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# Selenium micro-encapsulation: Innovative strategies for supplementing plant-based beverages

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ABSTRACT

Selenium (Se) is an essential micronutrient for humans, and one of the new strategies to increase its intake is to fortify foodstuff with Se-bioaccessible species. This study aimed to produce Se-microparticles (selenate, selenite and Se-organic) using combined methods of microencapsulation; fortify commercial plant-based beverages (PBBs); evaluate the stability of microparticles; and estimate the bioaccessibility of Se and its contribution to the Recommended Daily Intake (RDA). Samples and bioaccessible fractions were submitted to acid digestion and Se levels (total and bioaccessible) were quantified by ICP-MS. The bioaccessibility of the Se-microparticles produced was estimated using an *in vitro* method under standardized conditions (INFOGEST). During the storage period, the microparticles maintained their stability and similar size. The PBBs fortified with Se-microparticles presented high bioaccessibility (77–94 %); being superior, in comparison, to the traditional fortification (Se-isolated salts). Thus, the results suggest promising perspectives for Se-supplementation in PBBs to meet nutritional needs effectively.

## 1. Introduction

Selenium (Se) is an essential trace element necessary for humans and animals in small quantities. It performs several functions in the human body, such as: regulation of hormonal and immune systems, contributes to resistance against viral infections and the prevention of various types of cancer (di Dato et al., 2017; Schiavon et al., 2020). The ingestion of Se by the world population is variable and depends on the levels of this element found in the soil where food is produced; therefore, there is a highly unequal geographical distribution of Se, ranging from almost zero to 1250 mg $kg^{-1}$ in seleniferous soils (Grenha et al., 2023).

Foods rich in Se generally contain ingredients grown in soils abundant with this nutrient, or have been enriched with isolated salts. Nutrient fortification allows for the effective correction of insufficient or low intake. However, it is vital to evaluate the chemical form of Se used in alimentary supplementation, in order to ensure its availability for intestinal absorption. Se-inorganic salts, such as selenate and selenite sodium, are widely used as nutritional supplements and/or as additives for commercial food products (Chen et al., 2021; Grenha et al., 2023); and in supplementation/fortification of various dairy products (Cámara-Martos et al., 2019).

As an alternative to cow milk and its dairy products, there has been an increase in consumption of plant-based beverages (PBBs). Lactose intolerance, allergies to milk proteins, ethical considerations (animal welfare and environmental preservation), lifestyle changes and adoption of specific diets (vegetarian, vegan and flexitarian) are factors that drive the production and high consumption of PBBs (Welna et al., 2024). There has also recently been a significant increase in the marketing/consumption of PBBs made with soybeans, oats, hemp, coconut, rice, almonds and nuts (Manousi & Zachariadis, 2021); with the estimated market exceeding US$ 12.1 billion (Penha et al., 2021).

The nutrient contents of PBBs, such as proteins, carbohydrates, essential minerals (including Se) and bioactive components (vitamins, phenolic compounds, tannins), may vary according to the raw materials and technologies employed during their processing and storage (Munekata et al., 2020). Teixeira et al., 2024 studied the content of Se in commercial PBBs produced from almonds, cashew/Brazil nut, coconut, oats, peanuts, rice and soybeans; and reported values between <4.0 μg

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$kg^{-1}$ (detection limit) up to 226 μg $kg^{-1}$. Astolfi et al. (2020) and Orlando et al. (2020) identified non-measurable levels of Se in commercial PBBs produced with almonds, peanuts, rice, oats and soybeans.

The Recommended Daily Intake (RDI) of Se for children (≥ 4 years old) and adults is 55 μg/day (FDA, 2016; IOM, 2000). Although the RDA of Se is considered low, this trace element plays a vital role in metabolic pathways (glutathione peroxidase, for example) (de Lima et al., 2023; Kipp et al., 2015); and diets deficient in Se can induce dysfunctions in the body, therefore increasing susceptibility to chronic diseases (Schiavon et al., 2020).

However, to perform its functions in the body, Se must be available and absorbed at the end of the gastrointestinal digestion process. Static methods of *in vitro* gastrointestinal digestion, such as the INFOGEST protocol, are important tools to estimate the solubilization percentage of this trace element by the intestine. The bioaccessibility of micronutrients is directly influenced by the composition of the food; and includes proteins, fats, dietary fibers or even other inorganic elements (Grenha et al., 2023). In this context, microencapsulation offers a strategy to minimize interactions that may affect the stability of this trace element, enabling beyond protection, a controlled and/or sustained release (Fu et al., 2016).

The microencapsulation process has been applied to nutrients susceptible to interactions with food components, such as Se, enabling the creation of ingredients suitable for food applications. To produce microparticles, the compounds of interest are covered by a wall material, or dispersed in a polymer matrix (de Santos et al., 2021; Grenha et al., 2023). This process has several advantages, such as: the release of the active material in a controlled manner, prolonged shelf life and the protection of compounds with nutritional value (de Santos et al., 2021). Among the microencapsulation techniques applied in food, the following stand out: spray-drying, spray-chilling, in situ polymerization, coacervation, fluidized bed coating, freeze-drying and emulsification (de Santos et al., 2021). The choice of method depends on the properties of the material of interest, the desired characteristics of the microparticles (size, stability, solubility), the intended application, and the feasibility and cost of the process (Tolve et al., 2016).

Considering the information presented above, the objectives of this work are to produce microparticles of inorganic (sodium selenate and sodium selenite) and organic (Seleno-L-cystine) Se, through combined methods (spray-drying followed by spray-chilling) to fortify commercial PBBs of almonds, rice and soybeans; evaluate the stability of the produced microparticles; and to estimate the bioaccessible fraction of Se and its contribution to the RDI. For comparison purposes, cow milk was also enriched with the produced microparticles.

## 2. Material and methods

### 2.1. Obtainment of inorganic and organic Se-microparticles through spray-drying and incorporation into plant-based beverages

The Se-microparticles were obtained through combined methods, adapted from the study of Fadini et al. (2019). Sodium selenate and sodium selenite, Se-organic (S8295, S5261 and S45996, respectively; Sigma Aldrich, St. Louis, EUA), maltodextrin (MOR-REX 1910, DE 10, Ingredion, Brazil), gum arabic (Intantgum AA, Nexira, Brazil), carboxymethylcellulose (CMC) (GELYCEL F1 2000 S, Amitex, Brazil), surfactant polysorbate 80 (Tween 80, Synth, Brazil), vegetable fat (370B, Agropalma, Brazil) and fully hydrogenated palm fat (A. Azevedo Óleos Vegetais, Brazil) were utilized.

The microparticle production process comprised of three steps: initial microencapsulation of isolated Se salts (selenate and sodium selenite and Se-organic) through spray-drying (MI-SD) using maltodextrin as wall material, with a concentration of 1500 mg Se/g MI-SD (20 g/100 g total solids) under specific drying conditions (Tin = 150 ± 2 °C, Tout = 82 ± 3 °C), followed by microencapsulation of MI-SD by spray-chilling (M-SC) in a lipid mixture (melted at 80 ± 0.5 °C) obtaining a concentration of 150 μg Se/g M-SC (sprayed in a cold chamber at 7 ± 2 °C); then, the M-SC microparticles were dispersed in a solution composed of gum Arabic, CMC, surfactant Tween 80 and water, and dried by spray-drying, under the same drying conditions as MI-SD. This resulted in a concentration of 30 μg in Se/g of MF-SD. In Fig. 1, the general layered scheme of the microencapsulation procedure that was used through the combined methods (spray-drying and spray-chilling) is described.

The fortification of the drinks with the produced Se-microparticles was performed in order to provide 55 μg of Se in a 200 mL portion of each drink, which corresponds to 100 % of the RDI of Se for children (≥ 4 years old) and adults (FDA, 2016; IOM, 2000).

### 2.2. Characterization of Se-microparticles produced through spray-drying

The physical analyses described in items 2.2.1 and 2.2.2 were performed at the end of microparticle production (T0 - time zero) and after 6 months (TF - time final).

#### 2.2.1. Morphology, mean diameter and size distribution of microparticles

The morphology of the microparticles was analyzed by optical microscope (BX40, Olympus, Tokyo, Japan), and capturing images using a digital camera (Q-Capture, Olympus, Tokyo, Japan).

For the determination of mean diameter and size distribution, the MF-SD were suspended in absolute ethanol (99.7 % purity, Synth, São Paulo, Brazil) and the measurements were made by laser diffraction (LA 950-V2, HORIBA, Kyoto, Japan) (Alvim et al., 2016; Matos-Jr et al., 2015). The mean diameter measurements of the microparticles was represented by the diameter associated with 50 % of the accumulated distribution ($D_{50}$). The variability in distribution was evaluated by the polydispersity index (SPAN), calculated as SPAN = $(D_{90} - D_{10})/D_{50}$; where $D_{10}$, $D_{50}$ and $D_{90}$ represent the diameters corresponding to 10 %, 50 % and 90 % of accumulated distribution, respectively.

#### 2.2.2. Color determination, moisture, water activity ($a_w$) and encapsulation efficiency (EE%)

The color parameter was determined by a colorimeter (CR 410, Konica Minolta Inc., Osaka, Japan), in optimized conditions. To calculate the color variation during storage (ΔE) eq. (1) was used.

$$\Delta E = \left[(\Delta L^*)^2 + \left[(\Delta a^*)^2 + \left[(\Delta b^*)^2\right]0.5\right.\right. \quad (1)$$

where: ΔE is the magnitude of the color difference; ΔL* is the variation of the coordinate L*; Δa* is the variation of the coordinate a*; e Δb* is the variation of the coordinate b*.

The moisture content was determined by titration Karl Fischer (Titrino 785, Metrohm, Herisau, Switzerland) (Zenebon et al., 2008) and the water activity ($a_w$) was measured in an analyzer (AquaLab 4TEV, Decagon Devices Inc., Pullman, EUA) at 25 ± 0.5 °C.

The Concentration of Active (CA) in the microparticles (selenate, selenite and Se-organic) was determined at time zero and after 6 months of storage through quantifying the Se content after mineralization in block digester, at a maximum temperature of 130 °C in the presence of nitric acid and hydrogen peroxide (procedure described in item 2.3; Silva et al., 2020). The value of CA at time zero was used to determine the Encapsulation Efficiency (EE%), given by the percentage of active substance (Se) present in the microparticles after processing, compared to its initial amount in the liquid formulation (Alvim et al., 2016), according to the eq. (2):

$$EE(\%) = \frac{Quantified\ active\ substance\ after\ processing}{Active\ substance\ used\ before\ of\ processing}\ x\,100 \quad (2)$$

### 2.3. Quality control

The analytical method was validated considering the parameters:

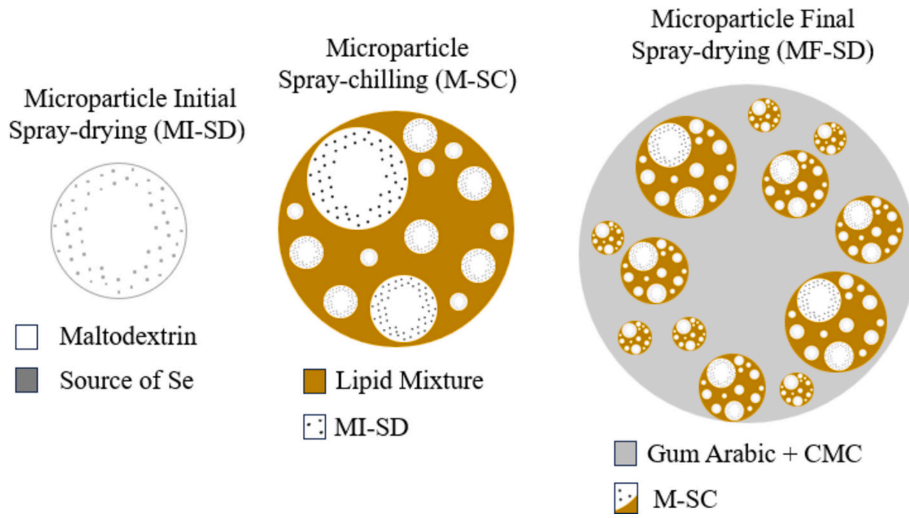


**Fig. 1.** Summary of the proposed microencapsulation procedure of selenium.

limit of detection (LOD), limit of quantification (LOQ), precision (coefficient of variation, CV) and accuracy (INMETRO, 2020; Teixeira et al., 2024). For total Se content and Se-bioaccessible extracts, the LOD was 2.1 and 10.5 μg $kg^{-1}$, respectively, while the LOQ was 4.0 and 20 μg $kg^{-1}$, respectively. Precision ($n = 7$) has a CV of 9 %. Accuracy (recovery) rates ranged from 95 to 111 %, both using certified reference materials (ERM-BD 151 - skimmed milk powder and NIST-SRM 1547 - peach leaves) and fortified experiments with 25 and 75 μg $kg^{-1}$ of Se.

### *2.4. Determination of the total content and percentage of bioaccessibility of Se*

The samples of fortified PBBs were mineralized in acid medium in the presence of 4 mL of concentrated nitric acid (Merck, Darmstadt, Germany) purified by sub-boiling distillation (Distillacid, Berghof, Eningen, Germany) and 2 mL of hydrogen peroxide (Merck, Darmstadt, Germany) in ultrasonic bath at 80 °C for 35 min (Fioravanti et al., 2020). Then, the digestate was cooled, purified water added to make up to 20 mL and filtered (PTFE, 0.45 μm, Agilent Technologies, Tokyo, Japan).

To evaluate the bioaccessibility estimation of Se, the standardized *in vitro* digestion procedure proposed by INFOGEST (Brodkorb et al., 2019) was used, considering an aliquot of 2.5 g of plant-based beverages. At the end of the gastrointestinal simulation, the content was centrifuged (3500 *g* at 4 °C for 30 min) and transferred to digestion tubes. After the reduction of volume in an oven at 100 °C for 24 h, the bioaccessible fractions were mineralized in a block digester at a maximum temperature of 130 °C for 4 h, with 4 mL of nitric acid and 2 mL of hydrogen peroxide (Silva et al., 2020). Finally, after cooling, the samples were transferred to 20 mL graduated tubes, filtered (PTFE, 0.45 μm, Agilent Technologies, Tokyo, Japan) and were analyzed with the use of an inductively coupled plasma mass spectrometer (ICP-MS) (iCAP RQ, Thermo Fisher Scientific™, Bremen, Germany) under optimal conditions: Air flow rate/Auxiliary air (14.0/0.8 L $min^{-1}$); He flow rate (5.0 mL $min^{-1}$); Nebulizer flow rate (Micromist; 0.98 L $min^{-1}$); dwell time (0.3 s/0.02 s IS); monitored isotopes ($^{77}Se$; $^{78}Se$; $^{82}Se$); and internal standards, IS (50 μg $L^{-1}$) ($^{72}Ge$, $^{74}Ge$; $^{115}In$) (Teixeira et al., 2024).

### *2.5. Contribution to the recommended daily intake (RDI) of Se*

The contribution to the RDI of Se was estimated considering the daily intake of 200 mL of plant-based beverages by children (≥ 4 years old) and adults; and the average levels of Se found in PBBs (Kroes et al., 2002). The RDI value considered was 55 μg/day, as established by the Food and Drug Administration (FDA, 2016) and Institute of Medicine (IOM, 2000).

### *2.6. Statistical analysis*

The results were evaluated using Analysis of Variance (ANOVA one-way), Tukey test (95 % confidence) and coefficient of variation (CV) using the Microsoft Office Excel (version 2016) software and Statgraphics Centurion XVI (StatPoint Technologies, Inc. USA, 2010).

## 3. Results and discussion

### *3.1. Production of microparticles*

In the development of this work, the microencapsulation technique was chosen to protect the different forms of Se (selenate, selenite and Se-organic) applied in PBBs until its absorption by the body.

The microencapsulation using maltodextrin as wall material through spray-drying was successfully performed in preliminary tests, however, the high solubility of this coating matrix made its application unfeasible in PBBs. Subsequently, the use of lipid microparticles (spray-chilling) was tested for vehiculation of Se in plant-based beverages; however, there was a poor distribution of Se in the samples, due to the low concentration and insolubility of the same what in the lipid used as wall material. Due to this, we opted for the use of combined methods: spray-drying followed by spray-chilling, with the first being for effective encapsulation of Se and the latter method to create a hydrophobic barrier, and consequently being insoluble in beverages. However, this insolubility caused the microparticles to separate from the PBBs. To overcome this problem a third layer containing stabilizing agents (CMC and gum arabic) was applied by spray-drying on the lipid microparticles, allowing the microparticles to stay dispersed properly in the PBBs. In all stages the microparticles were successfully obtained and the results of the characterization of the final samples are presented and discussed.

### *3.2. Characterization of the microparticles*

#### *3.2.1. Morphology, mean diameter and size distribution of microparticles*

The average size and distribution of selenate, selenite and Se-organic microparticles can be seen in Fig. 2 (A, B and C, respectively). For selenate and selenite, a statistical difference ($p < 0.05$) in minimum diameter ($D_{10}$) and maximum ($D_{90}$), mean diameter ($D_{50}$) and SPAN was observed, indicating slight agglomeration between the microparticles over time. Considering the global variation, the effect observed in the application of microparticles in plant-based beverages was considered

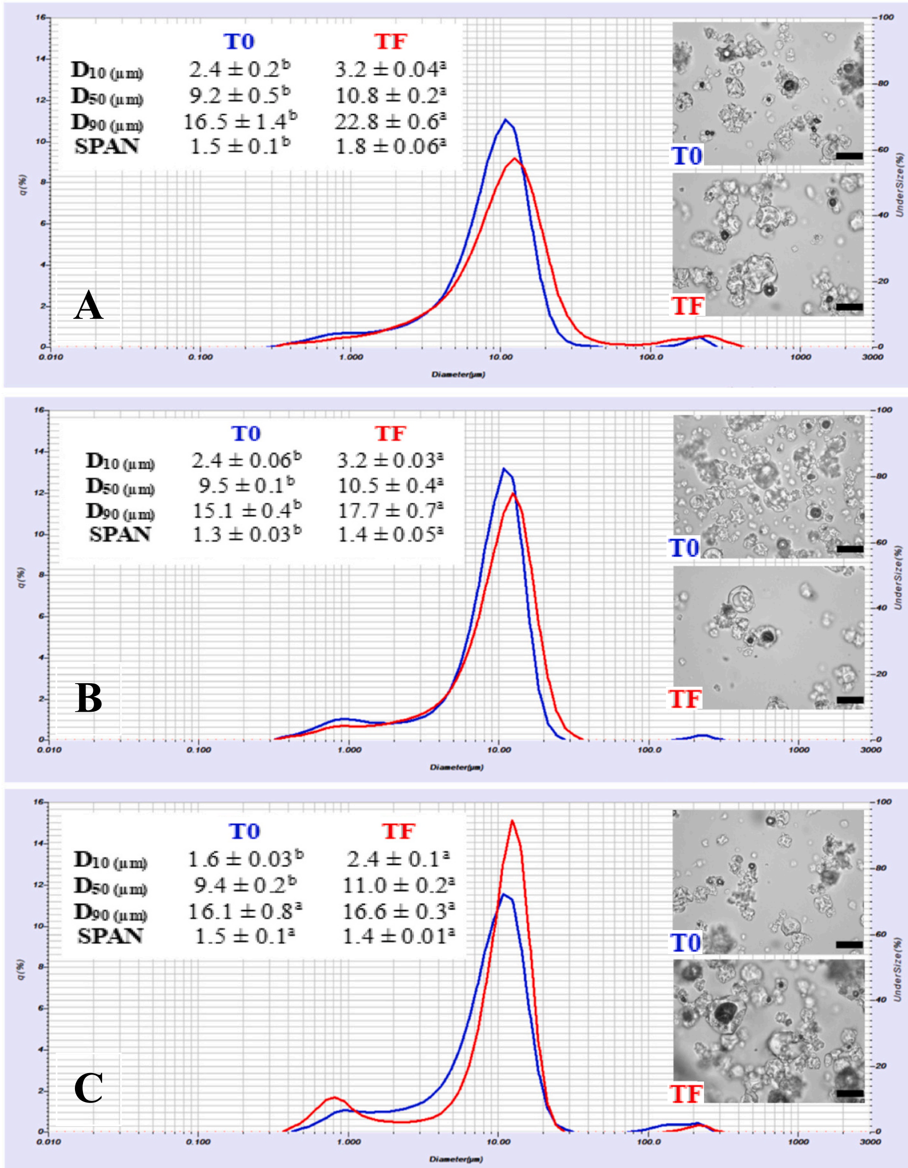


**Fig. 2.** Mean diameter (D50), accumulated diameters (μm), polydispersity index (SPAN) and size distribution curve measurements of microparticles containing sodium selenate (A), sodium selenite (B) and Se-organic (C).

despicable.

The curves indicate an increase in the size of the microparticles from the initial time (T0) to the final time (TF), and may indicate a slight agglomeration for the microparticles of sodium selenate (Fig. 2A) and sodium selenite (Fig. 2B), respectively. For Se-organic (Fig. 2C), the microparticles in TF were smaller when compared to the microparticles of T0.

The microparticles produced presented similar morphology, with high polydispersity and typical appearance of products obtained by combined methods (spray-drying and spray-chilling) at laboratory scale (Fadini et al., 2018). The combined method in three layers was applied in an attempt to overcome several challenges observed in the encapsulation of Se in PBBs (challenges discussed in item 3.1). Preliminary tests indicated the applicability of this procedure to enable a better distribution of the active in the microparticles, maintain the integrity of the microparticles in an aqueous system and allow for good dispersion of the final microparticles in plant-based beverages.

### *3.2.2. Color determination, moisture, water activity ($a_w$), concentration of active (CA) and encapsulation efficiency (EE%)*

In Table 1, the results of the parameters used for the characterization of the produced microparticles are given.

In general, the selenate, selenite and Se-organic microparticles produced showed high luminosity, with indexes higher than 92 % (very white with slight shade of yellow). For the luminosity index L, a slight darkening of the sample was observed between the initial time (T0) and the 6 months of storage (TF); in parameter a, samples all acquired a slight shade of green and for parameter b, all samples acquired a slight shade of yellow. The color variation (ΔE) between the initial and final time of the Se-microparticles was $2.2 \pm 0.4^a$; $1.6 \pm 0.2^b$ and $2.4 \pm 0.8^a$ for selenate, selenite and Se-organic, respectively; with a statistical difference for selenite ($p < 0.05$). However, when microparticles were applied in PBBs no visual differences for color were observed.

For the moisture results, a slight increase in percentages in all samples ($p < 0.05$) was observed, with obtained values of 8.5 %, 9.2 %, and 8.6 % for selenate, selenite and Se-organic (respectively), at the end of the storage period (6 months). However, no physical change was

**Table 1**

Color determination, moisture, activity water ($a_w$), concentration of active (CA) and encapsulation efficiency (EE%) results of produced microparticles.

| Determinations | | Time | MP Selenate | MPL Selenite | MPL Se-organic |
|---|---|---|---|---|---|
| Color | L | T0 | 94.9 ± 0.03$^a$ | 94.7 ± 0.03$^a$ | 94.6 ± 0.1$^a$ |
| | | TF | 93.0 ± 0.3$^b$ | 93.3 ± 0.2$^b$ | 92.5 ± 0.8$^b$ |
| | a | T0 | −0.17 ± 0.01$^a$ | −0.20 ± 0.01$^a$ | −0.14 ± 0.01$^a$ |
| | | TF | −0.25 ± 0.02$^b$ | −0.39 ± 0.03$^b$ | −0.22 ± 0.02$^b$ |
| | b | T0 | 5.8 ± 0.1$^b$ | 6.2 ± 0.1$^b$ | 5.8 ± 0.1$^b$ |
| | | TF | 6.9 ± 0.3$^a$ | 6.7 ± 0.1$^a$ | 7.0 ± 0.4$^a$ |
| Moisture (%) | | T0 | 6.9 ± 0.1$^b$ | 8.0 ± 0.2$^b$ | 7.5 ± 0.1$^b$ |
| | | TF | 8.5 ± 0.2$^a$ | 9.2 ± 0.3$^a$ | 8.6 ± 0.2$^a$ |
| $a_w$ | | T0 | 0.17 ± 0.004$^b$ | 0.25 ± 0.002$^b$ | 0.19 ± 0.003$^b$ |
| | | TF | 0.29 ± 0.01$^a$ | 0.37 ± 0.002$^a$ | 0.30 ± 0.01$^a$ |
| Concentration of Active (mg kg$^{-1}$) | | T0 | 22.9 ± 0.1$^a$ | 22.8 ± 0.04$^a$ | 8.7 ± 0.1$^a$ |
| | | TF | 22.7 ± 0.1$^a$ | 22.7 ± 0.1$^a$ | 8.6 ± 0.1$^a$ |
| Encapsulation efficiency (%) | | | 80.8 ± 2.0$^a$ | 81.8 ± 0.5$^a$ | 81.7 ± 0.6$^a$ |

MP: Microparticle; T0: time zero; TF: time final (6 months). The results are expressed as mean ± standard deviation ($n$ = 3). Different lowercase letters superscripted in the same column indicate significant differences ($p < 0.05$; ANOVA + Tukey's test) between microparticles at the initial time (T0) and final (TF).

observed in the microparticles that interfered with the protection capacity of the active compound. Corroborating with this result, there was no statistical difference (p < 0.05) in the determination of concentration of active (CA) between the initial and final time (6 months) for all microparticles studied.

Although the moisture and $a_w$ contents did not show direct correlation, the $a_w$ values at the final time (0.29, 0.37 and 0.30, for the of selenate, selenite and Se-organic microparticles, respectively) indicated satisfactory stability in relation to lipid oxidation and low probability of proliferation of pathogenic microorganisms ($a_w < 0.6$).

The CA between the initial and final time (6 months) showed no statistical change ($p < 0.05$) during the storage period, and had values at the final time (FT) of 22.7; 22.7 and 8.6 mg kg$^{-1}$ of Se for the microparticles of selenate, selenite and Se-organic, respectively. Thus, the CA in the microparticles remained stable during storage and subsequent application/fortification of plant-based beverages of almonds, rice and soybeans.

Adequate percentages of encapsulation efficiency (EE%) of >80 % were quantified for the microparticles produced; indicating that the parameters used in the microparticle production process did not cause significant changes to the active substance (Se). Fadini et al. (2019) studied the production of microparticles by spray-drying and reported that values of EE% between 60 and 90 % are considered satisfactory, being influenced by the food formulation, structural characteristics of the wall materials and the drying rate of the process. Alvim et al. (2016) and Nesterenko et al. (2014) also used the spray-drying technique to encapsulate ascorbic acid and vitamins and reported EE% of 97.8 % and 92 %, respectively; results close to those obtained in the present study.

### *3.3. Percentage of bioaccessibility in plant-based beverages fortified with inorganic and organic selenium*

The bioaccessibility tests were performed simulating gastrointestinal digestion *in vitro* through the oral, gastric and intestinal phases (Brodkorb et al., 2019; Egger et al., 2016). The digested content was centrifuged and the amount of Se present in the supernatant was considered as the portion of bioaccessible Se (Cámara et al., 2005).

In Fig. 3 (A, B and C), the bioaccessibility percentage of plant-based beverages of almonds, rice, soybeans are described, as well as whole milk (for comparison purposes) fortified with isolated salts and with microparticles of sodium selenate, sodium selenite and Se-organic.

Analyzing Fig. 3 (A, B and C), it is observed that the plant-based beverages fortified with the isolated salts of selenate and sodium selenite (Se-inorganic) presented bioaccessibility percentages ranging from 68.1 % (soy beverage) to 82.1 % (rice beverage). On the other hand, plant-based beverages fortified with Se-organic showed the lowest and highest percentage of bioaccessibility for Se, with values between 54.7 % and 88.7 % for plant-based beverages of soybean and rice, respectively.

Previously, studies had demonstrated the influence of the food composition in the bioaccessibility for Se naturally found in foodstuff. Singhato et al. (2022) studied the influence of cooking in Se-bioaccessibility in fishes from Thailand and reported values ranging from 48.8 to 58.5 % and 52.8 to 70.0 % for frying and boiling procedures, respectively. García-Conde et al. (2024) studied animal-based (dairy, chicken, fish, bovine and porcine meat) and plant-based foods (nuts, cereals, fruits, vegetables, legumes, oils and beverages) and reported higher values for Se bioaccessibility in animal-based food (93.6 ± 8.6 %) than in plant-based food (77.7 ± 20.4 %).

Recently, studies have investigated differences in the percentage of bioaccessibility of isolated Se-inorganic salts (selenate and sodium selenite) and Se-organic after supplementation in various food groups. Grenha et al. (2023) reported a higher bioaccessibility of organic forms (selenomethionine and methylselenocysteine) of Se when compared to inorganic salts (selenate and selenite sodium). Gómez-Jacinto et al. (2020) studied microalgae enriched with inorganic (selenate) and organic (selenomethionine) salts and reported bioaccessibility percentages of 79 % for samples supplemented with Se-organic, and about 2.5 % for supplementation with Se-inorganic. This behavior is due to the links between organic forms of Se and the amino acids (selenomethionine and selenocystine) highly absorbed by the organism (di Dato et al., 2017; Fairweather-Tait et al., 2010; Singhato et al., 2023). Previous research has also indicated that compounds, such as procyanidins and coenzyme Q10, reduce the bioaccessibility of Se, while vitamin C increases its bioaccessibility (Shilpashree et al., 2018; Zhang et al., 2020). In this context, caseins can also bind to various bioactive molecules and micronutrients, suggesting a potential system delivery of minerals after technological modifications (Casanova et al., 2021).

In this study, fortification with isolated Se-organic salt showed the highest variation in the bioaccessibility percentage among the plant-based beverages studied. Various factors can explain this fact, such as the interaction between Se and the various components of the PBBs studied and the lower solubility of the isolated Se-organic salt compared to the Se-inorganic.

The proposed microencapsulation process demonstrated a positive impact on the bioaccessibility percentages of the samples studied (Fig. 3, A, B and C). For all samples, after gastrointestinal simulation, the bioaccessibility of Se in plant beverages fortified with microparticles was higher when compared to those fortified with isolated salts of Se. This fact proves that the greater protection provided to the Se, until the intestinal phase by the gastro-resistant polymers employed were effective in protecting the Se until being absorbed by the organism. Grenha et al. (2023) fortified fermented milks with Se-microparticles produced by spray-drying and observed that the polymer contributed to the protection of the active compound from dietary interactions with other components of the food matrix, increasing its solubility. The bioaccessibility varied between 83.2 % and 90.6 %, 80.3 % and 93.8 % and 76.8 % and 93.0 % for PBBs and whole cow milk fortified with microparticles produced with sodium selenate, sodium selenite and Se-organic, respectively.

To date, this study is a pioneer in the fortification of PBBs through supplementation with inorganic and organic microparticles of Se. Alzate et al. (2010) studied the production of Se-inorganic microparticles (selenite) by spray-drying for the fortification of fermented milks, and reported bioaccessibility percentages of Se of up to 76 % (gastric phase). Grenha et al. (2023) used sodium selenite for the elaboration of microparticles of Se by spray-drying, to fortify yogurts with low levels of Se, and reported a higher percentage of bioaccessible Se when compared to the addition of this micronutrient in the form of free salt (maximum

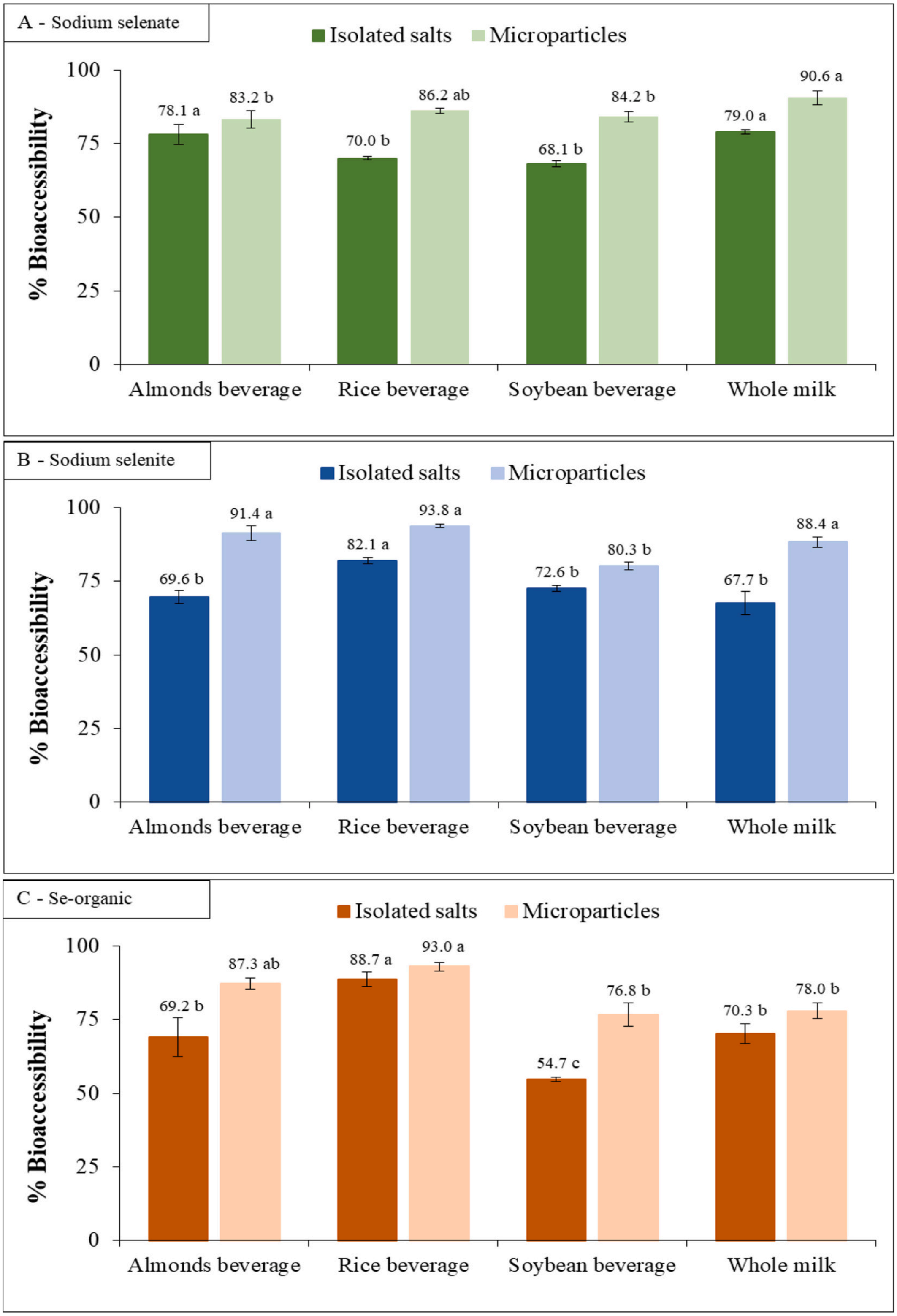


**Fig. 3.** Bioaccessibility percentage after *in vitro* digestion of plant-based beverages fortified with isolated salts and microparticles of sodium selenate (A), sodium selenite (B) and Se-organic (C).

percentages of 55 %).

### 3.4. Estimated intake of Se by consumption of plant-based beverages

The low consumption of Se is still a problem for the world population. Buffini et al. (2023) reported a Recommended Daily Intake (RDI) of Se below 55 μg/day in the Polish, Italian and Slovenian populations. Countries of the Middle East, such as Egypt, Iran and Turkey, also have lower intake rates of Se (Stoffaneller & Morse, 2015).

Teixeira et al., (2024) studied the percentage of RDI of Se in commercial PBBs of almonds and soybeans for different groups of individuals; and reported that these beverages contribute 1.6 % and 3.5 %

of the RDI to children (≥ 4 years) and adults, respectively. Orlando et al. (2020) reported that almond, rice and soy drinks are considered poor in Se, with a 240 mL serving providing less than 1 % of the RDI.

In Table 2, we can observe the variation in RDI percentages of PBBs of almonds, rice, soy and whole milk (for comparison purposes) fortified with isolated salts and with the microparticles produced. For the estimate evaluation the consumption of a glass (200 mL) of PBBs by children (≥ 4 years) and adults was considered, based on an RDI of 55 μg/day (FDA, 2016; IOM, 2000).

For PBBs fortified with the isolated salts, the percentages ranged from 52.8 % (soy PBB fortified with Se-organic) to 74.8 % (rice PBB fortified with sodium selenite). The contribution to the RDI of children (≥ 4 years) and adults was considered high, taking into account the consumption of only one 200 mL cup of these plant-based beverages. The variations observed in the percentages of RDI may be associated with the different formulations used to obtain these beverages, as well as the intrinsic characteristics of the ingredients used in these formulations.

In relation to beverages fortified with the microparticles produced, the results ranged from 96.1 % of the RDI (PBBs of rice fortified with the microparticle of Se-organic) to 131 % of the RDI (PBBs of almond fortified with microparticles of sodium selenate). It is remarkable that all PBBs fortified with microparticles of selenate, selenite and Se-organic showed higher estimates of daily absorption of Se, compared to beverages fortified with the salts isolated from this trace element. The microparticle polymer during *in vitro* digestion proved to be crucial for the protection of the active compound (Se), preventing unwanted nutritional interactions with other components of the matrices of PBBs (Grenha et al., 2023).

These findings indicate the effectiveness of the microparticles produced in promoting the bioaccessibility of Se, and offers valuable perspectives for the development of effective food fortification strategies in plant-based beverages. In addition, the proposed procedure has practical relevance in terms of effective contribution to the daily nutritional needs of Se for children and adults. Nevertheless, future studies are required to thoroughly examine the metabolism of Se-species, investigating potential interactions between selenium and other components of plant-based beverages that could affect its stability and intestinal absorption, such as phytates, oxalates, antioxidants and fibers.

**Table 2**
Results for recommended daily intake (RDI) of Se, considering the consumption of 200 mL of plant-based beverages by children (≥ 4 years) and adults.

| Plant-based beverages | Forms of Selenium | | BF (μg $kg^{-1}$) | % of RDI |
|---|---|---|---|---|
| Almonds | Isolated salts | Selenate | 184 ± 7[c] | 66.9 ± 2.5[c] |
| | | Selenite | 156 ± 4[d] | 56.9 ± 1.3[d] |
| | | Se-Organic | 159 ± 8[d] | 58.0 ± 2.8[d] |
| | Microparticles | Selenate | 360 ± 15[a] | 131 ± 5[a] |
| | | Selenite | 329 ± 0.2[b] | 120 ± 0.1[b] |
| | | Se-Organic | 308 ± 6[b] | 112 ± 2[b] |
| Rice | Isolated salts | Selenate | 186 ± 6[c] | 67.6 ± 2.4[c] |
| | | Selenite | 206 ± 4[b] | 74.8 ± 1.6[b] |
| | | Se-Organic | 176 ± 3[c] | 64.1 ± 1.2[c] |
| | Microparticles | Selenate | 274 ± 8[a] | 99.7 ± 3.0[a] |
| | | Selenite | 265 ± 0.5[a] | 96.4 ± 0.2[a] |
| | | Se-Organic | 264 ± 8[a] | 96.1 ± 3.0[a] |
| Soybean | Isolated salts | Selenate | 198 ± 3[b] | 72.1 ± 1.3[b] |
| | | Selenite | 199 ± 1[b] | 72.4 ± 0.3[b] |
| | | Se-Organic | 145 ± 1[c] | 52.8 ± 0.3[c] |
| | Microparticles | Selenate | 321 ± 13[a] | 117 ± 5[a] |
| | | Selenite | 309 ± 9[a] | 112 ± 3[a] |
| | | Se-Organic | 312 ± 14[a] | 113 ± 5[a] |
| Whole milk | Isolated salts | Selenate | 218 ± 8[c] | 79.2 ± 3.0[c] |
| | | Selenite | 215 ± 2[c] | 78.0 ± 0.6[c] |
| | | Se-Organic | 182 ± 9[c] | 66.3 ± 3.4[c] |
| | Microparticles | Selenate | 366 ± 13[ab] | 133 ± 5[ab] |
| | | Selenite | 376 ± 8[a] | 137 ± 3[a] |
| | | Se-Organic | 334 ± 21[b] | 121 ± 8[b] |

BF.: Bioaccessible fraction. The results are expressed as mean ± standard deviation (n = 3). Different lowercase letters superscripted in the same column indicate significant differences ($p < 0.05$) between samples fortified with isolated salts and with microparticles (selenate, selenite and Se-organic) for plant-based beverages of almonds, rice, soybean and whole milk. ANOVA + Tukey's test.

## 4. Conclusions

The selenate, selenite and Se-organic microparticles produced by combined methods (spray-drying and spray-chilling) were stable during the period evaluated, with only slight changes being observed (slight agglomeration and darkening) but with minor impacts for application in pant-based beverages. Additionally, all types of microparticles showed high luminosity, remained microbiologically safe and were effective in preserving the assets.

The evaluation assays for the total content and bioaccessibility of Se revealed that the PBBs fortified with isolated salts have a wide range of bioaccessibility (55 to 89 %). The use of fortification for plant-based beverages with Se-microparticles proved to be effective, with higher values of Se in the bioaccessible fraction (77 to 94 %) compared to traditional fortification processes (isolated salts).

The results obtained in this study offer valuable perspectives for the development of more efficient Se-fortification strategies in plant-based beverages, contributing to achieving daily nutritional needs and promoting public health.


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## CRediT authorship contribution statement

**José Luan da Paixão Teixeira:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ana Paula Rebellato:** Writing – review & editing, Visualization, Methodology. **Raquel Fernanda Milani:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Izabela Dutra Alvim:** Writing – review & editing, Visualization, Supervision, Methodology, Data curation. **Marcelo Antonio Morgano:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Funding acquisition.

## Declaration of competing interest

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

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.141993.

## Data availability

Data will be made available on request.

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