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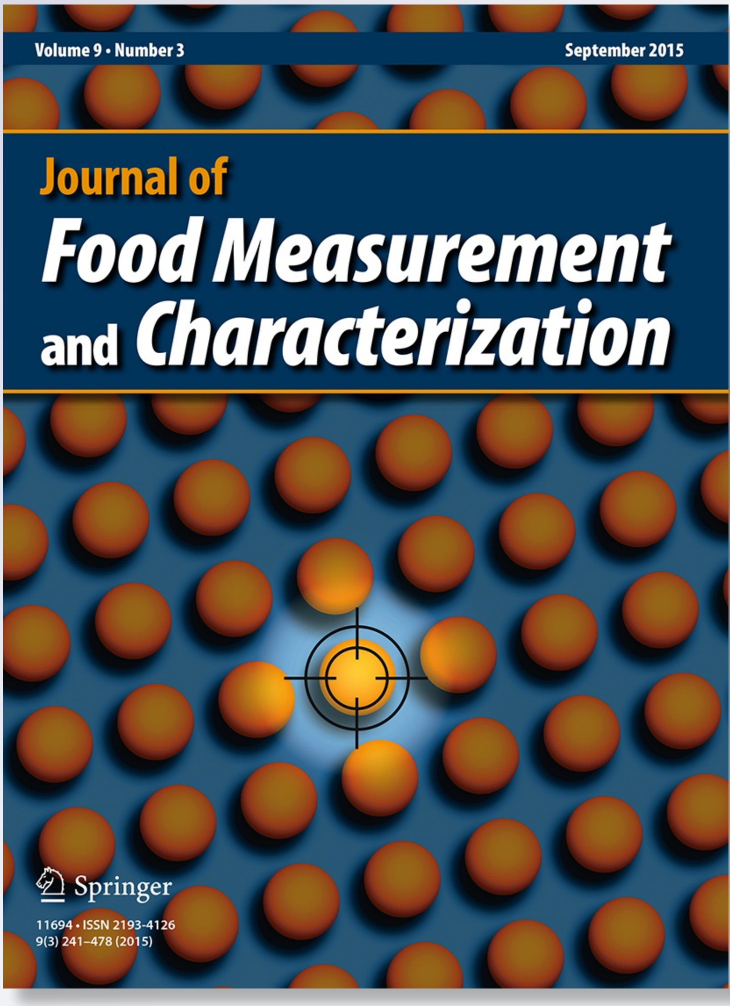

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ORIGINAL PAPER

Trace element concentrations in commercial gluten-free amaranth bars

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Abstract Gluten-free foods are specially designed to be consumed by people who suffer from celiac disease or some type of gluten intolerance. Few studies are currently available about trace element contents in gluten-free foods. This study aimed at analyzing the concentrations of 15 trace elements in commercial gluten-free amaranth bars by inductively coupled plasma optical emission spectrometry, after wet digestion by microwave system. The accuracy of the method was determined by the use of a standard reference material (NIST SRM 1570a Spinach Leaves) and recovery experiments, obtaining satisfactory values in all cases. The contents of the investigated trace elements in gluten-free amaranth bars were found to be in the range: Al (3.7–8.1 μg/g), Co (0.02–0.07 μg/g), Cr (0.05–0.25 μg/g), Cu (1.80–6.12 μg/g), Fe (7.0–14.5 μg/g), Mn (6.5–10.6 μg/g), Ni (0.30–0.78 μg/g), Pb (0.25–1.12 μg/g), Sr (1.4–2.7 μg/g), and Zn (4.7–9.8 μg/g). Some of the essential trace elements were found to have good nutritional contribution in accordance to daily nutrient recommendations for adults. The concentrations of non-essential elements such as Al, Ni and Sr, were very low in all samples. The levels of toxic elements such as As, Cd, Sb and Tl were not detected in any of the samples, and do not pose any threat to consumers. However, the Pb concentrations were above the safety limits in 8.9 % of the studied samples. Therefore, regular monitoring of this toxic metal should be conducted in the future, to ensure the quality of the gluten-free products for human health.

Keywords Snacks · Pseudo-cereal · ICP-OES · Nutrition · Celiac disease

Introduction

Cereal bars are considered a convenient and nutritious food for people who are concerned about their health. This food consists of cereal grains joined with a binder, forming a ready-to-eat snack with functional activity, which is available in a great variety of sizes and shapes to the consumer. Currently, there is an increasing interest in gluten-free cereal bars beyond their micronutrient contribution as a fortified food [1]. In this context, there is a growing attention to use alternative grains to fabricate high-quality gluten-free products, aiming at high nutritional values to attend dietary needs of people who follow a gluten-free diet [2].

A gluten-free diet must be strictly followed by people with gluten-related disorders. There are various types of gluten-related disorders, including celiac disease, gluten sensitivity, and wheat allergy. In the present, celiac disease is the most common lifelong dietary disorder worldwide. Celiac disease is a complex autoimmune enteropathy that occurs in genetically predisposed individuals following the inclusion of gluten in the diet. The reaction to gluten ingestion is an inflammation of the small intestine leading to the malabsorption of several important nutrients,

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including minerals (iron, calcium) and vitamins (folic acid) [3, 4]. Today, there is no cure for celiac disease and the only therapy is a strict gluten-free diet for life [5].

In recent years, the use of pseudo-cereals in gluten-free cereal bars has received much attention, especially the use of amaranth grains, due to their high nutritional composition, functional properties and low content of prolamins, which are the toxic proteins for celiacs [6]. Amaranth grains are considered a good source of minerals such as Fe, Mg and Ca [7]. This high content of essential minerals becomes important because gluten-free products provide poor contributions of these elements to the intake.

The food industry commonly uses unenriched flour instead of whole amaranth grains to prepare the cereal bars. Additionally, these cereal bars are elaborated together with other ingredients, which may offer poor contribution of minerals. Therefore, it is very important to control the trace element concentrations in the commercial gluten-free amaranth bars for two main reasons: (1) to provide actual information to consumers and nutritionists to support health conscious food choices, and (2) to corroborate the levels of toxic elements, which can affect the consumer health.

Several analytical techniques have been used to quantify trace element contents in food samples. Atomic absorption spectrometry (FAAS, GFAAS), atomic emission spectrometry (ICP-OES), and atomic mass spectrometry (ICP-MS) studies are reported most frequently [8–10]. In particular, ICP-OES is reliable and robust, allowing the simultaneous determination of major and trace elements in a broad, dynamic measurement range from µg/g to percentages with high linearity [11, 12].

Much information is available on the nutritional composition of amaranth seeds, but data on the trace element contents of gluten-free amaranth bars is limited. The aim of the present work was to measure 15 trace element concentrations (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sb, Se, Sr, Tl and Zn) in commercial gluten-free amaranth bars by the ICP-OES technique, after digestion in a closed-vessel microwave oven. The results obtained were compared with the critical levels specified by the Food and Nutrition Board for dietary intakes of nutritional elements [13] and by the European Food Safety Authority (EFSA) for non-essential elements [14–17].

Materials and methods

Sample collection

A total of 136 commercial gluten-free amaranth bars were purchased from the local Argentinean market at various times (between 2012 and 2013) for randomness purpose. The samples represented popular brands available in the market. The labels declared that these products are exported to other Latin-American countries and USA. The amaranth bars differ in their composition, but generally include dehydrated fruits and seeds of other botanical species such as chia, flax or sesame; also, they usually contain basic ingredients such as: crisped rice, sunflower oil, corn syrup, sorbitol, arabic gum, citric acid, among others. The sample characteristics and the declared ingredients are presented in Table 1. Three independent replicates of each sample were analyzed.

Reagents

Ultrapure deionized water (resistivity 18.2 MΩ cm^{-1}) was obtained by using a Milli-Q Pluswater purification system (Millipore Molsheim, France). Ultrapure grade 65 % (m/m) HNO_3 and 30 % (m/m) H_2O_2 were acquired from Sigma (St. Louis, MO, USA). Nitric acid was additionally cleaned by sub-boiling distillation. All materials were nitric acid-washed (10 %, v/v) for at least 3 days and then thoroughly rinsed with ultrapure water before use.

Working standard solutions were prepared by dilution of a multielement standard solution IV for ICP (in 10 % HNO_3) TraceCERT® (Sigma-Aldrich, St. Louis, MO, USA). This solution contains all the selected trace elements considered in the present study except Sb. The working solution of Sb was prepared from a single element standard solution for ICP (1000 µg/mL) TraceCERT® (Sigma-Aldrich, St. Louis, MO, USA).

Apparatus

The acid digestion of the amaranth bar samples was performed using a high-performance microwave digestion oven, Milestone® (Chicago, USA) model Ethos One with programmable power control (maximum power 1600 W) and segmented rotor HPR 1000/10 s (operating pressure up to 35 bar maximum; operating temperature 260 °C maximum) with ten 100 mL tetrafluoromethoxy vessels and a ceramic vessel jacket.

The ICP-OES measurements were performed with a simultaneous ICP spectrometer (VISTA PRO, Varian, Mulgrave, Australia), which was equipped with a solid-state detector. Nebulization of samples was performed with an ultrasonic nebulizer with a desolvation system (U-5000 AT, CETAC Technologies, Omaha, NE). The operating conditions are summarized in Table 2.

Sample preparation

All amaranth bar samples were grinded in a cryogenic mill (Spex model 6750, Metuchen, NJ, USA) to reduce particle sizes and increase the surface area for acid attack. The

Table 1 Gluten-free amaranth bar samples, ingredients and declared nutritional composition

Brand code	Product name	Ingredients	Declared nutritional composition (amount per container 23 g)
G-Apple	Gluten-free amaranth bar with dried green apple and amaranth seeds	Rice crisped yogurt bath, glucose, amaranth expanded, sunflower oil, dehydrated green apple, chia seed, corn bran, sorbitol, Arabic gum, fatty acid monoglycerides, citric acid, apple flavor	Energy value 100 kcal, carbohydrates 17 g, protein 1.2 g, total fat 3 g, saturated fat 1 g, trans fat 0 g, cholesterol 0 mg, dietary fiber 0.7 g, sodium 66 mg
Berries	Gluten-free amaranth bar with berries and whole amaranth seeds	Corn syrup, yogurt bath, amaranth expanded, organic sucrose, rice crisped, sunflower oil, dehydrated raspberry, corn bran, modified starch, Arabic gum, flax seeds, soy flour, citric acid, strawberry flavor	Energy value 90 kcal, carbohydrates 16 g, protein 1.4 g, total fat 2.3 g, saturated fat 0.8 g, trans fat 0 g, cholesterol 0 mg, dietary fiber 0.8 g, sodium 60 mg
R-Apple	Gluten-free amaranth bar with dried red apple pomace	Corn syrup, yogurt bath, sucrose, rice crisped, amaranth expanded, sunflower oil, sorbitol, corn bran, modified starch, arabic gum, lin seed, soy flour, citric acid, apple flavor	Energy value 90 kcal, carbohydrates 16 g, protein 1.4 g, total fat 2.3 g, saturated fat 0.8 g, trans fat 0 g, cholesterol 0 mg, dietary fiber 0.8 g, sodium 60 mg
Lemon	Gluten-free amaranth bar with dehydrated lemon and yogurt	Yogurt bath, sorbitol, rice crisped, amaranth expanded, Arabic gum, sunflower oil, dehydrated lemon, modified tapioca starch, soy flour, citric acid, lemon flavor	Energy value 78 kcal, carbohydrates 15 g, protein 1.4 g, total fat 1.5 g, saturated fat 0.4 g, trans fat 0 g, cholesterol 0 mg, dietary fiber 1.1 g, sodium 35 mg
Peach	Gluten-free amaranth bar with peach and amaranth seeds	Corn syrup, yogurt bath, organic sucrose, amaranth expanded, rice crisped, dehydrated peach, arabic gum, sunflower oil, corn bran, sorbitol, citric acid, peach flavor, lin seeds	Energy value 90 kcal, carbohydrates 16 g, protein 1.4 g, total fat 2.3 g, saturated fat 0.8 g, trans fat 0 g, cholesterol 0 mg, dietary fiber 0.8 g, sodium 60 mg
Straw	Gluten-free amaranth bar with dried strawberry	Glucose syrup, yogurt bath, organic sucrose, amaranth seeds, rice crisped, Arabic gum, sunflower oil, corn bran, sorbitol, dehydrated strawberry, citric acid	Energy value 90 kcal, carbohydrates 16 g, protein 1.3 g, total fat 2.5 g, saturated fat 0.8 g, trans fat 0 g, cholesterol 0 mg, dietary fiber 1.1 g, sodium 61 mg
Chocolate	Gluten-free amaranth bar with chocolate	Corn syrup, amaranth expanded, rice crisped, sugar bath, Arabic gum, bitter cocoa, sunflower oil, modified tapioca starch, sorbitol, soy flour, citric acid	Energy value 79 kcal, carbohydrates 15 g, protein 1.2 g, total fat 1.6 g, saturated fat 0.5 g, trans fat 0 g, cholesterol 0 mg, dietary fiber 1.1 g, sodium 42 mg
Sesame	Gluten-free amaranth bar with citric fruit, chia and sesame seeds	Yogurt bath, corn syrup, organic sucrose, amaranth expanded, sunflower oil, sorbitol, rice crisped, corn bran, sesame seeds, chia seeds, modified tapioca starch, Arabic gum, soy flour, citric acid, lemon flavor	Energy value 90 kcal, carbohydrates 15 g, protein 1.5 g, total fat 2.3 g, saturated fat 0.8 g, trans fat 0 g, cholesterol 0 mg, dietary fiber 0.9 g, sodium 55 mg

Table 2 The operating conditions for ICP-OES analysis

RF generator	40.68 MHz
Forward power	1.0 kW
Nebulizer	Ultrasonic
Plasma gas flow rate	8.5 L/min
Auxiliary gas flow rate	1.0 L/min
Sample gas flow rate	0.5 L/min
Sample uptake rate	1.5 mL/min
Observation height	15 mm
Sample uptake delay	30 s

digestion procedures were adapted from the Milestone SK-10 High Pressure Rotor application book (Application note HPR-AG-03). The Milestone microwave software automatically delivers the minimum power required to follow the defined temperature profile.

Approximately 500 mg of each sample were inserted directly into a microwave-closed vessel. Two milliliter of 30 % (m/m) H_2O_2 and 6 mL of HNO_3 (65 %) solutions were added to each vessel. An optimized temperature profile program was applied (Stage 1: 25–200 °C for 15 min, Stage 2: 200 °C for 15 min, Stage 3: 200–110 °C for 15 min, followed immediately by ventilation until the solutions reached room temperature). Finally, the digested samples were diluted to a final volume of 10 mL with ultrapure water. Three replicated digestions were made for each sample.

Measurements

All samples (digested and blank solutions) were measured in triplicate by direct nebulization. The emission lines for the ICP-OES analysis were chosen according to previous interference studies. The lines that exhibited low interference and high analytical signal and background ratios were selected. The selected wavelengths were as follow: Al: 237.312 nm, As 188.979 nm, Cd: 214.438 nm, Co: 238.892 nm, Cr: 267.716 nm, Cu: 324.754 nm, Fe: 259.940 nm, Mn: 257.610 nm, Ni: 231.604, Pb: 220.353 nm, Sb: 206.833 nm, Se: 196.022 nm, Sr: 421.552 nm, Tl: 190.802 nm and Zn: 213.856 nm. The calibration curves were obtained at five different concentration levels in triplicate. The coefficient of determination (R^2) values of such linear fit ranged from 0.9971 to 0.9990. Digested samples that were beyond the linear range were diluted and reanalyzed. Finally, the elemental contents were calculated from the measured data taking into consideration blanks, dilutions, and the mass of dried sample used for digestion, to obtain results of concentrations in dry weight.

Quality assurance

As no certified reference materials for amaranth bars were available, the accuracy of the method was evaluated by using a vegetable standard reference material (SRM 1570a: Spinach Leaves) from the National Institute of Standards and Technology (Gaithersburg, MD, USA). Digestion of this material was performed with the same decomposition procedure used for the analyzed samples. In order to further evaluate the proposed method, spike recovery studies were performed for all analytes in randomly selected gluten-free amaranth bars with green apple samples.

The precision of the method was evaluated by measuring the repeatability and reproducibility. In the repeatability test (within-day precision), a digested sample was analyzed three times within 1 day; and in the reproducibility test (day-to-day precision), sample digestion and ICP-OES analysis were studied by triplicate analyses of three samples on 3 days for a period of 3 weeks. The method detection limits (MDLs) for all the selected elements were obtained according to Thomsen et al. [18].

Statistical methods

The experimental results were subject to statistical analysis using MS Excel 2010 software. The means of the data were compared using a 2-sided paired *t* test at 5 % level of significance (95 % CI).

Results and discussion

ICP-OES performance

The analytical quality was verified by using the spinach leaves standard reference material (SRM 1570a), and the concentrations of ten elements were within the range of ±10 % of the certified values, demonstrating the quality of the method as presented in Table 3. In addition, the Student's *t* statistical test ($P = 0.05$) was performed in order to check if there were any significant differences between the certified and obtained values. For all the certified concentrations, no significant difference was observed.

The results of the recovery experiments for the fifteen elements are presented in Table 4. All spike recoveries were well within the allowed range of 85–115 % [19]. The obtained recoveries confirmed that no significant element loss occurred during the digestion process.

The capability of the method as a routine analysis method was estimated through the determination of the MDLs. In this work MDLs were determined according to FDA guidelines for method validations [20]. It is important to point out that MDLs are not the instrumental detection limits, since they take into account the entire procedure and refer to concentration levels in real samples, not in the final solution. The MDLs for all selected trace elements are shown in Table 4. As can be seen, all MDLs were sufficiently below the typical levels of interest for food samples.

Finally, repeatability and reproducibility data were obtained for all the analyzed elements. The repeatability data were generated on different days by using new instrument tuning and calibrations curves. The relative standard deviations were lower than 4.7 and 10.5 % for all the elements for repeatability and reproducibility data, respectively.

General features

The results obtained for the 15 trace elements analyzed in all the samples, grouped according to the different presentations considered, are summarized in Table 5. There were significant ($P < 0.05$) differences for all trace element concentrations between the gluten-free amaranth bars samples considered, depending on the different brands and formulations. However, similar trends in the order of decreasing trace element concentrations in all samples were observed, except for Sesame and Chocolate samples where Al was at higher levels than Zn. This singular regularity in the sequence of trace elements concentrations was observed despite the wide variety of formulations in most samples. The decreasing sequence of the mean elemental levels was: Fe > Mn > Zn > Al > Cu > Sr > Pb > Ni > Cr > Co.

The concentrations of Fe and Mn were usually between 5 and 15 µg/g, whereas the other trace elements were in the range 1–10 µg/g. In addition, Co, Cr, Ni and Pb concentrations were detectable in all samples, usually at levels below 1 µg/g. Finally, the As, Cd, Sb, Se and Tl contents were below the respective MDL in all samples.

Table 3 Recoveries (mean ± standard deviation, $n = 5$) obtained for the determination of trace elements in spinach leaves standard reference material (SRM 1570a)

	Certified value (mg/kg)	Found value (mg/kg)	Average recovery (%)
Al	310 ± 11	286 ± 15	92.2
As	0.068 ± 0.012	0.064 ± 0.015	94.1
Cd	2.89 ± 0.07	3.08 ± 0.09	103.1
Co	0.39 ± 0.05	0.37 ± 0.08	94.8
Cu	12.2 ± 0.6	11.9 ± 0.7	97.5
Mn	75.9 ± 1.9	75.0 ± 2.8	98.8
Ni	2.14 ± 0.10	2.22 ± 0.15	103.7
Se	0.117 ± 0.009	0.121 ± 0.010	103.4
Sr	55.6 ± 0.8	56.7 ± 5.3	101.9
Zn	82 ± 3	80 ± 5	97.5

All data were expressed on a dry weight basis

In summary, the measured elemental concentrations in the gluten-free samples were lower than trace element contents in amaranth whole grains [21, 22]. Two possible explanations for this finding may be: (1) the whole grains contain high levels of elements compared with flour and derived products because of the presence of the outer kernel layers, where elements are concentrated [23–25], or (2) the presence of other major ingredients in the amaranth bars composition with low mineral content such as rice crisped. Rice is a low-cost ingredient with low element contents compared to other gluten-free cereals [26].

Literature about the content of trace elements in gluten-free products based on amaranth is scarce. The only information available about mineral composition of gluten-free amaranth bars is provided by manufacturers. This information is limited on a number of elements such as Ca, Mg, K and Na. A recently published study by Orecchio et al. [27] examines the concentration of 20 trace elements in gluten-free products marketed in Italy. Nevertheless, gluten-free amaranth bars were not included among the samples studied in such research.

Trace elements in amaranth bar samples

The mean Fe and Zn levels in gluten-free amaranth bars were found to be 11.0 and 6.5 µg/g, respectively. In agreement with previous studies [27], Fe was the most abundant trace element in the gluten-free products studied. The Fe concentrations spanned in wide range, from 5.3 to 16.8 µg/g, whereas Zn concentrations ranged from 3.8 to 10.6 µg/g in the studied samples. The highest Fe and Zn levels were measured in gluten-free amaranth bars with dehydrated green apple samples (G-Apple). The lowest Fe and Zn concentrations were found in samples with berries (Berries). As expected, the Fe contents determined in all the gluten-free samples studied were significantly lower than the levels found in equivalent Fe fortified wheat food products [28]. Besides, the Fe and Zn levels were at similar orders than cereal flours produced in Finland, except for wheat flours in which the Fe content was considerably higher [29].

Iron and Zn are essential micronutrients for human growth, development, and maintenance of the immune system. Deficiencies in these two nutrients remain a global problem, especially among people who suffer celiac disease. In this work, Fe and Zn dietary intake, by body weight and gender, were also calculated considering the mean concentrations of these elements in the different brands and relating them to the consumption (46 g/day gluten-free amaranth bar based on manufacturer recommendations). The mean estimated dietary intake levels for Fe and Zn were of 506 and 299 µg/day, respectively. As can be seen from Table 6, the estimated daily intakes of Fe and Zn from the studied gluten-free amaranth bars for adults were less than 7 % for males and less than 4 % for females of the Dietary Reference Intake (DRI) [13]. Therefore, these gluten-free products provide inadequate contributions of Fe and Zn to the diet according to the range given by the DRIs. These low nutritional intakes are of great concern considering that celiac disease increases the risk of developing several malabsorption syndromes of these minerals. This is because such essential minerals from food are absorbed mainly in the upper intestines, the same part of the intestines that is damaged by gluten. In the literature, inadequate Fe and Zn contributions have been reported also for processed gluten-free products [30].

The Cu and Mn contents ranged between 0.85–6.29 and 6.2–10.9 µg/g, respectively. The maximum and minimum Cu concentrations in amaranth bars were found in R-Apple (6.1 µg/g) and Lemon (1.8 µg/g) samples, respectively. The highest Mn level was determined in G-Apple samples (10.2 µg/g), whereas the lowest was found in Berries samples (6.5 µg/g). The mean Cu and Mn concentrations found in this work were somewhat higher than those of the similar gluten-free products in Italy [27] and of bakery products (with gluten) from Finland [29], Brazil [31] and

Table 4 Recovery for spiked gluten-free amaranth bars with green apple ($n = 5$)

Element	MDL[a] (μg/g)	Added (μg/g)	Found (μg/g)	Recovery (%)
Al	0.62	0	4.02 ± 1.78	
		1.0	4.88 ± 0.277	97.4
		5.0	8.93 ± 0.044	99.0
As	0.188	0	<MDL	
		0.25	0.23 ± 0.054	92.0
		0.50	0.45 ± 0.041	90.0
Cd	0.063	0	<MDL	
		0.25	0.24 ± 0.038	96.0
		0.50	0.44 ± 0.034	88.0
Co	0.025	0	0.04 ± 0.011	
		0.25	0.30 ± 0.025	103.4
		0.50	0.57 ± 0.024	105.6
Cr	0.031	0	0.08 ± 0.011	
		0.25	0.32 ± 0.019	96.9
		0.50	0.51 ± 0.055	87.9
Cu	0.189	0	3.38 ± 0.057	
		1.0	4.40 ± 0.036	101.4
		5.0	8.42 ± 0.052	100.4
Fe	1.88	0	15.2 ± 1.05	
		5.0	19.9 ± 0.48	99.4
		10.0	27.7 ± 0.72	109.9
Mn	1.58	0	6.5 ± 0.95	
		1.0	7.4 ± 0.50	99.0
		5.0	11.3 ± 0.08	98.2
Ni	0.063	0	0.58 ± 0.023	
		0.25	0.79 ± 0.16	95.0
		0.50	0.99 ± 0.05	91.6
Pb	0.252	0	0.59 ± 0.08	
		0.25	0.86 ± 0.12	99.0
		0.50	1.10 ± 0.18	100.9
Sb	0.063	0	<MDL	
		0.25	0.26 ± 0.02	104.0
		0.50	0.45 ± 0.02	90.0
Se	0.157	0	<MDL	
		0.25	0.23 ± 0.01	92.0
		0.50	0.52 ± 0.05	104.0
Sr	0.094	0	1.85 ± 0.08	
		1.0	2.95 ± 0.05	103.5
		5.0	6.75 ± 0.12	98.5
Tl	0.031	0	<MDL	
		0.25	0.27 ± 0.01	108.0
		0.50	0.54 ± 0.02	108.0
Zn	0.125	0	6.27 ± 1.7	
		1.0	7.36 ± 0.05	101.2
		5.0	11.65 ± 0.08	103.4

[a] MDL: method detection limit calculated considering P = 0.01, $t_{Student}$ (theoretical value) = 3.14

Table 5 Results obtained for trace elements in gluten-free amaranth bar samples (mean ± SD, dry matter)

Element (μg/g)	Green apple $n = 18$	Berries $n = 16$	Red apple $n = 18$	Lemon $n = 12$	Peach $n = 18$	Strawberry $n = 18$	Chocolate $n = 18$	Sesame $n = 18$
Al	8.7 ± 1.21	4.3 ± 0.52	5.9 ± 0.68	3.6 ± 0.40	5.1 ± 0.60	4.8 ± 0.29	6.6 ± 0.64	7.7 ± 1.32
As	<0.188	<0.188	<0.188	<0.188	<0.188	<0.188	<0.188	<0.188
Cd	<0.063	<0.063	<0.063	<0.063	<0.063	<0.063	<0.063	<0.063
Co	0.07 ± 0.02	0.04 ± 0.03	0.04 ± 0.02	0.04 ± 0.03	0.06 ± 0.01	0.05 ± 0.03	0.02 ± 0.01	0.06 ± 0.03
Cr	0.16 ± 0.05	0.05 ± 0.03	0.12 ± 0.03	0.15 ± 0.04	0.25 ± 0.02	0.06 ± 0.02	0.08 ± 0.02	0.10 ± 0.04
Cu	4.78 ± 0.20	4.35 ± 0.11	6.12 ± 0.17	1.80 ± 0.95	1.95 ± 0.25	3.64 ± 1.22	3.25 ± 0.58	3.25 ± 0.55
Fe	14.5 ± 2.3	7.0 ± 1.7	12.8 ± 0.8	9.2 ± 1.3	10.9 ± 0.1	11.2 ± 0.5	10.3 ± 3.6	12.4 ± 2.2
Mn	10.2 ± 0.7	6.5 ± 0.3	7.0 ± 0.2	8.3 ± 0.5	7.3 ± 0.3	9.2 ± 0.3	7.7 ± 0.5	10.6 ± 0.1
Ni	0.75 ± 0.11	0.30 ± 0.06	0.52 ± 0.06	0.35 ± 0.07	0.35 ± 0.09	0.37 ± 0.03	0.32 ± 0.04	0.48 ± 0.05
Pb	0.28 ± 0.02	0.24 ± 0.10	0.90 ± 0.23	0.57 ± 0.02	1.08 ± 0.17	0.70 ± 0.04	0.65 ± 0.01	0.33 ± 0.02
Sb	<0.063	<0.063	<0.063	<0.063	<0.063	<0.063	<0.063	<0.063
Se	<0.157	<0.157	<0.157	<0.157	<0.157	<0.157	<0.157	<0.157
Sr	2.7 ± 0.03	1.4 ± 0.10	2.1 ± 0.32	1.6 ± 0.05	1.9 ± 0.02	2.0 ± 0.05	2.5 ± 0.05	2.3 ± 0.0
Tl	<0.031	<0.031	<0.031	<0.031	<0.031	<0.031	<0.031	<0.031
Zn	9.8 ± 0.8	4.7 ± 0.9	6.5 ± 0.7	5.0 ± 0.5	5.4 ± 0.2	7.0 ± 0.8	5.8 ± 0.3	7.7 ± 0.1

n is the number of independent determinations

Table 6 Percentage of contribution to dietary reference intakes (DRIs) values for adults considering a mean daily consumption of two gluten-free amaranth bars (46 g/day)

Element	DRI	% DRI							
		Green apple	Berries	Red apple	Lemon	Peach	Strawberry	Chocolate	Sesame
Cr									
Male	35 μg/day	21.0	6.6	15.8	19.7	32.9	7.9	10.5	13.1
Female	25 μg/day	29.4	9.2	22.1	27.6	46.0	11.0	14.7	18.4
Cu									
Male	0.9 mg/day	24.4	22.2	31.3	9.2	10.0	18.6	16.6	16.6
Female	0.9 mg/day	24.4	22.2	31.3	9.2	10.0	18.6	16.6	16.6
Fe									
Male	8 mg/day	8.3	4.0	7.4	5.3	6.3	6.4	5.9	7.1
Female	18 mg/day	3.7	1.8	3.3	2.4	2.8	2.9	2.6	3.2
Mn									
Male	2.3 mg/day	20.4	13.0	14.0	16.6	14.6	18.4	15.4	21.2
Female	1.8 mg/day	26.1	16.6	17.9	21.2	18.7	23.5	19.7	27.1
Zn									
Male	11 mg/day	4.1	2.0	2.7	2.1	2.3	2.9	2.4	3.2
Female	8 mg/day	5.6	2.7	3.7	2.9	3.1	4.0	3.3	4.4

France [32]. Copper and Mn are essential for humans since they are involved in several enzymatic systems and in the protection against oxidative processes. Contribution to the DRI of the analyzed samples ranged from 9.2 to 31.1 % for Cu; 13.0 to 21.2 % (male) and 16.6 to 27.1 % (female) for Mn (Table 6).

The mean contents of Cr and Co were 0.12 and 0.04 μg/g, respectively. The Co and Cr levels were similar to those found in gluten-free products marketed in Italy [27]. Chromium and Co were slightly more abundant in samples containing dried fruits in their formulation (Table 5), such as Peach and G-Apple samples. The high levels of these trace elements may be due to the contribution of dried fruits included in their formulation. There is scarce information about the Co and Cr contents in the dried fruits that were used to elaborate the amaranth bars. However, Altundag et al. reported that dried fruits may be used as a good dietary source of Co and Cr [33].

Low Cr and Co levels occur naturally in a wide variety of vegetables, fruits and foods. Cr and Co are essential nutrients in humans. Chromium(III) is known to enhance the action of insulin, a hormone critical to the metabolism and storage of carbohydrates, fat, and proteins in the body [17]. Cobalt is an integral part of vitamin B_{12}. This vitamin is the only source of Co actively used by the body. According to the acceptable intake (AI) for Cr (Table 6) developed by the Institute of Medicine of the National Academy of Sciences, the average mean dairy intake from the Peach samples contributes about 46 and 32 % of AI for adult women and men, respectively. On the other hand, although Co is an essential element, the Food and Nutrition Board has not developed DRI values for Co. Instead, the requirement for Co is implicit in the recommendation for vitamin B_{12}.

The mean concentrations of Ni in gluten-free amaranth samples were 0.43 µg/g. The minimum and maximum Ni levels ranged between 0.24 µg/g in Berries to 0.86 µg/g in GApple samples. These Ni concentrations are in agreement with those reported for cereal products in France: 0.55 µg/g (breakfast cereals) and 0.47 µg/g (miscellaneous cereals) [34]. So far, Ni is a metal with no clear identified biological function in humans. The tolerable upper intake (UL) for Ni has not been established due to the lack of adequate dose–response data. Nevertheless, the dose of 8 µg/kg b.w. (500 µg/day) has been reported to cause flare-ups of hand eczema in sensitized subjects [14]. Our results suggest that the studied gluten-free foods could only contribute with less than 8 % of the proposed dose.

The concentration of Al in all analyzed gluten-free amaranth bar samples was within 3.0–9.9 µg/g. The mean value of Al in GApple and Sesame amaranth bars showed higher mean concentrations (7.7–8.7 µg/g) than samples with Lemon (3.6 µg/g). The results reported for Al in this study are similar to those reported for cereal products from France in 2012: mean Al concentrations of 5.3 µg/g [35]. In addition, the Al level determined in this work was significantly lower than the Al levels reported in cereal products from Finland: 40–60 µg/g [29].

A daily consumption of two G-Apple amaranth bars (46 g/day) contributes only 3.7 % of the tolerable weekly intake (TWI) for Al (1 mg/kg b.w./week) proposed by EFSA [16, 33]. This recently established TWI is a conservative assessment of the level that can be consumed every week over a lifetime without any appreciable risk to health.

The highest Sr contents were found in G-Apple (2.7 µg/g) and the lowest in the Berries (1.4 µg/g) samples, with detectable concentrations in all studied brands, following the order from highest to lowest of: G-Apple > Chocolate > Sesame > R-Apple > Straw > Peach > Lemon > Berries. The Sr average concentrations were similar to those found in the literature [27]. Strontium is considered non-toxic, and no essentiality has been established for this metal.

Finally, the toxicity of gluten-free products is of much greater concern today than ever before, especially considering the population for which these products are designed. In this work, the concentrations of toxic elements As, Cd, Sb, and Tl were below the respective MDL, except for Pb (Table 5). The lowest and the highest Pb concentrations were 0.24 µg/g in Berries and 1.08 µg/g in Peach amaranth bar samples. The Pb contents in seven Peach and five R-Apple samples were exceptionally higher than in other samples (levels greater than 1.10 µg/g). In a recent study, Orecchio [27] reported significantly lower Pb contents in foods for celiac people in Italy (0.001–0.080 µg/g). Moreover, in comparison with Pb concentrations reported in foods derived from gluten-containing cereals in France [32], or infant cereals in Spain [36], the results obtained in this study were also higher.

The central nervous system is the main target organ for Pb toxicity. The most concerning is the chronic toxicity of Pb due to its long half-life in the body. Considering the benchmark dose level ($BMDL_{01}$) for adults reported by EFSA [15], 0.63 µg/kg b.w./day, at least 12 samples can contribute significant levels of this element (levels above 50 µg/day). These results show that some gluten-free samples present higher contamination levels than permitted. In addition, a recent study has been reported that several species of the gender *Amaranthus* are able to accumulate high concentrations of Pb in their tissues [37]. Therefore, the high metal levels found in several analyzed products indicate the importance of strengthening the regulation and monitoring of the manufacture of the studied gluten-free products.

Conclusions

This study was developed in order to provide information on trace element concentrations in commercial gluten-free amaranth bars. The results showed that the contributions of essential elements, Cu, Mn and Cr, were appropriate for adults according to the DRIs established for these elements. Although different manufacturers claim that the amaranth bars provide great amounts of minerals, their real contribution of Fe and Zn were below 8 % of the respective DRI.

Accordingly, the enrichment with Fe and Zn of these gluten-free products may be suggested.

The levels of toxic trace elements As, Cd, Sb, and Tl in the analyzed samples were very low and could not pose any threat to the consuming population. However, levels of Pb exceeded the safety limits in almost 10 % of the analyzed samples. Therefore, regular monitoring of Pb concentrations in the future should be recommended in order to ensure the quality of products containing amaranth and to protect human health.

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Conflict of interest Melisa J. Hidalgo, Sonia C. Sgroppo, José M. Camiña, Eduardo J. Marchevsky and Roberto G. Pellerano declare they have no conflict of interest.

Ethical standard This article does not contain any studies with humans or animal subjects.

References

1. M. Dean, R. Shepherd, A. Arvola, M. Vassallo, M. Winkelmann, E. Claupein, L. Lähteenmäki, M.M. Raats, A. Saba, J. Cereal Sci. (2007). doi:10.1016/j.jcs.2007.06.007
2. W. Marcason, J. Am. Diet. Assoc. (2011). doi:10.1016/j.jada.2011.09.030
3. G.A. Gaesser, S.S. Angadi, J. Acad. Nutr. Diet. (2012). doi:10.1016/j.jand.2012.06.009
4. E. Gallagher, T.R. Gormley, E.K. Arendt, Trends Food Sci. Technol. (2004). doi:10.1016/j.tifs.2003.09.012
5. S. Case, Gastroenterology (2005). doi:10.1053/j.gastro.2005.02.020
6. C. Ballabio, F. Uberti, C. Di Lorenzo, A. Brandolini, E. Penas, P. Restani, J. Agric. Food Chem. (2011). doi:10.1021/jf2041824
7. N. Singh, P. Singh, Flour and Breads and their Fortification, in *Health and Disease Prevention*, ed. by V. Preedy, R. Watson, V. Patel (Academic Press, San Diego, 2011), pp. 101–111
8. P. Pohl, E. Stelmach, M. Welna, A. Szymczycha-Madeja, Food Anal. Methods (2012). doi:10.1007/s12161-012-9467-6
9. E.J. Llorent-Martínez, M.L. Fernández-de Córdova, P. Ortega-Barrales, A. Ruiz-Medina, Microchem. J. (2013). doi:10.1016/j.microc.2013.05.016
10. F.C. Bressy, G.B. Brito, I.S. Barbosa, L.S.G. Teixeira, M.G.A. Korn, Microchem. J. (2013). doi:10.1016/j.microc.2012.03.010
11. S.S. Mitić, M.V. Obradović, M.N. Mitić, D.A. Kostić, A.N. Pavlović, S.B. Tošić, M.D. Stojković, Food Anal. Methods (2012). doi:10.1007/s12161-011-9232-2
12. D. Kara, Food Chem. (2009). doi:10.1016/j.foodchem.2008.09.054
13. National Research Council, *Dietary Reference Intakes: The Essential Guide to Nutrient Requirements* (The National Academies Press, Washington, 2006)
14. EFSA (European Food Safety Authority), EFSA J. (2005). doi:10.2903/j.efsa.2005.146
15. EFSA (European Food Safety Authority), EFSA J. (2005). doi:10.2903/j.efsa.2010.1570
16. EFSA (European Food Safety Authority), EFSA J. (2005). doi:10.2903/j.efsa.2011.2157
17. EFSA (European Food Safety Authority), EFSA J. (2005). doi:10.2903/j.efsa.2014.3595
18. V. Thomsen, D. Schatzlein, D. Mercuro, Spectroscopy **18**(12), 112–114 (2003)
19. M. Welna, A. Szymczycha-Madeja, P. Pohl, in *Wide Spectra of Quality Control*, ed. by I. Akyar (Intech, Rijeka, 2011), pp. 53–70
20. FDA U.S., ORA Laboratory Manual—Volume II—Methods, Method Verification and Validation ORA-LAB.5.4.5 (Food and Drug Administration U.S., 2014), http://www.fda.gov/ScienceResearch/FieldScience/ucm171877.htm. Accessed 6 Apr 2015
21. E.G. Aguilar, M.A. Cantarelli, E.J. Marchevsky, N.L. Escudero, J.M. Camiña, J. Agric. Food Chem. (2011). doi:10.1021/jf202610t
22. L. Alvarez-Jubete, E.K. Arendt, E. Gallagher, Trends Food Sci. Technol. (2010). doi:10.1016/j.tifs.2009.10.014
23. S. Ragaee, E.-S.M. Abdel-Aal, M. Noaman, Food Chem. (2006). doi:10.1016/j.foodchem.2005.04.039
24. F. Cubadda, F. Aureli, A. Raggi, M. Carcea, J. Cereal Sci. (2009). doi:10.1016/j.jcs.2008.07.008
25. D. Erba, A. Hidalgo, J. Bresciani, A. Brandolini, J. Cereal Sci. (2011). doi:10.1016/j.jcs.2011.06.011
26. A.S. Hager, A. Wolter, F. Jacob, E. Zannini, E.K. Arendt, J. Cereal Sci. (2012). doi:10.1016/j.jcs.2012.06.005
27. S. Orecchio, D. Amorello, M. Raso, S. Barreca, C. Lino, F. Di Gaudio, Microchem. J. (2014). doi:10.1016/j.microc.2014.04.011
28. R.P. Avegliano, V.A. Maihara, F.F. da Silva, J. Food Compos. Anal. (2011). doi:10.1016/j.jfca.2011.01.017
29. P. Ekholm, H. Reinivuo, P. Mattila, H. Pakkala, J. Koponen, A. Happonen, J. Hellström, M.-L. Ovaskainen, J. Food Compos. Anal. (2007). doi:10.1016/j.jfca.2007.02.007
30. L. Saturni, G. Ferretti, T. Bacchetti, Nutrients (2010). doi:10.3390/nu2010016
31. R.M. Padovani, D.M. Lima, F.A.B. Colugnati, D.B. Rodriguez-Amaya, J. Food Compos. Anal. (2007). doi:10.1016/j.jfca.2007.03.006
32. L. Noël, R. Chekri, S. Millour, C. Vastel, A. Kadar, V. Sirot, J.-C. Leblanc, T. Guérin, Food Chem. (2012). doi:10.1016/j.foodchem.2011.12.009
33. H. Altundag, M. Tuzen, Food Chem. Toxicol. (2011). doi:10.1016/j.fct.2011.07.064
34. J.-C. Leblanc, T. Guérin, L. Noël, G. Calamassi-Tran, J.-L. Volatier, P. Verger, Food Addit. Contam. (2005). doi:10.1080/02652030500135367
35. N. Arnich, V. Sirot, G. Rivière, J. Jean, L. Noël, T. Guérin, J.-C. Leblanc, Food Chem. Toxicol. (2012). doi:10.1016/j.fct.2012.04.016
36. R. Hernández-Martínez, I. Navarro-Blasco, Food Control (2012). doi:10.1016/j.foodcont.2011.12.024
37. E.G. Aguilar, M.A. Cantarelli, E.J. Marchevsky, N.L. Escudero, J.M. Camiña, J. Chem. (2013). doi:10.1155/2013/138391