

Discovery, The Student Journal of Dale Bumpers College of Agricultural, Food and Life Sciences

Volume 19

Article 7

Fall 2018

Nutraceutical snack prepared from sprouted rough rice and green gram and its physicochemical properties and in vitro glycemic index

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Recommended Citation

Browder, R., Hettiarachchy, N., & Horax, R. (2018). Nutraceutical snack prepared from sprouted rough rice and green gram and its physicochemical properties and in vitro glycemic index. *Discovery, The Student Journal of Dale Bumpers College of Agricultural, Food and Life Sciences*, 19(1), 6-15. Retrieved from <https://scholarworks.uark.edu/discoverymag/vol19/iss1/7>

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Nutraceutical snack prepared from sprouted rough rice and green gram and its physicochemical properties and in vitro glycemic index

Cover Page Footnote

Rachel M. Browder is a May 2018 Honors program graduate with a major in Food Science. Navam Hettiarachchy, a faculty mentor, University Professor, Department of Food Science in the Bumpers College of Agricultural, Food and Life Sciences. Corresponding Author Ronny Horax, Post-Doctoral Research Associate, Department of Food Science, in the Bumpers College of Agricultural, Food and Life Sciences.

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Meet the Student-Author

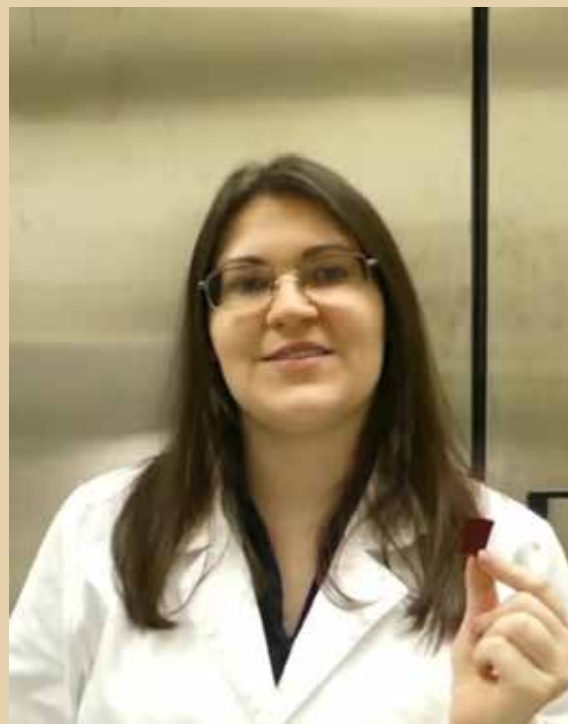


Rachel Browder

I am from Frisco, Texas. After graduating from high school in 2015, I attended Collin Community College, before transferring to the University of Arkansas in the summer 2016, where I graduated in May 2018 with a major in Food Science. While most of my time spent at the University was dedicated to my honors research project, I also had the opportunity to be part of the Food Science Club and two national food product development competitions. As a student, I interned with the food and beverage industry and helped develop protein beverage stabilization solutions with a special emphasis on protein hydrocolloid interactions. After graduation, I plan on working in the food and beverage industry in research and development, product development, or technical sales. I would like to thank Dr. Navam Hettiarachchy for serving as my honors mentor and advisor for this project and I wish to recognize her tireless persistent efforts and motivation, and Dr. Han-Seok Seo and Dr. Nick Anthony for serving as committee members. I would also like to acknowledge Dr. Ronny Horax for his help and technical assistance in completing this project.

Research at a Glance

- This study aims to develop a healthy and nutritional snack chip from germinated rice and germinated green gram (a combination that will have the 8 essential amino acids in right proportions) for health and wellness.
- Rough rice and green gram were germinated and then their nutritional composition, anti-nutrient components, and glycemic index (indicator of how foods affect blood sugar level) were evaluated. A lower glycemic index was observed.
- These chips are low in carbohydrates, rich in protein and nutrients, and may make a healthy alternative snack food.



Rachel holding a snack chip made from cranberry for a national product development competition.

Undergraduate Research Articles

Nutraceutical snack prepared from sprouted rough rice and green gram and its physicochemical properties and in vitro glycemic index

*Rachel M. Browder**, *Navam Hettiarachchy*[†], and *Ronny Horax*[§]

Abstract

Snacks make up a large portion of U.S. meals, but unhealthy snacks are a concern that can lead to being overweight or obese. Healthy alternatives can be germinated cereals and legumes, which undergo chemical compositional changes producing smaller size molecules for easier digestion and generate bioactives that can have health benefits. The objective of this research was to develop a healthy, nutritional snack chip from germinated rough rice and germinated green gram that will be easier for the body to digest, and provide much higher protein than conventional chips or crackers with low glycemic index. Rough rice and green gram were germinated for 1, 3, 5, and 7 days. There was a significant difference ($P < 0.05$) in the changes in nutrient composition, antinutrient (trypsin inhibitor and lipoxygenases) activities, and physical properties: increase of protein and lipids, decrease of starch, change in water activity, decrease in trypsin inhibitor, lipoxygenase-1, and lipoxygenase-3 activity, and decrease in glycemic index. The results indicate that snack chips prepared using sprouted rough rice and green gram are a healthier alternative to the snack chips currently on the market and have the potential for marketing and an impact on wellness.

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Introduction

People all over the world are changing their eating habits; many people are no longer eating the traditional three meals a day. For example, in the United States in 2016, snacks represented more than 50% of all eating and drinking occasions (Hartman, 2016). 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).

Germinating cereal grains is a way to reduce its anti-nutrients—such as lipoxigenase and trypsin inhibitor—which interfere with the human body's ability to digest grains (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 (Tortayeva et al., 2014). However, cereal grains do not form a complete nutritional protein due to the absence of 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.

Lysine and methionine, which are the limiting essential amino acids in rice and green gram, respectively, supplement each other and make a complete protein. Not only is the protein content of germinated rough rice higher than the protein content of brown rice, but the lipid content, γ -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 (Tortayeva et al., 2014; Kim et al., 2012; Lee et al., 2007; Moongngarm and Saetung, 2010; Saman et al., 2008).

The anti-nutrients in green gram greatly limit its 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

anti-nutrients 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).

Since 2016, more than 50% of the U.S. daily meals are made up of snacks, and the snacking industry sells hundreds of billions of dollars of snacks each year (Hartman, 2016; Nielson, 2014). 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, that still meets consumer demand for more nutritious and innovative snacks using local ingredients.

Materials and Methods

Rough rice was provided by Riceland Foods (Stuttgart, Arkansas, U.S.) 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, Pennsylvania, U.S.), Sigma Aldrich (St. Louis, Missouri, U.S.), and Fisher Scientific (Pittsburgh, Pennsylvania, U.S.).

Germination, Drying, Dehulling, and Milling

Rough rice (RR) (~100 g) was weighed, rinsed with deionized (DI) water, placed in a water bath (34 °C), and soaked for approximately 24 h in order to soften the hull. The softened RR was placed on the paper hydrated towels in a tray, sprayed with DI water, covered with a tray, and incubated in a humidifier (Hotpack, Philadelphia, Pennsylvania, U.S.) (27 °C, 100% humidity). The RR was germinated for a period of 7 days and germinated sprouts were collected at 1, 3, 5, and 7 days. The green gram (GG) underwent the same process as the RR, except for the soaking time was 2 h. The soaked GG (SGG) then went through the same procedure as the germinated RR (GRR).

The soaked RR (SRR), SGG, GRR, or germinated GG (GGG) in a metal tray were dried in an oven (Equatherm 267-914, Curtin Matheson Scientific Inc., Houston, Texas, U.S.) at 37 °C (~24 h), cooled, and refrigerated.

The sprouts were removed from GRR before being dehulled (STHU-35S Rice Huller, U-SHINE). The dehulled GRR combined with its sprouts and GGG were separately ground using a mill (Ika Universal Mill M20, Tekmar Company, Mason, Ohio, U.S.), and sifted through a 60-mesh strainer to obtain uniform particle size flours.

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 the

oven at 110 °C (~5 h) to constant weight. The percentage of moisture content was calculated as:

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

Protein Content of the Flours

The Kjeldahl Method 46-13.01 (AACC, 1990) was used to determine the protein. Each flour (~0.5 g) was digested with concentrated sulfuric acid, H₂SO₄ (5 mL), with Kjeldahl catalyst (0.5 tablet) using a digestion heater unit (Labconco 60011, Labconco Corp., Kansas City, Missouri, U.S.). Sodium hydroxide (NaOH) (40% w/v, 10 mL) was added to the digested sample (5.0 mL) and distilled using a Distillation unit (Labconco Corp., Kansas City, Missouri, U.S.) and 4% boric acid, H₃BO₃ containing methyl red/bromocresol green as an indicator was used as the receiver solution. The released ammonia, NH₃, was titrated with hydrochloric acid, HCl, and the nitrogen content was calculated as:

% Nitrogen =

$$\frac{\text{vol. HCl (mL)} \times M \text{ of HCl} \times \text{atom. wt. nitrogen} \times F}{\text{Mass dried flour (mg)}} \times 100$$

% Protein =

nitrogen-to-protein (N:P) conversion factor × % Nitrogen

where F was a dilution factor of 5 and the N:P conversion factors of 6.25 for RR (Tortayeva et al., 2014) and 6.40 for GG (Estrella, 2008) were used to calculate the percent protein content.

Lipids Content of the Flours

The soxhlet extraction procedure by the AACC (1990) was followed. A flour sample (2.0 g) in a Whatman filter paper No. 4 was placed in a thimble and a soxhlet apparatus was used for extracting the lipids. The petroleum ether that contained soluble lipid in the soxhlet was distilled to remove the petroleum ether. The lipid content was calculated using the equation:

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

Starch Content of the Flours

The AACC Method 76-13.01 (AACC International, 1999) was used to determine the starch content. A flour sample (~100 mg) was placed in a centrifuge tube with aqueous ethanol (80% v/v, 10 mL) and incubated for 5 min (80–85 °C). The tube was centrifuged for 10 min at 1800 g (~3000 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 vor-

tex 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 incubated in the water bath (100 °C) for 6 min with stirring. The tube was then placed in the water bath at 50 °C, amyloglucosidase (0.1 mL, 3300 U/mL) was added, vortexed, and incubated for 30 min (50 °C). The contents of the tube were transferred into a 100-mL volumetric flask and made up to 100 ml with distilled water. An aliquot of this solution was centrifuged at 3000 rpm (~1800 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 percent Starch was calculated using the following formula:

$$\begin{aligned} \text{Starch (\%)} &= \Delta_A \times F \times \frac{FV}{0.1} \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180} \\ &= \Delta_A \times \frac{F}{W} \times FV \times 0.9 \end{aligned}$$

where ΔA 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.

Water Activity of the Flours

A dew point water activity meter (AquaLab) was used to determined water activity (aw). The aw was automatically measured and recorded.

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 was prepared (140 mg), and Tween 20 (140 mg), DI water (8 mL), and NaOH (0.55 mL, 1.0 N) were added and diluted to 50 mL using DI water. 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 incubated (25 °C, 2 h) and 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 Model UV-1601, Kyoto, Japan) at ambient temperature and at the wavelength of 234 nm and 280 nm for lipoxygenase-1 and -3 activity determination, respectively. The non-germinated RR (NGRR) and non-germinated GG (NGGG) controls were set as 100%. The lipoxygenase-1 and -3 activities were calculated using the following formula:

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

Using AACC (1990) method 22-40.01 with modifications, 60-mesh flour (1 g) was added to NaOH (50 mL, 0.01 N, pH 8.4) and stirred for 3 hours. 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) was added into the sample solution and placed in the 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 NGRR flour (NGRRF) and NGGG flour (NGGGF) controls were set as 100%. The trypsin inhibitor activity was calculated using the following equation:

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

In vitro Glycemic Index of the Flours

The protocol described by Goñi 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 the water bath (40 °C) for 1 h 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 the water bath (37 °C). Every 30 min up to 3 h, an aliquot (1 mL) was taken and placed in the 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, St. Louis, Missouri, U.S.) 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:

$$\text{GI} = 0.862 * \text{HI} + 8.198.$$

Preparation of Snack Chips

The moisture, protein, lipids, and starch content, the trypsin inhibitor and lipoxygenase-1 and lipoxygenase-3 activity, and GI 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 (flour to water), baking soda (1.2%), and salt (1%) to form a dough, kneading, pressing and stretching until well mixed and passed through a pasta maker until ~1 mm. The flattened dough was cut into 2 × 2 cm chips and baked in an oven at 149 °C for 8 mins. The above process was repeated for the NGRRF and NGGGF, which served as the control snack chips (CSC).

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 analysis of variance utilizing JMP 13 Pro 2016 (SAS Institute Inc., Cary, N.C.). The values represented the means ± the standard deviation (SD) of each sample in triplicate. When a significant difference ($P < 0.05$) occurred, Student's *t*-test was performed to compare the means and differences considered significantly different ($P < 0.05$).

Results and Discussions

Proximate Nutrient Composition of the Rough Rice Flours

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 1). By day 5 and day 7, the protein content (%) had increased to 10.8% and 11.6%. 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 influence the

growth development in their hosts through fixation of N_2 (Hardoim et al., 2012). The increase in lipids could be due to the synthesis of structural lipids occurring during germination (Ching, 1972). 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. 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. The lower water activity relates to a higher amount of water being bound.

Proximate Nutrient Composition of the Green Gram Flours

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 2). This increase in protein throughout 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 nitro-

Table 1. Proximate nutrient composition (on dry weight basis) of non-germinated (NGRRF), soaked (SRRF), and germinated rough rice flours (GRRF).

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

[†] NGRRF = control non-germinated rough rice without soaking before being processed into flour.

[‡] SRRF = control non-germinated rough rice underwent soaking [water bath (34 °C), 24 h] before being processed into flour.

[§] Rough rice underwent soaking [water bath (34 °C), 24 hr] before being germinated and then processed into flour (GRRF).

[¶] Values are mean ± SD of triplicate analysis. Mean values followed by different letters in the same column are significantly different ($P < 0.05$).

Table 2. Proximate nutrient composition (on dry weight basis) of non-germinated (NGGGF), soaked (SGGF), and germinated green gram flours (GGGF).

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

[†] NGGGF = control non-germinated green gram without soaking before being processed into flour.

[‡] SGGF = control non-germinated green gram underwent soaking [water bath (34 °C), 2 h] before being processed into flour.

[§] Green gram underwent soaking [water bath (34 °C), 2 h] before being germinated and then processed into flour (GGGF).

[¶] Values are mean ± SD of triplicate analysis. Mean values followed by different letters in the same column are significantly different ($P < 0.05$).

gen-containing components, and takes photosynthesis-derived sugars and other nutritional factors from the sprouts (Glover and Lindemann, 2015). 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). The starch content decreased throughout the 7-day germination process, possibly as the radicles and plumules converted the starch into energy. 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. The moisture content of the 5-day and 7-day green gram flour was significantly different ($P < 0.05$). The water activity of the GGGF were all lower than the NGGGF (0.51), with the lowest being the SGGF (0.42).

Antinutrients of the Rough Rice Flours

In the RRF, the trypsin inhibitor activity (% , $P < 0.0001$), lipoxygenase-1 activity (% , $P < 0.0001$), and lipoxygenase-3 activity (% , $P < 0.0001$) had an overall significant difference ($P < 0.05$); all results are compared to the NGRRF, which was set at 100% (Table 3). Throughout the germination process of the RR, the trypsin inhibitor activity decreased from the NGRRF (100%) to 90.3% at the 7th day of germination. The 7-day GRRF had the lowest percentage of lipoxygenase-1 activity (62.6%) and lipoxygenase-3 activity (56.1%) followed by the 5-day GRRF (76.9% and 74.6% for lipoxygenase-1 and lipoxygenase-3 respectively). The decrease in the trypsin in-

hibitor, 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

The trypsin inhibitor activity (% , $P < 0.0001$), lipoxygenase-1 activity (% , $P < 0.0001$), and lipoxygenase-3 activity (% , $P < 0.0001$) of GGF had an overall significant difference ($P < 0.05$); all results are compared to the NGGGF, which was set at 100% (Table 4). The greatest percentage in decrease of the lipoxygenase-1 and lipoxygenase-3 activities occurred in the 7-day GGGF (78.9% and 63.6% for lipoxygenase-1 and lipoxygenase-3 respectively) followed by the 5-day GGGF (85.5% and 76.6% for lipoxygenase-1 and lipoxygenase-3 respectively). 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

The rough rice flour samples had overall significantly different ($P < 0.0001$) in vitro GI (Table 5). The in vitro GI among the NGRRF (49.46), the SRRF (49.32), and the 1-day GRRF (48.81) 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

Table 3. Trypsin inhibitor and lipoxygenase-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) [†]	100.0 ± 0.0 a [¶]	100.0 ± 0.0 a	100.0 ± 0.0 a
0-day (SRRF) [‡]	99.3 ± 0.6 ab	94.3 ± 1.6 b	95.7 ± 2.1 a
1-day [§]	99.2 ± 0.2 bc	92.4 ± 1.6 bc	92.4 ± 4.5 a
3-day [§]	97.1 ± 0.1 c	89.9 ± 1.3 c	83.1 ± 1.3 b
5-day [§]	94.8 ± 0.8 d	76.9 ± 1.6 d	74.6 ± 1.0 c
7-day [§]	90.3 ± 0.7 e	62.6 ± 1.7 e	56.1 ± 3.2 d
P-value	< 0.0001	< 0.0001	< 0.0001

[†] NGRRF = control non-germinated rough rice without soaking before being processed into flour.

[‡] SRRF = control non-germinated rough rice underwent soaking [water bath (34 °C), 24 h] before being processed into flour.

[§] Rough rice underwent soaking [water bath (34 °C), 24 h] before being germinated and then processed into flour (GRRF).

[¶] Values are mean ± SD of triplicate analysis. Mean values followed by different letters in the same column are significantly different ($P < 0.05$).

GRRF, and 5-day GRRF. The 5-day GRRF had the second lowest in vitro GI and was significantly different ($P < 0.05$) than the other rough rice flour samples. A lower GI indicates a slower digestion of the food.

The green gram flour samples had overall significantly different ($P < 0.0001$) in vitro GI (Table 5). 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 5-day GGGF. The 5-day GGGF had the second lowest in vitro GI and was significantly different ($P < 0.05$) than the other green gram flour samples.

In vitro Glycemic Index of the Snack Chips

The control snack chips (CSC) were found to have a higher in vitro GI (48.48 ± 0.17) and were significantly

Table 4. 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) [†]	100.0 ± 0.0 a [¶]	100.0 ± 0.0 a	100.0 ± 0.0 a
0 day (SGGF) [‡]	98.8 ± 0.4 ab	98.9 ± 1.7 a	98.7 ± 3.2 a
1-day [§]	97.8 ± 1.4 b	97.6 ± 1.6 a	95.8 ± 2.9 a
3-day [§]	91.9 ± 1.3 c	91.2 ± 1.7 b	90.0 ± 3.7 b
5-day [§]	85.1 ± 0.8 d	85.5 ± 0.5 c	76.6 ± 3.1 c
7-day [§]	76.1 ± 1.1 e	78.9 ± 2.0 d	63.6 ± 2.8 d
<i>P</i> -value	< 0.0001	< 0.0001	< 0.0001

[†] NGGGF = control non-germinated green gram without soaking before being processed into flour.

[‡] SGGF = control non-germinated green gram underwent soaking [water bath (34 °C), 2 h] before being processed into flour (GGGF).

[§] Green gram underwent soaking [water bath (34 °C), 2 h] before being germinated.

[¶] Values are mean ± SD of triplicate analysis. Mean values followed by different letters in the same column are significantly different ($P < 0.05$).

Table 5. In vitro glycemic index of non-germinated (NGRRF), soaked (SRRF), and germinated rough rice flours (GRRF) and non-germinated (NGGGF), soaked (SGGF), and germinated green gram flours (GGGF).

Germination	Rough Rice Flour [†]	Green Gram Flour [†]
0 day (NGF) [‡]	49.46 ± 0.39 a [#]	47.38 ± 0.13 a
0 day (SF) [§]	49.32 ± 0.59 a	47.55 ± 0.17 a
1-day [¶]	48.81 ± 0.33 ab	47.44 ± 0.26 a
3-day [¶]	48.22 ± 0.27 b	46.67 ± 0.14 b
5-day [¶]	47.57 ± 0.15 c	46.22 ± 0.24 c
7-day [¶]	46.48 ± 0.32 d	45.44 ± 0.08 d
<i>P</i> -value	< 0.0001	< 0.0001

[†] *In vitro* Glycemic Index (GI) of the flours were calculated using the best-curve fit equations and white bread (94.61 ± 0.00) as a reference.

[‡] NGF = control non-germinated rough rice and green gram without soaking before being processed into flour.

[§] SF = control non-germinated rough rice underwent soaking [water bath (34 °C), 24 h] and green gram underwent soaking [water bath (34 °C), 2 h] before being processed into flour.

[¶] Rough rice underwent soaking [water bath (34 °C), 24 h] and green gram underwent soaking [water bath (34 °C), 2 h] before being germinated and then processed into flour (GRRF, GGGF).

[#] Values are mean ± SD of triplicate analysis. Mean values followed by different letters in the same column are significantly different ($P < 0.05$).

different ($P = 0.0004$) than the sample snack chips (SSC), whose in vitro GI was 46.64 ± 0.22 . 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. Based on its GI value, this snack chip produced from germinated mung bean and rice could be considered as a low-GI food which is classified for the foods with the IG value of <55 (Famakin et al., 2016).

Conclusions

The protein (%) and lipids (%) content of germinated rough rice and germinated green gram was significantly different ($P < 0.0001$) overall 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 were 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 increase in the nutritional value of the GRRF and the GGGF compared to the RRF and the GGF control give optimal conditions 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.

Acknowledgements

This research was made possible by a State Undergraduate Research Fellowship (SURF) and Dale Bumpers Undergraduate Research and Creative Project Grant.

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