals All Natural Pure Cane Sugar Extra Fine Granulated, Lot # S1300-3-12:40, Imperial-Savannah LP, Sugar Land, TX, 16200.

Shortening was Golden Chef All-Purpose All Vegetable Shortening, which was comprised of partially hydrogenated soybean and cottonseed oil; Lot# 072312DE10105013.24, Stratus Foods LLC, Memphis, TN, 38016.

Baking soda used was Arm and Hammer Pure Baking Soda, containing sodium bicarbonate, Lot # BC303909, Arm and Hammer Division of Church and Dwight Co, Inc., 469 N. Harrison Street, Princeton, NJ, 08543.

Salt used was Diamond Crystal Fine All Natural Sea Salt 100% Pure California Sea Salt, Lot# 30012ADBAA, Cargill Inc., Minneapolis, MN, 55440.

Cookie Analysis

Moisture, Water Activity, and pH

Following color, spread factor and texture analyses, six cookies were ground in a food processor and analyzed for moisture, pH, and water activity. Additionally, cookie dough was measured for moisture and water activity.

Moisture

Moisture was determined using the AACC International Method 44-15.02, Moisture -Air Oven Methods with modifications (AACC 44-15.02, 2014). Briefly,
approximately 2 grams of sample were weighed on a Mettler Toledo top loading balance (model # PB303, Mettler-Toledo (Schweiz) GmbH, Im Langacher 44, 8606 Greifensee, Switzerland), in a 5.7 cm diameter x 1.6 cm deep round aluminum dish, and dried in a convection oven (Blue M Stabil Therm Model # OV-490A-2, Blue M Electric Company, Blue Island, IL, 60406) for 12 hours at 105°C. Sample dishes were transferred to a desiccator containing 4A Molecular Sieve (Delta Adsorbents, 24 Congress Circle West, Roselle, IL, 60172, www.deltaadsorbents.com) and were allowed to cool for at least 1 hour. Samples were then re-weighed after cooling, and percent moisture was calculated from the weight difference of loss on drying.

**Water Activity**

Water activity was determined using a Rotronic Hygroskop DT water activity meter (Rotronic AG, Grindelstrasse 6, CH-8303 Bassersdorf, Switzerland, www.rotronic.ch) set at 25.2°C. Approximately 2.5 grams of sample was evenly distributed in the sample cup, placed into a previously equilibrated water activity cell and allowed to re-equilibrate for at least 30 minutes before recording the equilibrium relative humidity.

**pH**

Determination of pH was made using an Accumet Model 10 pH meter (Thermo Fisher Scientific Inc., 81 Wyman Street, Waltham, MA 02454) using AACC International Method 02-52.01, Hydrogen-Ion Activity (pH)-Electrometric Method (AACC 02-52.01, 2014). Before measurements were taken, electrodes were standardized to pH 7.00 and 4.01. Ten grams of *B. alicastrum* seed powder was mixed with 100 mL
distilled water and agitated with a magnetic stirrer for 15 minutes. The suspension was allowed to settle for ten minutes and then decanted before pH measurements were taken.

**Spread Factor**

Spread factor was determined on cookies after baking and cooling for 30 minutes using AACC International Method 10-5.05 (AACC 10.50.05, 2014).

**Surface Color**

Immediately following size measurements, each cookie was evaluated for color in the geometric center of the cookie. Color was measured on the top and bottom of each cookie using a HunterLab Ultra Scan Pro Spectrophotometer and EasyMatch QC software (Hunter Associates Laboratory, Inc., 11491 Sunset Hills Road, Reston, VA, 20190, www.hunterlab.com). Prior to measuring color, the spectrophotometer was standardized using both a light trap (complete absence of reflectance color) and a standard white tile (USP1708). Color was also measured on flour and seed powder by placing it in a 58 x 55 x 14.3 mm cuvette (Minolta CR-A33a, Minolta Co, Ltd., Japan). International Commission on Illumination (CIE), L*, a* and b* color values were determined to provide a standard for comparison to other color values. CIE reflectance measures were taken using a C10 observer.

**Texture**

Cookies were analyzed for texture using a TA.XT Plus Texture Analyzer with Exponent Software (Stable Micro Systems, LTD., Vienna Court, Lammas Road, Godalming, Surrey GU7 1YL, UK, www.stablemicrosystems.com). A three point bend rig was used, which had a blade that measured 70 mm wide by 3 mm thick. The base gap
was set at 35 mm. The tests were run with a 5 kg load cell, 2.5 mm/sec pretest speed, 2.0 mm/sec test speed, 10 mm/sec post-test speed, and 15.0 mm distance. Test procedure was set according to the guidelines established by the American Institute of Baking Standard Procedure for Cookie Hardness (AIB, 2006). Each cookie was measured for three texture parameters: hardness or force to fracture in grams, brittleness/flexibility, or distance to deform before breaking, in mm, and toughness or force divided by distance.

Statistical Analysis

Data analysis was generated using SAS software, Version 9.3 of the SAS System for Windows Copyright © 2012 SAS Institute Inc. SAS and all other SAS Institute Inc. Product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA. Data were analyzed for statistical significance using the General Linear Model procedure of SAS software (SAS, 2012). Sources of variation in the data were Replacement level (0, 25, or 50 percent), country-of-origin (Guatemalan or Nicaraguan), seed-roasting (roasted or unroasted), and replication. Main effects of the model and their interactions were tested for statistical significance (P < 0.05) using the residual error. A Tukeys least square means statement was used to predict population margins for means separation at a significance level of 0.05.

Results and Discussion

Moisture, Water Activity, and pH

Moisture, pH, and water activity data for *B. alicastrum* seed powders, and the all-purpose white wheat flour used for this research, are presented in Table 15. Table 16 shows these same analyses for the all-purpose wheat flour cookies baked with unroasted
and roasted *B. alicastrum* seed powder from Guatemala and Nicaragua at replacement levels of 0%, 25%, and 50%. Table 16 also shows cookie weight, dough weight, and yield after baking.

Moisture levels were determined on all-purpose flour and seed powders to standardize the amount of moisture present in the cookie formulations. *B. alicastrum* seed powders, across all treatments, had significantly different levels of moisture (Table 15). Unroasted seed powder from Nicaragua had the highest moisture (11.75%) followed by unroasted Guatemalan seed powder (9.72%). Roasting caused a significant decrease in moisture content of the seed powder; however, this decrease appeared to occur uniformly, with roasted Nicaraguan seed powder having greater moisture content (8.78%) than Guatemalan (7.32%). All-purpose flour had a moisture content (9.76%) between unroasted Nicaraguan and unroasted Guatemalan seed powders. This moisture content is less than that reported by the USDA Nutrient Database (2014) for all-purpose white wheat flour #20581 (11.92%) (U. S. Department of Agriculture, 2014).

Dough moisture was measured to assure that there were no differences within and between treatments because dough moisture can effect final cookie spread and texture (Table 16). Dough moisture was the highest for cookies baked with unroasted Nicaraguan seed powder at the 50% replacement level (17.38%), and lowest for cookies baked at the 25% replacement level with unroasted Guatemalan seed powder (17.22%). While these differences were statistically significant, they represent a 0.16% difference from highest to lowest moisture content, which is less than the margin of error stated in the method (0.2% moisture) (AACC 44-15.02, 2014). Furthermore, small differences in dough
moisture are not considered to be of practical significance. There were no significant differences in dough moisture between and across 0% replacements (control) and 25% replacements.

Cookie moisture levels can effect storage time, texture, and sensory perception. Cookie moisture was the lowest in the control cookies (5.89% to 5.93%), and highest in cookies baked at the 50% replacement level (6.46% to 6.74%) (Table 16). At the 25% replacement level, moisture content of cookies baked with unroasted Nicaraguan (6.13%) and roasted Guatemalan (6.11%) seed powders were not significantly different than control cookies. However, moisture content of cookies baked at the 25% replacement level with unroasted Guatemalan (6.54%) or roasted Nicaraguan (6.63%) seed powder were the same as cookies baked at the 50% seed powder replacement level.

**Water Activity**

Water activity in flour and cookies can impact microbial growth and thus spoilage rates. There were statistically significant differences in water activity between all flour/seed powder treatments (Table 15). Water activity was significantly lower (p<0.05) in all-purpose wheat flour (0.45) than any of the *B. alicastrum* seed powders, roasted or unroasted. Unroasted seed powder from Nicaragua had the highest water activity (0.64), along with having the highest moisture content. While unroasted Guatemalan seed powder had the same moisture levels as all-purpose wheat flour, it had a significantly higher water activity (0.58) than all-purpose wheat flour (0.45). For cookie dough, water activity was highest in control cookie dough, although there was only a 0.03 difference in water activity from the highest control group (0.84) to the lowest water activity.
measured, which was in the dough of cookies containing roasted *B. alicastrum* from Guatemala and Nicaragua at the 50% replacement level (0.81) (Table 16). Cookie water activity ranged from the highest (0.53) in cookies baked with roasted Nicaraguan seed powder at the 25% and 50% replacement levels to the lowest (0.50) measured in three groups of control cookies, unroasted Nicaraguan and roasted Guatemalan seed powder cookies at the 25% replacement level, and unroasted and roasted Guatemalan seed powder cookies at the 50% replacement level. While these differences in water activity for *B. alicastrum* cookie dough and cookies were found to be statistically significant, it is not likely that they would be significant from a practical standpoint given that the differences are small.

**pH**

pH is a measure of acidity and is associated with cookie moisture and flavor. Measurements of pH show that overall, *B. alicastrum* seed powder has a lower pH than all-purpose wheat flour (6.17) (Table 15). Unroasted seed powder from Nicaragua (5.27) and Guatemala (5.23) had a higher pH than their roasted counterparts, with roasted seed powder from Nicaragua having the lowest pH (5.01). A similar trend was found in the cookies baked with *B. alicastrum* seed powder. Control cookies had the highest pH (9.52 to 9.53) and pH decreased as *B. alicastrum* flour replacement levels were increased. The lowest pH (7.30) was measured in cookies baked with roasted Nicaraguan seed powder at the 50% replacement level, which corresponds to the pH measurements of the *B. alicastrum* seed powder. *B. alicastrum*, because it has weak acidic properties, may have
buffering capacity against the baking soda in the cookie formulation. As the percentage of *B. alicastrum* seed powder is increased, the pH of cookies moves closer to neutral.

**Dough Weight, Cookie Weight and Percent Yield**

Dough weight ranged from approximately 24.3 to 24.9 g, while cookie weight ranged from approximately 21.9 to 22.4 g (Table 16). There were no significant differences (p>0.05) in dough or cookie weights. When percent cooked yield was calculated, control cookies had slightly lower yield (89.2 to 89.5%) than the cookies baked with *B. alicastrum* seed powders (89.5 to 90.3%). This is likely due to the higher moisture content of the cookies baked with *B. alicastrum* seed powder. Cookies baked with roasted Nicaraguan *B. alicastrum* seed powder at the 50% replacement level had the highest yield percentage (90.30%) along with the highest moisture percentage (6.74%).

**Spread Factor**

Cookie diameter and spread factor are standard measurements for evaluating the quality of soft wheat flour; a larger diameter or spread factor is a measure of better quality cookie flour (Kaldy, Rubenthaler, Kereliuk, Berhow, & Vandercook, 1991). As the percentage of *B. alicastrum* seed powder in the cookie formulation was increased, the width of the cookies decreased significantly (p<0.05) (Table 17). There was a larger decrease in width for cookies baked with roasted *B. alicastrum* seed powder as compared to unroasted seed powder at both the 25% and 50% replacement levels. The largest cookie width was measured in the control cookies (7.12 cm) while the smallest was measured in cookies baked with roasted Nicaraguan seed powder (6.36 cm). Width
measurements decreased when adjusted to a constant atmospheric pressure basis; however, statistical differences between treatments followed the same pattern.

Thickness was similar for control cookies (1.00 to 1.02 cm) and cookies baked with *B. alicastrum* seed powder at the 25% replacement level (1.00 to 1.01 cm), with the exception of cookies baked with unroasted Nicaraguan seed powder (0.99 cm), which were the thinnest cookies among all treatments (Table 17). Cookies baked at the 50% replacement level were slightly thicker, but were not significantly different from control cookies or cookies baked at the 25% replacement level; with the exception of cookies baked with unroasted Nicaraguan seed powder at the 25% level. The thickest cookies measured were baked with roasted Guatemalan seed powder at the 50% replacement level (1.07 cm). These cookies had a similar thickness to all other cookies baked at the 50% replacement level, as well as one group of controls.

Spread factor was determined by dividing width by thickness (spread ratio) and multiplying by 10 (AACC 10.50.05, 2014). As replacement levels of *B. alicastrum* seed powder increased, the spread factor of cookies decreased (Table 17). Control cookies and cookies baked at the 25% replacement level with unroasted Nicaraguan and roasted Guatemalan seed powders did not differ in spread factor (approximately 68.2 to 70.4). These cookies, at the 25% replacement level, differed from control cookies when measured for width. The lowest spread factor (approximately 62.1) was measured in cookies baked at the 50% replacement level with roasted Nicaraguan seed powder. These cookies differed by a spread factor of 8.3 from control cookies, which had the highest spread factor (approximately 70.4). Cookies baked with unroasted Nicaraguan seed
powder at the 50% replacement level had the smallest width and largest thickness. When spread factor was corrected to a constant atmospheric pressure basis, values were diminished, yet patterns of statistical significance remained relatively unchanged.

**Surface Color**

Surface color of *B. alicastrum* seed powder and cookies was measured because appearance is often the only quality attribute available at the point-of-purchase. The color of food has an effect on perceived flavor and overall acceptability. Surface color for *B. alicastrum* seed powder is shown in Table 18 and surface color for cookies baked with the seed powder is shown in Table 19. All-purpose white wheat flour (L* = 93.03) was significantly lighter than any of the *B. alicastrum* seed powders (L* = 61.77 to 71.59). As expected, lightness values decreased by one to nearly seven points (L* scale), when seeds were roasted. Unroasted Guatemalan seed powder (L*= 71.59) was the lightest among all seed powder treatments and became slightly but significantly darker when roasted (L*= 70.58). The roasted seed powder from Guatemala was lighter in color than either the unroasted (L* = 68.54) or roasted seed powder (L* = 61.77) from Nicaragua. Redness (a*) values were also lowest in all-purpose white wheat flour (a* = 0.16) when compared to *B. alicastrum* seed powders (Table 18). Unroasted Guatemalan seed powder (a* = 3.71) had a lower redness value than unroasted Nicaraguan seed powder (a* = 4.28). When seed powders were roasted, redness values increased, by 1.2 to 3.7 points (a* scale), with roasted Nicaraguan seed powder having the highest redness value (a* = 7.95). Similarly, yellowness was lowest in all-purpose white wheat flour (b* = 9.31). Unroasted Guatemalan seed powder had a lower yellowness value (b* = 18.68) than
unroasted Nicaraguan seed powder ($b^* = 19.61$). When these seeds were roasted, yellowness values increased, but there was no significant difference ($p>0.05$) in yellowness values between roasted Guatemalan seed powder ($b^* = 21.55$) and roasted Nicaraguan seed powder ($b^* = 21.32$).

Surface color values measured in cookies followed a similar pattern to that found in the all-purpose white wheat flour and *B. alicastrum* seed powder (Table 19). Top lightness values for the control cookies were the highest ($L^* = 79.58$ to 79.75) among all treatments ($L^* = 42.15$ to 79.75). As *B. alicastrum* seed powder replacement levels increased, lightness values for cookie top and bottom decreased. Lightness values were consistently lower for the bottom of the cookie when compared to cookie top measurements, likely due to the conductivity of the pan. Cookies baked at the 50% replacement level using roasted Nicaraguan seed powder had the lowest top ($L^* = 42.15$) and bottom ($L^* = 38.32$) $L^*$ values measured. Among the *B. alicastrum* seed powder replacements, cookies baked at the 25% replacement level using unroasted Nicaraguan seed powder had the highest L* value for cookie tops ($L^* = 60.02$). This result is unexpected as unroasted seed powder from Guatemala had a higher L* value than unroasted seed powder from Nicaragua. Lightness values decreased when seed was roasted. For example, cookies baked at the 25% replacement level using Guatemalan seed powder had lower top lightness values when roasted ($L^* = 53.19$), as compared to cookies baked with unroasted seed powder ($L^* = 58.05$). Redness ($a^*$) top values were lowest in control cookies ($a^* = 0.55$ to 0.60). Cookie redness increased as *B. alicastrum* seed powder replacement levels in the formulation increased. Redness values of cookies
also increased when seeds were roasted. Redness was higher for cookie bottoms than it
was for cookie tops. The highest redness top value was measured in cookies baked at the
50% replacement level using roasted Nicaraguan seed powder (a* = 11.38). The lowest
top redness value among *B. alicastrum* seed powder replacement cookies was measured
in cookies baked at the 25% replacement level using unroasted Guatemalan seed powder
(a* = 6.42). Cookie yellowness also increased as level of *B. alicastrum* seed powder in
the formulation increased and when seeds were roasted. Yellowness values were
consistently higher for the bottom versus the top of cookies. The lowest cookie
yellowness values were measured in control cookie tops (b* = 24.57 to 24.41). The
highest yellowness top value was measured in cookies baked at the 50% replacement
level using unroasted Nicaraguan seed powder (b* = 27.63). Amongst the *B. alicastrum*
seed powder cookies, those baked with roasted Nicaraguan seed powder at the 50%
replacement level had the lowest yellowness top values (b* = 25.12). Roasting had a
small effect on yellowness in the 50% replacement cookies, with roasted seed powder
cookies having a lower yellowness value than unroasted seed powder cookies. Overall,
cookies baked with *B. alicastrum* seed powder replacements are darker, redder and more
yellow than cookies baked solely of all-purpose white wheat flour. Lightness values
decrease while redness and yellowness values increase as *B. alicastrum* seed powder
replacement levels increase, and when seeds are roasted. Across all treatments, color
values measured at the top of the cookie had higher lightness values and lower redness
and yellowness values that the bottom of those same cookies.
Texture

Cookie texture was measured because it is an important factor for consumer acceptance. Texture was measured as hardness, brittleness, and toughness. Hardness, the force required to break a cookie (kg), was greatest in the control cookies (4.84 to 5.13 kg force) (Table 20). As replacement levels of *B. alicastrum* seed powder increased, cookie hardness decreased, with the softest cookies being those baked with roasted Guatemalan seed powder at the 50% replacement level (3.62 kg force); however there was no significant difference between these cookies and cookies baked using unroasted Guatemalan seed powder at the 50% replacement level (3.92 kg force). At the 25% replacement level, there were no significant differences in hardness due to county of origin or roast. The hardness of control cookies (4.84 kg force), baked alongside roasted Nicaraguan seed powder treatments, was similar to the hardness of cookies baked with unroasted (4.54 kg force) and roasted (4.53 kg force) Nicaraguan seed powder at the 25% replacement level. At the 50% replacement level, hardness decreased significantly although cookies baked with unroasted Nicaraguan seed powder (4.07 kg force) at this level had similar hardness values to cookies baked with unroasted Guatemalan seed powder at the 25% level (4.38 kg force).

Brittleness is measured as the distance to deform the cookie before it breaks. A higher brittleness value (mm) denotes a less brittle cookie. Control cookies were inconsistent in brittleness measures with values ranging from 1.57 to 1.71 mm. Control cookies baked at the same time as the unroasted Nicaraguan seed powder treatment (1.71 mm) had a significantly higher brittleness value than controls baked at the same time as
the roasted Nicaragua seed powder treatment (1.57 mm). There were no significant differences in brittleness between any of the cookies baked at the 25% replacement level (p>0.05). These cookies also had similar brittleness values as three of the control groups, as well as all cookies baked at the 50% replacement level, with the exception of unroasted Guatemalan seed powder. Cookies baked with unroasted Guatemalan seed powder at the 50% replacement level had the lowest numerical brittleness value (1.51 mm), denoting these cookies as the most brittle among all treatments. These cookies were not significantly different from the following: controls baked at the same time as unroasted Guatemalan (1.58 mm) and roasted Nicaraguan seed powder (1.57 mm) cookies, cookies baked with unroasted (1.62 mm) and roasted (1.65 mm) seed powder from Guatemalan at the 25% replacement level, cookies baked with unroasted Nicaraguan seed powder at the 25% (1.57 mm) and 50% (1.58 mm) replacement level and cookies baked with roasted Nicaraguan seed powder at the 50% replacement level (1.61 mm) (Table 20).

Toughness, which is calculated by dividing hardness by brittleness, decreased as B. alicastrum seed powder replacement levels increased, although there was some overlap between treatment groups (Table 20). Control cookies had the highest toughness scores and were consistent among treatments (3.05 to 3.19 kg/mm). Toughness values in the 25% replacement treatment group were lower than controls, yet consistent among this treatment group. Cookies baked with unroasted Nicaraguan seed powder at the 25% replacement level (2.98 kg/mm) had similar toughness values as controls (Table 20). Toughness was also consistent among all the 50% replacement level treatments, with the
exception of cookies baked with roasted Guatemalan seed powder (2.13 kg/mm), which had the lowest toughness values and were significantly different from all other treatments. Cookies baked at the 50% replacement level with roasted (2.48 kg/mm) and unroasted (2.60 kg/mm) Nicaraguan seed powder and unroasted Guatemalan seed powder (2.13 kg/mm) had similar toughness values as all cookies baked at the 25% replacement level, with the exception or cookies baked with unroasted Nicaraguan seed powder (2.98 kg/mm).

**Conclusions**

When unroasted and roasted *B. alicastrum* seed powders from two countries (Guatemala and Nicaragua), at two replacement levels (25% and 50%), were used to produce cookies, significant differences were found among the physical properties of moisture, water activity, pH, spread factor, surface color and texture of the flours, dough, and cookies when compared to traditional wheat flour.

*B. alicastrum* seeds, when obtained from the original source, and ground into a powder, had inconsistent moisture and these inconsistencies were reflected in the roasting process. These moisture level inconsistencies could be addressed in the drying process of *B. alicastrum* seed after harvest and during storage, or product formulations could be adjusted to account for moisture differences, as demonstrated by this research. While moisture levels in dough were consistent across treatments, final cookie moisture was not. Cookies baked with *B. alicastrum* seed powder had higher moisture percentages than controls and this is likely due to composition of the seed powder compared to traditional wheat flour. Although *B. alicastrum* cookies had higher moisture contents, there was
little difference in water activity between treatments. Final dough weight and cookie weight were unchanged within and between treatments. Control cookies had a more basic pH (9.52) than cookies baked with *B. alicastrum* seed powder, and as replacement levels increased, cookie pH moved closer to neutral (7.30). The pH of *B. alicastrum* seed powder ranged from 5.01-5.27, making it more acidic than traditional wheat flour. This could create a buffering effect in the cookie.

As replacement levels of *B. alicastrum* seed powder increased, cookie widths and spread factors decreased, while cookie thickness remained relatively unchanged. This decrease in width was also seen when using amaranth, pumpkin and fine milled pulse flours (Giami et al., 2005; Sindhuja et al., 2005; Zucco et al., 2011; Yamsaengsung et al., 2012). Spread ratios decreased in cookies baked with fonio, cowpea and quinoa flours (Lorenz & Coulter, 1991; McWatters et al., 2003). Previous research has shown that thickness either increased (Yamsaengsung et al., 2012; Zucco et al., 2011) or decreased (Giami et al., 2005; Khouryieh & Aramouni, 2012; Zucco et al., 2011) with the addition of non-wheat flours or powders. Overall there was a 0.76 cm difference from the widest control cookie (7.12 cm), to the smallest cookie, baked from roasted Nicaraguan seed powder at the 50% replacement level (6.36 cm). There was only a 0.06 cm difference in thickness between the thickest cookie, baked at the 50% replacement level with roasted Guatemalan seed powder (1.05 cm), and the thinnest, baked at the 25% replacement level with unroasted seed from Nicaragua (0.99 cm). While the differences in width and spread factor were significant, notably between controls and cookies baked at the 50% replacement level, they may not pose practical challenges to production or manufacturing
cookies with added *B. alicastrum* seed powder. Essentially, the end product would be a cookie that is slightly smaller with equal thickness and weight to that of a cookie baked entirely of wheat flour.

*B. alicastrum* seed powder is considerably darker than all-purpose white wheat flour, and takes on an even darker color profile once roasted. Therefore, it was expected that the addition of this seed powder would result in cookies of a darker color. As anticipated, top colors for cookies became darker as seed powder replacements of *B. alicastrum* increased. *B. alicastrum* cookies also had higher redness and yellowness values when compared to control cookies. The lightness value of control cookies is also likely higher due to bleaching of all-purpose flour. In nearly all the previous studies reviewed, additions of non-wheat flours resulted in decreased lightness values in cookies. While cookies formulated with *B. alicastrum* seed powder exhibited darker color than control cookies, this should not have a negative effect on consumer perception, as darker cookies made with chocolate, coffee, or molasses are readily available in the current market. Furthermore, dextrose, the purpose of which is to aid in cookie browning, was omitted from the cookie formulation. This omission was made to determine the color changes attributed solely to the addition of *B. alicastrum* seed powder. Had dextrose been added to the control cookies, it would have resulted in a darker color, and may have been closer in lightness values to the *B. alicastrum* seed powder cookies.

Cookie hardness was greatest in the control cookies and decreased as *B. alicastrum* seed powder replacement levels increased, although there was some overlap in hardness values between control cookies and cookies baked at the 25% replacement
level. Cookies baked at the 50% replacement level had lower hardness values than controls and those baked at the 25% replacement level. Roasting had little effect on hardness values. While cookies baked at the 50% replacement level may not give the characteristic snap of a sugar snap cookie, the texture of these cookies should still be acceptable to consumers. Cookie brittleness remained fairly consistent within and between treatments, which demonstrates that *B. alicastrum* seed powder replacements had little effect on the brittleness of the cookie. While control cookies had a significantly higher toughness value, cookies baked at the 25% and 50% replacement level showed little difference in toughness. Overall, *B. alicastrum* seed powder replacements produced a cookie that had less hardness and toughness than an all wheat flour cookie, with similar brittleness.

While *B. alicastrum* seed powder replacements yield a darker cookie with slightly reduced diameter and a softer texture, it should be a suitable replacement for all-purpose white wheat flour from a consumer acceptance perspective. Further research, using a sensory panel, is necessary to correlate the technical data of color, spread factor, and texture to sensory perception and consumer acceptance. Sindjuha et al. (2005) replaced white wheat flour with amaranth flour and found similar changes in cookies. Spread ratio and hardness decreased as amaranth flour replacement levels increased. When these cookies were evaluated by a sensory panel, compared to controls formulated with 100% percent wheat flour, cookies baked with 25%, 30% and 35% percent amaranth flour scored highest for subjective texture. Cookies baked with 25% and 35% amaranth flour replacement scored highest for mouthfeel. Cookies baked with 25% and 30% amaranth
flour received the highest scores for overall quality (Sindhuja et al., 2005). Giami et al. (2005) found that there was a decrease in spread factor and hardness when cookies were formulated by substituting pumpkin seed flour for white wheat flour. These changes became significant when replacement level reached 20%. At the 20% replacement level, a sensory panel found texture and overall acceptability to be significantly less favorable than cookies baked with 0-15% pumpkin seed flour; however panelists found cookies baked at the 15% replacement level to be as acceptable as control cookies. Cookies at the 15% replacement level were no different in spread factor or hardness when compared to controls (Giami et al., 2005). McWatters et al. (2003) replaced fonio flour for white wheat flour in cookie formulations at 50% and 75%. Fonio replacements resulted in decreased spread factor, hardness, and L* values. When these cookies were scored by a sensory panel, cookies baked with 50% fonio scored the same as 100% wheat flour cookies on appearance, color, flavor, texture and overall acceptability (McWatters et al., 2003). Few studies combine cookie flour replacement with a sensory evaluation component to assess the real world implications of laboratory measurements. When parameters are similar, sensory evaluation may vary as demonstrated above. While significant technical differences were found between control cookies and cookies baked with B. alicastrum seed powder, B. alicastrum cookies should still meet consumer acceptability standards. Based on the data and information presented here is can be said that B. alicastrum seed powders are a viable indigenous replacement to wheat flour in a sugar snap cookie formulation.
Future Research Recommendations

- Sensory panel evaluation of products made with *B. alicastrum* seed powder to determine consumer acceptance.

- Research to determine the particle size breakdown of *B. alicastrum* seed powder to determine the most suitable particle size for baked goods and other products such as drink mixes.

- Further studies on the feasibility of using *B. alicastrum* seed powder in various baked products, e.g. cakes, biscuits (American), tortillas, crackers etc.
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http://dx.doi.org/10.1094/AACCIntMethod-02-52.01


http://dx.doi.org/10.1094/AACCIntMethod-10-50.05


http://dx.doi.org/10.1094/AACCIntMethod-44-15.02


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<table>
<thead>
<tr>
<th>Replacement</th>
<th>Percent Replacement</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Chickpea</td>
<td>0, 20, 40, 60, 80, 100</td>
<td>Yamsaengsung, R., Berghofer, E., &amp; Schoenlechner, R. (2012).¹</td>
</tr>
<tr>
<td>Flaxseed</td>
<td>6, 12, 18</td>
<td>Khouryieh, H., &amp; Aramouni, F. (2012).²</td>
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<tr>
<td>Navy Bean, Pinto Bean, Green Lentil</td>
<td>25, 50, 75, 100 (fine milled and coarse milled)</td>
<td>Zucco, F., Borsuk, Y., &amp; Arntfield, S. D. (2011).³</td>
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<tr>
<td>Black Rice</td>
<td>5, 10, 15, 20</td>
<td>Lee, J. H., Kim, G. H., &amp; Kim, Y. S. (2005).⁵</td>
</tr>
<tr>
<td>White Fonio, California Blackeye Cowpea</td>
<td>50% fonio or cowpea/50% wheat, equal quantities (33%) wheat, fonio, and cowpea, 25% wheat/75% fonio, 25% wheat/50% fonio/25% cowpea, 75% fonio/25% cowpea, 50% fonio/50% cowpea</td>
<td>McWatters, K. H., Ouedraogo, J. B., Resurreccion, A. V., Hung, Y., &amp; Phillips, R. D. (2003)⁸</td>
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<tr>
<td>Quinoa</td>
<td>5, 10, 20, 30 (high and low spread wheat cookie flours)</td>
<td>Lorenz &amp; Coulter (1991)⁹</td>
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¹ Yamsaengsung, R., Berghofer, E., & Schoenlechner, R. (2012). Physical properties and sensory acceptability of cookies made from chickpea addition to white wheat or whole wheat flour compared to gluten-free amaranth or buckwheat flour. *International Journal of Food Science & Technology*, 47(10), 2221-2227.


Table 15: Moisture, pH, and water activity of unroasted and roasted *Brosimum alicastrum* seed powder from Guatemala and Nicaragua, and all-purpose (AP) flour.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Guatemala</th>
<th>Nicaragua</th>
<th>AP Flour</th>
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<td></td>
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<td>Roasted</td>
<td>Unroasted</td>
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<td>0.51d±0.01</td>
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<td>pH</td>
<td>5.23c±0.01</td>
<td>5.17d±0.02</td>
<td>5.27b±0.01</td>
</tr>
</tbody>
</table>

a–d Means ± standard deviation in a row without common superscripts are significantly different at P<0.05. N=9 for all variables except those associated with AP Flour where N=36

1 Moisture is expressed as a percentage (g/100g)
Table 16: Moisture, weight, water activity (a\textsubscript{w}), and pH of all-purpose wheat flour cookies baked with unroasted and roasted Brosimum alicastrum seed powder from Guatemala (GTM) and Nicaragua (NIC) at replacement levels of 0\%, 25\%, and 50\%.

<table>
<thead>
<tr>
<th>Level</th>
<th>0% Control</th>
<th>25%</th>
<th>50%</th>
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<td></td>
<td>Unroasted</td>
<td>Roasted</td>
<td>Unroasted</td>
</tr>
<tr>
<td></td>
<td>GTM</td>
<td>NIC</td>
<td>GTM</td>
</tr>
<tr>
<td>Dough Moisture(^1) (%)</td>
<td>17.26(^b) ±0.05</td>
<td>17.28(^ab) ±0.05</td>
<td>17.26(^b) ±0.08</td>
</tr>
<tr>
<td>Cookie Moisture(^2) (%)</td>
<td>5.93(^c) ±0.32</td>
<td>5.89(^c) ±0.15</td>
<td>5.94(^c) ±0.11</td>
</tr>
<tr>
<td>Dough (a\textsubscript{w})(^3)</td>
<td>0.84(^a) ±0.01</td>
<td>0.83(^ab) ±0.01</td>
<td>0.83(^bc) ±0.01</td>
</tr>
<tr>
<td>Cookie (a\textsubscript{w})(^4)</td>
<td>0.50(^bc) ±0.02</td>
<td>0.50(^bc) ±0.02</td>
<td>0.49(^c) ±0.01</td>
</tr>
<tr>
<td>Cookie pH(^5)</td>
<td>9.52(^a) ±0.02</td>
<td>9.52(^a) ±0.02</td>
<td>9.52(^a) ±0.02</td>
</tr>
<tr>
<td>Dough Weight(^6) (g)</td>
<td>24.86 ±0.35</td>
<td>24.79 ±0.12</td>
<td>24.74 ±0.31</td>
</tr>
<tr>
<td>Cookie Weight(^7) (g)</td>
<td>22.18 ±0.38</td>
<td>22.19 ±0.01</td>
<td>22.10 ±0.28</td>
</tr>
<tr>
<td>Yield(^8) (%)</td>
<td>89.22(^b) ±0.33</td>
<td>89.49(^ab) ±0.41</td>
<td>89.32(^b) ±0.06</td>
</tr>
</tbody>
</table>

\(^a\)\(^b\) Means ± standard deviation in a row without common superscripts are significantly different at \(P\leq0.05\). Each treatment was baked alongside its own control.

\(^1\)All dough from a replicate was sampled in triplicate for three replicates. Moisture calculated as loss on drying. \(N=9\)

\(^2\)All cookies from a replicate were combined and sampled in triplicate for three replicates. Moisture calculated as loss on drying. \(N=9\)

\(^3\)Water Activity. All dough from a replicate was sampled in triplicate for three replicates. \(N=9\)

\(^4\)Water activity. All cookies from a replicate were combined and sampled in triplicate for three replicates. \(N=9\)

\(^5\)All cookies from a replicate were combined and sampled in triplicate for three replicates. \(N=9\)

\(^6\)One replicate of dough for six cookies was weighed together and divided by 6 for three replicates. \(N=3\)

\(^7\)One replicate of six cookies was weighed together and divided by 6 for three replicates. \(N=3\)

\(^8\)Cookie weight divided by dough weight multiplied by 100. \(N=3\)
Table 17: Width, thickness, and spread factor for all-purpose wheat flour cookies baked with unroasted and roasted *Brosimum alicastrum* seed powder from Guatemala (GTM) and Nicaragua (NIC) at replacement levels of 0%, 25%, and 50%.

<table>
<thead>
<tr>
<th>Level</th>
<th>0%</th>
<th>25%</th>
<th>50%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unroasted</td>
<td>Roasted</td>
<td>Unroasted</td>
</tr>
<tr>
<td>Country</td>
<td>GTM</td>
<td>NIC</td>
<td>GTM</td>
</tr>
<tr>
<td>Width</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unroasted</td>
<td>7.05&lt;sup&gt;ab&lt;/sup&gt; ±0.05</td>
<td>7.03&lt;sup&gt;b&lt;/sup&gt; ±0.04</td>
<td>7.05&lt;sup&gt;ab&lt;/sup&gt; ±0.06</td>
</tr>
<tr>
<td>Roasted</td>
<td>6.83&lt;sup&gt;c&lt;/sup&gt; ±0.05</td>
<td>6.57&lt;sup&gt;e&lt;/sup&gt; ±0.02</td>
<td>6.67&lt;sup&gt;d&lt;/sup&gt; ±0.02</td>
</tr>
<tr>
<td>Width Corrected&lt;sup&gt;1&lt;/sup&gt;</td>
<td>7.00&lt;sup&gt;ab&lt;/sup&gt; ±0.05</td>
<td>6.96&lt;sup&gt;b&lt;/sup&gt; ±0.04</td>
<td>6.98&lt;sup&gt;ab&lt;/sup&gt; ±0.06</td>
</tr>
<tr>
<td>Thickness</td>
<td>1.01&lt;sup&gt;bc&lt;/sup&gt; ±0.02</td>
<td>1.00&lt;sup&gt;bc&lt;/sup&gt; ±0.02</td>
<td>1.01&lt;sup&gt;bc&lt;/sup&gt; ±0.02</td>
</tr>
<tr>
<td>Corrected&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thickness Corrected&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;bc&lt;/sup&gt; ±0.02</td>
<td>1.02&lt;sup&gt;ab&lt;/sup&gt; ±0.02</td>
<td>1.03&lt;sup&gt;bc&lt;/sup&gt; ±0.02</td>
</tr>
<tr>
<td>Spread Factor</td>
<td>70.1&lt;sup&gt;a&lt;/sup&gt; ±1.4</td>
<td>70.4&lt;sup&gt;a&lt;/sup&gt; ±1.7</td>
<td>69.7&lt;sup&gt;a&lt;/sup&gt; ±1.3</td>
</tr>
<tr>
<td>Spread Factor Corrected&lt;sup&gt;1,2&lt;/sup&gt;</td>
<td>68.6&lt;sup&gt;a&lt;/sup&gt; ±1.6</td>
<td>68.5&lt;sup&gt;a&lt;/sup&gt; ±1.8</td>
<td>68.0&lt;sup&gt;ab&lt;/sup&gt; ±1.2</td>
</tr>
</tbody>
</table>

<sup>a-f</sup> Means ± standard deviation in a row without common superscripts are significantly different at P<0.05. N=6 for all variables. Each treatment was baked alongside its own control. Width and thickness are expressed in cm.

<sup>1</sup>Correction factors were used to adjust to a constant atmospheric pressure basis, based on elevation and barometric pressure adjusted to sea level.

<sup>2</sup>Spread Factor is calculated by dividing width by thickness and multiplying by 10.
Table 18: Color values\(^1\) for unroasted and roasted *Brosimum alicastrum* seed powder from Guatemala and Nicaragua, and all-purpose (AP) flour.

<table>
<thead>
<tr>
<th>Color Values</th>
<th>Guatemala</th>
<th>Nicaragua</th>
<th>AP Flour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unroasted</td>
<td>Roasted</td>
<td>Unroasted</td>
</tr>
<tr>
<td>L*</td>
<td>71.59(^{b})±0.08</td>
<td>70.58(^{c})±0.17</td>
<td>68.54(^{d})±0.10</td>
</tr>
<tr>
<td>a*</td>
<td>3.71(^{d})±0.02</td>
<td>4.95(^{b})±0.05</td>
<td>4.28(^{c})±0.03</td>
</tr>
<tr>
<td>b*</td>
<td>18.68(^{c})±0.06</td>
<td>21.55(^{a})±0.10</td>
<td>19.61(^{b})±0.06</td>
</tr>
</tbody>
</table>

\(^{a-e}\) Means ± standard error in a row without common superscripts are significantly different at \(P<0.05\). N=9 for all variables except those associated with AP Flour where N=36

\(^1\)C.I. E. Color Values, 10° observer/illuminant C, where L* denotes lightness, a* denotes redness, and b* denotes yellowness.
Table 19: Color values\(^1\) for all-purpose wheat flour cookies baked\(^2\) with unroasted and roasted Brosimum alicastrum seed powder from Guatemala (GTM) and Nicaragua (NIC) at replacement levels of 0%, 25%, and 50%.

<table>
<thead>
<tr>
<th>Level</th>
<th>Roast</th>
<th>Country</th>
<th>Color Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cookie Top</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(L^*)</td>
</tr>
<tr>
<td>0%</td>
<td>Unroasted</td>
<td>GTM</td>
<td>79.66(^a)±0.08</td>
</tr>
<tr>
<td></td>
<td>NIC</td>
<td></td>
<td>79.58(^a)±0.06</td>
</tr>
<tr>
<td></td>
<td>Roasted</td>
<td>GTM</td>
<td>79.71(^a)±0.08</td>
</tr>
<tr>
<td></td>
<td>NIC</td>
<td></td>
<td>79.75(^a)±0.06</td>
</tr>
<tr>
<td>25%</td>
<td>Unroasted</td>
<td>GTM</td>
<td>58.05(^c)±0.09</td>
</tr>
<tr>
<td></td>
<td>NIC</td>
<td></td>
<td>60.02(^b)±0.13</td>
</tr>
<tr>
<td></td>
<td>Roasted</td>
<td>GTM</td>
<td>53.19(^d)±0.11</td>
</tr>
<tr>
<td></td>
<td>NIC</td>
<td></td>
<td>51.27(^f)±0.08</td>
</tr>
<tr>
<td>50%</td>
<td>Unroasted</td>
<td>GTM</td>
<td>51.24(^f)±0.08</td>
</tr>
<tr>
<td></td>
<td>NIC</td>
<td></td>
<td>52.61(^e)±0.10</td>
</tr>
<tr>
<td></td>
<td>Roasted</td>
<td>GTM</td>
<td>46.08(^g)±0.17</td>
</tr>
<tr>
<td></td>
<td>NIC</td>
<td></td>
<td>42.15(^b)±0.15</td>
</tr>
</tbody>
</table>

\(^{ab}\) Means ± standard error in a column without common superscripts are significantly different at \(P \leq 0.05\). \(N=18\) for all variables. Each treatment was baked alongside its own control.

\(^1\)C.I. E. Color Values 10° Observer/Illuminant C, where \(L^*\) denotes lightness, \(a^*\) denotes redness, and \(b^*\) denotes yellowness.

\(^2\)Average bake temperature ± standard deviation was 190.1±1.64°C, for 9 minutes.
Table 20: Texture values for all-purpose wheat flour cookies baked with unroasted and roasted *Brosimum alicastrum* seed powder from Guatemala (GTM) and Nicaragua (NIC) at replacements levels of 0%, 25%, and 50%.

<table>
<thead>
<tr>
<th>Level</th>
<th>Treatment</th>
<th>Country</th>
<th>Hardness&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Britteness&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Toughness&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>Unroasted</td>
<td>GTM</td>
<td>5.01&lt;sup&gt;a&lt;/sup&gt;±0.13</td>
<td>1.58&lt;sup&gt;abc&lt;/sup&gt;±0.05</td>
<td>3.19&lt;sup&gt;a&lt;/sup&gt;±0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NIC</td>
<td>5.13&lt;sup&gt;a&lt;/sup&gt;±0.11</td>
<td>1.71&lt;sup&gt;a&lt;/sup&gt;±0.06</td>
<td>3.05&lt;sup&gt;a&lt;/sup&gt;±0.11</td>
</tr>
<tr>
<td></td>
<td>Roasted</td>
<td>GTM</td>
<td>5.05&lt;sup&gt;a&lt;/sup&gt;±0.12</td>
<td>1.69&lt;sup&gt;ab&lt;/sup&gt;±0.07</td>
<td>3.07&lt;sup&gt;a&lt;/sup&gt;±0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NIC</td>
<td>4.84&lt;sup&gt;ab&lt;/sup&gt;±0.11</td>
<td>1.57&lt;sup&gt;bc&lt;/sup&gt;±0.04</td>
<td>3.12&lt;sup&gt;a&lt;/sup&gt;±0.12</td>
</tr>
<tr>
<td>25%</td>
<td>Unroasted</td>
<td>GTM</td>
<td>4.38&lt;sup&gt;cd&lt;/sup&gt;±0.12</td>
<td>1.62&lt;sup&gt;abc&lt;/sup&gt;±0.04</td>
<td>2.72&lt;sup&gt;bc&lt;/sup&gt;±0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NIC</td>
<td>4.54&lt;sup&gt;bc&lt;/sup&gt;±0.12</td>
<td>1.57&lt;sup&gt;abc&lt;/sup&gt;±0.06</td>
<td>2.98&lt;sup&gt;ab&lt;/sup&gt;±0.16</td>
</tr>
<tr>
<td></td>
<td>Roasted</td>
<td>GTM</td>
<td>4.46&lt;sup&gt;bc&lt;/sup&gt;±0.12</td>
<td>1.65&lt;sup&gt;abc&lt;/sup&gt;±0.05</td>
<td>2.72&lt;sup&gt;bc&lt;/sup&gt;±0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NIC</td>
<td>4.53&lt;sup&gt;bc&lt;/sup&gt;±0.11</td>
<td>1.68&lt;sup&gt;ab&lt;/sup&gt;±0.05</td>
<td>2.73&lt;sup&gt;bc&lt;/sup&gt;±0.10</td>
</tr>
<tr>
<td>50%</td>
<td>Unroasted</td>
<td>GTM</td>
<td>3.92&lt;sup&gt;ef&lt;/sup&gt;±0.10</td>
<td>1.51&lt;sup&gt;c&lt;/sup&gt;±0.04</td>
<td>2.61&lt;sup&gt;c&lt;/sup&gt;±0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NIC</td>
<td>4.07&lt;sup&gt;de&lt;/sup&gt;±0.11</td>
<td>1.58&lt;sup&gt;abc&lt;/sup&gt;±0.04</td>
<td>2.60&lt;sup&gt;c&lt;/sup&gt;±0.11</td>
</tr>
<tr>
<td></td>
<td>Roasted</td>
<td>GTM</td>
<td>3.62&lt;sup&gt;f&lt;/sup&gt;±0.11</td>
<td>1.70&lt;sup&gt;ab&lt;/sup&gt;±0.05</td>
<td>2.13&lt;sup&gt;d&lt;/sup&gt;±0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NIC</td>
<td>3.96&lt;sup&gt;e&lt;/sup&gt;±0.10</td>
<td>1.61&lt;sup&gt;abc&lt;/sup&gt;±0.04</td>
<td>2.48&lt;sup&gt;c&lt;/sup&gt;±0.10</td>
</tr>
</tbody>
</table>

<sup>a-f</sup> Means ± standard error in a column without common superscripts are significantly different at P<0.05. N=18 for all variables. Each treatment was baked alongside its own control.

<sup>1</sup>Force required to break cookie in kilograms (kg).

<sup>2</sup>Distance required to break cookie in millimeters (mm).

<sup>3</sup>Calculated by dividing hardness by brittleness (kg/mm).
APPENDICES
APPENDIX A: *Brosimum alicastrum* Harvest Locations.
APPENDIX B: Roasting Protocol for *Brosimum alicastrum* Seeds.

Roasting protocol for Guatemalan *Brosimum alicastrum* seeds.

<table>
<thead>
<tr>
<th>Time/Minutes</th>
<th>Temperature/ Celsius</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:00</td>
<td>93.3</td>
<td>Flame reduced to 48% when seed was dropped at 0:00</td>
</tr>
<tr>
<td>2:00</td>
<td>82.8</td>
<td></td>
</tr>
<tr>
<td>3:00</td>
<td>94.4</td>
<td>Flame reduced to 25% at 3:00</td>
</tr>
<tr>
<td>4:00</td>
<td>110.0</td>
<td>Flame reduced to 20% at 4:35</td>
</tr>
<tr>
<td>5:00</td>
<td>125.0</td>
<td>Flame reduced to 18% at 5:30</td>
</tr>
<tr>
<td>6:00</td>
<td>137.2</td>
<td></td>
</tr>
<tr>
<td>7:00</td>
<td>145.0</td>
<td>Flame increased to 20% at 7:15</td>
</tr>
<tr>
<td>8:00</td>
<td>151.7</td>
<td></td>
</tr>
<tr>
<td>8:30</td>
<td>155.0</td>
<td></td>
</tr>
<tr>
<td>9:00</td>
<td>157.2</td>
<td></td>
</tr>
<tr>
<td>9:28</td>
<td>160.0</td>
<td>Seed is dumped from roaster and air cooled</td>
</tr>
</tbody>
</table>

Roasting protocol for Nicaraguan *Brosimum alicastrum* seeds.

<table>
<thead>
<tr>
<th>Time / Minutes</th>
<th>Temperature/ Celsius</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:00</td>
<td>96.7</td>
<td>Flame reduced to 50% at 0:30</td>
</tr>
<tr>
<td>2:00</td>
<td>82.8</td>
<td></td>
</tr>
<tr>
<td>3:00</td>
<td>93.3</td>
<td>Flame reduced to 25% at 3:00</td>
</tr>
<tr>
<td>4:00</td>
<td>108.9</td>
<td>Flame reduced to 20% at 4:42</td>
</tr>
<tr>
<td>5:00</td>
<td>121.7</td>
<td></td>
</tr>
<tr>
<td>6:00</td>
<td>136.7</td>
<td></td>
</tr>
<tr>
<td>7:00</td>
<td>148.3</td>
<td></td>
</tr>
<tr>
<td>8:00</td>
<td>157.2</td>
<td></td>
</tr>
<tr>
<td>8:30</td>
<td>160.0</td>
<td>Seed is dumped from roaster and air cooled</td>
</tr>
</tbody>
</table>
APPENDIX C: Comparison of Nicaraguan *Brosimum alicastrum* Seed Powder to USDA Nutrients for All-Purpose White Wheat Flour and Yellow Whole Grain Cornmeal.

Appendix C.1: Nutrient comparison\(^1\) of unroasted and roasted *Brosimum alicastrum* seed powder from Nicaragua to USDA\(^2\) Nutrient Database nutrients for all-purpose flour (AP\(^3\)) and cornmeal\(^4\).

<table>
<thead>
<tr>
<th></th>
<th>Unroasted</th>
<th>Roasted</th>
<th>AP(^3)</th>
<th>Cornmeal(^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture g/100g</td>
<td>11.75</td>
<td>8.78</td>
<td>11.92</td>
<td>10.26</td>
</tr>
<tr>
<td>Protein g/100g</td>
<td>11.74</td>
<td>12.14</td>
<td>11.73</td>
<td>9.05</td>
</tr>
<tr>
<td>Fat g/100g</td>
<td>0.58</td>
<td>0.64</td>
<td>1.11</td>
<td>4.00</td>
</tr>
<tr>
<td>Carbohydrate(^5) g/100g</td>
<td>83.61</td>
<td>82.62</td>
<td>86.64</td>
<td>85.68</td>
</tr>
<tr>
<td>Ash g/100g</td>
<td>4.1</td>
<td>4.6</td>
<td>0.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Total Sugar g/100g</td>
<td>7.66</td>
<td>3.98</td>
<td>0.31</td>
<td>0.71</td>
</tr>
<tr>
<td>NDF(^6) g/100g</td>
<td>9.8</td>
<td>11.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ADF(^7) g/100g</td>
<td>6.5</td>
<td>8.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dietary Fiber</td>
<td>-</td>
<td>-</td>
<td>3.1</td>
<td>8.1</td>
</tr>
<tr>
<td>Vitamin A(^8) μg/100g</td>
<td>899</td>
<td>1820</td>
<td>1</td>
<td>178</td>
</tr>
<tr>
<td>Vitamin C(^9) mg/100g</td>
<td>41</td>
<td>23</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Calcium mg/100g</td>
<td>189</td>
<td>190</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>Copper mg/100g</td>
<td>0.9</td>
<td>1.0</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Iron mg/100g</td>
<td>6.1</td>
<td>5.6</td>
<td>5.3</td>
<td>3.8</td>
</tr>
<tr>
<td>Magnesium mg/100g</td>
<td>200</td>
<td>208</td>
<td>25</td>
<td>142</td>
</tr>
<tr>
<td>Manganese mg/100g</td>
<td>0.6</td>
<td>0.6</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Phosphorus mg/100g</td>
<td>163</td>
<td>171</td>
<td>123</td>
<td>269</td>
</tr>
<tr>
<td>Potassium mg/100g</td>
<td>1439</td>
<td>1498</td>
<td>121</td>
<td>320</td>
</tr>
<tr>
<td>Zinc mg/100g</td>
<td>2.7</td>
<td>2.9</td>
<td>0.8</td>
<td>2.0</td>
</tr>
<tr>
<td>Sodium mg/100g</td>
<td>18.1</td>
<td>11.7</td>
<td>2.3</td>
<td>39.0</td>
</tr>
</tbody>
</table>

\(^1\)Data reported on a dry weight basis, with the exception of moisture data. USDA data was converted to dry weight basis.

\(^2\)United States Department of Agriculture

\(^3\)USDA Nutrient Database: Wheat flour, white, all-purpose, enriched, unbleached, 20581.

\(^4\)USDA Nutrient Database: Cornmeal, whole grain, yellow, 20020.

\(^5\)By Difference

\(^6\)Neutral Detergent Fiber.

\(^7\)Acid Detergent Fiber

\(^8\)As total carotenoids.

\(^9\)As ascorbic acid
Appendix C.2: Comparison of indispensable amino acids\(^1\) of unroasted and roasted *Brosimum alicastrum* seed powder from Nicaragua to USDA\(^2\) Nutrient Database nutrients for all-purpose flour (AP\(^3\)) and cornmeal\(^4\).

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Unroasted</th>
<th>Roasted</th>
<th>AP(^3)</th>
<th>Cornmeal(^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>0.11</td>
<td>0.11</td>
<td>0.26</td>
<td>0.28</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.33</td>
<td>0.28</td>
<td>0.41</td>
<td>0.32</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.45</td>
<td>0.43</td>
<td>0.81</td>
<td>1.11</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.50(^5)</td>
<td>0.34(^5)</td>
<td>0.26</td>
<td>0.25</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.05</td>
<td>0.03</td>
<td>0.21</td>
<td>0.19</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.33</td>
<td>0.33</td>
<td>0.59</td>
<td>0.44</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.31</td>
<td>0.31</td>
<td>0.32</td>
<td>0.34</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.09</td>
<td>0.08</td>
<td>0.14</td>
<td>0.06</td>
</tr>
<tr>
<td>Valine</td>
<td>0.40</td>
<td>0.36</td>
<td>0.47</td>
<td>0.46</td>
</tr>
</tbody>
</table>

\(^1\)Data reported on a dry weight basis (g/100g). USDA Data converted to dry weight basis.  
\(^2\)United States Department of Agriculture  
\(^3\)USDA Nutrient Database: Wheat flour, white, all-purpose, enriched, unbleached, 20581.  
\(^4\)USDA Nutrient Database: Cornmeal, whole grain, yellow, 20020.  
\(^5\)Lysine + Hydroxylysine