

Gluten-Free Breakfast Cereal Prepared with Agroindustrial by-Products: Physical, Chemical, Microbiological Aspects and Sensory Acceptance

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Abstract

Develop foods that meet the needs of athletes and individuals with celiac disease and that serve as an alternative for the use of unconventional raw materials. Accordingly, the objective of this study was to develop and characterize a gluten-free breakfast cereal formulated with rice, passion fruit and milk by-products and to test whether its physical, chemical, and microbiological characteristics and sensory acceptance are adequate for commercialization. The experimental gluten-free cereal exhibited an expansion index of 2.56, specific volume of 1.6mL g⁻¹ and chromaticity coordinate a* of 7.06. It is also a source of protein (7.55 g 100 g⁻¹), has a low lipid content (0.97 g 100 g⁻¹), and is rich in dietary fiber (6.12 g 100 g⁻¹), a third of which is soluble, providing functional value to the product. In the sensory analysis, the developed product scored average on acceptance, remaining above "I neither liked nor disliked it" and "I moderately liked it", which is considered as accepted, scoring higher than 4 in all attributes and 52% on purchase intention. The use of rice, passion fruit and milk by-products was found to be an alternative for the preparation of gluten-free extruded breakfast cereal, producing a final product with high nutritional value. The cereal met the recommended daily intake (RDI) requirements for six essential amino acids according to the FAO standards and contained 85.29, 0.78 and 39.65% of the RDI for the amino acids threonine, histidine and lysine, respectively. In addition to containing no trans-fatty acids and 20% of the mono-unsaturated fatty acid requirement, one portion of the cereal meets the Fe and Zn RDI requirements for adults.

Keywords: By-products; Extrusion; Centesimal composition; Amino acids; Fatty acids

Introduction

Food processing waste and by-products are promising sources of natural compounds, such as dietary fibers, antioxidants, essential fatty acids, antimicrobials and minerals that can be used to aggregate value to food because of their technological, nutritional and functional properties. Rice (*Oryza sativa*) is one of the three most produced and consumed cereals in the world. A portion of rice production is processed, resulting in the waste named "broken rice", which generates considerable yield loss during the production of polished grain. This by-product has been transformed into rice flour, which may partially or completely substitute for wheat flour in many foods or act as the basis for new ingredients, such as mixed flours and modified starches [1-3]. According to Molina-Infante, et al. [4], gluten sensitivity is an emerging disease with unknown prevalence. Passion fruit peel flour is considered an important source of dietary fiber [5]. Individuals with high consumption of dietary fiber apparently have lower risk of developing cardiac diseases, stroke, arterial hypertension, diabetes, obesity and certain gastrointestinal diseases.

Whey originates from cheese production, a process that involves the separation of two main proteins, casein and lactalbumin, which remain in the liquid fraction that constitutes the whey. Previous studies have demonstrated that whey proteins, such as casein, are absorbed more rapidly than other proteins. Broken rice grains (BRG), passion fruit peel and whey are gluten-free by-products, which makes them of note for the preparation of food products destined for consumption by celiac or gluten-intolerant consumers. In turn, extrusion technology allows the use of mixtures of these by-products and their transformation into industrialized food ready for consumption, such as breakfast cereals. Therefore, the objective of this study was to evaluate the physical, chemical, and microbiological characteristics and sensory acceptance of an experimental gluten-free breakfast cereal formulated with BRG, passion fruit peel flour (PPPF) and whey powder (WP) to develop and evaluate a product that meets the dietary needs of patients with celiac disease and those who practice physical activities.

Materials and Methods

Raw materials

The raw materials used for the formulation of the experimental breakfast cereal were BRG (mixture of IRGA 417 and IRGA 424 cultivars), 2013 harvest, provided by the Empresa Arroz Cristal, located at Aparecida de Goiânia, Goiás, Brazil; PFPF of the brand Natural Life, obtained in the local market of Rio Verde, Goiás, Brazil; and WP, provided by Indústria de Laticínios Italc, located in Santa Helena de Goiás, Goiás, Brazil.

Breakfast cereal formulation and processing

A gluten-free breakfast cereal was prepared with a mixture of BRG, PFPF and WP (87:03:10), homogenized in a Y-type homogenizer (*Tecnal, TE 201/05, Piracicaba, Brazil*) for 15 min, and packaged at 15g/100 g⁻¹ of moisture with a manual pulverizer in low-density polyethylene (LDPE) bags and stored at 5°C for 12 h, until the time of extrusion. Thermoplastic extrusion was performed in a simple-screw extruder (*Inbramaq, PQ-30, Ribeirão Preto-SP, Brazil*). The processing conditions were defined in preliminary tests and were maintained at the following constant values: motor rotation of 250 rpm (60 Hz); circular die opening 4 mm in diameter, pre-die

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with 22 holes; screw with three entrances, 30 mm in length, and screw compression rate of 3:1; helical jacket with 335 g min⁻¹ feed rate; and temperatures in the first, second and third heating zones equal to 40, 60 and 85 °C, respectively. Then, the cereals were dried in an oven at 80°C for 40 min, cooled and packaged in LDPE bags until the analyses were performed.

Grain size of the raw materials

The grain sizes of the raw materials (BRG, PFPF and WP) were determined according to the AOAC [6] method, modifying the set of sieves, using a vibrating separator (Bertel) and a set of sieves from 10 to 100 mesh.

Chemical composition of the raw materials and breakfast cereal

The moisture was measured from the mass loss of the sample heated in an oven at 105°C to constant weight. The proteins were measured using the Kjeldahl method for the determination of total nitrogen, which was converted into crude protein by a factor of 6.38 for WP, 6.25 for PFPF and 5.25 for BRG. The lipid content was measured using the Soxhlet method; the ash content, by carbonization followed by complete incineration in a muffle at 550°C; and the total, soluble and insoluble dietary fiber, using the enzyme-gravimetric method. All methods are recommended by the AOAC [6]. The carbohydrates were calculated by the differences method, subtracting from one hundred the values of the water, ash, protein and lipid contents. The total energetic value was estimated according to the Atwater conversion values, for which the carbohydrate (minus the dietary fiber content) and protein content was multiplied by four and the lipid content by nine, and the sum of the products was calculated. To determine the fatty acids, the lipids were extracted from the breakfast cereal using the hydrolysis method, adding pyrogallol acid to minimize the oxidative degradation of fatty acids. The fat was extracted using ether, and fatty acid methyl esters (FAMES) were obtained through a reaction with boron trifluoride (BF₃) in methanol. The FAMES were quantitatively measured by capillary gas chromatography using C 11:0 as an internal standard. The saturated and monounsaturated fats were calculated by adding their respective fatty acids and were expressed as triglyceride equivalents. Monounsaturated fats included only the cis form. For the chromatographic analysis, the following parameters were used: helium carrier gas; injector temperature of 225°C, detector temperature of 285°C and initial column temperature of 100°C (isotherm) for 4 min, and final column temperature of 240°C; and rate of 3°C/min, according to the AOAC [6] method.

The minerals (Ca²⁺, P²⁺, K⁺, Mg²⁺, Fe²⁺, and Zn²⁺) were quantified by atomic absorption spectrophotometry, with instrument parameters (lamp, wavelength, lamp current and gap width) specific to each nutrient, according to the official AOAC [6] method, with nitric-perchloric digestion. The amino acid profile was determined using a chromatographer, after acid hydrolysis. A sample containing approximately 25 mg of protein was processed according to the general recommendations by White, et al. [7] and Hagen, et al. [8]. All of the analyses were performed in triplicate.

Physical characteristics of the gluten-free breakfast cereal

The water activity (Aw) of the breakfast cereal was determined at 25 ± 4°C, using an Aqua Lab CX-2 Water Activity System instrument; the instrumental color parameters, according to the system CIEL L*, a* and b*, in a colorimeter (*Color Quest, XE, Reston, USA*), with fixed hue angle at 10° and standard illuminant D65, corresponding to

natural daylight; the expansion index (EI), from the ratio between the diameter of the extruded products and the diameter of the extruder output hole (4 mm), measured with a paquimeter (*Digital Caliper, Messen, Danyang, China*); the volume, by the millet seed displacement method; the mass, in a semi-analytical scale; and the specific volume, from the ratio between the mean volume and the mass of cereals. The ultimate tensile strength, or hardness, was measured using a TA-XT2 texturometer (Stable Micro Systems, Surrey, England), with a 5-kg load cell, equipped with Texture Expert® software for data collection and analysis. The 2-cm-long samples were cut along a single axis using a probe with guillotine-type straight blade, using the methodology described by Chang, et al. [9], with a pre-test velocity of 2.0 mm s⁻¹, test velocity of 2 mm s⁻¹, post-test velocity of 5 mm s⁻¹, probe calibration distance of 5 mm, force threshold of 5 g and measurement under shear stress. All analyses were performed in 10 randomly selected cereals.

Microbiological risk and acceptance of the breakfast cereal

The coliform counts were performed at 35 and 45°C, according to APHA [10]. The sensory evaluation of the developed breakfast cereal was performed by 60 untrained tasters of both genders, ages 18 to 60 years. The seven-point hedonic scale acceptance test (7 = I really liked it; 6 = I liked it a lot; 5 = I moderately liked it; 4 = I neither liked nor disliked it; 3 = I moderately disliked it; 2 = I disliked it a lot; 1 = I really disliked it) was applied, and it was established that for the acceptance of the breakfast cereal, the limit score would be 4. Initially, each taster received the sample, served in a disposable plate, and a cup of cold milk (50 mL), so each taster could evaluate the breakfast cereal mixed or not with the milk, according to his or her preference. The sample was evaluated according to the attributes color, aroma, texture, flavor, and purchase intention. For this purpose, a five-point structured scale (1 = I would certainly not buy it; 5 = I would certainly buy it) was used [11]. The study was submitted to and approved by the Committee of Ethics in Research of the Goiano Federal Institute (IF Goiano) under protocol n. 040/2013. The acceptance test participants signed an informed consent form confirming they were instructed as to the study purpose, as well as the risks and benefits.

Data analysis

The data were analyzed using analysis of variance (ANOVA), and the means were compared by Tukey's test at 5% probability, using the program Assisat (2013), version 7.6.

Results and Discussion

Grain size of the raw materials

The grain size analysis of the WP indicated that all particles were smaller than 0.297 mm, with almost one fifth of the sample consisting of fine grains (<0.147 mm). The largest grain size was observed for BRG, for which almost all particles were larger than 0.841 mm because this raw material was not ground before the extrusion step to facilitate gravity feeding to the equipment [12]. The PFPF consisted of intermediate particles between 0.297 and 0.595 mm. Grain size irregularity can negatively affect physical characteristics, such as the hardness and expansion of extruded products, whereas homogeneity promotes adequate, uniform cooking of the raw material during the extrusion process, preventing hardness and partial cooking. Such problems can lead to undesirable particles with different cooking degrees, compromising the quality of the extruded product's appearance and palatability [13]. However, the grain size irregularity of the raw materials in this study did not negatively affect the expansion, texture or appearance of the breakfast cereals produced.

Chemical composition of the raw materials and breakfast cereal

The low moisture values found for the by-products are desirable (Table 1) because moisture levels lower than 14 g 100 g⁻¹ avoid microbial development and increase chemical and enzymatic stability and the useful life of products [14]. The centesimal composition of the WP was similar to the values reported by the technical specification sheet of the dairy company that supplied the product, which established as standards a maximum of 3 g 100 g⁻¹ of moisture and 8.5 g 100 g⁻¹ of ashes, minimum of 11.0 g 100 g⁻¹ of protein, and maximum of 1.5 g 100 g⁻¹ of lipids.

The ash content of BRG was 142% higher than that found for rice flour by Carvalho, et al. [15], whereas the lipid content was similar, and the protein content was 13.3% lower. The moisture, ash, protein and lipid contents of PFPF were, respectively, 6%, 19%, 24% and 8.5% lower than the values found by Souza, et al. [16], whereas the ash, protein and lipid contents were, respectively, 7.3%, 14% and 58.6% higher than those found by Vernaza et al. [5]. PFPF is a food rich in fiber, of which 77.24% was insoluble and 27.76% was soluble (Table 1). The insoluble fraction is related to an increase in fecal matter, therefore ensuring intestinal peristalsis, avoiding constipation and eliminating the risk of hemorrhoids and diverticulitis. The soluble fraction, in turn, has beneficial effects on insulin metabolism and cholesterol and can be consumed by diabetics because it exerts a hyperglycemic effect by delaying gastric emptying, therefore reducing intestinal transit and glucose absorption [17]. Because the amount of soluble dietary fiber in PFPF is high, it is classified as a functional food. The WP had the highest energy value among the by-products, as it is an efficient source of calories for the biological functions of the body; further, it provides a sweet flavor to the breakfast cereal. When present in the formulation of the breakfast cereal, WP increased the moisture content after extrusion, most likely because lactose is capable of retaining water in the product due to its hygroscopic character. The high ash content of the breakfast cereal was caused by the presence of PFPF and WP, which also contain high ash contents (Table 1).

The lipid content of the breakfast cereals was low, reflecting the low content of this compound in the raw materials. Compared to the values found by Silva, et al. [18] in manioc starch and whey powder breakfast cereal (0.7574g 100 g⁻¹), the lipid content in the breakfast cereals produced in this study was only 26% higher. The gluten-free breakfast cereal made with rice, passion fruit and milk by-products may be considered a protein food (Table 1). The fortification of extruded products with proteins from selected sources can improve health and promote the quality of snacks and breakfast cereals [19]. Fibers belong to the group of biologically active compounds, and their consumption is of fundamental importance to health. The gluten-free breakfast cereal is considered to have "high fiber content" (Table 1). In turn, the incorporation of fibers in breakfast cereals may cause texture problems, thus decreasing consumer acceptance. These texture problems are partially caused by deterioration of the product's microstructure, one of the primary quality attributes of extruded breakfast cereals [20]. The carbohydrate content and the total energy value obtained in the breakfast cereal were expressive. The gluten-free breakfast cereal exhibited an adequate balance of essential amino acids when compared to the recommendation by the FAO/WHO [21] for adults (Table 2), most likely because of the addition of WP and BRG. However, contents of the amino acids threonine, histidine and lysine remained lower than the values recommended by the FAO/WHO [21] for this product. Haraguchi, et al. [22] reported that whey soluble proteins have an excellent amino acid profile, characterizing

them as having high biological value because of their bioactive peptides, which confer different functional properties. The different amino acids, especially those with branched chains, favor anabolism as well as protein catabolism reduction, promoting muscle gain and reducing the loss of muscle mass during weight loss. Studies have demonstrated that only the essential amino acids, especially leucine, are necessary to stimulate protein synthesis. The gluten-free breakfast cereal had a higher content of unsaturated fatty acids than that of saturated fatty acids (Table 3). This is a positive relationship because saturated fatty acids increase cholesterolemia, reducing hepatic receptors and consequently inhibiting plasma removal of LDL, whereas unsaturated fatty acids exert protecting effects and can reduce LDL and triglyceride levels in the blood [23]. Polyunsaturated fatty acids play an important role in many physiological processes, and because they are not synthesized by the human body, they must be supplied by food. Minerals are important for many physiological functions of the human body. More than 100 mg of minerals (Na, Mg, K, Ca, P, and Cl) and less than 100 mg of microminerals (Fe, Cu, and Zn) are necessary to meet the recommended daily intake (RDI) [24]. According to the RDI [25] of minerals for adults, the necessary amount of calcium and phosphorus is 800 mg, potassium 1,950 to 5,900 mg, magnesium 300 mg, iron 14 mg, and zinc 15 mg. Thus, the amounts of iron and zinc present in the gluten-free breakfast cereal meet the daily intake value for adults, considering 100 g of cereal consumed daily. According to Lacerda, et al. [26] iron deficiency is one of the main public health

Component ¹	BRG	PFPF	WP	Breakfast Cereal
Moisture ²	10.52 ± 0.06	5.73 ± 0.01	3.96 ± 0.02	5.38±0.02
Ashes ²	0.58 ± 0.01	6.62 ± 0.02	7.27 ± 0.02	1.38±0.01
Protein ²	8.96 ± 0.02	8.93 ± 0.01	13.50 ± 0.01	7.55±0.07
Lipids ²	0.76 ± 0.01	1.45 ± 0.01	1.38 ± 0.02	0.97±0.02
TDF ²	3.85 ± 0.01	53.94 ± 0.03	-	6.12±0.01
SDF ²	2.95 ± 0.02	14.87 ± 0.01	-	2.2±0.01
IDF ²	0.9 ± 0.05	39.06 ± 0.03	-	3.91±0.01
Carbohydrates ²	79.18	77.27	73.89	84.72
Energy value ³	359.4	357.85	361.98	353.33

¹Mean value with standard deviation; ²g.100 g⁻¹; ³kcal.100 g⁻¹;TDF (Total Dietary Fiber); SDF (Soluble Dietary Fiber); IDF (Insoluble Dietary Fiber).

Table 1: Centesimal compositions and energy values of broken rice grains (BRG), passion fruit peel flour (PFPF), whey powder (WP) and gluten-free breakfast cereal.

Amino Acid	Breakfast Cereal	FAO/WHO
Phenylalanine + Tyrosine	0.71	0.63
Leucine	0.66	0.66
Glycine	0.32	
Isoleucine	0.36	0.28
Arginine	0.58	
Alanine	0.41	
Tryptophan	0.11	0.11
Methionine + Cystine	0.28	0.25
Valine	0.49	0.35
Proline	0.36	
Serine	0.37	
Threonine	0.29	0.34
Histidine	0.15	0.19
Lysine	0.23	0.58
Taurine	<0.10	
Total amino acids	7.34	

Table 2: Amino acid profiles of the breakfast cereal (g. 100 g⁻¹) and FAO/WHO [21] essential amino acid recommendation

Fatty Acids (g.100 g ⁻¹)		Minerals (mg/kg)	
Polyunsaturated Fatty Acids	0.3	Calcium	80
Trans Fatty Acids	0	Phosphorus	190
Monounsaturated Fatty Acids	0.2	Potassium	370
Saturated Fatty Acids	0.31	Magnesium	60
Unsaturated Fatty Acids	0.49	Iron	33.16
		Zinc	17.86

Table 3: Fatty acid and mineral profile of the breakfast cereal.

problems in developing countries and is one of the main factors that cause anemia, affecting up to 46% and 48% of children and pregnant women, respectively, worldwide. In Brazil, anemia is the health problem most strongly related to micronutrient deficiency [27]. The calcium, potassium, magnesium and phosphorus contents in the breakfast cereal are below the recommended RDI for adults (Table 3); however, in a varied diet, such minerals might come from other food sources, thus reinforcing the need for a balanced diet.

Conclusions

New studies are necessary to accurately evaluate the effectiveness of gluten-free breakfast cereal components. The enrichment of food with whey protein would facilitate its consumption and reduce environmental damage; in addition, it is an excellent source of essential amino acids and is safe for consumption by those affected by celiac disease.

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Ethical Approval

Informed consent was obtained from all individual participants included in the study.

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