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ASSESSMENT OF METALS AND THEIR ESTIMATED DAILY INTAKES IN VARIOUS NUTS

SUMMARY

Nuts have received increased public attention in recent years as important sources of essential elements. Information on the levels of elements in edible nuts is useful to consumers. Content of metals was analysed in various types of nuts from Sarajevo markets, Bosnia and Herzegovina. The samples included almond, walnuts, Brazil walnuts, Indian walnuts, Macadamia, hazelnuts and pistachios. Metals cadmium, chromium, iron, manganese, lead and zinc (Cd, Cr, Fe, Mn, Pb and Zn) were determined by flame atomic absorption spectrometry (FAAS). Moreover, estimated daily intake of all analysed metals and carcinogenic risk over a lifetime exposure to Pb has been calculated.

The metal levels in nuts ranged as follows: 1.53-6.95 mgCd/kg, 1.97-7.92 mgCr/kg, 32.03-97.70 mgFe/kg, 6.48-30.58 mgMn/kg, 0.42-1.38 mgPb/kg, 31.30-50.23 mgZn/kg. By concentrations in nuts, in most cases, the metals were arranged as the following diminishing series: Fe > Pb > Zn >Mn > Cr > Cd. Estimated daily intakes for Cd, Cr, Fe, Mn, Pb and Zn were calculated and varied from 0.0002 mg/kg/day for Pb to 0.0489 mg/kg/day for Fe. Carcinogenic risk for Pb was lower than 10^{-6} indicating the risk of cancer due to exposure to Pb through nuts consumption is in an acceptable range. The results provide important information on the nutritional values of nut samples at the Sarajevo market. In most cases, the content of metal was in accordance with the contents given in the previously published papers.

Keywords: Nuts, Metals, Contamination, Daily Intake, Carcinogenic Risk.

INTRODUCTION

Food crops like fruits, vegetables and nuts cultivated in contaminated lands can accumulate toxic heavy metals. Human exposure to toxic metals goes through ingestion of contaminated food and water and by inhalation of air pollutants or contaminated soil particles (Davarynejad et al. 2013; Onyedikachi et al. 2018). In the past years, the increasing demand of food safety has stimulated research regarding the risk associated with consumption of contaminated foodstuffs by

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heavy metals and pesticides or toxins (D'Mello 2003). Nuts are widely used in the food industry such as baking, confectionery, candy and chocolate products, ice-cream and dairy (Özkutlu et al. 2013). Nuts have received increased public attention in recent years as important sources of essential elements, and information on the levels of elements in edible nuts is useful to consumers (Yin et al. 2015). Bioactive compounds, such as essential fatty acids, essential amino acids, antioxidants, essential mineral and vitamins are present in high concentrations in nuts. Thus, health benefits which we have by consuming nuts are blood pressure and body weight control, reduction of blood cholesterol and triglycerides, as well as prevention of coronary heart disease (Moreda-Piñeiro et al. 2016). Determination of some elements in nuts is not only necessary in evaluating the dietary intake of the essential elements, but also useful in detecting heavy metal contamination in food (Cabrera et al. 2003; Chung et al. 2013; Yin et al. 2015).

Nuts are rich in micronutrients such as niacin and folic acid, vitamins (E and B6) and minerals (Ca, Cr, Mg, Mn, Cu, Fe, Zn, Se, P and K) (Kafaoglu et al. 2016). Fe, Cu, Cr and Zn are essential micronutrients for human health. They play an important role in human metabolism, and interest in essential micronutrients is increasing together with reports of relationships between trace element status and oxidative diseases (Cabrera et al. 2003). Some heavy metals, if present even in very low concentrations in food products, can cause human health problems. Information about the dietary intake of such metals is very important to assess risks to consumers (Lanre-Iyanda and Adekunle 2012). Also, the determination of trace elements and minerals in foodstuffs is an important part of toxicological and nutritional analyses (Cabrera et al. 2003). Biotoxic effects of heavy metals depend upon the oxidation states and concentrations of heavy metals, mode of deposition and kind of sources (Bukvić et al. 2013).

The aim of this study was to analyse the metals cadmium, chromium, iron, manganese, lead and zinc (Cd, Cr, Fe, Mn, Pb, Zn) concentrations in different types of nuts marketed in Sarajevo: almond, walnuts, Brazil walnuts, Indian walnuts, Macadamia, hazelnuts and pistachios. Metals were analysed by flame atomic absorption spectrometry (FAAS). In addition, estimated daily intakes of metals from nuts samples and estimated carcinogenic risk over a lifetime exposure to Pb were also calculated.

MATERIAL AND METHODS

Sample collection and analysis

Nuts samples were collected from different Sarajevo (Bosnia and Herzegovina) markets. Samples of nuts were collected randomly in sufficient quantities to provide a representative samples. Samples were cut to small pieces and dried at 105ºC, then powdered and stored in plastic bags for metals analysis. Metals in nuts samples were extracted following the acid digestion procedure: a total of about 1 g (± 0.1 mg) of dry nuts was weight directly in Teflon digestion vessels. Following the addition of 30 mL 65% (v/v) HNO_3, after the evaporation of nitrogen oxides, the vessel was sealed and allowed to react at 50ºC for 12 h. The digest was transferred to a volumetric flask and filled up with Milli-Q water to 50 mL. Metals content of commonly consumed nuts were determined by FAAS using an atomic absorption spectrometer model AA240FS, Varian (Australia). All samples and blanks were prepared in triplicate and measurement for each replicate was repeated thrice. All the chemicals and reagents were of analytical grade and were purchased from Merck (Germany). Standard solutions of metals were also provided by Merck (Germany). Milli-Q water was used throughout the whole experiment. All of the volumetric glassware used were soaked in 10% (v/v) HNO_3 overnight and rinsed with Milli-Q water before use. The sample analyses were performed in triplicate and the standard deviation was calculated. The method detection limits (LOD) were calculated as three times the standard deviation of the blank signal. The LOD values were: Cr (0.006 mg/L), Mn (0.002 mg/L), Fe (0.006 mg/L), Pb (0.01 mg/L), Ni (0.01 mg/L), Cd (0.002 mg/L), Zn (0.001 mg/L). Spiked samples were used to test the methods at varying concentrations of analyte and the percent recovery was calculated. The acceptable recoveries of known additions ranged between 89-108%. Metal concentrations are expressed as the mean value (mg/kg) ± standard deviation (SD) of three subsamples collected from the same source.

Estimated daily intake of metals (EDI)

A recommended dietary intake (RDI), sometimes referred to as recommended daily intake, is the average daily intake level of a particular nutrient that is likely to meet the nutrient requirements of 97-98% of healthy individuals in a particular life stage or gender group. Estimated daily intake (mg/kg/day) is calculated based on the assumptions that: a) body weight is 60 kg and b) daily intake of nuts is 30 g. Estimated daily intake were calculated using (mg/kg/day) = metal concentrations in nuts × 30/1000/60 (Ozbas et al. 2013).

Carcinogenic risk (CR)

Carcinogenic risk (CR) shows the probability of developing cancer over a lifetime due to exposure to a potential carcinogen for an individual. The United States Environmental Protection Agency (USEPA 2010) provided a cancer slope factor (CSF) for the calculation of the cancer risk over a lifetime exposure to Pb. The estimation of the cancer risk is calculated using the Equation 1 given by USEPA: $CR = CSF \times ED$ /1/

where, CSF is the carcinogenic slope factor of 0.0085 (mg/kg/day) for Pb set by USEPA (2010). Acceptable risk levels for carcinogens range from 10^{-4} (risk of developing cancer over a human lifetime is 1 in 10000) to 10^{-6} (risk of developing cancer over a human lifetime is 1 in 1000000).

RESULTS AND DISCUSSION

Nuts are known to be a concentrated food for major elements and they are an interesting food for trace elements for the human diet. A small number of published data are available on the metal contamination in nuts on different market sites.

Moisture content in nuts. The knowledge of real moisture distribution of nut and their components is an essential requirement for obtaining desired high-quality products and designing of efficient processing operations (Khir et al. 2013). Like many other products, nuts must have moisture content where they are dry enough to meet customer quality specifications but not so dry that they break during shipment. For example, moisture content in walnuts is a sensitive parameter. Target moisture content is around 4.6% (Nielsen 2010). The Food and Drug Administration regulations for tree nuts define a safe moisture level (moisture content that does not support fungal growth) which is typically 8% on a wet basis (Khir et al. 2013). Moisture content (%) in nuts is presented in Table 1. Moisture content in all analysed samples ranged from 1.62% (Macadamia) to 4.26% (Indian walnuts). Thus, the obtained values were common values for the moisture content in such nutritional products.

Table1. Moisture content (%) in nuts

Sample	Moisture content (%)
Almond	4.06
Walnuts	3.78
Brazil walnuts	3.02
Indian walnuts	4.26
Macadamia	1.62
Hazelnuts	3.77
Pistachios	3.47

Metal content in nuts. All metal concentrations were determined on a dry weight basis. Metal contents in different types of nuts marketed in Sarajevo are presented in Table 2.

Table 2. Metal concentration (mg/kg ± standard deviation) in different types of nuts (n = 3)

Metal concentration (mg/kg) ± standard deviation							
Metal	Sample						
	Brazil walnuts	Pistachios	Indian walnuts	Almond	Walnuts	Hazelnuts	Macadamia
Cd	1.53±0.36	2.64±0.31	2.91±0.03	3.35±0.08	3.85±0.19	6.95±2.78	4.81±0.15
Cr	6.46±4.15	7.92±0.82	5.58±0.85	2.90±1.51	7.17±1.16	3.34±1.09	1.97±0.30
Fe	40.96±28.37	97.7±21.90	69.5±28.11	32.03±15.96	70.16±17.5	19.39±3.39	54.50±7.78
Mn	6.48±4.37	7.37±1.67	14.88±1.10	10.88±2.10	24.36±1.07	11.02±5.21	30.56±2.40
Pb	0.61±0.26	0.42±0.04	0.53±0.01	0.70±0.02	0.64±0.03	1.38±0.20	0.84±0.08
Zn	33.08±17.12	36.43±0.60	50.23±3.60	37.1±16.00	41.6±2.52	49.91±19.70	31.3±4.04

By concentrations in nuts, in most cases, the metals were arranged as the following diminishing series: Fe > Pb > Zn > Mn > Cr > Cd. Published data on the content of metals in nuts around the world are presented in Table 3.

Table 3. Literature values of metal content in nuts from various parts of the world

Samples	Cd (mg/kg)	Cr (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
Brazil walnut	n.a.	n.a.	21.29±1.36 [6]	11.98±0.16 [6]	n.a.	32.06±1.5 [6]
Pistachio	0.07±0.01 [3] 0.45±0.23 [5] n.d. [4]	0.60 - 1.86 [1] 0.15±0.08 [3] 0.37 - 0.41 [4]	24.92±3.87 [6] 88.67±6.49 [5] 70.0 - 75.6 [4]	6.0±1.2 [3] 9.11±0.23 [6]	<LOD [3] 11.62±1.42 [5] 0.15 - 0.25 [4]	15±3.3 [3] 16.81±0.65 [6] 67.24±30.25 [5] 30.2 - 36.7 [4]
Almond	0.04±0.01 [3] 0.24 [7] 0.48±0.24 [5] n.d.-0.012 [4]	0.31±0.038 [3] 0.39-0.49 [4]	20.57±1.92 [6] 62.10±5.86 [5] 40.3-48.7 [4]	14±4.5 [3] 21.13±0.94 [6]	<LOD [3] 1.02 [7] 2.81±0.56 [5] 0.26-0.37 [4]	24±4.0 [3] 27.31±0.98 [6] 19.16±16.21 [5] 27.9-50.0 [4]
Walnut	0.02±0.00 [3] 24.23±1.62 [6] 0.49±0.32 [5] n.d.-0.009 [4]	<LOD [3] 0.001 [8] 0.30-0.38 [4]	22.08±1.01 [6] 57.42±22.24 [5] 0.064 [8] 20.0-24.4 [4]	10±0.12 [3] 0.012 [8]	<LOD [3] 3.43±0.22 [5] 0.20-0.26 [4]	20±1.1 [3] 23.25±0.50 [6] 15.21±12.86 [5] 25.6-39.7 [4]
Hazelnut	0.24±0.00 [3] 0.01-0.03 [2]	0.22±0.02 [3] 0.02-0.05 [2]	25.95±1.02 [6] 35.1-49.4 [2]	33±9.8 [3] 53.47±1.73 [6] 41.1-116.81 [2]	0.17±0.14 [3] 0.02-0.07 [2]	15±0.82 [3] 16.02±0.15 [6] 23.5-35.5 [2]
Macadamia	0.05±0.00 [3] 0.46±0.23 [5]	0.090±0.060 [3]	57.68±8.23 [5]	11.6±0.17 [3]	0.18±0.02 [3] <LOD [5]	8.6±1.5 [3] 9.01±7.64 [5]
Indian Walnut	n.a.					

[1]- (Davarynejad *et al.* 2013); [2]- (Özkutlu *et al.* 2013); [3]- (Yin *et al.* 2015); [4]- (Cabrera *et al.* 2003); [5]- (Chung *et al.* 2013); [6]- (Kafaoglu *et al.* 2016); [7]- (Sattar *et al.* 1989) [8]- (Edem *et al.* 2008); n.a. - not available

Cadmium has no known biological functions (ATSDR 2008). It interferes with some essential function of Zn, thereby inhibiting nutrient utilization and enzyme reactions. Cadmium catalyses oxidation reactions and generates free-radical damage of tissue (Lanre-Iyanda and Adekunle 2012). Concentrations of Cd in nuts samples were found between 1.53 (Brazil walnuts) and 6.95 mg/kg (hazelnuts). If comparing the results from this study with results from other countries shows that the Cd content was similar or slightly higher in this study. According to the Ordinance on Maximum Permissible Quantities for Certain Contaminants in Food (Official Gazette of BiH 2014) the maximum permissible amount of Cd for example in wheat and rice (there is no defined maximum permissible amount in nuts) is 0.20 mg/kg of wet weight.

Chromium concentrations in human tissues decline with age, except for the lungs in which Cr accumulates. Parity, juvenile diabetes, and coronary artery disease are associated with low Cr content rations in serum or hair (Kafaoglu et al. 2016). Significant variation in Cr concentration was found among the nut samples. Values ranged from 1.97 mg/kg in Macadamia to 7.92 mg/kg in pistachios and were slightly higher in regard to previous published results given in Table 3. The results have shown that pistachio is an excellent source of Cr.

Consuming 3.5 g of pistachio per day covers the adequate daily intake of an adult (woman). The intake of too much of Cr can cause harmful health effects for instance hepatitis, ulcers, gastritis and lung cancer (Davarynejad et al. 2013).

Iron as essential metal plays a vital role in human physiology mainly in the formation of hemoglobin, electron and oxygen transport in human body (Nejabat et al. 2017). Fe concentrations ranged from 19.39 (hazelnut) to 97.70 mg/kg in pistachios. According to results of Fe content in nuts and comparing these results with recommended daily Fe intake, it seems that consumption of around 80 g/day of pistachios can provide the total Fe need in the human body of men. Samples were arranged as the following diminishing series by Fe concentrations: pistachios > walnuts > Indian walnuts > Macadamia > Brazil walnuts > almond > hazelnuts.

Manganese serves as a co-factor for many metabolic and physiological functions (Yin et al. 2015). Concentrations of Mn in nuts samples were found between 6.48 mg/kg in Brazil walnuts and 30.56 mg/kg in Macadamia. Comparing the results from this study with adequate daily Mn intake value, it seems that consumption of 15 g/day of Macadamia or walnuts can provide the total Mn need of woman.

Toxic compounds find their way into food during manufacture, transportation or storage, these include largely heavy metals. Lead presence in food may be influenced by industries, heavily travelled highways, and urban communities in combination with Pb supplied through irrigation water, pesticides and fertilizers (Cabrera et al. 2003). Pb is one of the representative elements whose levels in the environment represent a reliable index of environmental pollution (Yin et al. 2015). Pb levels in this study ranged from 0.42 (pistachios) to 1.38 mg/kg (hazelnuts) and were similar to the values from previously published data (Table 3). The carcinogenic risk over a lifetime exposure to Pb was calculated and presented in Table 4. Generally, the values of CR lower than 10^{-6} are considered as negligible, above 10^{-4} are considered to be unacceptable and lying in between 10^{-6} and 10^{-4} are considered as acceptable range (Atique et al. 2017). In this study, CR for Pb was lower than 10^{-6} indicating the risk of cancer due to exposure to Pb through nuts consumption is in an acceptable range.

The net delivery of Zn to an organism is a function of its bioavailability and of the total amount of this element in foods. Zn levels determined in nuts ranged from 31.33 mg/kg in Macadamia to 50.23 mg/kg in Indian walnuts. According to results of Zn content in nuts and comparing these results with recommended daily Zn intake, consumption of about 140 g of Indian walnuts can provide the total Zn need in the human body of woman. By comparing the obtained results with results from other countries it is shown that the Zn content was very similar or higher in the case of Macadamia walnuts.

Estimated daily intakes of metals from nuts. The estimated daily intake of metals (Cd, Cr, Fe, Mn, Pb and Zn) depended on both the metal concentration level and the amount of consumption of nuts. Results of estimated daily intakes for all metals from nuts samples are presented in Table 5.

Table 4. Estimated carcinogenic risk over a lifetime exposure to Pb

Pb/ Sample	Carcinogenic risk (CR)
Brazil Walnut	2.59E-06
Pistachio	1.79E-06
Indian Walnut	2.25E-06
Almond	2.98E-06
Walnut	2.72E-06
Hazelnut	5.87E-06
Macadamia	3.57E-06

Table 5. Estimated daily intakes of metals from nuts

	Estimated daily intakes (mg/kg/day)					
Metal/ Sample	Cd	Cr	Fe	Mn	Pb	Zn
Brazil Walnut	0.0008	0.0032	0.0205	0.0032	0.0003	0.0165
Pistachio	0.0013	0.0040	0.0489	0.0037	0.0002	0.0182
Indian Walnut	0.0015	0.0028	0.0348	0.0074	0.0003	0.0251
Almond	0.0017	0.0015	0.0160	0.0054	0.0004	0.0186
Walnut	0.0019	0.0036	0.0351	0.0122	0.0003	0.0208
Hazelnut	0.0035	0.0017	0.0097	0.0055	0.0007	0.0250
Macadamia	0.0024	0.0010	0.0273	0.0153	0.0004	0.0157

It was shown that the calculated EDI values for daily average consumption (30 g) ranged from 0.0002 mg/kg/day for Pb in pistachio to 0.0489 mg/kg/day for Fe in pistachio. The recommended daily intake of Fe for adults ranges from 8 mg/day for men and 18 mg/day for women, for Zn from 9.4 mg/day for men and 6.8 mg/day for woman, adequate intake of Cr is 35 μg/day for men and 25 μg/day for woman and for Mn is 2.3 mg/day for men and 1.8 mg/day for woman (Official Gazette of BiH 2014).

CONCLUSIONS

Food contamination with heavy metals may come from food processing and packaging, therefore, monitoring of nuts from markets is very important. For this study nut samples were collected from markets in Sarajevo. In spite of the known major mineral composition of K, P, Mn, Ca, Mg, Fe and other in nuts, there is scarce literature on trace and heavy element contents like Cd, Cr, Pb and Zn. Total elemental concentrations of Cd, Cr, Fe, Mn, Pb and Zn in nuts have been determined in several types of nut, including almond, walnuts, Brazil walnuts, Indian walnuts, Macadamia, hazelnuts and pistachios.

The results provide important information on the nutritional values of nut samples at the Sarajevo market. In most cases, the content of metal was in accordance with the contents given in the previously published papers. The calculated carcinogenic risk for Pb is indicating that the risk of cancer due to exposure to Pb through nuts consumption is in an acceptable range.

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