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Speciation of Arsenic in Different Types of Nuts by Ion Chromatography−Inductively Coupled Plasma Mass Spectrometry

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In this work the quantitative determination and analytical speciation of arsenic were undertaken in different types of nuts, randomly purchased from local markets. The hardness of the whole nuts and high lipid content made the preparation of this material difficult for analysis. The lack of sample homogeneity caused irreproducible results. To improve the precision of analysis, arsenic was determined separately in nut oil and in the defatted sample. The lipids were extracted from the ground sample with the two portions of a mixture of chloroform and methanol (2:1). The defatted material was dried and ground again, yielding a fine powder. The nut oil was obtained by combining the two organic extracts and by evaporating the solvents. The two nut fractions were microwave digested, and total arsenic was determined by inductively coupled plasma mass spectrometry (ICP-MS). The results obtained for oils from different types of nuts showed element concentration in the range 2.9−16.9 ng g^{-1}. Lower levels of arsenic were found in defatted material (<0.1 ng g^{-1} with the exception of Brazil nuts purchased with and without shells, 3.0 and 2.8 ng g^{-1} respectively). For speciation analysis of arsenic in nut oils, elemental species were extracted from 2 g of oil with 12 mL of chloroform/methanol (2:1) and 8 mL of deionized water. The aqueous layer, containing polar arsenic species, was evaporated and the residue dissolved and analyzed by ion chromatography−ICP-MS. The anion exchange chromatography enabled separation of As(III), dimethylarsinic acid (DMAs(V)), monomethylarsonic acid (MMAs(V)), and As(V) within 8 min. Several types of nuts were analyzed, including walnuts, Brazil nuts, almonds, cashews, pine nuts, peanuts, pistachio nuts, and sunflower seeds. The recovery for the speciation procedure was in the range 72.7−90.6%. The primary species found in the oil extracts were As(III) and As(V). The arsenic concentration levels in these two species were 0.7−12.7 and 0.5−4.3 ng g^{-1}, respectively. The contribution of As in DMAs(V) ranged from 0.1 ± 0.1 ng g^{-1} in walnuts to 1.3 ± 0.3 ng g^{-1} in pine nuts. MMAs(V) was not detected in almonds, peanuts, pine nuts, sunflower seeds, or walnuts, and the highest concentration was found in pistachio nuts (0.5 ± 0.2 ng g^{-1}).

KEYWORDS: IC−ICP-MS; arsenic; speciation; nut oil

INTRODUCTION

The emission of various arsenicals to the environment resulting in biologically mediated transformations and accumulation in different organisms may increase health risk (*1*). The important sources of arsenic exposure for humans are dietary products and drinking water. Because the toxicity of arsenic is species dependent, quantitative information on different arsenic forms in foods is needed (*2*). The majority of element speciation studies have been focused on marine organisms that accumulate and metabolize arsenic (*3*−*6*). Depending on the type of organism, the primary metabolites identified were arsenobetaine, arsenocholine, and arsenosugars (*7*−*11*). In these organisms, the total arsenic concentration levels reported ranged from 1 to 100 μg g^{-1} (*3*, *5*, *6*). In comparison, lower concentration levels found in food products of terrestrial origin are in the nanogram per gram range, and speciation analysis has rarely been undertaken (*6*, *12*−*16*). In the studies performed in vegetables (carrot) (*14*, *17*) and crops (*13*, *18*, *19*), the inorganic forms of arsenic (As(III), As(V)) and two methylated forms, dimethylarsinic acid (DMAs(V)) and monomethylarsonic acid (MMAs(V)), were observed. In mushrooms from a contaminated area, arsenocholine was also detected (*16*, *20*).

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When speciation studies are undertaken in biological material, sample pretreatment becomes critical. A variety of extraction procedures have been tested, depending on the sample composition and arsenic species of interest (*21*). Most commonly, water or methanol/water extracts were analyzed (*17*, *22*−*24*). Other solvent mixtures used for the extraction of arsenic species from marine biological samples were chloroform/methanol or chloroform/methanol/water (*3*, *10*, *11*, *25*). Different extraction conditions have also been explored, including room temperature mixing, Soxhlet extraction, supercritical fluid extraction, extraction assisted by sonication or microwave radiation (MAE), and accelerated solvent extraction (ASE) (*3*, *14*, *26*−*29*). However, the application of elevated temperature and/or pressure is controversial, because these factors could cause a species or distribution modification.

The hyphenated techniques that couple liquid chromatography with element-selective detection are preferentially used in studies on arsenic speciation. The polarity or ion charge of most common species (As(V), As(III), DMAs(V), MMAs(V), arsenobetaine (AsB)) can be controlled by choosing adequate pH conditions (*11*, *30*). Thus, the chromatographic separation is usually achieved by anion exchange (*13*, *31*) or ion-pairing mechanisms (*11*, *32*, *33*). Heitkemper et al. (*13*) obtained the resolution of the five species listed above on the anion exchange column with a carbonate buffer (pH 10) as the mobile phase. The separation time was 17 min, but it could be reduced to 10 min by lowering the pH of the mobile phase to 6.3 (phosphate buffer). It should be noted however that, at lower pH, As(III) coeluted with AsB. On the other hand, a versatile ion-pairing high-pressure liquid chromatography−inductively coupled plasma mass spectrometry (HPLC−ICP-MS) procedure for fast determination of As(V), MMAs(V), As(III), DMAs(V), and AsB was proposed (*11*). After sample acidification (10 mmol L^{-1} phosphoric acid), the five arsenic species were resolved within 4 min using hexanesulfonic acid (pH 4.5) as an ion-pairing reagent. The feasibility of the method for the analysis of different biological samples was demonstrated.

For on-line detection/quantification of arsenic in the column effluent, ICP-MS and hydride generation atomic absorption (HG-AAS) and atomic fluorescence (HG-AFS) spectrometries were used (*21*, *32*, *34*). The important advantages of ICP-MS over other detection methods are the outstanding specificity assured by stable isotope analysis and very low detection limits.

The aim of the present work was to assess the distribution and speciation of arsenic in different types of nuts. These are healthy dietary products of high nutritional value, related to the presence of proteins (10−20%), unsaturated lipids (50−70%), antioxidant vitamins, and also some essential inorganic micronutrients (Mg, Mn, Zn) (*35*). The nuts analyzed were randomly purchased in the local market and included almonds, Brazil nuts, cashews, peanuts, pine nuts, pistachios, sunflower seeds, and walnuts. For total element determination, microwave-assisted acid digestion and ICP-MS were used. Speciation analysis was carried out using the ion chromatography (IC)−ICP-MS system (*36*, *37*). The only other report found for arsenic in similar biological materials focused on total element determination in sunflower oil (*38*). Thus, the results presented here provide novel analytical information on this difficult biological material and might have possible toxicological relevance.

EXPERIMENTAL PROCEDURES

Instrumentation. HPLC was done with an Agilent Technology (Palo Alto, CA) series 1100 liquid chromatograph equipped with an autosampler, a diode-array detector, and a Chemstation. The chromatographic columns were a Hamilton PRP-X100 (4.6 mm × 250 mm) and a C18 Alltima (4.6 mm × 150 mm, 5 μm).

Table 1. Instrumental Operating Conditions for Total Arsenic Determination and for Analytical Speciation

Ion-Pairing HPLC Parameters (*11*)	
column	C18 Alltima, 4.6 mm × 150 mm, 5 μm
mobile phase	5 mmol L^{-1} hexanesulfonic acid, 5 mmol L^{-1} citric acid/NaOH, pH 4.5
flow rate	0.9 mL min^{-1}
injected volume	20 μL

IC Parameters (*13*)	
column	Hamilton PRP-X100 (4.6 mm × 250 mm)
mobile phase	10 mmol L^{-1} $NH_4H_2PO_4$, 10 mmol L^{-1} NH_4NO_3, pH 6.3 adjusted with NH_3(aq)
flow rate	1 mL min^{-1}
injected volume	50 μL

ICP-MS Parameters	
forward power	1300 W
nebulizer gas flow rate	0.8 L min^{-1}
dwell time	100 ms
isotopes monitored	^{75}As, ^{72}Ge

Microwave Digestion Program

step	power,[a] %	ramp time, min	hold time, min
1	25	5	2
2	45	5	2
3	55	5	2
4	65	5	2

[a] Maximum power 950 W.

An Agilent 7500s inductively coupled plasma-mass spectrometer connected to a concentric nebulizer and Scott-type double-pass spray chamber was used for arsenic-specific detection. The solution eluted from the column was introduced on-line to ICP-MS instrument. The instrumental operation conditions are given in **Table 1**.

A model MES 1000 closed vessel microwave digestion system (CEM, Matthews), a Chermle Z 230 centrifuge (National Labnet, Woodbridge, NJ), a Rotavap RE 111 (Buchi Laboratoriums Technik AG, Schwitzerland), and a model RC5C centrifuge (Sorvall Insruments DuPont) were used.

Reagents and Samples. Doubly deionized water was used (18.2 MΩ cm), prepared by passing deionized water through a NanoPure treatment system (Barnstead, Boston, MA). Analytical-reagent-grade chemicals and HPLC-grade methanol and chloroform (Fisher Scientific, Pittsburgh, PA) were used.

Inorganic arsenic standards, 1000 μg mL^{-1} As as As_2O_3 in 2% HCl and $H_3AsO_4 \cdot {}^1/_2H_2O$ in 2% HNO_3, were kindly provided by the U.S. Food and Drug Administration (originally purchased from Spex Industries, Metuchen, NJ). DMAs(V) and disodium methylarsenate (MMAs(V)) were from Chem Service (West Chester, PA). AsB was acquired from the Department of Chemistry, University of British Columbia (Vancouver, Canada). All stock standard solutions were made on the basis of arsenic content, and their concentrations were validated using NIST 1643c (*11*).

The mobile phase for anion exchange chromatography was 10 mmol L^{-1} ammonium phosphate monobasic and 10 mmol L^{-1} ammonium nitrate at pH 6.3 (adjusted with sodium hydroxide) prepared from Sigma reagents. For ion-pairing HPLC the mobile phase was 5 mmol L^{-1} hexanesulfonic acid in 5 mmol L^{-1} citric acid (pH 4.5 adjusted with sodium hydroxide), prepared from Fisher and Sigma reagents, respectively.

Solutions of the following Sigma reagents were used: nitric acid, hydrogen peroxide, phosphoric acid, ammonium hydroxide.

DORM-2 (dogfish muscle tissue) was a reference material purchased from the National Research Council (NRCC, Ottawa, Ontario, Canada).

The nuts analyzed in this work were purchased in the local market and were the following: walnuts (1, white; 2, black), Brazil nuts (1, sold with shells; 2, without shells), almonds, cashews, pine nuts, pistachio nuts (not processed), peanuts, and sunflower seeds (salted).

Procedures. *Determination of Total Arsenic in Defatted Nuts and in Nut Oil*. About 20 g of each type of nut was ground in a coffee mill, 100 mL of chloroform/methanol (2:1) was added, and the mixture was shaken vigorously. After filtration and drying (room temperature) the nuts were again ground, and the extraction was repeated. Three subsamples of defatted nut powder were precisely weighed (1.0 g) and transferred to PTFE vessels. Digestion was performed with 10 mL of aqueous nitric acid (50% (v/v)) in a microwave oven (detailed program in **Table 1**). The chloroform/methanol fractions were combined and the solvents evaporated (Rotavap). An aliquot (1 g) of the obtained oil was digested with a 9:1 mixture of 50% (v/v) nitric acid/30% (v/v) hydrogen peroxide in a microwave oven using the same program (**Table 1**). In the two types of digests, total arsenic was determined by ICP-MS using an internal standard of germanium (10 μg L^{-1}) for analytical signal correction and the two-point standard addition technique (three replicates).

Speciation of Arsenic in the Lipid Fraction of Nuts. To the subsample of nut oil (2 g) was added 12 mL of chloroform/methanol (2:1), and the mixture was placed in an ultrasonic bath (30 min). Then, 8 mL of deionized water was added and the aqueous layer collected after phase separation. The solvents were evaporated, and the residue was dissolved in 1 mL of diluted phosphoric acid (25 mmol L^{-1}). This solution was introduced to IC−ICP-MS for arsenic speciation. The anion exchange separation was achieved using the method developed by Heitkemper et al. (*13*) (**Table 1**). In each case, the analysis was carried out in triplicate.

RESULTS AND DISCUSSION

Initially, total arsenic determination was undertaken. When whole nuts were ground, greasy particles of different sizes were obtained. This physical appearance and the lack of homogeneity made the manipulation and weighing of the analytical sample difficult. As might be expected, highly irreproducible results were obtained. To improve analytical performance, arsenic was determined separately in the defatted samples and the oil fractions. Lipids were extracted from the ground nuts with the mixture chloroform/methanol (2:1). After the first extraction, the nuts were dried and ground again, and the second extraction was performed. The third grinding yielded a fine powder of defatted nuts. The nut oil was obtained by evaporation of solvents from the combined organic extracts. For each type of nut, the defatted material and the oil fraction were microwave digested, and the analysis of total arsenic was carried out by ICP-MS. For validation purposes, the reference material (DORM-2) was analyzed for total arsenic. The obtained result, 17.3 ± 1.6 μg g^{-1}, was in good agreement with the certified value (18.0 ± 1.1 μg g^{-1}).

The results obtained for oil fractions showed the arsenic levels in different types of nuts in the range 2.9−16.9 ng g^{-1} (**Table 2**). Much lower levels of this element were found in defatted material (<0.1 ng g^{-1} with the exception of Brazil nuts with and without shells, 3.0 and 2.8 ng g^{-1}, respectively). Generally, the total concentration levels in nuts were similar to those reported for other terrestrial plant products (*13*) and lower than in marine organisms (*3*, *5*, *6*), suggesting the lack of bioaccumulation of arsenic in nuts. A significantly higher contribution of arsenic in the oil fraction should be ascribed to the polarity of its species (solubility in chloroform/methanol mixture). Because of the arsenic in oil, elemental speciation in the nut fraction was studied. The extraction of arsenic species was achieved with a mixture of chloroform, methanol, and water. This solvent composition was selected to ensure efficient extraction of polar As species (*11*, *39*) and also to eliminate lipids before sample introduction to the chromatographic system. For preconcentration of the analytes and for elimination of methanol, the water/methanol phase was evaporated (Rotavap) and the residue dissolved in 1 mL of phosphoric acid, 25 mmol L^{-1}. As already mentioned in the Introduction, the primary species found in terrestrial plant products were As(III), As(V), MMAs(V), and DMAs(V) (*13*, *14*, *17*−*19*). Logically, these same species were expected to be present in nuts.

Table 2. Results of Arsenic Determination in the Digested Nut Oils and the Results of Speciation Analysis in This Fraction

type of nut	concn of As found in the form of different species,[a] ng g^{-1}					speciation recovery,[b] %	total as concn in digested oil[a]
	As(III)	DMAs(V)	MMAs(V)	As(V)	Â		
almond	3.3 ± 0.3	0.2 ± 0.1	nf[c]	2.6 ± 0.4	6.1	76.3	8.0 ± 1.5
Brazil 1	1.8 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	2.4 ± 0.4	4.5	75.0	6.0 ± 0.5
Brazil 2	2.9 ± 0.2	0.5 ± 0.2	0.1 ± 0.1	4.3 ± 0.5	7.8	81.3	9.6 ± 2.1
cashew	12.7 ± 0.9	0.2 ± 0.1	0.2 ± 0.1	1.4 ± 0.5	14.5	85.8	16.9 ± 0.7
peanut	0.7 ± 0.2	0.3 ± 0.1	nf	1.2 ± 0.5	2.2	75.9	2.9 ± 0.6
pine	6.3 ± 0.7	1.3 ± 0.3	nf	0.5 ± 0.3	8.1	84.4	9.6 ± 0.7
pistachio	4.2 ± 0.5	0.4 ± 0.1	0.5 ± 0.2	2.1 ± 0.4	7.2	72.7	9.9 ± 1.0
sunflower	0.9 ± 0.3	0.1 ± 0.1	nf	1.4 ± 0.3	2.4	77.4	3.1 ± 0.5
walnut 1	12.7 ± 0.8	0.4 ± 0.2	nf	1.4 ± 0.5	14.5	90.6	16.0 ± 2.4
walnut 2	4.2 ± 0.6	0.1 ± 0.1	nf	0.9 ± 0.5	5.2	74.3	7.0 ± 0.8

[a] Mean results ± SD from triplicate analyses are expressed as nanograms of As per gram of nut oil. [b] Ratio between the sum of As species eluted from the column and total As in the digested nut oil, expressed as percentage. [c] nf = not found.

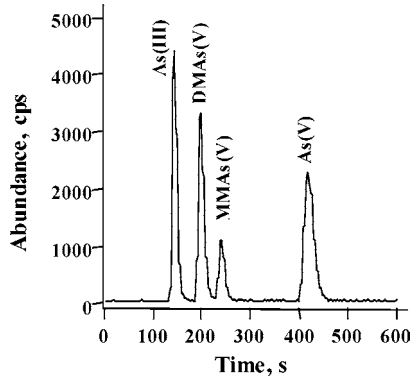

Figure 1. IC–ICP-MS chromatograms of arsenic standards according to the following elution order: As(III), 40 μg L^{-1} As; DMAs(V), 40 μg L^{-1} As; MMAs(V), 12 μg L^{-1} As; As(V), 40 μg L^{-1} As. The instrumental operating conditions and the conditions of anion exchange separation are detailed in **Table 1**.

In the first approach, the chromatographic separation was performed by ion-pairing HPLC. As reported elsewhere, the fast baseline separation of the five standards was obtained with the following elution order: As(V), t_{ret} = 83 s; MMAs(V), t_{ret} = 99 s; As(III), t_{ret} = 130 s; DMAs(V), t_{ret} = 166 s; AsB, t_{ret} = 208 s (*11*). However, in the analysis of nut extracts the early eluting peaks were broadened, suggesting possible matrix effects. It was verified by using the $m/z = 35$ channel that these interferences were not caused by the formation of $ArCl^+$. The ion-pairing HPLC was used in this work to screen the samples for the possible presence of AsB, and this species was not detected in any sample.

In the application of anion exchange separation proposed by Heitkemper (phosphate buffer, pH 6.3) (*13*), good resolution of arsenic species was obtained in nut extracts (sharp, symmetric peaks). The coelution of As(III) and AsB reported in the cited work was not important owing to the absence of AsB in the samples, so this separation procedure was selected for the analysis of nuts. The chromatogram obtained for four arsenic

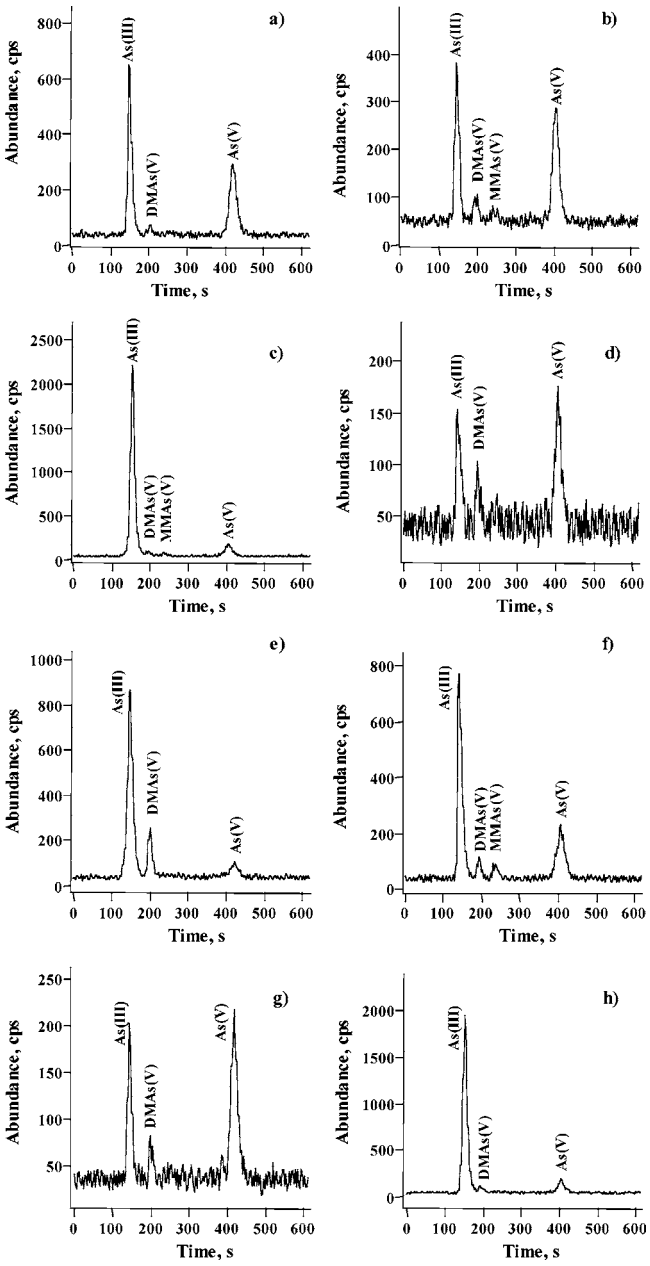

Figure 2. Typical chromatograms obtained in the analysis of nut oils: (a) almond, (b) Brazil 1, (c) cashew, (d) peanut, (e) pine nuts, (f) pistachio, (g) sunflower, (h) walnut 1. The instrumental operating conditions and the conditions of anion exchange separation are detailed in **Table 1**.

compounds is presented in **Figure 1** (instrumental operating conditions given in **Table 1**). In agreement with the results reported previously, the elution order was As(III) (t_{ret} = 149 s), DMAs(V) (t_{ret} = 201 s), MMAs(V) (t_{ret} = 240 s), and As-(V) (t_{ret} = 417 s). Calibration was carried out using the mixed standard solutions in the concentration range 0−60 μg L^{-1} As in each compound ($r^2 > 0.997$). The quantification limits were evaluated on the basis of six standard deviations of the noise level, and these were 0.13 ng L^{-1} for As(III), 0.16 ng L^{-1} for DMAs(V), 0.15 ng L^{-1} for MMAs(V), and 0.24 ng L^{-1} for As(V). Correction for possible argon chloride interference at m/z = 75 was not necessary. To check the stability of arsenic compounds in nuts during the speciation procedure, a spiking experiment was performed. To do so, the aliquot (50 μL) of the highest calibration standard solution (60 μg L^{-1} As in each compound) was added to the subsample (2 g) of the oil from Brazil nuts (Brazil nuts 1), and the speciation procedure was performed. The recoveries obtained for As(III), DMAs(V), MMAs(V), and As(V) were 85.1%, 87.6%, 86.3%, and 88.8%, respectively. These relatively low values were ascribed to incomplete extraction of arsenic species from nut oil, which was confirmed by comparing total As in digested oils with the sum of As species determined by IC−ICP-MS (see the speciation recovery data in **Table 2**).

Several nut extracts were analyzed by IC−ICP-MS. Arsenic speciation was carried out in almonds, Brazil nuts (purchased with (1) and without (2) shells), cashews, peanuts, pine nuts, pistachios, sunflower seeds, and walnuts (white (1) and black (2)). Typical chromatograms are presented in **Figure 2**. The speciation recoveries were in the range 72.7−90.6% (**Table 2**). The primary species found in all oil samples were As(III) and As(V). The quantitative results are presented in **Table 2**. As can be observed, arsenic in the form of As(III) ranged from 0.7 ± 0.2 to 12.7 ± 0.9 ng g^{-1}; the lowest level was obtained in peanuts, the highest in cashews and in white walnuts. For As(V), the contribution in nut oil extracts ranged from 0.5 ± 0.3 ng g^{-1} in pine nuts to 4.3 ± 0.5 ng g^{-1} in Brazil nuts 2. A tendency was observed toward higher levels of As(III) in the not-processed nuts (cashews, almonds, pine nuts, walnuts) as compared to those processed (salted peanuts and sunflower seeds) (**Table 2**). Two methylated compounds were detected. The contribution of As in the form of DMAs(V) ranged from 0.1 ± 0.1 to 1.3 ± 0.3 ng g^{-1}, and the highest concentration was found in the oil from pine nuts. The MMAs(V) was not detected in almonds, peanuts, pine nuts, sunflower seeds, or walnuts. The highest concentration was found in pistachio nuts (0.5 ± 0.2 ng g^{-1}).

CONCLUSIONS

The analysis of arsenic in nuts carried out in this work indicated that, for off the shelf products we obtained locally, there were relatively low (ng g^{-1}) levels of As, similar to the levels reported for other terrestrial plant materials. Quantitative determination of total arsenic in whole nut samples at these low levels was not achieved due to the lack of homogeneity of the ground sample. The nut samples were fractionated by solvent extraction, and a significantly higher total arsenic was found in the oil fractions relative to defatted nuts. Speciation analysis was done with the nut oils by anion exchange ICP-MS. To separate polar arsenic species from lipids, the oil samples were extracted with a chloroform/methanol/water mixture. The water/methanol phase was evaporated to eliminate methanol and to concentrate the arsenic compounds. The recovery for the speciation procedure ranged from 72.7% to 90.6%. The primary species found in different types of nuts were As(III) and As-(V). Lower concentrations of DMAs(V) and MMAs(V) were also found. Generalization about arsenic species in nuts cannot be made without more extensive sampling.

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