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Inorganic contaminants and micronutrients in foods consumed by the vegetarian Portuguese population

Sandra Gueifão¹ , Andreia Rego^{1,2} , Inês Delgado^{1,2} , Marta Ventura^{1,3} , Inês Coelho^{1*} ¹Department of Food and Nutrition, National Institute of Health Doutor Ricardo Jorge (INSA IP), 1649-016 Lisboa, Portugal²Department of Chemical Engineering (DEQ), Instituto Superior Técnico, 1049-001 Lisboa, Portugal³MARE—Marine and Environmental Sciences Centre, Department of Environmental Sciences and Engineering, NOVA School of Science and Technology (NOVA FCT), NOVA University Lisbon, 2829-516 Caparica, Portugal***Correspondence:** Inês Coelho, Department of Food and Nutrition, National Institute of Health Doutor Ricardo Jorge (INSA IP), Avenida Padre Cruz, 1649-016 Lisboa, Portugal. ines.coelho@insa.min-saude.pt**Academic Editor:** Maria Tsimidou, Aristotle University of Thessaloniki, Greece**Received:** June 7, 2024 **Accepted:** July 18, 2024 **Published:** September 24, 2024**Cite this article:** Gueifão S, Rego A, Delgado I, Ventura M, Coelho I. Inorganic contaminants and micronutrients in foods consumed by the vegetarian Portuguese population. *Explor Foods Foodomics*. 2024;2:555–69. <https://doi.org/10.37349/eff.2024.00051>

Abstract

Aim: The present study aimed to determine the profile of micronutrients and inorganic contaminants in the plant-based and dairy food products most consumed by the Portuguese population.**Methods:** The sampling plan followed the Total Diet Studies (TDS) methodology and included representative samples of the Portuguese vegetarian diet, chosen based on the National Food and Physical Activity Survey. Five main food groups were selected: Grains and grain-based products ($n = 48$); Dairy products ($n = 60$); Products for non-standard diets ($n = 72$); Pulses, dried fruits, and oilseeds ($n = 132$); and Fruiting vegetables ($n = 12$). The sampling plan included 324 individual samples, prepared as 27 pooled samples for laboratory analyses. Each pooled sample was analyzed for 13 elements: As, Cd, Co, Cr, Cu, I, Li, Mn, Mo, Pb, Se, Sr, and Zn. The methodology used for the analysis was ICP-MS with samples previously subjected to acid digestion through closed vessel microwave-assisted, except for I, which required an alkaline extraction.**Results:** The results of this study indicate that food samples from the group of Pulses, dried fruits, and oilseeds had the highest content of Mn, Zn, Mo, and Se. On the other hand, Products for non-standard diets and Dairy products had the highest content of Cu and I, respectively. The As, Cd, and Pb levels were below the limit of quantification for most of the analyzed samples from every food group.**Conclusions:** This research enabled updating the Portuguese Food Composition Database (FCD), underscoring the importance of regular dietary assessments and the role of FCDs and TDS in safeguarding public health by ensuring nutritional adequacy and safety in the food supply.

Keywords

Vegetarian diet, Total Diet Study, inorganic contaminants, micronutrients, plant-based products, dairy products, ICP-MS

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Introduction

Over the past decade, dietary habits have changed, increasing the popularity of vegetarian and vegan diets [1]. Some advantages have been associated with these diets as these consumers typically present a lower risk of ischemic heart disease, type 2 diabetes, hypertension, certain types of cancer, and obesity [2]. Nonetheless, since there are restrictions on the consumption of certain food groups, it has become pertinent to understand whether the foods consumed by vegetarians can meet the dietary reference value (DRV) of micronutrients and whether the levels of contaminants present in these foods are safe. Among the different types of vegetarian diets, vegans do not consume any animal products, including eggs, dairy products, meat, and fish. In contrast, vegetarians exclude meat and fish from their diet but consume animal-derived products, such as milk and eggs [1].

Total Diet Studies (TDS) are a globally recognized public health tool for assessing dietary exposure linked to beneficial and harmful food substances. The World Health Organization (WHO) has endorsed and highlighted TDS as the most cost-effective method for estimating population risk assessment of food contaminants, instilling confidence in its credibility [3]. The essential principles of TDS are representativeness of the whole diet, preparation and analyses of food as consumed by the targeted population, pooling the food, and combining the occurrence data with the consumption data to estimate the dietary exposure of the population [4]. TDS provides a harmonized approach to ensuring data comparability between countries. The first TDS pilot study in Portugal collected samples between 2014 and 2016 and included 1,070 food items from selected food groups to cover the overall diet of the population [5]. However, it did not focus on specific diets, such as the vegetarian diet, characteristic of smaller population groups.

From a public health perspective, promoting diets that include the recommended amount of nutrients is crucial. This ensures the health and well-being of the population and guarantees that the levels of toxic substances do not exceed the maximum levels established in the legislation [6].

The human body requires only small amounts of micronutrients. However, their deficiency may seriously compromise human health. Ingestion is the principal route of human exposure to many trace elements, which can be divided according to their importance for human health.

Essential elements such as copper (Cu), iodine (I), manganese (Mn), molybdenum (Mo), selenium (Se), and zinc (Zn) play active roles in biological functions. They are fundamental components of several enzymes (Cu, Mn, Mo) and hormones (I) or are effective antioxidant agents also contributing to toxicity mitigation (Se, Zn) [7–13]. Pulses, cereal grains, and grain products are the main dietary contributors of several trace elements [14]. For I, in particular, the most significant source is food, especially dairy products, fish, and eggs [15]. Failure to consume these types of food could lead to I deficiencies. The WHO encourages the population to use iodized salt, which is particularly relevant in diets with a low or absent consumption of I-rich foods [16]. An incorrect intake of I can lead to the development of hypothyroidism and brain damage, resulting in mental retardation [8, 9].

Non-essential elements, such as lithium (Li) and strontium (Sr), are not considered micronutrients since they do not have any proven biological function in the human body. However, these two elements have proved beneficial in medical applications under controlled dosages. While Li has been extensively used in pharmaceuticals for the treatment of bipolar disease, Sr is effective in the treatment of postmenopausal osteoporosis and knee osteoarthritis [17, 18].

Other elements, like chromium (Cr) and cobalt (Co), play a role in human health depending on the dose or the chemical form in which they are present in foodstuffs. Cr is a natural element that occurs in different oxidation states. The most common form is the trivalent form, an essential nutrient in dietary supplements, which has been demonstrated to be involved in glucose metabolism, where it plays a vital role as a cofactor that could affect the metabolism of glucose, lipids, and proteins. Cr has also been shown to improve immune function [19, 20]. On the other hand, the hexavalent form is considered a toxic industrial pollutant [21]. Co, a transition metal, is essential in vitamin B12 metabolism as Co (III). However, exposure to high levels of Co compounds could be toxic and may entail adverse human health effects [22].

In contrast, toxic elements like arsenic (As), cadmium (Cd), and lead (Pb) occur in the environment through natural or anthropogenic sources, and their acute and chronic exposures are associated with severe disease, particularly cancer risk (As and Cd) and neurotoxicity (Pb), due to its bioaccumulation capability. Food, including crops, is one of the main sources of human exposure [23–25]. These three contaminants are included in the top ten chemicals of major concern for public health and are prioritized substances in TDS exposure [3, 26–28].

This work aimed to characterize trace elements (micronutrients and inorganic contaminants) in the plant-based and dairy food products most consumed by the Portuguese population, in alignment with the necessities of the Portuguese Food Composition Database (FCD). It provides crucial information and analytical data produced in compliance with the criteria for integrating the FCD, filling an existing gap in Portuguese FCD information.

Materials and methods

Sampling plan

The sampling plan implemented in this work was based on the pilot TDS-Exposure project [5] and was designed to fill the previously identified lack of information in the Portuguese FCD. The TDS methodology is a multi-phase process that begins with selecting a representative market basket based on a national food consumption survey. This is followed by collecting foods at the retail level, processing food for consumption, and pooling food into representative food groups. TDS pooled samples comprising twelve subsamples are homogenized and analyzed for harmful and beneficial chemical substances [4]. All samples of this study were collected to represent the Portuguese diet and the foods that are available on the market for consumption.

The analytical results obtained under rigorous quality control schemes assure that the data obtained comply with the criteria for integrating the Portuguese FCD.

Food selection

The food list was composed of samples of foods consumed by the vegetarian population and available on the Portuguese market. The selection of samples was based on three criteria: 1) consumer responses to the National Food, Nutrition, and Physical Activity Survey (IAN-AF) [29]; 2) non-overlapping of samples with the first harmonized TDS in Portugal [5, 30–33]; and 3) lack of data in the Portuguese FCD.

For this study, five main food groups were analyzed: Grains and grain-based products ($n = 48$); Dairy products ($n = 60$); Products for non-standard diets ($n = 72$); Pulses, dried fruits, and oilseeds ($n = 132$); and Fruiting vegetables ($n = 12$).

The sampling plan included 324 food items collected and prepared as 27 pooled samples for laboratory analyses. Each TDS pooled sample comprises 12 sub-samples, previously homogenized before the analyses of harmful and beneficial chemical substances. Eleven pooled samples of Pulses, dried fruits, and oilseeds, six samples of products for Non-standard diets, and four samples of Grains and grain-based products were analyzed as purchased (raw), which is not per the TDS methodology where samples are analyzed as consumed. However, this option provided information for essential data currently absent in the Portuguese FCD.

Instrumentation

All trace elements were analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Thermo X series II, Thermo Fisher Scientific, Waltham, MA, USA) combined with an autosampler (CETAC ASX-520) (CETAC Technologies, Omaha, Nebraska, USA). Each pooled sample was analyzed for 13 chemical elements resorting to the following isotopes: ^{75}As , ^{111}Cd , ^{59}Co , ^{52}Cr , ^{65}Cu , ^{127}I , ^7Li , ^{55}Mn , ^{95}Mo , ^{208}Pb , ^{77}Se , ^{88}Sr and ^{66}Zn .

A tuning solution of 10 $\mu\text{g/L}$, containing Li, Ba, Be, Bi, Ce, Co, In, Pb, Tl, and U (TUNE 01-25) (Analytika, Prague, Czech Republic), was used daily to evaluate and optimize the ICP-MS's performance.

Reagents

All reagents were of high analytical grade. The ultra-pure water level I, was obtained from a Milli-Q Element system from Millipore (Millipore Corporation, Saint-Quentin, France).

The nitric acid (HNO₃) 65% was purified for acid digestion using a sub-boiling distillation system (Milestone SubPUR). All solutions and samples were prepared with a 2% (v/v) HNO₃ solution. The hydrogen peroxide (H₂O₂) was of trace analysis grade (Merck KGAA, Darmstadt, Germany). The ICP stock standards were multi or single-element containing 1,000 mg/L (SCP Science, Marktoberdorf, Germany). Internal standards of germanium, indium, and yttrium solutions (1,000 mg/L) were from Inorganic Ventures (Christiansburg, Virginia). For alkaline extraction, used for the determination of I, tetramethylammonium hydroxide (TMAH) (25% v/v) was acquired from Fluka, Honeywell (Bucharest, Romania). All solutions and samples were prepared in a 0.5% (v/v) TMAH solution. Working standard solutions of I were prepared from single-element high-purity ICP stock standard containing 1,000 mg/L of I (Inorganic Ventures, Christiansburg, Virginia). Internal standard solutions of rhodium (10 mg/L) and tellurium (1,000 mg/L) were acquired from Inorganic Ventures (Christiansburg, Virginia) and Merck (Darmstadt, Germany), respectively. Pancreatin from porcine pancreas (Sigma Aldrich, Darmstadt, Germany) was used to pre-treat all samples from the Grains and grain-based products group, and eight samples from the Pulses, dried fruits, and oilseeds group (except chia seeds, miso, and soy dessert). To wash up the ICP-MS sample introduction system, a solution with Triton® (Merck, Darmstadt, Germany) and ammonium hydroxide 30% (Avantor Performance Materials, Netherlands) was used.

Methodology of sample preparation

The determination of I was based on EN 15111:2007 [34], using an alkaline extraction with a graphite heating block digestion system (DigiPREP, SCP Science, Courtaboeuf, France). Approximately 0.6 g of each sample was weighed and placed on the heating block for 3 hours at 90°C, as described in Delgado et al. [33]. To eliminate starch, a pancreatin solution of 2% (v/v) was added to certain samples and left overnight at 37°C before TMAH extraction.

The determination of the remaining trace elements (As, Cd, Co, Cr, Cu, Li, Mn, Mo, Pb, Se, Sr, and Zn) was based on EN 15763:2009 [35]. Before analysis, samples were previously subjected to acid digestion through a closed vessel microwave-assisted digestion system. Samples were weighed into Teflon vessels, and a mixture of concentrated HNO₃, H₂O₂, and ultrapure water in a 4:1:3 ratio was added. A four-step digestion cycle was performed: step one: 25 min to 90°C, maintain for 5 min; step two: 15 min to 180°C, maintain for 10 min; step three: 5 min to 210°C, maintain for 12 min and step four: 5 min to 90°C, maintain for 6 min.

Quality assurance

Analytical results were obtained under quality assurance conditions supported by the requirements described in EN ISO/IEC 17025:2017 [36], such as precision, accuracy, limit of quantification (LOQ), limit of detection (LOD), selectivity, spiked samples, and an external quality control (EQC) program (Table 1). All samples were analyzed in triplicate. Results were expressed as the mean of triplicates and the respective standard deviation.

Table 1. Dietary reference values (DRV) for essential elements

Nutrient	Gender	AI	AR	References
Cu	Male	1.6 mg/day	-	[7]
I	Both genders	150 µg/day	-	[37]
Mn	Both genders	3 mg/day	-	[11]
Mo	Both genders	65 µg/day	-	[10]
Se	Both genders	70 µg/day	-	[38]
Zn	Male	-	11 mg/day*	[39]

*: levels of phytate intake (LPI)—900 mg/day; AI: adequate intake; AR: average requirement

Dietary reference values

The DRV of a healthy adult (≥ 18 years old) for each essential element was obtained from European Food Safety Authority (EFSA) and is presented in Table 1 [7, 10, 11, 37–39]. Values for males were used when there was a sex-based difference, and for Zn, the DRV value reflects a consumption level of 900 mg/day of phytate intake [39].

The contribution of each food for the daily intake of micronutrients (% DRV) was calculated assuming a portion size of 100 g/day for all foodstuffs, according to the following equation:

$$\% DRV = \frac{[EE]}{DRV} \times 100$$

where, % DRV—contribution of each foodstuff for the daily intake of micronutrients in percentage, [EE]—concentration of essential element in mg/100g, DRV—dietary reference values in mg/day.

Results

Table 2 presents the performance metrics attained under rigorous quality control conditions. The working ranges established for each element align well with the characteristics of the matrices studied, ensuring precise and accurate quantification within specified limits. Across all elements, repeatability, expressed as relative standard deviation (RSD), remained consistently below 10%, underscoring the high precision of the analytical measurements. Uncertainties were calculated annually based on analytical internal quality control data from various matrices, demonstrating a robust and reliable performance. The laboratory participated in EQC programs for all elements in similar foods (milk powder, cereal, biscuit breakfast, and rice), excluding Li and Sr, for which EQC programs were unavailable. The Z-scores obtained translate a deviation from the expected value, within the acceptability criteria. Notably, spiked sample recovery rates fell within the range of 80% to 120%, affirming the accuracy of the measurements.

Table 2. Figures of merit

Elements	LOD ($\mu\text{g/L}$)	LOQ ($\mu\text{g/L}$)	Work range ($\mu\text{g/L}$)	Repeatability (RSD, %)	Recovery (%)	Uncertainty ⁽¹⁾ (%)	EQC (Z-score)
As	0.06	0.21	0.25–2.5	≤ 8.3	89–119	21–25	–0.1
Cd	0.03	0.09	0.25–2.5	≤ 5.6	93–117	20–25	–1.0
Co	0.06	0.19	0.25–2.5	≤ 5.4	87–107	18–23	0.2
Cr	0.13	0.42	0.5–5.0	≤ 10	94–102	20–28	1.9
Cu	0.11	0.35	0.5–5.0	≤ 8.3	93–104	16–20	0.9
I	0.27	0.90	1–10	≤ 8.4	89–111	17–27	–0.1
Li	0.06	0.19	0.25–2.5	≤ 8.4	100–114	26	n.a.
Mn	0.12	0.42	0.5–5.0	≤ 8.0	88–112	22–26	0.7
Mo	0.10	0.34	0.5–5.0	≤ 7.0	104–120	21–30	0.2
Pb	0.11	0.37	0.5–5.0	≤ 3.2	93–114	22–32	–0.5
Se	0.13	0.44	0.5–5.0	≤ 7.8	84–107	21–24	0.1
Sr	0.12	0.41	0.5–5.0	≤ 7.6	95–117	20–23	n.a.
Zn	1.1	3.7	5–50	≤ 9.8	81–99	22–29	–0.8

⁽¹⁾ Expanded uncertainty (coverage factor of 2). EQC: external quality control; LOD: limit of detection; LOQ: limit of quantification; n.a.: not available; RSD: relative standard deviation

This study aimed to analyze the concentration of various elements in different food groups, covering potentially harmful elements and essential nutrients. The analyses included five food groups: Grains and grain-based products; Dairy products; Products for non-standard diets; Pulses, dried fruits, and oilseeds; and Fruiting vegetables. The results are presented in two tables. Table 3 focuses on essential elements (Cu, I, Mn, Mo, Se, Zn) along with their contribution to the % DRV for adults. Table 4 covers non-essential and toxic elements (As, Cd, Cr, Co, Li, Sr).

Table 3. Levels of essential elements (mg/kg) in foods consumed by vegetarian populations

Food group	Food item	Cu				I				Mn				Mo				Se				Zn			
		Min	Max	Mean ± SD	% DRV	Min	Max	Mean ± SD	% DRV	Min	Max	Mean ± SD	% DRV	Min	Max	Mean ± SD	% DRV	Min	Max	Mean ± SD	% DRV	Min	Max	Mean ± SD	% DRV
Grains and grain-based products	Buckwheat			5.04 ± 0.06	31.5			< LOQ (0.010)	-			15.3 ± 0.4	51.1			0.48 ± 0.01	74.2			0.033 ± 0.001	4.69			21.5 ± 0.4	37.5
	Bulgur wheat	3.76	5.04	4.8 ± 0.3	29.9	-	-	0.0132 ± 0.0004	0.88	5.2	28	13.7 ± 0.3	45.8	0.113	0.77	0.58 ± 0.02	89.1	0.033	0.057	0.035 ± 0.001	4.94	19.9	27.9	22 ± 1	53.5
	Spelt flour			4.3 ± 0.2	26.8			< LOQ (0.010)	-			28 ± 1	92.9			0.77 ± 0.04	118			0.057 ± 0.003	8.18			27.9 ± 0.7	35.1
	Millet			3.76 ± 0.05	23.5			< LOQ (0.047)	-			5.2 ± 0.1	17.3			0.113 ± 0.003	17.5			0.035 ± 0.001	4.98			19.9 ± 0.3	29.3
Dairy products	Fresh semi-fat cow's cheese			0.17 ± 0.02	1.04			0.28 ± 0.01	18.7			0.107 ± 0.009	0.36			0.079 ± 0.004	12.2			0.111 ± 0.006	15.9			15.9 ± 0.7	14.5
	Fresh low-fat cow's cheese			0.179 ± 0.009	1.12			0.52 ± 0.02	34.9			0.122 ± 0.001	0.41			0.074 ± 0.005	11.5			0.091 ± 0.007	13.1			15.9 ± 0.2	14.5
	Fresh goat cheese	0.071	0.339	0.34 ± 0.03	2.12	0.144	0.534	0.534 ± 0.005	35.6	0.039	0.42	0.42 ± 0.02	1.40	0.021	0.079	0.021 ± 0.001	3.18	0.024	0.125	0.125 ± 0.003	17.9	2.77	15.9	14 ± 1	12.5
	Unsweetened natural Greek yogurt			0.071 ± 0.003	0.45			0.172 ± 0.003	11.5			0.039 ± 0.001	0.13			0.056 ± 0.001	8.67			0.027 ± 0.001	3.81			2.83 ± 0.08	2.57
	Greek yogurt with fruit			0.085 ± 0.005	0.53			0.144 ± 0.001	9.59			0.37 ± 0.01	1.24			0.074 ± 0.004	11.3			0.024 ± 0.001	3.43			2.77 ± 0.09	2.52
Products for non-standard diets	Miso			3.5 ± 0.1	22.1			< LOQ (0.047)	-			9.4 ± 0.3	31.2			4.11 ± 0.09	12.2			0.31 ± 0.01	44.8			12.6 ± 0.5	11.4
	Textured soy protein			19.5 ± 0.9	122			< LOQ (0.010)	-			44 ± 2	148			0.16 ± 0.01	11.5			0.142 ± 0.003	20.3			52 ± 4	46.9
	Soy yogurt			1.24 ± 0.05	7.72			< LOQ (0.010)	-			2.5 ± 0.1	8.28			0.199 ± 0.002	3.18			< LOQ (0.020)	1.78			2.2 ± 0.1	2.00
	Soy sausage	1.24	19.5	2.04 ± 0.05	12.8	0.027	0.114	0.027 ± 0.001	1.79	2.44	44	3.31 ± 0.05	11.1	0.124	4.11	0.229 ± 0.003	8.67	0.067	0.31	0.15 ± 0.01	21.5	2.2	52	6.3 ± 0.3	5.76
	Seitan			2.25 ± 0.01	14.1			0.114 ± 0.001	7.61			6.8 ± 0.1	22.5			0.124 ± 0.001	11.3			0.067 ± 0.001	9.56			12.97 ± 0.02	11.8
	Soy dessert			1.61 ± 0.03	10.1			< LOQ (0.010)	-			2.44 ± 0.04	8.12			0.59 ± 0.02	35.6			< LOQ (0.020)	2.01			3.29 ± 0.08	2.99
Pulses, dried fruits and oilseeds	Chia seeds			17.7 ± 0.5	111			< LOQ (0.010)	-			56 ± 2	186			0.557 ± 0.01	85.8			0.636 ± 0.003	90.9			63 ± 6	57.3
	Brazil nuts			18.6 ± 0.9	116			< LOQ (0.010)	-			12.1 ± 0.5	40.3			0.025 ± 0.001	3.91			2.15 ± 0.07	307			41 ± 2	37.2
	Pumpkin seeds			10.5 ± 0.2	65.7			< LOQ (0.047)	-			34 ± 1	115			1.77 ± 0.07	273			0.14 ± 0.01	19.6			41.2 ± 0.5	37.5
	Sunflower seeds			19.4 ± 0.3	122			< LOQ (0.047)	-			23.7 ± 0.3	79.0			0.29 ± 0.01	44.9			0.127 ± 0.007	18.2			58.8 ± 0.3	53.5
	Red lentils (dry)			8.4 ± 0.2	52.6			< LOQ (0.047)	-			10.9 ± 0.4	36.5			2.6 ± 0.1	404			0.166 ± 0.004	23.6			38.6 ± 0.8	35.1
	White bean	6.5	19.4	9.5 ± 0.6	59.1	-	-	< LOQ (0.047)	-	10.9	56	17 ± 1	56.3	0.025	5.4	4.1 ± 0.1	624	0.028	2.15	0.17 ± 0.01	24.1	24	63	32 ± 3	29.3
	Catarino bean			7.1 ± 0.2	44.5			< LOQ (0.047)	-			15.3 ± 0.3	50.9			1.11 ± 0.04	171			0.028 ± 0.002	3.98			27.3 ± 0.4	24.8
	Red bean			7.4 ± 0.4	46.2			< LOQ (0.047)	-			12.4 ± 0.2	41.5			5.4 ± 0.3	823			0.151 ± 0.006	21.6			29 ± 2	26.1
	Black-eyed pea			7.5 ± 0.1	47.0			< LOQ (0.047)	-			12.8 ± 0.2	42.8			1.22 ± 0.07	187			0.49 ± 0.03	70.6			34.2 ± 0.9	31.1
	Butter bean			6.5 ± 0.3	40.7			< LOQ (0.047)	-			13.9 ± 0.6	46.3			2.07 ± 0.04	319			0.085 ± 0.002	12.1			30 ± 1	27.6
Black bean			7.7 ± 0.2	48.4			n.a.				13.2 ± 0.6	44.1			1.6 ± 0.2	248			0.088 ± 0.003	12.6			24 ± 2	21.7	
Fruiting vegetables	Goji berries	-	-	7.9 ± 0.3	49.2	-	-	0.0179 ± 0.0002	1.19	-	-	8.1 ± 0.4	27.1	-	-	0.232 ± 0.008	35.6	-	-	0.032 ± 0.002	4.52	-	-	10.9 ± 0.4	9.93

% DRV: contribution of each foodstuff for the daily intake of micronutrients in percentage; LOQ: limit of quantification

Table 4. Levels of non-essential and toxic elements (mg/kg) in foods consumed by vegetarian populations

Food group	Food item	As			Cd			Cr			Co			Li			Sr		
		Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD
Grains and grain-based products	Buckwheat			< LOQ (0.010)			0.0248 ± 0.0004			0.091 ± 0.002			0.059 ± 0.002			< LOQ (0.010)			0.39 ± 0.01
	Bulgur wheat	-	-	0.0124 ± 0.0001	0.0137	0.027	0.0137 ± 0.0003	0.0409	0.142	0.142 ± 0.005	0.0106	0.059	0.0113 ± 0.0004	-	-	0.026 ± 0.001	0.187	2.9	2.9 ± 0.1
	Spelt flour			< LOQ (0.010)			0.027 ± 0.001			0.076 ± 0.005			0.0106 ± 0.0003			< LOQ (0.010)			1.33 ± 0.03
	Millet			< LOQ (0.010)			< LOQ (0.010)			0.0409 ± 0.0004			0.053 ± 0.002			< LOQ (0.010)			0.187 ± 0.007
Dairy products	Fresh semi-fat cow's cheese			0.0148 ± 0.0009			< LOQ (0.010)			0.11 ± 0.01			< LOQ (0.010)			< LOQ (0.010)			1.24 ± 0.09
	Fresh low-fat cow's cheese			0.0159 ± 0.0003			< LOQ (0.010)			0.063 ± 0.003			< LOQ (0.010)			< LOQ (0.010)			1.35 ± 0.02
	Fresh goat cheese	0.0148	0.021	0.021 ± 0.002	-	-	< LOQ(0.010)	0.023	0.119	0.119 ± 0.008	-	-	< LOQ (0.010)	-	-	0.012 ± 0.001	0.457	2.5	2.5 ± 0.1
	Unsweetened natural Greek yogurt			< LOQ (0.010)			< LOQ (0.010)			0.023 ± 0.002			< LOQ (0.010)			< LOQ (0.010)			0.457 ± 0.002
	Greek yogurt with fruit			< LOQ (0.010)			< LOQ (0.010)			0.0285 ± 0.0005			< LOQ (0.010)			< LOQ (0.010)			0.62 ± 0.04
Products for non-standard diets	Miso			0.069 ± 0.003			0.012 ± 0.001			0.294 ± 0.001			0.0204 ± 0.0004			0.038 ± 0.001			5.0 ± 0.2
	Textured soy protein			0.0105 ± 0.0004			0.041 ± 0.001			0.285 ± 0.009			0.204 ± 0.003			0.0127 ± 0.0001			6.8 ± 0.3
	Soy yogurt			< LOQ (0.010)			< LOQ (0.010)			< LOQ (0.020)			< LOQ (0.010)			0.0149 ± 0.0005			0.79 ± 0.05
	Soy sausage	0.0105	0.069	0.028 ± 0.002	0.0173	0.041	< LOQ (0.010)	0.124	0.294	0.124 ± 0.009	0.0151	0.204	0.0151 ± 0.0003	0.0127	0.038	0.036 ± 0.001	0.79	6.85	3.08 ± 0.02
	Seitan			0.0191 ± 0.0009			0.0173 ± 0.0002			0.125 ± 0.006			< LOQ (0.010)			0.0251 ± 0.0002			1.27 ± 0.02
	Soy dessert			< LOQ (0.010)			< LOQ (0.010)			0.176 ± 0.005			0.031 ± 0.001			0.01746 ± 0.00003			1.02 ± 0.01
Pulses, dried fruits and oilseeds	Chia seeds			< LOQ (0.010)			< LOQ (0.010)			0.31 ± 0.03			0.28 ± 0.01			0.022 ± 0.001			43 ± 2
	Brazil nuts			< LOQ (0.010)			< LOQ (0.010)			0.27 ± 0.03			0.94 ± 0.03			< LOQ (0.010)			198 ± 8
	Pumpkin seeds			0.0183 ± 0.0005			< LOQ (0.010)			0.31 ± 0.02			0.054 ± 0.002			0.018 ± 0.001			7.4 ± 0.3
	Sunflower seeds			< LOQ (0.010)			0.29 ± 0.01			0.22 ± 0.02			0.088 ± 0.004			< LOQ (0.010)			3.1 ± 0.1
	Red lentils (dry)			< LOQ (0.010)			< LOQ (0.010)			0.056 ± 0.002			0.093 ± 0.003			0.017 ± 0.001			0.98 ± 0.04
	White bean	-	-	< LOQ (0.010)	-	-	< LOQ (0.010)	0.056	0.35	0.114 ± 0.005	0.054	0.94	0.213 ± 0.003	0.017	0.027	< LOQ (0.010)	0.98	198	9.1 ± 0.3
	Catarino bean			< LOQ (0.010)			< LOQ (0.010)			0.35 ± 0.03			0.212 ± 0.008			< LOQ (0.010)			6.7 ± 0.3
	Red bean			< LOQ (0.010)			< LOQ (0.010)			0.291 ± 0.009			0.179 ± 0.009			0.027 ± 0.002			6.8 ± 0.4
	Black-eyed pea			< LOQ (0.010)			< LOQ (0.010)			0.15 ± 0.01			0.115 ± 0.006			< LOQ (0.010)			3.4 ± 0.1
	Butter bean			< LOQ (0.010)			< LOQ (0.010)			0.086 ± 0.003			0.26 ± 0.02			0.018 ± 0.001			8.4 ± 0.6
	Black bean			< LOQ (0.010)			< LOQ (0.010)			0.23 ± 0.02			0.192 ± 0.005			< LOQ (0.010)			4.6 ± 0.4
Fruiting vegetables	Goji berries	-	-	0.019 ± 0.001	-	-	0.037 ± 0.002	-	-	0.105 ± 0.004	-	-	0.047 ± 0.003	-	-	1.10 ± 0.03	-	-	4.5 ± 0.3

LOQ: limit of quantification

Pb levels were below the LOQ (0.020 mg/kg) in all samples except for goji berries (0.025 ± 0.001 mg/kg) and, therefore, were not included in [Table 4](#).

Discussion

Copper

In all food samples analyzed, the Cu levels ranged from 0.071 ± 0.003 mg/kg in unsweetened natural Greek yogurt to 19.4 ± 0.3 mg/kg in sunflower seeds and 19.5 ± 0.9 mg/kg in textured soy protein. This study found the highest % DRV values for Cu in samples of textured soy protein and sunflower seeds with 122%, followed by Brazil nuts with 116%. As such, the consumption of 100 g/day of these foodstuffs exceeds the DRV value of Cu ([Table 1](#)). On the other hand, the lowest % DRV values for Cu were found in Dairy products, which ranged from 0.45% unsweetened natural Greek yogurt to 2.12% fresh goat cheese. This means that the consumption of 100 g/day of these food samples alone is deficient in achieving the DRV value of Cu ([Table 1](#)).

The copper levels observed in this study are largely in line with the literature and FCDs, strongly validating our findings [[40–42](#)]. However, there is an exception for textured soy protein (19.5 ± 0.9 mg/kg), which is higher in the present study than reported in Australian FCD (2.3 mg/kg) [[40](#)].

Iodine

Approximately 65% of the food samples analyzed had an I content below the LOQ. The quantified samples ranged between 0.0132 ± 0.0004 mg/kg (bulgur wheat) and 0.534 ± 0.005 mg/kg (fresh goat cheese). Within quantifiable samples the contributions to the DRV were in general low, varying between 0.88% in bulgur wheat and 35.6% in fresh goat cheese. The consumption of 100 g/day of each food sample is deficient in supplying the DRV value of I ([Table 1](#)).

The I levels observed in this study for all the analyzed samples align with the values previously reported in other FCDs [[40–44](#)].

Except for the Dairy products group, the foodstuffs that integrate vegetarian diets are known for not having high iodine content in their constitution [[45, 46](#)]. Therefore, including other methods for obtaining iodine becomes essential for public health. Strategies such as fortifying crops or other foodstuffs are already being applied in different countries. Also, a worldwide proposed solution is the use of iodized salt during food preparation, thus increasing the consumption of this element [[47](#)].

Manganese

The level of Mn in the analyzed samples ranged from 0.039 ± 0.001 mg/kg (unsweetened natural Greek yogurt) to 56 ± 2 mg/kg (chia seeds). The main contributors to the DRV of Mn are chia seeds, textured soy protein, and pumpkin seeds, with a % DRV of 186%, 148%, and 115%, respectively, which means that the intake of 100 g/day of these foodstuffs exceeds the DRV value of Mn ([Table 1](#)). On the other hand, the lowest levels of Mn were observed in the Dairy products. The samples that contribute the least to the DRV of Mn are unsweetened natural Greek yogurt and fresh semi-fat cow's cheese, with contributions of 0.13% and 0.36%, respectively; meaning that the consumption of 100 g/day of this food group is deficient to the DRV value of Mn ([Table 1](#)).

The Mn levels observed in this study for the majority of the analyzed samples are in line with values reported in FCDs. Textured soy protein (44 ± 2 mg/kg) and soy yogurt samples (2.5 ± 0.1 mg/kg) are an exception since values reported in the Australian FCD (6 mg/kg and 0.9 mg/kg, respectively) are lower than the present study [[40–42](#)].

Molybdenum

The Mo levels in all analyzed food groups ranged from 0.021 ± 0.001 mg/kg (fresh goat cheese) to 5.4 ± 0.3 mg/kg (red bean). The principal contributors to the DRV of Mo belong to the Pulses, dried fruits, and oilseeds food group, with a % DRV of 823% (red bean), 624% (white bean), 404% (dry red lentils), 319%

(butter bean), and 273% (pumpkin seeds). These results provide evidence that the intake of 100 g/day of these pulses significantly exceeds the DRV value of Mo (Table 1), although the tolerable upper intake level of Mo is 2 mg/day. Dairy products and Products for non-standard diets food groups along with Brazil nuts presented the lowest % DRV of Mo.

The Mo values are aligned with those reported in the Danish, Australian, and American FCDs, except for the miso sample (4.11 ± 0.09 mg/kg) and soy yogurt sample (0.199 ± 0.002 mg/kg), which are higher in the present study. The reported values for these samples in the Australian FCD are 0.83 mg/kg and 0.052 mg/kg, respectively [40–42].

Selenium

The level of Se was below the LOQ (0.020 mg/kg) in two samples, namely soy yogurt and soy desert. The quantifiable samples ranged from 0.024 ± 0.001 mg/kg (Greek yogurt with fruit) to 2.15 ± 0.07 mg/kg (Brazil nuts). The principal contributor to the DRV of Se is the Brazil nuts sample, with a contribution of 307%; this result provides evidence that the intake of 100 g/day of Brazil nuts significantly exceeds the DRV value of Se but does not reach the upper limit of 300 µg/day for adults [48] (Table 1).

The Se values align with those reported in the Danish, Australian, and American FCDs for most analyzed samples. On the other hand, buckwheat (0.033 ± 0.001 mg/kg), millet (0.035 ± 0.001 mg/kg), seitan (0.067 ± 0.001 mg/kg) and Brazil nuts (2.15 ± 0.07 mg/kg) revealed lower values of Se than other FCDs (0.2 mg/kg, 0.16 mg/kg, 0.318 mg/kg, and 19.2 mg/kg respectively). In contrast, miso (0.31 ± 0.01 mg/kg), white bean (0.17 ± 0.01 mg/kg), and red bean (0.151 ± 0.006 mg/kg) samples showed values higher than other FCDs (0.010 mg/kg, 0.0227 mg/kg, and 0.040 mg/kg respectively) [40–42].

Zinc

The Zn values in the present study ranged from 2.2 ± 0.1 mg/kg (soy yogurt) to 63 ± 6 mg/kg (chia seeds). The main contributors to the DRV of Zn are chia seeds, sunflower seeds, and bulgur wheat, with contributions of 57.3%, 53.5%, and 53.5%, respectively. However, the consumption of 100 g/day of these samples alone is not sufficient to achieve the DRV of Zn. On the other hand, the lowest contributors to the DRV of Zn were soy dessert, soy yogurt, Greek yogurt with fruit, and unsweetened natural Greek yogurt samples, with % DRV below 3%.

The Zn results for all analyzed samples are in agreement with those reported in the Danish, Australian, and American FCDs, except for the textured soy protein sample (52 ± 4 mg/kg), whose value was reported in the Australian FCD as 14 mg/kg [40–42].

Arsenic

The levels of As were below the LOQ (0.010 mg/kg) in 63% of the analyzed samples. The highest level of As was found in miso with a reported concentration of 0.069 ± 0.003 mg/kg. All Grains and grain-based products were below the LOQ, except bulgur wheat (0.0124 ± 0.0001 mg/kg). Similar values can be found in the literature for bulgur wheat (0.012 mg/kg), but the ones available for millet (0.079 mg/kg) and buckwheat (0.061 mg/kg) are higher compared to the current study [40, 41]. Amongst the food group Pulses, dried fruits, oilseeds, and spices, only pumpkin seeds showed quantifiable values (0.0183 ± 0.0005 mg/kg), in line with those reported in the Australian FCD (0.019 mg/kg) [41].

As is classified as a group I carcinogen [49]. Its toxicity is highly dependent on the chemical species present in foodstuffs. Currently, there is no legally established maximum level in any of the matrices under study for total or inorganic As. Observing the low As levels found for all matrices under study, there is no need to pursue further speciation studies.

Cadmium

The levels of Cd were below the LOQ (0.010 mg/kg) in 70% of the analyzed samples, including in all Dairy products.

From the food group Pulses, dried fruits, and oilseeds only sunflower seeds presented quantifiable values (0.29 ± 0.01 mg/kg), with results higher than the ones reported in the literature ($0.17\text{--}0.19$ mg/kg) [40, 41].

Following sunflower seeds, the highest levels of Cd were in textured soy protein (0.041 ± 0.001 mg/kg) followed by goji berries (0.037 ± 0.002 mg/kg). In Grains and grain-based products, the results varied from below the LOQ (0.010 mg/kg) in millet to 0.027 mg/kg in spelt flour. These results are lower than the ones reported in FCD, which range from 0.0281 mg/kg in millet to 0.045 mg/kg in spelt flour [40].

Cd is a carcinogen compound for humans, and food is the main route of exposure for the non-smoking population. The EFSA established a tolerable weekly intake (TWI) for Cd of 2.5 $\mu\text{g}/\text{kg}$ body weight [25]. Sunflower seeds showed the highest Cd level in the present study. An adult of 70 kg would have to consume over 0.6 kg of this food item to exceed the TWI.

Chromium

The levels of Cr were above the LOQ (0.020 mg/kg) for all samples except soy yogurt. The unsweetened natural Greek yogurt sample had the lowest Cr value (0.023 ± 0.002 mg/kg), while the highest level of Cr was observed in the Catarino bean (0.35 ± 0.03 mg/kg).

The Cr values observed in this study align with those reported in the Danish and Australian FCDs for most of the analyzed samples. However, regarding buckwheat (0.091 ± 0.002 mg/kg), fresh goat cheese (0.119 ± 0.008 mg/kg), Greek yogurt with fruit (0.0285 ± 0.0005 mg/kg), seitan (0.125 ± 0.006 mg/kg), miso (0.294 ± 0.001 mg/kg), pumpkin seeds (0.31 ± 0.02 mg/kg), and white bean (0.114 ± 0.005 mg/kg), this study revealed higher values of Cr than the Danish and Australian FCDs (0.014 mg/kg, 0.0031 mg/kg, 0.0005 mg/kg, 0.040 mg/kg, 0.028 mg/kg, 0.080 mg/kg, and 0.0586 mg/kg, respectively) [40, 41].

Cobalt

The Co amounts were below the LOQ (0.010 mg/kg) in 25% of the analyzed samples, including all analyzed Dairy products. The lowest level was observed in spelt flour (0.0106 ± 0.0003 mg/kg), while the highest amount of Co was present in the Brazil nuts (0.94 ± 0.03 mg/kg). The majority of the analyzed samples were higher when compared to similar studies reported in the literature [50–52]. However, Lahhob et al. [53] state that humans consume between 5 and 50 $\mu\text{g}/\text{day}$.

Regarding Co amounts in Grain and grain products, the samples included in this food group are within the range reported by Udeh et al. [54].

Exposure to high levels of Co could be hazardous to human health. Therefore, the International Agency for Research on Cancer (IARC) has classified cobalt and cobalt compounds without tungsten as Group 2B—possibly carcinogenic to humans, while cobalt with tungsten as Group 2A—probably carcinogenic to humans [55].

Lithium

Around 42% of the food samples analyzed were below the LOQ (0.010 mg/kg). Within the quantifiable samples, the lowest result was found in fresh goat cheese (Dairy products), with 0.012 ± 0.001 mg/kg, and the highest mean levels were found in goji berries (Fruiting vegetables), with 1.10 ± 0.03 mg/kg. The sample of bulgur wheat achieved 0.026 ± 0.001 mg/kg and was the only sample above the LOQ within the food group Grains and grain-based products. On the other hand, the food group Products for non-standard diets presented all samples with levels of Li higher than the LOQ.

The levels of Li observed in this study are lower than those reported in the literature. Szklarska et al. [17], refers to Li content for different food groups such as Cereals with 4.4 mg/kg, Vegetables with 2.3 mg/kg, Dairy products with 0.5 mg/kg, and Nuts with 8.8 mg/kg. On the other hand, Iordache et al. [56] showed that in Pulses, dried fruits, oilseeds, and spices such as pulses, they found 1.08 ± 1.08 mg/kg, and in Dairy products, they found 0.35 ± 0.55 mg/kg, in Pulses, dried fruits, oilseeds and spices such as beans they found 2.66 ± 1.10 mg/kg, lentils 1.89 ± 1.19 mg/kg.

Strontium

The levels of Sr ranged from 0.187 ± 0.007 mg/kg (millet) to 198 ± 8 mg/kg (Brazil nuts). The concentration of Sr in food is highly influenced by geographical location, in particular concerning the levels of Sr in soils [18].

The result obtained for millet samples can be compared with the findings of Udeh et al. [54], who analyzed various millet varieties and reported significantly higher Sr concentrations than those observed in this study, with values ranging from 25–33 mg/kg.

Dairy products showed Sr concentrations ranging from 0.457 ± 0.002 mg/kg in unsweetened natural Greek yogurt to 2.5 ± 0.1 mg/kg in fresh goat cheese. The results obtained are consistent with those observed in the literature, for cheeses from the Canary Islands, mainland Spain, and Europe, which were between 1.59 and 7.73 mg/kg [57].

In the Pulses, dried fruits and oilseeds category, chia seeds and Brazil nuts showed the highest Sr concentrations at 43 ± 2 mg/kg and 198 ± 8 mg/kg, respectively. According to Lemire et al. [58], the Sr concentration in Brazil nuts from four communities in Brazil reported a maximum value of 172.6 mg/kg, similar to the one obtained in this study.

According to Bertoldi et al. [59], Sr content in Italian goji berries ranged between 0.40 and 10.20 mg/kg, while in non-Italian goji berries, ranged between 1.26 and 13.05 mg/kg. Compared to these findings, our study's goji berries (4.5 ± 0.3 mg/kg), are in the range reported by Bertoldi et al. [59].

Based on the analysis of trace elements in various food groups, this study provides insights into the levels of essential nutrients and potentially harmful elements in commonly consumed plant-based and dairy products within the Portuguese diet. The findings demonstrate that while the consumption of 100 g of certain foods, such as chia seeds, textured soy protein, and Brazil nuts, significantly exceeds the DRV for essential elements like Cu, Mn, Mo, and Se, others, particularly within dairy products, fall short of these nutritional benchmarks. These results highlighted the importance of dietary diversity and the potential need for supplementation in vegetarian and vegan diets to ensure adequate nutrient intake. Nonetheless, the present study covers a limited number of food items. Similar studies must continue, to increase the number and diversity of foodstuffs analyzed thus covering the majority of food items commonly consumed in vegetarian diets that also contribute to the DRV.

The study also emphasizes the relatively low levels of toxic elements such as Pb, As, and Cd in most analyzed food samples, with only a few exceptions like goji berries and sunflower seeds. However, these elements are not dangerous to human health at the concentrations found in the foods tested.

The majority of these results align with existing FCDs from other countries. Characterizing the analyzed micronutrients (Cu, I, Mn, Se, Mo, and Zn) in the plant-based and dairy products consumed by the Portuguese population was fundamental to bridging the existing gap in the Portuguese FCD. Overall, this research underscores the importance of regular dietary assessments and the role of FCDs and TDS in safeguarding public health by ensuring both nutritional adequacy and safety in the food supply.

Abbreviations

% DRV: contribution of each foodstuff for the daily intake of micronutrients in percentage

DRV: dietary reference value

EQC: external quality control

FCD: Food Composition Database

ICP-MS: Inductively Coupled Plasma Mass Spectrometry

LOQ: limit of quantification

TDS: Total Diet Studies

TMAH: tetramethylammonium hydroxide

Declarations

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Author contributions

SG, AR, ID, MV: Conceptualization, Investigation, Writing—original draft, Writing—review & editing. IC: Conceptualization, Investigation, Writing—original draft, Writing—review & editing, Supervision. All authors read and approved the submitted version.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publication

Not applicable.

Availability of data and materials

Data from the present manuscript will be made available upon request.

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