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Trace elements in plant-based meat available in Brazil: Total content, bioaccessibility and risk assessment

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ABSTRACT

There is limited data on trace elements in plant-based foods, especially meat analogues. This study assessed the presence of Al, Cr, Co, Ni, As, Se, Mo, Cd, Sb, Ba, Hg, and Pb in plant-based products intended as meat substitutes using ICP-MS. In addition, estimated exposure and bioaccessibility (%B) of these elements, following the INFOGEST 2.0 protocol were also evaluated. Al, Cr, Co, Ni, Se, Mo, and Ba were found in all samples, while Cd, Pb, Hg, and As remained at low levels. Principal Component Analysis (PCA) grouped the samples into seven categories based on trace element profiles. Exposure estimates indicated no concern for adult consumption, though further research is recommended for children. In five samples, aluminum exceeded 100 % of the Tolerable Weekly Intake (TWI), while cobalt and nickel surpassed 50 % of the Health-Based Guidance Value (HBGV) and Tolerable Daily Intake (TDI), respectively, in one sample. The Margin of Exposure (MOE) for lead ranged from 1 to 10 in thirteen samples. The study also found that trace element bioaccessibility varies depending on the food's composition. These unprecedented results reinforce the need for further research to better understand the potential risks associated with the consumption of plant-based meat substitutes, especially for child consumers.

1. Introduction

Large food companies and startups have introduced new plant-based protein products to the market aimed at replacing animal-based products (Alasi et al., 2024; Ihsan et al., 2024). However, this sector still faces challenges related to attracting new consumers, achieving cost alignment with animal-based products, and building the perception of these products as nutritious and safe for human health (Gómez-Luciano et al., 2019; Banach et al., 2022; Penna Franca et al., 2022; Alasi et al., 2024).

Plant-based meat substitutes are designed to mimic the flavor and sensory experience of meat, featuring similar appearance, nutritional characteristics, and aromas (Penna Franca et al., 2022). Plant protein sources include cereals (rice, wheat, and corn), legumes (peas, beans, chickpeas, lentils, and lupins), vegetables (jackfruit, water hyacinth, pumpkin, amaranth, mulberry, and moringa), and oilseeds (soy, canola, and sunflower). To achieve the desired texture and structure in these new foods, protein extraction techniques were refined, taking into account cultural, nutritional, and sensory aspects (Alasi et al., 2024).

Although there is evidence demonstrating the benefits of a plant-based diet (Miller et al., 2024), the development of plant-based foods that mimic animal products often requires saturated fats and sodium, classifying them as ultra-processed foods and raising concerns among consumers. Furthermore, the production of plant-derived proteins involves numerous processing steps, which can introduce microbiological, chemical, and allergenic contaminants (Lin et al., 2023; Tan et al., 2024).

The presence of inorganic contaminants in plant-based foods can be a concern for food safety. Although naturally occurring in the environment (soil and air), these contaminants pose a potential threat to environmental quality and human health (Afonne and Ifediba, 2020; Tian et al., 2023). During plant growth, essential nutrients and inorganic biostimulants, such as Se, Co, Al, and certain lanthanides, are necessary (Ayub et al., 2022). These elements can be toxic depending on their concentration and chemical form (organic or inorganic; oxidation state) (IOM, 2000; EFSA, 2008, 2012b, 2023). Institutions and agencies such as Codex Alimentarius, European Commission (EU), and the Brazilian

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Health Regulatory Agency (ANVISA) set tolerance limits for elements like As, Cd, Pb, and Hg in various food categories to protect consumer health (Brasil, 2022a, 2022b; FAO/WHO, 2022; EU, 2023).

Studies on products intended to substitute milk and yogurt showed significant variation in trace element concentrations, mainly due to differences in ingredient lists among similar products, highlighting the importance of research to ensure food safety (Fioravanti et al., 2023; Rebellato et al., 2023a; Teixeira et al., 2024a, 2024b). In a study on inorganic contaminants in plant-based beverages marketed in Brazil, Fioravanti et al. (2023) reported safe intake levels of these plant-based beverages for elements such as Al, Cr, Co, As, Cd, and Pb. However, for Ni, Mo, and Ba, exposure assessment calculations for children raised concerns regarding the consumption of these beverages. Regarding the element Se, Teixeira et al. (2024a) indicated that its concentration is linked to the raw materials used, and that consuming plant-based beverages containing Brazil nuts may pose a risk to both children and adults due to high intake contributions relative to the tolerable upper intake level.

To assess exposure to inorganic elements, it is crucial not only to determine the total content of elements in foods but also to evaluate their absorbable fraction, or bioaccessibility, within the food (Brodkorb et al., 2019; Fioravanti et al., 2020). Bioaccessibility is defined as the fraction of the element that is released from the food matrix after ingestion and solubilized in the intestinal lumen and tract, which can be determined through *in vitro* solubility. *In vitro* digestion models are recognized alternatives to *in vivo* experiments, with no ethical restrictions, providing accurate results in a short period of time (Thomas, 2013; Ren et al., 2014; Hernández-Pellón et al., 2018; Astolfi et al., 2020; Kgabi and Ambushe, 2021; Román-Ochoa et al., 2021), such as the standardized method proposed by the INFOGEST group (Brodkorb et al., 2019).

Data on trace elements in plant-based foods are still scarce, and no studies were found that quantify these elements in plant-based foods intended as meat analogues. Given this context, the aim of this study was to evaluate the presence of trace elements Al, Cr, Co, Ni, As, Se, Mo, Cd, Sb, Ba, Hg, and Pb in plant-based foods marketed in Brazil that are intended as meat substitutes, using the ICP-MS method. To assess food safety, an exposure estimate was calculated and bioaccessibility was evaluated through simulated *in vitro* digestion following the standardized INFOGEST 2.0 protocol.

2. Materials and methods

2.1. Sampling

A total of 27 plant-based foods (PB) intended as substitutes for meat and derivatives were acquired in supermarkets in the city of Campinas: plant-based chicken (PBC, n = 4); plant-based ground beef (PBGB, n = 4); plant-based kibbeh (PBK, n = 2); ready-to-eat plant-based penne pasta (PBP, n = 2); plant-based burger (PBB, n = 9); plant-based breaded fish fillet (PBF, n = 4); and plant-based codfish cake (PBFC, n = 2). All brands have national reach in the Brazilian commerce and the sampling considered products' availability, flavors, and at least two different batches of each product. For comparison of bioaccessible content, six animal-based samples were also acquired: chicken fillet (C), ground beef (GB), kibbeh (K), mixed burger (chicken and beef) (B), breaded tilapia fillet (F), and codfish cake (FC).

All samples were purchased frozen from supermarkets in Campinas (Brazil) and stored in their original packaging in a freezer until analysis. Sample identification and key label information are detailed in Table 1.

2.2. Instrumentation

For trace element determination, an ICP-MS (iCAP RQ, Thermo Scientific, Bremen, Germany) was used under the following optimized conditions: Power = 1550 W; Argon flow rate = 14.0 L min^{-1}; Auxiliary

Table 1
Identification and main information from sample labels (Plant-based, n = 27; Meat and meat products, n = 6).

Sample	Brand	Commercial name	Ingredients (without additives)	Portion * (g)
PBC-a1	a	Plant-Based Chicken	Water, soy protein concentrate, pea protein, chickpea protein, vegetable oil, plant fibers, natural chicken flavor, spices.	80
PBC-a2			Water, soy protein concentrate, pea protein, sunflower vegetable oil, plant fibers, yeast extract, chicken-flavored seasoning.	
PBC-e1 PBC-e2	e	Plant-Based Chicken Fillet	Water, soy protein, vegetable oil, fava bean protein, gluten, bamboo fiber, minerals (Fe and Zn), vitamins (A, B9, B12), strawberry extract, tomato extract.	80
PBGB-a1 PBGB-a2	a	Plant-Based Ground Meat	Water, protein blend (textured soy protein, soy protein isolate, and pea protein), coconut fat, canola oil, beet powder.	80
PBGB-e1 PBGB-e2	e	Plant-Based Ground Meat	Water, pea protein, vegetable oil, coconut oil, bamboo fiber, fava bean protein, cocoa powder, onion powder, beet powder concentrate, garlic powder, spinach powder, black pepper powder, minerals (Fe and Zn), vitamins (A, B9, B12).	80
PBK-b1 PBK-b2	b	Plant-Based Kibbeh	Water, soy protein, wheat, vegetable oil, onion, gluten, garlic, malt extract, parsley, Fe, vitamin B12.	80
PBP-b1 PBP-b2	b	Plant-Based Penne	Water, enriched wheat semolina, annatto, turmeric, soy protein, vegetable oil, pea flour, chickpea protein, onion, beet flour, Fe, vitamin B12, tomato pulp, tomato, cashew nuts, seasonings.	100
PBB-a1	a	Plant-Based Burger with Beef Flavor	Water, protein blend (textured soy protein, soy protein isolate, pea protein, and chickpea flour), coconut fat, canola oil, beet powder	80
PBB-a2 PBB-a3			Water, protein blend (textured soy protein, soy protein isolate, and pea protein), vegetable fat, salt, beet powder.	
PBB-b1 PBB-b2	b	Plant-Based Burger with Beef Flavor	Water, soy protein, vegetable oil, vegetable fat, wheat gluten, pea protein, salt, onion, garlic, iron, vitamin B12.	80
PBB-c1 PBB-c2	c	Plant-Based Burger with Beef Flavor	Water, textured pea protein, coconut fat, modified starch, onion, yeast extract, beet powder, Himalayan pink salt, iron, vitamins (B1, B2, B3, B6, B9, B12, B7), black pepper, garlic.	120
PBB-c3 PBB-c4	c	Plant-Based Burger with Chicken Flavor	Water, textured pea protein, coconut fat, modified starch, onion,	100

(continued on next page)

Table 1 (*continued*)

Sample	Brand	Commercial name	Ingredients (without additives)	Portion * (g)
			yeast extract, Himalayan pink salt, iron, vitamins (B1, B2, B3, B6, B9, B12, B7), black pepper, garlic.	
PBF-b1 **PBF-b2**	b	Breaded Fish Strips	Water, breadcrumbs, soy protein, vegetable oil, vegetable fat, rice flour, cassava starch, corn flour, onion, gluten, wheat flour, wheat protein, salt, garlic, black pepper, dill, white pepper, iron, vitamin B12.	130
PBF-b3 **PBF-b4**	b	Breaded Fish fillet	Water, vegetable oil, breadcrumbs, soy protein, vegetable fat, onion, wheat gluten, wheat protein, rice flour, salt, cassava starch, corn flour, wheat flour, garlic, dill, black pepper, iron, vitamin B12, white pepper.	80
PBFC-d1 **PBFC-d2**	d	Codfish cake	Water, textured pea protein, pea protein isolate, rice protein isolate, lentil protein concentrate, potato, green jackfruit, rice flour, olive oil, sunflower oil, cassava starch, corn flour, garlic, onion, black pepper, parsley, Himalayan pink salt, bamboo fiber, rosemary extract	80
C	f	Chicken Fillet	Chicken Fillet	-
GB	f	Ground Beef	Chicken Fillet	-
K	f	Kibbeh	Beef, water (25 %), beef fat, whole wheat for kibbeh, textured soy protein (3.9 %), spices, onion, mint, garlic, black pepper, and white pepper, breadcrumbs, meat broth, salt, natural flavors.	-
B	g	Mixed Burger (Chicken and Beef)	Chicken with skin, animal fat, water (9.5 %), beef, soy protein (3 %), onion, collagen fiber (animal), salt, maltodextrin, potassium chloride, paprika, sugar, coriander.	-
F	g	Breaded Tilapia Fillet	Tilapia fillet (*Oreochromis niloticus*), enriched wheat flour with iron and folic acid, corn flour, salt, sugar, garlic, onion, pepper, cottonseed oil.	-
FC	f	Codfish cake	Cod (*Gadus morhua*) 35 %, water (30 %), potato flakes, antioxidants, preservatives, natural flavor, dehydrated egg, salt, onion, parsley, garlic, white pepper, nutmeg, and turmeric.	-

* Recommend portion from labelling.

argon flow rate = 0.80 L min^{-1}; Helium flow rate = 5.00 L min^{-1}; Micromist nebulizer flow rate = 0.98 L min^{-1}; Dwell time = 0.3 s and 0.02 s; monitored isotopes: ^{27}Al, ^{53}Cr, ^{59}Co, ^{60}Ni, ^{75}As, ^{78}Se, ^{97}Mo, ^{111}Cd, ^{123}Sb, ^{137}Ba, ^{202}Hg, ^{208}Pb; and internal standards (IS) at a concentration of 50 μg L^{-1}: ^{72}Ge, ^{103}Rh, ^{115}In, ^{209}Bi, ^{195}Pt.

For sample mineralization, a closed microwave-assisted system (Start D, Milestone, Sorisole, Italy) was employed at 1000 W power with the following settings: (a) ambient temperature to 120 °C for 15 minutes; (b) maintaining a constant temperature of 120 °C for 3 minutes; (c) temperature increase from 120 °C to 170 °C for 10 minutes; (d) maintaining a constant temperature of 170 °C for 15 minutes. For bioaccessibility assays, a Dubnoff-type water bath (Nova Técnica, Piracicaba, Brazil), pH meter (Ohaus, Parsippany, NJ, USA), and refrigerated centrifuge (Eppendorf, Hamburg, Germany) were used.

2.3. Reagents and materials

Reagents of analytical grade or higher were used: reverse osmosis purified water (Gehaka, São Paulo, Brazil), concentrated nitric acid (HNO_3) purified by sub boiling distillation (Distillacid, Berghof, Eningen, Germany), 30 % hydrogen peroxide (H_2O_2), and 37 % hydrochloric acid (HCl) (Merck, Darmstadt, Germany), sodium hydroxide (NaOH), potassium chloride (KCl), monopotassium phosphate (KH_2PO_4), sodium bicarbonate ($NaHCO_3$), sodium chloride (NaCl), magnesium chloride hexahydrate ($MgC_{l2}(H_2O)_6$), ammonium carbonate ($(NH_4)_2CO_3$), calcium chloride dihydrate ($CaCl_2$ (H_2O)), α-amylase from hog pancreas (50 U/mg), pepsin from porcine gastric mucosa (>3200 U/mg), pancreatin from porcine pancreas (8 USP) and bovine bile (Sigma Aldrich, St Louis, MO, USA). The argon and helium gases (Air Liquide, Nova Aparecida - Campinas, Brazil) were used for the ICP-MS analysis.

For quality control, a certified reference material SRM 1547 – Peach leaves (National Institute of Standards and Technology, Gaithersburg, USA) and analytical standards solutions of 100 or 1000 mg/L (Al, Cr, Co, Ni, Se, Cd, Sb, and Pb, brand Specsol-Quimlab, Jacareí, Brazil) were used. The samples were homogenized using an analytical mill (IKA, Staufen, Germany).

2.4. Sample mineralization

Approximately 0.5 g of each sample was weighed into a PTFE digestion tube, and 4 mL of HNO_3 was added. After resting overnight, 3 mL of H_2O_2 (30 %) and 1 mL of purified water were added. The containers were sealed and transferred to the microwave digester. After cooling, the content was transferred to a graduated tube, and the volume was adjusted to 20 mL with purified water, then filtered (PTFE, 0.45 μm). Assays were performed in triplicate, including analytical blanks.

2.5. Analytical quality control

The method was evaluated based on the following figures of merit: linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision (AOAC, 2016; INMETRO, 2020). Analytical curves were linear ($R^2 > 0.99$) for all elements, within the range of 5–1000 μg L^{-1} for Al and 0.1–100 μg L^{-1} for other elements.

Table 2
Results of figures of merit for the analyzed elements.

Element	LOD mg kg^{-1}	LOQ mg kg^{-1}	Accuracy (%)	Precision (CV, %)
Al	0.0600	0.2000	88 – 100	7.0
Cr	0.0024	0.0080	81 – 99	9.3
Co	0.0008	0.0032	92 – 95	1.3
Ni	0.0024	0.0080	80 – 91	6.0
As	0.0008	0.0032	76 – 83	4.1
Se	0.0008	0.0032	95 – 112	14
Mo	0.0024	0.0080	94 – 101	6.3
Cd	0.0004	0.0016	98 – 106	4.1
Sb	0.0008	0.0032	89 – 93	2.1
Ba	0.0024	0.0080	70 – 83	8.4
Hg	0.0016	0.0080	80 – 83	2.2
Pb	0.0024	0.0080	100 – 102	1.3

LOD = Limit of detection; LOQ = Limit of quantification; Accuracy: Certified reference material (NIST SRM 1547) for Al, Cr, Co, Ni, Se, Cd, Sb and Pb; and spiked experiments (0.005, 0.025 and 0.075 mg kg^{-1}) for As, Mo, Ba and Hg; Precision: CV = coefficient of variation (n = 10)

The LOD and LOQ, reported in Table 2, were estimated as LOD = 3 *s * f and LOQ = 5 *s * f; where s = standard deviation of 10 blank experiments and f = dilution factor (40x). To assess the accuracy of the method (Al, Cr, Co, Ni, Se, Cd, Sb, and Pb), a certified reference material was used, along with spiking/recovery tests (As, Mo, Ba, and Hg) at three levels: 0.005, 0.025, and 0.075 mg kg^{-1}. Table 2 shows recovery results ranging from 70 % (Ba) to 112 % (Se), with good agreement between the values and the SRM certification, meeting INMETRO (2020) criteria of 60–115 %. The precision of the method was evaluated by the coefficient of variation (CV%), which ranged from 1.3 % (Co and Pb) to 14 % (Se), in accordance with the established criterion (maximum CV% = 32) (INMETRO, 2020).

$$Bioaccessibility(\%) = \left(\frac{\text{element concentration in bioaccessible fraction}}{\text{total element concentration}}\right) X100 \quad (2)$$

2.6. Exposure assessment

For the calculation of the exposure, estimated per eating occasion (exposure per portion), to trace elements in the plant-based (PB) samples, the daily consumption of a portion (80, 100, 120, and 130 g) of plant-based meat analogues was considered, as described on the packaging (Table 1). The exposure per portion of trace elements was determined according to Eq. 1, using a standard body weight (bw) of 60 kg for adults and 15 kg for children:

$$Exposure\ per\ portion = \frac{[conc] * serving\ size}{bw} \quad (1)$$

Where:

Exposure per portion = mg kg^{-1} bw day; [conc] = trace element concentration (mg kg^{-1}); Serving size = portion of consumption (kg); bw = body weight (kg).

The results were compared with the Health-Based Guidance Values (HBGV) available in the literature to characterize the risk associated with exposure (IOM, 2000; EFSA, 2008, 2010, 2012a, 2012b, 2013, 2014, 2020, 2021, 2022, 2023; FAO/WHO, 2011, 2022), as shown in Table 4.

2.7. Bioaccessibility estimation

The estimation of bioaccessibility percentages for trace elements in the PB samples, as well as in meat and derivative samples, was evaluated by simulating *in vitro* digestion according to the INFOGEST 2.0 protocol (Brodkorb et al., 2019). For the procedure, enzymatic activities were determined and samples were prepared according to the instructions on the labels. The assays used a sample mass of 2.5 g (Rebellato et al., 2023b). Briefly, 2.5 g of the sample were weighed into a PTFE digestion tube. Then, 2.0 mL of simulated salivary fluid (SSF), 12.5 μL of $CaCl_2$ (0.3 M), 375 μL of SSF or α-amylase (for samples containing starch), and 112.5 μL of purified water were added. The mixture was homogenized by shaking for 2 minutes in a water bath at 37 °C. The oral bolus was then diluted with 4.0 mL of simulated gastric fluid (SGF) and the pH was adjusted to 3 with HCl (6 M). Next, 2.5 μL of $CaCl_2$, 333.5 μL of pepsin, and 464 μL of purified water were added, and the pH was readjusted, if necessary. The mixture was then incubated in a shaking water bath at 37 °C for 2 hours. The sample was kept in an ice bath to reach room temperature (± 10 minutes). For the intestinal phase, 4.0 mL of simulated intestinal fluid (SIF) were added and the pH was adjusted to 7.0 with NaOH (2.5 or 5 M). Then, 1.5 mL of freshly prepared bile solution, 20 μL of $CaCl_2$, 2.5 mL of freshly prepared pancreatin solution, and 1580 μL of purified water were added. The tube containing the sample, salts, and enzymes was pH-adjusted, if necessary, and placed in a shaking water bath at 37 °C for 2 hours.

After *in vitro* digestion (oral, gastric, and intestinal phases), the digested content was centrifuged at 4°C for 30 minutes (5000 rpm). The supernatant was transferred to a PTFE digestion tube, incubated at 100°C overnight (for volume reduction), and mineralized as described in 2.4. All experiments were performed in triplicate, with analytical blanks. Bioaccessibility percentages were calculated using Eq. 2.

2.8. Statistical analysis

The results for trace elements were evaluated using descriptive statistics (mean and standard deviation estimation), subjected to F-test (normality and variance), one-way analysis of variance (ANOVA), and Tukey's test (95 % confidence level), as well as multivariate analysis (Principal component analysis, PCA). For these analyses, Microsoft Office Excel (version 2019), Statistic 7.0 (StatSoft, Tulsa, USA), and Pirouette 3.11 (Infometrix, Woodinville, USA) were used.

3. Results and discussion

3.1. Total concentration of inorganic elements

Table 3 presents the results of the total trace elements in the plant-based samples. The elements Sb and Hg were not quantified in the samples, except for PBB-c2 (0.0048 ± 0.0003 mg kg^{-1}) and PBC-a1 (0.012 ± 0.001 mg kg^{-1}), respectively.

For a better understanding of the results, statistical analysis (one-way ANOVA and Tukey's test) was performed based on four distinct groups: i) Chicken (PBC-a1, PBC-a2, PBC-e1, PBC-e2), ii) Meat and related products (PBGB-a1, PBGB-a2, PBGB-e1, PBGB-e2, PBK-b1, PBK-b2, PBP-b1, PBP-b2), iii) Burger (PBB-a1, PBB-a2, PBB-a3, PBB-b1, PBB-b2, PBB-c1, PBB-c2, PBB-c3, PBB-c4), and iv) Fish and related products (PBF-b1, PBF-b2, PBF-b3, PBF-b4, PBFC-d1, PBFC-d2). Although the different products (chicken, meat, burger, and fish) showed significant differences, samples from the same manufacturer (different batches) were similar, particularly for Ba and Se in the chicken samples of brand "e" (PBC-e). The variation found in this study for the concentration of total trace elements in PB samples may be related to a lack of standardization in the formulation of these foods and the variability in the raw material sources (Shanmugam et al., 2023; Alasi et al., 2024; Tan et al., 2024).

Miller et al. (2024) reported a wide variation in the formulations of plant-based meat analogs, recommending that consumers check product labels to ensure alignment with their health and nutrition goals. In this study, a visual lack of homogeneity was observed among the samples (color, texture, moisture), even belonging to the same brand. Regarding the plant protein sources used, soy and its derivatives stood out as the primary protein source, followed by pea-based ingredients (Table 1).

The elements As, Cd, and Pb were quantified in most samples, observed in 63 % (n = 17), 78 % (n = 21), and 52 % (n = 14) of PB samples, respectively. Different results were found in studies on plant-based beverages and yogurts (Fioravanti et al., 2023; Rebellato et al., 2023a) and highly processed traditional meat products for As and Pb (Olmedo et al., 2024). In the present study, these elements were quantified for all five brands studied (a, b, c, d, e), but no studies on these trace elements in plant-based meat analogs were found for comparison

Table 3
Trace elements levels ($\bar{x}$ ± SD mg kg^{-1}) in plant-based products equivalent to chicken, meat and fish products.

Plant based similar to:	Sample	Al	Cr	Co	Ni	As	Se	Mo	Cd	Ba	Pb
		Mean ± standard deviation ($\bar{x}$ ± SD), mg kg^{-1}									
Chicken	**PBC-a1**	28.7 ± 2.4^{a}	0.43 ± 0.01^{a}	0.0036 ± 0.001^{a}	0.14 ± 0.01^{bc}	0.015 ± 0.001^{a}	0.015 ± 0.005^{ab}	2.93 ± 0.13^{a}	0.0048 ± 0.0002^{a}	1.30 ± 0.03^{a}	0.017 ± 0.003^{b}
	PBC-a2	27.4 ± 1.1^{a}	0.13 ± 0.10^{b}	0.008 ± 0.001^{b}	0.10 ± 0.02^{c}	0.0078 ± 0.0002^{b}	0.0090 ± 0.0005^{b}	0.72 ± 0.02^{b}	<LOQ	0.79 ± 0.02^{b}	0.038 ± 0.003^{a}
	PBC-e1	2.92 ± 0.26^{b}	0.13 ± 0.01^{b}	0.026 ± 0.002^{a}	0.18 ± 0.01^{a}	<LOQ	0.026 ± 0.005^{a}	0.42 ± 0.04^{c}	<LOQ	0.95 ± 0.14^{ab}	<LOQ
	PBC-e2	2.26 ± 0.02^{b}	0.09 ± 0.01^{c}	0.026 ± 0.001^{a}	0.158 ± 0.003^{ab}	<LOQ	0.023 ± 0.002^{a}	0.37 ± 0.05^{c}	<LOQ	1.02 ± 0.14^{a}	<LOQ
Meat and meat products	**PBGB-a1**	12.6 ± 0.4^{b}	0.461 ± 0.002^{b}	0.042 ± 0.001^{c}	0.26 ± 0.02^{b}	0.0077 ± 0.0004^{a}	0.041 ± 0.001^{b}	1.49 ± 0.04^{a}	0.0068 ± 0.0004^{a}	1.37 ± 0.10^{bc}	0.013 ± 0.003^{b}
	PBGB-a2	6.31 ± 0.72^{e}	0.12 ± 0.02^{d}	0.014 ± 0.001^{d}	0.14 ± 0.02^{c}	0.0040 ± 0.0006^{c}	0.0048 ± 0.0005^{c}	0.62 ± 0.01^{b}	0.0045 ± 0.0001 ^{cd}	1.48 ± 0.05^{abc}	0.013 ± 0.004^{b}
	PBGB-e1	7.75 ± 0.47^{e}	0.26 ± 0.03^{c}	0.053 ± 0.001^{b}	0.24 ± 0.02^{b}	0.0055 ± 0,0004^{b}	0.13 ± 0.01^{a}	0.45 ± 0.01^{d}	0.0053 ± 0.0003^{b}	1.26 ± 0.02^{c}	0.016 ± 0.001^{a}
	PBGB-e2	7.12 ± 0.77^{e}	0.15 ± 0.06^{d}	0.060 ± 0.005^{a}	0.17 ± 0.02^{c}	0.0042 ± 0.0006^{c}	0.13 ± 0.01^{a}	0.43 ± 0.02^{d}	0.0044 ± 0.0002^{d}	1.37 ± 0.05^{bc}	0.010 ± 0.001^{b}
	PBK-b1	14.55 ± 0.77^{a}	0.093 ± 0.006^{d}	0.020 ± 0.001 ^{cd}	0.08 ± 0.02^{d}	0.0040 ± 0.0002^{c}	0.0118 ± 0.0005^{bc}	0.47 ± 0.01^{d}	0.0051 ± 0.0003^{bc}	1.50 ± 0.16^{ab}	<LOQ
	PBK-b2	13.24 ± 0.42^{ab}	0.093 ± 0.003^{d}	0.0181 ± 0.0004^{d}	0.06 ± 0.01^{d}	0.0055 ± 0.0002^{b}	0.013 ± 0.001^{bc}	0.55 ± 0.01^{c}	0.0051 ± 0.0001^{bc}	1.89 ± 0.04^{a}	<LOQ
	PBP-b1	10.55 ± 1.18^{c}	0.59 ± 0.05^{a}	0.015 ± 0.001^{d}	0.40 ± 0.03^{a}	<LOQ	0.027 ± 0.001^{b}	0.20 ± 0.03 ^{f}	<LOQ	0.66 ± 0.02^{d}	<LOQ
	PBP-b2	9.30 ± 0.55 ^{cd}	0.23 ± 0.01^{c}	0.0161 ± 0.0001^{d}	0.21 ± 0.02^{bc}	<LOQ	0.014 ± 0.001^{bc}	0.30 ± 0.03^{e}	<LOQ	0.60 ± 0.01^{d}	<LOQ
Hamburgers	**PBB-a1**	16.37 ± 0.28^{c}	0.174 ± 0.003^{c}	0.024 ± 0.003^{de}	0.20 ± 0.03^{e}	0.0064 ± 0.0002^{b}	0.011 ± 0.001^{d}	0.81 ± 0.02^{d}	0.0064 ± 0.0001 ^{cd}	1.84 ± 0.09^{a}	<LOQ
	PBB-a2	9.82 ± 0.12^{d}	0.15 ± 0.01 ^{cd}	0.019 ± 0.004^{de}	0.205 ± 0.005^{e}	<LOQ	0.006 ± 0.002^{d}	0.63 ± 0.04^{e}	0.0047 ± 0.0003^{e}	1.32 ± 0.20^{c}	0.013 ± 0.004^{b}
	PBB-a3	8.03 ± 0.12^{de}	0.14 ± 0.04 ^{cd}	0.015 ± 0.001^{e}	0.22 ± 0.03^{e}	<LOQ	0.0042 ± 0.0005^{d}	0.59 ± 0.02^{e}	0.0037 ± 0.0003 ^{f}	1.24 ± 0.15^{c}	<LOQ
	PBB-b1	14.15 ± 1.47^{c}	0.13 ± 0.01 ^{cd}	0.0277 ± 0.0005^{d}	0.26 ± 0.03^{e}	<LOQ	0.029 ± 0.004^{c}	0.43 ± 0.02 ^{f}	0.0059 ± 0.0003^{d}	1.48 ± 0.11^{bc}	<LOQ
	PBB-b2	6.51 ± 0.28^{e}	0.098 ± 0.002^{d}	0.0157 ± 0.0003^{e}	0.12 ± 0.02 ^{f}	0.0055 ± 0.0003^{b}	0.0258 ± 0.0002^{c}	0.481 ± 0.001 ^{f}	0.0056 ± 0.0001^{d}	1.68 ± 0.04^{ab}	<LOQ
	PBB-c1	16.30 ± 0.92^{c}	0.25 ± 0.04^{b}	0.104 ± 0.002^{a}	0.97 ± 0.01^{a}	<LOQ	0.121 ± 0.004^{b}	1.01 ± 0.04^{bc}	0.0081 ± 0.0003^{a}	0.68 ± 0.03^{d}	0.008 ± 0.001^{c}
	PBB-c2	14.31 ± 1.19^{c}	0.166 ± 0.004^{c}	0.064 ± 0.001^{c}	0.73 ± 0.02^{c}	<LOQ	0.148 ± 0.009^{a}	1.48 ± 0.03^{a}	0.0081 ± 0.0003^{a}	0.79 ± 0.05^{d}	0.018 ± 0.001^{a}
	PBB-c3	22.87 ± 1.29^{b}	0.50 ± 0.03^{a}	0.088 ± 0.005^{b}	0.61 ± 0.02^{d}	0.009 ± 0.002^{a}	0.14 ± 0.01^{a}	0.95 ± 0.05^{c}	0.0069 ± 0.0005^{bc}	0.68 ± 0.04^{d}	0.018 ± 0.002^{a}
	PBB-c4	28.32 ± 1.05^{a}	0.28 ± 0.03^{b}	0.109 ± 0.006^{a}	0.87 ± 0.01^{b}	0.0068 ± 0.0002^{b}	0.13 ± 0.01^{ab}	1.08 ± 0.04^{b}	0.0074 ± 0.0004^{b}	0.72 ± 0.05^{d}	0.011 ± 0.001^{b}
Fish and fish products	**PBF-b1**	4.34 ± 0.07^{c}	0.37 ± 0.03^{c}	0.014 ± 0.001 ^{cd}	0.24 ± 0.02 ^{cd}	0.0041 ± 0.0002^{d}	0.012 ± 0.001^{c}	0.26 ± 0.01^{b}	0.0048 ± 0.0003^{ab}	1.49 ± 0.05^{a}	0.021 ± 0.004^{a}
	PBF-b2	4.46 ± 0.61^{c}	0.87 ± 0.03^{a}	0.027 ± 0.007^{b}	0.35 ± 0.06^{bc}	0.0051 ± 0.0001 ^{cd}	0.0066 ± 0.0002^{c}	0.28 ± 0.03^{b}	<LOQ	1.54 ± 0.08^{a}	<LOQ
	PBF-b3	9.12 ± 0.52^{b}	0.36 ± 0.04 ^{cd}	0.0225 ± 0.0004^{bc}	0.27 ± 0.02^{c}	<LOQ	0.013 ± 0.002^{c}	0.27 ± 0.02^{b}	0.0045 ± 0.0008^{b}	1.42 ± 0.15^{a}	<LOQ
	PBF-b4	2.94 ± 0.07^{c}	0.23 ± 0.03^{e}	0.0100 ± 0.0002^{d}	0.14 ± 0.01^{d}	0.006 ± 0.001^{c}	0.008 ± 0.001^{c}	0.39 ± 0.01^{b}	0.0046 ± 0.0003^{b}	1.47 ± 0.04^{a}	<LOQ
	PBFC-d1	9.73 ± 1.00^{b}	0.28 ± 0.01^{de}	0.029 ± 0.001^{b}	0.45 ± 0.07^{b}	0.015 ± 0.001^{a}	0.09 ± 0.01^{b}	0.75 ± 0.09^{a}	0.006 ± 0.001^{a}	0.49 ± 0.05^{b}	0.017 ± 0.004^{a}
	PBFC-d2	50.28 ± 0.69^{a}	0.54 ± 0.06^{b}	0.073 ± 0.006^{a}	1.096 ± 0.04^{a}	0.0129 ± 0.0002^{b}	0.131 ± 0.004^{a}	1.06 ± 0.06^{a}	0.006 ± 0.001^{ab}	0.67 ± 0.05^{b}	0.008 ± 0.001^{b}

Results are expressed as $\bar{x}$ ± SD, mean ± standard deviation (n = 3). Different letters for the same element (column) and same PB indicate significant difference ($p < 0.05$), according to Tukey's test at a 95 % confidence level. <LOQ: level below the limit of quantification.

purposes.

Fioravanti et al. (2023) observed the occurrence of As in rice-based plant beverages. A similar pattern was observed in this study: of the six samples containing rice flour or rice protein isolate in their ingredient list (Table 1, Fish Group), only one (PBF-b3) did not show quantifiable levels of this inorganic contaminant, possibly due to the high number of ingredients in the sample composition. The samples with the highest As concentrations were PBC-a1 (0.0149 mg kg^{-1}), PBFC-d1 (0.0150 mg kg^{-1}), and PBFC-d2 (0.0129 mg kg^{-1}), which contained chickpea and pea or rice and pea in their formulations.

Samples from brand b (PBK, PBP, PBB, PBF) are the only ones that list wheat and its derivatives in ingredient lists. Although studies have indicated the presence of Al, Ni, Cr, and Cd in wheat and its derivatives (Mathebula et al., 2017; Motta-Romero et al., 2023; Basaran, 2022), the present study was unable to correlate the concentration of the trace elements analyzed with these raw materials, probably due to the low amounts of wheat ingredients in the formulation.

Brazilian law (Brasil, 2022b) has established maximum tolerable limits (MTL) by food category: 0.20 mg kg^{-1} for Cd and Pb in soybeans, wheat and its derivatives; 0.20 mg kg^{-1} for As in wheat and its derivatives; 0.10 mg kg^{-1} for As and Cd, and 0.20 mg kg^{-1} for Pb in legumes (dry legume seeds), except soybeans. For Cd and Pb, European regulation 2023/915 (EU, 2023) has established maximum levels of 0.020 and 0.10 mg kg^{-1}, respectively, for legumes, and 0.15–0.20 mg kg^{-1} for wheat and its derivatives. For Hg, both Brazilian legislation (Brasil, 2022b) and European regulation 2023/915 (EU, 2023) have set MTLs for crustaceans, mollusks, and fish, ranging from 0.50 to 1.00 mg kg^{-1}. Based on the data presented in Table 3, all samples analyzed in this study were below the maximum levels established for these inorganic contaminants.

Olmedo et al. (2024) evaluated the concentration of eight elements, including Cd, Cr, Ni, Pb, and As, in samples of highly processed meat products purchased from supermarkets in the USA. The authors reported low levels of As, Cd, Cr, Ni, and Pb, all below the maximum limits set by international organizations. When comparing the average concentrations of Cd (0.00371 mg kg^{-1}), Cr (0.01839 mg kg^{-1}), and Ni (0.02225 mg kg^{-1}) reported by Olmedo et al. (2024), the PB samples analyzed in the present study exhibited higher levels of these elements.

The trace elements Al, Cr, Co, Ni, Se, Mo, and Ba were quantified in 100 % of the samples studied. The presence of Al, Se, Ni, and Co may be due to these elements that, in low concentrations, have biostimulants properties for plants. However, high levels of these elements may be a concern due to their toxicity in plants (Ayub et al., 2022).

3.2. Multivariate analysis

Principal component analysis (PCA) was used as a tool to classify plant-based meat analog samples based on the occurrence of trace elements. Since sampling considered brands with a national reach in Brazil, and it could be reflected in products obtained using raw materials from different sources or a lack of standardization in product formulations, even among products from the same brand. The PCA was constructed from the 27 PB samples (lines) and the levels of elements Al, Cr, Co, Ni, As, Se, Mo, Cd, Ba, and Pb (columns), resulting in a 27 × 10 matrix. Data

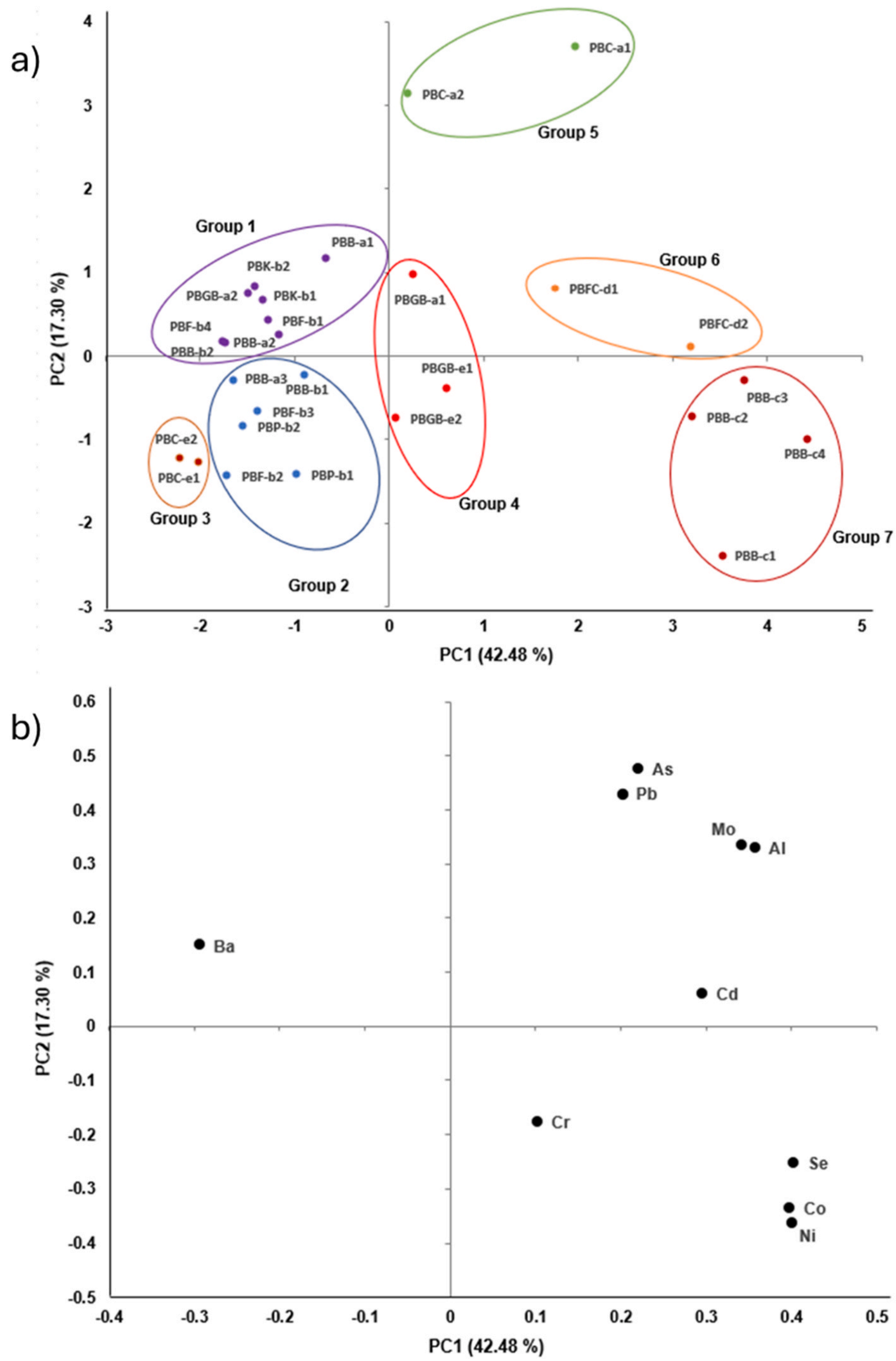

Fig. 1. Principal component analysis (PCA) of trace element levels in plant-based meat: a) scores and b) loading plots.

were autoscaled to normalize the importance of all variables studied. The graphical representation of the principal components is shown in Fig. 1, with 59.78 % of the variance explained by components PC1 and PC2 (loading and score plots). In the score plot, the samples were distributed into 7 distinct groups, according to their trace element composition, as follows:

- **Group 1**: Samples with high Ba levels, composed of meat analogues and burger samples from brands “a” and “b” (PBGB-a2, PBB-a1, PBB-a2, PBK-b1, PBK-b2, PBB-b2), as well as fish from brand “b” (PBF-b1 and PBF-b4). Only the plant-based kibbeh (PBK) had both batches from the same manufacturer classified similarly.

- **Group 2**: Samples with low levels of As, Cd, and Pb, which comprised six samples, including burger analogues from brands “a” and “b” (PBB-a3 and PBB-b1) and meat and fish analogues from brand “b” (PBP-b1, PBP-b2, PBF-b2, and PBF-b3). This group included pairs of batches from group 1, indicating variation in Cd and Pb concentrations between different batches of the same product (PBB-a, PBB-b and PBF-b).

- **Group 3**: Plant-based chicken analogues from brand “e” (PBC-e1 and PBC-e2). The sample composition differed from Group 4 due to the absence of cocoa and the use of soy protein in their ingredient list (Table 1), with low levels of As, Cd, and Pb.

- **Group 4**: Ground meat analogues (PBGB-a1, PBGB-e1, and PBGB-e2). These samples from brand “e” contain cocoa and pea protein in their formulation, which may contribute to higher concentrations of Co, As, Se, Cd, and Pb (Vanderschueren et al., 2021; Rovasi Adolfo et al., 2024; Godebo et al., 2024).

- **Group 5**: Samples with high levels of As and Mo. Composed solely of chicken analogues from brand “a” (PBC-a1 and PBC-a2), indicating this brand had a controlled source of plant-based ingredients for this product.

- **Group 6:** Samples with high levels of As and Cd. Composed solely of codfish cake analogues from brand “d” (PBFC-d1 and PBFC-d2), also indicating a controlled source of plant-based ingredients for this product.

- **Group 7**: Samples with high levels of Co, Ni, and Se and low Ba levels. Composed of burger analogues from brand “c” with meat (PBB-c1, PBB-c2) and chicken flavors (PBB-c3, PBB-c4). The samples acquired from brand “c” presented a distinguished composition from brands “a” and “b”, which were classified in Groups 1 and 2.

3.3. *Exposure assessment*

The exposure per portion was calculated for each PB food considering the portion recommended by the manufacturer (80–130 g, Table 1) and the standard weights of 60 kg (adult) and 15 kg (child). To characterize the risk associated with trace element exposure, the highest exposure per portion values obtained (mg kg^{-1} bw day) were compared to the reference values presented in Table 4 for each trace element. It is worth noting that portion size directly influences the exposure per portion calculation and that plant-based meat analogs are not the only dietary sources of these trace elements. The authors emphasize that only the exposure per portion and its % of contribution to the HBGV were calculated; therefore, this assessment does not include de % of contribution of plant-based meat products in relation to the diet as a whole.

The percentage contribution of samples to the HBGV for the trace elements Al, Ni, and Co, with at least one sample exceeding 50 % of the reference value, is shown in Fig. 2a and b. For Pb, Fig. 2c illustrates the margin of exposure values.

The highest exposure values calculated for Al were 0.047–0.189 mg kg^{-1} bw for adults and children, respectively, in sample PBB-c4 (Table 4). These values correspond to 17 % of the Provisional Tolerable Weekly Intake (PTWI) for adults and 66 % PTWI for children, equating to 33 % of the Tolerable Weekly Intake (TWI, adults) and 132 % TWI (children). It is important to note that, in addition to PBB-c4, four other PB samples showed contributions above 100 % of the Al TWI for children (Fig. 2a): PBC-a1 (106 %), PBC-a2 (102 %), PBB-c3 (107 %), and PBFC-d2 (109 %). Children generally represent the group with the highest potential Al exposure per kg of body weight, and studies suggest that high levels of dietary Al may be associated with developmental issues in infants and young children (EFSA, 2008; FAO/WHO, 2022).

As shown in Table 4, the plant-based burger PBB-c1 showed the highest exposure values for Co (0.0002 and 0.0008 mg kg^{-1} bw) and Ni (0.0019 and 0.0077 mg kg^{-1} bw) for adults and children, respectively. For adults, the calculated exposure per portion for Co corresponds to 11 % (Fig. 2b) of the HBGV for chronic exposure with toxic effects of Co (EFSA, 2012b). Extrapolating this reference value for children, the exposure per portion values ranged from 3 % (PBC-a2; PBF-b4) to 52 % (PBB-c1) of the HBGV. For Ni, the contribution of sample PBB-c1 corresponds to 15 % and 60 % of the Tolerable Daily Intake (TDI) (EFSA, 2020) for adults and children, respectively (Fig. 2b).

Table 4
Exposure per portion values of trace inorganic elements (mg kg^{-1} bw), considering the recommended portion (Table 1) for adults (body weight, bw = 60 kg) and children (bw = 15 kg).

Elements	Reference value and Health-based guidance values established		Population	Highest Exposure per portion (mg kg^{-1} bw day)	Sample
Al	PTWI	2 mg kg^{-1} bw (FAO/WHO, 2022)	Adults	0.047	PBB-c4
	TWI	1 mg kg^{-1} bw (EFSA, 2008)	Children	0.189	
Cr	TDI	Cr III 0.3 mg kg^{-1} bw (EFSA, 2014)	Adults	0.0019	PBF-b2
			Children	0.0075	
Co	Health-based guidance value	0.0016 mg kg^{-1} bw (EFSA, 2012b)	Adults	0.0002	PBB-c1
			Children	0.0008	
Ni	TDI	0.013 mg kg^{-1} bw (EFSA, 2020)	Adults	0.0019	PBB-c1
			Children	0.0077	
As	$BMDL_{0.5}$	i-As 0.003 mg kg^{-1} bw (FAO/WHO, 2011; EFSA, 2021)	Adults	0.00002	PBFC-d1
			Children	0.00008	
Se	UL	0.255 mg (EFSA, 2023)	Adults	0.0003	PBB-c2
	UL	0.4 mg (IOM, 2000)	Children	0.0012	
Mo	UL	0.6 mg (EFSA, 2013)	Adults	0.0030	PBB-c2
		0.1 mg (EFSA, 2013)	Children	0.0119	
Cd	PTMI	0.025 mg kg^{-1} bw (FAO/WHO, 2022)	Adults	0.00002	PBB-c2
	TWI	0.0025 mg kg^{-1} bw (EFSA, 2012a)	Children	0.00006	
Ba	TDI	0.2 mg kg^{-1} bw (EFSA, 2022)	Adults	0.0033	PBF-b2
			Children	0.0133	
Pb	$BMDL_{10}$	0.015 mg kg^{-1} bw (EFSA, 2010)	Adults	0.00005	PBC-a2
	$BMDL_{0.1}$	0.012 mg kg^{-1} bw (EFSA, 2010)	Children	0.00020	

PTWI = Provisional Tolerable Week Intake; TWI = Tolerable Week Intake; TDI = Tolerable Daily Intake; BMDL = Benchmark Dose Lower Limit; UL = Upper Intake Level; PTMI = Provisional Tolerable Monthly Intake.

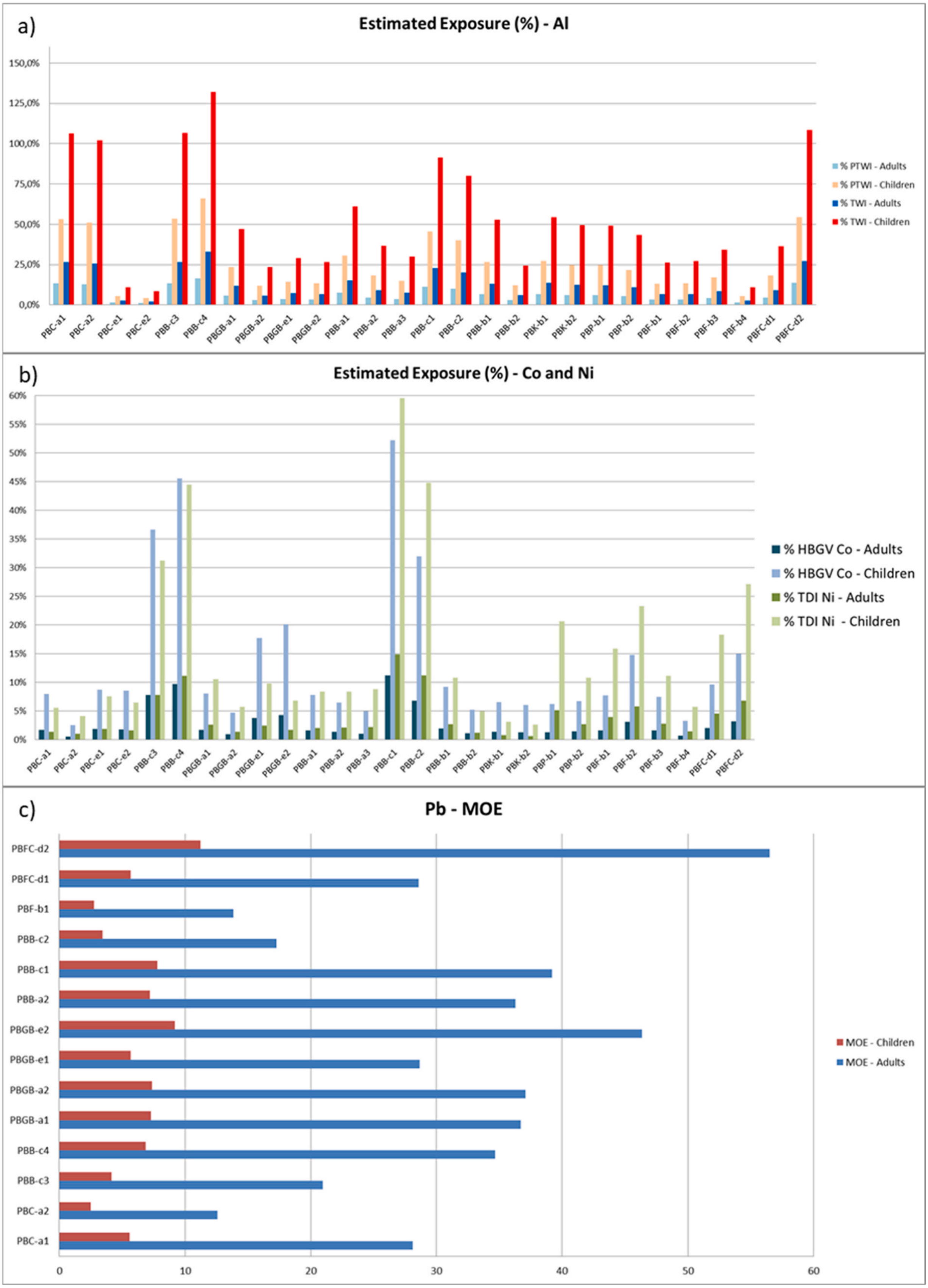

Fig. 2. a) Percentage (%) of PTWI (2 mg/kg bw) and TWI (1 mg/kg bw) for Al relative to the exposure per portion of the samples. PTWI = Provisional Tolerable Weekly Intake, TWI = Tolerable Weekly Intake; b) Percentage (%) of HBGV (0.006 mg/kg bw) for Co and TDI (0.013 mg/kg bw) for Ni relative to the exposure per portion of the samples. HBGV = Health-Based Guidance Value; TDI = Tolerable Daily Intake; c) MOE value relative to the exposure per portion obtained for the samples. MOE = Margins of Exposure; bw = body weight (adult = 60 kg; child = 15 kg); data not shown are below the limits of quantification (LOQ).

For the inorganic contaminant Pb, EFSA (2010) established a Benchmark Dose Lower Confidence Limit (BMDL), BMDL$_{01}$, of 0.012 mg Pb kg^{-1} bw day for children under six years old, corresponding to a dietary intake of 0.00050 mg kg^{-1} bw day. For adults, a BMDL$_{10}$ of 0.015 mg kg^{-1} bw day was set, corresponding to a dietary exposure of 0.00063 mg kg^{-1} bw day (EFSA, 2010). Among the samples studied, the plant-based chicken PBC-a2 showed the highest exposure values (0.00005 mg kg^{-1} bw for adults and 0.00020 mg kg^{-1} bw for children), as shown in Table 4.

The CONTAM panel established that Margins of Exposure (MOE) of 10 or greater would be sufficient that there was no appreciable risk of a clinically significant effect on Intelligence Quotient (IQ). For MOEs below 10 and greater than 1.0, the risk is likely to be low, but not such that it could be dismissed as of no potential concern. Fig. 2c presents the MOE results for samples in which Pb was quantified. For adults, all samples exhibited MOEs greater than 10, indicating no appreciable risk of a clinically significant effect. In contrast, for children, MOEs ranged between 1 and 10, except for the sample PBFC-d2 (MOE >10), indicating a likely low risk that nevertheless requires careful evaluation.

The calculated exposures for the trace elements Cr, As, Se, Mo, Cd, and Ba were considered low, as they represent only a minimal percentage of the HBGV available in the literature (IOM, 2000; EFSA, 2012a, 2013, 2014, 2021, 2022, 2023; FAO/WHO, 2011, 2022). As shown in Table 4, the estimated intakes for adults and children, respectively, were: 0.00002 and 0.00008 mg kg^{-1} bw for As (PBFC-d1), representing 1 % and 3 %, respectively, of the BMDL$_{0.5}$ (As) established for this element (FAO/WHO, 2011). The maximum values calculated for Cr (PBF-b2) and Ba (PBF-b2) were 0.0019 and 0.0033 mg kg^{-1} bw for adults, and 0.0075 and 0.0133 mg kg^{-1} bw for children (Table 4), corresponding to 0.6 % and 2.5 % of the TDI for Cr, and 1.7 % and 6.7 % of the TDI for Ba in adults and children, respectively (EFSA, 2014; EFSA, 2022). These contribution values are similar to those observed by Olmedo et al. (2024).

As shown in Table 4, the exposure from sample PBB-c2, for adults and children, respectively, were 0.0003 and 0.0012 mg kg^{-1} bw for Se, and 0.0030 and 0.0119 mg kg^{-1} bw for Mo. These results contributed between 1 % and 13 % of the Upper Intake Level (UL) stipulated for these elements (EFSA, 2013; EFSA, 2023). The results for the element Se are consistent with those reported by Teixeira et al. (2024a and 2024b) for plant-based beverages, except for those based on Brazil nuts.

Concerning the element Cd, the highest calculated exposure per portion values were also for the burger PBB-c2, which were 0.00002 and 0.00006 mg kg^{-1} bw (Table 4), corresponding to 5 % and 18 % of the TWI for adults and children, respectively, as established by EFSA (2012a). When considering the Provisional Tolerable Monthly Intake (PTMI) of 0.025 mg Cd kg^{-1} bw adopted by the FAO/WHO Joint Expert Committee on Food Additives (JECFA) (FAO/WHO, 2022), the Cd contribution in sample PBB-c1 accounted for 1.9 % and 7.8 % of the PTMI for adults and children, respectively.

3.4. Bioaccessibility of trace elements

To estimate the bioaccessibility (%B) of trace elements, six PB samples were selected, comprising the main plant-based meat products available in Brazil: Chicken (PBC-a1), Ground meat (PBGB-a1), Kibbeh (PBK-b2), Burger (PBB-a1), Fish (PBF-b4) and Codfish cake (PBFC-d2).

Table 5
Total content (T) and bioaccessible fraction (B content) of trace elements in meat and meat plant-based products, expressed as mean and standard deviation ($\bar{x}$ ± SD, n = 3).

Sample	Total content ($\bar{x}$ ± SD mg kg^{-1})									
	Al	Cr	Co	Ni	As	Se	Mo	Cd	Ba	Pb
C	0.98 ± 0.48	0.132 ± 0.004	<LOQ	0.064 ± 0.008	<LOQ	0.102 ± 0.002	0.051 ± 0.005	<LOQ	0.079 ± 0.006	0.009 ± 0.01
GB	0.82 ± 0.26	0.17 ± 0.02	0.007 ± 0.001	0.041 ± 0.009	<LOQ	0.100 ± 0.002	0.026 ± 0.001	<LOQ	0.072 ± 0.006	<LOQ
FC	2.35 ± 0.42	0.32 ± 0.005	0.007 ± 0.001	0.13 ± 0.02	0.26 ± 0.01	0.11 ± 0.01	0.031 ± 0.001	0.026 ± 0.001	0.17 ± 0.02	<LOQ
K	4.71 ± 0.29	0.19 ± 0.02	0.013 ± 0.001	0.18 ± 0.03	<LOQ	0.033 ± 0.002	0.46 ± 0.03	<LOQ	0.48 ± 0.05	<LOQ
B	6.62 ± 0.93	0.13 ± 0.03	0.013 ± 0.001	0.14 ± 0.01	<LOQ	0.087 ± 0.003	0.31 ± 0.01	<LOQ	0.26 ± 0.01	0.019 ± 0.001
F	5.40 ± 0.12	0.24 ± 0.05	0.012 ± 0.001	0.080 ± 0.004	0.012 ± 0.001	0.066 ± 0.003	0.052 ± 0.006	<LOQ	0.49 ± 0.04	0.004 ± 0.001
Sample	B content ($\bar{x}$ ± SD mg kg^{-1})									
	Al	Cr	Co	Ni	As	Se	Mo	Cd	Ba	Pb
C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.046 ± 0.001	<LOQ	0.014 ± 0.001	<LOQ
GB	0.12 ± 0.01	0.029 ± 0.002	0.004 ± 0.001	0.024 ± 0.004	<LOQ	0.07 ± 0.01	0.028 ± 0.003	<LOQ	0.016 ± 0.002	<LOQ
FC	0.51 ± 0.20	0.027 ± 0.002	<LOQ	0.05 ± 0.02	0.26 ± 0.002	0.09 ± 0.01	0.024 ± 0.001	0.013 ± 0.001	0.116 ± 0.0001	<LOQ
K	2.92 ± 1.95	0.042 ± 0.003	0.008 ± 0.001	0.08 ± 0.02	<LOQ	0.034 ± 0.008	0.28 ± 0.02	<LOQ	0.080 ± 0.004	<LOQ
B	1.86 ± 0.07	0.031 ± 0.001	0.009 ± 0.001	0.061 ± 0.004	<LOQ	0.039 ± 0.005	0.16 ± 0.01	<LOQ	0.027 ± 0.001	<LOQ
F	0.99 ± 0.07	0.035 ± 0.002	0.008 ± 0.001	0.014 ± 0.002	0.013 ± 0.001	0.065 ± 0.002	0.045 ± 0.005	<LOQ	0.12 ± 0.01	<LOQ
PBC-a1	2.67 ± 1.67	0.085 ± 0.004	0.021 ± 0.002	0.099 ± 0.003	<LOQ	<LOQ	2.31 ± 0.10	<LOQ	0.035 ± 0.001	<LOQ
PBGB-a1	1.49 ± 0.01	0.048 ± 0.003	0.027 ± 0.002	0.172 ± 0.008	<LOQ	0.043 ± 0.005	1.08 ± 0.11	<LOQ	0.076 ± 0.003	<LOQ
PBFC-d2	0.65 ± 0.07	0.037 ± 0.005	0.024 ± 0.002	0.41 ± 0.02	<LOQ	0.058 ± 0.009	0.15 ± 0.03	<LOQ	0.11 ± 0.01	<LOQ
PBK-b2	2.32 ± 0.64	0.036 ± 0.002	0.013 ± 0.001	0.05 ± 0.01	<LOQ	<LOQ	0.44 ± 0.02	<LOQ	0.24 ± 0.01	<LOQ
PBB-a1	1.89 ± 0.14	0.048 ± 0.002	0.015 ± 0.001	0.070 ± 0.003	<LOQ	<LOQ	0.50 ± 0.02	<LOQ	0.09 ± 0.01	<LOQ
PBF-b4	0.83 ± 0.01	0.053 ± 0.007	0.008 ± 0.002	0.067 ± 0.005	<LOQ	0.023 ± 0.007	0.27 ± 0.01	<LOQ	0.13 ± 0.01	<LOQ

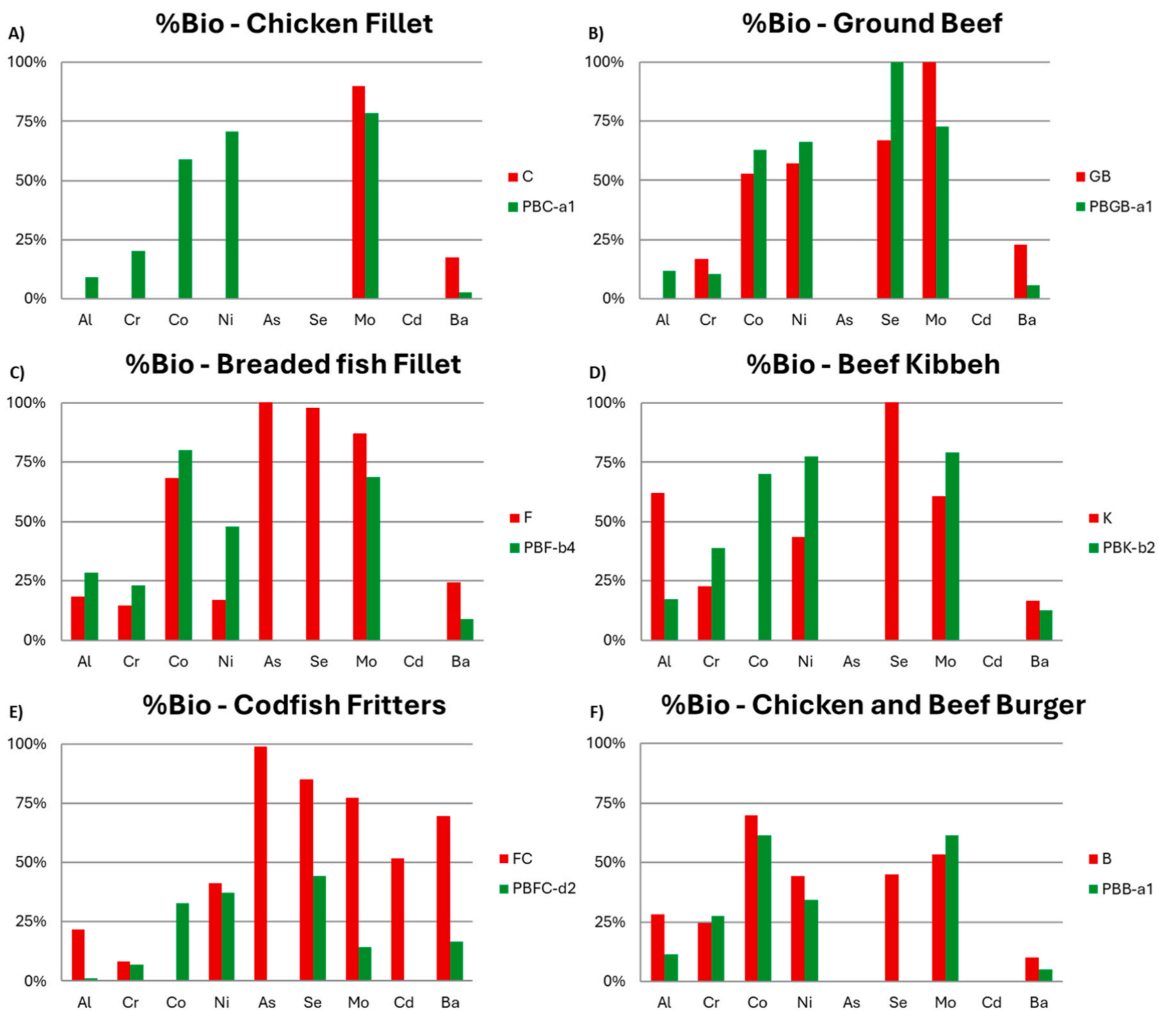

Fig. 3. Bioaccessibility (%) of trace elements: A) chicken fillet (C) and plant-based chicken (PBC-a1); B) ground beef (GB) and plant-based ground beef (PBGB-a1); C) breaded fish fillet (F) and plant-based breaded fish fillet (PBF-b4); D) kibbeh (K) and plant-based kibbeh (PBK-b2); E) codfish cakes (FC) and plant-based codfish cakes (PBFC-d2); F) Mixed burger (B) and plant-based burger (PBB-a1).

For comparison purposes, equivalent animal-based samples were analyzed: chicken fillet (C), ground beef (GB), kibbeh (K), mixed burger (B), breaded Tilapia fillet (F), and codfish cake (FC). The results are shown in Table 5 and Fig. 3.

In Fig. 3, differences between the PB and animal-based samples were observed. Generally, in PB samples, the trace elements Al (1–28 %), Cr (7–39 %), Co (33–80 %), Ni (34–77 %), Mo (14–79 %), and Ba (3–17 %) showed %B for the six samples evaluated, whereas in animal-based samples, only Mo (61–108 %) and Ba (10–70 %) showed %B for all samples studied. The trace elements Al and Cr demonstrated low bioaccessibility in 12 samples studied, ranging from 1 % (PBFC-d2) to 62 % (K) for Al and from 7 % (PBFC-d2) to 39 % (PBK-b2) for Cr. In addition, %B for Ba was also low, and notably the bioaccessibility in animal-based samples was higher than in plant-based samples (Fig. 3). For Co and Ni, the opposite trend was observed, except for the codfish cake (Ni) and burger (Co and Ni) samples, with %B for Co and Ni ranging from 33 % (PBFC-d2) to 80 % (PBF-b4) and from 17 % (F) to 77 % (PBK-b2), respectively. The samples PBF-b4 and PBK-b2 that declared vitamin B12 on their labels (Table 1) showed the highest bioaccessible fractions of 80 and 70 %, respectively, consistent with the high availability expected from vitamin B12 (Hazell, 1985).

Regarding the trace elements As and Cd, only fish-based foods showed a quantifiable soluble fraction: breaded Tilapia fillet F (As = 99 %) and codfish cake FC (As = 104 % and Cd = 52 %) (Fig. 3 C and E). The primary source of total As in the human diet is seafood. However, in these foods, As is predominantly present in its organic form, which is non-toxic (Zhang et al., 2002; Jackson and Punshon, 2015). Ciminelli et al. (2016) evaluated the total As content in the most consumed foods in Brazil (rice, beans, meats, fish, among others), and reported the highest total As levels (0.23 mg kg^{-1}) for fish samples when compared to the classes studied. The authors emphasized that the high proportion of organic As in fish is well established.

Concerning the codfish cake samples (Fig. 3E), the trace elements As, Se, Mo, Cd, and Ba showed bioaccessibility greater than 50 % in the animal-based sample (FC) when compared to the PBFC-d2. For the trace element Se, five animal-based samples (GB, F, K, FC, and B) and only two PB samples (PBGB-a1 and PBFC-d2) exhibited %B, with results ranging from 44 % (PBFC-d2) to 105 % (PBGB-a1 and K), respectively.

The *in vitro* digestion method simulates a sequence of events that occur during digestion in the human gastrointestinal tract, allowing us to estimate what percentage of nutrients will be available for absorption (bioaccessible content). Few studies have reported trace elements in plant-based foods, particularly in meat analogues. Previous research by our group has evaluated trace elements in plant-based foods including plant-based beverages (Fioravanti et al., 2023; Teixeira et al., 2024a, 2024b) and plant-based yogurts (Rebellato et al., 2023b), reporting results similar to those found in meat analogues, as follows: %B for Al (11–35 %), Cr (8–20 %), Mo (9–63 %), Co (51–107 %), Ba (9–92 %), and Ni (54–86 %) in plant-based beverages (Fioravanti et al., 2023); Se (63.5 % and 95.9 %) in plant-based beverages (Teixeira et al., 2024a, 2024b); and %B for Cr (8–64 %), Mo (>70 %), Co (11–89 %), Ba (12–76 %), and Ni (7–90 %) in plant-based yogurts (Rebellato et al., 2023b).

4. Conclusion

The trace elements Al, Cr, Co, Ni, Se, Mo, and Ba were quantified in 100 % of the samples studied, while the inorganic contaminants As, Cd, and Pb were found at levels below the maximum limits established by legislation. Considering adult consumption, the results indicated that the plant-based (PB) food samples are safe for the 12 trace elements evaluated. In turn, additional studies are recommended for child consumers. Some samples showed relevant trace element contributions to children's diets: five PB samples (two chicken-based foods, two burgers, and one codfish cake) showed significant contributions for Al (>100 % of TWI); Co and Ni with contributions > 50 % of the values established for a PB burger sample; and 13 PB samples with MOE between 1 and 10 for Pb.

The bioaccessibility (%B) for Se, Mo, and Ba was lower in PB foods compared to the meat products, while Co and Ni showed higher bioaccessibility in the plant-based samples. These differences are probably due to the influence of the raw material (plant-based or animal-based) and the composition of the food, emphasizing the impact that the replacement of animal source products by this new class of food can have on consumer health. The present findings are novel and underscore the need for additional studies to monitor the risks associated with children's consumption of plant-based foods.

CRediT authorship contribution statement

Bragotto Adriana Pavesi Arisseto: Writing – review & editing, Supervision, Resources, Conceptualization. **Morgano Marcelo Antônio:** Writing – review & editing, Supervision, Resources, Conceptualization. **Rebellato Ana Paula:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Milani Raquel Fernanda:** Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Fioravanti Maria Isabel Andrekowisk:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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