Journal of Trace Elements in Medicine and Biology 81 (2024) 127329

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

# Selenium in plant-based beverages: Total content, estimated bioaccessibility and contribution to daily intake

José Luan da Paixão Teixeira [a,*], Ana Paula Rebellato [a], Maria Isabel Andrekowisk Fioravanti [b], Raquel Fernanda Milani [a], Marcelo Antonio Morgano [a]

[a] Food Science and Quality Center, Institute of Food Technology, Av. Brazil, 2880, Jd. Chapadão, CEP.: 13070-178, Campinas, SP, Brazil
[b] Adolfo Lutz Institute, Street São Carlos, 720, Vila Industrial, CEP.: 13035-420, Campinas, SP, Brazil

ARTICLE INFO

*Keywords:*
Vegetable beverages
Ultrasound assisted acid digestion
ICP-MS
Bioaccessibility
INFOGEST
Reference daily intake

ABSTRACT

*Background:* The search for alternative protein sources has increased the consumption and commercialization of plant-based beverages (PBBs). This study aimed to determine the total Se content, estimate the bioaccessibility of selenium (Se) in commercial PBBs derived from different raw materials, and evaluate their contribution to the reference daily intake (RDI).
*Methods:* An ultrasound assisted acid digestion method and ICP-MS was used to determine Se, and the INFOGEST method to estimate the bioaccessible percentages. Validation of this method was also performed, and the parameters obtained were: LOD and LOQ were 2.1 and 4.0 μg/kg, respectively. For accuracy, recovery percentages ranged from 99 % and 111 % (certified reference materials), and 95 % and 101 % (spiked experiments for bioaccessible extracts as recoveries).
*Results:* The PBBs presented total Se content between 4 and 226 μg/kg. Bioaccessible percentages ranged from 63.5 % (mix of plant sources) to 95.9 % (produced with organic cashew nuts). Only one cashew nut PBBs supplied the daily demand of Se, representing 64.6 %, 75.3 % and 82.2 % of the RDI; for lactating and pregnant women, children (≥ 4 years) and adults, respectively.
*Conclusions:* The Se determination method through acid digestion assisted by ultrasound and ICP-MS was considered adequate for the PBBs samples. Se content varied according to the raw material used in sample preparation. High percentages (> 60 %) of bioaccessibility were observed and only one PBBs derived from organic cashew nuts supplied the recommended Se demand for different groups of individuals.

## 1. Introduction

The plant-based beverage (PBBs) market has grown significantly in recent years, reaching a value of USD 14 billion worldwide [1]. The consumption and commercialization of PBBs manufactured from nuts, cereals and seeds is already a reality in all parts of the world [2].

These beverages are the preferred alternative for consumers who are lactose intolerant and/or allergic to cow milk proteins; for people who follow vegetarian/vegan diets (flexitarians), and/or are concerned about environmental issues; and their consumption is also associated with the absence of cholesterol, as well as reduced caloric intake [2,3]. Moreover, PBBs can serve as an additional nutritious option for developing countries, where the supply of cow milk is insufficient.

In general, PBBs are prepared by disintegrating the plant material of various raw commodities, such as: almonds, peanuts, rice, oats, coconut, cashew nuts, and soy, among others. These present appreciable amounts of unsaturated fatty acids, phenolic compounds, vitamins and essential minerals (calcium, iron, magnesium, phosphorus, potassium, manganese, zinc, among others); affording health benefits to consumers [4–8]. However, information regarding selenium (Se) content in PBBs is scarce. In addition, knowledge of the Se fraction that can be absorbed by the body from these beverages is of great importance, considering all the benefits linked with intake of these particular nutrients.

In this sense, studies that evaluate the bioaccessible fractions of different food matrices have already been conducted using methods such as static *in vitro* gastrointestinal digestion; which determines the estimated mineral and trace element bioaccessibility of soluble fractions after centrifugation and/or dialysis [9]. The static method proposed by INFOGEST has been previously recommended in the literature. This method is a standardized *in vitro* simulation of the gastrointestinal

* Corresponding author.
*E-mail address:* joseluanp.teixeira@gmail.com (J.L.P. Teixeira).

https://doi.org/10.1016/j.jtemb.2023.127329
Received 13 July 2023; Received in revised form 9 October 2023; Accepted 21 October 2023
Available online 23 October 2023

conditions, and imitates the natural process using enzymes and/or reagents [10].

The evaluation of Se levels, and estimation of bioaccessibility, in different PBBs is fundamental for the adequate characterization of these beverages, since Se performs important functions in humans [2,5]. Regular intake of dietary Se can control oxidative stress, reduce the incidence of inflammation in the body and aid in lipid metabolism [11].

In contrast, low intake levels of dietary Se can lead to the emergence of different diseases, including: weakening of the immune system, hypothyroidism, cardiovascular diseases, reduced fertility in men and increased risk of various types of cancer [11,12]. In this context, the reference daily intake (RDI) of Se also needs to be monitored, considering that the deficiency in the RDI of Se is not only a reality in Brazil, but also in several parts of the world [13,14].

However, analytical instrumentation is one of the main challenges for Se determination in food matrices, due to low levels of concentration. In this context, the inductively coupled plasma mass spectrometer (ICP-MS) is one of the main systems used for Se determination in foods matrices [15–17]. On the other hand, wet mineralization using nitric acid and hydrogen peroxide is one of the most used methods for the decomposition of organic matter; being used for different food matrices [18,19].

Given the above, and taking into account the scarce or non-existent information available in the literature regarding this subject, the main objectives of this study are: (1) validate a method that determines Se content in PBBs through ultrasound assisted acid digestion and ICP-MS; (2) analyze total Se content in commercial PBBs produced from different raw materials; (3) estimate the bioaccessibility of Se in PBBs using the standardized *in vitro* digestion method described by INFOGEST; and (4) evaluate the contribution of PBBs to the RDI of Se for lactating and pregnant women, children and adults.

## 2. Material and methods

### 2.1. Reagents and solutions

For the analytical procedures, the following analytical grade reagents were used: water purified through reverse osmosis, with resistivity less than 18.2 MΩ cm (Gehaka, São Paulo, Brazil); 65 % concentrated nitric acid ($HNO_3$) (Merck, Darmstadt, Germany) purified by sub-boiling distillation (Distillacid, Berghof, Eningen, Germany) and 30 % v/v hydrogen peroxide ($H_2O_2$) (Merck, Darmstadt, Germany).

For the bioaccessibility assays using the standardized method proposed by INFOGEST, the following reagents were used: 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 ($MgCl_2(H_2O)_6$); ammonium carbonate ($(NH_4)_2CO_3$); dihydrate calcium chloride ($CaCl_2(H_2O)_2$); salivary α-amylase (50 U/mg); porcine pepsin (3843 U/mg); porcine pancreatin (7.3 U/mg) and bile salts (200 mg/mL); all sourced from Sigma and Aldrich, St. Louis, MO, United States.

Certified reference materials (CRM) used were: skimmed milk powder (ERM-BD 151, European Commission, Geel, Belgium), peach leaves (1547, NIST, Gaithersburg, EUA) and standard solutions of Se, Ge and In (1000 mg/L) (Quimlab, Jacareí, Brazil).

### 2.2. Equipment

An ultrasonic bath (Easy 180 H, Elma, Germany) and a digester block (Tecnal, Brazil) were used for the mineralization of PBBs samples. A centrifuge (5804 R, Eppendorf, Hamburg, Germany) was used to obtain the bioaccessible extracts, and an inductively coupled plasma mass spectrometer (ICP-MS) (iCAP RQ, Thermo Fisher Scientific™, Bremen, Germany) was used to determine the Se levels in PBBs under optimized operational conditions. Conditions followed Rebellato et al. [20], and were: Air flow rate/Auxiliary air (14.0/0.80 L/min); He flow rate (5.0 mL/min); Nebulizer flow rate (Micromist; 0.98 L/min); dwell time (0.3 s/0.02 s IS); monitored isotopes ($^{77}Se$; $^{78}Se$; $^{82}Se$); and internal standards, IS (50 μg/L) ($^{72}Ge$; $^{74}Ge$; $^{115}In$).

### 2.3. Plant-based beverages (PBBs)

All PBBs samples were purchased from commercial establishments in the city of Campinas (São Paulo, Brazil). Forty-two beverage samples containing different plant proteins were evaluated: five almond-based samples, seven from cashew nuts, five from coconut, five from oat, five from peanut, six from rice, five from soy and four samples from other vegetable sources (beverages consisting of plant protein and other plant ingredients). Table 1 indicates the described ingredients and main protein source of the different PBBs characterized in this study. For the acquisition of PBBs samples, different commercial brands were considered in different batches. For comparison purposes, two samples of cow milk were also evaluated. In total, forty-four samples were analyzed.

**Table 1**
Main protein source of plant-based beverage samples.

| Samples | Type | Main Ingredients / Source of Protein |
|---|---|---|
| L1 | Almonds | Almond paste. |
| L2 | | Almond paste. |
| L3 | | Almond paste, coconut cream and pea. |
| L4 | | Almond paste, coconut cream and pea. |
| L5 | | Almond paste, cocoa. |
| L6 | Cashew Nut | Organic cashew nuts and Brazil nuts (*Bertholletia excelsa*). |
| L7 | | Organic cashew nuts and Brazil nuts (*Bertholletia excelsa*). |
| L8 | | Cashew nuts. |
| L9 | | Organic cashew nuts. |
| L10 | | Cashew nut paste. |
| L11 | | Cashew nut paste. |
| L12 | | Cashew nut and cocoa. |
| L13 | Coconut | Coconut milk. |
| L14 | | Coconut cream and pea. |
| L15 | | Coconut cream. |
| L16 | | Coconut cream. |
| L17 | | Coconut milk. |
| L18 | Oat | Oat paste. |
| L19 | | Oat and vanilla. |
| L20 | | Integral oatmeal (16 %). |
| L21 | | Oat and natural cocoa. |
| L22 | | Oat, pea and cocoa powder. |
| L23 | Peanut | Peanut and coconut cream. |
| L24 | | Peanut paste. |
| L25 | | Peanut and cocoa. |
| L26 | | Peanut and oat flour. |
| L27 | | Peanut and oat flour. |
| L28 | Rice | Rice (6.7 %). |
| L29 | | Rice flour. |
| L30 | | Rice (8.0 %) and cocoa powder (2.0 %). |
| L31 | | Organic rice (14 %). |
| L32 | | Rice (6.7 %) and coconut powder (3.0 %). |
| L33 | | Organic rice. |
| L34 | Soy | Grain paste of soy. |
| L35 | | Grain paste of soy. |
| L36 | | Grain paste of soy. |
| L37 | | Soy extract and apple juice concentrate. |
| L38 | | Grain paste of soy. |
| L39 | *Mix of vegetable sources | Chicory fiber, soy, concentrated pineapple, cabbage juice and pea. |
| L40 | | Soy, pea, chicory fiber, cocoa powder, concentrated pineapple and cabbage juice. |
| L41 | | Chicory fiber, soy, concentrated pineapple, cabbage juice, pea and soluble coffee powder. |
| L42 | | Soy, pea, chicory fiber, cocoa powder, concentrated pineapple and cabbage juice. |
| L43 | Cow milk | Integral milk. |
| L44 | | Whey milk, integral milk, cocoa and barley malt extract. |

* Beverages consisting of plant protein and other plant ingredients.

### 2.4. Quality control

The analytical method was validated for the following parameters: linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy (recovery) and repeatability (precision); according to the guidelines of the Institute of Metrology, Standardization and Industrial Quality (DOQ-CGCRE-008) [21].

The linearity was evaluated using the correlation coefficient of the analytical curve for Se, obtained with 5 (five) points, and ranging from 0.1 to 100 μg/kg. The limits of detection and quantification were calculated as LOD = 3 * s * f, and LOQ = 5 * s * f, considering the standard deviation of the concentration of 10 blank experiments (s), and the dilution factor (f) for Se content and Se bioaccessible extracts: 40x and 200x, respectively. Certified reference materials (skimmed milk powder and peach leaves), and spiked experiments for bioaccessible extracts at 25 and 75 μg/kg, were used for accuracy (recovery). Repeatability (precision) was evaluated by the coefficient of variation (CV), obtained by analyzing 7 (seven) repetitions of the same PBBs sample on the same day.

The analytical curve was considered linear ($r^2 \geq 0.99$). The LOD and LOQ were 2.1 and 4.0 μg/kg, respectively. For accuracy, recovery percentages ranged from 99 ± 8 % for skimmed milk powder and 111 ± 6 % for peach leaves (certified reference materials), and 95 ± 1 % and 101 ± 5 % (spiked experiments). While, for repeatability (precision), the value of the coefficient of variation (CV) was 9 %, which corresponds to INMETRO specifications [21].

### 2.5. Determination of PBBs Se content

The samples were subjected to ultrasound-assisted acid digestion, as described by Rebellato et al. [22]. Approximately 0.5 g of sample was weighed into graduated tubes (50 mL). Then, 4 mL of $HNO_3$ was added, and left overnight. The following day, 2 mL of $H_2O_2$ was added and the tubes closed and transferred to an ultrasonic bath for 35 min at 80 °C. At the end of mineralization, the digestate was cooled to room temperature, volume was made up to 20 mL with ultrapure water and filtered using a 0.45 μm PTFE filter (Agilent Technologies, Tokyo, Japan). All mineralizations were performed in triplicate, including analytical blanks.

### 2.6. Estimation of Se bioaccessibility in PBBs

The estimate of Se bioaccessibility percentages found in the PBBs samples was determined (in triplicate) through the standardized *in vitro* digestion procedure proposed by INFOGEST, as reported by Brodkorb et al. [10]. The procedure was comprised of an oral, gastric, and intestinal phase, and utilized 2.5 g of PBBs samples.

To determine the Se content, after the centrifugation process, the supernatant was transferred into digestion tubes, and evaporated overnight in an oven at 80 ºC. The samples were mineralized in an open system, as proposed by Silva et al. [7], using two steps: first, 4 mL of $HNO_3$ was added to the digestion tubes and heated using a digester block set at 110 ºC for 2 h; subsequently, after cooling to room temperature, 2 mL of $H_2O_2$ was added, and heated again at 130 °C for 2 h. During the entire mineralization step, a watch glass was placed on top of the tubes, allowing for $HNO_3$ reflux and better contact between the samples and the oxidizing mixture. The *in vitro* digestion blanks were prepared with 2.5 g of ultrapure $H_2O$, as substitute for the samples, and the complete protocol was followed in its entirety.

### 2.7. Reference daily intake (RDI)

The RDI was calculated using the deterministic model [23], considering the maximum Se content observed from the different types of PBBs; as well as the RDI established by Normative Instruction 75 [24], and the FDA [25]. Results were expressed in μg/kg of Se.

The contribution to RDI percentage of Se was determined for different groups of individuals, which were: lactating and pregnant women, children (≥ 4 years old) and adults; in accordance with the recommendations expressed in Normative Instruction 75 [24], and by the FDA [25]. It is worth noting that, in order to carry out the RDI calculations, the consumption of one glass (200 mL) of PBBs was adopted for all samples; as all PBBs manufacturers characterized in this study regarded this amount as the recommended single serving size, and is also based on the nutritional labelling information.

### 2.8. Statistical analysis

The results obtained for the determinations of total content, estimation of bioaccessibility and RDI percentages of Se were evaluated with the following parameters: Analysis of Variance (ANOVA one-way), Tukey test (95 % confidence) and coefficient of variation (CV). For this, the following computational programs were used: Microsoft Office Excel (version 2016), Statistica 7.0 (StatSoft, EUA) and Statgraphics Centurion XVI.II (Statistics Graphics Corporation, EUA).

## 3. Results and discussion

### 3.1. Total selenium content of plant-based beverages

The levels (mean ± standard deviation) of Se found in the different

**Table 2**
Total selenium content of different plant-based beverages.

| Samples | Type | Total Se (μg/kg) | Range (μg/kg) |
|---|---|---|---|
| L1, L2, L4, L5 | Almonds | n.d. | <4.0 – 4.4 |
| L3 | | $4.4 \pm 0.05^{JK}$ | |
| L6 | Cashew Nuts (cashew and Brazil nuts) | $185.7 \pm 0.5^{bB}$ | 5.2 – 226.0 |
| L7 | | $226.0 \pm 4.1^{aA}$ | |
| L8 | | $17.7 \pm 0.5^{cE}$ | |
| L9 | | $22.9 \pm 0.7^{cD}$ | |
| L10 | | $5.2 \pm 0.2^{dIJK}$ | |
| L11 | | $8.8 \pm 0.1^{dFGHIJ}$ | |
| L12 | | $23.8 \pm 1.4^{cD}$ | |
| L13, L15, L16, L17 | Coconut | n.d. | <4.0 – 7.2 |
| L14 | | $7.2 \pm 0.8^{GHIJK}$ | |
| L18, L19 | Oat | n.d. | <4.0 – 29.2 |
| L20 | | $4.1 \pm 0.1^{bK}$ | |
| L21 | | $5.4 \pm 0.1^{bIJK}$ | |
| L22 | | $29.2 \pm 2.5^{aC}$ | |
| L23, L24, L25, L26, L27 | Peanut | n.d. | <4.0 |
| L28, L29, L30, L31, L32, L33 | Rice | n.d. | <4.0 |
| L34, L35, L36, L38 | Soy | n.d. | <4.0 – 9.7 |
| L37 | | $9.7 \pm 0.4^{FGHI}$ | |
| L39 | *Mix of vegetable sources | $10.6 \pm 0.4^{bFGH}$ | 10.6 – 20.9 |
| L40 | | $20.9 \pm 0.3^{aDE}$ | |
| L41 | | $12.3 \pm 0.3^{bF}$ | |
| L42 | | $20.1 \pm 1.6^{aDE}$ | |
| L43 | Cow milk | $10.8 \pm 0.6^{aFG}$ | 6.3 – 10.8 |
| L44 | | $6.3 \pm 0.9^{bHIJK}$ | |

[*] Beverages consisting of plant protein and other plant ingredients. Results are expressed as mean ± standard deviation (n = 3). Different lowercase superscript letters in the column indicate significant difference ($P < 0.05$) between samples of the same type of beverage. Different capital superscript letters in the column indicate significant difference ($P < 0.05$) between the different types of beverages. ANOVA + Tukey Test at 95 % of confidence; n.d.: not detected (<LOQ, < 4.0 μg/kg).

PBBs are shown in Table 2. The lowest and highest values of quantifiable Se were found in samples L20 (oats + calcium) (4.1 μg/kg) and in sample L7 (cashew nuts + Brazil nuts (*Bertholletia excelsa*) (226 μg/kg), respectively. Statistical differences ($P < 0.05$) were observed between samples belonging to the same group, as well as between the different groups evaluated.

All peanut and rice PBBs samples showed Se levels below the limit of quantification (< 4.0 μg/kg) and are considered poor in this nutrient. For PBBs samples derived from almonds, coconut and soy, only one beverage from each source presented Se content higher than the limit of quantification; with values of 4.4 (L3); 7.2 (L14) and 9.7 (L37) μg/kg, respectively. Corroborating the results expressed in this study, Astolfi et al. [4] and Orlando et al. [26] also reported unquantifiable levels of Se in almond, peanut, rice, oats and soy PBBs.

However, soy and oat PBBs samples show low levels of Se, being detected in only oat (L20, L21 and L22) and one of soy (L37). Silva et al. [27] evaluated the technological aspects of rice plant-based beverages, reporting that, although white rice has approximately 151 μg/kg of Se, the rice beverages derived did not maintain quantifiable levels of this trace element; as also observed in the present study. Similar behavior was also observed in PBBs samples of soy [28]. According to these authors, despite the levels of Se in soy reaching 800 μg/kg, in this study, the highest Se value observed from soy beverage was 9.7 μg/kg. In addition, oat beverages had quantifiable Se content, ranging from 4.1 to 29.2 μg/kg of Se; which were lower than the levels reported by Rybicka et al. [29] in oat flour (222 μg/kg).

On the other hand, the cashew nut beverages had the highest Se content, ranging from 5.2 to 226 μg/kg; with the highest being a mixture of cashew nuts and Brazil nuts (Pará nuts). Brazil nuts are known for having high Se content [26], and the results showed the highest levels were in L6 (185 μg/kg) and L7 (226 μg/kg). For the other samples, where the cashew nut ingredient was predominant, the maximum Se content obtained was 23.8 μg/kg (L12). Rico et al. [30] assessed the nutritional composition of cashew nuts *in natura* from different nationalities, and reported levels of 750 and 800 μg/kg of Se in Brazilian and Indian cashew nuts, respectively. Orlando et al. [26] investigated different vegetable-based beverages and found that the beverage made with cashew nuts presented the highest levels of Se, ranging from 19.6 to 160.5 μg/kg. Vasquez-Rojas et al. [8] studied the Se content in cashew nut vegetable drinks and reported maximum values of 150 μg/kg. Therefore, the cashew nut beverages reported in this work presented Se levels close to the studies mentioned by Orlando et al. [26] and Vasquez-Rojas et al. [8].

Four samples of plant-based beverages were also evaluated, which were not classified as almonds, cashew nut, coconut, oat, peanut, rice or soy beverages; having a list of ingredients different from those previously reported. In general, the samples consisted of a mixture of coconut and sunflower oils, soy and pea proteins, chicory fiber, pineapple and/or cabbage concentrated juices; and in some cases, the addition of soluble coffee powder (sample L41) and cocoa (L40 and L42). The results for these samples being that Se content ranged from 10.6 to 20.9 μg/kg.

The Se content was also evaluated in a chocolate beverage, as well as an integral milk UHT (ultra-high temperature) samples, for comparative purposes; with levels of 6.3 and 10.8 μg/kg determined, respectively. Assis et al. [31] studied different samples of cow milk and reported mean Se levels of 29 μg/kg. OKane et al. [32] and Ling et al. [33] reported mean Se contents of 18 and 46 μg/kg in cow milk samples from the United Kingdom and Estonia, respectively. Thus, the Se levels reported by the authors above are higher than those mentioned in this study. The Se content contained in cow milk samples showed variation, a fact that may be related to the different milk origins, due to the geographic diversity of the soils of these countries [31]; and the supplementation of Se in animal feed [32,34].

### 3.2. Bioaccessibility of selenium in plant-based beverages

The percentage Se bioaccessibility estimated from the different PBBs samples, according to the INFOGEST protocol, are shown in Table 3. The results were expressed in trace element solubility (μg/kg) and bioaccessibility (percentage of the soluble fraction in relation to the initial amount). The *in vitro* digestion method was conducted on PBBs samples with Se content above 20 μg/kg (Table 2), considering the LOQ of the method. Preliminary analysis showed that samples with Se content between 4 and 20 μg/kg would present bioaccessible extracts with non-quantifiable values, resulting in inaccurate Se bioaccessibility.

Considering the PBBs analyzed (almonds, cashew nuts, coconut, oats, peanuts, rice and soy), we observed that PBBs derived from cashew nuts showed significant results regarding the estimation of Se bioaccessibility (68.0–95.9 %). Samples prepared with oat and a mix of vegetables presented lower values, ranging from 63.5 % to 79.6 %. Analyzing the behavior of the cashew nut PBBs (Table 3), we observed that the L7 and L6 samples presented similar Se bioaccessibility percentages of 89.1 % and 95.3 %, respectively. This finding can be explained by the sample labelling: these samples presented the same set of ingredients (cashew and Brazil nuts) that are being marketed by the same manufacturer.

The L9 sample, comprised of only water and organic cashew nuts, showed the highest percentage of Se bioaccessibility among all the characterized samples; with a bioaccessible fraction of 21.9 μg/kg and a bioaccessibility percentage of 95.9 %.

On the other hand, lower values of Se bioaccessibility were found in the L12 sample, for bioaccessible fractions and bioaccessibility percentage; 16.2 μg/kg and 68.0 %, respectively. This sample being the only one among the cashew PBBs samples that contained cocoa in its list of ingredients. The Se bioaccessibility percentage in this sample seems to be affected by the presence of cocoa. Silva et al. [7] and Gibson and Newsham [35], estimated trace element bioaccessibility in several plant-based beverages, as well as in cocoa/powder chocolate; they reported that the concentration of phenolic compounds present in cocoa contributes to the decrease in trace element bioaccessibility in these matrices.

The PBBs produced from oats presented Se absorption estimates of 65 %. While the PBBs that were not classified in any of the main beverage groups of this study (almonds, cashew nuts, coconut, oats, peanuts, rice and soy) presented bioaccessibility percentages of 63.5 %

**Table 3**
Estimation of selenium bioaccessibility of different plant-based beverages through the standardized method proposed by INFOGEST.

| Type | Samples | Total content (μg/kg) | Bioaccessible fraction (μg/kg) | Bioaccessibility (%) |
|---|---|---|---|---|
| Cashew nuts | L6 | $185.7 \pm 0.5^{bB}$ | $176.9 \pm 0.5^{bB}$ | $95.3 \pm 0.2^{aA}$ |
| | L7 | $226.0 \pm 4.1^{aA}$ | $201.4 \pm 4.3^{aA}$ | $89.1 \pm 1.9^{bAB}$ |
| | L9 | $22.9 \pm 0.7^{cCD}$ | $21.9 \pm 0.1^{cC}$ | $95.9 \pm 0.4^{aA}$ |
| | L12 | $23.8 \pm 1.4^{cCD}$ | $16.2 \pm 1.9^{cCD}$ | $68.0 \pm 7.9^{cC}$ |
| Oat | L22 | $29.2 \pm 2.5^{C}$ | $19.0 \pm 0.3^{CD}$ | $65.0 \pm 1.0^{C}$ |
| *Mix of vegetable sources | L40 | $20.9 \pm 0.3^{aD}$ | $13.2 \pm 0.02^{bD}$ | $63.5 \pm 2.6^{bC}$ |
| | L42 | $20.1 \pm 1.6^{aD}$ | $16.0 \pm 0.8^{aCD}$ | $79.6 \pm 3.9^{aB}$ |

* Beverages consisting of plant protein and other plant ingredients. Results are expressed as mean ± standard deviation (n = 3). Different lowercase superscript letters in the column indicate significant difference ($P < 0.05$) between samples of the same type of beverage. Different capital superscript letters in the column indicate significant difference ($P < 0.05$) between the different types of beverages. ANOVA + Tukey Test at 95 % of confidence.

(L40) and 79.6 % (L42). As previously reported, samples L22, L40 and L42 also contained cocoa in their list of ingredients, favoring the decrease in Se bioaccessibility. In addition, the quantity of ingredients used in the preparation of these beverages may also explain the estimated Se absorption values in these samples, since trace element bioaccessibility is strictly related with the food matrix composition; as well as with possible complexation reactions, including Se with other nutrients present in the samples [36].

In general, the PBBs presented high Se bioaccessibility percentages, ranging from 63.5 % to 95.9 %. No studies were found in the literature that reports on the estimation of Se bioaccessibility from different PBBs (various raw materials) using the standardized method proposed by INFOGEST, making this study current and unprecedented. On the other hand, Se bioaccessibility percentages in plant species can be greater than 80 % [37,38].

The high bioaccessible percentages reported in the PBBs samples can be explained by the association of Se with soluble proteins (about 80 %), and their susceptibility to the action of digestive proteases [39]. Furthermore, differences in the chemical composition of Se can also affect the bioaccessibility of this trace element, since organic Se has greater solubility when compared to inorganic Se [18,39]. All these factors may have contributed to the high percentages Se bioaccessibility, as observed from the different PBBs samples.

### *3.3. Reference daily intake (RDI) of Se from plant-based beverages*

Table 4 shows the different PBBs Se contribution to the RDI of different groups of individuals considered healthy. The results were compared with the Se daily intake recommendations according to Brazilian legislation (Normative Instruction No. 75) [24] and the FDA [25]. The RDI refers to the highest amount of nutrients ingested without causing laughter and/or side effects.

The values were calculated using the highest Se values reported for each of the PBBs characterized in this study (presented in Table 2). As the PBBs from peanuts and rice did not present Se levels above the limits of quantification (< 4 μg/kg), they were not mentioned at this stage of the study and were considered deficient in this nutrient. Thus, for the other samples, the percentage of how much PBBs can contribute to the RDI for different groups of individuals was calculated.

**Table 4**
Percentage of the reference daily intake (RDI) of selenium for different groups of individuals (intake of 200 mL for the different PBBs, equivalent to 1 glass).

| Types | Samples | Se content (μg/kg) | % of RDI | | |
|---|---|---|---|---|---|
| | | | Pregnant women | Children (≥ 4 years) and adults | Lactating women |
| Almonds | L3 | 4.4 ± 0.05$^{d}$ | 1.5 ± 0.03$^{d}$ | 1.6 ± 0.01$^{d}$ | 1.3 ± 0.03$^{d}$ |
| Cashew nut | L7 | 226.0 ± 4.1$^{a}$ | 75.3 ± 1.4$^{a}$ | 82.2 ± 1.5$^{a}$ | 64.6 ± 1.2$^{a}$ |
| Coconut | L14 | 7.2 ± 0.8$^{d}$ | 2.4 ± 0.2$^{d}$ | 2.6 ± 0.3$^{d}$ | 2.1 ± 0.2$^{d}$ |
| Oat | L22 | 29.2 ± 2.5$^{b}$ | 9.7 ± 0.8$^{b}$ | 10.6 ± 0.9$^{b}$ | 8.3 ± 0.7$^{b}$ |
| Soy | L37 | 9.7 ± 0.4$^{d}$ | 3.2 ± 0.1$^{d}$ | 3.5 ± 0.1$^{d}$ | 2.8 ± 0.1$^{d}$ |
| *Mix of vegetable sources | L40 | 20.9 ± 0.3$^{c}$ | 7.0 ± 0.1$^{c}$ | 7.6 ± 0.1$^{c}$ | 6.0 ± 0.1$^{c}$ |
| Cow milk | L43 | 10.8 ± 0.6$^{d}$ | 3.6 ± 0.2$^{d}$ | 3.9 ± 0.2$^{d}$ | 3.1 ± 0.2$^{d}$ |

* Beverages consisting of plant protein and other plant ingredients. Results are expressed as mean ± standard deviation (n = 3). Different superscript letters in the columns indicate significant difference (P < 0.05) between the different types of beverages. ANOVA + Tukey Test at 95 % of confidence. For the % RDI calculations, the following RDI values were considered: pregnant women (60 μg) [24]; children (≥ 4 years) and adults (55 μg) [25] and lactating women (70 μg) [24] and [25].

Sample L7 is comprised of cashew nuts and Brazil nuts, and presented the highest RDI percentages for Se, since a 200 mL portion can contribute to 64.6 %, 75.3 % and 82.2 % of the RDI of Se for lactating and pregnant women, children (≥ 4 years) and adults, respectively. In this way, sample L7 presents itself as an excellent source of this trace element. Statistical differences (P < 0.05) were found between the samples.

The results presented in this work are close to those reported by Orlando et al. [26], who studied the contribution of different plant-based beverages based on cashew nuts (with or without adding Brazil nuts, coconut and cocoa), coconut, oats, soy, rice, almonds and peanuts. These authors reported that only the beverage made with cashew nuts combined with Brazil nuts was able to contribute to around 70 % of the RDI of Se for men and women considered healthy (whose recommendation is 55 μg). The second highest percentage among the other PBBs samples was attributed to coconut plant-based beverages, which contributed with only 10 % of the RDI of Se for this same target group.

In the present study, the second highest RDI percentage contribution of Se was found in the oat plant-based beverage (sample L22). The ingestion of this sample may contribute to 10.6 % of the RDI of Se, taking into account the adequate consumption of children (≥ 4 years) and adults; whose daily recommendation is 55 μg. On the other hand, PBBs derived from almonds (L3), coconut (L14), soy (L37) and the sample of mixed vegetable sources (L40), did not significantly contribute to the RDI supply of Se for the different groups of individuals; considering that RDI percentages below 10 % are regarded as poor for this important micronutrient. Similar behavior was also observed for the cow milk sample (L43).

In general, PBBs are produced with the aim of organoleptic similarity (appearance, texture, flavor and odor) to cow's milk, and/or as an alternative to juices and other beverages [26,40]. On the other hand, factors such as lactose intolerance, allergy to cow milk proteins, as well as changes in eating habits collaborate with this replacement [41]. However, appreciable amounts of Se are reported in different food groups (fish, viscera, Brazil nuts, eggs and cereals) [14]; with several groups of people mostly using PBBs consumption as the main protein component in their diet. However, as observed in this study, the PBBs produced from almonds, coconut, oats, peanuts, rice and soy did not contribute to meeting the daily Se intake established by current legislation in Brazil [24] and around the world [25].

The low consumption of Se still presents itself as a problem for several world populations, including Brazil. Se intake less than the recommended daily allowance (RDA) have been reported in the Polish, Italian and Slovenian populations (55 μg/daily) [14]. Stoffaneller and Morse [42] also found Se intake to be below the RDI set by the FDA [25] in several countries in Europe (Slovenia, Italy and Poland), and in the Middle East (Egypt, Iran and Turkey).

In this context, PBBs industries are increasingly supplying vegetable beverages enriched with essential proteins, vitamins, minerals and trace elements, including Se; with the aim of producing a nutritional composition equivalent to that of cow's milk. There are still few reports of Se supplementation in PBBs with its subsequent contribution in different groups of individuals. Therefore, more studies need to be carried out in order to guarantee consumers that the production of PBBs will meet their nutritional needs, especially in terms of Se content.

## 4. Conclusions

The total Se levels in the PBBs ranged from 4 to 226 μg/kg, with emphasis on the PBBs produced from cashew nuts and Brazil nuts. Bioaccessibility percentages ranged from 63.5 % to 95.9 %, attributed to the association of Se with soluble proteins, as well as the greater solubility of organic Se (higher incidence in foods, including PBBs) when compared to inorganic Se. Nevertheless, the consumption of PBBs derived from almonds, coconut, oats, peanuts, rice and soy did not contribute to meeting the reference daily intake for Se. These results are relevant, considering that there are no previous reports on that estimate Se bioaccessibility in plant-based beverages produced from different raw materials, and it is important to further studies involving fortification of plant-based beverages using this one trace element.

## Funding

This work was supported by the São Paulo Research Foundation - FAPESP [grant numbers 2022/06111–8 and 2022/07015–2]; the National Council for Scientific and Technological Development (CNPq) [grant numbers 306054/2020–5 and 407080/2021–0].

## CRedit authorship contribution statement

**José Luan da Paixão Teixeira**: Data curation, Conceptualization, Methodology, Formal analysis, Investigation, Validation, Funding acquisition, Writing - original draft, Writing – review & editing. **Ana Paula Rebellato**: Methodology, Visualization, Writing – review & editing. **Maria Isabel Andrekowisk Fioravanti**: Methodology, Visualization, Writing – review & editing. **Raquel Fernanda Milani**: Data curation, Methodology, Formal analysis, Investigation, Writing – review & editing. **Marcelo Antonio Morgano**: Investigation, Data curation, Supervision, Conceptualization, Revision, Writing - original draft, Writing – review & editing.

## 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.

## Acknowledgements

The authors would like to thank the Institute of Food Technology and the Food Science and Quality Center for providing the physical space of the Inorganics Laboratory, materials and reagents of laboratory, and all equipment necessary for the development of this research.

## References

[1] S. Sethi, S.K. Tyagi, R.K. Anurag, Plant-based milk alternatives an emerging segment of functional beverages: a review, J. Food Sci. Technol. 53 (2016) 3408–3423, https://doi.org/10.1007/s13197-016-2328-3.

[2] S. Chalupa-Krebzdak, C.J. Long, B.M. Bohrer, Nutrient density and nutritional value of milk and plant-based milk alternatives, Int. Dairy J. 87 (2018) 84–92, https://doi.org/10.1016/j.idairyj.2018.07.018.

[3] O.E. Mäkinen, V. Wanhalinna, E. Zannini, E.K. Arendt, Foods for special dietary needs: non-dairy plant-based milk substitutes and fermented dairy-type products, Crit. Rev. Food Sci. Nutr. 56 (2016) 339–349, https://doi.org/10.1080/10408398.2012.761950.

[4] M.L. Astolfi, E. Marconi, C. Protano, S. Canepari, Comparative elemental analysis of dairy milk and plant-based milk alternatives, Food Control 116 (2020) 1–11, https://doi.org/10.1016/j.foodcont.2020.107327.

[5] K. Briviba, V. Gräf, E. Walz, B. Guamis, P. Butz, Ultra high pressure homogenization of almond milk: physico-chemical and physiological effects, Food Chem. 192 (2016) 82–89, https://doi.org/10.1016/j.foodchem.2015.06.063.

[6] I. Felberg, R. Antoniassi, R. Deliza, S.C. de Freitas, R.C.Della Modesta, Soy and Brazil nut beverage: processing, composition, sensory, and color evaluation, Ciência e Tecnol. De. Aliment. 29 (2009) 609–617, https://doi.org/10.1590/s0101-20612009000300024.

[7] J.G.S. Silva, A.P. Rebellato, E.T. dos S. Caramês, R. Greiner, J.A.L. Pallone, In vitro digestion effect on mineral bioaccessibility and antioxidant bioactive compounds of plant-based beverages, Food Res. Int. 130 (2020) 1–10, https://doi.org/10.1016/j.foodres.2020.108993.

[8] W.V. Vasquez-Rojas, D. Martín, B. Miralles, I. Recio, T. Fornari, M.P. Cano, Composition of Brazil nut (Bertholletia excels HBK), its beverage and by-products: a healthy food and potential source of ingredients, Foods 10 (2021) 1–24, https://doi.org/10.3390/foods10123007.

[9] T. Bergillos-Meca, C. Cabrera-Vique, R. Artacho, M. Moreno-Montoro, M. Navarro-Alarcón, M. Olalla, R. Giménez, I. Seiquer, M.D. Ruiz-López, Does Lactobacillus plantarum or ultrafiltration process improve Ca, Mg, Zn and P bioavailability from fermented goats' milk? Food Chem. 187 (2015) 314–321, https://doi.org/10.1016/j.foodchem.2015.04.051.

[10] A. Brodkorb, L. Egger, M. Alminger, P. Alvito, R. Assunção, S. Ballance, T. Bohn, C. Bourlieu-Lacanal, R. Boutrou, F. Carrière, A. Clemente, M. Corredig, D. Dupont, C. Dufour, C. Edwards, M. Golding, S. Karakaya, B. Kirkhus, S. Le Feunteun, U. Lesmes, A. Macierzanka, A.R. Mackie, C. Martins, S. Marze, D.J. McClements, O. Ménard, M. Minekus, R. Portmann, C.N. Santos, I. Souchon, R.P. Singh, G. E. Vegarud, M.S.J. Wickham, W. Weitschies, I. Recio, INFOGEST static in vitro simulation of gastrointestinal food digestion, Nat. Protoc. 14 (2019) 991–1014, https://doi.org/10.1038/s41596-018-0119-1.

[11] D.B. Alcântara, A.P. Dionísio, A.G. Artur, B.K.S. Silveira, A.F. Lopes, J.A.C. Guedes, L.R. Luz, R.F. Nascimento, G.S. Lopes, H.H.M. Hermsdorff, G.J. Zocolo, Selenium in Brazil nuts: an overview of agronomical aspects, recent trends in analytical chemistry, and health outcomes, Food Chem. 372 (2022) 1–17, https://doi.org/10.1016/j.foodchem.2021.131207.

[12] M.C. Piñero, G. Otálora, J. Collado-González, J. López-Marín, F.M. del Amor, Effects triggered by foliar selenium application on growth, enzyme activities, mineral nutrients and carbohydrates in lettuce under an aquaculture system, Plant Physiol. Biochem. 180 (2022) 1–8, https://doi.org/10.1016/j.plaphy.2022.03.028.

[13] J.B. Ambrógi, R.P. Avegliano, V.A. Maihara, Essential element contents in food groups from the second Brazilian total diet study, J. Radioanal. Nucl. Chem. 307 (2016) 2209–2216, https://doi.org/10.1007/s10967-015-4591-6.

[14] M. Buffini, A.P. Nugent, J. Walton, A. Flynn, B.A. McNulty, Selenium intakes in the Irish adult population, J. Nutr. Sci. 12 (2023) 1–10, https://doi.org/10.1017/jns.2023.23.

[15] M. Esteki, Y. Vander Heyden, B. Farajmand, Y. Kolahderazi, Qualitative and quantitative analysis of peanut adulteration in almond powder samples using multi-elemental fingerprinting combined with multivariate data analysis methods, Food Control 82 (2017) 31–41, https://doi.org/10.1016/j.foodcont.2017.06.014.

[16] O. İçelli, E. Öz, S. Bakırdere, E. Nuroğlu, Analysis of conventionally and magnetic-field dried fruit and nuts for mycotoxins by high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) and trace elements by inductively coupled plasma–mass spectrometry (ICP-MS), Anal. Lett. 53 (2020) 735–745, https://doi.org/10.1080/00032719.2019.1669630.

[17] S. Kilic, M. Soylak, Determination of trace element contaminants in herbal teas using ICP-MS by different sample preparation method, J. Food Sci. Technol. 57 (2020) 927–933, https://doi.org/10.1007/s13197-019-04125-6.

[18] L. Gao, D. Luo, X. Hu, J. Wu, Se in Se-enriched peanut, and losses during peanut protein preparation, Int. J. Food Sci. Technol. 52 (2017) 843–850, https://doi.org/10.1111/ijfs.13347.

[19] A. Karasakal, Determination of trace and major elements in vegan milk and oils by ICP-OES after microwave digestion, Biol. Trace Elem. Res. 197 (2020) 683–693, https://doi.org/10.1007/s12011-019-02024-7.

[20] A.P. Rebellato, M.I.A. Fioravanti, R.F. Milani, M.A. Morgano, Inorganic contaminants in plant-based yogurts commercialized in Brazil, Int. J. Environ. Res. Public Health 20 (2023) 1–16, https://doi.org/10.3390/ijerph20043707.

[21] INMETRO, The National Institute of Metrology, Stardardization and Industrial Quality. (DOQ-CGCRE-008). Rio de Janeiro, Brazil, (2020).

[22] A.P. Rebellato, M.I.A. Fioravanti, R.F. Milani, M.A. Morgano, Composition and bioaccessibility of inorganic elements in plant-based yogurts, J. Food Compos. Anal. 123 (2023) 1–9, https://doi.org/10.1016/j.jfca.2023.105639.

[23] R. Kroes, D. Müller, J. Lambe, M.R.H. Löwik, J. Van Klaveren, J. Kleiner, R. Massey, S. Mayer, I. Urieta, P. Verger, A. Visconti, Assessment of intake from the diet, Food Chem. Toxicol. 40 (2002) 327–385, https://doi.org/10.1016/S0278-6915(01)00113-2.

[24] Brazil, Instrução Normativa - IN n 75, de 8 de Outubro de 2020. Ministério da Saúde. Brasília, Brazil, (2020).

[25] FDA, Department of Health and Human Services, Food and Drug Administration, Food Labeling: Revision of the Nutrition and Supplement Facts Labels, in: Final Rule. Federal Register, 81, 2016, pp. 33741–33999. ⟨https://www.federalregister.gov/d/2016-11867/p-5⟩.

[26] E.A. Orlando, J.G.S. Silva, J.A.L. Pallone, Bebidas À Base De Vegetais São Fontes De Selênio? Tecnol. De. Aliment.: Tópicos Físicos, Quím. e Biológicos - Vol. 1 (2020) 335–341, https://doi.org/10.37885/200800964.

[27] A.R.A. Silva, M.M.N. Silva, B.D. Ribeiro, Health issues and technological aspects of plant-based alternative milk, Food Res. Int. 131 (2020) 1–17, https://doi.org/10.1016/j.foodres.2019.108972.

[28] I. Felberg, R. Deliza, E.B. Gonçalves, R. Antoniassi, S.C. De Freitas, L.C. Cabral, Bebida Mista de Extrato de Soja Integral e Castanha-do-Brasil: Caracterização Físico-Química, Nutricional e Aceitabilidade do Consumidor, Alimentos e Nutrição, Araraquara 15 (2004) 163–174.

[29] I. Rybicka, M. Krawczyk, E. Stanisz, A. Gliszczyńska-Świgło, Selenium in Gluten-free Products, Plant Foods Hum. Nutr. 70 (2015) 128–134, https://doi.org/10.1007/s11130-015-0467-8.

[30] R. Rico, M. Bulló, J. Salas-Salvadó, Nutritional composition of raw fresh cashew (Anacardium occidentale L.) kernels from different origin, Food Sci. Nutr. 4 (2016) 329–338, https://doi.org/10.1002/fsn3.294.

[31] R.C. de Assis, M.D. de A. Nogueira, A.D.V. Pinheiro, R.S. Galdino, K.G. de S. Almondes, C.S.C. Maia, Determinação de selênio em leite bovino in natura provenientes do Ceará - Brasil, Res., Soc. Dev. 10 (6) (2021) 14, https://doi.org/10.33448/rsd-v10i6.16011.

[32] S.M. O'Kane, L.K. Pourshahidi, M.S. Mulhern, R.R. Weir, S. Hill, J. O'Reilly, D. Kmiotek, C. Deitrich, E.M. Mackle, E. Fitzgerald, C. Lowis, M. Johnston, J. J. Strain, A.J. Yeates, The effect of processing and seasonality on the iodine and selenium concentration of cow's milk produced in Northern Ireland (NI): implications for population dietary intake, Nutrients 10 (2018) 1–14, https://doi.org/10.3390/nu10030287.
[33] K. Ling, M. Henno, I. Jõudu, T. Püssa, H. Jaakson, M. Kass, D. Anton, M. Ots, Selenium supplementation of diets of dairy cows to produce Se-enriched cheese, Int. Dairy J. 71 (2017) 76–81, https://doi.org/10.1016/j.idairyj.2017.03.004.
[34] H.Y. Liu, W.Z. Zhu, B.Y. Lu, Z.H. Wei, D.X. Ren, Effect of feed selenium supplementation on milk selenium distribution and mozzarella quality, J. Dairy Sci. 98 (2015) 8359–8367, https://doi.org/10.3168/jds.2015-9676.
[35] M. Gibson, P. Newsham, Chocolate/Cacao, Food Sci. Culin. Arts (2018) 341–352, https://doi.org/10.1016/b978-0-12-811816-0.00017-8.
[36] M. Wang, J. Zhou, N. Pallarés, J.Manuel Castagnini, M. Carmen Collado, F. J. Barba, Evaluation of heavy metals, mycotoxins and mineral bioaccessibility through in vitro static digestion models of rainbow trout (Oncorhynchus mykiss) and sole (Dover sole) side stream extracts obtained by pressurized liquid extraction, Food Chem. 419 (2023) 1–9, https://doi.org/10.1016/j.foodchem.2023.136054.
[37] F. Cámara-martos, S. Obregón-cano, O. Mesa-plata, M.E. Cartea-gonzález, Quantification and in vitro bioaccessibility of glucosinolates and trace elements in Brassicaceae leafy vegetables, Food Chem. 339 (2021) 1–8, https://doi.org/10.1016/j.foodchem.2020.127860.
[38] J. Martínez-castro, A. De Haro-Bail, S. Obregón-Cano, I.M.G. Magdaleno, A. M. Ortega, F. Cámara-Martos, Bioaccessibility of glucosinolates, isothiocyanates and inorganic micronutrients in cruciferous vegetables through INFOGEST static *in vitro* digestion model, Food Res. Int. 166 (2023) 1–10, https://doi.org/10.1016/j.foodres.2023.112598.
[39] C. Afonso, C. Cardoso, A. Gomes-Bispo, I. Ferreira, A. Rego, I. Coelho, C. Motta, A. M. Prates, I. Castanheira, N.M. Bandarra, Fatty acids, selenium, and vitamin B12 in chub mackerel (Scomber colias) as nourishment considering seasonality and bioaccessibility as factors, Food Chem. 403 (2023) 1–8, https://doi.org/10.1016/j.foodchem.2022.134455.
[40] F. Wild, M. Czerny, A.M. Janssen, A.P.W. Kole, M. Zunabovic, K.J. Domig, The evolution of a plant-based alternative to meat, Agro Food Ind. Hi Tech. 25 (2014) 45–49.
[41] O.E. Mäkinen, T. Uniacke-Lowe, J.A. O'Mahony, E.K. Arendt, Physicochemical and acid gelation properties of commercial UHT-treated plant-based milk substitutes and lactose free bovine milk, Food Chem. 168 (2015) 630–638, https://doi.org/10.1016/j.foodchem.2014.07.036.
[42] R. Stoffaneller, N.L. Morse, A review of dietary selenium intake and selenium status in Europe and the Middle East, Nutrients 7 (2015) 1494–1537, https://doi.org/10.3390/nu7031494.