Food Research International 169 (2023) 112817

Contents lists available at ScienceDirect

Food Research International

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

Total Zn of foods and bioaccesible fractions in the small and large intestine after *in vitro* digestion and fermentation with fecal material of healthy adults and children: Influence of culinary techniques

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ARTICLE INFO

Keywords:
total Zn
released Zn amounts from foods
Zn bioaccesibility
Healthy adults
Healthy children
Culinary techniques

ABSTRACT

The healthy status of human beings is associated with an appropriate nutritional status in Zn, which must firstly be bioavailable. We measured the total Zn amount and its bioaccesibility in raw foods and after cooking by common culinary techniques. These foods were submitted to an *in vitro* digestion and fermentation with faecal inocula from healthy adults and children to evaluate Zn bioaccesibility in the small and large intestine. Mean total Zn amount provided by foods was 8.080 μg/g. Zn amount released from food in the small intestine was significantly different among several food groups and lower in raw vegetal foods compared to cooked ones (frying, roasting and grilling; $p < 0.05$); the same behaviour was found in the large intestine for healthy children. Zn bioaccesibility in the large intestine varied statistically according to the subjects' idiosyncrasies, and was higher in healthy children ($p < 0.05$) probably due to growth demands and different composition of the colonic microbiota. In healthy adults and children, the bioaccesible fractions were 33.0 ± 20.4 % for the small intestine, 16.4 ± 22.0 and 59.6 ± 29.9% for the large one, and the non-bioaccessible ones 50.6 ± 19.9 and 7.4 ± 9.1%, respectively.

1. Introduction

Zinc is an essential element related to proper growth, development and cell replication in the human organism (Sing, Prasad, & Aalbersberg, 2016; Banaszak, Górna, & Przysławski, 2021; Obaid et al., 2022), as well as to the immune system, cognitive ability, sexual maturation and tissue repair (Arreondo Olguín, Ruz Ortiz, Olivares Grohnet, & Castillo Durán, 2017; Kumar et al., 2022). In this sense, Zn is present in many tissues of the human body (Tamura, 2021) as part of at least 2,800 proteins, so most cellular processes are dependent on this element (Maret, 2011).

In developing countries, deficiencies and malnutrition problems have been reported in pregnant or lactating women and children, whose diet (mainly dependent on cereal-based plant foods) is related with low Zn utilisation due to the high levels of phytates, polyphenols, fiber, etc. (Al Hasan et al., 2016; Sing, Prasad, & Aalbersberg, 2016). Although it is important to know the total amount of Zn in foods, more relevant is to know its bioavailability (Navarro, & Wood, 2003; Velasco-Reynold, Navarro-Alarcon, López-G de la Serrana, Perez-Valero, & Lopez-Martinez, 2008). The bioavailability of Zn (Zn-BA) can be assessed directly by *in vivo* methods in experimental animals or more precisely in humans. As a preliminary step, *in vitro* methods for the assessment of Zn-BA are available (Rebellato, Siqueira Silva, Probio de Moraes, Trajano, & Lima Pallone, 2022), using oral-gastrointestinal digestion simulation systems (small intestine bioaccessibility), and colonic fermentation after contact of digestion solids with faecal inocula from volunteer human donors (large intestine bioaccessibility; Navajas-Porras et al., 2020; Pérez-Burillo et al., 2021a,b).

Gut microbiota rapidly adapt their composition to the macronutrient composition of the diet (Pérez-Burillo et al., 2018a) as well as to Zn metabolism (Skalny et al., 2022). For example, high-fat diets have been shown in humans and animals to alter the gut microbiota by decreasing

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https://doi.org/10.1016/j.foodres.2023.112817
Received 12 December 2022; Received in revised form 25 February 2023; Accepted 11 April 2023
Available online 17 April 2023

the production of short-chain fatty acids (SCFA; Agans et al., 2018). Such microbiota modulation by diet and food components would alter fermentative processes, generating different metabolites, which could modify the molecules that act as ligands, affecting the Zn-BA in the large intestine (Pérez-Burillo et al., 2018a, 2021a). This has also been reported for long-term Zn fortification doses (Chen et al., 2021) and for Zn supplementation in obese male Wistar rats (Squizani et al., 2022).

On the other hand, the Zn-BA in foods can be influenced by different factors such as their origin, food group, composition (Sing, Prasad, & Aalbersberg, 2016; Ramírez-Ojeda, Moreno-Rojas, & Cámara-Martos, 2018) and culinary techniques applied (Alegría-Torán, Barbera-Sáez, & Cilla-Tatay, 2015; Yadav, Kaur, Malaviya, Saini, & Anjum, 2019; Ganguly, Sabikhi, & Singh, 2022; Kumar et al, 2022; Skalickova et al., 2022). Different home cooking techniques applied to foods of animal (Navajas-Porras et al., 2021) and plant origin (Pérez-Burillo et al., 2019; Navajas-Porras et al., 2021) have been shown to modify their composition and antioxidant capacity, and that of the human gut microbiota.

Taking together all this information, *in vitro* digestion and fermentation methods could possibly determine different Zn-BA values for different food groups submitted to home cooking technologies, as well as for different human beings (i.e. healthy normal weight adults and children) that have different gut microbiota, which are likely to interact differently with food components related to Zn-BA in the large intestine (Pérez-Burillo et al., 2021a). It is therefore mandatory, as it has not been studied before, to evaluate the bioaccessible fractions of Zn in animal- and plant-based foods submitted to different culinary techniques (raw form, frying, roasting, toasting, boiling and grilling), in the small and large intestine (in healthy adults and children with normal weight), as well as the non-bioaccessible ones. The ultimate aim of this research is to achieve greater health benefits, and to prevent the development of diseases and Zn malnutrition states, by finding out which foods have more bioaccessible Zn fractions, and the influence of home cooking techniques on them.

2. Materials and methods

2.1. Food samples and culinary techniques

A total of 159 samples of the most commonly consumed foods (Supplementary Table) were studied belonging 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 °C for 8 min. Roasting was prepared at 180 °C 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 °C for 20 min. Grilling was prepared at a rate of 0.5:1 (oil: food) at 220– 250 °C for 3 min. Frying and grilling used extra virgin olive oil (EVOO) as cooking medium. Cooking times and food/medium rates were acquired from Navajas-Porras et al., 2020, 2021. Some plant and animal foods were also analysed 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, Rufián-Henares, & Pastoriza, 2019).

2.2. Stool samples from healthy adults and children

For two groups of healthy subjects included in the study the eligibility criteria of the European project Stance4Health were performed (Dello Russo et al., 2022): a) Adult subjects ($n = 3$): apparently healthy adults aged 20–65 years, with body mass index (BMI) between 20–28 kg/m^2, stable weight with the exclusion criteria previously reported (Dello Russo et al., 2022); b) Normal weight children ($n = 3$) aged 6–11 years, with BMI > 5th and < 85th percentile for age, gender and height with the exclusion criteria stated elsewhere (Dello Russo et al., 2022).

Stool sample containers were provided to healthy adults and children and collected with the necessary faeces to perform the *in vitro* fermentation procedure (Dello Russo et al., 2022).

2.3. In vitro digestion and fermentation method

In order to mimic physiological processes in the human gut, all foods were submitted to an *in vitro* digestion followed by an *in vitro* fermentation process previously developed (Pérez-Burillo et al., 2018b). Three phases were used for the gastrointestinal *in vitro* digestion procedure, namely oral phase (5 min at 37 °C with alpha-amylase 75 U/mL, pH 7.0), gastric phase (2 h at 37 °C with pepsin 2,000 U/mL at pH 3.0) and intestinal phase (2 h at 37 °C with pancreatin 13.37 mg/mL at pH 7.0).

The fermentation was carried out using fecal samples from six healthy donors The solid residue obtained after *in vitro* gastrointestinal digestion plus 10% of the digestion supernatant was fermented (500 mg) (Pérez-Burillo et al., 2021a). After *in vitro* digestion-fermentation three different fractions were obtained: digestion supernatant (fraction available for Zn absorption at the small intestine: released Zn amounts from the food matrix [R-ZnA] in the small intestine), fermentation supernatant (fraction available for Zn absorption at the large intestine: bioaccesibility in the large intestine for healthy adults and children) and fermentation solid residue (Zn fraction not available for absorption and excreted with feces: non-bioaccesible Zn fraction for healthy adults and children). The sum of the Zn levels corresponding to the 3 fractions analysed has facilitated the determination of the average amount of Zn present in the analysed foods.

2.4. Food mineralisation and analysis of Zn in the digestion and fermentation fractions

For the determination of Zn content in the digestion and fermentation supernatants of foodstuffs studied, 250 mg were weighed into 25 mL falcon tubes. Then, 3.5 mL of 33% HNO_3 was added and the samples were kept for 96 h for complete mineralisation. The mineralised samples were diluted to 25 mL with reagent grade water (Milli-Q water prepared with the R015 Milli-Q system, Waters, Medford, MA, USA) to obtain the analytical dissolution. Then, Zn was measured by inductively coupled plasma mass spectrometry (ICP-MS/MS; Agilent 8900 Triple Quadrupole ICP-MS/MS, Agilent Technologies Inc., Santa Clara, CA, USA), for which a calibration curve was prepared by serial dilutions from a standard solution of 1,000 mg/L of Zn in HNO_3 at 1 % (ppm; Merck; Darmstatdt, Germany). For the measurement, the Internal Standard Kit (Ge, Ir, Rh Sc; ISC Science, batch 20210712) was used.

For Zn determination in fermentation solids and in some fermentation supernatants from foods (which were not completely mineralised by the cold mineralisation technique described above) between 50 and 100 mg were weighed into borosilicate tubes, followed by the addition of 3 mL of 65% HNO_3. On the other hand, 0.5 mL of 65% HNO_3 together with 2.5 mL of Milli-Q water were added to the used microwave Teflon digestion vessels, into which the borosilicate tubes with the samples to be mineralised were placed. The Teflon digestion vessels were placed in the rotor of the microwave digester (Multiwave 5000 with Rotor 24HVT50, Anton Parr GmbH, Graz, Austria) used. A suitable time–temperature programme was optimised beforehand (Table 1).

Table 1
Optimised procedure for the mineralisation of the samples of the fermentation solids and supernatants obtained in the digestion-fermentation process of foods.

Step	Temperature (C)	Heating ramp (min)	Temperature maintenance (min)	Fan power
1	150	15	5	1
2	180	5	10	1
3	70	–	–	3

The mineralised samples obtained were diluted to 40 mLl with Milli-Q water in 50 mL falcon tubes, obtaining the analytical solutions in which the concentrations of Zn present were determined as previously reported. In each of the batches, four blanks were prepared with the reagents used along the mineralisation process described. The measurements were carried out using the linear calibration method, and in triplicate for each of the samples analysed.

Prior to the determination of Zn concentrations using the ICP-MS technique, the analytical parameters of the procedure were checked. The limit of detection (LOD), after measuring Zn in 10 blanks was 0.42 μg/L. To check the accuracy and precision of the method (n = 10), reference standards certified in Zn such as "Bovine muscle powder n° 8414″, and "Citrus leaves powder n° 1515″ were used, both certified by the National Institute for Standards and Technology (NIST; Gaithersburg, MD, USA); the concentrations found were 145 ± 2. 5 and 12.5 ± 0.68 μg/g for certified levels of 142.0 ± 14.0 and 12.7 ± 0.85 μg/g, respectively, with no significant differences ($p > 0.05$). Additionally, recovery experiments after the addition of increasing Zn amounts to the food samples before the preparation and analysis process, were also done (Cervera-Mata et al., 2019). The calculated recoveries for Zn ranged between 98.5 and 101.3 %. All reported values corresponded to mean and range values found for both cold and microwave mineralization methods.

2.5. Statistical analysis

In the statistical analysis of the data obtained, the SPSS statistical programme (SPSS 25.0, Chicago, IL) was used. Data were expressed as mean Zn values ± standard deviation. 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 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 (ANOVA). Otherwise, non-parametric methods such the Kruskal-Wallis' test (for data from multiple independent samples) and the Mann-Whitney's test (for data comparison of two independent samples) were used.

3. Results and discussion

3.1. Zn amounts released from the food matrix in the small intestine after an in vitro digestion

Table 2 shows the mean released Zn amounts (R-ZnA) in small intestine (±SD) after *in vitro* digestion of the different food groups. Statistically significant differences were observed between many of plant food groups considered (Fig. 1A; $p < 0.001$), with the highest Zn values corresponding to nuts, beverages and vegetables, and the lowest to other plant foods and cereals. In our work we found lower Zn values in cereals than in vegetables and fruits, which could be correlated with the high levels of phytates present (Khouzam, Pohl, & Lobinski, 2011). Other authors (Lestienne, Besançon, Caporiccio, Lullien-Péllerin, & Tréche, 2005) also observed an association between improved *in vitro* Zn availability and decreased phytate content in pearl millet flour.

On the other hand, those plants with higher protein and fibre content (cereals, legumes and nuts) had significantly lower Zn-BA than those with lower protein content (vegetables, fruits and other vegetal foods; $p < 0.001$). This result could be related to that of Pereira et al. (2018), who reported that Zn tends to form insoluble chelates with plant components of high protein content (such as phytates, oxalic acid, fibre, carbonates and polyphenols) impairing the solubility and absorption of Zn, and therefore Zn-BA. Other researchers found considerably lower Zn-BA values in cereals (4.52%) than in legumes (12.32%; Singh, Prasad, & Aalbersberg, 2016).

Table 2
Mean Zn bioaccesibility (Zn-BA) values* (±standard deviation [SD]; %; and into parenthesis, mean Zn amount (±SD) released from the food matrix after digestion/fermentation; μg/g) in the small and large intestine of healthy adults ($n = 3$) and children ($n = 3$) in the different groups of plant- and animal-based foods.

Food group	Zn amount released in small intestine	Zn-BA and Zn amount released in large intestine in adults	Zn-BA and Zn-amount released in large intestine in children
Nuts	(4.00 ± 3.51)^{ab}	34.2 ± 32.8 (4.87 ± 6.37)^{a}	66.6 ± 25.3 (10.7 ± 7.09^{b})
Cereals	(1.26 ± 0.506)^{a}	20.8 ± 22.5 (1.73 ± 2.48)^{b}	56.4 ± 27.3 (4.21 ± 3.24)^{ab}
Fruits	(2.01 ± 0.610)^{ab}	16.3 ± 25.9 (1.86 ± 4.08)^{ac}	48.2 ± 31.2 (4.63 ± 4.43)^{bc}
Vegetables	(2.42 ± 1.10)^{ab}	15.7 ± 23.3 (1.56 ± 3.20)^{ac}	62.5 ± 20.8 (7.18 ± 5.03)^{bc}
Legumes	(1.68 ± 0.714)^{ab}	6.69 ± 15.4 (0.497 ± 1.16)^{ac}	57.8 ± 26.7 (5.19 ± 3.88)^{bc}
Oils	(1.60 ± 0.711)^{ab}	0.072 ± 0.227 (0.003 ± 0.008)^{ac}	1.29 ± 1.60 (0.022 ± 0.019)^{bc}
Beverages	(1.50 ± 0.175)	0.170 ± 0.443 (0.008 ± 0.020)	0.64 ± 0.63 (0.018 ± 0.017)
Other plant foods	(3.02 ± 0.839)^{ab}	6.70 ± 16.4 (0.603 ± 1.48)^{a}	39.9 ± 39.5 (6.14 ± 7.52)^{b}
Dairy	(2.37 ± 1.03)	1.82 ± 6.28 (0.041 ± 0.140)	50.5 ± 40.4 (5.27 ± 6.42)
Chicken	(1.91 ± 1.07)	5.84 ± 16.3 (0.088 ± 0.233)	62.0 ± 28.8 (3.97 ± 3.09)
Beef	(6.12 ± 2.77)^{a}	4.16 ± 8.68 (0.354 ± 0.800^{ab})	45.8 ± 24.6 (6.91 ± 5.62)^{b}
Salmon	(1.81 ± 0.592)	11.4 ± 18.5 (1.129 ± 3.087)	55.7 ± 25.6 (4.00 ± 3.29)
Cod	(0.83 ± 0.304)^{ab}	4.72 ± 7.78 (0.203 ± 0.372^{ac})	58.6 ± 32.5 (4.19 ± 3.58)^{bc}
Gouda cheese	(6.52 ± 1.85)^{a}	5.61 ± 9.14 (0.584 ± 1.100^{ab})	39.3 ± 24.8 (6.26 ± 5.27)^{b}
Egg	(3.94 ± 1.863)^{a}	4.87 ± 6.39 (0.306 ± 0.381^{ab})	40.4 ± 27.6 (4.50 ± 4.18)^{b}
Lamb	(2.83 ± 0.850)^{a}	2.28 ± 7.91 (0.147 ± 0.512^{ab})	31.2 ± 32.4 (3.03 ± 4.05)^{b}
Pork	(2.31 ± 1.12)^{a}	4.75 ± 15.0 (0.268 ± 0.929^{ab})	42.7 ± 32.4 (3.65 ± 3.19)^{b}

*Similar letters in the same row shows the existence of statistically significant differences ($p < 0.05$) among small and large intestine Zn amounts released from the matrix food for adults and children ($p < 0.05$), and therefore Zn-BA values.

Similarly, for animal-based foods, R-ZnA were significantly different ($p = 0.002$; Fig. 1B). Amiard et al. (2008) pointed out that most products of marine origin, before consumption, are processed by culinary techniques, which accelerate the degradation and denaturation of proteins and water loss (and thus soluble Zn) negatively affecting the Zn-BA. On the other hand, R-ZnA for pork and beef (2.31 ± 1.12 and 6.12 ± 2.77 μg/g, respectively) were allocated in the range of dialyzed Zn levels measured in pork and beef meat products reported by other authors (1.40–4.30 and 2.90–11.1 μg/g, respectively; Rebellato, Siqueira Silva, Probio de Moraes, Trajano, & Lima Pallone, 2022).

The R-ZnA of animal foods was not significantly different from that of plant foods. Contrarily Hunt (2002) stated that a plant-based diet decreases Zn absorption.

In nuts, peanuts had the highest Zn-BA in the small intestine (86.1 ± 26.3%). In cereals, higher Zn-BA values were measured in non-wholemeal grain ones (16.4 ± 5.68%), remarking among them the bread (17.8 ± 8.03%). In fruits, the highest Zn-BA values were measured in orange (34.5 ± 6.41%) which was significantly higher than the lowest one found in apple (17.1 ± 3.02%). In vegetables, the highest Zn-BA values were measured in garlic (51.4 ± 18.3%) and potatoes (43.6 ± 10.4%) which were both significantly higher than the lowest ones found in eggplant (20.3 ± 2.64%) and onion (21.8 ± 7.23%). In legumes higher Zn-BA was found for beans (25.3 ± 10.7%) in comparison to lentils (16.4 ± 4.46%).

Other studies have highlighted the influence of food processing on

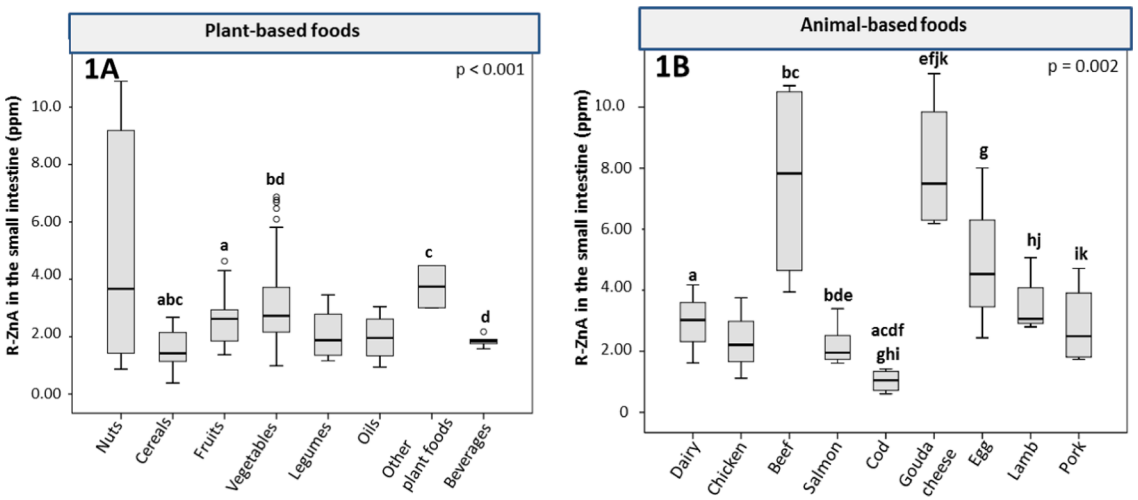

Fig. 1. Released Zn amounts (R-ZnA) from foods (μg/g; ppm) in the small intestine: (1A) in plant foods; (1B) in animal foods. Values are expressed as mean value for each food group considered. The presence of equal letters on the boxplots and outlayer values (represented as * and °) for R-ZnA of food groups shows the existence of statistically significant differences ($p < 0.05$).

Zn-BA (Yadav, Kaur, Malaviya, Saini, & Anjum, 2019; Ganguly et al., 2022; Huertas, Allwood, Hancock, & Stewart, 2022; Skalickova et al., 2022). In raw plant foods (some of them were processed foods but not submitted in the laboratory to the home culinary techniques compared; Fig. 2A), R-ZnA was statistically lower than that found in foods submitted to frying, roasting and grilling ($p < 0.05$), which could be related to the destruction of anti-nutritional factors present in plant foods. Skalickova et al. (2022) found that cooking pea flour decreased the Zn content by insolubilisation with its chelators. Similarly, other authors (Ramírez-Ojeda, Moreno-Rojas, & Cámara-Martos, 2018) observed in legumes that Zn concentrations after cooking were lower, due to migration to the cooking water; however, their bioaccessibility increased with respect to soaked legumes, due to the possible destruction of the anti-nutritional components. Similarly, other researchers noticed that in boiled beans a notable amount of Zn was lost in cooking water (Huertas et al., 2022). In extruded legumes, only 50% of the cultivars had increased Zn-BA, despite degradation of inositol hexaphosphate, possibly due to its tetra- and triphosphate forms, which still have the potential to inhibit Zn availability (Yadav, Kaur, Malaviya, Saini, & Anjum, 2019). On the contrary, in the present study the cooking technique used did not influence R-ZnA in animal-based foods (Fig. 2A, 2B). Galán, González, & Drago (2013) reported lower Zn-BA in cooked foods *vs.* raw foods, due to its interaction with other food components that interfere with its release along the digestive processes.

In raw plant-based foods, R-ZnA was significantly lower compared to those submitted to hot air heating techniques (roasting, toasting and grilling; Fig. 2C), which was not observed in animal-based foods (Fig. 2D). Finally, when comparing raw plant-based foods with cooked ones considered globally, R-ZnA were significantly lower (Fig. 2E) in raw foods. Similarly, Pandab, Jaiswala, and Lakshmi (2019) also reported a higher Zn-BA in high protein kheer mix when a previous combination of enzymatic and moist heat treatment was performed. In higher protein plant foods, no significant differences were observed according to the temperature used for cooking: high (frying, toasting, roasting and grilling) or low (boiling; Fig. 2F).

3.2. Zn-BA of foods in the large intestine of healthy adults after in vitro fermentation

With regard to the Zn-BA in the large intestine of healthy adults, statistically significant differences were observed in several plant food groups considered (Table 2; Fig. 3A; $p < 0.001$). The highest values were found for nuts and cereals, and the lowest for oils and other plant foods.

In plant-based foods with higher protein and fibre content, Zn-BA in the large intestine (contrary to the small intestine) was significantly higher ($p = 0.002$). In these foods there would be a more intense fermentative process by the colonic microbiota (Flint, Duncan, Scott, & Louis, 2015), generating new ligands that facilitate the formation of soluble Zn chelates, and therefore a rise in Zn-BA.

In animal foods, Zn-BA levels in the large intestine of healthy adults were not significantly different among the different food groups ($p > 0.05$; Fig. 3B). However, animal-based foods had significantly lower Zn-BA levels than plant-based foods ($p = 0.002$; Fig. 3C). Gut microbial functionality is usually assessed by the SCFA production, as the main products of microbial fermentation (Flint, Duncan, Scott, & Louis, 2015). High fibre, low fat and low meat diets have been shown to improve gut microbial functionality by increasing SCFA production (De Filippo et al., 2010; Fernandes, Su, Rahat-Rozenbloom, Wolever, & Comelli, 2014). In the present study, the increased Zn-BA in the large intestine of healthy adults on plant-based foods shows that fermentative processes enhanced by colonic microbiota empower the emergence of metabolites that enhanced Zn-BA. These results suggest the need for future studies to correlate the SCFA produced during fermentation of plant foods with Zn-BA values.

The values of Zn-BA in plant foods in the large intestine increased (respect to those corresponding to the small intestine) only in nuts, and decreased in fruits, vegetables, legumes, oils and other plant foods significantly ($p < 0.05$; Table 2). Similarly, in most of animal foods also R-ZnA in the large intestine were significantly lower than those determined in the small intestine specifically in beef, cod, gouda cheese, egg, lamb and pork ($p < 0.05$).

Contrarily to that found in the small intestine, in nut mix the Zn-BA (86.1 ± 26.3%) in the large intestine of adults was higher than that of peanuts (6.64 ± 15.0%). In cereals, the highest Zn-BA values in the large intestine were measured in wholemeal breakfast cereals (43.1 ± 41.3%) and bread (30.11 ± 23.4%) and the lowest in breakfast cereals (4.53 ± 8.56%), and whole rice (6.30 ± 6.20%) and rice (6.77 ± 10.4%). In fruits, the highest Zn-BA values were measured in apple (23.8 ± 29.5%) and orange (23.7 ± 33.0%) and the lowest ones in plum (6.41 ± 11.2%) and peach (8.7 ± 17.3%). In vegetables, the highest Zn-BA values were measured in tomatoes (58.1 ± 15.3%) and garlic (54.9 ± 6.6%) which were both significantly higher ($p < 0.05$) than the lowest one found in capsicum (9.63 ± 21.5%). Additionally, it is remarkable that Zn was not bioaccesible from potatoes and sweet potatoes in the large intestine of adults. In legumes higher Zn-BA was found for beans (25.3 ± 10.7%) in comparison to lentils (16.4 ± 4.46%).

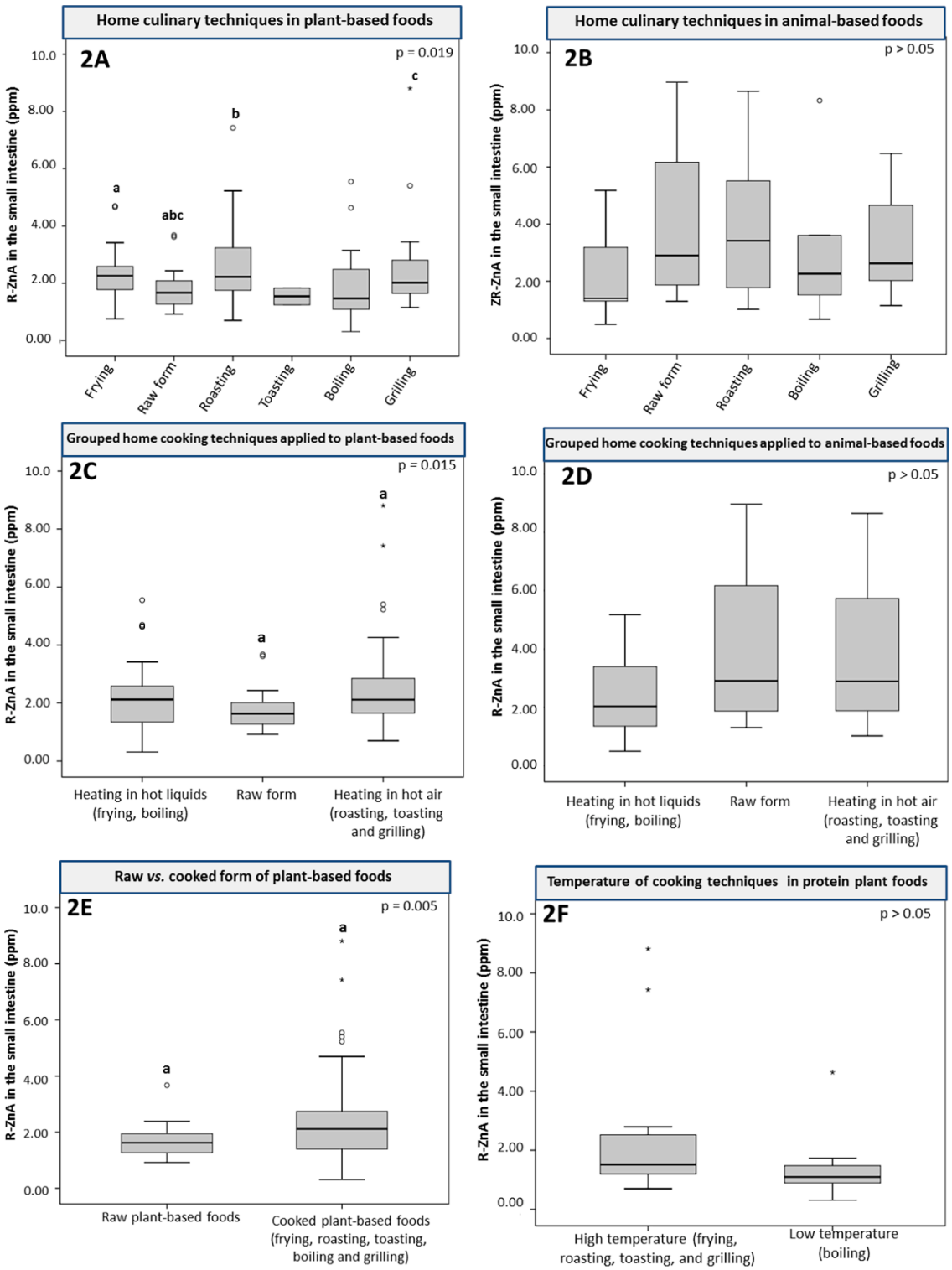

Fig. 2. Released Zn amounts (R-ZnA) from foods (μg/g; ppm) in the small intestine depending on the culinary techniques: (2A) in plant foods; (2B) in animal foods; (2C) in raw plant foods *vs.* those heated in hot liquids (oil: frying; water: boiling) or hot air (roasting, toasting and grilling); (2D) in raw animal foods *vs.* those heated in hot liquids (oil: frying; water: boiling) or hot air (roasting, toasting and grilling); (2E) in raw *vs.* cooked plant foods; (2F) in protein plant foods (legumes, cereals and nuts) according to the temperature of culinary techniques (high temperature: frying, roasting, toasting and grilling; low temperature: boiling). Values are expressed as mean value for each food group considered. The presence of equal letters on the boxplots and outlayer (represented as * and °) for R-ZnA values of food groups shows the existence of statistically significant differences ($p < 0.05$).

It has been observed that the Zn-BA of adult 1 was significantly higher ($p < 0.001$; Fig. 3D). This interindividual variability of Zn-BA in the large intestine would be related to different metabolites, which would facilitate the formation of soluble chelates with Zn, due to the different microorganisms that make up the specific gut microbiota of each adult (Pérez-Burillo et al., 2021a). 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

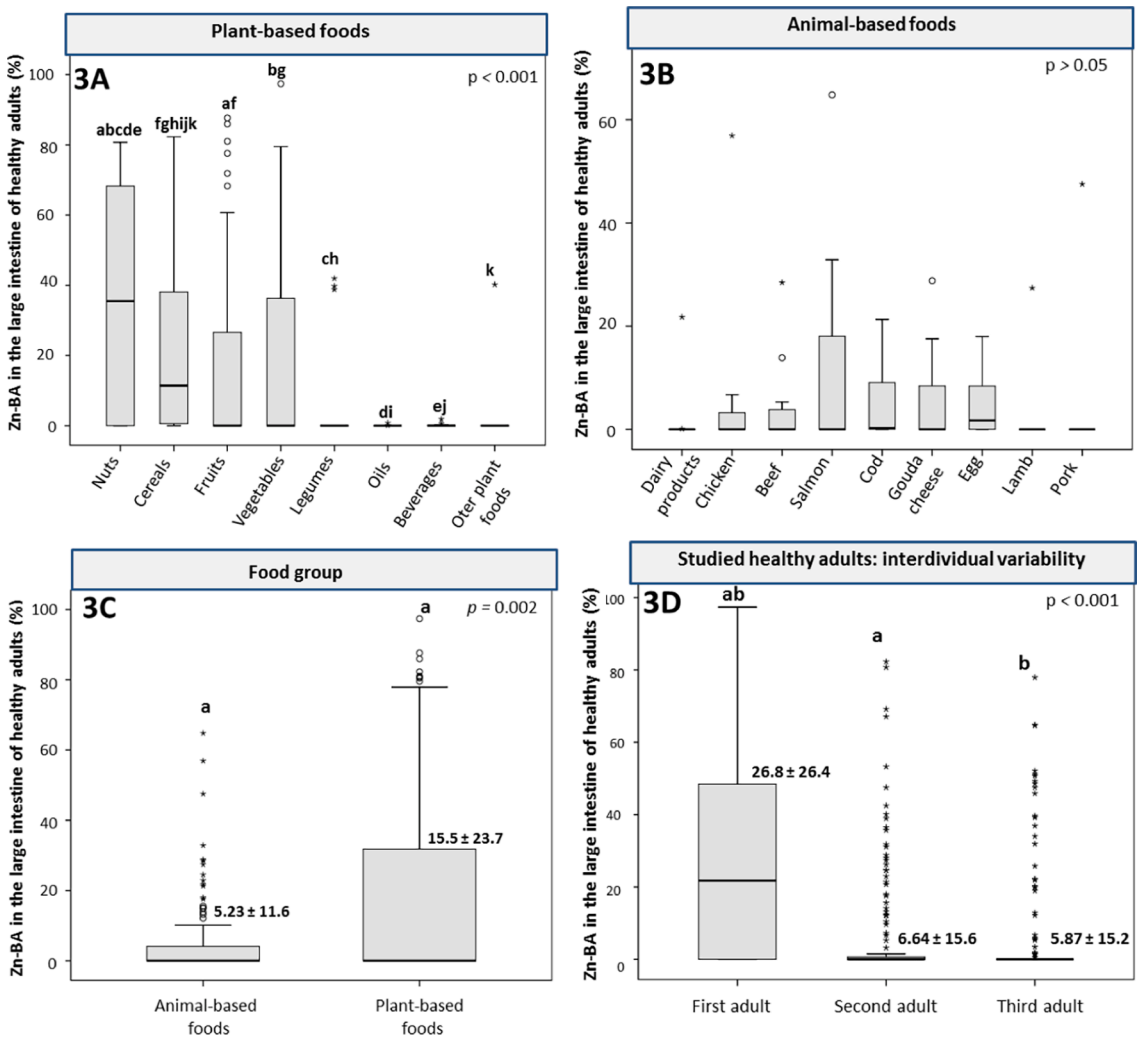

Fig. 3. Zn bioaccesibility (Zn-BA) values of foods (%) in the large intestine of healthy adults; (3A) in plant-based foods; (3B) in animal-based foods; (3C) in food groups; (3D) in adults included in the study (interindividual variability: $n = 3$). Values are expressed as mean value for each food group considered. The presence of equal letters on the boxplots and outlayer values (represented as * and °) for Zn-BA values of food groups shows the existence of statistically significant differences ($p < 0.05$).

Zn-BA.

When considering all foods (Fig. 4A) or dividing them into plant-based (Fig. 4B) or animal-based (Fig. 4C) foods, there were no significant differences which shows no influence of the culinary techniques used ($p > 0.05$).

3.3. Zn bioaccesibility of foods in the large intestine of healthy children after in vitro fermentation

In plant-based foods, there were significant differences ($p < 0.001$) in Zn-BA values in the large intestine of healthy children among many of the food groups considered (Fig. 5A). The highest values were found for nuts and vegetables, and the lowest for oils and beverages. In vegetables, Zn-BA was significantly higher than in cereals, fruits, oils and beverages which would be related to the different contents of dietary fibre, phytates, polyphenols, resistant starch, and to Maillard reaction products (Pérez-Burillo et al., 2018a). The above components constitute the important fraction of ingested food that escapes digestion in the small intestine, which together with bile acids and enzymes released into the small intestine, reaches the colon where they are fermented by the gut microbiota (Agans et al., 2018), resulting in different Zn-BA values for different plant-based food groups.

Differently to that found for adults, Zn-BA in the large intestine of healthy children was not influenced by plant foods, when grouped into those with the highest and lowest protein and fibre content ($p > 0.05$). In foods of animal origin, the Zn-BA values in the large intestine in children and healthy adults were not significantly different between the different food groups ($p > 0.05$; Fig. 5B, 3B, respectively).

As in healthy adults, animal–based foods showed significantly lower Zn-BA values in healthy children compared to those determined for plant-based foods ($p = 0.048$; Fig. 5C). Thus, Zn-BA in plant foods in the large intestine of healthy children increased in nuts, cereals, fruits, vegetables and legumes, and decreased in oils and other plan foods significantly ($p < 0.05$; Table 2), with respect to those measured in the small intestine. Also, Zn-BA values of plant foods were significantly higher ($p < 0.05$) in cereals, fruits, vegetables, legumes, oils and other plant foods, compared to those of healthy adults (Table 2). Therefore, the fermentative processes of plant foods are very different, as the faecal inocula used to estimate Zn-BA correspond to healthy children or adults.

Similarly, for most of animal foods, Zn-BA values in the large intestine of healthy children were significantly higher than those of healthy adults for beef, cod, gouda cheese, egg, lamb and pork ($p < 0.05$). Therefore, future studies would be needed to correlate the differential composition of the gut microbiota (between healthy children and adults) and the higher Zn-BA in the large intestine (Table 2).

In nut mix the Zn-BA (86.1 ± 26.3%) in the large intestine of

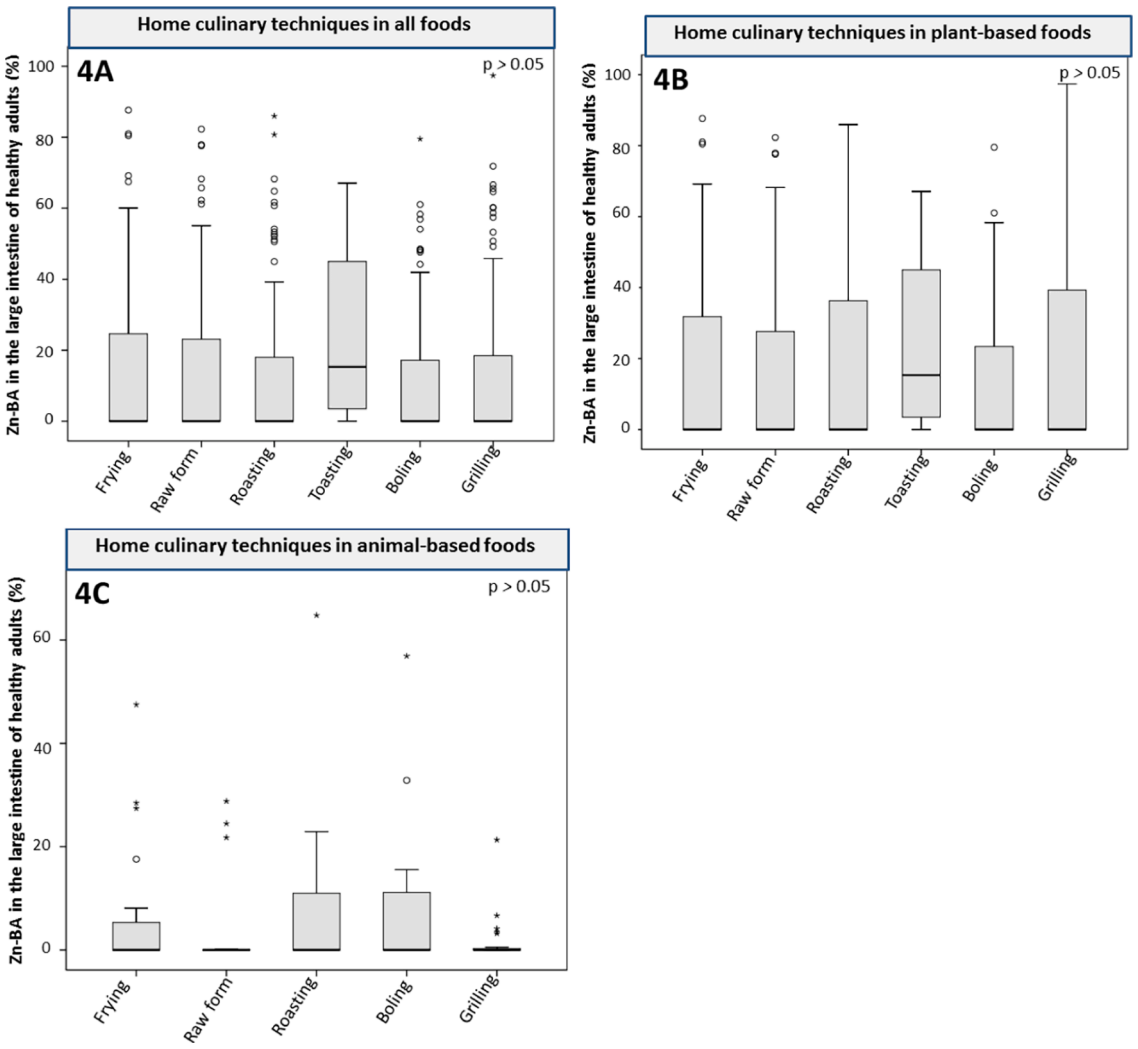

Fig. 4. Zn bioaccesibility (Zn-BA) values in foods (%) in the large intestine of healthy adults depending on the culinary techniques: (4A) in all foods; (4B) in plant foods; (4C) in animal foods. Values are expressed as mean value for each food group considered. The presence of equal letters on the boxplots and outlayer values (represented as * and °) for Zn-BA values of food groups shows the existence of statistically significant differences ($p < 0.05$).

children was significantly higher than that of peanuts (50.6 ± 26.1%), which in turn was considerably higher than that measured in the small intestine. In cereals, the highest Zn-BA values in the large intestine of children were measured in wholemeal penne (85.6 ± 12.4%) and wholemeal rice (63.7 ± 1.34%) which were significantly higher ($p < 0.05$) than the lowest one found in rice (27.0 ± 9.73%). It should be noted that the Zn-BA of wholemeal rice and rice and of wholemeal cereals and non-wholemeal cereals, in the large intestine of children is significantly and considerably higher than that measured for adults. In fruits, like in the large intestine of adults but with values significantly higher ($p < 0.05$), the highest Zn-BA values were also found in apple (77.8 ± 5.1%) and orange (59.1 ± 29.9%) and the lowest ones in plum (20.8 ± 25.3%) and peach (30.4 ± 32.2%). In vegetables, the highest Zn-BA values were measured in onion (80.6. ± 2.88%) and cauliflower (84.1 ± 4.34%) which were both significantly higher ($p < 0.05$) than the lowest ones found in potatoes (25.7 ± 8.86%) and garlic (39.7 ± 11.5%). In legumes higher Zn-BA was found for beans (58.7 ± 2.99%) in comparison to lentils (23.8 ± 20.7%).

For all foods, Zn-BA in the large intestine of healthy children showed inter-individual variation (Fig. 5D), with that of child 1 being significantly lower ($p < 0.001$).

When considering all foods together (Fig. 6A), or dividing them into their plant (Fig. 6B) or animal (Fig. 6C) origin, there were significant differences depending on the culinary techniques used ($p < 0.05$) in Zn-BA. Frying was related to the highest Zn-BA values in all foods (Fig. 6A), plant- (Fig. 6B) or animal-based (Fig. 6C) foods. In all foods, in their raw form Zn-BA was significantly lower than those submitted to frying, roasting and boiling ($p < 0.05$, Fig. 6A), as also was observed for plant-based foods only for frying and roasting ($p < 0.05$; Fig. 5B). In animal-based foods those submitted to boiling showed significantly higher values than those cooked by roasting and grilling ($p < 0.05$, Fig. 6C). These results could be related to intense thermal food processing (grilling, roasting and frying) which increases the levels of melanoidins (Martin et al., 2009; Pérez-Burillo et al., 2018a), that after being fermented by the gut microbiota could increase beneficial gut bacteria and butyrate production (Pérez-Burillo et al., 2018a).

Zn-BA values of raw plant foods in the large intestine of healthy children were lower than when they were submitted to frying and roasting (Fig. 6B, 2A; respectively), a result not seen for the Zn-BA in the large intestine of healthy adults. This finding reinforces the different behaviour of plant foods submitted to different cooking techniques depending on whether the fermentation process is carried out with inocula from healthy children or adults.

In raw plant-based foods, Zn-BA values were significantly lower than those obtained after cooking in liquid media or in hot air (Fig. 6D). In the small intestine, lower levels of Zn-BA in the raw form of plant-based foods were only seen when compared to foods heated by hot air heating techniques (Fig. 2C). It has been indicated that roasting applied to

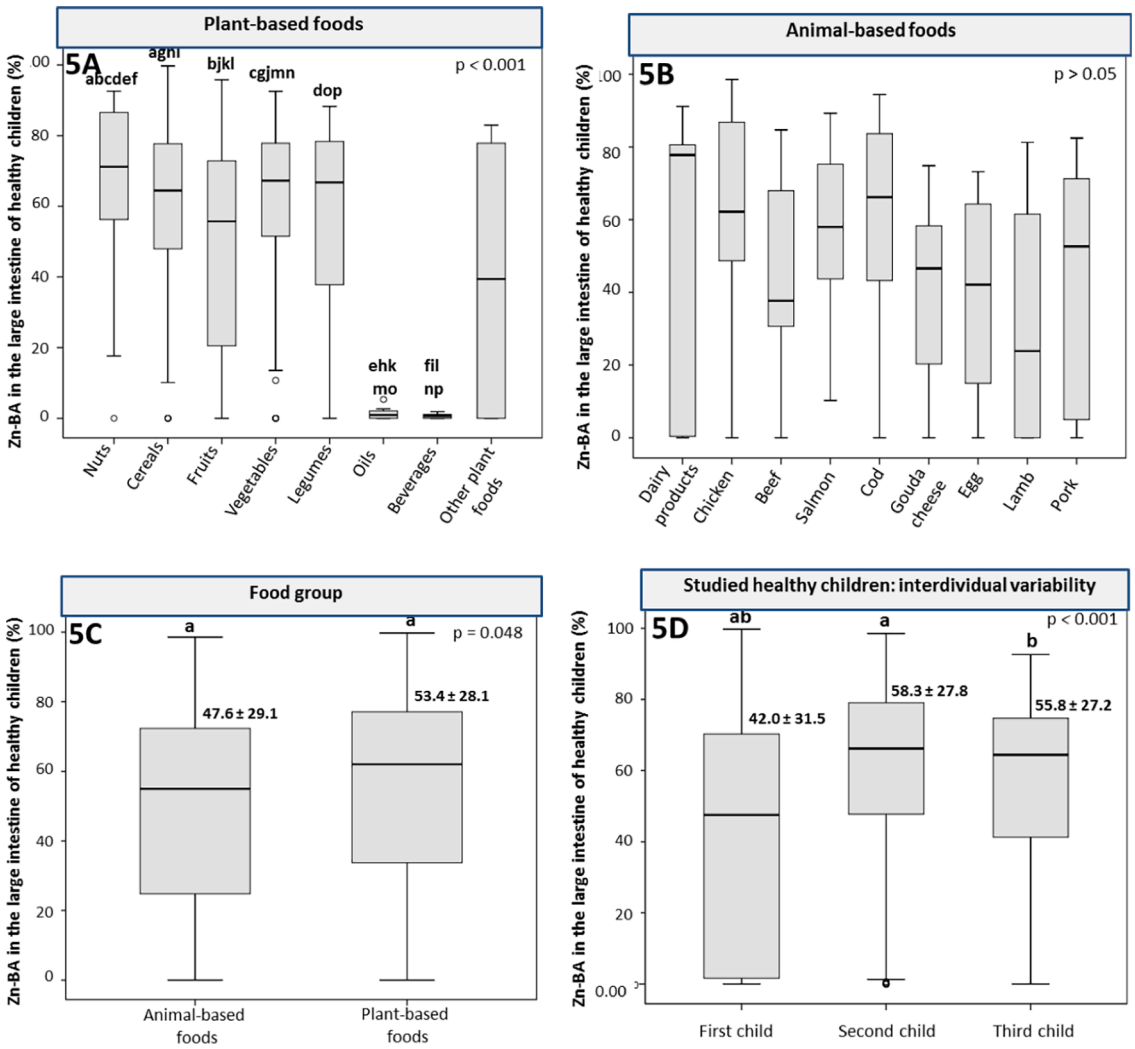

Fig. 5. Zn bioaccesibility (Zn-BA) values of foods (%) in the large intestine of healthy children; (5A) in plant-based foods; (5B) in animal-based foods; (5C) in food groups; (5D) in children included in the study (interindividual variability; $n = 3$). Values are expressed as mean value for each food group considered. The presence of equal letters on the boxplots and outlayer values (represented as * and °) for Zn-BA values of food groups shows the existence of statistically significant differences ($p < 0.05$).

cocoa causes changes in the functionality and composition of the gut microbial communities, associated with loss of polyphenols, decrease in carbohydrates and chemical browning with increase of furfural and 5-hydroxy-methylfurfural with temperature, and formation of melanoidins (Maldonado-Mateus et al., 2021). The changes observed in plant-derived foods could facilitate the appearance of new ligands that induce the increase in Zn-BA after fermentation with faecal material of health children found in the present study (Fig. 6D).

In foods of animal origin, Zn-BA values of foods cooked in liquid media were significantly higher or those using hot air (Fig. 6E), which was not found in the small intestine (Fig. 2D), nor in the large intestine of healthy adults.

These findings, especially in plant-based foods fermented with faeces of healthy children, indicate that Zn-BA values are modified, depending on the type of food, culinary technique and thermal treatment applied. Therefore, the correlation of indicators of Maillard reaction like furosine, 5-hydroxymethy-furfural and furfural (and the associated gut microbiota composition) with the Zn-BA in specific food groups should be performed in future studies.

3.4. Total amount of Zn and distribution between bioaccessible and non-bioaccessible fractions

The mean total amount of Zn from all foods was 8.080 μg/g (Fig. 7). Zn-BA in the large intestine of healthy children was significantly higher than that of the small intestine ($p < 0.001$), and that determined in the large intestine of healthy adults ($p < 0.001$). The non-bioavailable Zn fraction was also significantly lower in healthy children than in healthy adults ($p < 0.001$; Fig. 7).

If the Zn fractions were distributed as a percentage of the total Zn intake, the Zn-BA in the small intestine was 33.0 ± 20.4, and in the large intestine 16.4 ± 22.0, and 59.6 ± 29.9%, % for healthy adults and children, respectively; the non-bioaccessible Zn fractions in the large intestine were 50.6 ± 19.9 % and 7.39 ± 9.12%, %, respectively. Therefore, the total bioaccessible fraction of Zn was 92.61% for children and 49.44% for adults. Others authors (Laquinta, Rodríguez, & Machado, 2023) concluded that Zn bioaccesible fraction was higher than 66% in the analysed foods, being the beef a major Zn source (as we have also found). The higher Zn-BA in the large intestine of children could be related to the higher body demands for Zn during the growth stage, such as childhood, as well as to its participation in the regulation of body metabolism and maturation, as it is part of many essential enzymes and proteins of a developing organism (Sing, Prasad, &

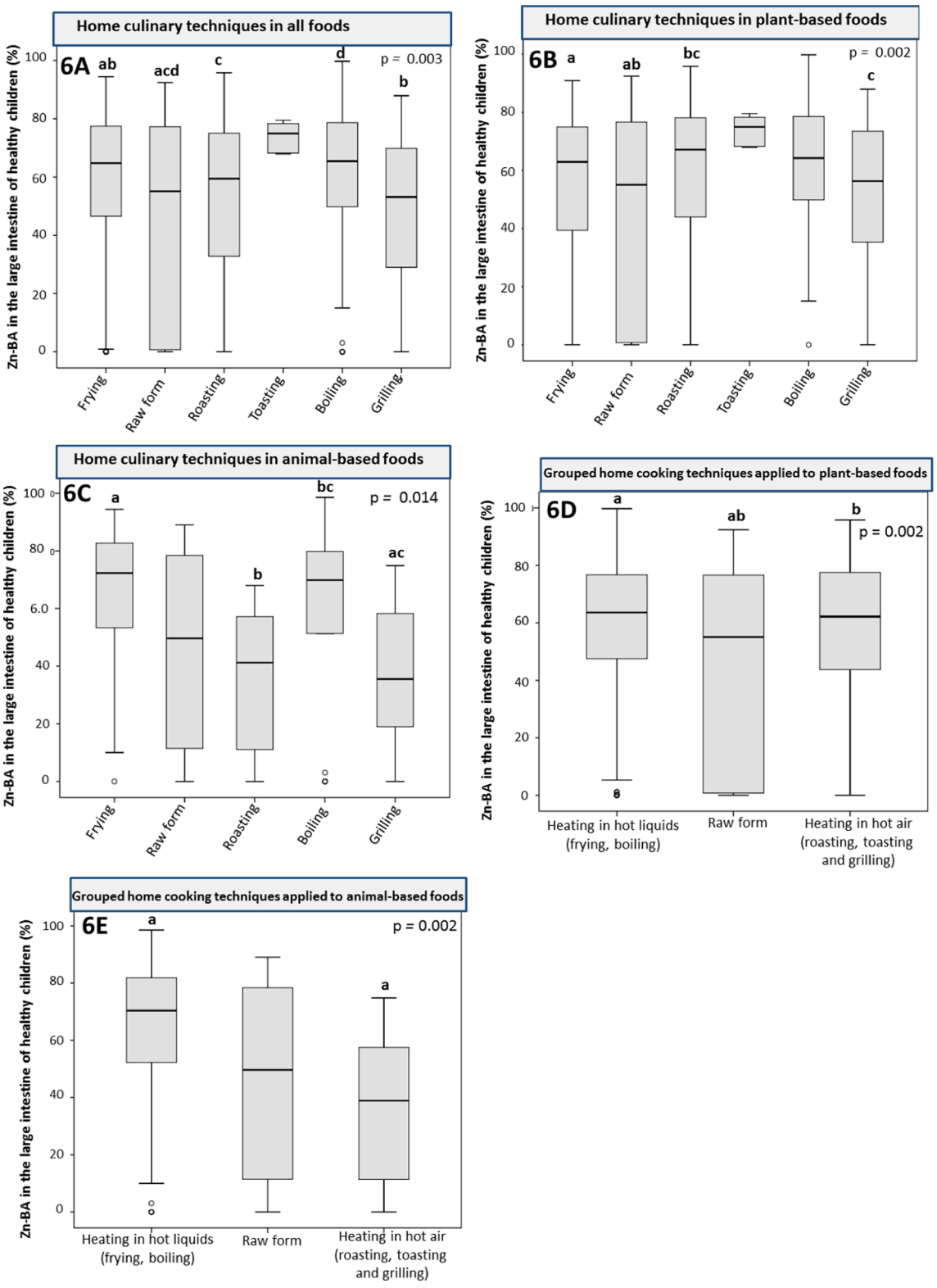

Fig. 6. Zn bioaccesibility (Zn-BA) values in foods (%) in the large intestine of healthy children depending on the culinary technique; (6A) in plant foods; (6B) in animal foods; (6C) in raw plant foods *vs.* those heated in hot liquids (frying and boiling) or hot air (roasting, toasting and grilling); (6D) in raw animal foods *vs.* those heated in hot liquids (oil; frying; water: boiling) or hot air (roasting, toasting and grilling); (6E) in raw *vs.* cooked plant foods. Values are expressed as mean value for each food group considered. The presence of equal letters on the boxplots and outlayer values (represented as * and °) for Zn-BA values of food groups shows the existence of statistically significant differences ($p < 0.05$).

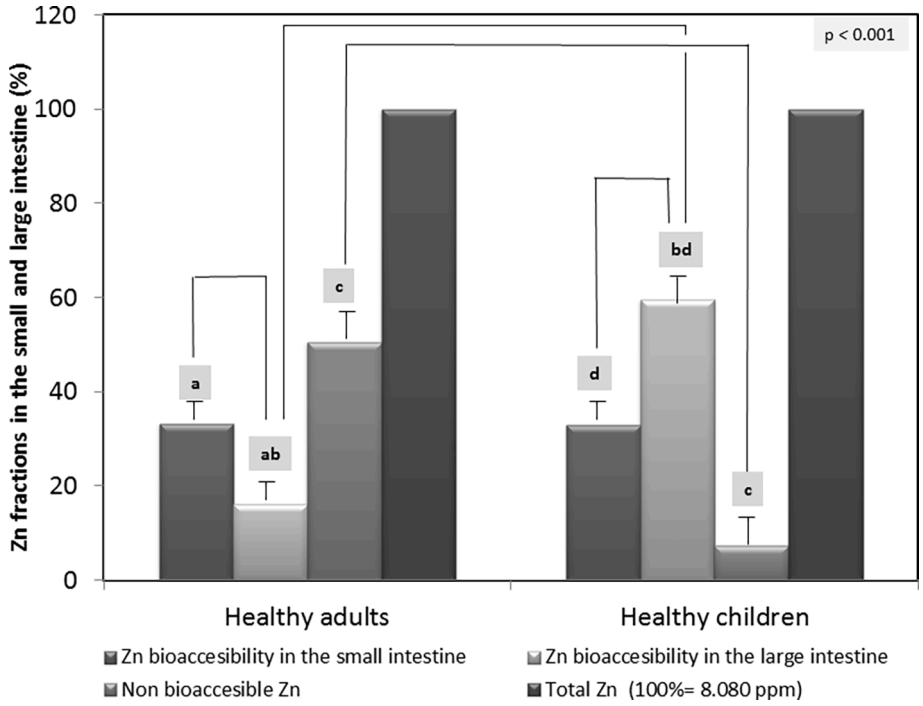

Fig. 7. Total amount of Zn (μg/g; ppm) and distribution between bioaccessible (in the small and large intestine; %) and non bioaccesible fractions (%) in healthy adults and children. Values are expressed as mean value for total Zn amount and different Zn fractions considered. The presence of equal letters on the bars of different Zn fractions shows the existence of statistically significant differences ($p < 0.05$).

Aalbersberg, 2016; Arreondo Olguín, Ruz Ortiz, Olivares Grohnet, & Castillo Durán, 2017; Banaszak, Górna, & Przysławski, 2021; Obaid et al, 2022). Therefore, as others reported (Skalny et al., 2022) both Zn and gut microbiota and their interaction would be involved in the regulation of the physiological functions differently in healthy children and adults.

The ranges of adequate daily intakes of Zn are lower in childhood (5.5–10.7 and 4.4–8.9 mg/day, in boys and girls, respectively, for the studied age range) than in healthy adults (9.4–16.2 and 6.2–10. 2 mg/day, in males and females, respectively) as reported by EFSA (2017). Our results indicate a higher Zn-BA in the large intestine of healthy children, and thus possibly higher Zn bioavailability. Nonetheless, it should be noted that the contribution of a specific food to the Zn content of a diet depends on its Zn concentration and serving size, not just bioaccessibility.

There are gaps in our current knowledge, as to which components of the gut microbiota are associated with the improved large intestinal Zn-BA after *in vitro* fermentation. Others researchers identified significant changes in gut microbial composition and metabolites concentration in a wheat-based diet biofortified with Zn administered to rooster (Reed et al., 2018) and in altered Zn-fed mice (Chen et al., 2021), and that both Zn excessive or deficient diets could lead to a dysbiosis (Chen et al., 2021). Given the differential composition between the different food groups included in our study, it would be necessary to analyse the food groups individually, and to scale up the *in vitro* studies to in vivo rodent animal models and human volunteers as well. This would allow a more accurate understanding of the specific components of the constituent foods in the diet, associated with the distribution of species, genera and families of microorganisms of the colonic microbiota related to Zn-BA, in order to ensure more personalised nutritional recommendations.

4. Conclusions

Mean total Zn amount provided by foods was 8.080 μg/g. The Zn amount released from foods in the small intestine was significantly different among several food groups and lower in raw vegetable foods compared to cooked foods as it was also found after *in vitro* fermentation with faeces of healthy children. Zn-BA after *in vitro* fermentation varied according to the subjects' idiosyncrasies, and was higher in healthy children, especially for cereals, due to growth demands and different composition of the gut microbiota. In healthy adults and children, the bioaccessible fractions were 33.0 ± 20.4 for the small intestine, and 16.4 ± 22.0 and 59.6 ± 29.9%% for the large one, while the non-bioaccessible fractions reached 50.6 ± 19.9 % and 7.39 ± 9.12%, respectively. Based on the results included in this manuscript, the positive influence on the Zn-BA of plant foods has been underestimated since the major role exerted by the gastrointestinal system in the release and transformation of molecules acting as Zn ligands (especially revealed by fermenting these foods with faecal material from healthy children) has not been considered.

CRediT authorship contribution statement

Úrsula García-Conde: Formal analysis, Investigation, Validation, Writing – original draft. **Miguel Navarro-Alarcón:** Formal analysis, Methodology, Validation, Writing – original draft, Data curation. **Beatriz Navajas-Porras:** Investigation, Methodology, Validation. **Daniel Hinojosa-Nogueira:** Investigation, Methodology. **Adriana Delgado-Osorio:** Investigation, Methodology. **Sergio Pérez-Burillo:** Investigation, Methodology, Validation, Data curation. **Silvia Pastoriza:** Conceptualization, Writing – original draft. **Miguel Navarro- Moreno:** Formal analysis, Investigation. **José-Ángel Rufián- Henares:** Conceptualization, Investigation, Visualization, Project administration, Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

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

Acknowledgements

This work was supported by the European Research Commission (Research Executive Agency) under the research project Stance4Health under Grant Contract N^{o} 816303 and by the Plan propio de Investigación y Transferencia of the University of Granada 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.foodres.2023.112817.

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