Food Research International 245 (2026) 120892



Contents lists available at ScienceDirect

Food Research International

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





Plant-based beverages supplemented with Se-microparticles: a comprehensive study of absorption and gastrointestinal simulation using static and dynamic *in vitro* digestion models☆

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ARTICLE INFO

Keywords:
Plant-based beverages
Selenium
Microencapsulation
Bioaccessibility
Bioavailability estimate
INFOGEST
SHIME

ABSTRACT

This study evaluated and compared the physicochemical properties, antioxidant capacity, and Selenium (Se) bioaccessibility and intestinal absorption (bioavailability estimate) of almond, rice, and soy beverages fortified with microencapsulated organic (Selenium-L-cystine) or inorganic (sodium selenate) Se. These were compared against a traditional homemade Brazil nut beverage, naturally rich in this mineral. Supplementation produced matrix-dependent effects, notably slight reductions in L* (lightness) and consistent increases in b* (yellowness). After *in vitro* digestion, antioxidant activity and total polyphenol content increased, indicating digestion-driven release of bioactive compounds. Se bioaccessibility, assessed *via* static and dynamic models, varied significantly by food matrix. In the static digestion approach, almond beverages showed high soluble fractions (84–93%), rice lower values (63–70%), soy intermediate levels (67–79%), and Brazil nut beverage reached approximately 97%. In the dynamic system, bioaccessibility was generally higher for all drinks (almonds 89–93%; rice 70–81%; soy 61–97%), with Brazil nut exceeding 100%. Caco-2 assays, used to estimate the intestinal absorption component of Se bioavailability through transepithelial transport cells, indicated the highest Se absorption in rice beverages (~9–10%), while Brazil nut beverage, despite its high Se content, showed the lowest absorption rate (~2%). Findings highlight the dominant influence of matrix composition over Se chemical form on Se bioaccessibility and intestinal absorption, supporting the importance of considering both food matrix and Se species when designing fortified plan-based beverages.

1. Introduction

The production and marketing of plant-based beverages (PBBs) has shown exponential growth in recent years (Vaikma et al., 2021). The plant-based milk market is rapidly growing, with revenues projected to reach US$ 35 billion by 2028 (Mekanna et al., 2024) and exceed US$ 62 billion by 2030 (Karoui & Bouaicha, 2024; Sharma et al., 2024). Lactose intolerant and/or consumers allergic to cow milk proteins, followers of vegetarian and vegan diets, as well as individuals concerned about environmental and sustainability issues drive the production and high consumption of these vegetable drinks (Teixeira et al., 2025).

Despite this worldwide expansion, studies show that PBBs have relatively low levels of essential minerals, including calcium, iron, zinc and especially selenium (Se); when compared to cow's milk and other food sources (Orlando et al., 2020). PBBs generally have low Se levels and a safe dose range for Se enhancement. Almonds, rice, and soy

☆ This article is part of a Special issue entitled: 'SLACAN 2025' published in Food Research International.

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https://doi.org/10.1016/j.foodres.2026.120892
Received 23 April 2026; Received in revised form 17 September 2026; Accepted 22 September 2026
Available online 23 September 2026
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vegetables beverages are currently among the most widely consumed PBBs and they are easily available in the market, representing oilseeds, cereals, and legumes, respectively, with differing substantially in their protein, lipid, carbohydrate, phenolic, and phytochemical compositions (Silva et al., 2020; Stöckl et al., 2024). These compositional differences may influence Se stability during fortification and its interactions with the food matrix, potentially affecting its gastrointestinal release, bioaccessibility, and intestinal uptake (Moreda-Piñeiro et al., 2017). On the other hand, the relatively low content of essential minerals present in these drinks offer an opportunity for safe fortification strategies to enhance their nutritional value and their potential as sources of micronutrients (Orlando et al., 2020; Teixeira et al., 2025; Vaikma et al., 2021). Therefore, these PBBs have the potential to serve as effective carriers of bioactive compounds, representing a promising tool in fortification programs.

Se is an essential mineral for the proper functioning of the body, acting as a cofactor of antioxidant enzymes, modulator of the immune system and regulator of essential metabolic processes (Singhato et al., 2023). Deficiency of Se can cause thyroid dysfunction, impaired immune response, and increased risk of cardiovascular disease and chronic inflammatory processes (Alcântara et al., 2022). Considering the low Se levels in foods, most adults hardly reach the recommended daily intake (RDI) of 55 μg, leaving the average population intake below this threshold. Few foods are considered naturally rich in Se, such as some cereals and the oleaginous Brazil nut (Pedro et al., 2025), which is traditionally used to prepare a typical homemade Brazilian beverage. Consequently, this naturally Se-rich Brazil nut drink serves as an excellent reference matrix for evaluating and designing new Se-fortified plant-based beverages (Orlando et al., 2020; Pedro et al., 2025). Given this scenario, the fortification of PBBs with different sources of Se emerges as a technological and nutritional strategy, able to contribute to the adequate supply of this important essential micronutrient. Although previous studies have reported the Se content and bioaccessible fractions in different plant-based beverages (Alcântara et al., 2022; Orlando et al., 2020; Silva Junior et al., 2017), comparative investigations integrating intestinal Se absorption using static and dynamic *in vitro* models remain scarce.

Therefore, the central challenge lies not only in the controlled enrichment of PBBs with Se, but also in accurately estimating the bioaccessibility and bioavailability of this mineral. Bioaccessibility refers to the fraction of nutrient released from the food matrix during gastrointestinal digestion and made available for intestinal absorption, while bioavailability encompasses the fraction that is absorbed and becomes available for physiological functions (Grundy et al., 2025). *In vitro* models combining simulated gastrointestinal digestion with differentiated Caco-2 cell monolayers are widely recognized as a practical approach for estimating the intestinal absorption stage of bioavailability, as they reproduce transepithelial transport across the intestinal epithelium; however, they do not measure complete systemic bioavailability because post-absorptive processes, including tissue distribution, metabolism, and excretion, are beyond the scope of the model (Etcheverry et al., 2012; Grundy et al., 2025; Sandberg, 2010).

On the other hand, the chemical form of Se is a key factor influencing its behavior in food matrices and its potential bioaccessibility (Singhato et al., 2023). Inorganic sources of Se, such as selenate and selenite, often exhibit lower intestinal absorption and tissue retention in the body; while organic sources can bind to amino acids (selenomethionine and selenocysteine, for example) being more easily absorbed (Alcântara et al., 2022; Gao et al., 2017; Lavu et al., 2016). In the present study, the salts of sodium selenate (inorganic) and Selenium-L-cystine (organic) were strategically chosen to supplement PBBs due to their contrasting metabolic behavior, allowing for a comparative analysis of the influence of chemical forms on the bioaccessible and bioavailable fractions of Se in these plant beverages. Taken together, these aspects highlight that the effectiveness of the fortification process depends not only on the chemical form of Se added, but primarily on the bioaccessible fraction of this nutrient. In this sense, microencapsulation emerges as a promising technique capable of protecting Se, controlling its release, and enhancing its stability and bioaccessibility within complex matrices such as PBBs (Teixeira et al., 2025). Although multilayer Se microparticles have been previously developed and technologically characterized (Fadini et al., 2019; Teixeira et al., 2025), and almond, rice and soy plant-based beverages have recently been investigated as matrices for Se microparticle fortification, the specific influence of these contrasting plant-based matrices on the gastrointestinal behavior of Se remains insufficiently understood. In particular, their effects on Se bioaccessibility and intestinal uptake have not been investigated using complementary static and dynamic *in vitro* digestion models coupled with Caco-2 intestinal transport assays. The application of *in vitro* digestion methods is an essential tool to estimate the bioaccessibility or the intestinal absorption component of bioavailability of Se in different food matrices, including the PBBs.

Different *in vitro* digestion models have been applied to estimate mineral bioaccessibility, and are classified into either static and dynamic models (Xia et al., 2017). Digestion in static models takes place in a single stationary support where foods, digestive enzymes and electrolytes are accumulated and processed in batches (Brodkorb et al., 2019); while dynamic models incorporate factors such as pH changes, peristaltic movements, gastric emptying and secretion flow rates to simulate the dynamic physiological conditions of the human digestive system (Dupont et al., 2019; Teixeira et al., 2022). In several scientific publications, the use of static and dynamic models to simulate *in vitro* digestion in food matrices has already been reported (Teixeira et al., 2022; Teixeira et al., 2025; Vellido-Perez et al., 2021). The INFOGEST protocol, internationally standardized and developed by an international consortium of researchers, is a consensus method for static *in vitro* gastrointestinal digestion and is currently the most adopted methodology for simulating *in vivo* digestive conditions (Brodkorb et al., 2019; Egger et al., 2016). It maintains physiologically relevant parameters (*e.g.*, pH, residence times, enzyme activities, bile salt concentrations and secretion volumes), which ensures reproducibility and comparability across studies (Minekus et al., 2014). Furthermore, the INFOGEST protocol parameters are often used to calibrate and validate dynamic gastrointestinal systems, making compatible and complementary to models such as SHIME system (Simulator of the Human Intestinal Microbial Ecosystem).

For the nutrient estimated bioavailability, especially minerals, combined models of *in vitro* digestion and cultured cells from the human gastrointestinal tract, the Caco-2 cells, have been successfully applied. Originating from a colon carcinoma, these cells, under defined culture conditions, undergo differentiation to become polarized enterocytes, developing microvilli and acquiring characteristics similar to the epithelial cells of the small intestine (Pardo & Seiquer, 2021). This combination of procedures offers some advantages over animal models by providing a highly controlled environment to simulate mineral digestion and absorption, minimizing biological (Ben Hlel et al., 2019). Therefore, *in vitro* gastrointestinal digestion coupled with Caco-2 transport assays provides a suitable approach for estimating Se intestinal uptake from different food matrices (Teixeira et al., 2025).

In summary, we hypothesize that the distinct structural composition of the PBB matrix exerts a predominant influence over Se release and cellular transport, an effect which could be modulated by the specific Se chemical species (organic *vs.* inorganic). Considering the controlled-release properties of the Se microparticles, we further hypothesize that matrix-dependent interactions with the encapsulation system contribute to the observed differences in Se bioaccessibility and cellular uptake. Therefore, the central aim of this study was to determine how different plant-based beverage matrices (almond, rice, and soy) influence the gastrointestinal fate, bioaccessibility and cellular uptake of Se delivered as organic or inorganic Se microparticles, comparing them with a traditional Brazil nut beverage as a natural reference. To test these hypotheses, we characterized the physicochemical properties of the

beverages (color and proximal composition), antioxidant capacity, and Caco-2 cellular absorption. Furthermore, we adopted and comparatively analyzed both static and dynamic (SHIME system) *in vitro* gastrointestinal digestion models, using physiologically-based INFOGEST protocol parameters, to investigate the influence of complementary digestion approaches on Se bioaccessibility and to provide a comprehensively assessment this nutrient gastrointestinal behavior.

2. Material and methods

2.1. Chemicals

All chemical reagents used for mineral and *in vitro* digestion analyses were procured from Sigma-Aldrich (St Louis, USA) and of analytical grade. The ABTS was obtained from Amresco (Solon, USA). 2,4,6-Tri(2-pyridyl)-*s*-triazine (TPTZ) and iron (III) chloride for the ferric reducing power (FRAP) assay were from Fluka Chemicals (Fluka Chemicals, Madrid, Spain). Methanol and acetone were provided by VWR (Barcelona, Spain). All chemicals used were of high purity or analytical reagent grade. Bidistilled deionized water (Milli-Q purification system, Millipore, Bedford, MA, USA) was used.

2.2. Acquisition of commercial beverages, preparation of organic and inorganic Se microparticles and formulation of supplemented PBBs and Brazil nut beverage

The sampling process focused on PBB with distinctive nutritional content, such as carbohydrates, protein and lipids. Commercial almond, rice, and soy PBBs were purchased from local commercial establishments in the city of Campinas (São Paulo, Brazil), considering the most representative commercial brands available on the market. For each PBB, two distinct commercial batches were evaluated and pooled to obtain a single sample for each beverage type. Proximate composition, color, and antioxidant activity analyses were performed in triplicate using the pooled samples. Bioaccessibility and intestinal absorption assays were performed in triplicate from the pooled sample of each beverage type, with each digestion replicate carried out on a different day.

For PBB fortification, Se microparticles obtained through combined methods were prepared according to Fadini et al. (2019) and Teixeira et al. (2025), and involved three sequential steps: initial microencapsulation of Se salts (sodium selenate and selenium-L-cystine; Sigma-Aldrich, St Louis, USA) by Spray-Drying (MI-SD) using maltodextrin as wall material; microencapsulation of MI-SD by Spray-Chilling (M-SC) in a lipid mixture; and finally, microencapsulation of M-SC by Spray-Drying (MF-SD) using arabic gum and carboxymethylcellulose as wall materials. The same encapsulation procedure and wall materials were used for both Se forms. The sequential application of the methods ensured the distribution, integrity and dispersion of microparticles in vegetable beverages. Selenium content of the microparticles was quantified by ICP-MS, as detailed in the corresponding analytical section, yielding 10.4 μg/g for selenium-L-cystine microparticles and 34.2 μg/g for sodium selenate microparticles. The PBBs were fortified with each type of microparticle (organic and inorganic) to provide a target selenium amount of 55 μg per 200 mL of beverage, corresponding 100% of the recommended daily intake (RDI) of selenium. This fortification level was well below the tolerable upper intake level (UL) established by the European Food Safety Authority (EFSA; 255 μg/day) (Turck et al., 2023) and the Institute of Medicine (400 μg/day) (IOM, 2000). To achieve this level of fortification, 2.644 g of selenium-L-cystine (Se-ORG) microparticles and 0.804 g of sodium selenate (Se-INORG) microparticles were added per 100 mL of beverage, reflecting the different measured Se concentrations of the two microparticle preparations. A detailed characterization of the microparticles was reported in our previous study (Teixeira et al., 2025). Briefly, the produced microparticles exhibited typical morphology of particles obtained by combined spray-drying and spray-chilling, predominantly spherical multilayer particles with mean diameters ranging from approximately 26 to 32 μm, low moisture content and water activity, and controlled Se release under simulated gastrointestinal conditions, with protection during the gastric phase followed by enhanced release during the intestinal stage. The encapsulation efficiencies were above 80%, with satisfactory storage stability, maintaining selenium content throughout the evaluated period. These characteristics supported the selection of this multilayer delivery system for the present study, allowing the evaluation of Se bioaccessibility and cellular uptake under different PBBs matrices while minimizing variability associated with microparticle production.

The Brazil nut (*Bertholletia excelsa*) beverage was prepared from Se-rich seeds grown in Belém-PA (Brazil), according to Teixeira, Milani, and Morgano (2024) as follows: overnight immersion in Milli-Q water at room temperature using a solid-to-liquid ratio of 1:4 (*w/v*) for 17 h, homogenization in a domestic processor. Selenium-fortified PBBs and Brazil nut beverage were freshly prepared each week for analyses.

2.3. Characterization of PBBs: moisture, ash, fat, protein, energy and color

For nutritional composition determination in commercial PBBs supplemented with Se-ORG and Se-INORG and the Brazil nut drink, percentages of moisture, ash, fat and protein were evaluated. The percentage of moisture (method 934.01) and total ash content (method 942.05) were determined according to official methods described by AOAC (2000). Total fat was extracted using a mixture of chloroform: methanol (2:1) and quantified by the Soxhlet method (AOAC, 2000, method 963.15). Determination of total nitrogen followed the Dumas method, using the LECO Truspec CN equipment (LECO Corporation, St. Joseph, USA) under the manufacturer's recommended conditions and with a conversion factor of 6.25. The gross energy of the samples was measured using an isoperibolic bomb calorimeter (Parr Instrument Co., Moline, IL, USA). All determinations were performed in triplicate.

For color measurement, a portable colorimeter (CR-400, Konica Minolta, Japan), calibrated with D65 standard white plate and an observation geometry of 10° (manufacturer's recommended conditions) was used. The samples were gently homogenized and transferred to quartz vats for analysis. The CIE Lab L* (lightness component, from black to white), the chromatic coordinates a* (positive values indicate the red component and negative values the green component) and b* (positive values indicate the yellow component and negative values the blue component) parameters were recorded. From these coordinates, the chroma value ($C^* = (a^{*2} + b^{*2})^{0.5}$) was calculated. Each value reported was the average of in triplicate performed at 22–24 °C, and always under the same lighting conditions.

2.4. Static in vitro gastrointestinal digestion

The *in vitro* gastrointestinal digestion experiments were carried out in triplicate at minimum, using the standardized static procedure proposed by INFOGEST protocol (Brodkorb et al., 2019; Egger et al., 2016). The *in vitro* digestion followed three sequential steps representing the oral, gastric, and intestinal phases. At the oral stage, 5 g of beverage was combined with 5 g of simulated salivary fluid (SSF). As the PBBs are liquid matrices, salivary α-amylase was not included due to an extremely short oral transit time, according to the standardized INFOGEST consensus protocol (Brodkorb et al., 2019). The mixture was incubated at 37 °C for 2 min under gentle agitation. For the gastric phase, the oral bolus was mixed with simulated gastric fluid (SGF) and the pH was adjusted to 3.0. Porcine pepsin (3359 U/mg) was added to reach a final activity of 40,000 U, and the digestion proceeded at 37 °C with agitation for 2 h. The resulting chyme was then brought to pH 7.0 prior to the intestinal phase, in which simulated intestinal fluid (SIF), pancreatin (4000 U; 5.18 U trypsin/mg), and bile salts (160 mM) were incorporated. This mixture was incubated for 2 h at 37 °C with shaking (80

rpm). Digestion was stopped by snap-freezing the samples. After thawing on ice, the digesta were centrifuged (3500 ×*g* at 4 °C for 45 min) to obtain the bioaccessible fraction (BF) and the residual fraction (RF). The percentages (%) of BF and RF were calculated based on the initial quantities present in the beverages. While, recovery was calculated as the sum of percentages of BF and RF. A blank digestion was carried out by substituting the beverage with Milli-Q water. Aliquots of BF were stored at −80 °C in light-protected tubes under nitrogen and used for antioxidant and cell culture assays. For cell experiments, the BF was further filtered with a 30 kDa cutoff membrane (Amicon ultra-15, Millipore).

2.5. Antioxidant activity and total phenolic content

For antioxidant capacity determination, the ABTS and FRAP methods were adopted: the ABTS method, which evaluates the capacity of neutralization of free radicals (Jiménez-Escrig et al., 2003) and the FRAP method, which analyzes the capacity of samples to reduce ferric ions to ferrous (Benzie & Strain, 1996). Additionally, the total polyphenol content was determined by the Folin-Ciocalteu method, adapted by Saura-Calixto and Goñi (2006).

These procedures were applied to both the chemical extracts of commercial beverages, as well as to the bioaccessible fractions obtained after *in vitro* gastrointestinal digestion (static model). For chemical extraction, 1 mL of fresh sample was placed in a tube and 5 mL of acidic methanol/water (50:50 *v*/v, pH 2) were added. The tube was thoroughly shaken at room temperature for 1 h and centrifuged at 2500 *g* for 10 min, and the supernatant recovered. Five mL of acetone/water (70:30, *v*/v) was added to the residue, and the shaking and centrifugation steps were repeated. The methanolic and aqueous–acetone extracts were then combined and the volume made up to 10 mL. For antioxidant activity and total phenolic content measurements, appropriate dilutions of chemical extract and bioaccessible fractions were made when necessary.

The ABTS+·was produced as described by Delgado-Andrade et al. (2024). For the analyses, 40 μL of the sample extract, blank or Trolox standard, and 200 μL of 5 mM phosphate buffer (pH 8.4) were added with 60 μL of diluted ABTS+·solution. The absorbance reading was taken at 10 min using a Victor X5 microplate reader (Perkin Elmer, Massachusetts, USA). Aqueous solutions of Trolox were used for calibration (15–125 μM). Results were expressed as μmol equivalents of Trolox (TEAC)/L beverage.

The FRAP method was performed by preparing the reagent according to Seiquer et al. (2015). For the analysis, 20 μL of the sample extract, blank, or Trolox standard was added to 280 μL of warmed FRAP reagent (37 °C) and incubated at 37 °C for 30 min in darkness, and the absorbance was read at 595 nm. The same aqueous solutions of Trolox previously mentioned were used for calibration. Results were expressed as μmol equivalents of Trolox (TEAC)/L beverage.

Total phenolic compounds (TPCs) were determined following the Folin–Ciocalteau colorimetric method (Seiquer et al., 2015). For the analysis, 10 μL of the sample extract, blank, or gallic acid standard and 10 μL of Folin–Ciocalteau reagent were mixed and allowed to stand for 3 min. Then, 200 μL of sodium carbonate solution (75 g/L) was added; the volume made up to 250 μL with Milli-Q water, mixed, and allowed to stand in the dark for 60 min. The absorbance was measured at 750 nm against a standard curve of gallic acid (25–250 mg/L). Results were expressed as mmol equivalents of gallic acid (GAE)/L beverage. Each measurement was carried out in triplicate.

2.6. Experiments with Caco-2 cells

The cells were acquired from the European Collection of Authenticated Cell Cultures (ECACC) through the Cell Bank of the University of Granada (Spain). The cells were maintained by serial passage in plastic vials of 75 cm^3 (every 3 days), cultured with DMEM medium (Dulbecco's Modified Eagle's Medium) with heat-inactivated fetal bovine serum (FBS) (10%), $NaHCO_3$ (3.7 g/L), nonessential amino acids (1%), HEPES (15 mM), bovine insulin (0.1 UI/mL), and 1% antibiotic-antimycotic solution. The condition of the cell incubator was an air/CO_2 atmosphere (95:5) with 90% humidity and a temperature of 37 °C, with fresh medium provided every 3 days.

To evaluate the intestinal transport of selenium, the cells were trypsinized and seeded in permeable polycarbonate supports (Transwell bi-chamber plates, 24 mm diameter, 4.7 cm^2 area and 3 μm pore size) at a density of 3.0 × 10^5 cells/insert, with 2.5 mL of DMEM culture medium in the basal chamber and 1.5 mL in the apical chamber (Seiquer et al., 2015). The culture medium was changed every two days, and the cells were used for transport experiments after 20–21 days of culture, when they were differentiated into mature enterocytes. Cell monolayer integrity during differentiation of Caco-2 cells was assessed by measuring the transepithelial electrical resistance (TEER), using a Millicell electrical resistance system (Millipore, Bedford, MA), as described by Pardo and Seiquer (2021). Immediately before the absorption experiments, the differentiated monolayers showed mean TEER values of 780 Ω.cm^2 on average. Cell-culture experiments were performed in three independent assays, and three inserts were used per treatment.

To ensure cell viability, only purified BF (digestion enzymes and free macromolecular compounds) were used for the cell experiments, at a dilution of 1:2 (v:v) with DMEM without FBS; since cell viability after 2 h of exposure to purified samples under such conditions was never <85–90%, as assessed by the MTT assay. On the day of the transport assays, the culture medium was removed from both the apical and basolateral compartments, and the cell monolayers were rinsed with pre-warmed Hank's balanced salt solution (HBSS, 37 °C). Subsequently, 2.5 mL of Mg-free transport buffer (130 mmol/L NaCl, 10 mmol/L KCl, 5 mmol/L glucose, and 50 mmol/L HEPES; pH 7.0) was added to the basolateral side, while 1.5 mL of the diluted BF sample was applied to the apical side. The cells were incubated at 37 °C for 2 h. After incubation, the apical solution and the insert containing the cell monolayer were removed. To determine mineral transport, the basolateral buffer was recovered and wells were rinsed with deionized water to ensure full sample retrieval. Selenium concentrations were quantified by ICP-MS, as described below. Results of selenium absorption were expressed as μg/well and as percentage from the experimental solution added to the apical chamber.

2.7. Dynamic gastrointestinal digestion model (SHIME system) adapted to the INFOGEST protocol

For the *in vitro* digestion dynamic model, the SHIME system (Simulator of the Human Intestinal Microbial Ecosystem; ProDigest, Ghent, Belgium) was used. The procedure consisted of an oral phase (carried out externally to the SHIME unit), followed by gastric and intestinal phases. During the gastric and small intestinal stages (duodenum and jejunum), the system provided precise control of pH and temperature, allowing the simulation of physiologically relevant conditions under a standardized fasting state configuration according to the validated SHIME protocol originally proposed by Molly et al. (1993). The salivary, gastric and intestinal fluids were prepared according to recommendations described by Tamargo et al. (2022), and the INFOGEST protocol (Brodkorb et al., 2019; Egger et al., 2016). During the gastric phase (2 h), pH was gradually reduced from 5 to 2, while during the small intestine phase (2 h) pH was maintained at 7.5 to mimic *in vivo* physiological environments. The reactors were kept under continuous stirring at 37 °C and anaerobic conditions were ensured by nitrogen (N_2) flushing. The pH was automatically regulated throughout the digestion process *via* controlled addition of 0.5 N HCl or 0.5 N NaOH by the SHIME system. Digestion was stopped by snap-freezing the samples. After thawing on ice, the digesta were centrifuged (3500*g* at 4 °C for 45 min) to obtain the bioaccessible fraction (BF) and the residual fraction (RF). A blank digestion was carried out by substituting the beverage with Milli-Q water. Aliquots of BF were stored at −80 °C until the moment of

analysis.

2.8. Selenium analysis by ICP-MS

Selenium was analyzed in supplemented beverages, and in the BF and RF obtained after *in vitro* gastrointestinal digestion and cell culture solutions. The samples were previously wet digested in an acid medium, as described in Haro et al. (2020). The presence of impurities in reagents was controlled including a blank without sample with only the acids used for mineralization.

The selenium content in PBBs, BF, RF and in solutions coming from the cell culture assays were determined by inductively coupled plasma mass spectrometry (ICP-MS), with a Perkin Elmer NexION 2000B instrument (PerkinElmer, Waltham, USA) equipped with a triple cone interface and a quadrupole ion deflector, operated in standard mode (^{82}Se), using argon for plasma formation (auxiliary flow, 1.2 L/min; plasma flow, 18 L/min; nebulizer flow, 0.88 L/min) and Rh as internal standard. A calibration curve was built with serial dilutions from analytical standards (Perkin Elmer, Massachusetts, USA) prepared using sub-distilled HNO_3 and Milli-Q water. Standards for calibration were prepared within the lineal range for Se measurement, ranging from 0 to 1500 μg/L; the correlation coefficient of the standard curve was always ≥0.999. The limit of detection (LOD) was within the range of 0.01 to 0.1 μg/L. The analyses were performed in triplicate.

2.9. Statistical analysis

Statistical analyses were performed using analysis of variance (ANOVA). One-way ANOVA was applied to evaluate the effect of selenium supplementation within each type of plant-based beverage. For each type of supplemented beverage, data from static and dynamic *in vitro* digestions were individually compared using a Student's *t*-test (95% confidence level). In addition, for compositional, color, and antioxidant data, two-way ANOVA was conducted to assess the effects of beverage type (almond, rice, and soy), supplementation type (commercial, Se-ORG, and Se-INORG), and their interaction. For bioaccessibility and Caco-2 transport data, two-way ANOVA was conducted including only the supplemented almond, rice, and soy beverages to assess the effects of beverage type, Se forms (organic *vs.* inorganic), and their interaction. The Brazil nut beverage was not included in either two-way ANOVA. When significant differences were detected ($p < 0.05$), mean separation was carried out using the LSD *post hoc* test (95% confidence level). All analyses were performed using Statgraphics Centurion XVI.II (Statistical Graphics Corporation, USA) and Microsoft Excel (version 2016).

3. Results and discussion

3.1. Proximate composition of vegetable beverages

Table 1 reports the nutritional composition of commercial almond, rice, and soy PBBs, their selenium-fortified variants (organic and inorganic forms), and the naturally Se-rich Brazil nut beverage produced in the laboratory. A two-way ANOVA confirmed a highly significant effect of the type of beverage on all five determined nutritional indices ($p < 0.0001$).

The almond beverage presented the highest moisture values (> 92%), while the Brazil nut beverage presented the lowest value (84%). This lower moisture content in the Brazil nut beverage can be attributed to its artisanal formulation, lacking the industrial homogenization processes that enhance suspension stability and water retention in commercial PBBs (Xie et al., 2023). In the same line, Vogelsang-O'Dwyer et al. (2021) studied vegetable beverages submitted to different homogenization pressures and showed that the intensity of the process influences the physicochemical stability and water retention of the beverages. Supplementation of PBBs significantly decreased the moisture content in all three commercial beverages (almond, rice, and soy) when compared to their commercial counterparts. This reduction confirms the effective incorporation of solids into the PBB matrix *via* the added microparticles.

Regarding the ash content, the soy beverage, both commercial and supplemented displayed the highest ash content (0.82–0.85%), reflecting the superior intrinsic mineral density of soybeans and the additive effect with the Se supplementation. In a recent study, the mineral profile of several plant-based beverages, including soy-based drinks, was assessed. The authors reported that soy beverages contain significantly higher levels of calcium, magnesium, copper, and phosphorus (Antunes et al., 2025). The two-way ANOVA showed that ash content was significantly influenced by both the type of beverage and the type of supplementation. Moreover, the significant interaction between these factors supports the absence of differences in ash content among the soy-based PBBs. The Brazil nut PBB had the lowest ash content (0.31%) among all the PBBs and the highest fat percentage (12.30%), far from

Table 1
Proximate composition and energy value of commercial and selenium-supplemented plant-based beverages.

Beverage	Supplementation	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Energy (kcal/100 mL)
	Commercial	94.41 ± 0.01 c	0.79 ± 0.01 b	0.99 ± 0.01 b	0.60 ± 0.01	25.91 ± 0.03 a
ALMOND	ORG	92.28 ± 0.03 a	0.75 ± 0.01 a	0.94 ± 0.01 a	0.62 ± 0.03	34.80 ± 0.10 c
	INORG	93.79 ± 0.01 b	0.78 ± 0.01 b	1.02 ± 0.01 b	0.62 ± 0.03	28.20 ± 0.10 b
p	–	**<0.0001**	**0.0204**	**0.0095**	0.7459	**<0.0001**
	Commercial	92.51 ± 0.01 c	0.59 ± 0.01 b	1.28 ± 0.01	0.41 ± 0.01 a	33.46 ± 0.04 a
RICE	ORG	90.54 ± 0.01 a	0.56 ± 0.01 a	1.27 ± 0.01	0.43 ± 0.01 b	41.90 ± 0.10 c
	INORG	91.98 ± 0.02 b	0.55 ± 0.01 a	1.28 ± 0.01	0.41 ± 0.01 a	36.00 ± 0.10 b
p	–	**<0.0001**	**0.0089**	0.6272	**0.0134**	**<0.0001**
	Commercial	91.09 ± 0.01 c	0.84 ± 0.01	1.47 ± 0.01	3.25 ± 0.01	47.40 ± 0.10 a
SOY	ORG	89.10 ± 0.01 a	0.82 ± 0.02	1.46 ± 0.01	3.23 ± 0.01	55.65 ± 0.01 c
	INORG	90.48 ± 0.01 b	0.85 ± 0.01	1.46 ± 0.01	3.26 ± 0.01	49.90 ± 0.04 b
p	–	**<0.0001**	0.2020	0.4055	0.0928	**<0.0001**
BRAZIL NUT	–	83.96 ± 0.01	0.31 ± 0.01	12.30 ± 0.20	3.30 ± 0.06	132.13 ± 0.04
Two-way ANOVA*	TYPE OF BEVERAGE	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	SUPPLEMENTATION	**<0.0001**	**0.0021**	**0.0018**	0.4366	**<0.0001**
	TB × S	**<0.0001**	**0.0333**	**0.0025**	0.1783	**0.0001**

Mean ± SD ($n = 3$). ORG: supplementation with Se-ORG; INORG: supplementation with Se-INORG; TB: type of beverage; S: supplementation; SD: standard deviation. For each type of PBBs, different superscript letters within a column indicate significant differences among commercial beverages and supplemented PBBs (One-way ANOVA followed by the LSD test, $p < 0.05$); their absence indicates no significant differences ($p \geq 0.05$).

* Two-way ANOVA to study the effect of the type of beverage (almond, rice, and soy), supplementation (commercial, Se-ORG, and Se-INORG) and interaction between both factors ($p < 0.05$). The Brazil nut beverage was not included in this analysis.

values found in the commercial PBBs (<2%). This result is consistent with the high natural concentration of lipids in Brazil nuts, especially unsaturated fatty acids such as oleic and linoleic (Correa et al., 2024). In soy and rice beverages, supplementation with Se did not significantly alter the fat content, indicating maintenance of colloidal stability.

In the almond beverage, Se-ORG supplementation resulted in a significantly lower fat content (0.94%) compared to the commercial (0.99%) and Se-INORG supplemented (1.02%) PBBs. This suggests that the organic microencapsulation matrix could modify the colloidal stability of the almond emulsion, potentially altering fat extraction efficiency (Molet-Rodríguez et al., 2018). As described for moisture and ash, the fat content was significantly influenced by the type of beverage, the supplementation type ($p = 0.0018$), and the interaction between these factors. The protein content differed significantly between types of beverages ($p = 0.0000$), with soybean beverages having the highest values, followed by almond and rice drinks. No significant effect of Se supplementation on the overall protein concentration was detected. These findings align with the well-established higher protein quality of soybeans compared to almonds and rice (Olías et al., 2023) and their comparative analysis of various plant-based beverages. Pointke et al. (2022) reported that soy drinks contain protein levels comparable to cow milk (3.0–3.2 g/100 g), whereas almond and rice beverages exhibit markedly lower protein concentrations (≤ 2,0 g/100 g). The beverage formulated from Brazil nuts exhibited a protein content comparable to that of soy-based drinks; however, the protein derived from nuts is generally characterized as having low to moderate biological value (Lackey & Fleming, 2021).

The energy value showed highly significant differences based on both the type of sample and the type of supplementation. In all cases, the use of Se-ORG statistically increased the caloric content of the beverages. The Brazil nut beverage presented the highest energy value (132.13 kcal/100 mL), reflecting its high fat and protein levels; while the almond beverage showed the lowest (25.91–34.80 kcal/100 mL). Silva et al. (2024) reported a lower energy value for Brazil nut beverage (46 kcal/100 mL), which could be due to the use of a different variety of nut. Our findings are in line with Smith et al. (2022), who reported that plant-based beverages with higher lipid and protein contents (*e.g.*, oilseeds such as soy and cashew) typically exceed 50 kcal/100 mL, whereas almond and rice beverages generally remain at ≤30–40 kcal/100 mL.

In general, the proximate composition (moisture, ash, protein, and lipids) reported by the plant-based beverages analyzed in this study can contribute to different gastrointestinal behaviors, influencing the bioaccessible estimate and subsequent cellular absorption of selenium. Variations in protein and lipid content affect mineral-matrix interactions during digestion, highlighting the importance of considering the physicochemical composition of plant-based beverages for a correct interpretation of the bioaccessibility and bioavailability results of this mineral (Olías et al., 2023; Pointke et al., 2022; Smith et al., 2022; Teixeira, Rebellato, et al., 2024).

3.2. Color measurements CIE lab

Color is the first quality parameter observed by consumers in a food product and often determines whether or not they choose it. Color is critical for food acceptance, including plant beverages, since it is strongly related with flavor, freshness and general quality. Color coordinates (L*, a* and b*) in the PBBs showed significant effects, for both type of beverage and the type of supplementation, as well as interactions between these factors (Table 2).

A general statistically significant reduction in L* (lightness), was observed upon Se-fortification, being most pronounced with Se-ORG. This slight darkening is consistent with an increase in light scattering and changes in the refractive index within the colloidal system, as Daszkiewicz et al. (2024) described when analyzing UHT milk and PBBs. On the other hand, Silva et al. (2024) reported that the type of formulation and processing of PBBs made from almonds, rice and soybeans affect the luminosity values of the final drinks.

For the a* parameter (redness trend), inorganic supplementation reduced the values in almond and rice beverages, whereas organic supplements only decreased redness in the rice drink, and no changes were observed in the soy beverage. These beverages showed the highest values of a*, indicating the predominance of reddish tones. Color changes derived from Se supplementation can be attributed to colloidal optical effects, chromatic characteristics of the materials used in the microencapsulation process, variations in composition and unit operations developed in the preparation of PPBs (McClements, 2020). The two-way ANOVA confirmed the importance of the matrix and type of supplementation on this parameter.

For the b* parameter (yellowness), there was a significant increase in all supplemented PBBs, especially those supplemented with Se-ORG.

Table 2
CIE Lab color coordinates in commercial and supplemented PBBs made from almonds, rice, soybean and traditional Brazil nut.

Beverage	Supplementation	L*	a*	b*	C*
	Commercial	63.21 ± 0.03 b	1.63 ± 0.01 b	4.46 ± 0.01 a	4.75 ± 0.01 a
ALMOND	ORG	61.80 ± 0.01 a	1.66 ± 0.01 c	4.77 ± 0.01 c	5.05 ± 0.01 c
	INORG	63.30 ± 0.01 c	1.53 ± 0.01 a	4.71 ± 0.01 b	4.95 ± 0.01 b
p	–	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	Commercial	67.83 ± 0.01 c	2.17 ± 0.01 c	2.49 ± 0.01 a	3.31 ± 0.01 a
RICE	ORG	66.76 ± 0.01 a	2.13 ± 0.01 b	2.93 ± 0.01 c	3.62 ± 0.01 b
	INORG	67.38 ± 0.01 b	2.06 ± 0.01 a	2.60 ± 0.01 b	3.32 ± 0.01 a
p	–	**<0.0001**	**0.0001**	**<0.0001**	**<0.0001**
	Commercial	65.97 ± 0.03 b	3.83 ± 0.02	6.43 ± 0.01 a	7.49 ± 0.01 a
SOY	ORG	65.34 ± 0.03 a	3.82 ± 0.01	6.78 ± 0.01 c	7.78 ± 0.01 c
	INORG	65.91 ± 0.01 b	3.82 ± 0.01	6.51 ± 0.01 b	7.55 ± 0.01 b
p	–	**<0.0001**	0.7663	**<0.0001**	**<0.0001**
BRAZIL NUT	–	76.90 ± 0.20	3.18 ± 0.01	−0.14 ± 0.01	3.19 ± 0.01
Two-way ANOVA*	TYPE OF BEVERAGE	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	SUPPLEMENTATION	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	TB × S	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

Mean ± SD ($n = 3$). ORG: supplementation with Se-ORG; INORG: supplementation with Se-INORG; L*: lightness; a*: redness; b*: yellowness; C*: chroma; TB: type of beverage; S: supplementation; SD: standard deviation.
For each type of PBBs, different superscript letters within a column indicate significant differences among commercial beverages and supplemented PBBs (One-way ANOVA followed by the LSD test, $p < 0.05$); their absence indicates no significant differences ($p \geq 0.05$).
* Two-way ANOVA to study the effect of the type of beverage (almond, rice, and soy), supplementation (commercial, Se-ORG, and Se-INORG) and interaction between both factors ($p < 0.05$). The Brazil nut beverage was not included in this analysis.

Otálora et al. (2023) associated this effect with the addition of maltodextrin in the microencapsulation process, while da Silva Júnior et al. (2023) and Oliveira et al. (2023) reported that the spray-drying procedure can intensify yellowish tones.

The chroma index (C*), related with color saturation and intensity, also increased with supplementation, especially with Se-ORG, while remaining lower in Brazil nut beverage (Table 2). The C* values in soy beverages were 40% higher than those of almond beverages, and more than twice those observed in rice beverages, which is consistent with the highest a* and b* coordinates in this matrix. The more intense color of the soy beverage (especially due to higher values of b*), may be related with the presence of pigments, mainly carotenoids and anthocyanins, released from their seed coats during blending (Tobolková & Durec, 2023). Moreover, colored compounds derived from the Maillard reaction formed in soy drinks (Olías et al., 2023) may contribute to this more intense color.

3.3. Determination of antioxidant activity in vegetable beverages

The results of the antioxidant activity analysis (Table 3) and the total polyphenol con-tent revealed a significant effect of both the type of beverage and the supplementation in the studied parameters, as well as a significant interaction between both factors.

Supplementation with Se-ORG resulted in matrix-dependent effects on the antioxidant capacity of commercial PBBs. Regarding ABTS, different trends were observed among matrices. In the almond beverage, Se-ORG significantly increased the ABTS values compared to the non-supplemented control, whereas Se-INORG did not show any significant effect. In contrast, for both rice and soy beverages, significant differences were identified between the two chemical forms, with Se-ORG leading to a more pronounced increase than Se-INORG, although both forms successfully outperformed their respective controls. Regarding the FRAP assay, a highly specific behavior was also noted. In the rice matrix, supplementation resulted in lower values compared to the control. Remarkably, in the almond beverage, FRAP value was significantly reduced in the presence of Se-INORG compared to the non-supplemented beverage, while Se-ORG produced a modest increase. This result is consistent with findings in foods like Chinese kale, where the enrichment with Se-ORG showed higher ABTS antioxidant activity than with Se-INORG (Leamsamrong et al., 2019). Experiments carried out in animals fed diets supplemented with Se, also support the better antioxidant capacity of organic compared with inorganic sources (Huang et al., 2023). However, it is chemically essential to clarify that selenium species do not possess direct, chemical *in vitro* radical scavenging or reducing capacity (Fairweather-Tait et al., 2011; Roman et al., 2014). Therefore, these variations cannot be fully attributed to a direct chemical antioxidant effect of the mineral itself, but rather to minor colloidal interactions or synergistic stabilization of endogenous plant compounds in the assay medium (McClements, 2020; Molet-Rodríguez et al., 2018).

In both rice and soy beverages, a marked increase in antioxidant capacity measured by ABTS was observed upon supplementation with selenium, whether in organic (Se-ORG) or inorganic (Se-INORG) form, with the effect being more pronounced for the organic one. Previous results have shown that rice-based beverages can exhibit significant antioxidant activity, especially when enriched with bioactive compounds or prepared using whole ingredients, which is reflected in elevated ABTS and total phenolic values (da Silva et al., 2023). Soy-based beverages demonstrated the highest ABTS antioxidant potential among supplemented PBBs, particularly when supplemented with Se-ORG. It is worth mentioning that the Brazil nut drink displayed the highest free radical scavenging capacity among all studied beverages and a remarkable concentration of total polyphenols, only surpassed by soybean beverages, confirming their natural richness in bioactive compounds. These values exceed those reported in previous studies (McClements, 2020; Vasquez-Rojas et al., 2023), likely due to differences in preparation methods or raw material origin. Two-way ANOVA confirmed that antioxidant activity and total polyphenol content are significantly influenced by both the type of beverage matrix and the form of selenium supplementation.

After *in vitro* static digestion, significant increase in ABTS, FRAP, and total polyphenols values were observed in the bioaccessible fractions compared to undigested extracts. This phenomenon has been widely documented in vegetable beverages and other matrices (Delgado-

Table 3
Determination of antioxidant activity and total phenolic compounds in commercial and supplemented PBBs made from almonds, rice, soybean, and traditional Brazil nut.

Beverage	Supplementation	Initial content in the commercial PBBs			Bioaccessible fraction after digestion (static model)		
		ABTS μmoles TEAC/L	FRAP μmoles TEAC/L	Polyphenols mmoles GAE/L	ABTS μmoles TEAC/L	FRAP μmoles TEAC/L	Polyphenols mmoles GAE/L
ALMOND	Commercial	192 ± 5 a	26 ± 1 b	0.07 ± 0.01 b	2480 ± 7 a	1292 ± 41 a	20.7 ± 0.3 c
	ORG	245 ± 2 b	30 ± 1 c	0.08 ± 0.01 c	2518 ± 10 b	1618 ± 51 c	18.9 ± 0.1 a
	INORG	185 ± 2 a	19 ± 1 a	0.06 ± 0.01 a	2590 ± 5 c	1474 ± 49 b	20.0 ± 0.2 b
p	–	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0008**	**0.0001**
RICE	Commercial	171 ± 4 a	49 ± 1 b	0.08 ± 0.01 b	2534 ± 6 b	2213 ± 21 c	20.6 ± 0.2 b
	ORG	254 ± 3 c	44 ± 1 a	0.08 ± 0.01 b	2495 ± 6 a	2120 ± 33 b	20.5 ± 0.1 b
	INORG	195 ± 4 b	43 ± 1 a	0.07 ± 0.02 a	2488 ± 9 a	1719 ± 31 a	19.0 ± 0.3 a
p	–	**<0.0001**	**0.0002**	**0.0006**	**0.0006**	**<0.0001**	**0.0003**
SOY	Commercial	580 ± 1 a	665 ± 10 b	1.51 ± 0.02 a	1721 ± 10	3458 ± 87	26.2 ± 0.4 b
	ORG	714 ± 1 c	609 ± 8 a	1.67 ± 0.04 b	1729 ± 11	3400 ± 76	23.1 ± 0.2 a
	INORG	628 ± 1 b	710 ± 14 c	1.45 ± 0.06 a	1688 ± 25	3348 ± 104	25.7 ± 0.1 b
p	–	**<0.0001**	**<0.0001**	**0.0047**	0.2158	0.6958	**<0.0001**
BRAZIL NUT	–	844 ± 9	303 ± 5	0.68 ± 0.02	564 ± 18	4635 ± 144	24.0 ± 0.2
Two-way ANOVA*	TYPE OF BEVERAGE	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	SUPPLEMENTATION	**<0.0001**	**<0.0001**	**0.0002**	0.5174	**0.0007**	**<0.0001**
	TB × S	**<0.0001**	**<0.0001**	**0.0003**	**<0.0001**	**<0.0001**	**<0.0001**

Mean ± SD ($n = 3$). TEAC/L: Trolox Equivalent Antioxidant Capacity/L; GAE/L: Gallic Acid Equivalents/L; ORG: supplementation with Se-ORG; INORG: supplementation with Se-INORG; TB: type of beverage; S: supplementation; SD: standard deviation.
For each type of PBBs, different superscript letters within a column indicate significant differences among commercial beverages and supplemented PBBs (One-way ANOVA followed by the LSD test, $p < 0.05$); their absence indicates no significant differences ($p \geq 0.05$).
* Two-way ANOVA to study the effect of the type of beverage (almond, rice, and soy), supplementation (commercial, Se-ORG, and Se-INORG) and interaction between both factors ($p < 0.05$). The Brazil nut beverage was not included in this analysis.

Andrade et al., 2024; Delgado-Andrade et al., 2025) is mediated by the enzymatic hydrolysis and pH-induced release of matrix-bound phenolic compounds (Silva et al., 2020). Simultaneously, the progressive enzymatic cleavage of major native storage proteins (such as glycinin and β-conglycinin in soy, amandine in almond, and excelsin in Brazil nut) by pepsin and pancreatin releases a wide array of short-chain, low-molecular-weight bioactive peptides. These peptides, particularly those rich in hydrophobic or aromatic residues (such as TYR, TRP, PHE, HIS, CYS, and MET), act as potent proton/electron donors and metal chelators, able to contribute to the elevated post-digestion ABTS and FRAP values (Zou et al., 2016).

In addition, chemical transformations, such as glycoside hydrolysis and phenolic decomposition, generate lower molecular weight metabolites with enhanced reducing capacity (Luo et al., 2022). Findings in the same line were reported in fractions of rice bran and other vegetable matrices, confirming that gastrointestinal digestion intensifies the antioxidant potential through mobilization of previously inaccessible phenolic compounds (Wanyo et al., 2024). Additionally, the disruption of colloidal structures, including lipid emulsions and protein-polyphenol interactions, may further enhance the progressive release of antioxidant compounds during digestion (Collins et al., 2024). This effect is particularly striking in vegetable matrices, such as soybeans and rice, but also in almonds, whose lipid and phenolic fraction of the peel is released more intensively after digestion; contributing to the substantial increase of antioxidant activity (Li et al., 2023).

Similar to the other beverages, soybean PBBs markedly increased their ABTS and FRAP antioxidant potential after digestion, but selenium supplementation did not affect these parameters. A similar pattern was observed for total polyphenol content. This lack of correlation further suggests that the bioaccessible antioxidant capacity is predominantly mediated by the synergistic release of soy isoflavones and digestive peptide fragments, rather than by the presence of the mineral itself. Brazil nut beverages displayed a distinct pattern: prior to digestion, antioxidant activity was dominated by free radical scavenging, whereas post-digestion, reducing capacity (FRAP) became predominant. This highlights the beverage's potential as a functional food with high antioxidant capacity, where both pre-existing and newly released or transformed compounds after digestion contribute to its activity. In general, bioaccessible polyphenols significantly increased after digestion in all beverages, with very interesting results in the case of the Brazil nut beverage (24.0 ± 0.2 mmoles GAE/L), which suggests an efficient release of these compounds and natural nut peptides during gastrointestinal transit. Overall, although both forms of selenium supplementation significantly improved antioxidant capacity and polyphenol content in the undigested PBBs, Se-ORG generally produced slightly higher values in the bioaccessible fraction. Since both selenium species were encapsulated using identical wall materials, these antioxidant differences cannot be attributed to the polymer framework nor to the added mass variation, as these coating ingredients lack intrinsic radical scavenging activity. In conclusion, while some differences were detected among supplementation groups, the post-digestion antioxidant activity is an independent matrix-driven phenomenon. The marked increase in bioaccessible antioxidant capacity is predominantly mediated by the mobilization of native plant polyphenols and the simultaneous release of hydrolyzed bioactive peptides rather than the added mineral itself, as selenium's antioxidant role is strictly physiological, mediated through its incorporation into selenoenzymes like glutathione peroxidase, rather than a direct chemical one (Fairweather-Tait et al., 2011; Roman et al., 2014).

3.4. Se bioaccessibility using the static model

For Se supplementation, plant-based beverages were fortified using multilayer Se microparticles previously characterized (Teixeira et al., 2025), minimizing differences in particle morphology, encapsulation efficiency and physicochemical stability. The same encapsulation procedure and wall materials were used for both Se forms; however, different amounts of Se-ORG and Se-INORG microparticles were required to provide the same target Se dose (55 μg/200 mL), due to their different measured Se concentrations. Therefore, variations in Se bioaccessibility observed among the PBBs were interpreted primarily in relation to matrix-dependent interactions affecting microparticle hydration, wall disintegration and Se release during gastrointestinal digestion. Consequently, Se bioaccessibility should be interpreted as the combined outcome of the controlled-release characteristics of the multilayer delivery system, and the physicochemical composition of each beverage matrix. Within this matrix-dependent framework, the Brazil nut beverage requires a specific interpretation, as it was included as a natural selenium-rich reference. The Brazil nut beverage was studied to enable a comparison between the naturally occurring selenium and the selenium delivered through controlled fortification, rather than as a direct comparison of equivalent plant matrices. Although Brazil nuts differ from almond, rice and soy beverages in botanical origin and intrinsic composition, their exceptionally high selenium content provides a valuable reference for evaluating the gastrointestinal behavior of naturally occurring selenium compared with selenium supplied through an engineered delivery system (Dumont et al., 2006; Fairweather-Tait et al., 2011;Orlando et al., 2020; Vasquez-Rojas et al., 2023). While matrix-matched comparisons would provide further insights into the isolated influence of food composition on selenium bioaccessibility, the low selenium content of non-fortified plant-based beverages limits their suitability for comparison with fortified formulations. Therefore, the present design aimed to investigate whether distinct plant-based beverage matrices modulate selenium bioaccessibility while providing complementary insight into the behavior of a naturally selenium-enriched food source.

In Table 4, the Se bioaccessibility (static model) in commercial and supplemented PBBs (almonds, rice and soy) is shown. As mentioned previously, the fortification level was selected to approximately provide the recommended daily intake (RDI) of selenium (55 μg) in a 200 mL serving. Natural selenium was below the limit of detection (n.d.) in all commercial beverages prior to fortification. After fortification with Se-ORG and Se-INORG, the overall Se concentration in the beverages averaged 0.42 μg/mL, ranging from 0.38 to 0.45 μg/mL. In the almond beverage, Se-ORG supplementation resulted in slight but statistically significant higher Se values than the Se-INORG one. In the rice and soy beverages, no significant differences were observed between the forms of supplementation. As expected, the Brazil nut drink contained substantially higher Se (6.25 μg/mL beverage), approximately 15 times the mean level in the supplemented PBBs. This confirms that Brazil nuts are among the richest natural sources of Se, although the final content depends strongly on nut variety, agronomic practices, and environmental conditions (Silva Junior et al., 2017). The selenium concentration measured in the Brazil nut beverage was considerably higher than previously reported by other authors (Orlando et al., 2020; Vasquez-Rojas et al., 2023).

For the almond beverage, the bioaccessible Se fraction varied between 84 and 93%, with significant differences between the organic and inorganic forms. Both forms exhibited high Se accessibility, highlighting the almond matrix as an efficient facilitator of mineral solubilization. This is likely associated with the physicochemical properties of almonds, which are rich in phenolic compounds and some fatty acids that could enhance mineral incorporation into the micellar phase during the simulated intestinal digestion. Li et al. (2023) reported that the lipid and protein composition of oilseeds and seeds strongly influence mineral release during *in vitro* digestion assays, supporting the idea that these matrices can act as facilitators of mineral transport to the micellar phase. Specifically, the lower bioaccessibility of organic selenium (84%) compared to inorganic selenate (93%) can be attributed to hydrophobic partitioning. Se-ORG exhibits hydrophobic affinity toward the lipid-protein interface of the almond emulsion, which may contribute to partial entrapment (Molet-Rodríguez et al., 2018; Zhang et al., 2020).

Table 4
Bioaccessibility of selenium (Se) after static *in vitro* digestion of commercial and supplemented PBBs made from almonds, rice, soybean and traditional Brazil nut.

Beverage	Supplementation	Initial (μg/mL beverage)	BF (μg/mL beverage)	BF (%)	RF (μg/mL beverage)	RF (%)	Recovery (%)[†]
	Commercial	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ALMOND	ORG	0.45 ± 0.01 b	0.38 ± 0.01	84.4 ± 2.0 a	0.11 ± 0.01 b	24.4 ± 0.1 b	109 ± 1 b
	INORG	0.42 ± 0.01 a	0.39 ± 0.01	93.0 ± 1.0 b	0.04 ± 0.01 a	9.5 ± 0.3 a	102 ± 1 a
p	–	**0.0003**	0.2048	**0.0140**	**<0.0001**	**<0.0001**	**0.0193**
	Commercial	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RICE	ORG	0.43 ± 0.01	0.30 ± 0.01 b	70.0 ± 1.0 b	0.07 ± 0.01	16.3 ± 0.6 b	86 ± 1 b
	INORG	0.43 ± 0.01	0.27 ± 0.01 a	62.8 ± 2.0 a	0.06 ± 0.01	13.9 ± 0.1 a	77 ± 2 a
p	–	0.9670	**0.0142**	**0.0143**	0.0948	0.0894	**0.0121**
	Commercial	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
SOY	ORG	0.39 ± 0.01	0.26 ± 0.01 a	66.7 ± 0.2 a	0.09 ± 0.01 b	23.1 ± 0.6 b	90 ± 1
	INORG	0.38 ± 0.01	0.30 ± 0.01 b	79.0 ± 4.0 b	0.02 ± 0.01 a	5.3 ± 0.1 a	84 ± 4
p	–	0.3568	**0.0177**	**0.0437**	**<0.0001**	**<0.0001**	0.5101
BRAZIL NUT	–	6.25 ± 0.07	6.08 ± 0.02	97.3 ± 0.3	0.11 ± 0.01	1.8 ± 0.1	99 ± 1
	TYPE OF BEVERAGE	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Two-way ANOVA[*]	SUPPLEMENTATION	0.0841	0.2126	**0.0270**	**<0.0001**	**<0.0001**	**0.0030**
	TB × S	0.3206	**0.0003**	**0.0010**	**<0.0001**	**<0.0001**	0.4143

Mean ± SD (n = 3; three different experimental days). ORG: supplementation with Se-ORG; INORG: supplementation with Se-INORG; TB: type of beverage; S: supplementation; SD: standard deviation. BF: bioaccessible fraction; RF: residual fraction. The percentages (%) of BF and RF were calculated based on the initial quantities present in the beverages. Recovery was calculated as the sum of BF and RF.
For each type of PBBs, different superscript letters within a column indicate significant differences among commercial beverages and supplemented PBBs (One-way ANOVA followed by the LSD test, $p < 0.05$); their absence indicates no significant differences ($p \geq 0.05$).
[†] Total recoveries exceeding 100% in the almond beverage are attributed to cumulative analytical uncertainty inherent to trace-level sequential processing, alongside minor ICP-MS matrix-induced signal enhancement caused by the high lipid content of the natural sample.
[*] Two-way ANOVA to study the effect of the type of beverage, supplementation (Se-ORG and Se-INORG), and interaction between both factors using only the supplemented almond, rice, and soy beverages ($p < 0.05$). The Brazil nut beverage was not included in this analysis.

Conversely, Se-INORG is highly hydrophilic and charged (Roman et al., 2014), remaining fully dissolved in the aqueous bioaccessible phase. Additionally, organic selenium may establish weak hydrogen bonds and hydrophobic interactions with almond polyphenols, such as flavonoids and proanthocyanidins, stabilizing its co-precipitation within the insoluble matrix (Xu et al., 2026), while inorganic selenate does not participate in these non-covalent associations. Although the microparticles were previously shown to promote controlled Se release during digestion (Teixeira et al., 2025), the composition of the almond beverage may further modulate the hydration and disintegration of the encapsulating wall materials, contributing to the observed differences between Se species.

In the rice beverage, the Se bioaccessible fraction was lower (63–70%), with Se-ORG supplementation showing higher bioaccessibility than Se-INORG. The lower bioaccessibility is explained by the high content of starch, fiber and phytates, which form complexes with metals and reduce their solubility during digestion (Sęczyk et al., 2021; Yu et al., 2024). It is well-known that the composition of the food vehicle (starch *vs.* lipids/proteins) is a critical determinant of bioaccessibility (Ośko et al., 2024). Furthermore, the chemical form of Se plays a role: organic compounds are more readily incorporated into micelles during digestion, whereas inorganic salts interact more strongly with inhibitory compounds such as the phytates and fibers highly present in the rice matrix, limiting their solubility (An et al., 2025; de Lima et al., 2023). Finally, the higher Se-ORG bioaccessibility in rice beverages likely results from the synergy between matrix interactions and microencapsulation, which ensures gastric protection and enhanced intestinal release (Rasera et al., 2022). This finding reinforces that the performance of the encapsulated Se cannot be interpreted independently of the food vehicle, as the starch-rich rice matrix appears to influence the release dynamics of the previously characterizes microparticles during digestion.

In the soy beverage, the Se bioaccessible fraction was significantly higher with Se-INORG supplementation compared to Se-ORG (79 *vs.* 67%). This difference could be linked to the complex interactions between soy reserve proteins, phenolic compounds and phytates, which often reduce the mobility of organic mineral forms, whereas inorganic species such as selenate tend to remain more available during the simulated intestinal phase (Bawiec et al., 2023). Specifically, the lower bioaccessibility of organic selenium is likely driven by soy proteins, such as glycinin, rich in reactive sulfhydryl groups that can undergo disulfide exchange with selenium-L-cystine, covalently trapping the mineral in the insoluble fraction (Hondal et al., 2013; Ruan et al., 2015). Conversely, inorganic selenate experiences strong electrostatic repulsion with negatively charged soy proteins and phytates at intestinal pH, keeping it soluble in the bioaccessible fraction (Prattley & Stanley, 1982). Furthermore, soy isoflavones and other endogenous polyphenols can interact non-covalently with the organic selenium, assisting in its retention within the colloidal digestive precipitate (Xu et al., 2026; Yang et al., 2025). In contrast, the negative charges of both inorganic selenate and intestinal polyphenolic species prevent any significant interaction, preserving selenate solubility (Prattley & Stanley, 1982). Ultimately, this interactions highlight that the structural conformation of soy proteins, which are rich in functional ligand groups, directly influences the retention or release of micronutrients during digestion (Kohli & Singha, 2024). Thus, the lower bioaccessibility observed for Se-ORG in the soy beverage is likely associated with the combined effect of protein-Se interactions and matrix-dependent modulation of microparticle disintegration rather than limitations of the encapsulation system itself.

Interestingly, the Brazil nut beverage presented an exceptionally high soluble fraction (97.3%). This reinforces its suitability as a natural, bioaccessible Se source. This performance can be attributed to the fact that Brazil nuts store Se predominantly as selenomethionine, a highly stable and absorbable species (Singhato et al., 2023).

Regarding residual fractions, the Se-ORG forms consistently showed higher insolubility than Se-INORG in all beverages. In the almond beverage, the residual fraction of Se-ORG reached 25%, likely reflecting stronger interactions with lipids, proteins, and phytates (Bawiec et al., 2023; Yu et al., 2024). In the rice beverage, although Se-ORG showed a slightly higher residual fraction compared to Se-INORG (16.3 *vs.* 13.9%; statistically significant difference), this increase did not translate into a significant difference in bioaccessibility, indicating that matrix-related factors may be more relevant than the specific Se forms. For soy beverages, the higher residual Se observed with the organic supplementation confirms that the organic form tends to remain more

insoluble in protein- and phytate-rich matrices (Fairweather-Tait et al., 2011). By contrast, the Brazil nut beverage showed minimal residual Se (< 2%), consistent with the predominance of highly soluble and bioaccessible selenomethionine (Singhato et al., 2023). This Brazil nut beverage showed recovery close to 100%, reinforcing the stability and bioaccessibility of natural Se (Cardoso et al., 2017).

In summary, the two-way ANOVA confirmed that both the beverage matrix and the chemical form of Se exert a significant effect on bioaccessibility ($p < 0.0001$), indicating that Se release is not an intrinsic property of the Se species alone, but a result of the dynamic interaction between the matrix composition and the micronutrient. While oleaginous matrices facilitated Se bioaccessibility, the starchy rice matrix presented greater challenges to Se release. Given the inconsistent performance of Se species across matrices, successful fortification requires a tailored approach considering the overall composition of the selected vehicle. Overall, the present findings demonstrate that Se release is governed by a dynamic interaction between Se species and the composition of the beverage matrix. Thus, the effectiveness of Se fortification should be interpreted not only according to the chemical form of the mineral but also considering matrix-dependent modulation of microparticle behavior during gastrointestinal digestion and the different amounts of microparticles required to achieve the same target Se dose.

3.5. Intestinal absorption (bioavailability estimate) of se in vegetable beverages using the static digestion model combined with Caco-2 cells

The results of Se absorption from the bioaccessible fractions of supplemented PBBs after static digestion in Caco-2 cells are presented in Table 5. Although the Caco-2 model represents an established *in vitro* approach to estimate the intestinal absorption component of bioavailability, the transport data presented here should be interpreted within the scope of intestinal absorption rather than complete systemic bioavailability (Grundy et al., 2024; Grundy et al., 2025). Since all beverages were fortified with the same microparticles, differences observed in Se transport across Caco-2 cells are expected to reflect the characteristics of the bioaccessible fraction generated during digestion rather than variability in microparticle production. Thus, intestinal uptake represents the cumulative consequence of matrix-dependent Se release together with the intrinsic transport mechanisms associated with each Se species.

Table 5
Intestinal absorption (bioavailability estimate) of Selenium in Caco-2 cells from bioaccessible fractions of commercial and supplemented PBBs made from almonds, rice, soybean and traditional Brazil nut.

Beverage	Supplementation	Initial (ng/well)	Transported (ng/well)	Transported (%)
ALMOND	ORG	257 ± 12 a	12.8 ± 0.5 a	5.0 ± 0.2 a
	INORG	293 ± 1 b	17.1 ± 0.3 b	5.8 ± 0.1 b
p	–	**0.0349**	**0.0028**	**0.0209**
RICE	ORG	153 ± 4	15.0 ± 0.5	9.8 ± 0.3
	INORG	160 ± 3	15.2 ± 0.2	9.5 ± 0.1
p	–	0.1939	0.6070	0.7855
SOY	ORG	301 ± 5	17.0 ± 1.0	5.7 ± 0.2
	INORG	297 ± 47	15.0 ± 1.0	5.1 ± 0.4
p	–	0.9363	0.2509	0.3323
BRAZIL NUT	–	1156 ± 22	28.0 ± 1.0	2.4 ± 0.1
Two-way ANOVA*	TYPE OF BEVERAGE	**<0.0001**	0.2099	**<0.0001**
	SUPPLEMENTATION	0.4311	0.0963	0.5975
	TB × S	0.5921	**0.0018**	**0.0438**

Mean ± SD ($n = 3$; three different experimental days). ORG: supplementation with Se-ORG; INORG: supplementation with Se-INORG; TB: type of beverage; S: supplementation; SD: standard deviation.
For each type of PBBs, different superscript letters within a column indicate significant differences among commercial beverages and supplemented PBBs (One-way ANOVA followed by the LSD test, $p < 0.05$); their absence indicates no significant differences ($p \geq 0.05$).
* Two-way ANOVA to study the effect of the type of beverage, supplementation (Se-ORG and Se-INORG), and interaction between both factors using only the supplemented almond, rice, and soy beverages ($p < 0.05$). The Brazil nut beverage was not included in this analysis.

In the almond beverage, Se-INORG supplementation showed significantly higher initial Se content compared to Se-ORG (293 *vs.* 257 ng/total), likely due to its higher solubility after *in vitro* digestion. Consistently, the absolute amount and percentage of Se absorbed by Caco-2 cells was significantly greater for Se-INORG, indicating enhanced intestinal absorption of the inorganic form in the almond matrix. This difference can be attributed to the higher solubility and reduced interaction of Se-INORG with food matrix components such as fibers and polyphenols, which facilitates more efficient transport across intestinal cells and minimizes complexation (Bawiec et al., 2023). These findings are in agreement with previous studies reporting that inorganic mineral forms, due to their simpler molecular structure, are less prone to form stable complexes with food macromolecules (da Silva et al., 2017).

In contrast, no significant differences were observed between Se-ORG and Se-INORG in rice beverages regarding the initial apical contents, the absolute amount transported or the absorption percentage, that was higher than that observed with the other beverages. This outcome may be attributed to the relatively simple composition of this matrix and a limited presence of antinutritional factors (phytates) compared to other vegetal matrices (Omodara et al., 2025). Consequently, Se intestinal absorption in rice beverages appears comparable regardless of the chemical forms. A similar pattern was exhibited by the soy beverage, where Se absorption was also unaffected by the form of supplementation. This may result from the equivalent solubilization of both organic and inorganic Se during *in vitro* digestion, as well as from the potential saturation of intestinal transport pathways, including amino acid or ion-mediated mechanisms (Roman et al., 2014).

In the Brazil nut beverage, the absolute amount of Se transported across Caco-2 cells was substantially higher than the supplemented PBBs. Nevertheless, this corresponds to only 2.4% of the initial Se placed in the apical chamber, a lower relative transport rate compared to the supplemented beverages. Thus, while the digested Brazil nut beverage exhibited high absolute Se transport, it showed a lower absorption percentage. These differences should be interpreted with caution, given that the Brazil nut beverage naturally contained a substantially higher Se concentration than the supplemented PBBs, resulting in a higher Se dose in the Caco-2 cell experiments, limiting direct comparison of Se transport percentages between the beverages. This limited relative transport may also reflect saturation of intestinal transport mechanisms under the high Se load present in the Brazil nut matrix. Although these varying initial doses introduce a comparative constraint due to pathway saturation, this experimental design was intentionally maintained to mimic physiological conditions, reflecting the actual matrix-dependent solubilization. In any case, the high absolute Se transport observed further reinforces the potential of Brazil nuts as a natural source of absorbable selenium, consistent with the predominance of highly soluble selenomethionine in this matrix (Singhato et al., 2023).

The two-way ANOVA revealed that the type of beverage matrix had a significant effect on both initial Se content and absorption percentage ($p < 0.0001$), indicating that the intrinsic characteristics of each food matrix directly modulate Se absorption. In contrast, the form of supplementation (ORG *vs.* INORG) did not exert a significant overall effect on Se absorption. However, a significant interaction between the matrix and the chemical form was identified, suggesting that the effectiveness of supplementation depends on the specific beverage matrix. This effect was particularly evident in the almond beverage, where Se-INORG exhibited a significant advantage over Se-ORG. In summary, Se intestinal absorption is not determined solely by its chemical form, but results from a complex interplay with the composition of the food matrix, in

agreement with previous studies on mineral absorption from plant-based matrices (Bawiec et al., 2023; da Silva et al., 2017). Although the present study did not determine release kinetics separately for each beverage matrix, previous characterization demonstrated the controlled-release behavior of the microparticles under gastrointestinal conditions (Teixeira et al., 2025). Therefore, differences in Se uptake observed among beverages may partially reflect matrix-dependent modulation of microparticle disintegration and Se release, representing an important mechanism to be explored in future studies.

3.6. Bioaccessibility of Se using the dynamic model (SHIME system): comparison with the static model

The Table 6 shows the results obtained from the *in vitro* dynamic digestion model (SHIME system) to evaluate selenium bioaccessibility.

For the rice beverage, Se-ORG exhibited higher bioaccessibility than Se-INORG (81.4 *vs.* 69.8%;), whereas the opposite trend was observed for almond (88.9 *vs.* 92.8%) and soy plant-based beverages (61.5 *vs.* 97.4%). The Brazil nut beverage yielded an apparent soluble fraction exceeding 100%, consistent with the predominance of selenomethionine in this matrix, although certain analytical overestimation or ion enhancement by a matrix effect cannot be discarded, as discussed below. Two-way ANOVA indicated that beverage matrix significantly influenced bioaccessible selenium; while the effect of the chemical form was matrix-dependent, generally favoring Se-INORG in almond and soy beverages, but Se-ORG in rice and Brazil nut matrices. This disparity underscores the differential value of natural selenium enrichment *versus* artificial reinforcement. While artificial reinforcement supplies a controlled dose to comply with the RDI, the Brazil nut beverage provides a complex food system benchmark. Despite a 15-fold higher initial mineral load, this natural matrix allows for a better understanding of how selenium forms and complex chemical interactions affect solubility during dynamic digestion, driving the distinct behaviors observed between natural and fortified drinks.

A comparison between static and dynamic *in vitro* digestion models (Fig. 1) revealed matrix- and chemical form-dependent trends. The dynamic digestion model, which incorporates peristaltic movements, continuous enzyme flow and replenishment of digestive fluids and gradual pH changes, generally increased the Se bioaccessible fraction compared with the static protocol; particularly for rice and Brazil nut beverages. For instance, Se-ORG in rice increased from 70% under the static model to 81% under the dynamic model. This significant improvement highlights that the combination of gradual pH shifts and peristaltic shear in the SHIME system is highly effective in disintegrating the starch-rich rice matrix compared to the static model. Meanwhile, the bioaccessible fraction in the Brazil nut beverage increased from 97.3% to 114.4%, suggesting a more complete release under physiologically realistic conditions. However, rather than reflecting a true enhanced release or an actual physiological value superior to the initial selenium content, this apparent recovery exceeding 100% must be interpreted cautiously as an artifact driven by analytical uncertainty and matrix-induced signal enhancement in the ICP-MS system. For soy beverages, Se bioaccessibility with the INORG supplement was substantially

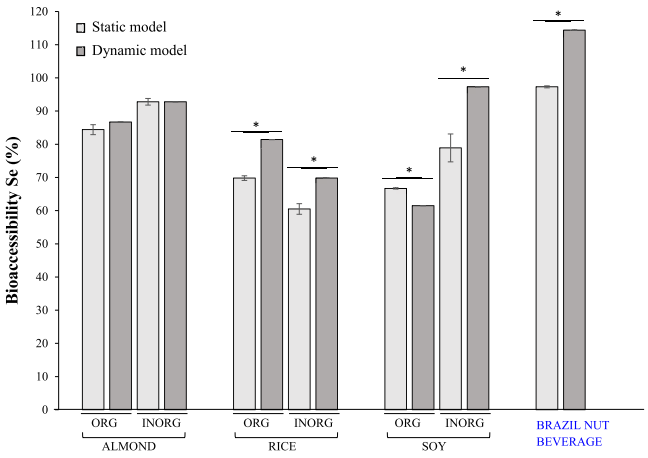


Fig. 1. Selenium bioaccessibility (%) in supplemented PBBs made from almonds, rice, soybean and traditional Brazil nut. Static *vs.* dynamic *in vitro* digestion models. Mean ± SD ($n = 3$). Superscript asterisks indicate significant difference between models (Student's *t*-test, $p < 0.05$).

Table 6
Bioaccessibility of selenium (Se) after dynamic *in vitro* digestion of commercial and supplemented PBBs made from almonds, rice, soybean and traditional Brazil nut.

Beverage	Supplementation	Initial (μg/mL beverage)	BF (μg/mL beverage)	BF (%)†	RF (μg/mL beverage)	RF (%)	Recovery (%)†
	Commercial	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ALMOND	ORG	0.45 ± 0.01 b	0.40 ± 0.01	88.9 ± 0.1 a	0.06 ± 0.01 b	13.3 ± 0.1 b	102 ± 1 b
	INORG	0.42 ± 0.01 a	0.39 ± 0.02	92.8 ± 0.1 b	0.02 ± 0.01 a	4.8 ± 0.1 a	98 ± 1 a
p	–	**0.0003**	0.1489	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	Commercial	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RICE	ORG	0.43 ± 0.01	0.35 ± 0.01 b	81.4 ± 0.1 b	0.03 ± 0.01	7.0 ± 0.1	88 ± 1 b
	INORG	0.43 ± 0.01	0.30 ± 0.01 a	69.8 ± 0.1 a	0.03 ± 0.01	7.0 ± 0.1	77 ± 1 a
p	–	0.9670	**0.0202**	**<0.0001**	0.2378	0.2548	**<0.0001**
	Commercial	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
SOY	ORG	0.39 ± 0.01	0.24 ± 0.01 a	61.5 ± 0.1 a	0.08 ± 0.01 b	20.5 ± 0.1 b	82 ± 1 a
	INORG	0.38 ± 0.01	0.37 ± 0.01 b	97.4 ± 0.1 b	0.01 ± 0.01 a	2.6 ± 0.1 a	100 ± 1 b
p	–	0.3568	**0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
BRAZIL NUT	–	6.25 ± 0.07	7.15 ± 0.08	114.4 ± 0.1	0.08 ± 0.01	1.3 ± 0.01	116 ± 1
	TYPE OF BEVERAGE	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Two-way ANOVA*	SUPPLEMENTATION	0.0841	**0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	TB × S	0.3206	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

Mean ± SD ($n = 3$; three different experimental days). ORG: supplementation with Se-ORG; INORG: supplementation with Se-INORG; TB: type of beverage; S: supplementation; SD: standard deviation. BF: bioaccessible fraction; RF: residual fraction. The percentages (%) of BF and RF were calculated based on the initial quantities present in the beverages. Recovery was calculated as the sum of BF and RF.

For each type of PBBs, different superscript letters within a column indicate significant differences among commercial beverages and supplemented PBBs (One-way ANOVA followed by the LSD test, $p < 0.05$); their absence indicates no significant differences ($p \geq 0.05$).

† BF (%) values and total recoveries exceeding 100% in the Brazil nut beverage are attributed to cumulative analytical uncertainty inherent to trace-level sequential processing, alongside minor ICP-MS matrix-induced signal enhancement caused by the high lipid content of the natural sample.

* Two-way ANOVA to study the effect of the type of beverage, supplementation (Se-ORG and Se-INORG), and interaction between both factors using only the supplemented almond, rice, and soy beverages ($p < 0.05$). The Brazil nut beverage was not included in this analysis.

enhanced by the dynamic model, whereas the Se-ORG-supplemented soy beverage showed a decrease compared with the static protocol. Almond beverages exhibited similar bioaccessible Se percentages between models for both Se-ORG and Se-INORG supplementation. Recovery values in the dynamic system approached or exceeded 100%, particularly for Brazil-nut and Se-ORG treatments. These recovery deviations from 100% in specific matrices are recognized phenomena in trace element complex analysis in ICP-MS. The high concentrations of organic carbon and lipids present in the digested Brazil nut matrix led to 'carbon-induced signal enhancement' in the argon plasma, which increases the ionization efficiency of Se, leading to a slight analytical overestimation (Kovačevič & Goessler, 2005). Therefore, these deviations are primarily attributed to matrix-induced concentration effects in ICP-MS, rather than actual experimental bias in the SHIME system.

These findings align with previous comparative assessments, showing that dynamic digestion systems reproduce more comprehensively the gastrointestinal processes than static models by incorporating gradual pH changes, continuous secretion of digestive fluids and peristaltic mixing, thereby providing a more physiologically representative assessment of nutrient release (Dupont et al., 2019; Oško et al., 2024).

An important methodological consideration is that the SHIME system experiments were conducted using the standardized fasted-state configuration originally described by Molly et al. (1993), following a validated and widely adopted dynamic gastrointestinal protocol. This setup was intentionally selected to ensure methodological consistency and reproducible comparisons across different beverage matrices while minimizing physiological variability between experimental runs. However, when plant-based beverages are consumed as meal replacements, fed-state physiological conditions may be more representative. Under such fed-state conditions, the transient rise in gastric pH, delayed gastric emptying, alterations in digestive secretions, and interactions between dietary proteins, lipids, and encapsulated compounds may modify microparticle disintegration and selenium release during digestion (Abbeele et al., 2010; Brodkorb et al., 2019; Dupont et al., 2019; Jantratid et al., 2008; Minekus et al., 2014; Teixeira et al., 2022). Consequently, although the fasted-state configuration provides a standardized framework for comparative assessment, the absolute selenium bioaccessibility values reported here may differ from those expected under fed-state conditions. Nevertheless, since all beverage matrices were evaluated under identical experimental conditions using the same validated protocol, the comparative interpretation of matrix-dependent effects remains valid. Future studies employing fed-state dynamic digestion protocols would further enhance the physiological relevance and external validity of these findings.

4. Conclusions

This study advances the technological application of selenium microparticles by demonstrating their behavior in plant-based beverages through complementary static and dynamic digestion models and Caco-2 intestinal transport assays. The investigation demonstrates that the food matrix is the primary determinant of selenium bioaccessibility and intestinal absorption (bioavailability estimate) in plant-based beverages, exerting a greater influence than the chemical form of the mineral. Our results confirm that almond and soybean beverage matrices favor inorganic selenium, whereas the rice beverage matrix is more suitable for organic selenium, with dynamic digestion models providing more physiologically representative insights than static protocols. Collectively, these findings underscore that plant-based beverages are effective vehicles for selenium fortification; however, optimization requires a tailored approach considering the matrix composition and microencapsulation strategy. Furthermore, a study on a Brazil nut beverage allowed a comparative analysis of naturally occurring selenium in the beverage *vs.* selenium obtained through fortification with microparticles. Brazil nut beverage should be considered separately from fortified PBBs, due to its naturally high selenium content and the resulting differences in selenium exposure. While Brazil nut beverage is naturally rich in selenium, our Caco-2 findings demonstrate relatively low intestinal absorption rates from this drink, indicating that controlled fortification strategies provide a more predictable and safer approach to delivering nutritionally relevant levels of selenium in various plant-based beverages. More broadly, this study highlights that successful micronutrient fortification depends on the interaction between food matrix composition, selenium species, and the performance of the previously validated multilayer delivery system. This integrated framework provides valuable guidance for the rational development of fortified functional foods and supports the broader application of dynamic digestion models in future studies to improve the estimation of micronutrient bioaccessibility.

Funding/financial statement

This work was supported by the São Paulo Research Foundation - FAPESP [grant numbers 2022/06111-8 and 2023/11364-5]; the National Council for Scientific and Technological Development (CNPq) [grant number 304152/2024-2] and the Studies and Projects Financing Agency (FINEP) [grants number 1310/24 and 1050/24].

CRediT authorship contribution statement

José Luan da Paixão Teixeira: Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Raquel Fernanda Milani:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Izabela Dutra Alvim:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Marcelo Antonio Morgano:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Data curation, Conceptualization. **Ascensión Rueda-Robles:** Writing – review & editing, Investigation, Data curation, Conceptualization. **Jesús Lozano-Sánchez:** Writing – review & editing, Supervision, Data curation, Conceptualization. **Isabel Seiquer:** Writing – review & editing, Supervision, Investigation, Data curation, Conceptualization. **Cristina Delgado-Andrade:** Writing – review & editing, Supervision, Investigation, Data curation, Conceptualization.

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.

Acknowledgements

The authors would like to thank the Institute of Food Technology for providing materials, reagents, and all equipment necessary for the development of this study. We also thank the Department of Physiology and Biochemistry of Animal Nutrition, Estación Experimental del Zaidín (CSIC, Spain) and the Department of Food Science and Nutrition, University of Granada (Spain) for their valuable support and collaboration during the experimental trials.

Data availability

Data will be made available on request.

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