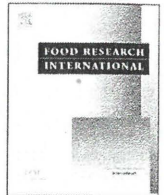


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*In vitro* digestion effect on mineral bioaccessibility and antioxidant bioactive compounds of plant-based beverages



Joyce Grazielle Siqueira Silva<sup>a</sup>, Ana Paula Rebellato<sup>a</sup>, Elem Tamirys dos Santos Caramês<sup>a</sup>, Ralf Greiner<sup>b</sup>, Juliana Azevedo Lima Pallone<sup>a,\*</sup>

<sup>a</sup> Department of Food Science, School of Food Engineering, University of Campinas, Campinas, São Paulo, Brazil

<sup>b</sup> Department of Food Technology and Bioprocess Engineering, Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Karlsruhe, Germany

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ABSTRACT

Consumption of plant-based beverages (PBB) is a growing trend; and have been used as viable substitutes for dairy based products. To date, no study has comparatively analyzed mineral composition and effect of *in vitro* digestion on the bioaccessibility of different PBB. The aim of this research was to investigate the content of essential minerals (calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn)) and to estimate the effect of *in vitro* digestion in plant-based beverages, and their antioxidant bioactive compounds (phenolic compounds and antioxidant capacity). Moreover, the presence of antinutritional factors, such as myo-inositol phosphates fractions, were evaluated. Samples of PBB (rice, cashew nut, almond, peanut, coconut, oat, soy, blended or not with another ingredients, fortified with minerals or naturally present) and milk for comparison were evaluated. TPC ranged from 0.2 mg GAEq/L for coconut to 12.4 mg GAEq/L for rice and, the antioxidant capacity (DPPH) ranged from 3.1 to 306.5 μmol TE/L for samples containing peanut and oat, respectively. Only a few samples presented myo-inositol phosphates fractions in their composition, mostly IP5 and IP6, especially cashew nut beverages. Mineral content showed a wide range for Ca, ranging from 10 to 1697.33 mg/L for rice and coconut, respectively. The Mg content ranged from 6.29 to 251.23–268.43 mg/L for rice and cashew nut beverages, respectively. Fe content ranged from 0.76 mg/L to 12.89 mg/L for the samples of rice. Zinc content ranged from 0.57 mg/L to 8.13 mg/L for samples of oat and soy, respectively. Significant variation was observed for Ca (8.2–306.6 mg/L) and Mg (1.9–107.4 mg/L) dialyzed between the beverages, with lower concentrations of Fe (1.0 mg/L) and Zn (0.5 mg/L) in dialyzed fractions. This study provides at least 975 analytically determined laboratory results, providing important information for characterization and comparison of different plant-based beverages.

1. Introduction

Plant-based beverages (PBB) have stood out and are a growing worldwide trend in the food sector, they are used as a substitute for cow's milk or as an alternative to juices and other beverages (Sethi, Tyagi, & Anurag, 2016). In addition, factors such as lactose intolerance, cow's milk allergy, heart disease (caused by high cholesterol levels), as well as vegetarian, vegan, and flexitarian diets have contributed to the increased consumption (Chalupa-Krebzdak, Long, & Bohrer, 2018; Diarra, Nong, & Jie, 2005; Mäkinen, Uniacke-Lowe, O'Mahony, & Arendt, 2015; Sethi et al., 2016). Due to the impact of this demand, food industries are investing in the development of new non-dairy products based on nuts, cereals and seeds, as well as almond, oat, rice, soy and coconut beverages, among others (MINTEL, 2016; Tecnavio,

2015)

In this context, the nutritional properties of the PBB may vary according to the raw material used, type of processing, and compounds used in fortification, among others. These factors may influence aspects such as; particle size, rheology, stability, color and composition of macro and micronutrients (Jeske & Arendt, 2018; Jeske, Zannini, & Arendt, 2017). Thus, depending on the composition of the raw material used in its preparation, bioactive substances such as phenolic compounds, fibers, flavonoids, vitamins and minerals may be present in the final product, and consequently, provide health benefits (Briviba, Gräf, Walz, Guamis, & Butz, 2016).

Studies have shown that oats, rice, cashews, Brazil nuts, and almonds are rich in phenolic compounds (45–456 mg gallic acid equivalent/100 g) and have proven antioxidant capacity (Chang,

\* Corresponding author.

E-mail address: [jpallone@unicamp.br](mailto:jpallone@unicamp.br) (J.A.L. Pallone).

Alasalvar, Bolling, & Shahidi, 2016; Gong et al., 2017; John & Shahidi, 2010; Kaur, Whitson, Ashton, Katopo, & Kasapis, 2018; Kornsteiner, Wagner, & Elmadfa, 2006). In addition to phenolic compounds and antioxidant capacity, PBB can naturally have in their composition minerals such as; phosphorus (P), potassium (K), zinc (Zn), manganese (Mn), copper (Cu), sodium (Na), selenium (Se), calcium (Ca), magnesium (Mg) and iron (Fe), which are characterized as nutrients (Felberg, Antoniassi, Deliza, Freitas, & Modesta, 2009; dos Santos, 2015). Thus, the evaluation of nutritional and functional properties (such as essential mineral content and bioaccessibility) and content of phenolic compounds are required for proper characterization of these beverages (Briviba et al., 2016; Codina-Torrella, Guamis, Ferragut, & Trujillo, 2017; Faccin, Miotto, Vieira, Barreto, & Amante, 2009; dos Santos, 2015).

Essential minerals, including Ca, Fe, Mg and Zn, have an important role in the proper functioning of the body (Quintaes & Diez-Garcia, 2015). Therefore, the ingestion of foods that contain bioaccessible minerals is essential for the body to perform vital functions. Bioaccessibility studies are evaluated by *in vitro* digestion assays, such as the dialysis method; which involves nutrient transport through a semi-permeable membrane of defined pore size, similar to the intestinal pore, and simulates the transition time of the food during digestion, with consideration to changes in pH, agitation and temperature similar to what occurs in the body (Alegría-Torán, Barberá-Sáez, & Cilla-Tatay, 2015; Miller, Schrickler, Rasmussen, & Van Campen, 1981; Sahuquillo, Barberá, & Farré, 2003). However, the bioaccessibility may be affected by different chemical forms of minerals and their interaction with vegetable components used in beverage production (Chen et al., 2005; Mäkinen et al., 2015; Zhao, Martin, & Weaver, 2005).

Some components that may be present in beverages, in addition to nutritional compounds are *myo*-inositol phosphates (IP3, IP4, IP5, IP6), considered antinutritional substances that may interfere with essential mineral absorption (Zhang, Önnings, Öste, Gramatkovski, & Hulthén, 2007). The negative effect attributed to *myo*-inositol phosphates in the absorption of some nutrients is due to six reactive phosphate groups in their chemical structure, which may form complexes with divalent cations such as  $Mg^{2+}$ ,  $Mn^{2+}$ ,  $Fe^{2+}$ ,  $Cu^{2+}$ ,  $Zn^{2+}$  and  $Ca^{2+}$  (Greiner, Konietzky, & Jany, 2006; Kumar, Sinha, Makkar, & Becker, 2010). Some compounds like oxalates, tannins, fibers, bivalent cations (in excess) and fatty acids (in excess) could also interfere in the availability of minerals. However, *myo*-inositol phosphates are one of the most intrusive compounds related to mineral absorption reduction (Amalraj & Pius, 2015; Bosscher, Van Caillie-Bertrand, Van Cauwenbergh, & Deelstra, 2003).

Studies have already detected *myo*-inositol phosphates in almonds, cashew nuts, Brazil nuts, rice and oats (Duong, Clark, Lapsley, & Pegg, 2017a, 2017b; Zhang et al., 2007). However, information about the *myo*-inositol phosphate concentrations of plant-based beverages is scarce. Bernat, Chafer, Chiralt, and Gonzalez-Martinez (2014) and Singhal, Baker, and Baker (2017) defined minerals in PBB and compared the different beverages according to their mineral content and vitamins, based on available label information and in databases. Other than these, comparative analytical studies of different PBB considering mineral content and *in vitro* digestion effects on bioaccessibility, total phenolic compounds, antioxidant activity and *myo*-inositol phosphates are scarce.

Few studies have evaluated the mineral bioaccessibility in samples such as soy beverages (Theodoropoulos, Turatti, Greiner, Macedo, & Pallone, 2018), but they did not compare different types of beverages composed of different raw materials, making the current study novel research.

The aims of this study are to investigate the content of essential minerals (calcium, iron, magnesium and zinc); to estimate the effect of *in vitro* digestion on mineral bioaccessibility in PBB; determine the presence of antinutritional factors in PBB (*myo*-inositol phosphates fractions) and possible effects on mineral bioaccessibility; antioxidant

capacity and phenolic compound content and effects on mineral bioaccessibility in PBB. Moreover, the obtained analytical data was evaluated by multivariate analysis.

## 2. Material and methods

### 2.1. Samples

24 commercial samples of liquid and powder PBB were evaluated (Supplementary material 1). Samples included, 7 rice samples, 5 cashew nut samples, 5 coconut samples, 3 oat samples, 2 almond samples, one sample of peanut and one soy. Samples were either blended or not with other ingredients, or fortified with added minerals or not. One sample of sterilized cows milk was also evaluated for comparison with the PBB, totaling 25 samples. The samples obtained were available commercially in Campinas, São Paulo, Brazil. The study evaluated 12 brands of PBB and one brand of milk. The powder samples were prepared with ultra-pure water according to label instructions and used in all the analysis. For the *myo*-inositol phosphates analysis, the samples were freeze-dried for further analysis.

### 2.2. Evaluation of total phenolic compounds and antioxidant capacity

#### 2.2.1. Sample preparation

Sample preparation for total phenolic compounds (TPC) and antioxidant capacity determination was performed according to the method proposed by Varga, Jójárt, Fónad, Mihály, and Palágyi (2018), with some modifications. For the extraction step, a total 4 mL of 80% methanol solution was added at 400  $\mu$ L increments to each sample, followed by manual shaking in a 15 mL falcon tube. The tubes were then placed for 10 min in an ultrasound bath (Model: Unique, Brand: Catel) and centrifuged for 5 min at 6000 rpm (Refrigerated Centrifuge SL - 706, SOLAB). After centrifugation, the supernatant (extract) was collected for analysis.

#### 2.2.2. Phenolic compounds and antioxidant capacity determination

The TPC content was determined according to the method described by Singleton and Rossi (1965). In a dark room, the reaction was performed using 85  $\mu$ L sample extract, 43  $\mu$ L Folin-Ciocalteu reagent (1N) and 212  $\mu$ L  $Na_2CO_3$  (75 g/L). After 25 min of reaction, the absorbance was read on 96-well microplate in a multi-mode microplate (BMG Labtech, Germany, model Fluostar Omega) at 725 nm. Analytical curves with a gallic acid standard (standard antioxidant) at concentrations from 1 to 50 mg/L were performed. TPC contents were expressed in mg of GAEq/L sample. Analyses were performed in triplicate, and the limit of quantification was 1 mg/L.

Antioxidant capacity was performed by the DPPH (2,2-Diphenyl-1-picrylhydrazyl, Sigma Aldrich, USA) Scavenging Assay as proposed by Sánchez-Moreno, Larrauri, and Saura-Calixto (1998), with modifications. A calibration curve was determined with Trolox standard solution (6-hydroxy-2,5,7-tetramethylchroman-2-carboxylic acid, Sigma Aldrich, USA) diluted in methanol at concentrations from 0.6 mM to 20 mM. The extracts of each sample or trolox solution (85  $\mu$ L) were added of 312  $\mu$ M of DPPH reagent in methanol (25 mg/L) for the assay. Absorbance values during the kinetic were measured at 515 nm in the multi-mode microplate (BMG Labtech, Germany, model Fluostar Omega). All samples were analyzed in triplicate and DPPH results were expressed as  $\mu$ mol Trolox Equivalent per liter of sample ( $\mu$ mol TE/L). The limit of quantification was considered as 0.6 mM.

### 2.3. Myo-inositol phosphates quantification

Samples were freeze-dried (for 24 h up to a pressure of < 200  $\mu$ Hg) and maintained at  $-40$  °C until *myo*-inositol phosphate (InsP6, InsP5, InsP4, InsP3) quantification. 1 g of the freeze-dried samples were extracted with 2.4% (w/w) HCl for 3 h at 22 °C, according to Feitosa et al.

**Table 1**  
Total phenolic compounds, antioxidant capacity myo-inositol fractions and molar ratio.

Type	Sample	Total phenolic compounds	Antioxidant capacity	Myo-Inositol fractions			Myo-Inositol molar ratios			
		TPC (mg GAE/L)	DPPH ( $\mu\text{mol TE/L}$ )	IP4 ( $\mu\text{mol/L}$ )	IP5 ( $\mu\text{mol/L}$ )	IP6 ( $\mu\text{mol/L}$ )	(IP5 + IP6):Ca	(IP5 + IP6):Fe	(IP5 + IP6):Zn	(IP5 + IP6):Mg
Rice	1	1,10 $\pm$ 0,08 jklm	7,82 $\pm$ 1,82 e	< LOQ	< LOQ	< LOQ	ND	ND	ND	ND
	2	0,91 $\pm$ 0,20 klm	283,55 $\pm$ 18,81 a	< LOQ	< LOQ	< LOQ	ND	ND	ND	ND
	3	1,88 $\pm$ 1,10 hijk	301,93 $\pm$ 1,94 a	< LOQ	< LOQ	< LOQ	ND	ND	ND	ND
	4	1,48 $\pm$ 0,03 ijkl	ND	< LOQ	< LOQ	< LOQ	ND	ND	ND	ND
	5	1,07 $\pm$ 0,16 jklm	28,58 $\pm$ 2,53 de	< LOQ	< LOQ	< LOQ	ND	ND	ND	ND
	6	1,55 $\pm$ 0,14 ijkl	150,21 $\pm$ 2,82 b	< LOQ	< LOQ	< LOQ	ND	ND	ND	ND
	7	12,39 $\pm$ 0,82 a	112,25 $\pm$ 16,36 c	< LOQ	< LOQ	< LOQ	ND	ND	ND	ND
Cashew nut	8	3,21 $\pm$ 0,10 f	146,77 $\pm$ 4,78 b	< LOQ	0,4 $\pm$ 0,02 b	0,67 $\pm$ 0,02 c	0,80	15,28	24,72	0,13
	9	4,76 $\pm$ 0,05 e	297,19 $\pm$ 3,25 a	< LOQ	0,59 $\pm$ 0,02 ab	1,07 $\pm$ 0,08 b	0,99	20,33	27,76	0,16
	10	4,24 $\pm$ 0,14 e	300,21 $\pm$ 0,37 a	< LOQ	0,54 $\pm$ 0,05 b	0,76 $\pm$ 0,02 c	1,15	18,29	18,36	0,16
	11	8,27 $\pm$ 0,24 e	46,83 $\pm$ 3,58 d	< LOQ	0,67 $\pm$ 0,02 a	0,82 $\pm$ 0,08 c	0,61	8,06	26,05	0,13
	12	2,98 $\pm$ 0,11 fg	151,75 $\pm$ 0,73 b	2,05 $\pm$ 0,04	0,56 $\pm$ 0,01 ab	0,69 $\pm$ 0,03 c	0,03	18,42	37,84	0,18
Almond	13	1,96 $\pm$ 0,00 ghij	304,83 $\pm$ 0,97 a	< LOQ	< LOQ	< LOQ	ND	ND	ND	ND
	14	0,53 $\pm$ 0,11 lm	151,64 $\pm$ 4,32 b	< LOQ	< LOQ	0,17 $\pm$ 0,02 d	0,03	ND	ND	0,13
Peanut	15	6,74 $\pm$ 0,03 d	ND	< LOQ	0,32 $\pm$ 0,04 c	1,46 $\pm$ 0,03 a	0,05	12,76	28,73	0,26
Coconut	16	1,90 $\pm$ 0,03 hijk	306,23 $\pm$ 0,37 a	< LOQ	< LOQ	< LOQ	ND	ND	ND	ND
	17	1,47 $\pm$ 0,11 ijkl	3,07 $\pm$ 0,56 e	< LOQ	< LOQ	< LOQ	ND	ND	ND	ND
	18	0,21 $\pm$ 0,05 m	290,61 $\pm$ 30,95 a	< LOQ	< LOQ	< LOQ	ND	ND	ND	ND
	19	7,39 $\pm$ 0,05 cd	299,99 $\pm$ 1,29 a	< LOQ	< LOQ	< LOQ	ND	ND	ND	ND
	20	7,77 $\pm$ 0,36 e	12,59 $\pm$ 1,71 e	< LOQ	< LOQ	< LOQ	ND	ND	ND	ND
Oat	21	4,77 $\pm$ 0,16 e	151,96 $\pm$ 0,37 b	< LOQ	< LOQ	< LOQ	ND	ND	ND	ND
	22	2,36 $\pm$ 0,20 fghi	ND	< LOQ	< LOQ	< LOQ	ND	ND	ND	ND
	23	2,65 $\pm$ 0,30 fgh	306,46 $\pm$ 2,40 a	< LOQ	< LOQ	< LOQ	ND	ND	ND	ND
Soy	24	10,99 $\pm$ 0,30 b	150,32 $\pm$ 3,08 b	< LOQ	< LOQ	0,77 $\pm$ 0,02 c	ND	12,83	6,05	0,10
Milk	25	4,79 $\pm$ 0,37 e	298,05 $\pm$ 1,29 a	< LOQ	< LOQ	< LOQ	ND	ND	ND	ND

Results are expressed as mean  $\pm$  standard deviation,  $n = 3$ . Mean with different letters in the same column indicate significant difference ( $p < 0.05$ ) as determinate using one way ANOVA and Tukey test at 95% of confidence.

TPC; Total phenolic compounds, DPPH; antioxidant capacity and myo-inositol fractions (IP4; IP5; IP6). TPC (mg GAE/L) expressed as mg gallic acid equivalent per liter of sample. DPPH ( $\mu\text{mol TE/L}$ ) expressed as micromoles of Trolox equivalent per liter of sample. IP3 content was under the LOQ.

(2018).

The extracts were centrifuged at 30.000g for 30 min to remove any solid particles. Thereafter, the clear supernatants were diluted with water (1:25) and applied to a column containing AG1-X8, 100-200 mesh resin. The column was washed with 10 volumes (10 times the column volume) of water and 10 volumes of 25 mM HCL. The *myo*-inositol phosphates were eluted with 5 volumes of 2 M HCL. The eluate obtained was concentrated in a vacuum evaporator till dry and the residue was dissolved in 10 mL water. 20  $\mu$ L of the samples were chromatographed on Ultrasep ES 100 RP18 (2  $\times$  250 mm) to quantify the *myo*-inositol phosphates. The column was run at 45° C and 0.2 mL min<sup>-1</sup> with an eluant consisting of formic acid:methanol:water:tetrabutylammonium hydroxide (44:56:5:1.5 v/v), pH 4.25, as described by Sandberg and Ahderinne (1986). A standard mixture of the individual *myo*-inositol phosphate esters (InsP3–InsP6) was used. The limits of detection and quantification for the method were of  $8.0 \times 10^{-9}$  and  $12 \times 10^{-9}$  mol/L, respectively.

#### 2.4. Method validation for essential mineral evaluation by FAAS

The method was validated using samples of rice and cashew nut beverages and according to the parameters of sensitivity, linearity, limit of detection and quantification, accuracy, and recovery as suggested by the guidelines on validation (AOAC, 2016; Brasil, 2016).

Prior to analysis approximately 500 mL of each sample was homogenized in a blender (Philips Wallita). Powder samples were prepared with ultra-pure water according to label and used during the analysis. The mineralization step was performed according to Silva, Orlando, Rebellato, and Pallone (2017), with modifications. Sample volumes were adjusted to 4 mL and 6 mL of nitric acid added, then left to stand for one night and then 2 mL of hydrogen peroxide (30%) added. The mixture was mineralized for 4 h at 130 °C on a digester block and Ca, Mg, Fe and Zn were evaluated by flame atomic absorption spectrometer (FAAS), model AAnalyst 200. Mineralized samples were placed into a nebulizer and mixed with air-acetylene flame (2.5/10 L h<sup>-1</sup>) at approximately 2000 °C. A deuterium lamp was used for correction of background radiation and hollow cathode lamps for determination of iron (248.3 nm), calcium (422.67 nm), magnesium (285.21 nm), and zinc (213.86 nm) (PerkinElmer).

#### 2.5. In vitro assay for bioaccessibility estimation of essential minerals

The *in vitro* assay to estimate bioaccessibility was performed using the dialysis method according to Jovani, Barbera, Farre, and Aguilera (2001) and Luten et al. (1996), with modifications. Approximately 20 mL of each beverage was added to a 250 mL erlenmeyer flask and the pH adjusted to 2.0 by adding 6 mol L<sup>-1</sup> HCL. For the gastric digestion step, 0.6 g of freshly prepared pepsin solution (1.6 g in 10 mL 0.1 mol L<sup>-1</sup> HCL) was added, the flasks were mixed and incubated at 37 °C for 2 h. The digests were cooled, in an ice bath, for the titratable acidity determination. After that, a dialysis bag (Sigma-Aldrich, 35x21x30 mm, porosity of 25 Å, pore size of 14,000 Da) containing water (25 mL) and NaHCO<sub>3</sub> equivalent to the titratable acidity was added to the flasks and incubated for 37 °C for 30 min. Then a mixture of 5 g of a bile-pancreatin solution (0.4% of pancreatin (w/v) and 2.5% of bile extract (w/v) in 0.1 mol L<sup>-1</sup> NaHCO<sub>3</sub>) were added and the incubation proceeded for 2 h at 37 °C.

The dialyzed fractions content were transferred into a falcon tube, acidified with 1 mL HNO<sub>3</sub> 65% v/v and the mineral content determined by FAAS.

#### 2.6. Statistical analysis

The data was performed in triplicate and the results were evaluated with an analysis of variance (one-way ANOVA) and Tukey test, with 95% of confidence using the software Statistica 8.0 (StatSoft, Inc.,

Tulsa, USA). The multivariate analysis was conducted by Principal Component Analysis (PCA) and performed using the MatLab R2019a (MathWorks, USA) with PLS-toolbox version 8.6 (Eigenvector Research Inc, 2010) to evaluate all the results performed.

### 3. Results and discussion

#### 3.1. Evaluation of total phenolic compounds, antioxidant capacity and *myo*-inositol phosphates

Total phenolic compounds, antioxidant capacity and *myo*-inositol phosphates were evaluated in the 24 samples of plant-based beverages and also in the cow's milk sample and the results obtained were presented in Table 1.

##### 3.1.1. Total phenolic compounds

The total phenolic compounds (TPC) were significantly different between evaluated samples. The TPC ranged from 0.20 mg GAEq/L for sample 18 (coconut) to 12.39 mg GAEq/L for sample 7 (rice) (Table 1). A study about the phenolic compounds in different types of rice, was performed by Setyaningsih, Saputro, Carrera, Palma, and Garcia-Barroso (2019), they found a total of 13.49 mg/kg of TPC in white rice, similar to the value determined in sample 7 of rice beverage (12.39 mg GAEq/L), but higher than the values determined for the others rice beverage samples analyzed in this study. However, sample 7 has in its composition quinoa and cocoa, which are known to be plants with high amounts of total phenolic compounds (Abderrahim et al., 2015). Cacao has a relevant amount of flavonols, such as epicatechin and catechin and methylxanthines (such as theobromine); while quinoa contains a good profile of phenolic acids such as vanilic, chlorogenic and caffeic acids as well as some flavonoids (Zięba, Makarewicz-Wujec, & Kozłowska-Wojciechowska, 2019).

Rodríguez-Roque, Rojas-Grañ, Elez-Martínez, and Martín-Belloso (2013) determined TPC in soy milk and found 61.4 mg/100 mL, which is higher than the found in this research (10.99 mg/L). The TPC in soymilk was already determined in other studies and ranged from 96 to 320 mg of gallic acid equivalent/100 g (Tyug, Prasad, & Ismail, 2010). These results have a wide range once it depends on the soybean variety, climatic and soils conditions, besides that the amount of grain used to produce the beverage can also be a relevant variable once it is not standardized.

The raw materials used in the production of PBB such as almonds, cashew nuts, rice and soybeans could present antioxidant activity and different profiles of phenolic compounds, such as flavonoids, iso-flavones, phenolic acids, and others (Herbello-Hermelo et al., 2018). TPC values found in the phenol-explorer database show that some of these raw materials could present a high variation in the TPC according to the different raw materials, origin and processes applied. For almonds the TPC ranged from 126,8 to 418,00 mg/100 g; oats from 1,46 to 389,70 mg/100 g; peanuts from 395 to 420 mg/100 g and cashew nuts from 137 to 274 mg/100 g (Phenol-Explorer, 2019).

Therefore, the different contents of TPC found in this research for the PBB could be associated to several factors: the type, origin or amount of the raw material used during the process (since many companies do not declare the amount of raw material used in production); and the proportion of water added and presence of possible additives, which could cause an alteration of the contents of phenolic compounds and other compounds (Herbello-Hermelo et al., 2018; Mendes et al., 2019; Rodríguez-Roque et al., 2013).

##### 3.1.2. Antioxidant capacity (DPPH)

The DPPH method is based on a measure of the consumption of DPPH radical by an antioxidant compound, in this case by a bioactive compound present in the samples (Souza, Oliveira, Oliveira, Moraes, & Abarza, 2015).

In this context, the antioxidant capacity (DPPH) ranged from 3.07 to

306.46  $\mu\text{mol TE/L}$  for samples 17 (peanut) and 23 (oat), respectively. Exception was observed for samples 4 (rice), 15 (peanut) and 22 (oat) where antioxidant capacity was not detected (Table 1). Rice samples, 2 (283.55  $\mu\text{mol TE/L}$ ) and 3 (301.93  $\mu\text{mol TE/L}$ ); cashew nut, 9 (297.19  $\mu\text{mol TE/L}$ ) and 10 (300.21  $\mu\text{mol TE/L}$ ); almond samples, 13 (304.83  $\mu\text{mol TE/L}$ ); coconut samples 16 (306.23  $\mu\text{mol TE/L}$ ), 18 (290.61  $\mu\text{mol TE/L}$ ) and 19 (299.99  $\mu\text{mol TE/L}$ ) and oat sample 23 (306.46  $\mu\text{mol TE/L}$ ) presented the highest DPPH values and showed no significant difference ( $p < 0.05$ ) in relation to cow's milk (sample 25). In addition, it is relevant that at least one sample of each PBB type presented in our study about 300  $\mu\text{mol TE/L}$ , proving that, as expected, these beverages present some antioxidant capacity.

Sudjaroen, Thongkao, and Suwannahong (2018) determined the antioxidant capacity in cashew nut shell waste through DPPH assay and found an antioxidant capacity of 57.1  $\mu\text{MET/mL}$ , higher than the "DPPH values" determined in the cashew nut beverages.

It is important to highlight that antioxidant assays can be classified according to the base mechanism; Hydrogen Atom Transfer (HAT) or Single Electron Transfer (SET). The DPPH assay was used in this study combines these two mechanisms; the DPPH radical reacts fast through electron transfer and slowly with hydrogen atom transfer. In this context, each type of compound reacts in its own way, phenols (without an aromatic ring) present fast reactions, while phenols with aromatic rings and acid groups present slow reactions. Once the DPPH radical site hinders the access by phenolic compounds, all the reactions are expected to slow, especially HAT (Schaich, Tian, & Xie, 2015).

The composition of the PBB evaluated in this research is distinct from each other, not only the raw matrices, but also by the ingredients used by the producers. The samples with the highest value of TPC are not the same that showed the highest values of antioxidant capacity by DPPH assay. This fact could be related to the matrix effects, DPPH assay limitations and the unknown bioactive compound composition of each sample. The identification of the phenolic compounds profile was not performed in this study, we conducted a preliminary study about the total phenolic compounds of the samples.

In this context, we observed that there is a large variation on the antioxidant capacity and composition of evaluated beverages. The antioxidant capacity could be low (ND) or high according to the PBB chosen for consumption.

### 3.1.3. Quantification of myo-inositol phosphates

The myo-inositol phosphates (IP3, IP4, IP5, IP6) were evaluated in samples of PBB (Table 1). The myo-inositol trisphosphate (IP3) could not be detected in the beverages. The myo-inositol tetrakisphosphate (IP4) was only observed in the non-blended cashew nut beverage ( $2.05 \pm 0.04 \mu\text{mol/L}$ ) of the brand D (sample 12). The concentrations of myo-inositol pentakisphosphate (IP5) ranged from 0.32 to 0.66  $\mu\text{mol/L}$ , for the samples 15 (peanut) and 11 (cashew nut), respectively. Except for the peanuts, all the samples that presented IP5 fractions were from the cashew nut class, blended or not with other components.

A myo-inositol hexakisphosphate (IP6, phytate) was observed in all the cashew nut beverages, one almond, peanut and soy. The IP6 concentration ranged from 0.16 to 1.46  $\mu\text{mol/L}$ . The lowest concentration was found in the almond beverage and the highest in the peanut beverage. All the cashew nut beverages and the soy beverage were considered equal.

The antinutritional effect of myo-inositol hexaphosphate (IP6), or phytic acid, and also IP5, in reducing or inhibiting the absorption of minerals are known. These compounds actively bind to divalent cations such as Ca, Fe and Zn, forming insoluble complexes and as a consequence decrease the absorption of these minerals in the body (Hurrell, 2004; Sandberg et al., 1999). Thus, mineral availability can also be estimated by evaluating the phytate (IP5 + IP6)/mineral molar ratio and can be used as a tool to predict the inhibitory effect of this antinutrient on mineral bioavailability (Sánchez-Moya et al., 2019;

Udomkun et al., 2019).

In literature, few studies were found about PBB and the impact of phytates on mineral bioaccessibility. In this context, we evaluated the effect of molar proportions of phytates (IP5 + IP6) on mineral availability (Fe, Ca, Mg and Zn). In our study, only cashew nuts (8 to 12), almond (14), peanut (15) and soy (24) samples presented phytates (IP5 + IP6)/mineral molar ratio values. For the molar ratio [phytates]:[iron] the values ranged from 8.06 to 20.33; for zinc, the [phytate]:[zinc] molar ratio ranged from 6.05 to 37.84, while the molar ratio [phytate]:[calcium] ranged from 0.03 to 1.15 among the samples of cashew nuts, almond, peanut and soy.

To predict mineral availability, it is ideal that the [phytate]:[iron] molar ratio is less than 0.4 (Hurrell, 2004). Although Hurrell et al. (1992) indicated that there is a strong phytate inhibiting effect on iron even when the molar ratio is 0.2. Therefore, in the current study the Fe bioaccessibility of all the cashew nut, peanut and soy samples could be negatively affected by phytates.

The suggested critical value of the [phytate]:[calcium] molar ratio is less than 0.17 (Sánchez-Moya et al., 2019; Udomkun et al., 2019; Umeta, West, & Fufa, 2005). However, Morris and Ellis (1985) mentioned that a [phytate]:[calcium]  $> 0.24$  M ratio is already responsible for decreasing calcium absorption. Then, the Ca bioaccessibility of the cashew nut samples (8–11) could be negatively affected by phytates.

The molar ratio [phytate]:[magnesium] ranged from 0.10 to 0.26, and these values were considered too low to interfere with Mg bioaccessibility (Israr, Frazier, & Gordon, 2013). Zinc availability is correlated when the [phytate]:[zinc] molar ratio is less than 18 (Sánchez-Moya et al., 2019). Although, Ellis et al. (1987) recommended a molar ratio  $\leq 10$  [phytate]:[zinc] for adequate bioavailability of zinc. In this context, only soy (sample 24) was not influenced by the antinutritional effect of phytic acid.

Theodoropoulos et al. (2018) studied soy beverages and observed that myo-inositol fractions (IP3, IP4, IP5, IP6) were present in almost all the soy beverages studied. It ranged from not detected (ND) to 0.2  $\mu\text{mol/g}$  for IP3, 0.2 to 0.9  $\mu\text{mol/g}$  to IP4, 0.2 to 5.2  $\mu\text{mol/g}$  to IP5 and 0.49 to 19.7  $\mu\text{mol/g}$  for IP6. These results are congruent with the results found in our research, where IP6 was also the most prevalent component in the samples with a mean concentration of  $11.88 \pm 0.28 \mu\text{mol/g}$ .

The myo-inositol fractions were evaluated in almond meal and almond brown skins and six forms of myo-inositol phosphates were detected, they ranged from 8 to 12  $\mu\text{mol/g}$  in the meal and 5 to 14  $\mu\text{mol/g}$  in the brown skins and IP6 was the dominant form (Duong, Clark, Lapsley, & Pegg, 2017b). Other research detected myo-inositol phosphates in cashew nuts (5.02  $\mu\text{mol/g}$ ), Brazil nuts (20.08  $\mu\text{mol/g}$ ), rice (97.36  $\mu\text{mol/g}$ ) and oats (54.47 mg/240 g) (Zhang et al., 2007). However, in this research we did not observed myo-inositol phosphates in rice and oats beverages; probably due the amount of raw material, and variations of, used in production process and dilution with water (moisture variation).

### 3.2. Essential mineral content and in vitro digestion for bioaccessibility estimation of Ca, Fe, Mg and Zn

#### 3.2.1. Method validation for calcium, iron, magnesium and zinc in PBB

The parameter evaluation for linearity and sensitivity were made using analytical curves (five to seven points) for each element, Ca (0.5–5.0 mg/L), Fe (0.1–2 mg/L), Mg (0.025–0.5 mg/L), and Zn (0.05–0.35 mg/L). The curves were linear with suitable percentages of explained variance ( $R^2 > 0.99$ ). The regressions were considered significant through ANOVA evaluation and no lack of fit for the linear models were observed.

The limits of detection (LOD) and quantification (LOQ) values for the minerals were Fe, Ca, Mg and Zn were 0.04; 0.45; 0.01 and 0.05 mg/L, respectively. The LOQ were 0.11; 0.81; 0.02 and 0.07 mg/L for Fe, Ca, Mg and Zn, respectively.

Precision and accuracy were evaluated in rice and cashew nut beverages. Precision was evaluated using parameters of repeatability and intermediate precision. The repeatability was considered appropriate for the analysis, due to the relative standard deviation (RSD) obtained for 7 repetitions of each mineral (RSD rice values: Ca 3.46, Fe 1.95, Mg 4.76 and Zn 4.76; RSD cashew nut values: Ca 4.57, Fe 3.84, Mg 4.76 and Zn 4.76) being lower than the maximum RSD established by the guidelines of validation (AOAC, 2016; Brasil, 2016). According to the guidelines, samples with concentrations of up to 1, 10 and 100 ppm and 0.1% of analyte, the RSD should be 11, 7.3, 5.3 and 3.7, respectively. Intermediate precision, was assessed using ANOVA on three different days and was not significantly different ( $P > 0.05$ ), with 95% of confidence.

The accuracy assay was performed according to INMETRO (Brasil, 2016) and was evaluated through recovery tests for all minerals. The rice and cashew nut beverage samples were fortified with 50, 100% or a known concentration of each mineral, regarding the initial value present in the samples studied. If residual carbon was less than 0.1% in the beverages after mineralization, according Gouveia, Silva, Costa, Nogueira, and Nóbrega (2001), then the digestion procedure was considered efficient for analysis of plant-based beverages by FAAS.

### 3.2.2. Mineral content and effect of *in vitro* digestion on bioaccessibility

Several minerals in PBB were evaluated, including calcium, magnesium, iron and zinc and bioaccessibility was assessed through *in vitro* digestion assay. The analyses were also carried out for a milk sample for comparison.

The total calcium content and dialyzed fractions were evaluated in the samples and compared (Table 2).

Results show a high variation in the calcium content of the beverages, ranging from 10.00 mg/L to 1697.33 mg/L, for samples 1 (rice) and 20 (coconut), respectively. The highest amounts were observed in samples that were fortified with some calcium compound (some brands used calcium lactate, calcium phosphate, calcium carbonate and calcium from algae). Some of them, sample 6 (rice), sample 12 (cashew nut), sample 13 (almond), sample 15 (peanut), sample 20 (coconut),

samples 21 and 23 (oat) had a calcium content equal to milk (1531.0 mg/L), but all of were fortified with calcium. They also presented more calcium than soy, a more common PBB in the markets. When evaluating Ca fortified samples, we observed that despite the different types of calcium, there was significant variation between the fortified samples.

A similar comparison was observed by Chalupa-Krebzdak et al. (2018). They evaluated the USDA Food Composition Database of common PBB products (soy, coconut, almond, and cashew nut) and observed that the Ca content was highly variable. The fortified beverages contained higher total Ca when compared to milk, but non-fortified PBB had drastically lower levels of calcium, ranging from 0 to 188 mg/100 g for non-fortified and fortified, respectively.

The dialyzed calcium ranged from 8.2 to 306.6 mg/L, for the samples 18 (coconut) and 24 (soy), respectively, corresponding to 1.6 and 45.9% of calcium dialyzed. For other beverages the Ca dialyzed was under the LOQ. Some beverages such as samples 2, 4, 5 (rice), 12 (cashew nut) and 24 (soy) had higher amounts and percentages of dialyzed calcium than milk; with 13.1, 15.4, 21.2, 17.2 and 45.9% of dialyzed calcium and only 7.10% in milk.

Theodoropoulos et al. (2018) demonstrated that soy beverages have a percentage of Ca dialysis ranging from 3.3 to 5.4%, values lower than what was found in the current study (45.9%), likely due the type of calcium used for fortification. Besides that, they also observed that samples treated with phytase, had a decrease in IP fractions, which increased the dialyzed Ca percentage.

The differences between the dialyzed calcium might be related to the interactions of Ca with the other compounds of the food matrix, and may also be affected by the calcium compounds used during fortification (Chalupa-Krebzdak et al., 2018). The PBB studied were fortified with calcium carbonate, tricalcium phosphate, calcium lactate and calcium from algae. Studies observed that different compounds (chemical forms) of calcium had different behaviors in Ca bioaccessibility, are affected by pH, molecular interactions with other compounds of the digestive tract, and other factors (Lorieau et al., 2018). Other studies show that calcium carbonate in puffed rice extrudates and rice noodles

**Table 2**  
Content of total, dialyzed and percentage of dialysis for calcium and magnesium.

Type	Sample	Calcium			Magnesium		
		Total Ca (mg/L)	Dialyzed Ca (mg/L)	Dialysis (%)	Total Mg (mg/L)	Dialyzed Mg (mg/L)	Dialysis (%)
Rice	1	10.00 ± 0.85 i	< LOQ	ND	12.63 ± 0.24 h	2.46 ± 0.21 j	9.5
	2	1149.33 ± 48.01 d	151.15 ± 7.94 cd	13.2	27.69 ± 2.30 ijh	8.91 ± 0.62 ij	34.8
	3	170.80 ± 12.12 hi	15.81 ± 1.68 f	9.3	122.80 ± 9.44 fgh	11.24 ± 1.79 ij	9.2
	4	1091.50 ± 97.10 d	168.30 ± 17.50 bcd	15.4	6.29 ± 0.47 h	1.73 ± 0.21 j	30.2
	5	858.20 ± 44.15 ef	182.02 ± 7.49 bc	21.2	24.82 ± 2.34 ijh	12.32 ± 0.19 ij	53.2
	6	1619.00 ± 68.61 ab	89.52 ± 9.82 e	5.5	48.99 ± 3.25 ij	19.84 ± 0.89 ghi	35.7
	7	142.43 ± 0.67 hi	< LOQ	ND	31.37 ± 1.14 ijh	15.73 ± 1.91 hi	56.6
Cashew nut	8	53.70 ± 3.25 i	< LOQ	ND	196.33 ± 13.76 bc	35.13 ± 8.09 def	17.7
	9	67.10 ± 2.00 hi	< LOQ	ND	251.23 ± 14.20 a	40.03 ± 1.54 de	15.4
	10	45.34 ± 0.11 i	< LOQ	ND	195.30 ± 13.95 bc	31.21 ± 0.23 efg	13.3
	11	97.21 ± 2.14 hi	< LOQ	ND	268.43 ± 2.83 a	45.88 ± 4.59 cd	17.1
	12	1637.33 ± 107.87 ab	281.31 ± 25.36 a	17.2	168.33 ± 9.40 cd	60.93 ± 8.16 b	31.3
Almond	13	1482.67 ± 152.18 bc	89.82 ± 9.66 e	6.1	153.67 ± 15.77 def	42.51 ± 4.31 de	24.1
	14	266.47 ± 21.37 h	9.44 ± 0.38 f	3.5	32.98 ± 2.66 ijh	8.73 ± 1.12 ij	22.5
	15	1641.70 ± 135.36 ab	143.97 ± 12.72 d	8.8	169.60 ± 17.44 cd	44.74 ± 1.01 cd	23.6
Peanut	16	35.46 ± 2.86 i	8.25 ± 0.60 f	23.3	157.60 ± 21.66 de	56.20 ± 1.25 bc	30.4
	17	876.70 ± 10.48 e	34.94 ± 4.22 f	3.9	26.69 ± 1.40 ijh	8.07 ± 1.40 ij	27.5
Coconut	18	511.80 ± 24.79 g	8.21 ± 0.51 f	1.6	131.63 ± 5.62 efg	107.42 ± 10.65 a	65.3
	19	527.90 ± 30.22 g	10.91 ± 0.59 f	2.1	206.83 ± 20.91 b	62.43 ± 0.76 b	25.7
	20	1697.33 ± 92.59 a	197.11 ± 4.72 b	11.6	121.16 ± 8.59 gh	42.98 ± 3.37 de	32.9
	21	1477.33 ± 121.96 bc	78.40 ± 6.31 e	5.3	56.00 ± 2.34 i	19.09 ± 1.33 ghi	29.6
	22	139.43 ± 1.19 hi	< LOQ	ND	22.01 ± 2.25 jh	3.30 ± 0.55 j	14.2
	23	1359.67 ± 72.86 c	32.69 ± 0.74 f	2.4	92.60 ± 5.96 h	26.39 ± 4.06 fgh	27.1
Soy	24	668.00 ± 18.19 fg	306.65 ± 13.57 a	45.9	179.37 ± 6.29 bcd	60.02 ± 6.30 b	28.1
Milk	25	1531.00 ± 33.06 abc	108.64 ± 11.22 e	7.1	94.08 ± 3.52 h	34.60 ± 2.07 def	35.6

Results are expressed as mean ± standard deviation,  $n = 3$ . Mean with different letters in the same column indicate significant difference ( $p < 0.05$ ) as determinate using one way ANOVA and Tukey test at 95% of confidence.

are more bioaccessible than tricalcium phosphate, but not in comparative studies of bioaccessibility of all different calcium compounds (Janve & Singhal, 2018).

However, it is not possible to determine the exact influence of each calcium compound on bioaccessibility in PBB, because some brands do not inform which type of calcium is used. This information could ensure that future studies can be performed in order to estimate the best kind of calcium source for plant-based beverages.

Despite fortification and different calcium compounds, phenolic compounds and phytates could affect Ca bioaccessibility (Vitali, Vedin, Dragojević, & Šebečić, 2008). In our research we observed that TPC could influence Ca bioaccessibility and that most of the samples with phytates are related to low or undetected Ca bioaccessibility, as observed for most of the cashew nut samples.

The total magnesium content ranged from 6.29 to 251.23–268.43 mg/L for samples 4 (rice), 9 and 11 (cashew nut with Brazil nut and cashew nut with cacao), respectively (Table 2). The samples with the highest amounts of Mg were in the cashew nut class and with no added Mg. However, they contained Brazil nut and cacao with Mg also in their composition, likely leading to the differences in Mg from the other beverages due to the presence of these raw materials (Cardoso, Duarte, Reis, & Cozzolino, 2017).

Coconut samples 18 and 19 are from the same brand and Mg was added, but despite this, it did not have the highest Mg content when compared with the beverages of cashew nuts, which naturally have a high Mg content. Some beverages from almond, peanut, coconut, soy and all the cashew nut class had higher amounts of Mg when compared with milk.

It was possible to quantify dialyzed Mg in all the samples, and it ranged from 1.90 to 107.41 mg/L for the samples 4 (rice) and 18 (coconut), respectively. This corresponds to 30.2 to 65.3% of dialysis. This means that the sample with the lowest Mg content also had the lowest amount of dialyzed Mg, but the beverage with the highest content of total Mg was not the same with the highest Mg dialyzed.

Sample 18 had the highest dialyzed Mg (65.3%) and is fortified with Mg, despite this, sample 19, also from the same brand and fortified with Mg only had 25.7% of dialyzed Mg. Sample 19 has in its composition cacao, which could be related to the low Mg dialyzed when compared to the sample without cacao (sample 18), probably because cacao is known as source of phenolic compounds, that are related to the decrease of mineral bioaccessibility (Gibson, Newsham, Gibson, & Newsham, 2018).

The comparison between samples fortified with Mg compounds and ones that have naturally occurring Mg, show that both samples have bioaccessible Mg considered are equal, as observed for samples 19 (fortified) and 12 (naturally occurring), therefore naturally occurring Mg samples could have Mg dialyzed amounts similar to samples fortified with Mg salts. This might be related to the other compounds present in the samples which could interact with the minerals. Sample 12 is not fortified with Mg and has less total Mg content (compared to sample 19), however it has a lower TPC (2.98 mg GAE/L) when compared with sample 19 (7.77 mg GAE/L); possibly due the added cacao which is a source of phenolic compounds (5624.23 mg/100 g) (Phenol-Explorer, , 2019). Therefore, reinforcing the hypothesis that TPC may decrease the absorption of Mg. Furthermore, sample 19 has cacao in its composition, which is a source of phenolic compounds (5624.23 mg/100 g).

The total content of iron and zinc are presented in the Table 3.

Total iron content ranged from 0.76 mg/L to 12.89 mg/L for the samples 4 (rice) and 6 (rice), respectively. The difference in iron levels between the two samples is that sample 4 is not fortified while the sample 6 has iron added. The following samples contained high iron content; 11 (cashew nut) and 20 (coconut) with 10.32 and 10.50 mg/L, with no significant difference. These have not been fortified with iron, however, they have cacao as a common additive.

Dialysis assays showed that the samples had of dialyzed iron

Table 3  
Content of total iron and zinc.

Type	Sample	Iron Total Fe (mg/L)	Zinc Total Zn (mg/L)
Rice	1	< LOQ	< LOQ
	2	< LOQ	0.86 ± 0.05 k
	3	0.92 ± 0.04 hi	< LOQ
	4	0.77 ± 0.00 i	< LOQ
	5	0.87 ± 0.09 i	1.47 ± 0.07 hij
	6	12.89 ± 0.92 a	1.10 ± 0.08 ijk
	7	1.59 ± 0.15 ghi	1.78 ± 0.05 gh
Cashew nut	8	3.91 ± 0.20 de	2.83 ± 0.32 d
	9	4.56 ± 0.57 d	3.91 ± 0.27 c
	10	3.97 ± 0.17 d	4.63 ± 0.017 b
	11	10.32 ± 0.26 b	3.74 ± 0.02 c
	12	3.79 ± 0.20 de	2.16 ± 0.10 efg
Almond	13	2.13 ± 0.23 fgh	1.68 ± 0.27 ghi
	14	< LOQ	< LOQ
Peanut	15	7.79 ± 0.47 c	4.05 ± 0.21 bc
Coconut	16	3.79 ± 0.24 de	1.59 ± 0.05 ghi
	17	< LOQ	0.65 ± 0.04 k
	18	< LOQ	2.16 ± 0.06 efg
	19	2.68 ± 0.14 efg	2.43 ± 0.14 def
	20	10.51 ± 1.04 b	1.97 ± 0.12 fgh
	21	2.13 ± 0.16 fgh	0.58 ± 0.06 k
Oat	22	< LOQ	0.65 ± 0.03 k
	23	1.91 ± 0.06 ghi	< LOQ
Soy Milk	24	3.35 ± 0.27 def	8.32 ± 0.63 a
	25	< LOQ	2.65 ± 0.19 de

Results are expressed as mean ± standard deviation,  $n = 3$ . Mean with different letters in the same column indicate significant difference ( $p < 0.05$ ) as determinate using one way ANOVA and Tukey test at 95% of confidence. Values for dialyzed (mg/L) and percentage of dialysis (%) for iron and zinc were under the LOQ.

amounts under the LOQ. Only sample 16 (coconut) had 1.02 mg/L ± 0.15 of dialyzed Fe, which represented 31.76% of dialyzed iron. According to the PCA results (Fig. 1), the low dialysis percentage of iron could be related to samples with high amounts of total and dialyzed Ca, Zn, and with levels of IP4, IP5 and IP6, suggesting competition between the minerals and a negative influence of the *myo*-inositol phosphates. Strain and Cashman (2009) related that a competition between divalent minerals could occur, as Fe, Ca and Zn might decrease Fe bioaccessibility.

Zinc content ranged from 0.57 mg/L to 8.13 mg/L for samples 21 (oat) and 24 (soy), respectively. Other beverages with high zinc content were cashew nuts (8, 9, 10, 11) and coconut beverages (18 and 19) fortified with zinc. However, it was not possible to quantify Zn for some samples of rice, almond and oat, as the values were under the LOQ for the method.

The soy beverage was the only that had an amount of Zn dialyzed, with 0.51 mg/L ± 0.03, representing 6.15% of zinc dialyzed. This could be related to the initial amount of zinc in the samples and also due to possible interactions of zinc with the matrices in the sample. Other studies analyzed Fe and Zn in soy beverages, naturally occurring or added, and observed an increase in the bioaccessibility of Fe and Zn in the beverages when smaller amounts of *myo*-inositol phosphates were present (Theodoropoulos et al., 2018).

From the results of essential minerals content analysis, we estimated the contribution of minerals Ca, Mg, Fe and Zn towards the Recommended Daily Intake (RDI) was calculated from 200 mL of PBB and compared to equal amounts of traditionally consumed or know beverages of cow's milk and soybean, as they are traditionally consumed and known beverages. According to the combined Food Agriculture Organization and World Health report (FAO/OMS, 2001) the approximate RDI for Ca, Mg, Fe and Zn is 1000 mg, 400 mg, 18 mg and 8 mg, respectively.

Thus, considering the samples with the highest contents of each mineral, the ingestion of one portion of coconut (sample 20) will

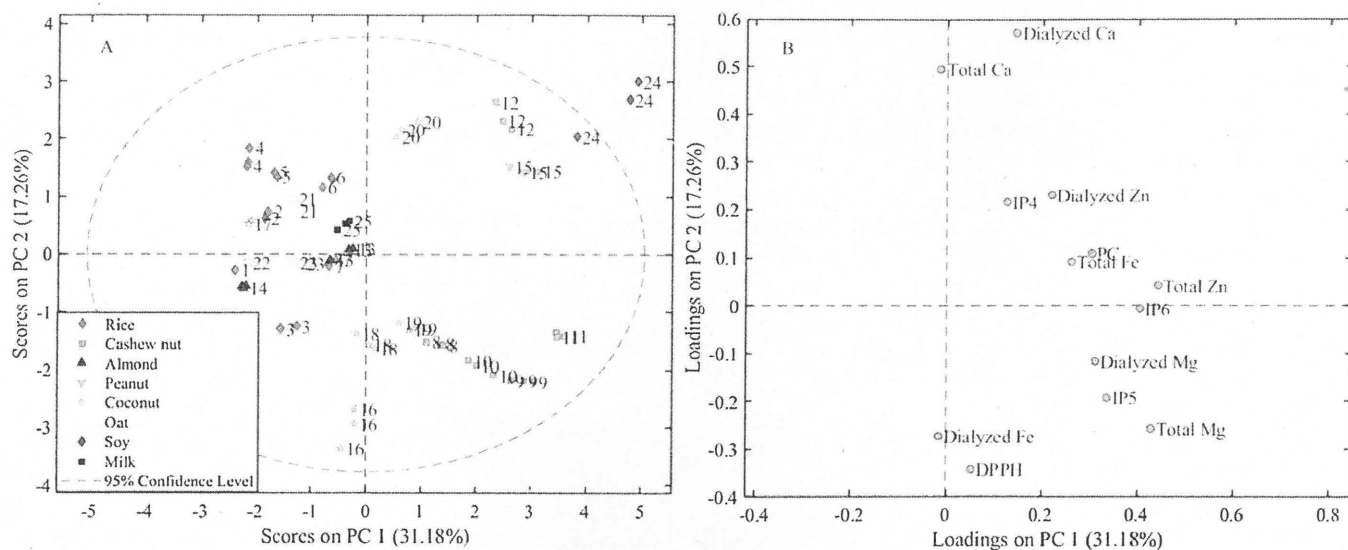


Fig. 1. Principal Component Analysis for evaluation of total and bioaccessible minerals, phenolic compounds, antioxidant capacity, and *myo*-inositol phosphates. Graphs of scores (A) and loadings (B).

contribute 33.9% of Ca RDI, while cow and soybean milks will contribute 30.6 and 13.4%, respectively. For Mg, intake of a portion of cashew (sample 11) will contribute 13.4%, while intake of a portion of cow's milk and soy will contribute 4.7 and 9.0% of the RDI. Regarding iron, intake of a single portion of cow's milk will not contribute to the RDI due to the low content of this mineral in its composition, while soy and rice milk (sample 6) may contribute to 3.3 and 13.0% of the RDI, respectively. Considering zinc, intake of a portion of cow's milk and soybeans will contribute 2.7 and 8.3% of RDI, respectively, while cashew (sample 10) will contribute 4.63% of RDI for this mineral.

The Principal Component Analysis (PCA) was performed as an exploratory analysis to evaluate all data obtained and determine possible correlations between the samples and the variables studied (Fig. 1).

The PCA was built using the 25 samples in triplicate and with 13 variables (TPC, antioxidant capacity, IP4, IP5, IP6, total calcium, iron, magnesium, zinc and dialyzed calcium, iron, magnesium and zinc), resulting in a  $75 \times 13$  matrix, representing 975 assays. The data was auto-scaled, to give equal importance to all variables with differing magnitudes.

The first and second PC's together represent 48.44% of the total explained variance. Other PC's were tested and they did not contribute to the increase of total explained variance, which indicates that other components could have more impact in sample differentiation. The graph of scores display how the samples are distributed. The graph indicates the formation of distinct groups (coconut, cashew nut, peanut, soy) and one large group composed of mixed sample types. The loadings graph indicates the variables and influences leading to the separation of groups. PC1 separated rice, almond, oats, and milk samples and one sample of coconut (17) from the coconut, cashew nuts, peanut and soy beverages groups.

The main group of cashew nut is related to high amounts of total and dialyzed Mg and also the presence of IP5 and IP6. On the other hand, the samples of rice, almond, and oats were separated, probably because they contained, in general, low amounts of all variables. Therefore, it is possible differentiate samples of coconut, cashew nut, peanut and soy from the other samples. According to the PCA, milk did not have considerable differences and was unable to be distinguished from the PBB. In addition, cashew nut samples (not fortified with Ca) have in their composition IP5 and IP6, and that the Ca dialyzed was not detected in all of them, indicating a possible negative effect on bioaccessibility of this mineral influenced by this antinutritional factor.

Differentiation between beverages was achieved possibly not only by raw material used, but also by the presence of different ingredients

that were added or absent in the formulation; mineral fortification (Ca, Fe, Mg, Zn, Se), different chemical types of fortificant compounds (calcium carbonate, calcium lactate, calcium phosphate, calcium from algae), addition of vitamins and other compounds such as sweeteners, maltodextrin, polydextrose, starch, gums, thickeners, and stabilizers, might also have influence in the sample discrimination.

According to the properties evaluated in this study, the composition of the PBB could vary notably according to different sample composition and production process, the ingredients added, raw material used and percentage of raw material added. This is in accordance with the results of Chalupa-Krebzdzak et al. (2018) and (Jeske et al., 2017) that evaluated some properties of PBB.

#### 4. Conclusions

The study demonstrated that some samples of PBB have bioactive potential, according the total phenolic compound content and antioxidant capacity in their composition. The sample with the highest TPC was a rice beverage with 12.39 mg GAEq/L, considered higher than milk; and the highest antioxidant capacity was an oat beverage, with 306.46  $\mu\text{mol TE/L}$ , considered equal to milk. They also have low or no detectable *myo*-inositol phosphates content; however, samples of cashew nuts had IP fractions that could bind with minerals.

It was possible to establish a method for mineral evaluation (Ca, Fe, Mg and Zn) in different PBB. The method presented suitable parameters for sensitivity, linearity, limit of detection and quantification, accuracy, and recovery for the mineral quantification in plant-based beverages.

In addition, the content and bioaccessibility of essential minerals evaluated varied widely; samples of PBB not fortified with Ca presented less amount of Ca content than cow's milk, however samples that were fortified presented comparable values for total Ca and more Ca bioaccessibility than cow's milk. The beverages presented high amounts of Mg content and dialyzed Mg, with also Fe and Zn in their composition; however, low amounts of dialyzed Fe and Zn were verified. Regarding the type of raw material used in the production process and beverage composition, it was possible to demonstrate that PBB are able to provide a higher percentage of RDI than cow's milk, with either fortified or non-fortified samples. Indicating that PBB are a viable alternative, and are able to deliver the equal amount or better than the RDI of regular cow's milk.

Therefore, the substitution of milk for PBB should be carefully evaluated, according to the RDI of each mineral. In addition, consumers are able to determine nutritional value due to the available information



of beverage composition (ingredients used), by evaluating how much of the raw material was added and presence of other ingredients that may or may not improve the nutritional value of this kind of product.

### CRedit authorship contribution statement

**Joyce Grazielle Siqueira Silva:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing - original draft, Writing - review & editing. **Ana Paula Rebellato:** Data curation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Elem Tamirys dos Santos Caramés:** Formal analysis, Methodology, Writing - original draft. **Ralf Greiner:** Funding acquisition, Resources, Supervision, Writing - review & editing. **Juliana Azevedo Lima Pallone:** Conceptualization, Funding acquisition, Resources, Supervision, Visualization, Writing - review & editing.

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### Declaration of Competing Interest

The authors declare none conflict of interest.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2020.108993>.

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