



# Effects of green coffee bean flour fortification on the chemical and nutritional properties of gluten-free cake

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## Abstract

The objective of this study was the application of green coffee bean flour in gluten-free cakes with different percentages (4, 8 and 15% (w/w)), to evaluate the optimal value for fortification, and the products were characterized based on their centesimal composition and bioactive compounds (caffeine and total phenolic compounds). Significant differences ( $p \leq 0.05$ ) were observed in the content of lipids, total dietary fiber, insoluble fiber, energy values, sodium, caffeine, and total phenolics, mainly in the product in which 15% (w/w) green coffee bean flour was added. Caffeine content was only detected and quantified in products with >8% (w/w) green coffee bean flour, whereas the total phenolic content was detected and quantified in products with >4% (w/w) green coffee bean flour. Thus, fortification of these products with 15% green coffee bean flour promoted a higher content of total dietary fiber and lower content of lipids, calories, sodium, and increased bioactive compounds. Thus, green coffee bean flour is an excellent alternative for the production of innovative foods.

**Keywords** Bioactive compounds · Caffeine · Food insecurity · Fortification · Gluten free cake · Green coffee flour

## Introduction

The coffee fruit process produces several by-products, such

as coffee pulp, parchment (endocarp), silver film, mucilage, spent coffee and green coffee beans [1, 2]. According to the International Coffee Organization, green coffee is defined as beans that are naked and processed before undergoing the roasting process and the seed (endosperm) is dried from the coffee fruit [3, 4]. When the coffee beans are roasted, they produce one of the most consumed beverages worldwide, with an average of 1.3 kg year<sup>-1</sup> of coffee per capita [5, 6]. In northern Europe, consumption reaches 6 kg year<sup>-1</sup>, whereas in Brazil, which is the largest producer and exporter in the world and has a tradition of growing coffee (*Coffea* sp.), consumption is 5.8 kg year<sup>-1</sup> [6, 7].

Green coffee beans contain important bioactive compounds such as the essential fatty acid linoleic (omega-6) [8, 9], which represents 46% of total free lipids and chlorogenic acids (5-O-caffeoylquinic acid), an aromatic plant metabolite. These components have various functions in the human body, including anticarcinogenic, antibacterial, and antioxidant activities, and prevent the initiation of  $\beta$ -cleavage reaction propagation (fatty acid decomposition pathway and generating oxidative rancidity) [10, 11, 12, 13, 14].

Caffeine (1,3,7-trimethylxanthine), the main methylxanthine in coffee, is an alkaloid that acts on the kidneys, cardiac and skeletal muscles, and the central nervous system,

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reducing tiredness and increasing the level of alertness [8, 15].

When green coffee is incorporated into other products, it increases the concentration of polyphenols, antioxidants, and total phenolics [10], which reduces the loss of polyunsaturated fatty acids [16], formation of acrylamide [17], and syneresis rate [18], demonstrating excellent sensory properties [19] and high bioaccessibility and bioavailability [20]. These bioactive compounds in green coffee beans are classified as functional ingredients and can be important raw materials for making nutritious and fortified foods that can solve a worldwide challenge [20, 21]. In 2019, approximately 820 million people suffered from food insecurity and with the Covid-19 pandemic, approximately 130 million people will go into extreme poverty and 265 million will be chronically hungry [22, 23].

In addition to food safety, another major global challenge of a technological nature is the development of gluten-free food products that provide sensory quality, better nutritional quality, and the potential to improve the quality of life of patients with celiac disease [24–26]. Gluten-free products do not use flours composed of two groups of proteins: gliadins and glutenins, being flours from white wheat, whole-meal wheat, spelt, barley, and rye [27, 28]. This study aims to reduce food insecurity and evaluate the centesimal composition and bioactive compounds (caffeine and total phenolic compounds) of gluten-free cake fortified with green coffee bean flour to present an alternative food for people with celiac disease.

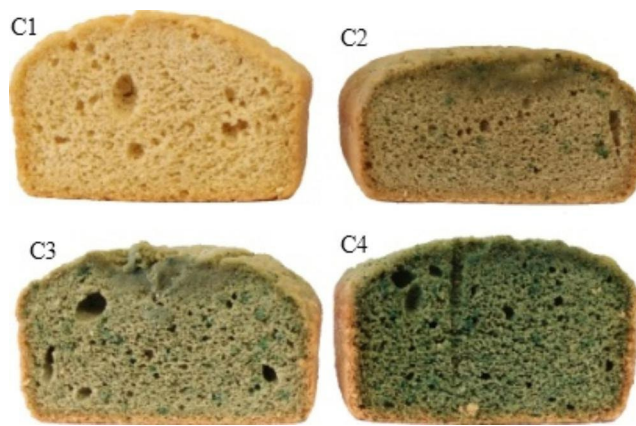
## Materials and methods

Green coffee beans (*Coffea arabica*) were purchased from the city of Americana, São Paulo, Brazil, ground in a knife mill, and sieved through a granulometric sieve (20 mesh) for standardization. Deionized water (resistivity > 18.2 M $\Omega$  cm at 25 °C) and analytical grade chemicals were used.

### Confection of cakes

Green coffee bean flour obtained from grinding was used to make the cakes. In this study was used a 45:50 ratio [29] between green coffee bean flour and rice flour in cakes made with a fortification of 4% (C2), 8% (C3), and 15% (C4) (w/w) and prepared a control cake using 100% rice flour (C1) (Fig. 1). All cakes contained equal amounts of margarine without salt (75 g), eggs (162 g), vanilla (10 g), and baking powder (12 g).

The cakes were developed at the Food Laboratory of the Technology College of Piracicaba. To prepare the cake dough, chicken eggs were transferred to a planetary mixer



**Fig. 1** Cross section of elaborated gluten-free cakes (100% rice flour (C1) and fortified with 4% (C2), 8% (C3), and 15% (C4) (w/w) green coffee bean flour)

(Kenwood, Kmix KMX50 model) and mixed (10 min). Thereafter, margarine without salt, vanilla, rice flour or green coffee bean flour, and baking powder were added and mixed to a homogeneous mass. The cakes were baked in a rectangular mold with dimensions of 10 cm x 20 cm in a Sonetto inox domestic electric oven preheated to 200 °C for 20 min. Once baked, the cakes were cooled to room temperature and wrapped in a polyethylene plastic.

All samples, C1, C2, C3, and C4, were pre-dried in an oven for 24 h at 60 °C, macerated, and used in subsequent analyses.

For the analysis of total phenolics and caffeine, extracts were prepared using 5.5% (w/v) of the samples in contact for 5 min with water at 93 °C [30].

### Analysis of centesimal composition

To determine the centesimal composition, the analytical methodology described by the Adolfo Lutz Institute was followed: the moisture content was determined in an oven at 105 °C until a constant weight was obtained [31]; the ash content was determined through muffle furnace incineration at 550 °C for 4 h or until the ash was completely white [30]; the sodium content was determined using the Mohr argentometric method [31]; the lipids or fat were determined through hot ether extraction in Soxhlet [31]; the proteins were determined using the Kjeldahl method, which was based on the determination of nitrogen and multiplication by the conversion factor (6.25) of nitrogen into protein [31]; the total dietary fiber was determined using the enzymatic-gravimetric method [32]; and the carbohydrates were determined by the difference (100 g - moisture, protein, lipid, ash, and total dietary fiber content) [31]. Energy values were determined using Atwater factors for lipids, proteins, and carbohydrates (9, 4, and 4 kcal g<sup>-1</sup>, respectively) [33].

## Chromatographic analysis of caffeine

Caffeine was analyzed using high-performance liquid chromatography (PerkinElmer<sup>®</sup>, Flexar model), with a diode array detector. A PerkinElmer<sup>®</sup> Brownie Analytical C8 column (100 mm x 4.6 mm – 5 μm) was used to inject a volume of 20 μL per sample. The mobile phase in isocratic mode, consisted of water and methanol (60:40 v/v) and a flow rate of 1.0 mL min<sup>-1</sup> on ultraviolet detection at 274 nm [31, 34]. The samples were filtered through a 0.45 μm cellulose acetate membrane and diluted 1:50 (v/v). For caffeine quantification, a calibration curve was obtained based on analytical standards of known concentrations, in triplicate, and a linear response was observed in the range of 0.50–20.0 mg L<sup>-1</sup> of caffeine and described by the following equation: Area = -3502.16 + 43615.16 C (mg L<sup>-1</sup>), linearity (R<sup>2</sup>) of 0.999, and detection and quantification limits of 0.20 and 0.50 mg L<sup>-1</sup>, respectively.

## Analysis of total phenolic compounds

Phenolic compounds were analyzed using the Folin–Ciocalteu method with gallic acid as the analytical standard and spectrophotometric absorbance measurement at 770 nm (AGILENT, model Cary 60 equipped cuvette with an optical path of 1 cm) [35]. The extract aliquot (600 μL) of the diluted sample (1:50 (v/v)) was transferred to a 15 mL Falcon<sup>®</sup> tube with 3000 μL of 10% (v/v) Folin–Ciocalteu reagent. After 5 min, 2250 μL of 7.5% potassium carbonate solution (w/v) was added. The mixture was stored at room temperature and protected from light for 40 min for subsequent spectrophotometric measurements. In the quantification of total phenolic compounds, the calibration curve was constructed based on the gallic acid standard, with known concentrations, in triplicate, and a linear response was observed in the range of 0.50–40.0 mg L<sup>-1</sup> of gallic acid and described by the following equation: A = 0.0018 + 0.0115 C

( mg L<sup>-1</sup> ), R<sup>2</sup> of 0.999, and detection and quantification limits of 0.20 and 0.50 mg L<sup>-1</sup>, respectively.

## Statistical analysis

The content of the investigated compounds (dry matter, moisture, ash, lipids, proteins, total dietary fiber, carbohydrates, energy values, sodium, caffeine, and total phenolic compounds) was calculated. The results were statistically analyzed using analysis of variance (ANOVA) and Tukey's test, and values with  $p \leq 0.05$  were considered statistically significant. The analyses were performed in triplicate, and the results are expressed as the mean ± standard deviation.

Principal component analysis (PCA) was used to group the samples according to the contents of the investigated compounds. This allowed the identification of the variables that most significantly affected the sample.

The OriginPro 2021 (Student Version 9.8.0.200) was used for analysis of ANOVA, Tukey's test, and PCA.

The limits of detection and quantification (LOQ) were estimated using the ratio of the blank standard deviation or the linear coefficient between the slope value of 3.3 and 10, respectively. Furthermore, for the LOQ (first point of the calibration curve), a relative standard deviation of less than 20% was adopted as an additional criterion to guarantee the reliability and quality of the results [36].

## Results and discussion

In Fig. 1, the cakes C1, C2, C3, and C4 presented aeration in their masses, and this aeration may be related: (I) with the use of chemical yeast, as mentioned in the cake preparation method [37]; (II) with the stirring time that the eggs remain. Because the eggs are great aeration agents, the longer the stirring time, the smaller the size of the air bubbles, and the more the amount present in the dough surrounded by the

**Table 1** Centesimal composition of the cakes on a wet basis in different compositions: 100% rice flour (C1) and fortified with 4% (C2), 8% (C3), and 15% (C4) (w/w) green coffee bean flour (n = 3)

Composition	C1	C2	C3	C4
	(g 100 g <sup>-1</sup> )			
Dry matter	61.372 ± 0.149 <sup>a</sup>	60.729 ± 0.159 <sup>a</sup>	58.608 ± 0.076 <sup>a</sup>	60.382 ± 0.063 <sup>a</sup>
Moisture	38.628 ± 0.149 <sup>a</sup>	39.271 ± 0.159 <sup>a</sup>	41.392 ± 0.076 <sup>a</sup>	39.618 ± 0.063 <sup>a</sup>
Ashes	2.923 ± 0.031 <sup>a</sup>	2.455 ± 0.019 <sup>a</sup>	2.299 ± 0.010 <sup>a</sup>	2.647 ± 0.013 <sup>a</sup>
Lipids	20.823 ± 1.609 <sup>a</sup>	20.975 ± 1.906 <sup>a</sup>	18.630 ± 3.113 <sup>ab</sup>	18.155 ± 2.466 <sup>b</sup>
Proteins	7.822 ± 0.046 <sup>a</sup>	7.678 ± 0.021 <sup>a</sup>	7.506 ± 0.265 <sup>a</sup>	7.667 ± 0.287 <sup>a</sup>
Total dietary fiber	4.669 ± 0.828 <sup>a</sup>	5.078 ± 0.517 <sup>a</sup>	6.558 ± 0.874 <sup>ab</sup>	8.471 ± 1.286 <sup>b</sup>
Soluble fiber	1.233 ± 0.824 <sup>a</sup>	0.936 ± 0.296 <sup>a</sup>	1.008 ± 0.211 <sup>a</sup>	1.143 ± 0.576 <sup>a</sup>
Insoluble fiber	3.435 ± 0.063 <sup>a</sup>	4.142 ± 0.324 <sup>a</sup>	5.549 ± 0.933 <sup>ab</sup>	7.328 ± 0.737 <sup>b</sup>
Carbohydrate available	25.145 ± 1.232 <sup>a</sup>	24.545 ± 1.799 <sup>a</sup>	23.616 ± 3.650 <sup>a</sup>	23.443 ± 2.535 <sup>a</sup>
Energy values	319.232 ± 11.025 <sup>a</sup>	317.661 ± 10.019 <sup>a</sup>	292.156 ± 15.577 <sup>b</sup>	287.831 ± 14.168 <sup>c</sup>

\*Means followed by the same letter, on the line, do not differ by Tukey's test at 95% confidence level ( $\alpha = 0.05$ )

egg proteins [38]; (III) owing to the protein content presented in the pasta, it is therefore possible that these proteins present can mimic the stable network that is formed when gluten is present, similar to the study by Krupa-Kozak et al. When using broccoli flour to make gluten-free bread, they realized that the bread exhibited aeration and could be due to the proteins from the broccoli flour [39]. It is not possible to determine the situation that was the case in this study, as cake C1 also presented aeration and was not fortified with green coffee bean flour; however, it is likely that in the case of cakes C2, C3, and C4, the flour was responsible because the studies of Guglielmetti et al., Littardi et al., and Rios et al., presented breads with aerated doughs [40–42]. However, specific studies should be conducted to assess whether the proteins present in green coffee bean flours can actually mimic the stable gluten network.

The results obtained from the analysis of cake products made with and without green coffee bean flour are summarized in Tables 1 and 2.

When evaluating the contents of dry matter, moisture, ash, proteins, soluble fiber, and carbohydrates in the cakes (Table 1), no statistically significant differences were observed, demonstrating that cakes enriched with 4, 8 and 15% green coffee bean flour did not change those characteristics in relation to the non-fortified product (C1). The change in these parameters was not noticeable because when evaluating the centesimal composition of rice flour and green coffee bean flour [43–46], we observed that these parameters have similar values. Even at the levels at which green coffee bean flour has a higher value than rice flour, the concentration of fortification is not sufficient to promote the overlap of green coffee bean flour with rice flour.

The lipid content, total dietary fiber, and insoluble fiber (Table 1), were not significantly different between products C1, C2, and C3. However, in product C4, there was a reduction of 2.7% in the lipid content, an increase of 3.8% in the total dietary fiber content, and 3.9% in the insoluble fiber content in relation to the control product (C1), mainly in relation to total and insoluble dietary fiber, which are components generally lacking in gluten-free products [47]. These characteristics were due to the addition of 15% green coffee bean flour, demonstrating the impact on these components in the final product. Furthermore, according to RDC n° 54/2012 [48], products C1 and C2 can be considered a

source of total dietary fiber, and products C3 and C4 are rich in total dietary fiber because they have contents in the range of 3–6 g and greater than 6 g of total dietary fiber in 100 g of product, respectively. Consequently, fortification with green coffee bean flour increases the dietary fiber content in gluten-free products, thus favoring the intake of this component for people with celiac diseases because gluten-free products are generally poor in this component [37, 41].

Regarding the energy value of the products (Table 1), in products C1 and C2 there were no statistically significant differences, whereas there were some differences for products C3 and C4. The largest difference was observed in product C4, which presented 31.40 kcal less than the control product (C1). This result demonstrates that when green coffee bean flour is added, it can reduce the caloric content of the cake products according to the energy values.

The sodium content (Table 2) exhibited a significant difference between all products, with the highlight being the product with the addition of 15% (C4) green coffee bean flour, where there was a reduction of 31.34% in relation to the control product (C1). In addition, product C1 can be considered to have a low sodium content, and C2, C3, and C4 are very low because they contain 80 and 40 mg of sodium in 100 g of product, respectively, according to RDC n° 54/2012 [48].

In products C1 and C2, caffeine content was not detected (Table 2), in the case of product C1, possibly because there was no addition of green coffee bean flour in the product and also the concentration present in it may be less than the detection limit, which is the possible reason for not detecting caffeine content in product C2. However, products C3 and C4, which were fortified with 8 and 15% green coffee bean flour, respectively, presented significant levels of caffeine. Notably, the products were within the regulations of Brazil [49, 50], where food products cannot exceed the maximum limit of 200 mg of caffeine; therefore, the daily intake of food products should not exceed the limit of 400 mg of caffeine. This difference is due to the daily consumption of other food sources by the Brazilian population, thus ensuring consumer safety.

The total phenolic compound content in products made with green coffee (Table 2) was not detected in C1, and no significant difference were observed among C2, C3, and C4. However, fortification with green coffee bean flour adds

**Table 2** Content of the bioactive compounds of the cakes on a wet basis in different compositions: 100% rice flour (C1) and fortified with 4% (C2), 8% (C3), and 15% (C4) (w/w) green coffee bean flour (n=3)

Composition	C1	C2	C3	C4
	(mg 100 g <sup>-1</sup> )			
Sodium	41.624 ± 0.010 <sup>a</sup>	25.745 ± 0.010 <sup>b</sup>	14.940 ± 0.010 <sup>c</sup>	10.287 ± 0.010 <sup>d</sup>
Caffeine	ND**	ND**	40.476 ± 2.009 <sup>a</sup>	58.646 ± 1.909 <sup>b</sup>
Total phenolics*	ND**	48.283 ± 3.350 <sup>a</sup>	58.120 ± 4.469 <sup>a</sup>	57.627 ± 3.353 <sup>a</sup>

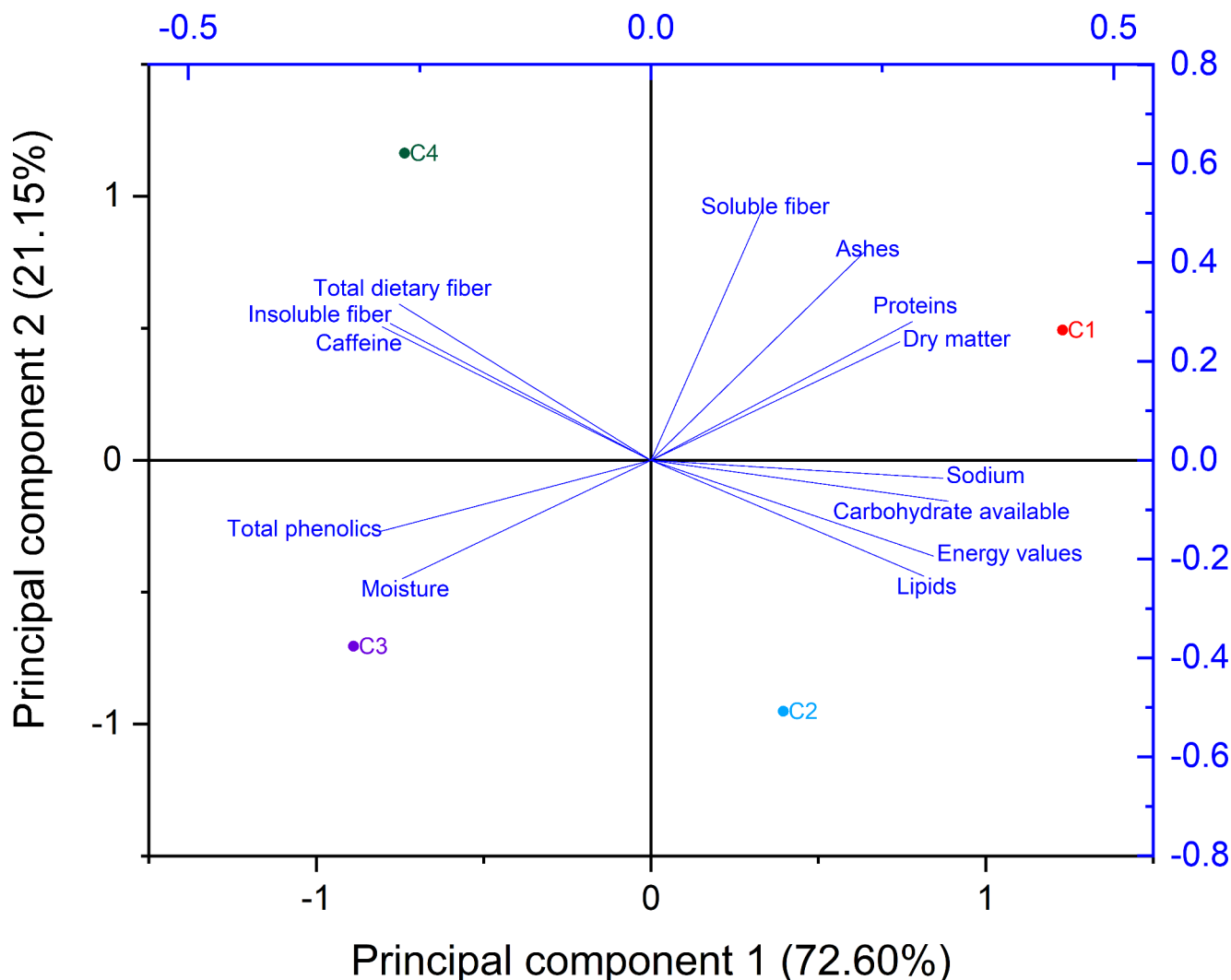
\*Results in mg of gallic acid. \*\*Not detected. \*\*\*Averages followed by the same letter, on the line, do not differ by Tukey's test at 95% confidence level ( $\alpha=0.05$ )

these chemical species to cakes, thus promoting food with better properties. The reason for the lack of significant difference among the cakes (C2, C3, and C4) may be related to the similar amount between the cakes in the parameters of proteins, carbohydrates and lipids. Because their contents are similar, it promotes the similarity of the contents of the total phenolic compounds, due to the interaction of phenolic compounds with protein, carbohydrate and lipid molecules, as these interactions protect phenolic compounds from oxidative degradation [51, 52].

Owing to the scarcity of previous studies on the application of food products made with green coffee bean flour, it was not possible to compare the results obtained in this study. However, the study conducted by Moreno et al. [53], which used 6% coffee pulp (a by-product of the coffee industry), obtained significant differences in moisture, lipid, ash and dietary fiber content in relation to the control product.

Desai et al. [2], who used 3, 6, 10, and 12% green coffee spent (a by-product of the coffee industry), obtained significant differences in moisture, ash, lipids, proteins (except for 3%), and dietary fiber (with the exception of the 3% percentage) in relation to the control product. Notably, the use of by-products from the coffee industry provides fortification of manufactured products. Recalling that these authors used wheat flour, which exhibits different properties from rice flour, this study shows the importance of studies on coffee fruit by-products applied in the fortification of food products because there are very few related studies in the literature.

Another important factor to be considered is that the compounds present in green coffee beans have several attributes such as: stimulating the central nervous system and cardiac muscles [10]; antioxidant, antimicrobial, antihypertensive, neuroprotective, anticarcinogenic, antimutagenic,



**Fig. 2** Biplot of the first two principal components that explain 93.75% of the total variation for the samples (100% rice flour (C1) and fortified with 4% (C2), 8% (C3), and 15% (C4) (w/w) green coffee bean flour). PC1 explains 71.49% of the total data variance, whereas PC2 explains 21.21%

antibacterial, anti-inflammatory, hepatoprotective, antiviral, free radical scavenger, body weight control, antipyretic and cardioprotective properties [10–13]; inhibition of fat accumulation, cyclooxygenase-2, production of nitric oxide and lipoxygenase [10, 12, 13]; action on lipid metabolism and glucose metabolism [12]; protection from DNA damage [54]; positive factor against Parkinson's and Alzheimer's disease [11, 54, 55]; and can help improve polycystic ovary syndrome [56], because they are highly absorbed and metabolized by the human body.

### Principal component analysis

In the PCA illustrated in Fig. 2, it can be observed that the first two main components (PC1: 72.60% and PC2: 21.15%) were responsible for 93.75% of the total variation. Each sample occupies a region on the graph (showing a distinction between them) and it characterized by the closest vectors.

Products C1 and C2 were best represented by the attributes: dry matter, ash, lipids, proteins, soluble fiber, available carbohydrates, energy value, and sodium. Moisture, total dietary fiber, insoluble fiber, caffeine, and total phenolics better represented products C3 and C4. However, according to Tukey's test, at 95% confidence level, dry matter, moisture, ash, proteins, soluble fiber, and available carbohydrates were not factors of high influence, because there was no significant difference among the products.

The addition of green coffee bean flour to rice flour significantly influenced the amount of lipids, total dietary fiber, insoluble fiber, energy values, sodium, caffeine, and total phenolics, particularly in the C4 product (i.e., 15% coffee bean flour addition). Calories and sodium contents were lower in products fortified with green coffee bean flour, and it was possible to add higher levels of total dietary fiber, insoluble fiber, caffeine, and total phenolics.

### Conclusions

Owing to the results obtained in this study and the numerous benefits of green coffee, 15% green coffee bean flour should be used to enrich gluten-free food products. In addition, it should be noted that the use of green coffee bean flour increased the total dietary fiber content, mainly insoluble fiber, in a gluten-free product in which these components are usually missing, thus assisting in the fortification of this macronutrient in products for people with celiac disease. Moreover, promoting an increase in bioactive compounds, such as caffeine and total phenolics, also yields a product with a lower lipid content, lower caloric value and sodium,

and it can be presented as an alternative to functional food to meet the global challenges of insecure food and technology.

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### Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

**Author statement** Winston Pinheiro Claro Gomes: Sampling, Experimental analysis and Writing. Juliana Angelo Pires – Sampling, Experimental analysis and Writing. Natalia Navarro Teixeira – Sampling, Experimental analysis and Writing. Gisele Gonçalves Bortoleto – Experimental analysis and Review. Erika Maria Roel Gutierrez – Experimental analysis and Review. Wanessa Melchert Mattos – Supervision, Review, Editing and Project administration. All authors read and approved the final manuscript.

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