Selenium bioaccessibility after in vitro digestion/fermentation of foods differs in adults and children

Úrsula García-Conde a, Miguel Navarro-Alarcón a,b,*, Beatriz Navajas-Porras a,b, Daniel Hinojosa-Nogueira a,b, Adriana Delgado-Osorio a,b, Miguel Navarro-Moreno a,b, Sergio Pérez-Burillo a,b, Silvia Pastoriza a,b, Konstantinos Douros c, José Ángel Rufián-Henares a,b,d

a Departamento de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Granada, Spain
b Instituto de Nutrición y Tecnología de Los Alimentos, INIYA (IBIS), Universidad de Granada, Spain
c Pediatric Allergy and Respiratory Unit, 3rd Department of Pediatrics, “Attikon” University Hospital, National and Kapodistrian University of Athens, School of Medicine, 11527, Athens, Greece
d Instituto de Investigación Biosanitaria Ibs.GRANADA, Universidad de Granada, Granada, Spain

ABSTRACT

Selenium (Se) as essential element regulates the immune, endocrine, reproductive and neurological systems through selenoproteins. More important than its content, is the fraction available to be absorbed (bioaccessibility) to exert its important metabolic functions. The objective of this study was to determine the bioaccessibility of Se (Se-BA) in multiple foods by an in vitro digestion/fermentation method. Samples were subjected to homemade culinary techniques and fermented with feces from healthy adults (HE-AD), and healthy (HE-CH) and unhealthy children (GRD-CH and OB-CH; 38.0(±24.9) and 34.2(±24.6) %, respectively). In plant-based foods, higher Se-BA values were found in the large intestine (34.0(±24.6) and 35.8(±28.1) %, respectively) could be related to greater demands on growth and specific fermentative microbiota.

1. Introduction

Se is an essential element that behaves as an enzymatic cofactor of at least 25 enzymes (Zhao et al., 2023) such as glutathione peroxidases, thioredoxin reductases, thyrone-S’ desydases and different selenoproteins. Through these enzymes, Se prevents oxidative stress and inflammatory processes (Navarro-Alarcón & Gil-Hernández, 2017). Despite this, it can cause toxicity when reaching high concentrations in the body.

Se, before exerting its important metabolic function (bioactivity), has to be released from the food matrix in the gastrointestinal tract (bioaccessibility) and then absorbed (bioavailability) (Thiry et al., 2012). Multiple in vivo methods exist for the evaluation of bioavailability in experimental animals, or more physiologically in humans. However, these methods have limitations as they must be performed on each food individually, which would result in a very monotonous diet, and it is also very difficult to differentiate between the Se released from the body pool into the intestinal lumen, and the Se present in the food that is not absorbed and eliminated in feces. Additionally, there are different in vitro methods available for the prior evaluation of Se-BA in

* Corresponding author. Departamento de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Granada, Spain.
E-mail address: nalarcon@ugr.es (M. Navarro-Alarcón).
such as Se are needed (Larvie et al., 2019). This element also regulates adipocyte differentiation (Tinkov et al., 2020; Watanabe et al., 2021). immune system activity, neuronal development and activity, and inflammation characteristic of obesity, adequate levels of antioxidants could modulate whey protein-induced allergic responses (Zhao et al., 2023).

In children, an adequate nutritional status in Se is especially relevant, and its deficiency affects cardiovascular health (Keshan disease), osteoarticular system (Kashin-Beck disease) (Hossain et al., 2021) and future growth and health (Zymbo et al., 2022). In this sense, celiac disease is a genetic predisposition to chronic gluten intolerance in 1% of the Western populations. The malabsorption associated with celiac disease causes a deficit of Se, which (as a cofactor of iodothyronine deiodinase) would affect thyroid health (Stazi & Trinti, 2010). Therefore, the control of Se availability in celiac disease and associated autoimmune thyroid diseases could be an effective treatment (Stazi & Trinti, 2010). Thyroid disorders have shown intestinal dysbiosis with altered Se absorption, immune response and damage of the intestinal mucosa due to its permeability to pro-inflammatory antigens (Knezevic et al., 2020). In young celiacs with high adherence to a gluten-free diet (>6 months of follow-up) Se consumption was lower than in the control group (Nestares et al., 2020). Newly diagnosed celiac children had blood levels of Se similar to those following a gluten-free diet 6 months and ≥12 months (McGrogan et al., 2021). In spite of this, it is relevant to eliminate gluten from the diet in celiac children, since nutritional deficiency due to malabsorption problems in the small intestine and inflammation, compromises their optimal growth and predisposes to future diseases (Coussens & Werb, 2002).

On the other hand, an inverse relationship has been established between Se intake and total body fat percentage (De Castro Santos et al., 2021). It has been referred that to combat the oxidative stress and inflammation characteristic of obesity, adequate levels of antioxidants such as Se are needed (Larvie et al., 2019). This element also regulates immune system activity, neuronal development and activity, and adipocyte differentiation (Tinkov et al., 2020; Watanabe et al., 2021). To control hypercholesterolemia in obese subjects, more than 50% of subjects take statins to decrease the activity of the enzyme hydroxy-methyl-glutaryl-coenzyme A reductase (HMGCoAR; Watanabe et al., 2021). This enzyme is an intermediate pathway in selenoprotein synthesis which could be associated with the decrease in erythrocyte peroxidase activity indicated in obese adults (Fontenelle et al., 2022). In humans, there is controversy as low serum/plasma Se levels are associated with obesity (Arnaud et al., 2006; Soares de Oliveira et al., 2021), and inversely with high blood concentrations and its biomarkers (Tinkov et al., 2021).

Cow’s milk protein allergy is an adverse immune response that affects 2–3% of children (D’Auria et al., 2019) and predisposes to asthmatic problems and atopic dermatitis (Santos et al., 2010). Katila et al. (2001) reported a high risk of Se deficiency in allergic children by presenting lower plasma levels. Others (Ferreira et al., 2021) report that Se can modulate allergic responses by influencing the composition and colonization of the microbiome. In female mice, oral Se supplementation could modulate whey protein-induced allergic responses (Zhao et al., 2021). Contrarily, Maslin et al. (2018) observed that adolescents with food allergy had higher Se intakes. In recent years, in order to improve the quality of life of allergic patients, additional strategies are being proposed, such as dietary supplementation with organic Se (selenomethionine: SeMet; and selenocysteine: SeCys) (Zhao et al., 2023).

Considering all the information reported above, and using an in vitro digestion/fermentation method, in this study we have determined the total amount of Se and its distribution between bioaccessible fractions in the small and large intestine (selenium bioaccessibility in the small intestine, Se-BASI; and selenium bioaccessibility in the large intestine, Se-BALI, respectively) and non-bioaccessible fractions, in different foods cooked with distinct home cooking techniques (raw, frying, roasting, toasting, boiling and grilling). For the fermentation process stool samples from healthy adults, healthy children and unhealthy children (with gluten related disorders, obesity or allergy/intolerance to cow’s milk proteins) have been used. So, the general objective of this study was to know the foods with the highest Se-BASI and Se-BALI, and more specifically if home culinary techniques used in their cooking, the stage of life or the presence of a series of pathologies in childhood influence the Se-BA. The ultimate goal would be to ensure the adequate supply of bioaccessible Se at different life stages/pathological situations in order to guarantee optimal growth and health status.

2. Materials and methods

2.1. Food samples and culinary techniques

A total of 159 samples of the most commonly consumed foods were studied (García-Conde et al., 2023). The studied foods belonged to the following groups: a) plant foods like nuts, cereals, fruits, vegetables, legumes, oils, beverages and other plant foods; b) animal foods as dairy, meat, fish, gouda cheese and egg. Foods were obtained, stored and submitted to different culinary treatments: frying, roasting, toasting, boiling or grilling. Frying was prepared at a rate of 5:1 (oil:food) at 180 ºC for 8 min. Roasting was prepared at 180 ºC for 10 min. Toasting was performed in a Grunkel TS140H toaster at the fourth level for 3 min at 900 W following the manufacturer’s instructions. Boiling was prepared at a rate of 5:1 (water: food) at 100 ºC for 20 min. Grilling was prepared at a rate of 0.5:1 (oil: food) at 220–250 ºC for 3 min. Frying and grilling used extra virgin olive oil as cooking medium. Cooking times and food/medium rates were acquired from Navajas-Porras et al. (2020). Some plant and animal foods were also analyzed in their raw form since they are usually consumed in that way. Vegetables were cut in different sizes to achieve the same texture for the same cooking time (Pérez-Burillo et al., 2019).

2.2. Study participants

Healthy adults (HE-AD, n = 10) were compared with healthy children (HE-CH, n = 10). In addition, three groups of unhealthy children, i.e. with gluten related disorders (GRD-CH, n = 10), obesity (OB-CH, n = 10) and allergy/intolerance to cow’s milk proteins (AICM-CH, n = 10) were also compared with HE-CH. The eligibility criteria of the European project Stance4Health were used for both adults and children (Dello Russo et al., 2022). Apparently healthy adults aged 20–65 years, with body mass index (BMI) between 20 and 28 kg/m², stable weight with the exclusion criteria previously reported (Dello Russo et al., 2022) were included in the study. On the other hand, all the children were 8–10 years old. The BMI of children was comprised between the 5th and 85th percentile for their gender, height and age. For the obese children, their BMI was above the 95th percentile for sex, weight and age. A common exclusion criterion for both adults and children was taking antibiotics or probiotics in the previous 3 months to the beginning of the study. Children and adults diagnosed of chronic gastrointestinal disorders or being on a special diet, were removed from the study.

Stool sample containers were provided to adults and children.
Enough fecal material was collected to perform the in vitro fermentation procedure. The informed consent document was signed by adults or legal representatives of children. That form included all of the information of the study as well as the exclusion and inclusion criteria. The study was conducted according to the guidelines of the Declaration of Helsinki. It was approved by the Ethics Committee of the University of Granada (protocol code 1080/CEIH/2020). Also, in Greece by the Scientific Committee of the University Hospital of Ioannina (Protocol number 382, Date June 4, 2020, Decision number 10/3-6-2020), the Scientific Committee of the University Hospital “Attikon” (Decision Number: 546/1-10-2020), and the Scientific Committee of the University Hospital of Patras (Decision Number: 360/22-7-20).

2.3. In vitro digestion and fermentation method of foods

In order to mimic physiological processes in the human gut, all plant- and animal-based food samples were subjected to an in vitro digestion process described in Pérez-Burillo, Ruflán-Henares, & Pastoriza, 2018 (which is an adaptation of the INFOGEST in vitro digestion protocol) with three phases: oral, gastric and intestinal; For each food, 5 g were added to Falcon tubes together with simulated salivary fluid (1:1, w/v) composed of KCl, KH₂PO₄, NaHCO₃, MgCl₂, NH₄Cl, (all from Sigma-Aldrich) and 75 U/mL α-amylase (from human saliva, Sigma-Aldrich). The mix was kept at 37 °C for 2 min in oscillation. Right after, 10 mL of simulated gastric fluid was added, mimicking the gastric juices content in KCl, KH₂PO₄, NaHCO₃, NaCl, MgCl₂, NH₄Cl, (all from Sigma-Aldrich) and pepsin (2000 U/mL, Sigma-Aldrich). The mix was kept at 37 °C for 2 h, at pH 3 in oscillation at 20 rpm (IKA Rocker 2D digital). Finally, 20 mL of simulated intestinal fluid was added, with equal content in salts (KCl, KH₂PO₄, NaHCO₃, NaCl, MgCl₂, and CaCl₂) bile salts (Sigma-Aldrich), and enzymes (67.2 mg/mL pancreatic, Sigma-Aldrich) than the intestinal juices. The mix was kept at 37 °C for 2 h, at pH 7, in oscillation. Once the intestinal phase was finished, tubes were kept in ice to stop enzymatic reactions and thereafter centrifuged at 8500 g for 10 min (Labnet Spectrafuge 24D). The supernatant, which represents the fraction available for absorption in the small intestine, was stored in 1 mL tubes at −80 °C until analysis. The solid pellet, which represents the not digested fraction that goes into the large intestine, and a 10% of the liquid fraction were used as in vitro fermentation substrate.

Next, an in vitro fermentation was carried out using fecal samples from donors (HE-AD, HE-CH, GRD-CH, OB-CH and AICM-CH). The solid residue previously obtained after in vitro oral-gastrointestinal digestion plus 10% of the digestion supernatant of foods was fermented (Pérez-Burillo et al., 2021). The fermentation was carried out at 37 °C for 20 h by the microbiota microorganisms present in the stool inocula of the different groups of subjects studied. Once the in vitro fermentation was finished, tubes were kept in ice to stop microbial reactions and thereafter centrifuged at 8500 g for 10 min (Labnet Spectrafuge 24D). The supernatant, which represents the fraction available for absorption in the large intestine, was stored in 1 mL tubes at −80 °C until analysis. The solid pellet, which represents the fraction not fermented and excreted with feces, was also stored in 1 mL tubes at −80 °C until analysis. After in vitro digestion-fermentation, three different fractions were obtained: a) digestion supernatant as fraction available for Se absorption at the small intestine, expressed as Se-BASI; b) fermentation supernatant as fraction available for Se absorption at the large intestine, expressed as Se-BALI; c) fermentation solid residue as Se fraction not available for absorption and excreted with feces, expressed as non-bioaccessible Se fraction. The sum of the Se levels corresponding to the 3 fractions analyzed allowed the determination of the average amount of Se present in the analyzed foods. The reported digestion-fermentation method was performed in triplicate for any food sample analyzed.

2.4. Food mineralization and analysis of Se

Se content in the digestion and fermentation supernatants, as well as in the fermentation residues of the foodstuffs studied, was determined by a previously described procedure (García-Conde et al., 2023). Teflon digestion vessels were used and placed in the rotor of the microwave digester for the sample mineralization (Multiwave 5000 with Rotor 24HVT50, Anton Parr GmbH, Graz, Austria). Next, in order to prepare the final analytical dissolution, the mineralized samples were diluted with reagent grade water (Milli-Q water prepared with the R015 Milli-Q system, Waters, Medford, MA, USA). Se concentrations were measured in that solution by inductively coupled plasma mass spectrometry technique (ICP-MS/MS; Agilent 8900, Agilent Technologies Inc., Santa Clara, CA, USA). With this aim, a calibration curve was prepared by serial dilutions from a standard Se solution of 1000 mg/L in HNO₃ at 1% (Merck; Darmstadt, Germany). For the Se measurement, the Internal Standard Kit (Ge, Ir, Rh Sc; ISC Science, batch 20,210,712) was used.

In each of the batches, four blanks were prepared with the reagents used in the mineralization process described. The measurements were carried out using the linear calibration method and in triplicate for each of the samples analyzed.

Analytical parameters of the procedure for the Se analysis in the food samples considered were carried out prior to the determination of element concentrations by ICP-MS. The limit of detection (LOD) was 0.135 µg/L. For standard reference material certified in Se such as 'Bovine muscle powder n° 8414' certified by the National Institute for Standards and Technology (NIST; Gaithersburg, MD, USA), we determined concentrations of 0.08±0.01 for certified levels of 0.08±0.01 µg/g. Additionally, for assaying the accuracy of the method recovery experiments were performed, obtaining calculated recoveries for Se that ranged between 98.2 and 101.1%.

2.5. Statistical analysis

In the statistical analysis of the data obtained, the SPSS statistical program (SPSS 25.0, Chicago, IL) was used. Data were expressed as mean Se values (±standard deviation, SD). The existence of statistically significant differences was set to a p value lower than 0.05 (p < 0.05).

For the statistical analysis in this in vitro study of the bioaccessible and non-bioaccessible Se fractions, the existence of homogeneity of variances by means of Levene’s test (p > 0.05) and the normal distribution of the results (p > 0.05) by means of the Kolmogorov-Smirnov test, was previously checked, in which case the parametric method of Student’s t-test was used in the analysis of variance. On the contrary, the non-parametric methods of Kruskall-Wallis as there were data from multiple independent samples, and the Mann-Whitney test for data comparison of two independent samples, were used.

3. Results and discussion

3.1. Comparison of partial (small vs. large intestine) and total Se-BA

In plant-based foods, the Se-BALI was significantly higher than in the small intestine (p < 0.01), contrary to what was found in foods of animal origin (Fig. 1). Furthermore, the Se-BASI in plant-based foods was significantly lower than that of animal-based foods, which in turn presented a Se-BALI significantly lower than that of plant-based foods (p < 0.01). Thus, the mean values of Se-BASI and Se-BALI differed depending on the animal or plant origin of the foods. The higher Se-BA values of plant-based foods in the large intestine are possibly related to the fermentative processes of the colonic microbiota present in the fecal inoculum, enabling the formation from undigested food components (mainly soluble fiber) of ligands like different short chain fatty acids (butyric, propionic and acetic acids; Pérez-Burillo, Pastoriza, et al., 2018) with which Se could possibly form soluble chelates, which should be addressed in future studies. Contrarily, other researchers observed
the highest Se-BA values in the small intestine, in Se-enriched foods and leaf and hemp cultures (Srikanth et al., 2016). These researchers further indicate that SeMet uptake by colonic microbiota is much more efficient than that of SeO₄²⁻ (Srikanth et al., 2016). It has been reported that the components present in foods associated with limiting the availability of micronutrients (such as Se and other minerals in the small intestine) are fiber, oxalic acid, phytates and polyphenolic compounds such as tannins (Viadel et al., 2006). However, there are other components derived from the fermentative processes of soluble fiber, such as short-chain fatty acids (SCFA), that could be involved in the formation of soluble chelates with certain elements such as Se, which could be associated with the higher Se-BALI observed in foods of plant origin. In fact, foods of plant origin have a higher amount of fermentable components (such as soluble fiber); the possible association of increased Se-BALI with soluble fiber content of specific food groups, as well as the colonic microbiota responsible for its fermentation, should be the subject of future studies. Other food components such as Maillard reaction products generated during heat treatment of foods (Ruñán-Henares, García-Villanova, & Guerra-Hernández, 2008), and even certain peptides released by enzymatic action during oral-gastrointestinal digestion, could also be related to the formation of insoluble complexes with Se, hindering its release in the small intestine, which would similarly need to be addressed in more specific and targeted studies in the future. In this sense, in relation to the influence of protein content in foods of vegetable origin grouped into high (legumes, nuts and cereals) and low (vegetables and fruits) content, we have found that there are no differences in the total Se-BA, nor in that corresponding to the small intestine (35.1 (±20.0)% vs. 37.2 (±27.0)%); however, there were no significant differences with respect to Se-BASI, with values of 35.8 (±22.9)% respectively (p > 0.05; Table S3).

In the case of foods of animal origin, the lower Se-BALI could be related to its uptake by the microbiota itself. The food component to which Se is mainly bound is protein, as it replaces sulphur in sulphur-containing amino acids due to a similar ionic radius (Navarro-Alarcon & Gil-Hernández, 2017). This is related to the higher Se-BASI in the small intestine in foods of animal origin, since soluble chelates would be formed with certain amino acids and peptides released by the enzymatic action, and consequently with the lower Se-BALI. In addition, as indicated above, the absence of soluble fiber related to fermentative processes in foods of animal origin must be considered, which would result in lower Se-BALI. In this regard, it has been indicated that the presence of inactive microbiota in the colon produced an increase in Se foods of animal origin in hemp and leaf (Srikanth Lavu et al., 2016). The highest levels of total Se foods corresponded to animal-based foods (82.5 (±97.5) μg/kg with salmon and dairy products standing out.

Fig. 1. Mean Se bioaccessibility values (%) in the small and large intestine of all plant- and animal-based foods.

In plant-based foods the mean Se contents were 44.3 (±55.6) μg/kg with those determined in other foods of plant origin (dark chocolate and hazelnut spread), oils (sunflower and olive oil) and legumes (lentils and kidney beans) standing out (Table S1). This finding is contrary to what is classically stated in relation to Se from animal sources, being less available than that from plants; however, it must be taken into account that classifying Se foods of animal origin in complex food matrices is very difficult, as it is influenced by factors such as the distribution of Se species, other components and even their origin (Thiry et al., 2012). In the case of cereals, the lowest total Se-BA values corresponded to bread, and the highest to whole grain cookies (p < 0.05; Table S2). The results are related to the different Se species present in the foods, with higher abundance of organic Se (SeCys and to a lesser extent SeMet) in animal-based foods. In plant-based foods, in addition to organic Se forms (SeMet, methyl-SeCys or γ-Glu-MeSeCys; Hossain et al., 2021), there are possibly higher levels of the inorganic ones (SeO₄²⁻ and SeO₂⁻⁻) which are less bioavailable (Rüder et al., 2010), since although SeO₄²⁻ is mostly absorbed close to 100%, it is nevertheless rapidly lost in urine (Gupta et al., 2021). In this regard, other researchers reported in foods that the biological transformation of SeO₄²⁻ into organic Se species ranges from 30 to 60% (appearing as MeSe-Cys), compared to 90% for SeO₂⁻⁻ (Ari et al., 2022). Others (Martínez et al., 2018) indicated that in chicken meat, the organic forms of Se are more bioavailable. On the other hand, it has been reported that organic Se species are considered nutritionally beneficial by their highly bioavailability usually higher than 90% (Ekumah et al., 2021). In line with this report, we have found mean total Se-BA values in animal-based foods also higher than 90% (93.6 (±8.58)). Future studies should address the partitioning of total Se into the different organic (SeCys and SeMet) and inorganic (SeO₂⁻⁻, SeO⁻, SeO₂⁻ and SeO₄²⁻) fractions as well as others using HPLC-ICP-MS technique.

Fig. 2A shows the Se-BASI and Se-BALI in different groups of plant-based foods (Table S1). There were statistically significant differences, with higher values in the bioaccessible fraction in the small intestine in cereals and by-products, fruits, oils and beverages (p < 0.05). However, in the nuts, vegetables and legumes groups, the bioaccessible fraction of Se in the large intestine was significantly higher (p < 0.05). As for foods of animal origin (Fig. 2B; Table S1), it was observed that Se-BA in dairy products, chicken, beef, salmon, cod and egg was significantly higher (p < 0.05). On the contrary, for lamb and pork the higher bioaccessibility was found in the large intestine (p < 0.05).

In relation to the specific cereal-based foods, it should be pointed out that in cereals and by-products (Fig. 3A) the Se-BASI was significantly higher in penne, whole grain penne, rice and whole grain rice (p < 0.05). On the contrary, in bread and whole grain breakfast cereals, the Se-BALI was statistically higher (p < 0.05; Table S2). Furthermore, it was observed that in whole-cereals (with a mean Se content of 36.3 (±15.1) μg/kg), the Se-BALI was significantly higher (37.7 (±18.9)%) than that corresponding to non-whole cereals (27.2 (±18.6)%; with mean Se levels of 32.8 (±8.72) μg/kg); however, there were no significant differences with respect to Se-BASI, with values of 35.3 (±16.7) and 35.8 (±22.9)%, respectively (p > 0.05; Table S3).

The Se-BASI, Se-BALI and the total Se, varies depending on the animal or vegetable origin, the category and the food considered and the different Se species present in it (Gupta et al., 2021). The different gastrointestinal transformations of several food components (carbohydrates, lipids and proteins) and different species of Se present in them by the enzymatic activity of the oral-gastrointestinal secretions and by the drastic pH changes, as well as interactions with diverse functional groups from other foods also influence the formation of chelates (Cabrero, Madrid & Cámara, 2004). On the other hand, Se extraction from food is only partial and the process of Se-BA estimation itself can alter the forms of Se available in the food. Therefore, optimization and standardization of bioaccessibility assessment methods is necessary (Gupta et al., 2021).

Table 1 shows that cereals with gluten had significantly higher mean values of Se content, total Se-BA and Se-BALI (p < 0.01). However,
The presence of equal lowercase letters on the bars shows the existence of statistically significant differences (p < 0.05).

**Table 1**

Mean Se contents (μg/kg; ± standard deviation, SD) and bioaccessibility values (%) ± SD) in the large and small intestine in cereals with gluten vs. free-gluten cereals.

<table>
<thead>
<tr>
<th>Cereal group</th>
<th>Total Se†</th>
<th>Se bioaccessibility in the small intestine†</th>
<th>Se bioaccessibility in the large intestine†</th>
<th>Total Se bioaccessibility†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gluten cereals*</td>
<td>36.2(±12.3)†</td>
<td>33.7(±20.7)†</td>
<td>35.1(±19.8)†</td>
<td>68.8(±24.3)†</td>
</tr>
<tr>
<td>Gluten-free cereals*</td>
<td>24.8(±7.48)†</td>
<td>47.1(±7.95)†</td>
<td>16.7(±12.3)†</td>
<td>63.8(±4.57)†</td>
</tr>
</tbody>
</table>

*Rows labelled with the same superscript lowercase letters for Se bioaccessibility values in the small and the large intestine for every cereal group denotes the existence of statistically significant differences (p < 0.05).

*Columns labelled with the same superscript capital letters for total Se and Se bioaccessibility values in different cereal groups denotes the existence of statistically significant differences (p < 0.05).

Gluten-free cereals (rice and whole grain rice) had significantly higher Se-BASI compared to those with gluten (47.1(±7.95) vs. 33.7(±20.7)†, respectively; p < 0.001). These results establish that cereals with gluten have higher Se levels as indicated by others (Rybicka et al., 2015) and more bioaccessible, being absorbed in both the small and large intestine. However, in gluten-free cereals Se absorption is lower, and occurs mainly in the small intestine. Therefore, gluten-free diets nutritionally provides deficient amounts of multiple micronutrients (Vici et al.,...
to which Se, which also presents lower bioaccessibility, should be added. Gluten is constituted by a series of amino acids arranged in a specific sequence (called epitopes) which are located in the prolamin fraction of different cereals (wheat, rye, ryegrass, oats and triticale). The elimination of gluten additionally produces the loss of multiple macro and micro nutrients, among which fiber has been indicated. In this sense, Vici et al. (2016) reported that a gluten-free diet is poor in alimentary fiber, in particular to the necessary avoidance of several kinds of foods naturally rich in fiber (like grains) and the low content of fiber of gluten-free products that are usually made with starches and/or refined flours. Therefore, Se-BASI in gluten-free cereals would be higher by the lower fiber content, which would be associated to the formation of insoluble complexes with Se.

On the other hand, we have found that Se-BASI is higher in gluten-free cereals, increasing approximately 50% with respect to that corresponding to cereals with gluten (Table 1). However, at the same time, the Se content is also reduced by about 50% in gluten-free cereals, so that the overall Se-BA in the small intestine (considering these two aspects) would be similar for gluten-free and gluten cereals. However, the Se-BA of the large intestine increases globally due to the fermentative processes associated with the presence of gluten and other components that have not been eliminated in cereals with gluten (like the reported fiber).

Additionally, it has been described that enzymatic action along in vitro studies of gastrointestinal digestion releases peptides from the proteins present in some foods (Moreno-Montoro et al., 2018), which can exert different biological functions. Some of the peptides released from gluten by digestive enzymes would be related to the formation of insoluble complexes with Se, negatively influencing Se-BA in the small intestine, which should have to be verified in future studies.

Fig. 4B, C and 3D show, for vegetables, fruits and dairy products, respectively, the Se-BASI and Se-BALI of the specific foods analyzed, and those for which there were statistically significant differences (p < 0.05; Table S6). Thus, the corresponding Se concentrations and specific Se-BA values are shown in Tables S4, S5 and S6, respectively). This finding shows the influence of the specific food matrix and its components on Se partitioning among its multiple species, and thus on Se-BA, as indicated by others (Bawiec et al., 2023; Thiry et al., 2012).

### 3.2. Influence of life stage (healthy adults vs. healthy children) on Se-BALI

Fig. 4A shows the distribution of Se in bioavailable and non-bioavailable fractions for all foods in the different groups of subjects. Fig. 4B shows that the Se-BALI in adults was significantly lower than that determined for healthy children (p < 0.05). Thus, in childhood, despite a lower dietary intake, the utilization of Se at the large intestine level is higher, probably because of the demands associated with growth (Zymabgo et al., 2022).

Table 2 shows the mean Se concentrations in all foods classified as plant- and animal-based foods, and the Se-BALI values of the subjects studied. As can be observed, the mean values of Se-BALI of plant-based foods in adults were significantly lower than those found in children (p < 0.001). The higher levels in children, and specifically in vegetables and legumes (Table 2), was possibly related to the metabolites generated by the fermentative action of the microorganisms that make up the specific intestinal microbiota in children with respect to that of adults (Pérez-Burillo et al., 2021); such microorganisms would enable the formation of soluble chelates with Se. In line with this, it has been previously stated (Diaz-Bone & Van de Wiele, 2010) that the gut microorganisms could moderate Se-BA by binding Se with some microbial metabolites released after fermentation. Further studies are needed to identify the keystone species in the gut microbiota and the metabolites obtained, by fermentation of specific food components that increase the bioaccessibility of the different Se species. The ultimate goal would be to achieve personalized nutrition associated with a healthier gut microbiota in people (Dello Russo et al., 2022) and to a greater extent, in groups vulnerable to Se deficiency situations such as children, pregnant women and the elderly, as it has been indicated that approximately one billion people in the world have selenium deficiency (Nothstein et al., 2016).

Table S7 shows that in plant foods with low protein content, Se-BALI was significantly lower in adults compared to those found in children (p < 0.05). Additionally, Table S8 (for cereals and by-products), S9 (for vegetables), S10 (for fruits) and S11 (for dairy products) include the mean Se concentrations and Se-BALI values, specifically in the individual foods included in each food category. Among all these foods, only for eggplant, onion and spinach the Se-BALI values were significantly lower in adults vs. children (p < 0.05).

Finally, Table S12 shows that in gluten-free cereals, Se-BA values in the large intestine were significantly lower in healthy children and adults compared to those with gluten (p < 0.05).

### 3.3. Influence of pathology on Se-BALI of children

The distribution of Se in bioavailable and non-bioavailable fractions in the large intestine in the groups of children is depicted in Fig. 4A. Se-BALI of healthy children was significantly higher than that determined for celiacs (p < 0.05; Fig. 4B). Therefore, the malabsorption associated with celiac disease would originate a Se deficit, as expressed by others (Stazi & Trianti, 2010). In the same line, it has been indicated that in thyroid disorders there is an intestinal dysbiosis with alteration in Se absorption, and exacerbation of inflammatory processes (Knezevic et al., 2020).

In cereals with gluten, Se-BA in all groups of healthy and unhealthy

**Fig. 4.** (4A) Non-bioaccessible and bioaccessible Se fractions in food in the small and large intestine of healthy adults, and healthy and unhealthy children. (4B) Mean Se bioaccessibility values (%) of all food in the large intestine of different groups of subjects.
Food Bioscience 59 (2024) 103964

Table 2

Mean Se contents (µg/kg; ± standard deviation, SD) and bioaccessibility (Se-BA) values in the large intestine (%; ±SD) of plant- and animal-based foods in the large intestine of healthy adults, and healthy and unhealthy children.

<table>
<thead>
<tr>
<th>Food group</th>
<th>Se (ppm)</th>
<th>Se-BA in HE-AD</th>
<th>Se-BA in HE-CH</th>
<th>Se-BA in GRD-CH</th>
<th>Se-BA in OB-CH</th>
<th>Se-BA in AICM-CH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant-based foods</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuts</td>
<td>41.4(±43.7)</td>
<td>49.7(±26.5)</td>
<td>64.9(±11.6)</td>
<td>53.2(±11.4)</td>
<td>50.5(±28.3)</td>
<td>60.5(±12.7)</td>
</tr>
<tr>
<td>Cereals</td>
<td>34.3(±12.4)</td>
<td>34.9(±24.3)</td>
<td>35.5(±18.3)</td>
<td>30.1(±18.1)</td>
<td>30.7(±19.5)</td>
<td>31.9(±16.3)</td>
</tr>
<tr>
<td>Fruits</td>
<td>60.0(±8.4)</td>
<td>39.5(±28.4)</td>
<td>43.7(±27.2)</td>
<td>41.2(±25.1)</td>
<td>37.4(±30.4)</td>
<td>44.3(±25.9)</td>
</tr>
<tr>
<td>Vegetables</td>
<td>38.5(±5.4)</td>
<td>41.9(±24.0)</td>
<td>53.1(±20.4)</td>
<td>47.2(±19.4)</td>
<td>44.2(±26.3)</td>
<td>53.1(±15.4)</td>
</tr>
<tr>
<td>Legumes</td>
<td>39.5(±19.4)</td>
<td>35.5(±26.8)</td>
<td>54.1(±18.9)</td>
<td>49.1(±18.7)</td>
<td>50.1(±21.9)</td>
<td>50.9(±16.1)</td>
</tr>
<tr>
<td>Olive oil</td>
<td>115.0(±105)</td>
<td>0.51(±0.81)</td>
<td>0.55(±0.90)</td>
<td>0.48(±0.56)</td>
<td>0.48(±0.85)</td>
<td>0.78(±1.14)</td>
</tr>
<tr>
<td>Beverages</td>
<td>12.3(±2.80)</td>
<td>1.40(±1.03)</td>
<td>1.56(±0.55)</td>
<td>1.28(±0.62)</td>
<td>1.23(±1.01)</td>
<td>1.11(±0.40)</td>
</tr>
<tr>
<td>Others</td>
<td>68.0(±11.8)</td>
<td>34.2(±34.1)</td>
<td>34.7(±30.9)</td>
<td>31.6(±30.2)</td>
<td>22.5(±30.2)</td>
<td>29.3(±27.5)</td>
</tr>
<tr>
<td>Animal-based foods</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy products</td>
<td>64.5(±36.6)</td>
<td>23.9(±24.1)</td>
<td>31.0(±27.8)</td>
<td>27.9(±25.8)</td>
<td>25.2(±24.0)</td>
<td>26.6(±26.2)</td>
</tr>
<tr>
<td>Chicken</td>
<td>67.5(±71.1)</td>
<td>41.2(±33.9)</td>
<td>42.9(±34.4)</td>
<td>47.0(±32.6)</td>
<td>41.7(±30.9)</td>
<td>45.2(±32.7)</td>
</tr>
<tr>
<td>Beef</td>
<td>91.0(±40.1)</td>
<td>10.5(±20.0)</td>
<td>20.6(±33.3)</td>
<td>18.0(±30.6)</td>
<td>19.3(±32.9)</td>
<td>19.9(±30.3)</td>
</tr>
<tr>
<td>Salmon</td>
<td>246(±176)</td>
<td>0.94(±1.16)</td>
<td>0.95(±0.83)</td>
<td>0.76(±0.73)</td>
<td>0.92(±1.00)</td>
<td>0.90(±0.96)</td>
</tr>
<tr>
<td>Cod</td>
<td>52.0(±10.2)</td>
<td>30.7(±13.6)</td>
<td>32.0(±14.8)</td>
<td>28.2(±19.7)</td>
<td>26.7(±15.2)</td>
<td>30.8(±10.7)</td>
</tr>
<tr>
<td>Egg</td>
<td>52.0(±25.7)</td>
<td>31.8(±23.4)</td>
<td>33.8(±20.3)</td>
<td>26.0(±17.9)</td>
<td>27.1(±21.9)</td>
<td>29.9(±18.4)</td>
</tr>
<tr>
<td>Lamb</td>
<td>31.2(±13.0)</td>
<td>55.4(±22.1)</td>
<td>49.8(±20.9)</td>
<td>53.8(±16.8)</td>
<td>48.4(±22.4)</td>
<td>52.7(±12.9)</td>
</tr>
<tr>
<td>Pork</td>
<td>31.8(±10.4)</td>
<td>48.0(±22.4)</td>
<td>50.2(±10.5)</td>
<td>52.2(±8.51)</td>
<td>50.2(±17.6)</td>
<td>50.0(±11.0)</td>
</tr>
</tbody>
</table>

**a** Healthy adults (HE-AD); healthy children (HE-CH); children with gluten related disorders (GRD-CH); children with obesity (OB-CH); children with allergy/ intolerance to cow’s milk proteins (AICM-CH).

**b** Rows labelled with the same superscript lowercase letters for Se bioaccessibility values in the large intestine in every food category for different groups of children (healthy and unhealthy children) denotes the existence of statistically significant differences (p < 0.05).

**c** Rows labelled with the same superscript capital letters for Se bioaccessibility values in every food category for healthy adults vs. healthy children denotes the existence of statistically significant differences (p < 0.05).

Children studied is significantly higher than in gluten-free cereals (p < 0.05; Table S12). In the mandatory follow-up of a gluten-free diet of celiac children, the lower Se-BA and lower Se content of gluten-free cereals could be associated with a Se-deficient nutritional status (Rybicka et al., 2015). This situation could compromise their future development and health. These results coincide with what was observed in women and in young celiacs following a gluten-free diet, in which there was a lower Se intake with respect to that of the control group (Nestares et al., 2020; Wild et al., 2010). Conversely, in newly diagnosed celiac children, Se blood levels were similar to those following a gluten-free diet for 6 months and ≥12 months (McGrogan et al., 2021). This result would be associated with a similar Se intake and bioavailability, with or without gluten-free diet follow-up. This controversy should be studied in depth in future studies, also taking into account the possible influence of variations in the composition of the gut microbiota in patients with celiac disease, depending on the specific diet followed (Constante et al., 2022), and its effects on Se bioavailability.

When comparing the values of Se-BALI in foods between celiacs and healthy children, celiac children had significantly lower values in nuts and vegetables (p < 0.05; Table 2), in foods of vegetable origin of both higher and lower protein content (p < 0.05, Table S7), and in vegetables, specifically in capsicum, eggplant and tomatoes (p < 0.05; Table S9).

In the case of obese children (Fig. 4B) Se-BALI was also significantly lower (p < 0.05) than that of healthy children. In this sense, some authors have found low serum and plasma Se levels in obese humans (Arnaud et al., 2006; Soares de Oliveira et al., 2021) and therefore with decreased Se bioavailability, a finding that others do not corroborate (Fontenelle et al., 2022; Watanabe et al., 2021). Recent studies have linked excess adiposity in obese adults to a decrease in erythrocyte glutathione peroxidase activity, as a measure of Se bioavailability (Fontenelle et al., 2022). Others indicate that there are no conclusive results on Se supplementation to prevent or alleviate obesity in humans (Tinkov et al., 2021). Further studies are needed to clarify the possible associations of Se species metabolism and bioavailability/bioaccessibility, and its regulation by the specific microbiota in obesity.

In foods, plant-based foods fermented with feces of OB-CHE showed significantly lower values (p < 0.05; Table S13) compared to HE-CH, specifically in vegetables (p < 0.05, Table 2) and those with lower protein content (p < 0.05, Table S7).

Comparing Se-BALI of allergic vs. healthy children, no significant differences were observed (Fig. 4B), although significantly lower values (p < 0.05; Table S9) were found for capsicum fermented with feces of allergic children. Other researchers (Kalita et al., 2001) do report that allergic children have lower plasma Se levels. The possible mechanism of action could be the modulation by Se of allergic responses through its influence on the composition and colonization of the microbiome (Ferreira et al., 2021). Moreover, in recent years, additional strategies for allergy control, such as dietary supplementation with organic Se (SeMet and SeCys), have been proposed in allergic patients (Zhao et al., 2023).

If Se-BA values were compared among the three unhealthy children (allergic, celiacs and obese), higher levels are found in allergic children (Fig. 4B) in foods of plant origin (p < 0.05; Table S13), specifically in the vegetable group (p < 0.05; Table 2) as well as in foods of plant origin with lower protein content (p < 0.05, Table S7). Additionally, significantly higher Se-BA values (p < 0.05) were also observed in nuts and other vegetal foods (Table 2), tomato and cabbage (Table S9), and apple (Table S10) fermented with feces from allergic children, with respect to obese. These results show a different behavior in relation to Se-BALI among the different groups of unhealthy children.

In celiacs and by-products there were no significant differences between the Se bioaccessibility between healthy and unhealthy (Table S8), except for whole cereals vs. non-whole cereals, with a significantly higher bioaccessibility in HE-CH (Table S14).

Different authors report the modulation of Se on the colonic microbiota (Sumner et al., 2019), by increasing beneficial lactic acid bacteria in experimental animals (Ren et al., 2016). Therefore, the decrease in Se-BALI observed in obese and celiac children (compared to HE-CH) could be associated with disbiosis. Future in vivo and in vitro studies should be planned on how the amount and species of Se present in foods can modulate the colonic microbiota towards bacterial genera with positive effects on human health.

3.4. Influence of home cooking technologies on Se-BA (Se-BASI vs. Se-BALI)

Regarding the effect of cooking technologies in Se-BALI, Fig. 5A shows that in plant-based foods, Se-BALI was significantly higher (p <
0.05) than in the small intestine for roasting, toasting, boiling and grilling techniques. On the contrary, in animal-based foods (Fig. 5B), frying, roasting and grilling released significantly higher Se levels in the small intestine ($p < 0.05$). If culinary techniques were grouped according to whether they are carried out in hot liquids (frying in oil and boiling in water) or hot air (roasting and grilling) vs. raw foods, it was observed a significantly higher ($p < 0.05$) Se-BASI after digestion of plant-based foods. The same result has been observed for animal foods (Fig. 5C).

Table S15 shows the mean Se concentrations, Se-BASI and Se-BALI of vegetable or animal foods grouped depending on the different home cooking techniques considered. Se-BASI of raw vegetable foods was significantly higher to the other cooking techniques ($p < 0.05$). The same was also observed for foods of animal origin, with the exception of grilling, for which the Se-BASI was significantly higher ($p < 0.05$). With respect to the Se-BALI the opposite occurs, with significantly lower values in the raw vegetables compared to roasting, boiling and grilling ($p < 0.05$); again, the same behavior was observed for animal foods, with the exception of grilling ($p < 0.05$).

The previous results show that culinary techniques of food preparation influence the Se-BA, although in a different way depending on whether selenium is released in the small or large intestine. In the small intestine, Se-BA decreases with cooking, probably due to the formation of ligands that form Se complexes, thus decreasing decrease solubility, and therefore bioaccessibility. However, the Se-BALI increases with culinary preparation, due to the additional fermentative processes of the undigested components exerted by the colonic microbiota, which enables the appearance of metabolites, including SCFA (Pérez-Burillo et al., 2021), which would act as ligands that generate more soluble chelates, a fact that should be addressed in future research. In this sense, it has been described (Palomo et al., 2014) that heat treatment of yogurt facilitates the expression of chaperones (such as heat shock proteins) and that the presence of Se negatively modulates their expression by bacteria of the genus *Lactobacillus*.

Additionally, we have also observed that the total Se-BA in foods cooked by boiling and frying is lower than that of those cooked by roasting and grilling, both in foods of plant- and animal-based foods. Therefore, heat treatments of higher intensity would facilitate greater protein denaturation and possibly increased enzymatic activity, as well as a more intense formation of Maillard reaction products, which both could be associated with the higher total Se-BA observed for roasting and grilling techniques.

All the above stated results highlight the need to increase the knowledge of food processing techniques, with a view to improving the effectiveness of bioaccessibility of bioactive compounds, such as Se when incorporated into the design of functional foods, as others previously reported (Cilla et al., 2018).

3.5. Influence of home cooking technologies on Se-BA (adults vs. children)

When the effect of cooking was studied grouping data depending on the life stage (adults vs. children), Se-BALI of fried, roasted and boiled plant foods fermented with feces of adults was significantly lower ($p < 0.05$) than that of healthy children (Table 3). In foods of animal origin, however, no statistically significant differences were observed for any of the culinary techniques studied ($p > 0.05$). If culinary techniques were grouped together, Se-BALI of healthy children was significantly higher ($p < 0.05$) than that of adults for foods of vegetable origin cooked with hot liquid and hot air (Table S16).

When comparisons were made among the different groups of children, Se-BALI of healthy children was higher ($p < 0.05$) than that of
celiac children for roasted, toasted and boiled plant-based foods (Table 3). If culinary techniques were grouped together, Se-BALI of healthy children was significantly higher (p < 0.05) than that of celiac children for foods of plant origin cooked with hot liquid and hot air (Table S16). Additionally, Se-BA in healthy children was higher than that of obese children for foods cooked with hot liquids (p < 0.05). No differences were observed for foods of animal origin (Table S16). A remarkable fact is that there were no significant differences in Se-BALI of the different groups of children considered for raw foods.

### 4. Conclusions

The Se-BALI in foods varies depending on their vegetal or animal origin, category and specific food, which relates to the different organic and inorganic Se species present. Animal-based foods have a higher total Se-BA, being larger in the small intestine. Se-BA in plant-based foods is lower, mostly established in the large intestine; this could be related to the fermentative processes of the gut microbiota, allowing the formation of microbial metabolites that would act as soluble ligands of Se, of greater bioaccessibility.

The higher Se-BALI in healthy children compared to adults could be related to the higher Se demands for growth of childhood. In obese and celiac children, Se-BALI is lower than that of healthy and allergic children; this fact may compromise their future development and health status, and is probably related to a specific gut microbiota signature, to be studied in the future. For only celiac children, the consumption of free-gluten cereals is recommended because of the associated loss of content and bioavailability of Se.

The cooking techniques of vegetable and animal foods grouped by heating in liquid media (frying and boiling) or with hot air (roasting and grilling) decrease Se-BASII, and enhance in the large intestine in comparison to raw foods. This result expresses the differential influence of the culinary techniques in the formation of insoluble Se chelates in the small intestine, and how the additional influence of the fermentative processes of the colonic microbiota generates more soluble and bioavailable chelates in the large intestine.

### Declaration of competing interest

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

### Data availability

Data will be made available on request.

### Acknowledgements

This work was supported by the European Research Commission (Research Executive Agency, European Union) under the research project Stance4Health under Grant Contract N° 816303 and by the Plan propio de Investigación y Transferencia de the University of Granada (Spain) under the program “Intensificación de la Investigación, modalidad B”. This work is part of the thesis of Úrsula García-Conde to obtain the PhD in the Nutrition and Food Science program at the University of Granada.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2024.103964.

### References


