

Toxic Trace Element Contents in Gluten-free Cereal Bars Marketed in Argentina

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Abstract Celiac disease is a food intolerance triggered by the ingestion of gluten-containing cereals; the only therapy is a strict gluten-free diet for life. In recent years, gluten-free cereal bars has achieved considerable attention as an interesting alternative food considering its several advantages, such as functional properties, nutritional contents, variety of presentations and flavors. The aim of this study was to determine the contents of three toxic trace elements (arsenic, cadmium and lead) in commercial gluten-free cereal bars marketed in Argentina by ICP-MS and assess the dietary intake of the three toxic elements compared with reference values. The results obtained indicated that the highest total concentration of arsenic is provided by the gluten-free cereal bars with green apple pomace (28.2 µg/kg) and the levels of cadmium were lower than 10 µg/kg or even below MDL in all samples. The lead contents in all bars with honey samples were exceptionally higher than in the other samples (levels greater than 130 µg/kg). The intake of available gluten-free cereal bars are generally safe for consumers, but particular attention should be paid to monitor the content of arsenic and lead gluten-free cereal bars to ensure the quality of the product. Further studies are necessary to achieve an exact risk evaluation, concerning the bioavailability of trace elements in gluten-free food sources.

Keywords: food analysis, trace elements, atomic spectrometry, arsenic, cadmium, lead

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1. Introduction

Celiac disease (CD) is a chronic systemic autoimmune disorder caused by a permanent intolerance to gluten proteins in genetically susceptible individuals. This disease is characterized by chronic inflammation of the intestinal mucosa, atrophy of intestinal villous and several clinical manifestations. Gluten proteins are a general term used to describe a mixture of storage proteins found in wheat, barley, rye and derivatives. Among other effects CD could cause problems with absorption of nutrients including micro and macroelements, and vitamins. [1,2] At present, the only treatment available for patients with CD is a lifelong exclusion from the diet of products containing gluten. This results in complete recovery for most individuals.

On the last years, the increased prevalence of celiac disease leads to an increased demand of gluten-free products. Among these products, gluten-free cereal bars have considerable advantages related to the functional properties, high nutritional profile, ready-to-eat, variety of presentations, and flavors. Gluten-free cereal bars can be

basically defined as a "mass" molded in a bar form, comprising a dry matrix of different types of cereals, linked by a binder mix them together [3]. They may also include seeds, fruit pieces, honey, chocolate, yogurt, or pseudocereal grains [4].

Currently, there is a limited amount of research focused on the trace element contents in gluten-free foods. In addition, the only available information about mineral composition is provided by the manufacturers. Some mineral trace elements, such as arsenic (As), cadmium (Cd) and lead (Pb) are toxic elements that can cause adverse effects in human health even at trace concentrations. Accordingly, reliable data on the toxic element levels are required not only for health professionals but also for research teams and for the food industry in order to propose possible nutritional improvements. The aim of the present work was to determine the arsenic, cadmium and lead concentrations in most gluten-free cereal bars marketed in Argentina. Furthermore, to evaluate the levels of these contaminants in terms of different types and cereal predominant, and to assess the dietary intake of the three toxic elements compared with reference values and tolerable weekly intake, as proposed by international organisms.

2. Material and Methods

2.1. Gluten-free Cereal Bar Samples

In this work, a total of 72 commercial gluten-free cereal bars marketed in Argentina from nine popular brands were analyzed; the labels of these products also declared that these products are exported to other Latin-American countries and USA. The sampling was carried out between May and July of 2014 for randomness. The cereal bars differ in their composition, but generally include dehydrated fruits and seeds of different botanical species such as amaranth, chia, flax or sesame; also, they usually contain basic ingredients such as: crisped rice, sunflower oil, corn syrup, sorbitol, arabic gum, citric acid, flavors, and others.

The samples studied were grouped according to the different main ingredients that enrich the cereal bar formulation: Apple (Rice based cereal bar with dried green apple pomace and yogurt), Blueberry (Rice, whole grain amaranth and blueberry juice from concentrate), Chocolate (Rice and whole amaranth based cereal bars with dark chocolate -unsweetened chocolate, cane sugar, cocoa butter-), Coconut (Soy crisp -soy protein, tapioca and calcium carbonate-, coconut, almonds and toasted coconut), Honey (Whole sesame and amaranth seeds with honey), Peach (Rice based cereal bar with chia seeds and dehydrated peach pulp), Peanut (Soy crisp, peanuts and peanut flour), Strawberry (Rice based cereal bar with dehydrated strawberry pulp and yogurt), and Vanilla (Puffed amaranth and chia seeds based cereal bar flavored with vanilla).

2.2. Chemical and Reagents

Ultrapure deionized water (resistivity $18.2 \text{ M}\Omega \text{ cm}^{-1}$) 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. Standard solutions were obtained by dilution of commercially prepared single element solutions Tracecert® (Sigma, MO, USA) prepared specifically for spectrometric analysis, (1000 mg/L). All materials were nitric acid-washed (10%, v/v) for at least 3 days and later thoroughly rinsed with ultrapure water before use.

2.3. Sample Preparation

A microwave oven (Milestone® model Ethos-ONE, Milestone Laboratory Systems, Italy) with rotor of high pressure (up to 100 bar) was used for sample digestion. About 500 mg of previously homogenized samples were weighted, transferred in Teflon vessels and 5 mL of HNO_3 (65%) and 2 mL H_2O_2 (30%) were added. The samples were digested in the microwave oven following a four successive stages of time and temperature: 1) 4 min 25 to 90°C, 2) 6 min 90°C, 3) 6 min 90 to 180°C and 4) 180°C 10 min. After digestion, the clear, colorless solution was transferred into volumetric flask and brought to final volume of 10 mL with deionized water.

2.4. Analytical Procedure

In this work, arsenic, cadmium and lead determination was achieved with an inductively-coupled plasma mass spectrometer Agilent 7700x (Agilent Technologies, Santa Clara, CA) equipped with a Babington nebulizer, a Peltier-cooled quartz spray chamber and a standard torch (2.5 mm i.d.). Before each experiment, the Inductively coupled plasma mass spectrometry (ICP-MS) was tuned using an aqueous multi-element standard solution of Li, Sc, Ge, Rh, In, Tb, Lu, Bi (Matrix: 10% HNO_3) for consistent sensitivity. Operating conditions and selected isotopes for simultaneous monitoring are summarized in Table 1.

Table 1. The Operating Conditions for ICP Mass Spectrometer

Plasma conditions	
RF power	1.55 kW
Plasma Ar flow rate	15 L/min
Auxiliary Ar flow rate	0.9 L/min
Nebulizer Ar flow rate	0.9 L/min
Collision gas (He)	3.9 mL/min
Instrument	
Sampler cone	Nickel, 1.0 mm orifice diameter
Skimmer cone	Nickel, 0.4 mm orifice diameter
Measured isotopes	
^{75}As ; $^{111,114}\text{Cd}$; $^{206,208}\text{Pb}$	

2.5. Quality Assurance

The external calibration technique was followed for the quantitative analysis of the samples. The calibration curves for all the analytes were built on 5 different concentrations. These were prepared in such a way that the concentrations all analyte elements in the samples were within the linear range of calibration curves and above the established lower linearity limit [5].

For all ICP-MS measurements an internal standard was added in order to quantify the elemental composition of the samples and correct for any instrument drift during analysis. An internal standard of 10 mg/L $^{115}\text{Indium}$ was added to each digest giving an internal standard concentration equivalent to 50 $\mu\text{g/L}$ after dilution. Indium was analyzed as another analyte by ICP-MS, obtaining a mean recovery value of $97.1 \pm 3.3\%$. In addition, the accuracy of the procedure was tested based on the analysis of spiked samples. The obtained results from spiked samples were 97.6, 99.4 and 99.2% for As, Cd and Pb, respectively.

The precision was evaluated using relative standard deviation of 10 independent measurements of a multi-elemental solution. Intraday variation and within-laboratory reproducibility did not exceed the limit of 10% ($n = 10$). These values fulfilled the required criteria.

The limit of detection of instrument (LOD) values were calculated as the concentrations corresponding to signals equal to 3 times the standard deviation of 10 reagents blank solutions. Additionally, limits of detection of the method values (MDL) were referred to the original samples ($\mu\text{g/kg}$), taking into consideration the amount of sample digested and the final dilution employed in the recommended procedure [6]. The MDLs obtained for As, Cd and Pb were 2.5, 0.3 and 1.6 $\mu\text{g/kg}$, respectively.

3. Results and Discussion

3.1. Toxic Trace Element Contents in Gluten-free Cereal Bars

The concentrations of As, Cd and Pb in 72 gluten free cereal bar marketed in Argentina were determined by analysis on ICP-MS. Table 2 presents the average concentrations of these elements in the studied samples grouped according to the different main ingredients. From the descriptive statistics of data the distribution of toxic element content showed a positive skew with scores clustered to the left at the low concentration values. This is common for trace element contents in foodstuffs, because they usually are present at low concentrations in foods. The most significant results will be discussed in more detail in following sub-sections.

Table 2. Concentrations of Arsenic, Cadmium and Lead (Mean \pm Std. Dev.) in Gluten-Free Cereal Bars ($\mu\text{g}/\text{kg}$)

Cereal bar	<i>n</i>	Arsenic	Cadmium	Lead
Apple	9	27.8 \pm 1.3	0.4 \pm 0.1	9.5 \pm 0.7
Blueberry	12	12.3 \pm 1.1	< 0.3	33.2 \pm 2.2
Chocolate	6	17.4 \pm 0.9	16.8 \pm 0.1	15.8 \pm 4.2
Coconut	6	10.5 \pm 1.2	4.3 \pm 0.1	13.7 \pm 1.1
Honey	6	6.5 \pm 1.5	< 0.3	145.3 \pm 10.8
Peach	12	14.6 \pm 2.1	< 0.3	12.4 \pm 0.5
Peanut	9	7.8 \pm 0.8	1.7 \pm 0.2	29.9 \pm 3.6
Strawberry	6	12.2 \pm 1.1	< 0.3	10.5 \pm 1.1
Vanilla	6	10.5 \pm 1.2	< 0.3	58.7 \pm 3.6

3.1.1. Arsenic

Arsenic is present in the environment as a naturally occurring substance or as a result of contamination from human activity. Arsenic may be present in many foods including grains, fruits, and vegetables where it is present due to absorption through the soil and water. There are two types of arsenic compounds in water, food, air, and soil: organic and inorganic (these together are referred to as "total arsenic"). The inorganic forms of arsenic are the forms that have been associated with long-term health effects. Long-term exposure to high levels of arsenic is associated with higher rates of skin, bladder, and lung cancers, as well as heart disease. The International Agency for Research on Cancer (IARC) classified arsenic as "carcinogenic to humans" (Group 1) based on sufficient evidence of carcinogenicity in humans [7]. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) withdrew the provisional tolerable intake (PTWI) for arsenic of 15 $\mu\text{g}/\text{kg}$ b.w. per week. Prior to that, in its 'Scientific Opinion on Arsenic in Food', the European Food Safety Authority (EFSA) panel had concluded that the PTWI value was no longer appropriate as the benchmark dose level (BMDL_{0.1}) between 0.3 and 8 $\mu\text{g}/\text{kg}$ b.w. per day for an increased risk of cancer of the lung, skin and bladder [8]. Benchmark dose is defined as a dose or concentration that produces a determined change in response rate of an adverse effect compared to background.

In this work, the arsenic content of gluten-free cereal bar samples had a range of < 2.5–28.2 $\mu\text{g}/\text{kg}$. The highest concentrations of arsenic were provided by the gluten-free cereal bars with green apple pomace. All the other gluten-free cereal bar samples studied showed very low concentrations (< 15 $\mu\text{g}/\text{kg}$) or even below MDL. This

finding may be explained, mainly due to the fact that the former group uses rice as the main component [9]. On the whole, within the same gluten-free cereal bar brand, the arsenic contents in the different formulations were relatively homogeneous, and even the extreme values did not exceed the levels normally found in the published literature (average arsenic content found in: gluten-free products produced in Italy was 23 $\mu\text{g}/\text{kg}$ [10] or gluten-free infant cereal marketed in Spain: 75 – 165 $\mu\text{g}/\text{kg}$ [11,12]).

3.1.2. Cadmium

Cadmium is generally accepted to be a carcinogen and nephrotoxic (Hall and Shannon, 2007; Huff et al., 2007), and as such is monitored and/or regulated in many food types. The Joint Expert Committee on Food Additives (FAO/WHO) initially set the provisional tolerable weekly intake (PTWI) of 7 $\mu\text{g}/\text{kg}$ b.w. per week for cadmium. However, in 2011, the EFSA Panel fixed the tolerable weekly intake, TWI value for cadmium at 2.5 $\mu\text{g}/\text{kg}$ b.w. per week [13].

The levels of cadmium were lower than 10 $\mu\text{g}/\text{kg}$ or even below MDL in all samples, except in gluten-free cereal bars with chocolate. These results are in good agreement with the levels obtained in previous study realized in cereal infant products marketed in Spain. [14] In the literature, previous studies proposed that the high levels of cadmium might be due to the enrichment of added raw materials such as cocoa (basic ingredient of chocolate) [15,16].

3.1.3. Lead

Lead toxicity causes hematological, gastrointestinal, and neurological dysfunction in adults and children. Although the main target of lead (Pb) toxicity is the red blood cell, Pb-associated changes in the nervous system, the kidney, and the reproductive system have also been described. The adverse health effects caused by low-level exposure to lead have been extensively documented and, therefore, most countries have stipulated allowable levels of lead in food and beverages, as food and beverages are among the major sources of lead in human body.

In this respect, the Joint Expert Committee on Food Additives (FAO/WHO) initially set the provisional tolerable weekly intake (PTWI) of 25 $\mu\text{g}/\text{kg}$ b.w. per week (WHO, 2000, 2006). Although, subsequently the expert committee concluded that the lead PTWI could no longer be considered health protective and withdrew it. In this regard, the EFSA Panel on Contaminants in the Food Chain (CONTAM) established the guidance value: Benchmark dose level (BMDL₀₁) for adults of 0.63 $\mu\text{g}/\text{kg}$ b.w. per day [17, 18].

In this work, the higher lead levels provided by gluten-free cereal bars with honey (138.5–152.8 $\mu\text{g}/\text{kg}$), bars with vanilla (48.7–55.6 $\mu\text{g}/\text{kg}$) bars with blueberry (30.2–36.8 $\mu\text{g}/\text{kg}$) and bars with peanut (25.3–32.5 $\mu\text{g}/\text{kg}$), exceed those values found in the other studied gluten-free cereal bars always under 20 $\mu\text{g}/\text{kg}$ of lead. However, the lead levels in all samples were found to be lower than maximum levels (0.2 mg/kg for cereals, legumes and pulses) set by EC (1881/2006) [19].

The lead contents in all bars with honey samples were exceptionally higher than in the other samples (levels greater than 130 $\mu\text{g}/\text{kg}$). In a recent study, Orecchio [10]

reported significantly lower lead contents in foods for celiac people in Italy (within 0.001-0.080 µg/g). Moreover, in comparison with lead concentrations reported in foods derived from gluten-containing cereals in France [20] or infant cereals in Spain [14], the results obtained in samples with honey were also higher.

3.2. Estimated Dietary Intake of Toxic Trace Elements

Weekly dietary intake was calculated using the arsenic, cadmium and lead concentration average value determined in the different gluten-free cereal bars. The estimated average dose ingested by adult end-users consuming the studied gluten-free cereal bars as such, was calculated according to the standard daily intake of two cereal bars per day (46 g/day – 322 g/week) as recommended by manufacturers and mean body weight (Males: 70 kg body weight, Females: 65 kg b.w.). Table 3 shows the data of the calculated amount of three elements supplied by gluten-free cereal bar studied according to the arsenic and lead reference values (BMDL₀₁), and TWI value for cadmium.

Table 3. Daily Intake Estimates for Toxic Elements From Gluten-Free Cereal Bars

	% Guidance value		
	Arsenic ^a	Cadmium ^b	Lead ^c
Apple	6.1	0.1	1.2
Blueberry	2.7	--	4.4
Chocolate	3.8	2.2	3.4
Coconut	2.3	0.8	1.8
Honey	1.4	--	15.8
Peach	3.2	--	1.6
Peanut	1.7	0.3	3.9
Strawberry	2.7	--	1.4
Vanilla	2.3	--	7.7

^aBMDL₀₁: 0.3 – 8 µg/kg b.w day

^bTWI: 2.5 µg/kg b.w. week

^cBMDL₀₁: 0.5 µg/kg b.w day

The estimated contribution from arsenic is highest from the mean concentration for samples with apple, and is 6.1% of the BMDL₀₁ for adults (70 kg b.w.). The greatest contribution from cadmium is 2.2% of the TWI for adults, estimated from mean contents in samples with chocolate. The highest estimate for lead intake is obtained from samples with honey, at 15.8% of the upper limit of BMDL₀₁ for adults. So, although none of these numbers exceed the established critical values, it is noteworthy that these estimates are only for cereal bar consumption. Contributions to the intake of these toxic elements from other food sources and drinking water will need to be considered to give a more robust picture of dietary exposure. In addition, it is important to point out that these thresholds limit values are estimated considering healthy people; currently there are not available reference values for persons who suffer the CD.

4. Conclusions

In this work, three toxic trace elements were determined in seventy-two commercial gluten-free cereal bar samples marketed in Argentina by using ICP-MS after microwave digestion. The results show that the intake of

available gluten-free cereal bars is generally safe for consumers, but particular attention should be paid to monitor the content of arsenic and lead gluten-free cereal bars to ensure the quality of the product. The findings of this study enlarge the knowledge of the toxic elemental profile of these gluten-free products and will be useful in the nutritional and toxicological fields. Regarding the risk for human health, further studies are necessary to achieve an exact risk evaluation, concerning the bioavailability of trace elements in gluten-free food sources.

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Statement of Competing Interests

The authors declare that they have no competing interest.

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