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Functional peanut butter stuffed snack development based on Brazilian pine (*Araucaria angustifolia)* and rice flours

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Abstract: This study aimed to develop peanut *butter* stuffed snacks (PBSS)based on Brazilian pine and rice flours by extrusion cooking and evaluating its nutritional composition, shelf life and prebiotic potential. Base blend (Brazilian pine and whole rice flour, refined sugar, soy protein extract, sodium chloride and sodium bicarbonate) were adjust to 14 % moisture and processed in a co-rotating twin-screw extruder. Afterwards, the snacks were handling stuffed with peanut smooth. PBSS was assessed both by nutrition composition (moisture, protein, ash, lipids, fiber, carbohydrate, and sodium) and water activity. Food safety was assessed by count of coliforms group, Bacillus cereus, coagulase positive Staphylococcus, sulfite-reducing Clostridium and Salmonella sp. In vitro assessment of PBSS prebiotic potential was either by PBSS medium (sterile distilled water, ground PBSS, and methylene blue colour indicator) acidification or probiotic lactobacilli growth. PBSS were highlighted for low sodium and carbohydrate foodstuffs. Furthermore, PBSS had higher proportion of fiber, proteins and lipids than other gluten-free snacks available in the marketplace.In fact, PBSS is a potential food forceliac, vegan, vegetarian and low-carbohydrate diet. PBSS also show some prebiotic potential observed by PBSS medium acidification due probiotic lactobacilli activity.Therefore, PBSSconsumptioncan be associated to improving gut microbiome as well.

Keywords:gluten-free food, vegan food, extrusion cooking, prebiotic, nutritional composition.

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I. Introduction

Araucaria angustifolia is an endangered native plant from rainforest. Brazilian pine seeds is broad use as basic food in human and animaldiet and also have an important economic role, providing both job and income source of many families [1-3]. Riceis the seed of the grass species *Oryza sativa* (Asian rice) or *Oryza glaberrima* (African rice). Brazil is among the largest African rice producers and it take important part of the Brazilians' diet[4, 5].

Peanuts have been introduced into food products encouraging its production and providing protein content, unsaturated fatty acids, vitamins and minerals. Furthermore, peanuts consumption is related to avoiding heart and cardiovascular disease, mortality and stroke [6].

Gluten-free diet is recommended for celiac people to avoid inflammatory responses to gluten proteins act against the mucosa of the small bowel. In addition, this diet may also soften the gut damage devices the irritable bowel syndrome.However, most people who are adopting this lifestyle are healthy individuals because they believe in the benefits of this health diet. Therefore, the consumption of gluten-free foods has been increasing significantly in the last 30 years. A database of gluten-free product composition based on the ingredients listed was constructed based on sixty foods representative of different food categories. It was found that almost all products had high carbohydrate content, trans fat and deficient in several nutrients, including dietary[7-9].

Extrusion cooking is an innovative technology arisen in last 25 years and can stimulates the consumption of several flours by turning they into ready-to-eat foods. This technology can allow the production of many differentiated food products and can be alternative to increasing the Brazilian pine seeds consumption. [10].Moreover, extrusion cooking is a potential technology to obtain Brazilian pine snack and the combination of rice with starch-rich seeds, as in the case of chestnut, in the extrusion cooking is interesting in relation to the functional, nutritional and sensory properties [11, 12].

Nut and fiber products consumption is clearly related to human health outcomes, but information about prebiotic activity in Brazilian pine seed is scarce yet. Its beneficial effects have been mainly attributed to nut fatty acid profiles and content of vegetable protein, fiber, vitamins, minerals, phytosterols and phenolics [13]. Prebiotic, probiotic and symbiotic foods is related with healthy eating habits to disease and illness prevention [14, 15].

This study aimed to develop peanutbutterstuffed snack(PBSS) based on Brazilian pine and rice flours by extrusion cooking and evaluating its nutritional composition, shelf life and prebiotic potential.

II. MATERIAL AND METHODS

Material

Brazilian pine seeds were obtained from the southern Brazilian in the state of Paraná under the authorization No. 30147-5 / 2016 of the Environment Ministry. African rice was purchase in the local market (Rio de Janeiro, Brazil). Seeds were dehulled in accordance with [16], dried at 50 °C and ground (< 400 *mesh*) through a hammer mill (TREU, 95-018-B, Rio de Janeiro, Brazil).

Shelled raw peanuts were roasted using an oven at 160 °C for 40 minutes and grinding by a double-knife cutter for 8 minutes. After cooling at 70 °C, brown sugar and palm fat wereadded and quickly knead to a smooth dough.

Extrusion conditions

The extrusion was conducted using a ClextralEvolum HT25 co-rotating, intermeshing twin-screw extruder (Clextral Inc., Firminy,France) with screw diameter of 25 mm, length:diameter ratio of 40:1and ten temperature zones.The front plate assembly (die) consisted of three parts: central manifold plate, distributor plate and holder-inserts plate.

Base blend (Brazilian pine and whole rice flours, refined sugar, soy protein extract, sodium chloride and sodium bicarbonate) were adjust to 14 % moisture in the mixer (VAEMS 40-2,Venâncio, Brazil) for 30 minutes one day before extrusion. Three factors (independent parameters) were considered: percentage of flour, screw speed and temperature in the last 3 heating zone (Table no 1). Afterwards, the snacks were handling stuffed to obtained PBSS.

Table no 1. Blend of flours and extrusion parameters to obtained Brazilian pine snacks.

Treatments	Independentvariables		
	X_1	X_2	X_3
1	10	650	105
2	30	650	120
3	50	550	120

X_1 - Brazilian pine flour (%), X_2 – speed (rpm) of the extruder screw, X_3 – temperature (°C) of the last 3 heating zone.

Nutrition composition and water activity assessment

Nutritional composition was determined according to the official methods of analysis [17], as following: moisture content (Method 925.09), total nitrogen (Method 2001.11, a conversion factor of 5.75 was used to convert total nitrogen to protein content), fat content (Method 945.38), ash content (Method 923.03), dietary fiber (Method 985.29). Carbohydrate composition and total energy were calculated according with RDC No. 360 of December 23, 2003. Sodium (Na) was determined by cavity microwave. Water activity (Aw) was measured on AquaLab 4TE (Decagon Devices, USA).

Food safety and shelf life determination

Microbiological safety was assessed by count of 35 and 45°C coliforms group (MPN/g), enumeration of *Bacillus cereus* (cfu/g), coagulase positive *Staphylococcus* (cfu/g) sulfite-reducing *Clostridium* (cfu/g) and *Salmonella* sp. (presence or absence/25 g).

Shelf life determination was performed by PBSS storing both at 23 and 35°C. Five snack packets were incubated at controlled temperature.On the0, 30, 60, 90, and 120 days, the pour plate method was used to enumeration of heterotrophic bacteria and yeast/molds in Plate Agar Count (HiMedia, Mumbai, India) and Potato Dextrose Agar (Oxoid, United Kingdom) acidified to pH 3.5 with tartaric acid in accordance with ISO/TS 11133-1:2009.

Growth potential of sporogenous pathogenic microorganisms

Bacillus cereus (INCQS 3) was used as sporogenous pathogenic strain. Dilutions 1:10, 1:20, 1:50, and 1:100 (v/v) were prepared in phosphate buffer pH 7.2 (PB) and the absorbance was read in a spectrophotometer (BEL Photonics, model 1105 SP, USA) at 480 nm wavelength. Simultaneously, viable cells were enumerated by plating aliquots of decimal serial dilutions on *Bacillus cereus* agar in accordance with Mossel (HiMedia, Mumbai, India). XY graph was plotted with viable *Bacillus cereus* number (cfu/ml) and Optical Densities (OD) to get linear regression. Working inoculum were obtained by growth overnight of *Bacillus cereus* at 30 °C for 24 hours in Brain-Heart Infusion broth (HiMedia, Mumbai, India). Cell suspension were centrifuged (2K15, Sigma Laborzentrifugen, Germany) at 6000*g* for 6 minutesand washed twice with PB. Medium broth fraction was thrown out, and bacterial pellet was washed twice with PB, and finally it was added with enough PB to get 10^5cfu/ml.

Exactly 10 g of PBSS was inoculated with *Bacillus cereus* inoculum (0.1 ml) to give a final concentration of *ca* 10^3cfu/g. PBSS packets were incubated at 30°C and the *Bacillus cereus* survivability was enumerated by drop test on Mossel agar (HiMedia, Mumbai, India) on the 0, 30, 60 and 90 days. Plates were incubated at 30°C for 48 hours and the colonies (cfu/ml) were counted by a colony counter (Phoenix, EUA).

Prebiotic potential assessment

Lactobacillus rhamnosus DTA 79, *Lactobacillus paracasei* DTA 83, *SalmonellaTiphymurium*DTA 41 and *Escherichia coli* ATCC 25922 were used as probiotics and pathogenic strains. PBSS prebiotic potential was assessed either by PBSS medium acidification or probiotic lactobacilli growth. Tubes were prepared with sterile distilled water and ground PBSS (1:10) and methylene blue colour indicator (1:1000). Each tube was inoculated with*ca* 10^6cfu/ml of either probiotic or pathogenic strain. All tubes were incubated at 36°C for 72 hours. After incubation, microbial survivability was enumerated on specific agar as follows: *Lactobacillus* - MRS (HiMedia, Mumbai, India); *Salmonella* Typhimurium – Enteric Hecktoen (Merck, France), and *Escherichia coli* - Eosin Methylene Blue (Merck, France). In addition, the tubes were observed to appearance of blue colour indicating microbial activity.

Statistical analysis

At minimum, all experimental measurements were conducted in triplicate. The statistical analyses were performed using Statistic software (version 13.0). Data were subjected to analysis of variance (ANOVA) at 95% confidence level ($p < 0.05$). Significantly different data sets were classified after post-hoc comparisons using Fisher’s Least Significant Difference (LSD) and Dunnet´stest.

III. RESULT

Nutrition composition

The Table no 2 shows the nutritional composition of PBSS and Brazilian pine seedswith different percentages of Brazilian pine flour.

Table no 2. Nutritional composition of Brazilian pine seedsand PBSS.

		Percentages of Brazilian pine in snacks		
Nutritional composition in 100g*	Brazilian pine seeds	10%	30%	50%
Moisture content (g)	47.53	0.19^b	1.37^a	1.87^a
Protein (g)	3.39	16.16^a	15.87^b	15.53^c
Ash (g)	1.46	1.59^b	1.62^{ab}	1.66^a
Lipids (g)	1.48	45.27^a	42.71^b	42.43^b
Fiber (g)	3.54	5.46	5.74	5.06
Carbohydrate (g)	42.60	31.33	32.69	33.46
Energy (kcal)	197.28	597.39	578.63	577.79
Na (mg)	-	60.21^c	74.28^b	81.65^a

*values in100g^{-1} samples; means in the same column with different superscript letters are significantly different at $p < 0.05$.

The Table no 3 shows the water activity values of the PBSS added with of Brazilian pine flour (10, 30, 50 %) on the 0, 30 and 60 days.

Table 3. Water activity (Aw) of the PBSS.

Brazilian pine flour	Water activity (days)		
	0	30	60
10%	0.458^{Ac}	0.451^{Ba}	0.446^{Ca}
30%	0.526^{Aa}	0.398^{Cb}	0.434^{Bb}
50%	0.508^{Ab}	0.432^{Bc}	0.419^{Cc}

*means in the same column and line with different superscript smalls and block letters, respectively, are significantly different at $p < 0.05$.

Food safety and shelf life determination

There were neither bacterial or yeast/molds growth inPBSS, the initial microorganism population keep in the same number during 120 days of storage either at 23 or 35 °C (Figure 1). In addition, there were no presence of pathogenic or food born pathogenic potential such as *Salmonella* sp, *Bacillus cereus*, *Escherichia coli* and positive coagulase *Staphylococcus*.

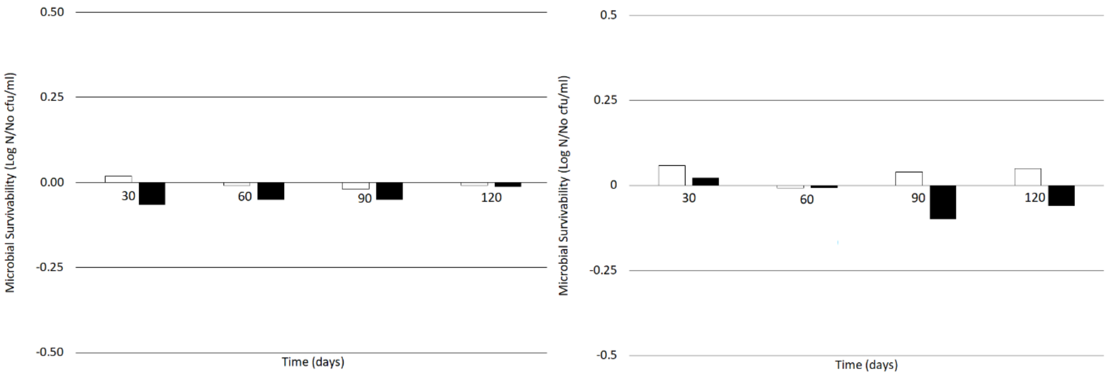

Figure 1 –Mesophilic aerobic bacteria total (□)and yeast and molds (■) survivability in peanutbutterstuffed snacks at 23 (left) and 35°C (right), respectively. Microbial enumeration (cfu/ml) cells were estimated subtracting the initial and final microbial in Plate Count Agar (HiMedia, Mambai, India) and Potato Dextrose Agar (Oxoid, United Kingdom) after 48 hours and 5 days of incubation, respectively. Initial population of mesophilic aerobic bacteria total and yeast/molds were 1,03 x 10^4cfu/g and 2,45 x 10^2cfu/g, respectively.

The Figure 2 shows the *Bacillus cereus* INCQS 3 survivability in PBSS during 90 days of storage at 30 °C.

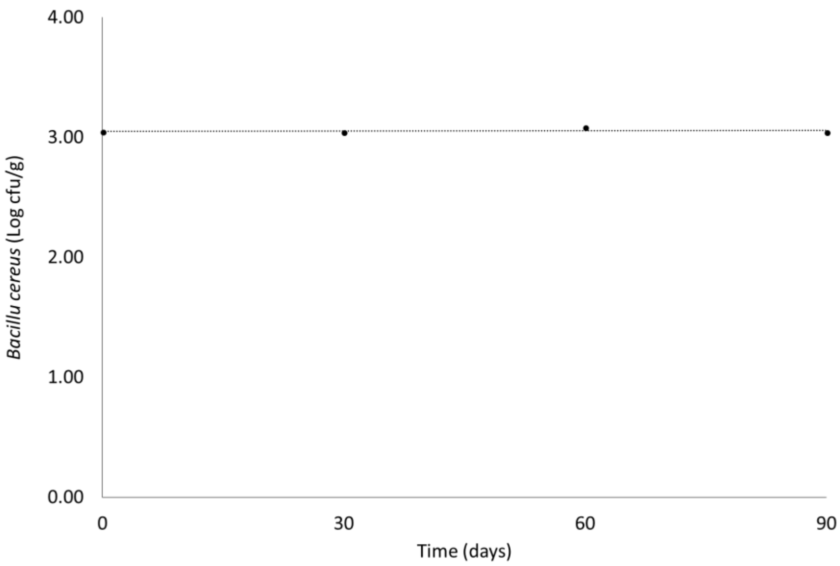

Figure 2 – Enumeration of *Bacillus cereus* INCQS 3 in peanutbutterstuffed snacks during 90 days of storage at 30°C.

Prebiotic Properties

Prebiotic potential of PBSSby *Lactobacillus rhamnosus* DTA 79, *Lactobacillus paracasei* DTA 83 growth. *Salmonella Typhimurium* DTA 41*and Escherichia coli* ATCC 25922were used as pathogenic and food born pathogenic strains to ensuring safety food(Figure 3).

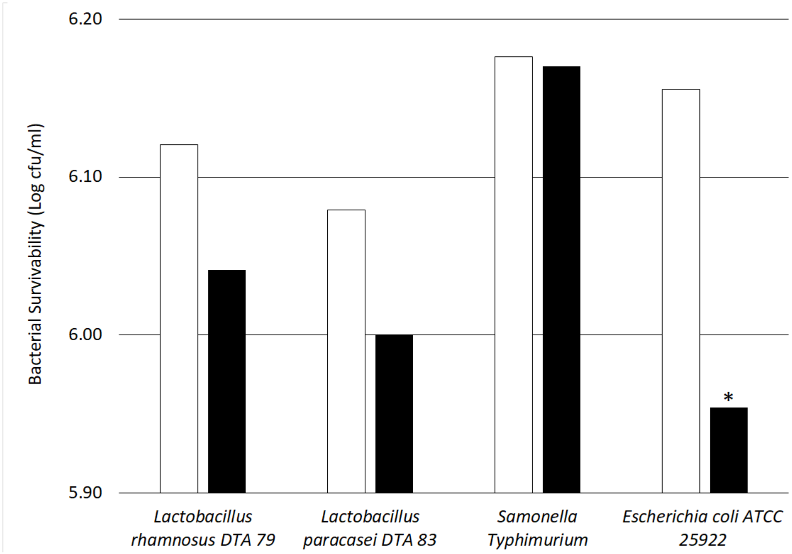

Figure 3 –Bacterial number (cfu/ml) was count before(□) and after(■) incubation of medium (sterile water, ground peanut butter stuffed snacks(1:10), and methylene blue colour indicator (1:1000)) at 36 °C for 72 hours. * indicates significant differences at a confidence level of 0.95 % by the Dunnet's test.

IV. DISCUSSION

Ratio between Brazilian pine and rice flour added in the base blend lead changes on nutritional composition of PBSS. Brazilian pine flour contributed to a higher content of ash and lipids, while rice and peanut directly affect the protein content. The overall nutritional composition of rice is: 12 % of moisture content, 7.2 % protein, 0.5 % ash, 0.6 % lipids, 0.6 % fiber, 79.7 % carbohydrate and 364 kcal [18]. Although there were no large variations, PBSS with 10% of Brazilian pine was the most attractive PBSS due low sodium. In fact, all PBSShad high proteins and fiber.

Feedingstuff intended for celiacs and healthy people should have the same content of energy, fat, protein, carbohydrates, including starch and sugars, fiber and cholesterol than ordinary foods. Brazilian pine snacks presented higher energy value when compared to the biscuits filled with chocolate.Whole-meal biscuits analyzed by [7], had highlighted by a higher protein content, due to the higher content of lipids, macromolecule representative of the peanut and palm fat of the filling. In fact, PBSSshowed lower carbohydrate content than the biscuits mentioned by [7]. In addition, most of the products showed a veryhigh Na content (> 400 mg Na / 100 g) and the PBSS were highlighted for low sodium foodstuffs (82 mg Na in 100 g).

Gluten-free and lactose-free snack, made with rice flour and filled with Brazil nut, peanut and cacao presents the following nutritional composition: energy value of 380kcal, 65.71 % carbohydrates, 3.43 % of proteins, 11.71 % of total fats, 2.0 % of dietary fiber and 68.57 mg/100 g of sodium. The PBSS based on10% of Brazilian pine flour had a lower sodium and carbohydrate content and higher proportion of fiber, proteins and lipids than gluten-free commercial snack.In fact, PBSS is a potential food for celiac, vegan, vegetarian and low carbohydrate diet consumers.

Aw is an important intrinsic parameter for microbial growth [19]. PBSS have a low Aw avoid the microbial growth. Aw is a physic property of the solvent and is dependent upon the composition and temperature of the product. It is measured by determining the water vapor pressure compared to the vapor pressure of pure water [20].

There was no *Bacillus cereus* growth in thePBSS during90 days of storage at 35°C. *Bacillus cereus* needs Aw greater than 0.95 to growth as vegetative cells. However, PBSS can serve as carry out *Bacillus cereus* sporous yet. Therefore, good manufacturing practices are still essential for the preparation of this type of food.

PBSSdid not stimulates the *lactobacilli* growth (cfu/ml). However, both *Lactobacillus rhamnosus*DTA 79and *Lactobacillus paracasei*DTA 83acidified well the PBSS medium showing some prebiotic properties. In addition, *SalmonellaTiphymurium*DTA 41 also had no growth and *Escherichia coli* ATCC 25922was significantly reduced after incubation at 36 °C for 72 hours. Starch content in the Brazilian pine snack is greatly reduced after the extrusion process while the content of slowly digestible starch is increased. However, snacks were considered prebiotic, which may have an influence on dietary fiber content [12].

V. CONCLUSION

PBSS is highlighted for low sodium and high protein content foodstuffs being a potential food to celiac and healthy diet. Withal, important beneficial togut microbiome can be related to its prebiotic potential.

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