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Investigation of major and trace elements and their distributions between lipid and non-lipid fractions in Brazil nuts by inductively coupled plasma atomic optical spectrometry

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ABSTRACT

Major (Ca, Mg, P) and trace (As, Al, Ba, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Se, Zn) elements were determined in Brazil nuts by means of inductively coupled plasma atomic optical spectrometry (ICP-OES) and five of them were measured for the first time. For measurement of As, Hg and Se levels, hydride generation was used as the sample treatment method. The element concentrations were compared with recommended dietary allowances and upper tolerable levels. The distributions of the elements between lipid and lipid-free fractions were investigated with the use of solvent extraction. Two extractants (petroleum ether and chloroform:methanol 2:1) were applied. Most of the Cr, Fe and Ni contents were found in the lipid fraction, while Ba, Ca, Cu, Mn, P and Zn were mainly bonded with defatted nut residue. Al, Mg, Se, Sr were only present in the defatted fraction.

1. Introduction

In recent years, there has been growing interest in evaluation of the macro and microelements in a variety of food samples. The importance of mineral composition is due to their nutritional properties and beneficial health effects, as well as their meeting of dietary guidelines required for a healthy diet. Knowledge of contents of the elements is needed, especially in food with nutritional potential. Most reported studies deal with the determination of total element contents. However, bioavailability and toxic properties of the elements depend on the chemical forms in which elements are present. Consequently, work dealing with element fractionation and speciation analysis has attracted increasing interest in recent years.

Nuts, especially Brazil nuts (USDA, 2004), are considered to be one of the most nutritious human foods, due to their high contents of proteins, carbohydrates, unsaturated lipids, vitamins and essential minerals. Brazil nuts (*Bertholletia excelsa*) have been of interest due to their exceptionally high, (by a substantial factor), Se level (Chang, Gutenmann, Reid, & Lisk, 1995; Chunhieng et al., 2004; Kannamkumarath, Wrobel, Wrobel, Vonderheide, & Caruso, 2002), which is a powerful antioxidant, essential nutrient and which has been proven to protect against heart disease and cancers. Since Se content of Brazil nuts was found to be protective against chemically induced tumors in rats (Ip & Lisk, 1994) the greatest attention has been paid to Se determination and Se-related nutritional research with reference to determination of total contents of other elements (Falandysz & Kotecka, 1993; Kannamkumarath, Wróbel, Wróbel, & Caruso, 2004; Kannamkumarath, Wuilloud, & Caruso, 2004; Tinggi & Reilly, 2000 and by Chunhieng et al., 2004). The Se distributions between different fractions (proteins, lipid extracts) as well as speciation analysis were the main subject of studies (Chunhieng et al., 2004; Dumont, De Pauw, Vanhaecke, & Cornelis, 2006; Vonderheide et al., 2002; Wrobel, Kannamkumarath, Wrobel, & Caruso, 2003). Extended speciation and fractionation investigations of Se for both, unshelled and shelled Brazil nuts have concerned mainly protein fractions. The protein extracts were obtained with the use of H_2O, NaOH, HCl as solvents or after enzymatic hydrolysis. As for Se, speciations of Mg, Fe, Co, Ag, Hg, Pb, Mo associated with proteins were carried out by Kannamkumarath, Wuilloud, et al. (2004). Investigation of As and P, bonded with lipids, was conducted by Chunhieng et al. (2004) and Kannamkumarath, Wróbel, et al. (2004). In the case of As, distribution of its various speciation forms between lipids and defatted nut residue was examined.

The most abundant fraction in Brazil nuts is lipids (fats) (more than 60% of the nut weight) (Venkatachalam & Sathe, 2006). Lipids, which are troublesome during sample preparation for element measurements, are often removed by solvent extraction using different organic solvents (Chunhieng et al., 2004; Dumont et al., 2006; Kannamkumarath et al., 2002; Vonderheide et al., 2002). Petroleum ether and chloroform:methanol mixture 2:1 were found

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to be suitable for this purpose (see Kannamkumarath et al., 2002; Venkatachalam & Sathe, 2006). This sample treatment step was found to be useful for Se determination, because Se was not detected in lipid extracts (Chunhieng et al., 2004; Kannamkumarath et al., 2002; Vonderheide et al., 2002).

Distributions of Se, P and As between lipid and lipid-free fractions of Brazil nuts have already been investigated. Therefore, it is important and interesting to study such distributions for other elements. This would also allow assessment of the suitability of the lipid removal procedure as a sample treatment step in multi-elemental analysis of the nut samples.

This study has been undertaken to determine concentrations of major and trace elements in Brazil nuts, including elements not investigated so far. The main attention was focused on distribution of these elements between the lipid extract and the defatted residue fraction. Concentrations of the elements associated with both fractions were measured and analyzed. Petroleum ether and chloroform:methanol (2:1) mixture served as the solvents for separation of the lipid part of the nuts. Inductively coupled plasma optical emission spectrometry was used for element determination. Hydride generation was the technique applied during As, Hg and Se concentration measurements.

2. Materials and methods

2.1. Samples and sample treatment procedures

2.1.1. General

Unshelled Brazil nuts were bought in a local market (Wroclaw, Poland). Before sample treatments, nuts were peeled, grated on a plastic grater and mixed. Digestion and extraction procedures recommended to prepare such food samples for analysis (see Kannamkumarath et al., 2002; Vonderheide et al., 2002) were employed here as follows:

2.1.2. Digestion of the raw samples

One gram of the grated powder of nuts was directly decomposed by a digestion with concentrated HNO_3/H_2O_2.

2.1.3. Fat separation

Fat was separated from the nuts by extraction with organic solvents: chloroform:methanol mixture (2:1) and petroleum ether. Two grams of the nuts were mixed with 10 ml of the extractant and shaken in an ultrasonic bath for 15 min. The extraction procedure was repeated 3 times. Combined lipid fractions were evaporated to dryness and remaining nut residues were left to dry for 24 h at room temperature (Kannamkumarath et al., 2002). Then dried residues of nuts were weighed to determine their weight loss and to evaluate fat contents.

2.1.4. Mineralization of the defatted nut samples and fat fractions

Dried residues and fat extracts were digested in the same way as described for the raw nut samples.

2.1.5. Pretreatment for Se determination

To ensure quantification of selenium hydride generation, Se(VI) was pre-reduced to Se(IV) with 6 M HCl by heating in a water bath at 90 °C for 30 min.

Five parallel samples were prepared for every analysis. With each set of digested and extracted samples, a blank sample was prepared and passed through all treatment procedures to make corrections of final analytical signals.

2.2. Reagents

All the chemicals were of analytical grade. Working standard solutions were prepared by dilution of a multielemental standard (Merck, Germany). The As, Se, Hg standards were prepared by dilution of concentrated single-element stocks. Freshly prepared $NaBH_4$ (POCh, Poland), stabilized in NaOH (POCh) solution, was used for hydride generation. The following solutions of Merck and POCh reagents were used: nitric acid, hydrochloric acid, hydrogen peroxide, methanol, chloroform and petroleum ether.

2.3. Measurement methods

Concentrations of the elements in nuts were measured with the use of the Jobin Yvon sequential inductively coupled plasma emission spectrometer (JY 38S). As, Hg and Se contents were determined by means of a hydride generation ICP-OES hyphenated system. The element lines used for measurements, as well as instrumental and operational parameters, are listed in Table 1.

3. Results and discussion

3.1. Total concentrations of the elements

Total concentrations of the elements (expressed as the means ± standard deviation) were measured in the Brazil nuts and are listed in Table 2. For comparison, the results reported earlier are included.

As far as it is known, Al, Cd, Cr, Ni and Sr in Brazil nuts are determined here for the first time although, for other types of nuts, concentrations of these elements, with the exception of Sr, have been measured (see Alasalvar, Shahidi, Liyanapathirana, & Ohshima, 2003; Cabrera, Lloris, Gimenéz, Olalla, & López, 2003; Momen, Zachariadis, Anthemidis, & Stratis, 2007; Vallilo, Tavares, Aued-Pimentel, Campos, & Moita Neto, 1999).

As can be seen from Table 2, our results (for most of the elements) are similar to those reported by Falandysz and Kotecka (1993), Kannamkumarath, Wuilloud, et al. (2004) and Tinggi and Reilly (2000). The mineral contents found by Chunhieng et al. (2004) (not cited in Table 2) were considerably higher (3–10 times) than those observed by others.

Dietary reference values for elements (i.e. recommended allowances/adequate daily intakes and upper tolerable intake levels for individuals in the 9–70 life stage group) cited in Table 2 can serve for evaluation of nutrition characteristics of examined Brazil nuts.

Table 1
The ICP operating conditions

Generator	40.68 MHz
Rf power	1000 W
Injector i.d.	2.5 mm
Spray chamber	Cyclonic
Nebuluzer	Concentric (Meinhard type)
Monochromator	1 m with 4320/2400 grooves/mm grating
Observation zone	12 mm above load coil
Ar flow rates	Plasma gas: 13 l/min Sheath gas: 0.2 l/min Carrier gas: 0.3 l/min
Solution uptake	0.75 ml/min for pneumatic nebulization 1.0 ml/min for hydride generation
Hydride generation condition	
$NaBH_4$	Concentration: 1.5% in 0.1 mol/l NaOH Uptake: 1.0 ml/min
Lines (wavelengths in nm)	Se I (196.1), Hg II (194.2), As I (197.3), Zn I (213.8), P I (214.9), Ni I (221.6), Cd I (228.8), Ba I (233.5), Mn I (259.4), Fe I (259.9), Cr I (267.7), Mg I (285.2), Ca I (317.9), Cu I (324.7), Al I(396.1), Sr I (407.8)

Table 2
Mineral contents in the analyzed Brazil nuts in comparison to recommended and tolerable dietary values

	Concentration (μg/g)		Dietary reference intakes (mg/d), (Food & Institute of Medicine, 1997; Food & Institute of Medicine, 2001)	
	This study	Falandysz and Kotecka (1993), Kannamkumarath et al. (2004a), Kannamkumarath et al. (2004b), and Tinggi and Reilly (2000)	Recommended dietary allowances/adequate daily dietary intake	Upper tolerable levels
P	7164 ± 212	4830–7250	700–1250	3000–4000
Mg	3321 ± 88	2470–3930	240–420	350
Ca	1630 ± 15	1560–2060	1000–1300	2500
Sr	115 ± 12			
Ba	66.2 ± 12.3	50–950		
Fe	55.8 ± 8.5	26–65	8–15	40–45
Zn	43.0 ± 0.3	38–48	8–11	23–40
Cu	19.6 ± 0.3	9.4–27	0.7–0.9	5–10
Mn	10.1 ± 0.2	5.2–12.2	1.8–2.3	6–11
Ni	8.82 ± 0.82			0.6–1.0
Cr	7.89 ± 1.06		0.02–0.035	
Al	5.33 ± 1.11			7000[b]
Cd	<0.005[a]			7[b]
As	<0.003[a]	0.0028–0.0096		15[b]
Hg	<0.002[a]	0.4		5[b]
	This study	Chang et al. (1995), Chunhieng et al. (2004), Dumont et al. (2006), Kannamkumarath et al. (2002), Tinggi and Reilly (2000), and Vonderheide et al. (2002)		
Se	10.0 ± 0.6	0.03–150	0.04–0.055	0.28–0.40

[a] Below limit of the detection (DL).
[b] Expressed (μg/kg body weight/week) on assumption of provisional permissible weekly intakes, Joint FAO/WHO, 2004.

A comparison of the measured and the dietary reference values indicates that Brazil nuts have high contents of essential elements, with absence of toxic types (As, Cd, Hg). These nuts are within safety levels for human consumption and can be considered as a supplement in the diet. In Table 2, for most of the elements, minimum and maximum concentrations obtained from various nut samples differ by up to 2–3 times. Only the discrepancies for Ba and Se were above 1 and 2 orders, respectively.

Ba and Sr concentrations are relatively high in Brazil nuts in comparison to other trace element contents. So, there is an urgent necessity to establish permissible daily intakes for metals such as Ba and Sr.

3.2. The distribution of the elements between lipid and defatted fractions

On the basis of extraction procedures, it was found here that lipid contents were 68.0 ± 0.6% and 68.2 ± 1.2%, using petroleum ether (PE) and chloroform:methanol 2:1 mixture (C + M), respectively. This indicated a good agreement in lipid content removal with the examined reagents. Similarly, Venkatachalam and Sathe (2006) reported 66.7 ± 1.2% lipid content using (PE) and Vonderheide et al. (2002) removed 67% of the lipids with the use of (C + M) mixture.

In the next step, defatted Brazil nut samples and lipid extracts were digested and total mineral contents were measured in both fractions. Relative standard deviations (RSD) were 1–20%, 3–10% and 7–15%, respectively for the concentration measurements in the digested raw nut samples, defatted residues and the lipid extracts. These RSD values reflect uncertainties in repeatability of appropriate analytical procedures used here. Generally, for each element, the sum of the concentrations in these two fractions was in a good agreement with the value obtained previously after raw sample digestion. Differences between these values did not exceed 5–10% and these differences were within limits of standard deviation uncertainties of the measurements.

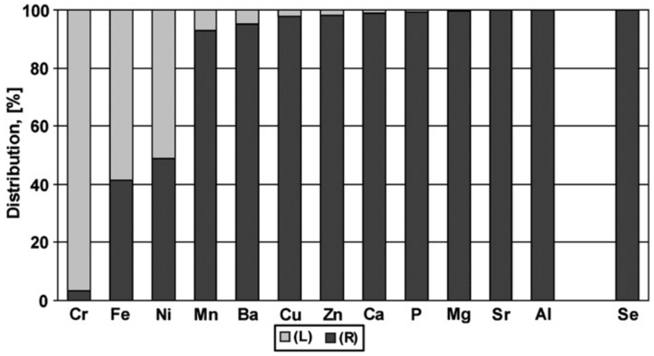

Fig. 1. Distribution of elements between lipid (L) and defatted residue (R) fractions after extraction with the use of petroleum ether as a solvent.

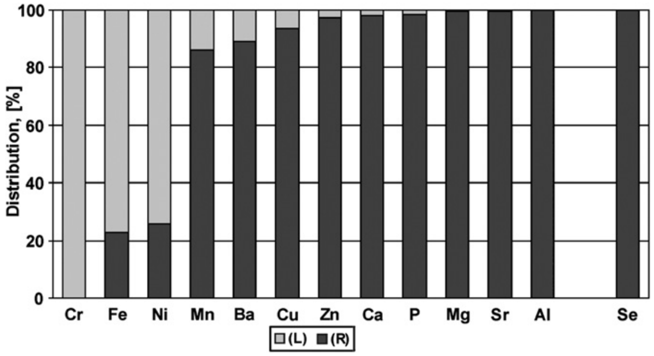

Fig. 2. Distribution of elements between lipid (L) and defatted residue (R) fractions after extraction with the use of chloroform:methanol 2:1 mixture as a solvent.

For transparency and clarity of the results of the fractionation, the element distributions were expressed as the percentages in the fractions and are presented in Figs. 1 and 2. Analyzed elements showed similar behaviour after treatment of the nut samples by the PE and C + M solvents. However, for most of the elements (with the exception of Al, Mg, Se and Sr) the extraction efficiencies were systematically higher with the use of the C + M mixture. This is surprising if one takes into account, that the same percentage of the sample (as lipids) was removed by both extractants. It can be explained by the extraction efficiencies of the extractants. Nevertheless, more advanced studies are necessary to explain such divergences in the quantitative distributions of the element in detail.

As can be seen in Figs. 1 and 2, Se was not found in the lipid fraction. This is in agreement with published reports on nuts, when lipids were removed by chloroform:methanol 2:1 and hexane (Chunhieng et al., 2004; Kannamkumarath et al., 2002; Vonderheide et al., 2002). In animal material samples (chicken tissues), Bohrer, Becker, Cícero do Nascimento, Dessuy, and Machado de Carvalho (2007) found ∼20% of the Se content in the fat fraction after lipid removal with the use of a methanol:dichloromethane (2:3) mixture. Al, Sr and Mg showed a fractionation similar to Se. About 100% of their total concentrations were also in the defatted residue. P, Ca, Zn, Cu, Ba and Mn may be considered as a second group of the elements. These elements were predominantly concentrated in the defatted fraction. In the lipid fraction, their contents were from about 10% for Mn to 1% for P and were slightly higher in the case of the C + M mixture than the PE solvent. Chunhieng et al. (2004) investigated P forms in Brazil nuts and observed that less than 2% of the P was in the lipid extract. Some of the elements, such as Ni, Fe and Cr (the third group), were mostly bonded with the lipid fraction. In the case of Cr, its total amount (in C + M mixture) and almost total (in PE solvent) was associated with the lipid fraction. It was observed that more than 50% of Ni and Fe contents were present in lipids, using PE while, with the use of C + M, amounts extracted were higher than 70%.

4. Conclusions

Concentrations of 16 elements were measured in the Brazil nuts and Al, Cd, Cr, Ni and Sr were determined here for the first time. Sr has not been measured so far in such food samples as nuts.

For most of the elements, their distributions between lipid and non-lipid fractions are investigated here for the first time. Our results showed that Cr (almost totally) and Ni and Fe (mainly) are bonded to lipids, while Mn, Ba, Cu, Zn, Ca and P are mostly found in the non-lipid fraction. Mg, Sr and Al, similarly to Se, are only associated with the lipid-free defatted nut residue.

With the use of petroleum ether and chloroform:methanol 2:1 as a solvents, the extraction efficiencies in lipid removal were the same (68 ± 0.6% and 68 ± 1%, respectively), while the element extraction efficiencies were different. The latter could be due to different affinities of the element forms for particular organic solvents. For more detailed explanations, additional studies in this area should be performed.

Defatting procedures by solvent extraction, although useful and justified for Se species investigation in nuts, can not be used for determination of such elements as Cr, Fe and Ni, as demonstrated here.

Significant contents of Sr and Ba, which are rather rarely determined in food, indicate that establishment of dietary reference values for the elements is an urgent problem.

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