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Nutraceutrical Snack Prepared From Sprouted Rough Rice and Green Gram and its Physichocemical Properties, In vitro Glycemic Index, and Sensory Attributes

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Nutraceutical Snack Prepared From Sprouted Rough Rice and Green Gram and its Physicochemical Properties, *In vitro* Glycemic Index, and Sensory Attributes

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Undergraduate Honors Thesis

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Keywords:

germination, rough rice, green gram, nutraceutical, snack chip, physicochemical, sensory, trypsin inhibitor, lipoxygenase

Abstract

Snacks make up a large portion of the U.S. daily meals, but unhealthy snacks may be causing consumers to become overweight or obese. A healthy alternatives are germinated cereals and legumes, which undergo chemical compositional changes producing smaller size molecules for easier digestion and generate bioactives. Therefore, the objective of this research was to develop a healthy and nutritional snack chip from germinated, Arkansas produced rough rice and germinated green gram that will be easier for the body to digest, provide much higher protein than conventional chips or crackers with low on the glycemic index, and still meet consumer demands for more nutritious and innovative snacks. Rough rice and green gram were soaked and germinated for 1, 3, 5, and 7 days. The germination showed a significant difference ($P < 0.05$) in the changes in nutrient composition and antinutrients: increase of protein and lipids, decrease of starch, change in moisture, change in water activity and decrease in trypsin inhibitor, lipoxygenase-1, lipoxygenase-3 activity. The germinated rice and green gram showed microbial counts around 10^4 which is within the usual acceptable counts. The *in vitro* glycemic index testing showed a decrease over the germination period tested for both rough rice and green gram. Color was also tested and showed a significant difference ($P < 0.05$) in difference in color. The germinated rice and green gram flours were made into a snack chip and underwent fracturability treatment, in which the chip made which germinated flours required almost twice the force in comparison to the chip made with non-germinated flours. 4-month shelf-life study showed a significant change in color after 3 months. A sensory evaluation by 74 subjects showed an increased acceptability for snack chips prepared from 5-day germinated rough rice and 5-day germinated green gram flours compared to snack chips prepared with non-germinated rough rice and green gram flours. The results indicate that snack chips prepared using sprouted rough rice and green gram is a healthier alternative to the

snack chips currently on the market due an increase in protein and lipids and a decrease in the glycemic index.

Introduction

People all over the world are changing their eating habits; many people are no longer sitting down to the traditional three meals a day. For example, in the United States in 2016, snacks represented more than 50 percent of all eating and drinking occasions (Hartman, 2016). The snack industry worldwide makes 374 billion dollars a year (Nielson, 2014). Since snacks are becoming an important part of people's daily diet, it is crucial for the snacking industry to produce healthier snacks as an alternative to the nutrients deficient from the traditional meals.

Many consumers are also demanding healthier and better-quality snacks. Frequent consumption of unhealthy snacks may be causing consumers to become overweight or obese and have other health issues, and it may be why Arkansas' obesity rate ranks 6th nationally in 2015 (Segal *et al*., 2016). In North America, 66% of consumers eat snacks to provide nutrition (Nielson, 2014). Roughly two thirds of consumers prefer snacks with low sugar, salt, fat, and calories and beneficial ingredients: fiber, protein, and whole grains (Nielson, 2014). Whole grains on the shelf today are typically made from cereal grains such as wheat, rye, or rice.

Cereal grains contain anti-nutrients, which are the grains natural protection from being eaten by pests or animals. These anti-nutrients—such as lipoxygenase and trypsin inhibitor—interfere with the human body's ability to digest grains. Germinating cereal grains is a way to reduce its anti-nutrients (Moongngarm and Saetung, 2010). During germination, the chemical composition of the grains changes drastically due to their biochemical activity, which provides essential compounds and energy for the formation of seedlings (Hettiarachchy, 2014). However, cereal grains do not form a complete protein due to their limiting essential amino acid lysine, but by combining a cereal grain with a legume—such as soybean, lentils, or green gram—they can form a complete protein.

This study utilized Arkansas' main crop: rice. Rice's limiting amino acid is lysine. Green gram's limiting amino acid is methionine. The limiting essential amino acids in rice and green gram supplemented each other and made it a complete protein.

Recently, germination of cereal and legume seeds has gained more attention due to their health benefits, and companies are allowed by the Food and Drug Administration (FDA) to make certain health claims on their labels (Donkor *et al.*, 2012; Hettiarachchy, 2014). Several studies have shown that during germination, the seeds' chemical compositions change drastically due to the biochemical activity used in creating sprouts. Simple sugars, peptides, the amino acids are produced by enzymatic hydrolysis of ~~la~~ carbohydrates and protein which improve the nutritional quality of the seeds (Donkor *et al.*, 2012; Hettiarachchy, 2014; Kim *et al.*, 2012; Moongngarm and Saetung, 2010). During germination seeds produce bioactive components such as ascorbic acid, tocopherols, tocotrienols, and phenolic compounds, and increase their antioxidant activities (Fernandez-Orozco *et al.*, 2008; Frias *et al.*, 2005).

Germinating rough rice is better than germinating brown rice due to the intact hull of rough rice keeping the seed germ protected and resulting in requiring less care during the germination process and producing higher germination yields, even though the germination period for rough rice is longer than milled brown rice (Moongngarm and Saetung, 2010). Also, when the hull is removed to produce brown rice, the embryo can be damaged and biological compounds like enzymes in the kernel deteriorate, causing oxidation due to the embryo being exposed to air and light, enzymatic and non-enzymatic reactions, and spoilage from the enzymes and microorganism having easy access to broken kernels or kernels missing the germ and resulting in a decreased concentration of bioactive compounds and nutrients compared to rough rice (Moongngarm and Saetung, 2010). Not only is the protein content of germinated rough rice higher than the protein

content of brown rice, but the lipid content, c-aminobutyric acid (GABA), dietary fiber, vitamin E, niacin, thiamine, and magnesium, and lysine, have been reported to be higher than those of brown rice due to germination increasing free sugars, crude protein, many essential amino acids including lysine, the limiting amino acid in rice, isoleucine, leucine, phenylalanine, threonine and valine, total free amino acids, and some bioactive substances (Hettiarachchy, 2014; Kim *et al.*, p. 2012; Lee *et al.*, 2007; Moongngarm and Saetung, 2010; Saman *et al.*, 2008).

The antinutrients in green gram greatly limit the protein digestibility and nutritional benefits, but this limitation can be overcome by germinating the green gram (Frias *et al.*, 2005; Mubarak 2005).

Many studies have shown a significant nutritive improvement in amino acids, digestible protein, carbohydrates, sugars, and antioxidants such as vitamins C and E in germinated green gram (Frias *et al.*, 2005; Fernandez-Orozco *et al.*, 2008; Mubarak 2005; Tang *et al.*, 2014). Also, studies have shown that germinated green gram has lower amounts of antinutrients such as trypsin inhibitors and reduced or eliminated amounts of indigestible factors such as phytic acid, stachyose, and raffinose (Fernandez-Orozco *et al.*, 2008; Mubarak 2005; Tang *et al.*, 2014). Germinated green gram has been found to promote digestion, eliminate toxins, significantly reduce blood pressure, and treat a common bacterial infection associated with gastroduodenal disease (Tang *et al.*, 2014). The health-promotion effect from germinated rough rice and germinated green gram can be utilized in creating a healthy snack with desirable attributes and sensory properties and contribute to reducing the obesity rate in Arkansas.

Since 2016, more than 50% of the U.S. daily meals are made up of snacks, and the snacking industry provides hundreds of billions of dollars of snacks each year (Hartman, 2016, Nielson, 2014). Many of the consumers are demanding more nutritious, innovative snacks that use local

ingredients and are filled with benefits. The purpose of this study was to develop a healthy and nutritional snack chip from germinated, Arkansas produced rough rice and germinated green gram that will be easier for the body to digest, much higher in protein than regular chips or crackers, low on the glycemic index, and still meet consumer demands for more nutritious and innovative snacks using local ingredients.

Objectives

1. Determine the optimal duration for germinating rough rice and green gram and prepare non-germinated and germinated rough rice and green gram flours.
2. Investigate the physicochemical characteristics, anti-nutrients, and *in vitro* Glycemic Index in flours made from germinated rough rice and green gram.
3. Prepare snack chips from germinated and non-germinated rough rice and green gram and determine physical characteristics, *in vitro* Glycemic Index, sensory properties and shelf-life study.

Materials

Rough rice was provided by Riceland Foods (Stuttgart, AR) and green gram seeds, baking soda, and salt were food grade purchased from a local store. All chemicals (analytical grade) for analysis were procured from VWR (Radnor, PA, USA), Sigma Aldrich (St. Louis, MO, USA), and Fisher Scientific (Pittsburg, PA, USA).

Methods

Objective 1: Determined the optimal duration for germinating rough rice and green gram and prepare non-germinated and germinated rough rice and green gram flours.

Germination

Rough rice (RR) (~100 g) was weighed then rinsed with deionized (DI) water, placed in a water bath (34 °C), and incubated for approximately 24 hr in order for the hull to soften and become elastic allowing the coleorhiza, the sheath covering the radicle or embryonic primary root, to elongate and emerge through the hull for the radicle and coleoptile, the primary leaf, to emerge during germination (Moldenhauer and Slaton, 2001). Then the soaked rough rice (SRR) was rinsed with DI water and then examined for unacceptable grains, grains showing evidence of the germ being damaged and were removed since these can promote decay in the germination process.

A plastic tray containing four hydrated paper towels was used as a bed for germination. The drained hydrated RR or soaked rough rice (SRR) was placed on the paper hydrated towels, sprayed with DI water, closed with four hydrated paper towels, and covered with an inverted plastic tray to prevent light exposure and placed inside an incubator at 27 °C at 100% humidity. After ~ every 24 hr, the germinated RR was examined and the damaged germs were removed. At ~ 72 hr of germination, the paper towels were replaced with new hydrated paper towels to prevent any contamination. Within two days the coleorhiza emerged from the hull and the grains deficient in coleorhiza were discarded. The RR was germinated for a period of 7 days and germinated sprouts were collected at 1, 3, 5, and 7 days, and either packaged inside a plastic bag and refrigerated or immediately underwent the drying procedure described below. The green gram (GG) underwent the same process as the RR, except for the soaking time was 2 hr. The soaked green gram (SGG) then went through the same procedure as the germinated RR (GRR).

Drying

The SRR, SGG, GRR, or germinated GG (GGG) in a metal tray was placed in oven (Equatherm 267-914, Curtin Matheson Scientific Inc) for ~ 24 hr (37 °C). Then the dried SRR, SGG, GRR, or GGG were cooled and refrigerated.

Dehulling and Milling

The GRR underwent abrasion against a 16-mesh sieve to remove sprouts. Then, the SRR and GRR were dehulled (STHU-35S Rice Huller, U-SHINE). The dehulled GRR was combined with sprouts and GGG were ground using a mill (Ika Universal Mill M20, Tekmar Company), and sifted through a 60-mesh strainer to obtain uniform particle size flours. Flours were made from non-germinated rough rice (NGRR) and the non-germinated green gram (NGGG) without soaking as above for comparison. There were 12 (twelve) sample flours: NGRR flour (NGRRF), SRR flour (SRRF), NGGG flours (NGGGF), and SGG flour (SGGF) as controls, and 1, 3, 5, and 7-day GRR flours (GRRF) as well as 1, 3, 5, and 7-day GGG flours (GGGF) as the germinated samples.

Objective 2: Investigated the physicochemical characteristics, anti-nutrients, and *in vitro* Glycemic Index in flours made from germinated rough rice and green gram.

Moisture Content of the Flours

Moisture contents of the sample flours were determined using the method approved by the AACC International (2000). Samples of the flours were placed in an oven (Equatherm 267-914, Curtin Matheson Scientific. Inc.) with a temperature of 110°C for 5 hr, weighed, and re-dried to constant weight. The percentage of moisture content was calculated as:

$$\text{Moisture (\%)} = \frac{\text{evaporated water weight}}{\text{sample weight}} * 100$$

Protein Content of the Flours

The Kjeldahl Method 46-13.01 (ACC International, 1990), routinely used in Dr. Hettiarachchy's laboratory, was used to determine the protein. Each flour (~ 0.5 g) was digested with concentrated sulfuric acid, H_2SO_4 (5 mL), and Kjeldahl catalyst (0.5 tablet) using a digestion heater unit (Labconco 60011, Labconco Corp., Kansas City, MO, USA), and then it was diluted to 25.0 mL using DI water. Sodium hydroxide (NaOH) (40% w/v, 10 mL) was added to the digested sample (5.0 mL) and distilled using a RapidStill Distillation unit (Labconco Corp., Kansas City, MO, USA) and 4% boric acid, H_3BO_3 containing methyl red/bromocresol green as an indicator was used as the receiver solution. The released ammonia, NH_3, was titrated with hydrochloric acid, HCl, and the nitrogen content was calculated as:

$$\% \text{ Nitrogen} = \frac{\text{volume HCl (mL) x M of HCl x atomic weight of nitrogen x F}}{\text{Mass of dried flour (mg)}} * 100$$

where F was a dilution factor of 5

$$\% \text{ Protein} = \text{nitrogen-to-protein (N:P) conversion factor x } \% \text{ Nitrogen}$$

using the N:P conversion factor of 6.25 for rice (Hettiarachchy, 2014) and 6.40 for green gram (Romo Estrella, 2008) to determine the protein content.

Lipids Content of the Flours

The soxhlet extraction procedure by the AACC (1990) was followed. Flour sample (2.0 g) was folded in a Whatman filter paper No. 4 and placed in a thimble and then the thimble was placed in a soxhlet tube. Petroleum ether (300 mL) was added into the soxhlet tube for lipid extraction and the sample was refluxed for 4 hr (45°C). The collected petroleum ether containing soluble lipid in the soxhlet flask was distilled to remove the petroleum ether. Then, the lipid content was calculated using the equation:

$$\text{Lipid (\%)} = \frac{\text{lipid weight}}{\text{sample weight}} * 100$$

Starch Content of the Flours

The AACC Method 76-13.01 (ACC International, 1999) was used to determine the starch content. Flour sample (~ 100 mg) was placed in a centrifuge tube with aqueous ethanol (80% v/v, 5 mL) and incubated for 5 min (80-85°C). The contents were mixed on a vortex stirrer and more aqueous ethanol (80%v/v, 5 mL) was added. The tube was centrifuged for 10 min at 1,800 *g* (~ 3,000 rpm) on a bench centrifuge. Then, the supernatant was discarded. The pellet was resuspended in aqueous ethanol (80%v/v, 10 mL), stirred on a vortex mixer, centrifuged as above, and the supernatant was carefully removed. Thermostable α-amylase (3 mL; 100 U/mL in sodium acetate buffer, pH 5.0) was added and the tube was incubated in a boiling water bath for 6 min, where the tube was stirred for 6 minutes. The tube was then placed in a 50°C, amyloglucosidase (0.1 mL, 3300 U/mL) was added, stirred on a vortex mixer, and incubated for 30 min (50°C). Using a funnel, the entire contents of the tube was transferred into a 100-mL volumetric flask. A wash bottle was used to carefully and thoroughly rinse the tube contents. The volume was adjusted using distilled DI water and mixed. An aliquot of this solution was centrifuged at 3,000 rpm (~ 1,800 *g*) for 10 min. The clear, undiluted supernatant was used for the assay. Duplicate aliquots (0.1 mL) of the supernatant were transferred to glass test tubes, GOPOD (glucose oxidase/ peroxidase) Reagent (3.0 mL) was added to each tube. D-glucose standard solution (0.1 mL; 1 mg D-glucose/mL) and DI water (0.1 mL) were included as standard and blank respectively. The tubes were incubated for 30 min (50°C). The absorbance for each sample and the standard was read at 510 nm against the blank. The % Starch was calculated using the following formula:

$$\text{Starch (\%)} = \Delta_A * F * \frac{FV}{0.1} * \frac{1}{1000} * \frac{100}{W} * \frac{162}{180}$$

$$= \Delta_A * \frac{F}{W} * FV * 0.9$$

where Δ_A is the absorbance against the blank, F is the conversion from absorbance to µg, FV is 100 mL, and W is the weight in mg of the flour analyzed.

Color Analysis of the Flours

Color analysis of 12 sample flours was performed using a CR-300 instrument. The "L*, a*, and b*" Hunter Lab system was used to determine the color difference of the flours. The total color difference (ΔE*) was calculated using the following equation (Calvo 2004):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Where $0 < \Delta E^* < 0.5$ is classified as "not noticeable," $0.5 < \Delta E^* < 1.5$ as "slightly noticeable," $1.5 < \Delta E^* < 3.0$ as "noticeable," $3.0 < \Delta E^* < 6.0$ as "well visible," and $6.0 < \Delta E^* < 12.0$ as "great." The NGRRF sample was used as a comparison for the different RR flour (RRF) sample colors and the NGGGF was used as a comparison for the different GG flour (GGF) colors.

Water Activity of the Flours

A dew point water activity meter (AquaLab) was used to determined water activity (a_w). The 12 sample flours were placed into a disposable sample cup before being placed in the instrument. The a_w was automatically measured and recorded.

Microbiological Evaluation

The total plate count (TPC) was evaluated for the sprout and flour samples. Tryptic soy agar (TSA) was used for TPC. The sample was dispersed and diluted in a serial dilution using a saline solution (0.85%) to 10^{-7} for the sprout samples and 10^{-3} for the flour samples before being spread-plated onto TSA plates and incubated for 48 hrs (35°C). The colonies were counted and recorded as colony forming units (CFU) per g.

Lipoxygenase and Trypsin Inhibitor Activity of the Flours

The method described by Zhu et al. (1996) with modifications was used to determine lipoxygenase activity. A linoleic acid stock solution prepared using linoleic acid (140 mg), Tween 20 (140 mg), and DI water (8 mL) was clarified with NaOH (0.55 mL, 1.0N) and diluted to 50 mL using DI water. Then the solution was diluted 1:40 with sodium borate buffer (0.2 M, pH 9.0) for the lipoxygenase-1 activity and with sodium phosphate buffer (0.2 M, pH 6.5) for lipoxygenase-3 activity determination. Dispersions containing sodium phosphate buffer (50 mL) and flour (1.0 g) were stirred and incubated for 2 hr (25°C). Then, the dispersions were centrifuged at 15,000 *g* for 30 min (20°C; Model J2-21, Beckman). The mixture of the supernatant (50 and 10 μL for lipoxygenase-1 and -3 activity determination, respectively) and substrate (2.5 mL) after 5 min incubation was transferred into a cuvette for absorbance reading using a UV-1601 spectrophotometer (Shimadzu) at ambient temperature and at the wavelength of 234 nm and 280 nm for lipoxygenase-1 and -3 activity determination, respectively. The NGRR and NGGG controls were set as 100%. The lipoxygenase-1 and -3 activities were calculated using the following formula:

$$\text{Lipoxygenase activity (\%)} = \frac{\text{absorbance sample}}{\text{absorbance control}} * 100$$

Using a method described by AACC (1990) with modifications, 60-mesh flour (1 g) was added to NaOH (50 mL, 0.01 N, pH 8.4) and stirred for 3 hr. The sample dispersion (1.4 mL) was diluted to 2 mL with DI water. Trypsin solution (4 mg, Porcine pancreas, Sigma, in 200 mL 0.001 M HCl) (2 mL) were added into the sample solution and placed in a water bath at 37°C. To start the reaction, 5 mL of BAPA (Na-benzoyl-DL-arginine 4-nitroanilide hydrochloride) solution (40 mg BAPA in 100 mL 0.05 M Tris buffer containing CaCl2, pH 8.2) was added. The reaction was stopped after 10 min by adding acetic acid solution (1 mL, 30% v/v), and the absorbance was

measured at 410 nm using the spectrophotometer at ambient temperature. The NGRRF and NGGGF controls were set as 100%. The trypsin inhibitor activity was calculated using the following equation:

$$\text{trypsin inhibitor activity (\%)} = \frac{\text{absorbance sample}}{\text{absorbance control}} * 100$$

***In vitro* Glycemic Index of the Flours**

The protocol described by Goni et al. (1997) was used to determine the *in vitro* Glycemic Index (GI). Flour samples (50 mg) in KCl-HCl buffer (10 mL, pH 1.5) were added with pepsin solution (0.2 mL; 0.1 g pepsin from porcine gastric mucosa per mL KCl-HCl buffer) and incubated in a warm water bath (40°C) for 1 hr. for protein digestion, and then diluted to 25 mL with Tris-Maleate buffer (pH 6.9). Then, α-amylase (5 mL; from *Aspergillus oryzae* in Tris-Maleate buffer containing 2.6 UI) was added and incubated in a water bath (37°C). Every 30 min up to 3 hr., an aliquot (1 mL) was taken and placed in a warm water bath (100°C) for 10 min. Then, sodium acetate buffer (3 mL, 0.4 M, pH 4.75) and amyloglucosidase (*Aspergillus niger*, 60 µL) were added and diluted to 5 mL with DI water. The samples were centrifuged at 20,000 g for 5 min, and the glucose content of the supernatants was determined using a glucose assay kit (Sigma) with the spectrophotometer at 540 nm. Using 0.9 as the conversion factor from glucose to starch, the starch digestion rate was calculated as the percentage of starch hydrolyzed at different times. The area under the hydrolysis curve was determined. The hydrolysis index (HI) was calculated as a relation between the area under the sample curve and the area under the reference curve (white bread). GI was calculated as:

$$GI = 0.862 * HI + 8.198.$$

Objective 3: Prepared snack chips from germinated and non-germinated rough rice and green gram and determine physical characteristics, *in vitro* Glycemic Index, sensory properties and shelf life study.

Preparation of Snack Chips

The germinating time of the RR and the GG from objective 1, and the moisture, protein, lipids, and starch content, the trypsin inhibitor and lipoxygenase-1 and lipoxygenase-3 activity, and GI from objective 2 were analyzed to determine the optimal germinating conditions of RR and GG for preparing the snack chips. Based on the results above, the 5-day GRRF and 5-day GGGF were considered as the optimized germinating time and picked to prepare the sample snack chips (SSC).

The experimental designs for the SSC were confined to using the 5-day GRRF and 5-day GGGF at a 1:1 ratio of water in respect to flour content. Water (40% based on the total flour), baking soda (1.2% based on the total flour), and salt (1% based on the total flour) was added to the flour mixture to form a dough, which was formed by kneading, pressing and stretching until well mixed and passed through a pasta maker until ~ 1 mm. The flattened dough was cut into 2x2 cm chips and baked in an oven at 149 ºC for 8 mins. The above process was repeated for the NGRRF and NGGGF, which acted as the control snack chips (CSC).

Color Analysis of the Snack Chips

Color analysis of the SSC was performed using a CR-300 instrument and the "L*, a*, and b*" Hunter Lab system as described in the objective 2 above, where the CSC were used as a comparison for the SSC.

In vitro Glycemic Index of the Snack Chips

As described in objective 2 above, the protocol described by Goni et al. (1997) was used to determine *in vitro* glycemic index of the SSC and CSC.

Texture Analysis to Determine the Fracturability of the Sample Snack Chips

Fracturability, a way in which consumers perceive the crunchiness of SSC and CSC, was determined using a TA/XT2 Texture Analyzer equipped with a crisp fracture base and TA-8 ¼" ball point and the following parameters were used: pre-test speed = 2.00 mm/sec, test speed = 1.00 mm/sec, post-test speed = 5.00 mm/sec, and distance = 5.0 mm. A graph with the maximum peak, which is equal to the fracturability (g) of the SSC and CSC, was computed by the instrument, and data from these graphs was extrapolated to give the maximum fracturability (g).

Evaluation of the Acceptability of the Sample Snack Chips Using Sensory Analysis

An approval form from the Institutional Review Board (IRB) was obtained before the sensory test was conducted. The SSC and the CSC were evaluated for sensory study using 74 voluntary panelists, male (21) and female (53).

The participants received a paper ballots accompanied with all sample plates to express their evaluation on samples' sensory attributes. Impression of appearance, aroma, hardness, cohesiveness, flavor, mouthfeel, aftertaste, and overall acceptability were measured on 9-point hedonic scale for each attribute. In addition, participants were asked to indicated their impressions of color, crispiness, and size on a 5-point "Just-About-Right" (JAR) scale. Also, participants were asked to indicate what they liked and disliked about the product from the following: appearance, surface color, color brightness, hardness (by touching), crispiness (by tasting), rice flavor, green gram flavor, mouthfeel, just-about-right of hardness intensity (by touching), just-about-right of crispiness intensity (by touching), sweet taste, sour taste, salty taste, just-about-right of taste

intensity (by tasting), bitter taste, balanced, crunchiness (by tasting), cohesiveness (by tasting), chewiness (by tasting), hardness (by tasting), size, and aroma. Between each sample, panelists took a short 30 sec break for palate cleansing with spring water and unsalted crackers.

Shelf-life Stability Study

The SSC and CSC were placed in a plastic bag and were stored at ambient temp within the lab. The SSC and CSC were tested for color and water activity at monthly intervals up to 4 months to determine their shelf life.

Statistical Analysis

Statistical analysis of the protein, moisture, and lipids content, water activity, lipoxygenases inhibitor activity, trypsin inhibitor activity, color, textural properties, and shelf-life study was performed using a one-way ANOVA utilizing JMP (JMP 13 Pro 2016). Statistical analysis of the sensory evaluation was performed using a two-way ANOVA utilizing JMP (JMP 13 Pro 2016). The values represented the means ± the standard deviation (SD) of each sample in triplicate. When a significant difference ($P < 0.05$) occurred, Student *t*-test was performed to compare the means and differences considered significantly different ($P < 0.05$).

Results and Discussions

Germinated Sprout Lengths

Germinated Rough Rice (GRR) Sprout Lengths

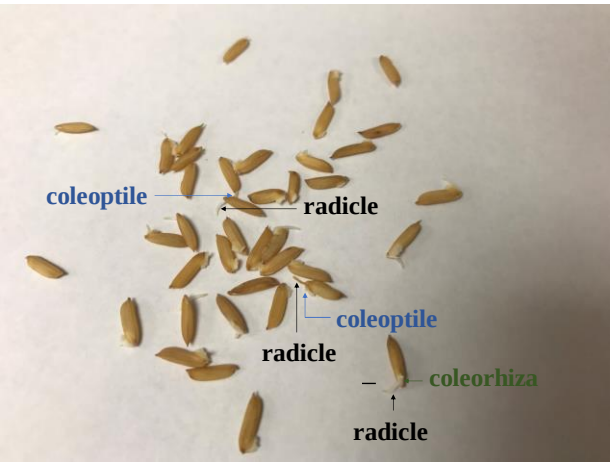

Figure 1: 1-day germinated rough rice (GRR).

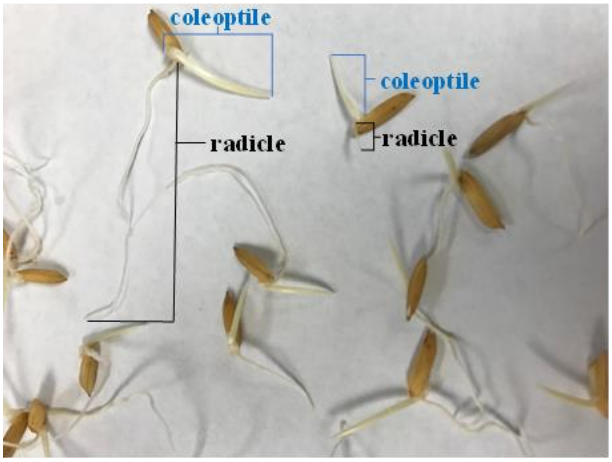

Figure 2: 3-Day germinated rough rice (GRR).

After the 24-hour soaking period and after 24 hours of germination, many of the RR grains showed the emergence of the coleorhiza from the seed coat or hull. The radicle was the first to emerge from the coleorhiza (Moldenhauer and Slaton, 2001). By three days of germination, the radicles showed a large variance in development (Fig. 2). Also, at 3 days of germinating, many of the radicles of RR grains had embedded themselves into the paper towels and emerged as a twisted tangle on the underside of the paper towels.

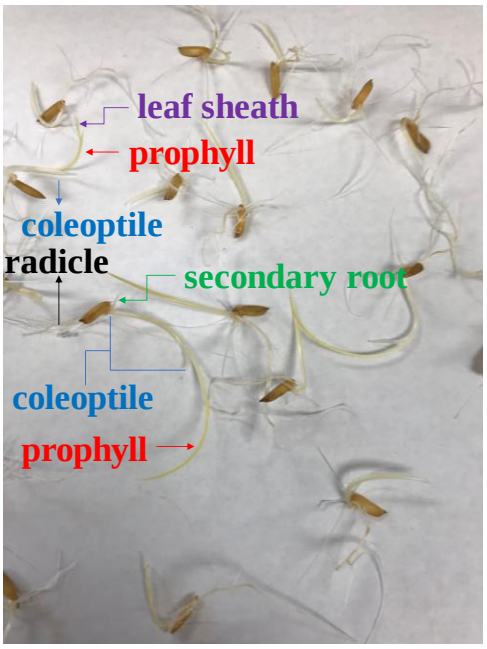

Figure 3: 5-day germinated rough rice (GRR).

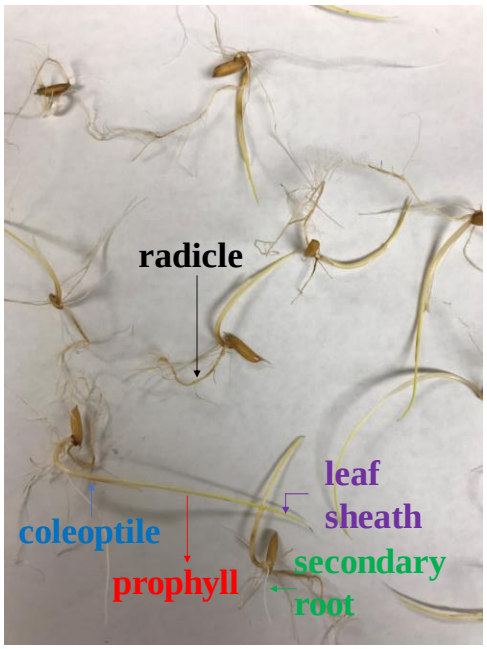

Figure 4: 7-Day germinated rough rice (GRR).

Between day 3 and day 5 of germination, lateral roots had formed off the radicles, and the coleoptile had undergone several changes: a formation of a secondary root from the base of the coleoptile, where nodal roots would eventually form; the emergence of the prophyll from the coleoptile; and in some germinating rice grains, a leaf sheath had emerged from the prophyll (Moldenhauer and Slaton, 2001). Between 5 days and 7 days of germination, the radicles and coleoptiles, underwent a significant amount of decay, which resulted in an overall decline in the rough rice sprouts (Fig. 3 and 4). So, in terms of the length of germination, the amount of healthy 5-day germinated RR (GRR) sprouts were significantly more than the amount of healthy 7-day GRR sprouts.

Table 1: The Radicle and Coleoptile Growth (length in mm) During Seven Days of Rough Rice (RR) Germination

Germination Days	Radicle (mm)	Coleoptile (mm)
1*	2.7 ± 1.8[1]f	0.2 ± 0.5[1]f
2*	17.2 ± 9.2[2]e	6.4 ± 3.1[2]e
3*	31.5 ± 10.3[2]d	15.6 ± 5.2[2]d
4*	45.2 ± 7.0[3]c	21.5 ± 6.4[3]c
5*	55.3 ± 14.7[3]b	32.6 ± 9.6[3]b
6*	75.2 ± 24.4[4]a	48.3 ± 11.6[4]a
7*	73.8 ± 28.4[4]a	52.6 ± 15.1[4]a
P-value	< 0.0001	< 0.0001

*Rough rice (RR) underwent soaking (water bath (34 ºC), 24 hr) before being germinated.
[1]Values are mean ± SD of 55 samples from 7 batches.
[2]Values are mean ± SD of 35 samples from 4 batches.
[3]Values are mean ± S of 30 samples from 3 batches.
[4]Values are mean ± SD of 20 samples from 2 batches.
Mean values followed by different letters in the same column are significantly different ($P < 0.05$).

The coleoptile and radicle growth during RR's first seven days of germination is shown, and the results are compared to the control or NGRR (Table 1). The length of the rice grains' radicle over the seven days of germination increased and had an overall significant difference ($P < 0.0001$), except for 6-day and 7-day germination ($P > 0.05$). The variance between the length of the radicles also increased with the exception of day 4. This may have been due to some of the radicles being damaged when the paper towels were changed. The length of the rice grains' coleoptile showed a significant difference during the first 5 days of germination. The length of the 6-day and 7-day coleoptiles were not significantly different ($P > 0.05$), though their lengths were significantly different ($P < 0.05$) from the length of the 1-day, 2-day, 3-day, 4-day, and 5-day coleoptiles.

Germinated Green Gram (GGG) Sprout Lengths

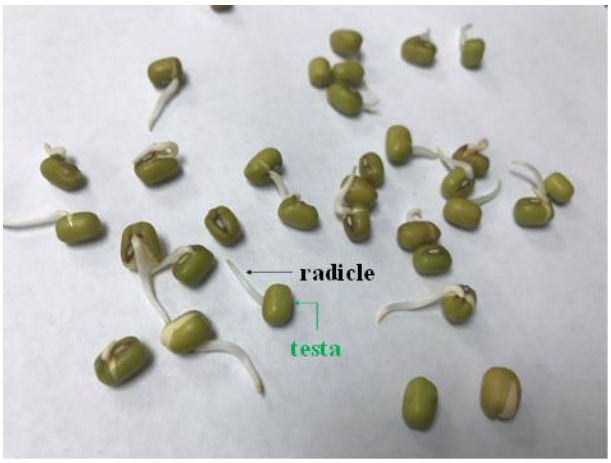

Figure 5: 1-day germinated green gram (GGG).

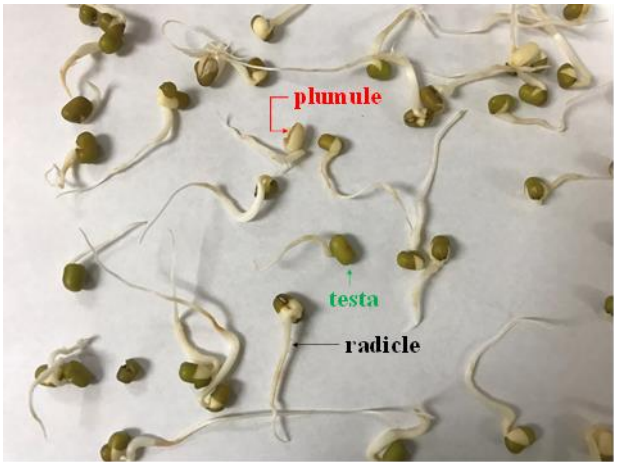

Figure 6: 3-Day germinated green gram (GGG).

After the 2-hour soaking period and after 24 hours of germinating in the water bath, several of the GG seed hulls or testas had split and showed the emergence of the radicle (Fig.5). By day three of germination, some GG seeds showed emergence of the plumule (Fig. 6).

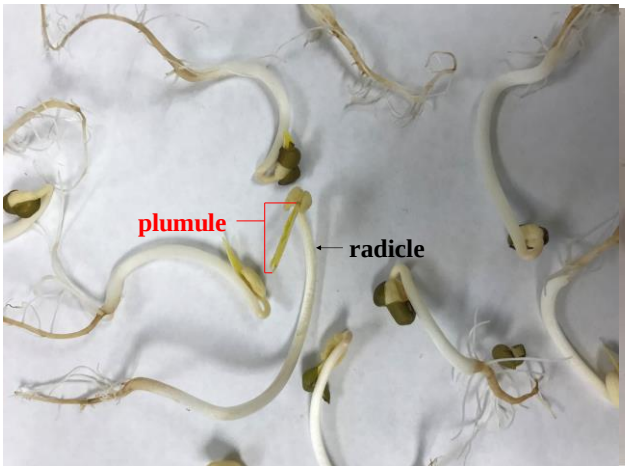

Figure 7: 5-day germinated green gram (GGG).

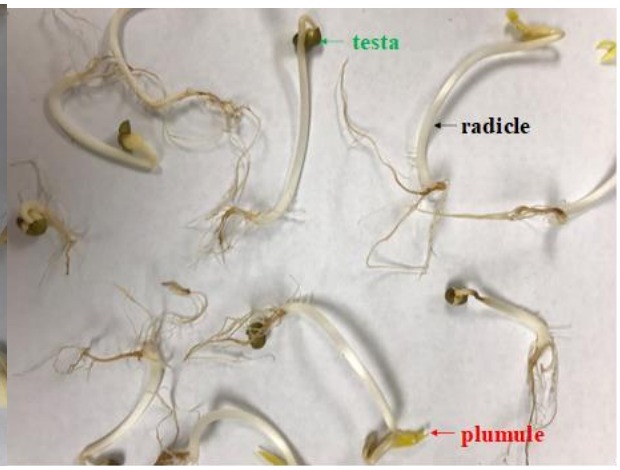

Figure 8: 7-Day germinated green gram (GGG).

By 5 days of germination, the plumule had emerged from the testa of several seeds, and the radicles had several root hairs on them (Fig.7). By day 7, some sprouts still contained the testa and did not show the plumule. Some of the radicles showed signs of decay (Fig. 8). Between 5

days and 7 days of germination, the radicles and coleoptiles, underwent a significant amount of decay, which resulted in an overall decline in the green gram (GG) sprouts (Fig. 3 and 4). So, in terms of the length of germination, the amount of healthy 5-day germinated GG (GRR) sprouts were significantly more than the amount of healthy 7-day GRR sprouts.

Table 2: The Radicle and Plumule Growth (length in mm) During Seven Days of Green Gram (GG) Germination

Germination Days	Radicle (mm)	Plumule (mm)
1*	10.7 ± 5.4[1]g	0.0 ± 0.0[1]d
2*	38.1 ± 14.2[2]f	0.0 ± 0.0[2]d
3*	78.9 ± 27.1[2]e	0.0 ± 0.0[2]d
4*	99.4 ± 36.8[3]d	3.0 ± 3.0[5]c
5*	141.6 ± 51.5[3]c	5.3 ± 5.1[5]bc
6*	173.3 ± 49.9[4]b	7.9 ± 5.3[5]ab
7*	206.2 ± 55.0[4]a	8.9 ± 4.0[5]a
P-value	< 0.0001	< 0.0001

*Green gram underwent soaking (water bath (34 ºC), 2 hr) before being germinated.
[1]Values are mean ± SD of 50 samples from 5 batches.
[2]Values are mean ± SD of 40 samples from 4 batches.
[3]Values are mean ± SD of 30 samples from 3 batches.
[4]Values are mean ± SD of 20 samples 2 batches.
[5]Values are mean ± SD of 10 samples 1 batch.
Mean values followed by different letters in the same column are significantly different ($P < 0.05$).

The GG's radicle and plumule growth during the first seven days of germination is shown; all results are compared to the control or NGG (Table 2). The length of the GG seeds' radicle showed a significant difference throughout the seven-day germination period. While the length of the GG seeds' plumule showed an overall significant difference ($P < 0.0001$), the 1-day, 2-day, and 3-day plumules were not significantly different ($P > 0.05$). The 5-day plumules were not significantly different ($P > 0.05$) from the 4-day and 6-day, although the 5-day plumules were significantly different ($P < 0.05$) from the 1-day, 2-day, 3-day, and 7-day plumules, and the 4-day plumules were significantly different ($P < 0.05$) than the 6-day plumules. The 6-day and 7-day plumules showed no significant difference ($P > 0.05$).

Proximate Nutrient Composition of the Flours

Proximate Nutrient Composition of the Rough Rice Flours

Table 3: Proximate Nutrient Composition (on dry weight basis) of Non-Germinated (NGRRF), Soaked (SRRF), and Germinated Rough Rice Flours (GRRF)

Germination	Protein (g/100g)	Lipids (g/100g)	Starch (g/100g)	Moisture (g/100g)	Water Activity
0-day (NGRRF)[1]	10.2 ± 0.3c	0.77 ± 0.20c	26.1 ± 0.9a	12.2 ± 0.2a	0.51 ± 0.01a
0-day (SRRF)[2]	9.6 ± 0.0e	1.09 ± 0.10c	25.9 ± 0.1a	8.4 ± 0.1c	0.34 ± 0.02e
1-day[3]	9.8 ± 0.1de	1.10 ± 0.17c	25.2 ± 0.1a	8.4 ± 0.2c	0.39 ± 0.01d
3-day[3]	10.1 ± 0.2cd	2.00 ± 0.43b	24.5 ± 1.5a	9.1 ± 0.1b	0.46 ± 0.01b
5-day[3]	10.8 ± 0.2b	2.30 ± 0.09b	22.6 ± 1.4b	7.7 ± 0.1d	0.41 ± 0.01c
7-day[3]	11.6 ± 0.0a	2.73 ± 0.20a	21.2 ± 0.6b	7.3 ± 0.1e	0.45 ± 0.00b
P-value	< 0.0001	< 0.0001	0.0002	< 0.0001	< 0.0001

[1]NGRRF = control non-germinated rough rice without soaking before being processed into flour.
[2]SRRF = control non-germinated rough rice underwent soaking (water bath (34 °C), 24 hr.) before being processed into flour.
[3]Rough rice underwent soaking (water bath (34 °C), 24 hr) before being germinated and processed into flour (GRRF).
Values are mean ± SD of triplicate analysis.
Mean values followed by different letters in the same column are significantly different ($P < 0.05$).

There is a significant difference ($P < 0.05$) in the proximate nutrient composition of GRRF, protein (%, $P < 0.0001$), lipids (%, $P < 0.0001$), and starch (%, $P = 0.0002$) along with moisture (%, $P < 0.0001$) and water activity ($P < 0.0001$); all results are compared to the NGRRF (Table 3). The protein content of RR slightly decreased during the soaking period, when the RR grains were undergoing changes in preparation for germinating, and then, increased until day 3 of germination, where the protein content was about the same as the NGRRF. There was no significant difference ($P > 0.05$) between the 0-day SRRF and the 1-day GRRF or between the NGRRF and the 3-day GRRF; however, the NGRRF and 3-day GRRF were significantly different ($P < 0.05$) than the SRRF and 1-day GRRF. By day 5 and day 7, the protein content (%) had increased to 10.8% and 11.6% respectively or approximately 0.6% and 14% respectively more than the NGRRF; both 5-day and 7-day GRRF were significantly different ($P < 0.05$) from the NGRRF as well as from each

other. The increase in protein content may be due to microbial endophytes, which have a symbiotic relationship with RR seeds and their emerging radicles and coleoptiles and may have influenced the growth development in their hosts through fixation of N_2 (Hardoim *et al*., 2012). Therefore, the 7-day GRRF had the most protein followed by the 5-day GRRF.

Although there was an overall significant difference ($P< 0.0001$) in the lipids content (%) over the seven-day germination period of RR, there was no significant difference ($P > 0.05$) between the NGRRF, SRRF, and 1-day GRRF or between the 3-day GRRF and 5-day GRRF. The lipids content (%) in the RRF increased over time starting with the SRRF. This increase in lipids could be due to the synthesis of structural lipids occurring during germination (Ching, 1972). So, the 7-day GRRF (2.73%) contained the most lipids (%) followed by the 5-day GRRF (2.3%).

There starch content (%) of the RRF showed an overall significant difference ($P = 0.0002$), but there was no significant difference ($P > 0.05$) between NGRRF, SRRF, 1-day GRRF, and 3-day GRRF or between the 5-day GRRF and 7-day GRRF. The starch content decreased starting with the SRRF, and by 5-day GRRF and 7-day GRRF, the starch content in the GRRF had decreased approximately 13% and 19% respectively versus the control. The decrease in the starch content could be due to the starch being hydrolyzed into free sugar, which could then be used as fuel for other metabolic functions. Therefore, the 7-day GRRF (21.2%) had the least amount of starch (%) followed by the 5-day GRRF (22.6%).

Although the overall moisture content (%) was significantly different ($P < 0.0001$), there was no significant difference ($P > 0.05$) between the SRRF and the 1-day GRRF. The moisture content (%) in the SRRF and GRRF were lower that the moisture (%) in the NGRRF, although the moisture content of the 3-day GRRF (9.1%) was greater and was significantly different ($P < 0.05$) than the moisture content of the SRRF (8.4%) or the 1-day GRRF (8.4%), but still much lower and

significantly different ($P < 0.05$) than the NGRRF. The moisture content of the 5-day GRRF (7.7%) and 7-day GRRF (7.3%) decreased by approximately 37% and 40% respectively and were significantly different ($P < 0.05$) compared to the NGRRF. So, the lowest percentage of moisture content was the 7-day GRRF (7.3%) followed by the 5- day GRRF (7.7%).

The water activity of the GRRF had an overall significant difference ($P < 0.0001$), although the water in the 3-day and 7-day GRRF showed no significant difference ($P > 0.05$). The water activity of the RRF decreased for the SRRF, 1, 3, 5, and 7-day GRRF and was significantly different ($P < 0.05$) when compared to the NGRRF (0.51). The water activity of the SRRF (0.34) was the lowest followed by 1-day GRRF (0.39), 5-day GRRF (0.41), 7-day GRRF (0.45), and 3-day GRRF (0.46). The lower water activity relates to a higher amount of water being bound.

Proximate Nutrient Composition of the Green Gram Flours

Table 4: Proximate Nutrient Composition (on dry weight basis) of Non-Germinated (NGGGF), Soaked (SGGF), and Germinated Green Gram Flours (GGGF)

Germination	Protein (g/100g)	Lipids (g/100g)	Starch (g/100g)	Moisture (g/100g)	Water Activity
0-day (NGGGF)[1]	27.6 ± 0.2d	0.84 ± 0.23d	52.4 ± 1.1a	10.4 ± 0.2d	0.51 ± 0.00a
0-day (SGGF)[2]	28.9 ± 0.2cd	0.94 ± 0.07d	50.7 ± 1.5a	8.6 ± 0.1e	0.42 ± 0.01e
1-day[3]	29.3 ± 0.3cd	1.13 ± 0.17d	47.9 ± 1.6b	8.9 ± 0.1e	0.48 ± 0.00c
3-day[3]	32.7 ± 0.5bc	2.36 ± 0.10c	44.8 ± 0.7c	11.1 ± 0.0c	0.44 ± 0.00d
5-day[3]	39.2 ± 0.1b	2.90 ± 0.19b	40.0 ± 1.1d	14.1 ± 0.2b	0.50 ± 0.00b
7-day[3]	44.3 ± 0.3a	5.68 ± 0.15a	35.7 ± 0.7e	12.2 ± 0.2a	0.45 ± 0.00d
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[1]NGGGF = control non-germinated green gram without soaking before being processed into flour.
[2]SGGF = control non-germinated green gram underwent soaking (water bath (34 °C), 2 hr.) before being processed into flour.
[3]Green gram underwent soaking (water bath (34 ºC), 2 hr.) before being germinated and processed into flour (GGGF).
Values are mean ± SD of triplicate analysis.
Mean values followed by different letters in the same column are significantly different ($P < 0.05$).

The proximate nutrient composition of GGF, protein ($P < 0.0001$), lipids ($P < 0.0001$), and starch ($P < 0.0001$) along with moisture ($P < 0.0001$) and water activity ($P < 0.0001$) had an overall

significant difference ($P < 0.05$); all results are compared to the NGGGF (Table 4). There was no significant difference ($P > 0.05$) between the protein content of the NGGGF, SGGF, and 1-day GGGF, between the SGGF, 1-day GGGF, and 3-day GGGF, or between the 3-day GGGF and 5-day GGGF. The protein content of the GGF increased over time starting with the SGGF (28.9%) and showed approximately a 61% increase in protein content by the 7-day GGGF (44.3%). This increase in protein during the duration of the sprouting period could be due to N-fixing rhizobia bacteria, which hold a symbiotic relationship with the green gram seeds and sprouts, produces NH_3 for the sprouts, which the sprouts use to manufacture protein and other nitrogen-containing components, and takes photosynthesis-derived sugars and other nutritional factors from the sprouts (Glover and Lindemann, 2015). So, the 7-day GGGF (44.3%) contained the most protein followed by the 5-day GGGF (39.2%).

Although the lipids content had an overall significant difference ($P < 0.0001$), between the NGGGF, SGGF, and 1-day GGGF, there was no significant difference ($P > 0.05$). The lipids content in the GGGF increased over time starting with the 0-day soaked GGGF (0.94%) and showed an increase to 5.68%. As with the GRRF, the increase of lipids could be due to the increase of structural lipids during germination (Ching, 1972). Therefore, the 7-day GGGF (5.68%) had the highest amount of lipids content (%) followed by the 5-day GGGF (2.9%).

The starch content of the GGGF had an overall significant difference ($P < 0.0001$), but between the NGGGF and the 0-day SGGF, there was no significant difference ($P > 0.05$). The starch content decreased throughout the 7-day germination process, possibly as the radicles and plumules converted the starch into energy, and the starch content in the 5-day and 7-day GGGF (40.0% and 35.7% respectively), which was also significantly different ($P < 0.05$), showed a decrease of approximately 24% and 32% respectively versus the control. As with the GRRF, the

decrease in the starch content could be due to the starch being hydrolyzed into free sugar, which could then be used as fuel for other metabolic functions. So, the starch (%) was lowest in the 7-day GGGF (35.7%) followed by the 5-day GGGF (40.0%).

The moisture content (%) showed an overall significant difference ($P < 0.0001$), although there was no significant difference ($P > 0.05$) between the SGGF and the 1-day GGGF. The moisture content was the lowest in the SGGF (8.6%) followed by 1-day GGGF (8.9%), the NGGGF (10.4%), 3-day GGGF (11.1%), 7-day GGGF (12.2%), and 5-day GGGF (14.1%). The moisture content of the 5-day and 7-day green gram flour was significantly different ($P < 0.05$) and increased by approximately 36% and 17% respectively. While the moisture (%) was lowest in the SGGF, the 7-day GGGF (12.2%) was lower than the 5-day GGGF (14.1%).

Even though the water activity had an overall significant difference ($P < 00001$) in the GGF, the 3-day GGGF was not significantly different ($P > 0.05$) than the 7-day GGGF. The water activity of the GGGF were all lower than the NGGGF (0.51), with the lowest being the SGGF (0.42) followed by the 3-day GGGF (0.44), the 7-day GGGF (0.45), 1-day GGGF (0.48), and 5-day GGGF (0.50).

Color Analysis of the Flours

Color Analysis of the Rough Rice Flours

Table 5: Color Analysis of Non-Germinated (NGRRF), Soaked (SRRF), and Germinated Rough Rice Flours (GRRF)

Germination	L*	a* (+/-)	b* (+/-)	ΔE*	Difference in appearance
0-day (NGRRF)[1]	88.47 ± 0.23c	0.45 ± 0.05a	7.96 ± 0.12d	0.00 ± 0.00	control
0-day (SRRF)[2]	89.27 ± 0.19b	0.41 ± 0.03ab	8.05 ± 0.10d	0.81 ± 0.41d	slightly noticeable[3]
1-day[3]	90.18 ± 0.31a	0.36 ± 0.05ab	7.77 ± 0.21d	1.73 ± 0.55c	noticeable[4]
3-day[3]	88.55 ± 0.28c	0.32 ± 0.05b	9.15 ± 0.08c	1.20 ± 0.07cd	slightly noticeable[3]
5-day[3]	86.20 ± 0.33d	0.02 ± 0.02c	11.99 ± 0.45b	4.65 ± 0.46b	well visible[5]
7-day[3]	83.73 ± 0.14e	0.34 ± 0.13ab	13.32 ± 0.27a	7.16 ± 0.23a	great[6]
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	

[1]NGRRF = control non-germinated rough rice flour without soaking.
[2]SRRF = control non-germinated rough rice flour with soaking (water bath (34 °C), 24 hr.).
[3]Rough rice underwent soaking (water bath (34 °C), 24 hr.) before being germinated and processed into flour (GRRF).
"not noticeable" = $0 < \Delta E < 0.5$; [3]slightly noticeable = $0.5 < \Delta E < 1.5$; [4]noticeable = $1.5 < \Delta E < 3$; [5]well visible = $3 < \Delta E < 6$; [6]great = $6 < \Delta E < 12$; "more than great" = $12 < \Delta E < 24$. ΔE* was calculated using chips from NGRRF as reference.
Values are mean ± SD of triplicate analysis.
Mean values followed by different letters in the same column are significantly different ($P < 0.05$).

The color difference in the SRRF and different GRRF compared to the NGRRF increased in noticeability and was overall significantly different ($P < 0.0001$) over the germination period starting with "slightly noticeable" ($0.5 < \Delta E < 1.5$) in the 0-day soaked RRF to "great" ($6 < \Delta E < 12$) in the 7-day GRRF with the exception of the 3-day GRRF, which was only "slightly noticeable" compared to the NGRRF. The SRRF and the 3-day GRRF showed "slightly noticeable" appearance and was not significantly different ($P > 0.05$). The 1-day GRRF had a "noticeable" ($1.5 < \Delta E < 3$) appearance while the 3-day GRRF had a "slightly noticeable" appearance, but the 1-day GRRF was not significantly different ($P > 0.05$) from the 3-day GRRF. The color difference between the flours could be due to the reactions taking place during

germination. The 7-day GRRF ("great," $6 < \Delta E < 12$) had the overall greatest difference in color compared to the NGRRF followed by the 5-day GRRF ("well visible," $3 < \Delta E < 6$).

For L*, where ΔL = difference in lightness and darkness (+ = lighter, - = darker), the overall RRF was significantly different ($P < 0.0001$). The 3-day GRRF (88.55) was slightly lighter but not significantly different ($P > 0.05$) than the NGRRF (88.47). The SRRF (89.27) and the 1-day (90.18) were lighter and significantly different ($P < 0.05$) than the NGRRF, while the 5-day GRRF (86.20) and the 7-day GRRF (83.73) were darker and significantly different ($P < 0.05$) than the NGRRF. The color difference between the flours could be due to the reactions taking place during germination, which caused a reduction in lightness compounds or an increase in darkness compounds.

For a*, where Δa = difference in red and green (+ = redder, - = greener), the overall different appearance of the RRF was significantly different ($P < 0.001$). Even though the 7-day GRRF (0.34) was 0.11 less or greener in appearance than the NGRRF (0.45), the 7-day GRRF was not significantly different ($P > 0.05$) than the NGRRF nor was the SRRF (0.41) or the 1-day GRRF (0.36) significantly different than the NGRRF. The SRRF, 1-day GRRF, 3-day GRRF (0.32), and 7-day GRRF were not significantly different. The 5-day GRRF (0.02) was greener and significantly different ($P < 0.05$) than the NGRRF. The color difference between the flours could be due to the reactions taking place during germination, which caused a reduction in red compounds or an increase in green compounds.

For b*, where Δb = difference in yellow and blue (+ = yellower, - = bluer), the overall difference in appearance of the RRF was significantly different ($P < 0.0001$). While the SRRF (8.05) was slightly yellower than the NGRRF (7.96) and the 1-day GRRF was slightly bluer than the NGRRF, neither were significantly different ($P > 0.05$). The 3-day GRRF (9.15), the 5-day

GRRF (11.99), and 7-day GRRF (13.32) were yellower and significantly different ($P < 0.05$) than the NGRRF. The color difference between the flours could be due to the reactions taking place during germination, which caused an increase in yellow compounds or a reduction in blue compounds.

Color Analysis of the Green Gram Flours

Table 6: Color Analysis of Non-Germinated (NGGGF), Soaked (SGGF), and Germinated Green Gram Flours (GGGF)

Germination	L*	a* (+/-)	b* (+/-)	ΔE*	Difference in appearance
0-day (NGGGF)[1]	88.21 ± 0.24a	-1.96 ± 0.01d	12.32 ± 0.21c	0.00 ± 0.00	control
0-day (SGGF)[2]	86.97 ± 0.02b	-1.88 ± 0.05d	11.91 ± 0.25d	1.30 ± 0.30e	slightly noticeable[3]
1-day[3]	88.45 ± 0.13a	-1.29 ± 0.04c	10.39 ± 0.11e	2.06 ± 0.24d	noticeable[4]
3-day[3]	81.62 ± 0.38c	-0.19 ± 0.04b	13.32 ± 0.05b	6.90 ± 0.30c	Great[5]
5-day[3]	74.47 ± 0.41e	1.27 ± 0.06a	15.17 ± 0.26a	14.40 ± 0.19a	more than great[6]
7-day[3]	75.91 ± 0.34d	1.27 ± 0.07a	13.50 ± 0.17b	12.77 ± 0.13b	more than great[6]
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	

[1]NGGGF = control non-germinated green gram flour without soaking.
[2]SGGF = control non-germinated green gram flour with soaking in water bath (34 °C) for 2 hr.
[3]Green gram underwent soaking (water bath (34 °C), 24 hr) before being germinated and processed into flour (GGGF).
not noticeable = $0 < \Delta E < 0.5$; [3]slightly noticeable = $0.5 < \Delta E < 1.5$; [4]noticeable = $1.5 < \Delta E < 3$; [5]well visible = $3 < \Delta E < 6$; [6]great = $6 < \Delta E < 12$; more than great = $12 < \Delta E < 24$. ΔE* was calculated using NGRRF as reference.
Values are mean ± SD of triplicate analysis.
Mean values followed by different letters in the same column are significantly different ($P < 0.05$).

The color difference in the different GGGF compared to the NGGGF increased in noticeability over the germination period starting with "slightly noticeable" ($0.5<\Delta E<1.5$) in the SGGF to "more than great" ($12 < \Delta E < 24$) in both the 5-day GGGF and 7-day GGGF and were significantly different ($P < 0.0001$. As with the GRRF, the color difference between the flours could be due to the reactions taking place after cell rupture.

For L*, where ΔL = difference in lightness and darkness (+ = lighter, - = darker), the overall GGF was significantly different ($P < 0.0001$). The 1-day GGGF (88.45) was slightly lighter but not significantly different ($P > 0.05$) than the NGGGF (88.21). The SGGF (86.97), the 3-day GGGF (81.62), 5-day GGGF (74.47) and the 7-day GGGF were darker and significantly different ($P < 0.05$) than the NGGGF. The color difference between the flours could be due to the reactions taking place during germination, which caused a reduction in lightness compounds or an increase in darkness compounds.

For a*, where Δa = difference in red and green (+ = redder, - = greener), the overall different appearance of the GGF was significantly different ($P < 0.001$). The 5-day GGGF (1.27) and the 7-day GGGF (1.27) were significantly different and redder in appearance than the NGGGF (0.45), the 5-day GGGF was not significantly different ($P > 0.05$) than the 7-day GGGF nor was the NGGGF (-1.96) significantly different ($P < 0.05$) than the SRRF (-1.88), although the NGGGF was slightly greener than the SGGF. The color difference between the flours could be due to the reactions taking place during germination, which caused a reduction in green compounds or an increase in red compounds. The color difference between the flours could be due to the reactions taking place during germination, which caused an increase in red compounds or a reduction in green compounds.

For b*, where Δb = difference in yellow and blue (+ = yellower, - = bluer), the overall difference in appearance of the GGF was significantly different ($P < 0.0001$), although the 3-day GGGF (13.32) was not significantly different than the 7-day GGGF (13.50). While the SGGF (11.91) and the 1-day GGGF (10.39) was significantly different ($P < 0.05$) less yellow than the NGGGF (12.32), the 3-day GGGF, 5-day GGGF (15.17), and the 7-day GGGF were significantly different ($P < 0.05$) and yellower than the NGGGF. The color difference between the flours could

be due to the reactions taking place during germination, which caused an increase in yellow compounds or a reduction in green compounds.

Microbiological Evaluation of Sprouts and Flour

Table 7: Microbiological Evaluation of Non-Germinated Rough Rice and Green Gram (NGRR and NGGG), Soaked Rough Rice and Green Gram (SRR and SGG), and Germinated Rough Rice and Green Gram (GRR and GGG) Flour Using Total Plate Count (TPC)

Germination	Rough Rice Flour (cfu/g)[1]	Green Gram Flour (cfu/g)[1]
0-day (NG)[2]	$9.2x10^3$	$9.7x10^3$
0-day (S)[3]	$2.3x10^4$	$2.3x10^4$
1-day[4]	$4.6x10^4$	$3.1x10^4$
3-day[4]	$3.9x10^4$	$2.4x10^4$
5-day[4]	$7.6x10^4$	$5.7x10^4$
7-day[4]	$1.0x10^5$	$9.5x10^4$

[1]cfu/g = colony forming units per gram.
[2]NG = control non-germinated rough rice (NGRR) or green gram (NGGG) without soaking.
[3]S = control non-germinated rough rice or green gram underwent soaking (water bath (34 °C), 24 hr. and 2 hr. respectively).
[4]Rough rice or green gram underwent soaking (water bath (34 °C), 24 hr. and 2 hr respectively) before being germinated (GRR or GGG respectively) and processed into flour.

The total plate count (TPC) was used in determining the microbes in the GRR sprouts and flour. The control for flour (9.2×10^3 cfu/g) had the least amount of microbiological growth or colony forming units (CFU). As the length of sprouting time increased, so did the CFU, with 7-day GRRF having the highest (1.0×10^5 cfu/g) followed by 5-day GRRF (7.6×10^4 cfu/g).

The TPC was used in determining the microbes in the GGG sprouts and flour. The control for the flour (9.7×10^3 cfu/g) had the least amount of microbiological growth or colony forming units (CFU). 7-day GGGF having the highest (9.5×10^4 cfu/g) followed by 5-day GGGF (5.7×10^4 cfu/g).

Antinutrients of the Flours

Antinutrients of the Rough Rice Flours

Table 8: Trypsin Inhibitor and Lypoxygenase-1 and -3 Activities (%) of Non-germinated (NGRRF), Soaked (SRRF), and Germinated Rough Rice Flours (GRRF)

Germination	Trypsin Inhibitor Activity	Lipoxygenase-1 Activity	Lipoxygenase-3 Activity
0-day (NGRRF)[1]	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
0-day (SRRF)[2]	99.3 ± 0.6ab	94.3 ± 1.6b	95.7 ± 2.1a
1-day[3]	99.2 ± 0.2bc	92.4 ± 1.6bc	92.4 ± 4.5a
3-day[3]	97.1 ± 0.1c	89.9 ± 1.3c	83.1 ± 1.3b
5-day[3]	94.8 ± 0.8d	76.9 ± 1.6d	74.6 ± 1.0c
7-day[3]	90.3 ± 0.7e	62.6 ± 1.7e	56.1 ± 3.2d
P-value	P < 0.0001	P < 0.0001	P < 0.0001

[1]NGRRF = control non-germinated rough rice without soaking before being processed into flour.
[2]SRRF = control non-germinated rough rice underwent soaking (water bath (34 °C), 24 hr) before being processed into flour.
[3]Rough rice underwent soaking (water bath (34 °C), 24 hr.) before being germinated and processed into flour (GRRF).
Values are mean ± SD of triplicate analysis.
Mean values followed by different letters in the same column are significantly different ($P < 0.05$).

In the RRF, the trypsin inhibitor activity (%, $P < 0.0001$), lipoxygenase-1 activity (%, $P < 0.0001$), and lipoxygenase-3 (%, $P < 0.0001$) had an overall significant difference ($P < 0.05$); all results are compared to the NGRRF, which was set at 100% (Table 5). Although the overall trypsin inhibitor activity (%) of the GRRF was significantly different ($P < 0.0001$), there was no significant difference ($P > 0.05$) between the NGRRF and the SRRF or between the SRRF and the 1-day GRRF or between the 1-day GRRF and the 3-day GRRF. Throughout the germination process of the RR, the trypsin inhibitor activity decreased from the NGRRF (100%) to the 7-day GRRF (90.3%) by 9.7%. Therefore, the 7-day GRRF (90.3%) had the lowest percentage of trypsin inhibitor activity followed by the 5-day GRRF (94.8%).

There was an overall significant difference ($P < 0.0001$) in the lipoxygenase-1 activity (%) when compared to the NGRRF; however, there was no significant difference ($P > 0.05$) between the SRRF and the 1-day GRRF or between the 1-day and the 3-day GRRF. Throughout the

germination process, there was a very significant decrease in the lipoxygenase-1 activity (%) from the NGRRF (100%) to the 7-day GRRF (62.6%) by 37.4% and was a significant difference ($P < 0.05$). So, the 7-day GRRF (62.6%) had the lowest percentage of lipoxygenase-1 activity followed by the 5-day GRRF (76.9%).

The lipoxygenase-3 activity (%) of the RRF was significantly different ($P < 0.0001$) overall, but there was no significant difference between the NGRRF, SRRF, and 1-day GRRF. The lipoxygenase-3 activity (%) from the control (100%) to the 7-day GRRF (56.1%) decreased by 43.9% and was significantly different ($P < 0.05$). Therefore, the 7-day GRRF (56.1%) had the lowest percentage of lipoxygenase-3 activity followed by the 5-day GRRF (74.6%).

The decrease in the trypsin inhibitor, lipoxygenase-1, and lipoxygenase-3 could be due to these enzymes, which are proteins, being hydrolyzed during germination. A decrease in trypsin inhibitor and lipoxygenase activities in germinated flours has the advantage of better digestion of proteins by the gastrointestinal system and preventing lipid oxidation (rancidity) in flours.

Antinutrients of the Green Gram Flours

Table 9: Trypsin Inhibitor and Lipoxygenase-1 and -3 Activities (%) of Non-Germinated (NGGGF), Soaked (SGGF), and Germinated Green Gram Flours (GGGF)

Germination	Trypsin Inhibitor Activity (g/100g)	Lipoxygenase-1 Activity (g/100g)	Lipoxygenase-3 Activity (g/100g)
0 day (NGGGF)[1]	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
0 day (SGGF)[2]	98.8 ± 0.4ab	98.9 ± 1.7a	98.7 ± 3.2a
1-day[3]	97.8 ± 1.4b	97.6 ± 1.6a	95.8 ± 2.9a
3-day[3]	91.9 ± 1.3c	91.2 ± 1.7b	90.0 ± 3.7b
5-day[3]	85.1 ± 0.8d	85.5 ± 0.5c	76.6 ± 3.1c
7-day[3]	76.1 ± 1.1e	78.9 ± 2.0d	63.6 ± 2.8d
P-value	P < 0.0001	P < 0.0001	P < 0.0001

[1]NGGGF = control non-germinated rough rice without soaking before being processed into flour.
[2]SGGF = control non-germinated rough rice underwent soaking (water bath (34 °C), 2 hr) before being processed into flour.
[3]Green gram underwent soaking (water bath (34 °C), 2 hr) before being germinated and processed into flour (GGGF).
Values are mean ± SD of triplicate analysis.
Mean values followed by different letters in the same column are significantly different ($P < 0.05$).

The trypsin inhibitor activity (%, $P < 0.0001$), lipoxygenase-1 activity (%, $P < 0.0001$), and lipoxygenase-3 (%, $P < 0.0001$) of GGF had an overall significant difference ($P < 0.05$); all results are compared to the NGGGF, which was set at 100% (Table 6). Although the overall trypsin inhibitor activity (%) of the GGF was significantly different ($P < 0.0001$), there was no significant difference ($P > 0.05$) between the NGGGF and the SGGF or between the SGGF and the 1-day GGGF. Throughout the germination process of the GG, the trypsin inhibitor activity (%) decreased from the NGGGF (100%) to the 7-day GGGF (76.1%) by 23.9% and was significantly different ($P < 0.05$). So, the 7-day GGGF (76.1%) had the lowest percentage of trypsin inhibitor activity followed by the 5-day GGGF (85.1%).

There was a decrease in lipoxygenase-1 activity (%) of the GGF between the NGGGF (100%), SGGF (98.9%), and the 1-day GGGF (97.6%) by a total of 2.4%, but the decrease was not significantly different ($P < 0.05$). However, the overall lipoxygenase-1 activity (%) of the GGF

was significantly different and decreased by approximately 21.1%, from the control (100%) to the 7-day GGGF (78.9%). Therefore, the largest decrease in lipoxygenase-1 activity (%) occurred in the 7-day GGGF (78.9%) followed by the 5-day GGGF (85.5%).

The lipoxygenase-1 activity (%) decreased by 4.2% from the NGGGF, SGGF, and the 1-day GGGF, but the decrease was not a significant difference. However, the overall lipoxygenase-1 activity (%) was a significant difference ($P < 0.0001$) and decreased from the NGGGF (100%) to the 7-day GGGF (63.6%) or by 36.4% So, the largest percentage in decrease of the lipoxygenase-1 activity occurred in the 7-day GGGF (63.6%) followed by the 5-day GGGF (76.6%).

As with the GRRF, the decrease in the trypsin inhibitor, lipoxygenase-1, and lipoxygenase-3 could be due to these enzymes being hydrolyzed during germination.

In vitro Glycemic Index of the Flours

In vitro Glycemic Index of the Rough Rice Flours

Table 10: *In vitro* Glycemic Index of Non-Germinated (NGRRF), Soaked (SRRF), and Germinated Rough Rice Flours (GRRF)

Germination	*In vitro* Glycemic Index[1]
0 day (NGRRF)[2]	49.46 ± 0.39a
0 day (SRRF)[3]	49.32 ± 0.59a
1-day[4]	48.81 ± 0.33ab
3-day[4]	48.22 ± 0.27b
5-day[4]	47.57 ± 0.55c
7-day[4]	46.48 ± 0.32d
P-value	< 0.0001

[1]*In vitro* Glycemic Index (GI) of the flours were calculated using the best-curve fit equations (Appendix Fig.1) and white bread (94.61 ± 0.00) as a reference.
[2]NGRRF = control non-germinated rough rice without soaking before being processed into flour.
[3]SRRF = control non-germinated rough rice underwent soaking (water bath (34 °C), 24 hr) before being processed into flour.
[4]Rough rice underwent soaking (water bath (34 ºC), 24 hr) before being germinated and processed into flour (GRRF).
Values are mean ± SD of triplicate analysis.
Mean values followed by different letters in the same column are significantly different ($P < 0.05$).

The *in vitro* GI of Non-Germinated (NGRRF), Soaked (SRRF), and Germinated Rough Rice Flours (GRRF) was determined using best-fit curve equations (Appendix Fig. 1) for starch hydrolysis and white bread (94.61 ± 0.00) as a reference. The *in vitro* GI of Non-Germinated (NGRRF), Soaked (SRRF), and Germinated Rough Rice Flours (GRRF) was overall significantly difference ($P < 0.0001$). The *in vitro* GI decreased between the NGRRF (49.46), the SRRF (49.32), and the 1-day GRRF (48.81) but was not significantly different ($P > 0.05$). However, the *in vitro* GI of the 7-day GRRF (46.48) was lower and significantly different ($P < 0.05$) from the *in vitro* GI of the NGRRF, SRRF, 1-day GRRF, 3-day GRRF, and 5-day GRRF. The 5-day GRRF had the second lowest *in vitro* GI and was significantly different ($P < 0.05$) from the other rough rice flour samples.

In vitro Glycemic Index of the Green Gram Flours

Table 11: *In vitro* Glycemic Index of Non-Germinated (NGGGF), Soaked (SGGF), and Germinated Green Gram Flours (GGGF)

Germination	*In vitro* Glycemic Index[1]
0 day (NGGGF)[2]	47.38 ± 0.13a
0 day (SGGF)[3]	47.55 ± 0.17a
1-day[4]	47.44 ± 0.26a
3-day[4]	46.67 ± 0.14b
5-day[4]	46.22 ± 0.24c
7-day[4]	45.44 ± 0.08d
P-value	< 0.0001

[1]*In vitro* Glycemic Index of the flours were calculated using the best-curve fit equations (Appendix Fig.1) and white bread (94.61 ± 0.00) as a reference.
[2]NGGGF = control non-germinated green gram without soaking before being processed into flour.
[3]SGGF = control non-germinated green gram underwent soaking (water bath (34 °C), 2 hr) before being processed into flour.
[4]Green gram underwent soaking (water bath (34 ºC), 2 hr) before being germinated and processed into flour (GGGF).
Values are mean ± SD of triplicate analysis.
Mean values followed by different letters in the same column are significantly different ($P < 0.05$).

Using best-fit curve equations (Appendix Fig. 1) for starch hydrolysis and white bread (94.61 ± 0.00) as a reference, the *in vitro* GI of the NGGGF (47.38), SGGF (47.55), 1-day GGGF

(47.44), 3-day GGGF (46.67), 5-day GGGF (46.22), and the 7-day GGGF (45.44) was overall significantly different ($P < 0.0001$). Although the SGGF and 1-day GGGF had a higher *in vitro* GI than the NGGGF, the *in vitro* GI was not significantly different ($P > 0.05$). The *in vitro* GI of the 7-day GGGF was lower and significantly different ($P < 0.05$) than the *in vitro* GI of the NGGGF, the SGGF, the 1-day GGGF, the 3-day GGGF, and the 5-day GGGF. The 5-day GGGF had the second lowest *in vitro* GI and was significantly different ($P < 0.05$) from the other green gram flour samples.

Determine Optimal Germination Time from Analysis of the Data for Use in Making Sample Snack Chip

From the "*Proximate Nutrient Composition of the Rough Rice Flours*" and "*Proximate Nutrient Composition of the Green Gram Flours*" sections above, within the rough rice group and the green gram group, the most protein (%) was the 7-day GRRF (11.6%) and the 7-day GGGF (44.3%) respectively followed by the 5-day GRRF (10.8%) and the 5-day GGGF (39.2%) respectively, the most lipids (%) was the 7-day GRRF (2.73%) and the 7-day GGGF (5.68%) respectively followed by the 5-day GRRF (2.3%) and the 5-day GGGF (2.9%) respectively, and the least amount of starch (%) was the 7-day GRRF (21.2%) and the 7-day GGGF (35.7%) respectively followed by the 5-day GRRF (22.6%) and the 5-day GGGF (40.0%) respectively. Within the rough rice group and the green gram group, the least amount of moisture (%) was the 7-day GRRF (7.3%) followed by the 5-day GRRF (7.7%) and the SGGF with the 7-day GGGF (12.2%) being lower than the 5-day GGGF (14.1%). Within the rough rice group and the green gram group, the least amount of water activity was the SRRF (0.34) with the 5-day GRRF (0.41) having less than the 7-day GRRF (0.45), and the SGGF (0.42) had the least amount of water activity with 7-day GGGF (0.45) having less than the 5-day GGGF (0.50).

From the "*Antinutrients of the Rough Rice Flours*" and "*Antinutrients of the Green Gram Flours*" sections above, within the rough rice group and the green gram group, the least amount of trypsin inhibitor activity (%) was the 7-day GRRF (90.3%) and the 7-day GGGF (76.1%) respectively followed by the 5-day GRRF (94.8%) and the 5-day GGGF (85.1%) respectively, the least amount of lipoxygenase-1 activity (%) was the 7-day GRRF (62.6%) and the 7-day GGGF (78.9%) respectively followed by the 5-day GRRF (76.9%) and the 5-day GGGF (85.5%) respectively, and the least amount of lipoxygenase-3 activity (%) was the 7-day GRRF (56.1%) and the 7-day GGGF (63.6%) respectively followed by the 5-day GRRF (74.6%) and the 5-day GGGF (76.6%) respectively.

From the *"In vitro Glycemic Index of the Rough Rice Flours*" and the *"In vitro Glycemic Index of the Green Gram Flours*" section above, within the rough rice group and the green gram group, the lowest amount of *in vitro* GI was the 7-day GRRF (46.48) and the 7-GGGF (45.44) respectively followed by the 5-day GRRF (47.57) and the 5-day GGGF (46.22). From the "*Color Analysis of the Rough Rice Flours*" and the "*Color Analysis of the Green Gram Flours*" sections above, within the rough rice group and the green gram group, 7-day GRRF ("great," $6 < \Delta E < 12$) had the overall greatest difference in color compared to the NGRRF followed by 5-day GRRF ("well visible," $3 < \Delta E < 6$), and the 5-day GGGF and 7-day GGGF ("more than great," $12<\Delta E<24$) had the overall greatest difference in color compared to the NGGGF.

The 7-day GRRF and the 7-day GGGF had the best overall values in the above categories. However, due to the physical degradation in the 7-day GRR sprouts and the 7-day GGG sprouts as discussed in the "*Germinated Rough Rice (GRR) Sprout Lengths*" and "*Germinated Green Gram (GGG) Sprout Lengths*" section above, the 5-day GRRF and the 5-day GGGF were picked

to prepare the sample snack chips (SSC), since they did not undergo physical degradation during germination and had the 2nd best overall values in the above categories.

Physicochemical Characteristics, *In vitro* Glycemic Index, and Textural Properties of the Sample Snack Chips

Color Analysis of the Snack Chips

Table 12: Color Analysis of the Sample (SCS) and Control (CSC) Snack Chips Prepared from 5-day Germinated Rough Rice and Green Gram Flour (GRRF and GGGF) and Non-Germinated Rough Rice and Green Gram Flour (NGRRF and NGGGF) Respectively

Snack Chips	L*	a* (+/-)	b* (+/-)	ΔE*	Difference in Appearance
Control snack chips[1]	94.27 ± 0.80	-7.99 ± 0.51	12.22 ± 2.73		
Sample snack chips[2]	86.96 ± 0.81	-0.15 ± 0.63	20.78 ± 2.38	13.71 ± 1.49	more than great
P-value	< 0.0001	< 0.0001	< 0.0001		

[1]control snack chips (CSC) using NGRRF (non-germinated rough rice flour) and NGGGF (non-germinated green gram flour).
[2]sample snack chips (SSC) using equal parts of 5-day GRRF (5-day germinated rough rice flour) and 5-day GGGF (germinated green gram flour).
not noticeable = $0 < \Delta E < 0.5$; slightly noticeable = $0.5 < \Delta E < 1.5$; noticeable = $1.5 < \Delta E < 3$; well visible = $3 < \Delta E < 6$; great = $6 < \Delta E < 12$; more than great = $12 < \Delta E < 24$. ΔE* was calculated using control chips from NGRRF and NGGGF as reference.
Values are mean ± SD of triplicate analysis.

The color of the SSC had an overall difference in appearance (ΔE) of "more than great" ($12<\Delta E<24$) when compared to the color of the control snack chips (CSC), which was prepared using NGRRF and NGGGF. Therefore, there is a large color change difference between the CSC and the SSC. Since the control is used to determine the difference in appearance, there are no other means besides the SSC in which to use to determine if there is a significant difference ($P < 0.05$) or not.

For L*, where ΔL = difference in lightness and darkness (+ = lighter, - = darker), the CSC (94.27) was significantly different ($P < 0.0001$) and lighter than the SSC (86.96). The color difference between the flours could be due to the reactions taking place during germination, which caused a reduction in lightness compounds or an increase in darkness compounds. For a*, where

Δa = difference in red and green (+ = redder, - = greener), the CSC (-7.99) was greener and significantly different (P < 0.0001) than the SSC (-0.15). The color difference between the flours could be due to the reactions taking place during germination, which caused an increase in red compounds or a reduction in green compounds. For b*, where Δb = difference in yellow and blue (+ = yellower, - = bluer), the SSC (20.78) was yellower and significantly different (P < 0.0001) than the CSC (12.22). The color difference between the flours could be due to the reactions taking place during germination, which caused an increase in yellow compounds or a reduction in blue compounds.

In vitro Glycemic Index of the Snack Chips

Table 13: *In vitro* Glycemic Index of the Sample (SCS) and Control (CSC) Snack Chips Prepared from 5-day Germinated Rough Rice and Green Gram Flour (GRRF and GGGF) and Non-Germinated Rough Rice and Green Gram Flour (NGRRF and NGGGF) Respectively

	Control Snack Chips[1]	Sample Snack Chips[2]	P-value
Glycemic Index[3]	48.48 ± 0.17	46.64 ± 0.22	0.0004

[1]control snack chips (CSC) using NGRRF (non-germinated rough rice flour) and NGGGF (non-germinated green gram flour).
[2]sample snack chips (SSC) using equal parts of 5-day GRRF (5-day germinated rough rice flour) and 5-day GGGF (germinated green gram flour).
[3]*In vitro* Glycemic Index of the flours were calculated using the best-curve fit equations (Appendix Fig.1) and white bread (94.61 ± 0.00) as a reference.
Values are mean ± SD of triplicate analysis.
Mean values followed by different letters in the same column are significantly different (P < 0.05).

Using best-fit curve equations (Appendix Fig. 1) and white bread (94.61 ± 0.00) as a reference, the control snack chips (CSC) was found to have a higher *in vitro* GI (48.48) and was significantly different (P = 0.0004) than the sample snack chips (SSC), whose *in vitro* GI was 46.64. This was expected since the 5-day GRRF and GGGF used to make the SSC had a lower *in vitro* GI than the NGRRF and NGGGF used to make the CSC.

Texture Analysis of the Snack Chips

Table 14: Texture Analysis of the Sample (SCS) and Control (CSC) Snack Chips Prepared from 5-day Germinated Rough Rice and Green Gram Flour (GRRF and GGGF) and Non-Germinated Rough Rice and Green Gram Flour (NGRRF and NGGGF) Respectively

	Control Snack Chips[1]	Sample Snack Chips[2]	P-value
Fracturability[3] (g)	621.13 ± 278.09	1103.34 ± 154.6	0.0040

[1]control snack chips (CSC) were prepared using equal parts of NGRRF (non-germinated rough rice flour) and NGGGF (non-germinated green gram flour).
[2]sample snack chips (SCS) were prepared using equal parts of 5-day GRRF (5-day germinated rough rice flour) and 5-day GGGF (germinated green gram flour).
[3]fracturability was used to measure the crunchiness of chips.
Note: Out of 10 values, removed the two lowest and two highest values.
Mean value is significantly different if $P < 0.05$.

Fracturability, a way in which consumers perceive the crunchiness of chips, was used in analyzing the texture of the SSC versus the texture of the CSC. The fracturability of the SSC (1103.34 g) was approximately 78% greater and significantly different ($P = 0.0040$) than the CSC (621.13 g).

Shelf-life Study

Color Analysis for Shelf-life Study

Table 15: Color Analysis of the Sample (SCS) and Control (CSC) Snack Chips Prepared from 5-day Germinated Rough Rice and Green Gram Flour (GRRF and GGGF) and Non-Germinated Rough Rice and Green Gram Flour (NGRRF and NGGGF) Respectively over Shelf-life Study Conducted over a 4-Month Period

Snack Chips	L*	a* (+/-)	b* (+/-)	ΔE*	Difference in Appearance
Control snack chips[1] (0 month)	94.27 ± 0.80a	-7.99 ± 0.51f	12.22 ± 2.73ef	0.00 ± 0.00	Control
Sample snack chips[2] (0 month)	86.96 ± 0.81bc	-0.15 ± 0.63c	20.78 ± 2.38c	13.71 ± 1.49a	More than great
Control snack chips[1] (1 month)	94.18 ± 0.81a	-8.14 ± 0.36f	13.42 ± 1.96de		
Sample snack chips[2] (1 month)	83.32 ± 1.10d	0.80 ± 0.48c	16.79 ± 3.98d	14.47 ± 1.31b	More than great
Control snack chips[1] (2 month)	94.40 ± 0.73a	-7.27 ± 0.31ef	9.05 ± 1.17f		
Sample snack chips[2] (2 month)	86.47 ± 2.28c	0.03 ± 1.20c	20.92 ± 2.84c	16.03 ± 3.83a	More than great
Control snack chips[1] (3 month)	87.29 ± 1.10bc	-5.90 ± 0.61d	21.13 ± 0.32bc		
Sample snack chips[2] (3 month)	69.88 ± 1.62e	3.73 ± 0.32b	24.66 ± 0.18ab	30.95 ± 1.79c	Extremely noticeable
Control snack	88.50 ± 0.34b	4.63 ± 0.27a	27.36 ± 0.56a		

chips[1] (4 month)					
Sample snack chips[2] (4 month)	69.15 ± 0.48e	-6.43 ± 0.44de	15.27 ± 1.31de	26.01 ± 0.90c	Extremely noticeable
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	

[1]control snack chips (CSC) were prepared using equal parts of NGRRF (non-germinated rough rice flour) and NGGGF (non-germinated green gram flour).
[2]sample snack chips (SSC) were prepared using equal parts of 5-day GRRF (5-day germinated rough rice flour) and 5-day GGGF (germinated green gram flour).
not noticeable = $0 < \Delta E < 0.5$; slightly noticeable = $0.5 < \Delta E < 1.5$; noticeable = $1.5 < \Delta E < 3$; well visible = $3 < \Delta E < 6$; great = $6 < \Delta E < 12$; more than great = $12 < \Delta E < 24$. ΔE^* was calculated using control chips from NGRRF and NGGGF as reference
Values are mean ± SD of triplicate analysis.
Mean values followed by different letters in the same column are significantly different ($P < 0.05$).

The overall color difference (ΔE) was significantly different ($P < 0.0001$) and changed from "more than great" ($12 < \Delta E < 24$) in the 0-month, 1- month, and 2-month SSC to "extremely noticeably" ($24 < \Delta E < 48$) in the 3-month and 4-month SSC. There was no significant different ($P > 0.05$) between the 0-month SSC (13.71, "more than great," $12 < \Delta E < 24$) and 2-month SSC (16.03, "more than great), but there was a significant difference ($P < 0.05$) between the 0-month SSC and the 1-month SSC (14.47, "more than great," $12 < \Delta E < 24$) and between the 1-month SSC and the 2-month SSC. There was also no significant difference ($P < 0.05$) between the 3-month SSC (30.95, "extremely noticeable," $24 < \Delta E < 48$) and 4-month SSC (26.01, "extremely noticeable," $24 < \Delta E < 48$).

For L*, where ΔL = difference in lightness and darkness (+ = lighter, - = darker), there was an overall significantly difference ($P < 0.0001$). There was no significant difference ($P > 0.05$) between the 0-month (94.27), 1- month (94.18), and 2-month (94.40) CSC, between the 3-month (87.29) and the 4-month (88.50) CSC and 0-month SSC (86.96), between the 0-month and 2-month (86.47) SSC and the 3-month CSC (87.29), or between the 3-month (69.88) and 4-month (69.15)

SSC. However, the 0-month CSC were lighter and significant different ($P > 0.05$) than the 4-month CSC and the 0-month SSC were lighter and significant different ($P > 0.05$) than the 4-month SSC.

For a*, where Δa = difference in red and green (+ = redder, - = greener), there was an overall significant difference ($P < 0.001$). Although the 1-month SSC (0.80) was redder than either the 2-month SSC (0.03) or the 0-month SSC (-0.15), there was no significant difference ($P > 0.05$). The 4-month SSC (-6.43) were greener than the 3-month CSC, but there was no significant difference ($P > 0.05$). Even though the 2-month CSC (-7.27) were greener than the 4-month SSC, there was no significant difference ($P > 0.05$). There was also no significant difference ($P > 0.05$) between the 2-month CSC (-7.27), 0-month CSC (-7.99), and the 1-month CSC (-8.15), even though both the 0-month and the 1-month CSC were greener than the 2-month CSC.

For b*, where Δb = difference in yellow and blue (+ = yellower, - = bluer), there was an overall significant difference ($P < 0.0001$), although there was no significant difference ($P > 0.05$) between the 3-month SSC (24.66) and the 4-month CSC (27.36), between the 3-month CSC (21.13) and the 3-month SSC, between the 0-month (20.78) and 2-month (20.92) SSC and the 3-month CSC, between the 1-month CSC (13.42) and the 1-month (16.79) and 4-month (15.27) SSC, and the 0-month (12.22) and 1-month CSC and the 4-month SSC, and between the 0-month and 2-month (9.05) CSC. The 4-month CSC were yellower and significantly different ($P < 0.05$) than the 0-month CSC, and the 0-month SSC were yellower and significantly different ($P < 0.05$) that the 4-month SSC.

Water Activity for Shelf-life Study

Table 16: Water Activity of the Sample (SCS) and Control (CSC) Snack Chips Prepared from 5-day Germinated Rough Rice and Green Gram Flour (GRRF and GGGF) and Non-Germinated Rough Rice and Green Gram Flour (NGRRF and NGGGF) Respectively over Shelf-life Study Conducted over a 4-Month Period

Snack Chips	A_w
Control snack chips[1] (0 Month)	0.47 ± 0.00a
Sample snack chips[2] (0 Month)	0.43 ± 0.00d
Control snack chips[1] (1 Month)	0.47 ± 0.00a
Sample snack chips[2] (1 Month)	0.43 ± 0.00de
Control snack chips[1] (2 Month)	0.47 ± 0.00ab
Sample snack chips[2] (2 Month)	0.43 ± 0.00de
Control snack chips[1] (3 Month)	0.46 ± 0.00bc
Sample snack chips[2] (3 Month)	0.42 ± 0.00e
Control snack chips[1] (4 Month)	0.45 ± 0.01c
Sample snack chips[2] (4 Month)	0.42 ± 0.01de
P-value	< 0.0001

[1]Control snack chips (CSC) were prepared using equal parts of NGRRF (non-germinated rough rice flour) and NGGGF (non-germinated green gram flour).
[2]Sample snack chips were prepared using equal parts of 5-day GRRF (5-day germinated rough rice flour) and 5-day GGGF (germinated green gram flour).
Mean values followed by different letters in the same column are significantly different ($P < 0.05$).

The A_w of the CSC (0.45 – 0.47) were higher and significantly different ($P < 0.0001$) than the A_w of the SSC (0.42 – 0.43). However, there was no significant difference ($P > 0.05$) between the 0-month (0,47), 1-month (0.47), and the 2-month (0.47) CSC, between the 2-month and 3-month (0.46) CSC, between the 3-month and 4-month (0.45) CSC, between the 0-month (0.43), 1-month (0.43), 2-month (0.43), and 4-month (0.42) SSC, and between the 1-month, 2-month, 3-month (0.42), and 4-month SSC. The A_w of the 0-month CSC were higher and significantly different ($P < 0.05$) than the A_w of the 0-month CSC, and the A_w of the 0-month SSC were higher and but not significantly different ($P > 0.05$) than the A_w of the 0-month CSC.

Sensory Analysis

Acceptability of the Sample Snack Chips Using a 9-point Hedonics Scale

Table 17: Evaluation[1] of the Acceptability of the Sample (SCS) and Control (CSC) Snack Chips Prepared from 5-day Germinated Rough Rice and Green Gram Flour (GRRF and GGGF) and Non-

Germinated Rough Rice and Green Gram Flour (NGRRF and NGGGF) Respectively Using a 9-Point Hedonics Scale for Sensory Attributes

Attribute	Control snack chips[2]	Sample snack chips[3]	Snack Chips P value	Gender P value	Snack Chips and Gender P value
Appearance	5.19 ± 1.30	6.22 ± 1.04	< 0.0001	0.4900	0.9469
Aroma	4.89 ± 1.11	5.38 ± 1.14	0.0053	0.1369	0.2740
Hardness	6.62 ± 1.09	5.92 ± 1.17	0.0056	0.1627	0.1576
Cohesiveness	6.21 ± 0.99	6.45 ± 1.24	0.1469	0.1307	0.5226
Flavor	3.82 ± 1.56	4.67 ± 1.52	0.0009	0.0102	0.4507
Mouthfeel	4.28 ± 1.31	5.03 ± 1.31	0.0027	0.0219	0.8213
Aftertaste	3.66 ± 1.54	3.95 ± 1.17	0.4255	0.1032	0.4255
Overall Acceptability	4.03 ± 1.45	4.92 ± 1.45	0.0003	0.0111	0.5017

[1]Evaluated by 74 volunteer panelists - male (21) and female (53).
[2]Control snack chips (CSC) were prepared using equal parts of non-germinated rough rice (NGRRF) and non-germinated green gram (NGGG) flour (1:1).
[3]Sample snack chips (SSC) were prepared using equal parts of 5-day germinated rough rice (GRRF) and 5-day germinated green gram (GGGF) flour (1:1).
Mean values are significantly different if $P < 0.05$.

The CSC were lower and significantly different ($P < 0.05$) than the SSC in the appearance ($P < 0.0001$) and aroma ($P = 0.0053$) attributes, although there was no significant difference between the gender ($P = 0.49$ and $P = 0.4900$) or between CSC, SSC, and gender ($P = 0.9469$ and $P = 0.2740$) in the appearance and aroma likeability respectively. The higher likeness of the SSC's appearance could be due the CSC being significantly greener than the SSC, as seen in the color analysis of the snack chips and the higher likeness of the SSC's aroma could be due to the metabolic changes happening during germination, which created more pleasant aroma compounds or reduced unpleasant compounds. However, there was no significant difference between males evaluating the CSC and the SSC vs females evaluating the CSC and the SSC.

The CSC were higher and significantly different ($P = 0.0056$) than the SSC in hardness likeability, but there was no significant difference between the gender ($P = 0.1627$) or between the CSC, SSC, and gender ($P = 0.1576$). This could be due to the SSC being denser than the CSC, as

seen in the texture analysis of the snack chips, and so the less hard CSC were liked more with no significant difference between the genders. Although the cohesiveness attribute was ranked higher in the SSC compared to the CSC, the likeability of the cohesiveness of the chips showed no significant difference (P = 0.1469) between the gender (P = 0.1307) or between the CSC, SSC, and the gender (P = 0.5226).

The CSC had a lower and significantly different (P < 0.05) than the SSC in flavor (P = 0.0009) and in mouthfeel (P = 0.0027) with the gender being significantly different (P = 0.0102 and P = 0.0219 respectively) but the CSC, SSC, and gender were not significantly different (P = 0.4507 and P = 0.8213 respectively). As with the aroma, the increase in flavor and mouthfeel likeability in the SSC could be due to the creation of more pleasant aroma compounds or reduction of unpleasant compounds happening during germination.

The likeness of the aftertaste was not significantly different between the CSC and the SSC (P = 0.4255), between the gender (P = 0.1032), or between the CSC, SSC, and the gender (P = 0.4255), and both of the samples were found to have a slightly to moderately unpleasant aftertaste. From this, one can conclude that the process of germination had little to no effect on the compounds giving the product an unpleasant aftertaste.

The overall acceptability was higher and significantly different between the SSC and the CSC (P = 0.0003) with the gender being significantly different (P = 0.0111) but was not significantly different between the CSC, SSC, and the gender (P = 0.5017), which means that the process of germination increased the acceptability of the product.

Acceptability of the Sample Snack Chips Using a 5-point Just-About-Right (JAR) Scale

Table 18: Evaluation[1] of the Acceptability of the Sample (SCS) and Control (CSC) Snack Chips Prepared from 5-day Germinated Rough Rice and Green Gram Flour (GRRF and GGGF) and Non-Germinated Rough Rice and Green Gram Flour (NGRRF and NGGGF) Respectively Using a 5-Point Just-About-Right (JAR) Scale for Color, Size, and Crispiness

Attribute	Control snack chips[2]	Sample snack chips[3]	Snack Chips P value	Gender P value	Snack Chips and Gender P value
Color	3.14 ± 0.67	3.16 ± 0.37	0.7375	0.3561	0.8847
Size	2.43 ± 0.60	2.39 ± 0.59	0.8987	0.9248	0.5740
Crispiness	3.01 ± 0.31	3.16 ± 0.69	0.0477	0.8684	0.3021

[1]Evaluated by 74 volunteer panelists - male (21) and female (53).
[2]Control snack chip made from equal parts of non-germinated rough rice (NGRRF) and non-germinated green gram (NGGG) flour (1:1).
[3]Sample snack chip made from equal parts of 5-day germinated rough rice (GRRF) and 5-day germinated green gram (GGGF) flour (1:1).
Mean values are significantly different if $P < 0.05$.

There was no significant difference ($P > 0.05$) in the bright or darkness of color between the CSC and the SCS ($P = 0.7375$), between the gender of those evaluating the color ($P = 0.3561$), or between the CSC, SCS, and gender ($P = 0.8847$), in the size between the CSC and the SCS ($P = 0.8987$), between the gender of those evaluating the size ($P = 0.9248$) or the CSC, SCS, and between the gender ($P = 0.5740$), nor in the crispiness between the CSC and the SSC ($P = 0.0876$), between the gender evaluating the crispiness ($P = 0.8684$), or between the CSC, SSC, and the gender ($P – 0.3021$) . The bright or darkness of color and the crispiness was thought to be just right in both of the samples. However, the size, 2cm x 2cm, was thought to be a little small in both the CSC and the SSC.

Acceptability of the Prepared Snack Chips Using Like and Dislike

Table 19: Evaluation[1] of the Acceptability of the Sample (SCS) and Control (CSC) Snack Chips Prepared from 5-day Germinated Rough Rice and Green Gram Flour (GRRF and GGGF) and Non-Germinated Rough Rice and Green Gram Flour (NGRRF and NGGGF) Respectively Using Like and Dislike for Sensory Attributes

Attribute	Like (%)[2]	
	Control[3]	Sample[4]
Surface color	9.5	28.4
Hardness (by touching)	24.3	43.2
Crispiness (by tasting)	78.4	43.2
Just-about-right of crispiness (by tasting)	51.4	24.3
Crunchiness (by tasting)	35.1	55.4
Hardness (by touching)	25.7	46.0

[1]Evaluated by 74 volunteer panelists - male (21) and female (53).
[2]Listeded top six highest percentage marked for the control snack chip and the sample snack chip.
[3]Control snack chips were prepared using equal parts of non-germinated rough rice (NGRRF) and non-germinated green gram (NGGG) flour (1:1).
[4]Sample snack chip were prepared using equal parts of 5-day germinated rough rice (GRRF) and 5-day germinated green gram (GGGF) flour (1:1).

A higher percentage of people disliked the appearance and surface color of the CSC compared to the SSC, which could be due to the CSC being significantly greener as stated above and in the color analysis of the snack chips. The hardness (by touching) was marked as disliked more in the SSC; however, it was also marked by a much higher percentage as liked than the CSC. A large percentage marked the crispiness (by tasting) as liked in the SSC and the CSC, which was higher. Both the hardness (by touching) and crispiness (by tasting) results could be due to the factorability of the SSC being notably harder than the CSC, as seen in the texture analysis of the snack chips. The just-about-right of hardness and crispiness intensity (by touching) had similar results as the hardness (by touching) and crispiness (by tasting), and as stated above, could be due to the difference in texture between the CSC and the SSC. A higher percentage of people marked the crunchiness as liked in the SSC and the CSC, with the SSC being higher. The hardness (by tasting) was higher liked and disliked in the SSC, with liked being marked more in the CSC and SSC.

Conclusion:

The proteins (%) and lipids (%) content of germinated rough rice and germinated green gram were significantly different ($P < 0.0001$) overall and increased over the germination period, the starch (%) content was significantly different ($P < 0.0001$) and decreased over the germination period, while the moisture (%) and water activity was significantly different ($P < 0.0001$) and decreased and increased over the germination period. The overall antinutrients, trypsin inhibitor, lipoxygenase-1, and lipoxygenase-3 activity (%), in both the germinated rough rice and green gram was significantly different ($P < 0.0001$) and decreased over the germination period. The *in vitro* glycemic index of the rough rice and green gram flours changed and was significantly different ($P < 0.0001$) over the length of the germination time.

The color analysis of the rough rice and green gram flours showed an overall significant different ($P < 0.0001$) and a color change of "slightly noticeable" to "great" and to "more than great" respectively. The microbiological evaluation for the flour showed a value ranging from 10^3 to 10^5 cfu/g.

The data was analyzed and the 5-day germinated rough rice and the 5-day germinated green gram was chosen to make prepared snack chips. The snack chips underwent *in vitro* glycemic index, where the *in vitro* glycemic index was lower and was significantly different ($P = 0.0004$) than a snack chip made with non-germinated rough rice and non-germinated green gram flours (control). A color analysis was performed on the snack and had a "more than great" appearance and while the significant difference for the ΔE^* could not be determined, the L^*, a^*, and b^* were significantly different ($P <0.0001$). The fracturability of the sample snack chips were higher and significant different ($P = 0.0040$) than the control. The sample snack chips underwent a color analysis for shelf-life study with the overall difference in appearance (ΔE^*), L^*, a^*, and b^* were

significantly different ($P < 0.0001$) and water activity for shelf-life study was overall significantly different ($P < 0.0001$).

The sample snack chips underwent a sensory 9-point hedonics evaluation by 74 volunteers and showed a higher likeability and was significantly different for appearance ($P < 0.0001$), aroma ($P = 0097$), flavor ($P = 0.0009$), mouthfeel ($P = 0.0027$), and overall acceptability ($P = 0.0003$) but with no significant difference between the gender except for the gender being significantly different for the flavor ($P = 0.0009$) and the mouthfeel ($P = 0.0027$) or between the CSC, SSC, and gender. The control snack chips had higher hardness and cohesiveness likeability and were significantly different ($P = 0.0056$) for hardness but not for cohesiveness compared to the sample snack chips, but there was no significant difference between the gender or between the CSC, SSC, and gender. There was no significant difference in the aftertaste, color, size, or crispiness between the CSC and the SSC, between the gender, or between the CSC, SSC, and the gender. Also, 78.4% of the participants indicated that they liked the crispiness (by tasting) of the control snack chips, while 55.4% liked the crunchiness (by tasting) of the sample snack chips from a given list.

The increase in the nutritional value of the GRRF and the GGGF compared to the RRF and the GGF control give optimal conditions in which to provide consumers with healthier and better-quality snacks. It also can fulfill consumers needs for snacks with increased protein and use local ingredients as well as additional health benefits. So, the use of GRRF and GGGF can be used in the growing snack market and meet the consumers demands for more nutritious and innovative snacks using local ingredients.

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Appendix
Starch Hydrolysis of NGRRF and GRRF

Appendix Figure 1: Best Curve Fit Equation and Coefficient of Determination for Starch Hydrolysis of White Bread, Non-germinated, Soaked and Germinated Rough Rice and Green Gram Flours, Control Snack Chip, and Sample Snack Chip

Flour	Best Curve Fit Equation	Coefficient of Determination
White bread – Trial 1	y = -0.0152180401243437x2 + 2.17372624975267x + 3.93327806290756	$R^2 = 0.97088$
White bread – Trial 2	y = -0.0158837730421474x2 + 2.24980230218711x + 4.56218535126726	$R^2 = 0.96294$
White bread – Trial 3	y = -0.0152459946484952x2 + 2.15717660544563x + 4.22196774881434	$R^2 = 0.96530$
Non-germinated rough rice[1] – Trial 1	y = -0.0015143583446433 6x2 + 0.313684598647436x + 0.656176267344151	$R^2 = 0.98068$
Non-germinated rough rice[1] – Trial 2	y = -0.0015788035214997 6x2 + 0.319850226765375x + 0.645400999997761	$R^2 = 0.98143$
Non-germinated rough rice[1] – Trial 3	y = -0.0016474512271770 2x2 + 0.332580386994526x + 0.624021313636355	$R^2 = 0.98382$
Soaked rough rice[2] – Trial 1	y = -0.0015897385713474 3x2 + 0.309377497926885x + 0.800592848809316	$R^2 = 0.96801$
Soaked rough rice[2] – Trial 2	y = -0.0014145932001681 1x2 + 0.293421475630069x + 1.06409612819765	$R^2 = 0.94420$
Soaked rough rice[2] – Trial 3	y = -0.0018970401062263 6x2 + 0.346178549494453x + 0.782164228413393	$R^2 = 0.97279$
Germinated 1-day rough rice[4] – Trial 1	y = -0.0015225354488204 3x2 + 0.306086295266833x + 0.700290746385534	$R^2 = 0.97599$
Germinated 1-day rough rice[4] – Trial 2	y = -0.0014520021816486 8x2 + 0.294773480709927x + 0.662582481847565	$R^2 = 0.97713$
Germinated 1-day rough rice[4] – Trial 3	y = -0.0013328248070948x2 + 0.284955302008787x + 0.852756281485121	$R^2 = 0.96283$

Germinated 3-day rough rice[4] – Trial 1	y = -0.0012492757230195x2 + 0.275053633303971x + 0.613054140668488	R^2 = 0.97981
Germinated 3-day rough rice[4] – Trial 2	y = -0.00119996643519127x2 + 0.266850173948528x + 0.626842704649917	R^2 = 0.97789
Germinated 3-day rough rice[4] – Trial 3	y = -0.00133220188034727x2 + 0.276114730133361x + 0.614551083576828	R^2 = 0.97820
Germinated 5-day rough rice[4] – Trial 1	y = -0.000938384969156714x2 + 0.240739303572673x + 0.562433601142352	R^2 = 0.98134
Germinated 5-day rough rice[4] – Trial 2	y = -0.000961626704238633x2 + 0.239382267004334x + 0.68192822211897	R^2 = 0.97166
Germinated 5-day rough rice[4] – Trial 3	y = -0.0007475364857405 1x2 + 0.22153419973198x + 0.618062424438591	R^2 = 0.97683
Germinated 7-day rough rice[4] – Trial 1	y = -0.000275485213739213x2 + 0.171775626351544x + 0.557751601970516	R^2 = 0.97894
Germinated 7-day rough rice[4] – Trial 2	y = -0.000183454652982321x2 + 0.167677552825844x + 0.308203817010352	R^2 = 0.99382
Germinated 7-day rough rice[4] – Trial 3	y = -0.0012514437269155x2 + 0.236110067434083x + 0.470555894188337	R^2 = 0.97983
Non-germinated green gram[1] – Trial 1	y = -0.0014509473668238x2 + 0.258334994791665x + 0.753828995778143	R^2 = 0.95355
Non-germinated green gram[1] – Trial 2	y = -0.0015215216302 4401x2 + 0.265830113249801x + 0.795993279881756	R^2 = 0.94960
Non-germinated green gram[1] – Trial 3	y = -0.0016949431244 9416x2 + 0.273380986499668x + 0.815381442975905	R^2 = 0.94199
Soaked green gram[3] – Trial 1	y = -0.0017364772406 1375x2 + 0.274421772999848x + 1.03780051556684	R^2 = 0.90629
Soaked green gram[3] – Trial 2	y = -0.0017402239963 6913x2 + 0.278895013262535x + 0.918407514164606	R^2 = 0.92929

Soaked green gram[3] – Trial 3	y = -0.0018860121308542 2x2 + 0.286561089079306x + 0.943351846846127	R² = 0.92122
Germinated 1-day green gram[5] – Trial 1	y = -0.0017816862176715 8x2 + 0.279216483199745x + 0.94044352037033	R² = 0.92292
Germinated 1-day green gram[5] – Trial 2	y = -0.0016326121376982 3x2 + 0.266522525670851x + 0.928958180761512	R² = 0.92419
Germinated 1-day green gram[5] – Trial 3	y = -0.0019018764095573 6x2 + 0.285947680862276x + 0.922623879060104	R² = 0.92235
Germinated 3-day green gram[5] – Trial 1	y = -0.0014715068935204 7x2 + 0.239217216037725x + 0.888699609416044	R² = 0.91408
Germinated 3-day green gram[5] – Trial 2	y = -0.0018441597178788x2 + 0.266641662987382x + 0.802669098622381	R² = 0.92548
Germinated 3-day green gram[5] – Trial 3	y = -0.0017567804553002x2 + 0.259642385093035x + 0.840074925412381	R² = 0.91898
Germinated 5-day green gram[5] – Trial 1	y = -0.0015790269553311 7x2 + 0.24457510602099x + 0.721354597330162	R² = 0.93833
Germinated 5-day green gram[5] – Trial 2	y = -0.0014325166216432x2 + 0.228880555133783x + 0.754741301722582	R² = 0.92869
Germinated 5-day green gram[5] – Trial 3	y = -0.0013595669785566 7x2 + 0.224721315255653x + 0.693681285059164	R² = 0.94097
Germinated 7-day green gram[5] – Trial 1	y = -0.0012520742657569 4x2 + 0.199741524240712x + 0.634779426568397	R² = 0.93322
Germinated 7-day green gram[5] – Trial 2	y = -0.0014016917823987x2 + 0.213632993871554x + 0.769022852449776	R² = 0.90782
Germinated 7-day green gram[5] – Trial 3	y = -0.0011813827900817 7x2 + 0.199320745551068x + 0.56734946164736	R² = 0.95127
Control Snack Chip[6] – Trial 1	y = -0.0019089260094735 5x2 + 0.319094619594377x + 0.558015430484353	R² = 0.98069
Control Snack Chip[6] – Trial 2	y = -0.0017577331416430 9x2 + 0.302215442077677x + 0.964946819075095	R² = 0.94147

Control Snack Chip[6] – Trial 3	y = -0.0016780340158721 9x2 + 0.299596865709066x + 0.789055633164921	$R^2 = 0.96202$
Sample Snack Chip[7] – Trial 1	y = -0.0012229402839507 6x2 + 0.235635871384202x + 0.512716073249955	$R^2 = 0.97682$
Sample Snack Chip[7] – Trial 2	y = -0.0011110745998551 6x2 + 0.223975436999907x + 0.567938220743265	$R^2 = 0.97078$
Sample Snack Chip[7] – Trial 3	y = -0.0012514437269155x2 + 0.236110067434083x + 0.470555894188337	$R^2 = 0.979827$

[1]Non-Germinated rough rice or green gram (control) without soaking before being processed into flour.
[2]Soaked rough rice = non-germinated rough rice (control) underwent soaking (water bath (34 °C), 24 hr) before being processed into flour.
[3]Soaked green gram = non-germinated green gram (control) underwent soaking (water bath (34 °C), 2 hr) before being processed into flour.
[4]Rough rice underwent soaking (water bath (34 ºC), 24 hr) before being germinated.
[5]Green gram underwent soaking (water bath (34 ºC), 2 hr) before being germinated.
[6]Control snack chips were prepared using equal parts of non-germinated rough rice (NGRRF) and non-germinated green gram (NGGG) flour (1:1).
[7]Sample snack chip were prepared using equal parts of 5-day germinated rough rice (GRRF) and 5-day germinated green gram (GGGF) flour (1:1).

Ballot

***Sample No.*_____**

Please evaluate the sample.

1 Please look at the sample closely. Concentrating on only the ***appearance*** of the ***sample***, which of the statements below best describes your ***impression*** of the **appearance**?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Live very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

2 Please look the sample closely. Considering only the ***color*** of the ***sample***, which of the statements below best describes your ***impression*** of the **color**?

Too light	Somewhat too light	Just about right	Somewhat too dark	Too dark
☐	☐	☐	☐	☐

3 Please look the sample closely. Considering only the ***size*** of the ***sample***, which of the statements below best describes your ***impression*** of the **size**?

Too small	Somewhat too small	Just about right	Somewhat too big	Too big
☐	☐	☐	☐	☐

4 Please sniff the aroma of the sample (but do not taste). Concentrating on only the ***aroma*** of the ***sample***, which of the statements below best describes your ***impression*** of the **aroma**?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Live very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

5 Please touch the sample (but do not taste). Considering the ***hardness*** of the ***sample,*** which of the statements below best describes your ***impression*** of the **hardness**?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Live very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

6 Please taste the sample. Considering the ***cohesiveness*** of the ***sample,*** which of the statements below best describes your ***impression*** of the **cohesiveness**?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Live very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

7 Please taste the sample. Considering only the ***crispiness*** of the ***sample***, which of the statements below best describes your ***impression*** of the **crispiness**?

Not crispy enough	Somewhat not crispy enough	Just about right	Somewhat too crispy	Too crispy
☐	☐	☐	☐	☐

8 Please taste the sample. Considering only the ***flavor*** of the ***sample***, which of the statements below best describes your ***impression*** of the **flavor**?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Live very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

9 Please taste the sample. Considering only the ***mouthfeel*** of the ***sample***, which of the statements below best describes your ***impression*** of the **mouthfeel**?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Live very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

10 Please taste the sample. Considering only the ***aftertaste*** of the ***sample***, which of the statements below best describes your ***impression*** of the **aftertaste**?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Live very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

11 Considering all things, which of the statements below best describes your ***overall acceptability*** of this product?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Live very much	Like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

6. What did you like about this product? Please check all that apply.

□ Appearance	□ Surface color	□ Color brightness
□ Hardness (by touching)	□ Crispiness (by tasting)	□ Rice flavor
□ Green gram flavor	□ Mouthfeel	□ Just-about-right of hardness intensity (by touching)
□ Just-about-right of crispiness intensity (by touching)	□ Sweet taste	□ Sour taste
□ Salty taste	□ Just-about-right of taste intensity (by tasting)	□ Bitter taste
□ Balanced	□ Crunchiness (by tasting)	□ Cohesiveness (by tasting)
□ Chewiness (by tasting)	□ Hardness (by tasting)	□ Size
□ Aroma		

7. What did you dislike about this product? Please check all that apply.

□ Appearance	□ Surface color	□ Color brightness
□ Hardness (by touching)	□ Crispiness (by tasting)	□ Rice flavor
□ Green gram flavor	□ Mouthfeel	□ Just-about-right of hardness intensity (by touching)
□ Just-about-right of crispiness intensity (by touching)	□ Sweet taste	□ Sour taste
□ Salty taste	□ Just-about-right of taste intensity (by tasting)	□ Bitter taste
□ Balanced	□ Crunchiness (by taste)	□ Cohesiveness (by tasting)
□ Chewiness (by tasting)	□ Hardness (by tasting)	□ Size
□ Aroma		