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Bioaccessibility of Cd, Cu, Fe, Mn, Pb, and Zn in Hazelnut and Walnut Kernels Investigated by an Enzymolysis Approach

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ABSTRACT: Bioaccessibility of four essential (Fe, Cu, Mn, Zn) and two toxic (Cd, Pb) elements in kernels of four walnut and four hazelnut cultivars was investigated using sequential enzymolysis approach and atomic absorption spectrometry. It was found that the assimilable part of elements was not dependent on nut cultivar. The bioaccessible fraction of Cu, Mn, and Zn was definitely higher for hazelnuts (62% Cu, 39% Mn, 58% Zn) than for walnuts (14% Cu, 21% Mn, 15% Zn). Bioaccessible Fe was 20−24% from its total content for both nut types. Solubility in the simulated intestinal juice is affected by both formation of stable soluble complexes and back sorption of dissolved elements on nut solid residues. Lead shows strong insolubility due to the high sorption affinity of lead ions to the insoluble fraction of nuts. Thus, walnuts and hazelnuts could act as effective biosorbents for lead detoxication.

KEYWORDS: *hazelnuts, walnuts, microelements, bioaccessibility, enzymolysis*

INTRODUCTION

Nuts have been considered health foods worldwide.[1] They provide a variety of bioactive compounds such as vegetable proteins, unsaturated essential fatty acids, fiber, tocopherols, phytosterols, phenolic compounds, and minerals.[2−6] Nut consumption leads to many positive health outcomes such as reduced incidences of coronary heart disease, reduced blood cholesterol content, regulated blood pressure, and protective effect on cancer.[7−9] Hazelnuts[10−12] and walnuts[13] are good sources of minerals. However, the reported data for the total concentrations of microelements provide little information about their possible nutritional value and toxic effects. To assess the real intake from these nuts means to assess their bioaccessibility defined as the amount of element converted to soluble forms in the gastrointestinal tract. A methodology based upon a sequential enzymolysis procedure[14−17] was developed to investigate the potentially bioaccessible part of microelements from different foods and food supplements. This in vitro method, which simulates food digestion in the gastrointestinal tract, has proved to be a very useful tool for investigating the bioaccessibility of essential elements from foods and the factors influencing it.[18−22] To the best of our knowledge no studies on the potential bioaccessibility of microelements from nuts have been published yet.

The objective of this study was to evaluate the bioaccessibility of four essential micronutrients (Fe, Cu, Mn, Zn) and two toxic microelements (Cd, Pb) from various varieties of walnut and hazelnut kernels using an enzymolysis approach. Further investigations were conducted to provide a reasonable explanation regarding the mechanism of the transformation of the microelements and their retention in soluble forms during the gastrointestinal digestion, including (i) evaluation of the soluble forms of the analytes using liquid phase extraction approach and (ii) investigation of the sorption properties of nuts before and after gastrointestinal treatment.

MATERIALS AND METHODS

Reagents and Chemicals. All reagents used were of analytical reagent grade (p.a. Merck, Darmstadt, Germany). Milli-Q water (Millipore, Bedford, MA, USA) was used throughout. Single-element 1 g/L stock standard solutions of Cd, Cu, Fe, Mn, Pb, and Zn from Merck were used for preparation of working aqueous standard solutions by appropriate dilution with 0.3% HNO_3. Multielement standard III dissolved in oil (Merck) with a concentration of 900 mg/L for all of the studied elements was used for determination of the element concentration in organic phase. The working organic standard solutions were prepared just before the measurements by diluting the multielement standard with xylene. Ammonium pyrollidine dithiocarbamate (APDC, p.a. Merck), sodium dodecyl sulfonate (NaDDS, p.a. Merck), trioctylmethyl ammonium chloride (TOMACl, p.a. Merck, 2% solution in xylene), and xylene (p.a. Merck) were used as received.

The simulated gastric juice was prepared as 1% (w/v) pepsin in 0.01 M HCl (pH 2). The simulated intestinal juice was prepared by mixing equal volumes of 3% (w/v) pancreatin plus 1% (w/v) amylase in water and 1.5 g/L bile salts in water. Both juices were prepared just before use. The enzymes and the bile salts were purchased from Sigma (pepsin, catalog no. P700; pancreatin, catalog no. P1750; amylase, catalog no. A3176; bile salts, catalog no. B8756).

Instrumentation. A Perkin-Elmer AAnalyst 400 (Waltham, MA, USA) flame atomic absorption spectrometer was used with an air−acetylene flame and hollow cathode lamps for Cu (324.8 nm), Fe (248.3 nm), Mn (279.5 nm), and Zn (213.9 nm). The instrumental parameters were optimized to obtain maximum signal-to-noise ratio

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Table 1. Total Content of Microelements in Hazelnut Cultivars and Their Dissolved Content in Simulated Gastric and Intestinal Fluids after Sequential Enzymatic Digestion under Gastric and Intestinal Conditions (Mean ± Standard Deviation)

			dissolved content (n = 2)[a]			
			gastric juice		intestinal juice	
element	hazelnut cultivar	total content (n = 3) (μg/g)	μg/g	%[b]	μg/g	%
Cd	Ata Baba	0.153 ± 0.006	0.041 ± 0.003	27	0.064 ± 0.005	42
Cd	Tonda Gentile	0.067 ± 0.003	0.018 ± 0.002	27	0.029 ± 0.002	43
Cd	Ran Trapezundski	0.167 ± 0.008	0.045 ± 0.003	27	0.075 ± 0.004	45
Cd	Halls Giant	0.183 ± 0.009	0.048 ± 0.003	26	0.077 ± 0.005	42
Cu	Ata Baba	16 ± 1	13.1 ± 0.7	82	10.7 ± 0.8	67
Cu	Tonda Gentile	12.5 ± 0.3	9.4 ± 0.5	75	7.8 ± 0.6	62
Cu	Ran Trapezundski	13.5 ± 0.3	10.5 ± 0.6	78	7.8 ± 0.6	58
Cu	Halls Giant	17 ± 1	13.9 ± 0.7	82	10.7 ± 0.8	63
Fe	Ata Baba	29 ± 2	1.7 ± 0.2	5.9	6.7 ± 0.2	23
Fe	Tonda Gentile	28 ± 2	0.9 ± 0.1	3.2	6.2 ± 0.3	22
Fe	Ran Trapezundski	32 ± 2	1.5 ± 0.2	4.7	7.7 ± 0.3	24
Fe	Halls Giant	29 ± 3	1.4 ± 0.1	4.8	6.7 ± 0.4	23
Mn	Ata Baba	21 ± 1	16.6 ± 0.7	79	8.0 ± 0.7	38
Mn	Tonda Gentile	34 ± 3	25.5 ± 0.9	75	13.3 ± 0.9	39
Mn	Ran Trapezundski	36 ± 3	28.1 ± 0.8	78	14.8 ± 0.9	41
Mn	Halls Giant	23 ± 2	17.9 ± 0.8	78	8.5 ± 0.8	37
Pb	Ata Baba	0.21 ± 0.03	0.016 ± 0.002	7.6	<0.002	
Pb	Tonda Gentile	0.078 ± 0.002	0.006 ± 0.001	7.7	<0.002	
Pb	Ran Trapezundski	0.11 ± 0.01	0.009 ± 0.001	8.2	<0.002	
Pb	Halls Giant	0.11 ± 0.01	0.009 ± 0.002	8.2	<0.002	
Zn	Ata Baba	24 ± 1	14.2 ± 0.5	59	13.7 ± 0.7	57
Zn	Tonda Gentile	24 ± 1	14.9 ± 0.6	62	14.4 ± 0.7	60
Zn	Ran Trapezundski	23 ± 1	13.8 ± 0.4	60	13.3 ± 0.6	58
Zn	Halls Giant	22 ± 1	13.0 ± 0.4	59	13.2 ± 0.5	60

[a] n = number of parallel determinations. [b] Soluble content of the elements as percent of their total content in hazelnut kernels.

for a standard analyte solution aspirated into the flame. Calibration with aqueous standard solutions was used for all flame AAS measurements.

The measurements of Cd and Pb as well as of Cu, Fe, and Mn in some fractions were performed using a Perkin-Elmer (Norwalk, CT, USA) Zeeman 3030 atomic absorption spectrometer with an HGA-600 atomizer. The light sources were an electrodeless discharge lamp for Cd (228.8 nm) and hollow cathode lamps for Cu, Fe, Mn, and Pb (283.3 nm). Uncoated graphite tubes with a platform were used for Cd and Pb and pyrolytically coated tubes for Cu, Fe, and Mn. As matrix modifier for determination of Cd and Pb, 10 μL of 2% (w/v) APDC in water was used.[23] It allowed pyrolysis temperatures of 400 °C for Cd and 800 °C for Pb. The atomization temperatures were 1000 °C for Cd and 1500 °C for Pb. Copper, iron, and manganese were determined without a modifier at 1000 °C pyrolysis and 2000 °C atomization temperatures. Autosampler AS-60 was used for injection of 10 μL aqueous sample solutions into the graphite tube. The organic solutions were injected manually. Only peak areas were used for quantification, applying the standard addition calibration mode.

Samples. The kernels of four walnut (*Juglans regia* L.) cultivars (Sheynovo, Izvor, Pedro, Hartley) and four hazelnut (*Corylus pontica* C. Koch) cultivars (Ata Baba, Tonda Gentile delle Laghne, Ran Trapezundski, Halls Giant) were used in this study. Samples of each cultivar were obtained from the Agricultural Experiment Station, Kardzhali (southeastern Bulgaria) collected during the 2010 harvest season. The harvest region is industrially polluted due to activities of a lead/zinc smelter located nearby. The collected nuts were air-dried, unshelled, milled, and packed in polyethylene bags and stored at 4 °C until the time of analysis.

Determination of Total Concentrations. For determination of the total microelements content, approximately 1 g of nut sample was accurately weighed into a 50 mL beaker and first digested with 20 mL of concentrated nitric acid for 12 h at room temperature (beaker covered with a watch glass). Then the samples were placed on a sand bath and heated at 150 °C for about 1 h. After cooling, cover glasses were removed, 5 mL of 30% (w/v) H_2O_2 was added, and the solutions were again heated on the sand bath until the volume reached about 4 mL and then quantitatively transferred into 25 mL volumetric flasks. From each sample three replicates were prepared together with corresponding blanks. The same procedure was applied for the determination of element content in the residues left after gastrointestinal digestion (residues preliminarily air-dried).

Bioaccessible Fraction. The methodology for investigation of bioaccessibility of the elements was adapted and modified from the procedures developed by Crews et al.[14] and later described by Olayinka et al.[15] and Dundar and Haswell.[16] Duplicate samples (5 g) were weighed accurately and homogenized with 50 mL of simulated gastric juice in a 200 mL cylindrical flask. Mixtures were incubated at 37 °C for 4 h in a shaking water bath. One of the gastric digests was centrifuged. The supernatant was filtered (0.45 μm Millipore filter) and stored in a refrigerator at 4 °C. The analysis of samples was performed within hours, and samples were never stored for >48 h.

The pH value of the second gastric digest was adjusted to 7.4 by adding drops of saturated $NaHCO_3$ solution. Then, 50 mL of simulated intestinal juice was added, and the mixtures were again

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Table 2. Total Content of Microelements in Walnut Cultivars and Their Dissolved Content in Simulated Gastric and Intestinal Fluids after Sequential Enzymatic Digestion under Gastric and Intestinal Conditions (Mean ± Standard Deviation)

			dissolved content (n = 2)[a]			
			gastric juice		intestinal juice	
element	walnut cultivar	total content (n = 3) (μg/g)	μg/g	%[b]	μg/g	%
Cd	Sheynovo	0.015 ± 0.001	0.0040 ± 0.0007	27	0.0066 ± 0.0005	44
Cd	Izvor	0.018 ± 0.002	0.0049 ± 0.0006	27	0.0077 ± 0.0007	43
Cd	Pedro	0.021 ± 0.003	0.0057 ± 0.0007	27	0.0094 ± 0.0007	45
Cd	Hartley	0.0097 ± 0.0006	0.0025 ± 0.0006	26	0.0041 ± 0.0006	42
Cu	Sheynovo	15.5 ± 0.6	3.7 ± 0.1	24	2.0 ± 0.1	13
Cu	Izvor	18.0 ± 0.4	4.7 ± 0.2	26	2.5 ± 0.2	14
Cu	Pedro	15.3 ± 0.3	4.1 ± 0.1	27	2.1 ± 0.2	14
Cu	Hartley	15.4 ± 0.4	4.0 ± 0.2	26	2.3 ± 0.2	15
Fe	Sheynovo	33 ± 2	1.2 ± 0.1	3.6	6.6 ± 0.2	20
Fe	Izvor	38 ± 3	1.7 ± 0.2	4.5	8.4 ± 0.4	22
Fe	Pedro	33 ± 2	1.9 ± 0.2	5.8	7.6 ± 0.3	23
Fe	Hartley	31 ± 2	1.5 ± 0.1	4.8	7.4 ± 0.4	24
Mn	Sheynovo	39 ± 1	27.3 ± 1.2	70	8.6 ± 0.7	22
Mn	Izvor	55 ± 4	36.8 ± 1.6	67	12.1 ± 0.9	22
Mn	Pedro	40 ± 1	26.8 ± 1.8	67	8.0 ± 0.6	20
Mn	Hartley	43 ± 2	29.7 ± 1.4	69	8.6 ± 0.8	20
Pb	Sheynovo	0.042 ± 0.005	<0.002		<0.02	
Pb	Izvor	0.064 ± 0.007	<0.002		<0.02	
Pb	Pedro	0.045 ± 0.005	<0.002		<0.02	
Pb	Hartley	0.094 ± 0.006	<0.002		<0.02	
Zn	Sheynovo	28 ± 2	7.8 ± 0.4	28	4.2 ± 0.6	15
Zn	Izvor	37 ± 1	10.7 ± 0.5	29	5.9 ± 0.7	16
Zn	Pedro	28 ± 1	7.6 ± 0.4	27	4.5 ± 0.6	16
Zn	Hartley	29 ± 1	8.1 ± 0.4	28	4.1 ± 0.6	14

[a]n = number of parallel determinations. [b]Soluble content of the elements as percent of their total content in walnut kernels.

incubated at 37 °C for 4 h in a shaking water bath. The intestinal digest was centrifuged; the supernatant was filtered through a 0.45 μm Millipore filter. As with the first digest, aliquots of the filtrate were analyzed by flame (Cu, Fe, Mn, Zn) or electrothermal (Cd, Pb) atomic absorption spectrometry (AAS) within 2 days. The experiment was repeated twice, always with corresponding blanks.

Liquid Phase Extraction. The supernatants obtained for assessment of the bioaccessible fraction of hazelnuts (see above) were immediately tested (after filtration) for the chemical forms of the dissolved elements by liquid phase extraction as described previously.[24] In brief, 5.0 mL aliquots of the studied supernatant were placed in four extraction tubes. Then, to the first tube were added 1 mL of 2% APDC plus 5 mL of xylene; to the second tube, 5 mL of xylene; to the third tube, 0.5 mL of 0.001 mol/L NaDDS plus 5 mL of xylene; and to the fourth tube, 5 mL of 2% TOMACl in xylene. The extraction was performed for 5 min. The concentrations of the elements were determined in both organic and aqueous phases by flame or electrothermal AAS.

Sorption. Two grams of milled raw nut sample and 2 g of the solid residue remaining after finishing the intestinal step of the enzymatic digestion were placed in 50 mL polyethylene centrifuge tubes. Then 20 mL of aqueous standard solution containing 1.5 ppm (C_i) of the studied analytes (pH value of the standard solution adjusted to pH 7.4 with diluted ammonia solution) was added to each tube, and the mixtures were shaken for 4 h at 37 °C in a shaking water bath. After centrifugation and filtration, the concentrations of the analytes in the supernatant (C_s) were measured by flame AAS. The sorption degree was calculated as $(C_i - C_s)/C_i$.

Quality Control and Assurance. Standard reference materials CRM NCS ZC73011 soybean and BCR-185R bovine liver were used to check the reliability of the results, because no nut materials with certified microelement content were available. The accuracy of the data for total element concentration has also been checked by analysis of spiked walnut (cv. Sheynovo, Pedro) and hazelnut (cv. Ata Baba, Halls Giant) samples. Recoveries from analysis of reference materials and spiked samples were in the range of 91−104%. No significant differences were registered between the certified and experimentally obtained mean values (t test, significance level of $p < 0.05$).

The accuracy of the measurements of element concentrations in different extracts was controlled by checking the mass balance, that is, by proving the total analyte recovery. The concentrations of the elements were determined (i) in the supernatants and in the corresponding residues (the balance of mass between the extracted (soluble fraction) and nonextracted content with the total concentration) and (ii) in the aqueous and in the organic phases obtained after liquid phase extraction. The total content of the elements before fractionation was compared with the sum content in the fractions. The analyte recovery was in the range of 87−116% and was judged to be satisfactory.

RESULTS AND DISCUSSION

Total Concentrations of Cd, Cu, Fe, Mn, Pb, and Zn in Hazelnut and Walnut Cultivars. The results obtained for the total content of Cd, Cu, Fe, Mn, Pb, and Zn in various hazelnut and walnut cultivars are given in Tables 1 and 2, respectively.

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Table 3. Potential Contribution of Hazelnuts and Walnuts to RDA of Elements Related to the Daily Consumption of 100 g of Nuts (RDA As Published by the U.S. Food and Drug Administration[32])

		potentially provided absolute amounts as % of RDA		bioaccessible content as % of RDA	
element	RDA (mg/day)	hazelnuts	walnuts	hazelnuts	walnuts
Cu	0.9	139−189	170−200	83−119	24−28
Fe	8	35−40	39−48	7.7−9.6	8.2−10.4
Mn	2.3	91−156	170−239	35−64	35−53
Zn	11	20−22	25−34	12−13	3.8−5.4

The concentration ranges for the total content of essential elements Cu, Fe, Mn, and Zn are similar to those reported from many authors for hazelnuts[10−12] and walnuts.[13] As seen from the results, the content of toxic elements Cd and Pb in some cultivars, particularly of hazelnuts (Table 1), is higher than the maximum admissible levels[25] for these elements (0.05 mg/kg Cd, 0.1 mg/kg Pb) due to air and soil pollution from a lead/zinc smelter located near the nut trees. This makes the investigations concerning the potential bioaccessibility of Cd and Pb extremely important for risk assessment.

Bioaccessible Fraction and Contribution to the Recommended Daily Allowance (RDA). Results of the in vitro bioaccessibility investigations using sequential enzymatic approach in relation to the total content of elements are given in Tables 1 and 2 for hazelnuts and walnuts, respectively. The presented results show that the solubilities of the microelements (as percent of their total content) in simulated gastric and intestinal juices are very similar for the four cultivars of each nut type. This is very likely related to the fact that all of the cultivars were harvested in one geographical region with similar soil and climatic characteristics. However, significant differences ($p > 0.05$) were observed between hazelnuts and walnuts. The solubility of Cu and Zn in gastric (75−82% Cu, 59−62% Zn) and in intestinal juices (58−67% Cu, 57−60% Zn) is higher in hazelnuts (Table 1) than in walnuts (Table 2). Whereas the solubility of Zn in both juices was approximately the same for hazelnuts, in the case of walnuts it decreased markedly during intestinal digestion, dropping from 27−29% in the gastric to 14−16% under intestinal conditions (Table 2). Approximately in the same proportion were also the concentrations of copper in the gastric (24−27% soluble Cu) and in the intestinal (13−15%) fluids of walnuts. The bioaccessibility of Cu in hazelnuts was similar to that obtained for bread (49−62%), cheese (52−67%),[20] and tea-biscuit samples (47−69%).[26] The results for zinc bioaccessibility from walnuts are in reasonably good agreement with the results obtained for cheese (12%).[20] The difference between the bioaccessibility of copper/zinc from hazelnuts (62%/59%) and walnuts (14%/16%) is difficult to explain. A possible reason might be the quite different fatty acid compositions of these nuts (75% monounsaturated fat in hazelnuts vs only 13% in walnuts[27,28]) if it could be supposed that monounsaturated fat more readily forms stable soluble copper and zinc complexes. However, further special investigations are needed to prove this assumption.

Manganese showed relatively high solubility under simulated gastric conditions (around 80% for hazelnuts and 70% for walnuts), but after the sequential intestinal step at least around 80% of Mn in walnuts and 60% in hazelnuts were found to be not assimilable. Similar was the bioaccessibility of Mn in bread.[20] It could be assumed that Mn at neutral pH precipitates or sorbs on the undissolved part of nut in the intestinal tract, because this element does not form soluble complexes (contrary to Cu and Fe) with the chelating compounds released in the intestinal juice during digestion.

The solubility of Fe under simulated stomach conditions was very low (3−6%) for both nut types. This is very likely due to the high content of phytates and phytosterols as has been stated also for bread and vegetables,[20] beans,[29] and lentils.[30] The phytic acid content in 100 g of hazelnut and walnut kernels varied between 650 and 1000 mg and the phytosterol content between 72 and 96 mg.[28] The soluble fraction of iron in the simulated intestinal fluid (∼23%) is higher than in the stomach (∼4%) for both hazelnut and walnut kernels (Tables 1 and 2). The enhancement of iron solubility in the intestine most probably could be explained with the formation of soluble, stable complexes of iron with organic ligands released in this stage of nut digestion.[31] The enhancement of Fe solubility in the intestinal tract, where the absorption is the greatest, is important for the delivery of this essential element to the body, particularly for people consuming food mainly of plant origin.

In the case of lead, the solubility was found to be very low. Lead was even not detectable in the gastrointestinal fluids (limit of detection = 2 ng/mL Pb), which means that >99% of lead was left insoluble. It is probably because of chelation with polyphenols, phytosterols, or di- and monoacylglycerols to which Pb gets bound. This means that despite the relatively high total Pb content, the bioaccessible fraction of this element is extremely low. Low lead bioaccessibility was found also for cocoa powder and related products.[18]

With the assessed bioaccessibility of elements as presented in Tables 1 and 2 taken into consideration, contributions of the intake of hazelnuts and walnuts to the RDA of Cu, Fe, Mn, and Zn were calculated (Table 3). It appears that the consumption of 100 g of hazelnut kernels can potentially supply to the human body up to 119% of the RDA for Cu, up to 64% for Mn, and up to 13% for Zn. The coverage of RDA for Fe was found to be around 8−10% for both nut types. The intake of Cu and Zn with 100 g of walnuts seems to be lower, that is, around 26% of the Cu RDA and 4.6% of the Zn RDA. In the case of Mn, the ingestion of 100 g of walnuts could potentially provide 35−53% of the RDA for this element. These results confirm the fact that nuts are a rich source of essential elements. Hazelnuts are a better source for Cu and Zn in comparison to walnuts.

Element Species in Bioaccessible Fractions. The chemical form of the studied elements soluble in simulated gastrointestinal fluids was evaluated for hazelnut varieties using various liquid phase extraction systems.[24] The chelating reagent APDC forms extractable complexes $[Me^{n+}(DTC)_n]$ with free metal ions or with metal ions existing as labile complexes with inorganic (chloride) or organic ligands (carboxylic acids). The neutral noncharged element complexes MeR (R, ligand) would be extracted into xylene in the absence of any additional reagent. If the elements exist in the supernatants as negatively charged complexes MeR^{n-}, they would be extracted with high recovery when the positively charged long-chain quaternary

Table 4. Soluble Element Species in the Simulated Gastric (G) and Gastrointestinal (I) Solutions Obtained after Sequential Enzymatic Digestion of Hazelnuts as Percent of Their Total Dissolved Content[a] (Mean of Three Replicates (SD))

		Cd		Cu		Fe		Mn		Zn	
extraction system	extractable form	G	I	G	I	G	I	G	I	G	I
APDC/xylene	Me^{n+}	>98	20 (2)	82 (1)	72 (3)	>95	3 (1)	>98	>98	>95	25 (2)
xylene	MeR	nd[b]		nd		nd		nd		nd	
TOMACl in xylene	MeR^{n-}	nd	77 (3)	18 (2)	28 (1)	nd	95 (2)	<0.4	<0.4	3 (1)	60 (3)
NaDDS/xylene	MeR^{n+}	nd		nd		nd		nd		3 (1)	7 (2)

[a]R, inorganic anions or polar organic compounds (carboxylic, aminocarboxylic acids). [b]nd, not detected.

ammonium salt TOMACl is used as counterion. The positively charged complexes of the elements MeR^{n+} would be extracted with a negatively charged big dodecylbenzenesulfonate (DDS) anion as counterion. Both counterions are responsible for the formation of extractable ion associate complexes $MeR^{n-}(TOMA^+)_n$ and $MeR^{n+}(DDS^-)_n$. The expected element species and the results from the liquid phase extraction study for hazelnuts are presented in Table 4. In the stomach the dissolved Cd, Cu, Fe, Mn, and Zn are present as free metal ions or their labile complexes with inorganic or organic ligands. In the intestinal juice only Mn does not change its form, being present mainly as manganese ion. Neutral complexes MeR were not detected. The main part of iron (95%), 77% of cadmium, 60% of zinc, and 28% of Cu are present in the intestinal solution as negatively charged complexes MeR^{n-}, most probably with carboxylic and aminocarboxylic acids as ligands (R) released from the digestion at the intestinal step.[31] The formation of soluble complexes with constituents of intestinal fluids explains the higher solubility of Cd and Fe in the intestine than in the stomach (Table 1). To the best of our knowledge, chemical speciation analysis of solutions obtained by sequential enzymatic treatment of foods has not been reported. However, our results confirm the importance of chelating constituents released from the foods for the solubility and, hence, for the bioaccessibility of microelements.[17]

Nuts as Sorbents. Our experiments showed that hazelnuts and walnuts act as biosorbents. The results obtained for the sorption properties of raw nuts and nut residues left after simulated gastrointestinal digestion are illustrated in Figures 1

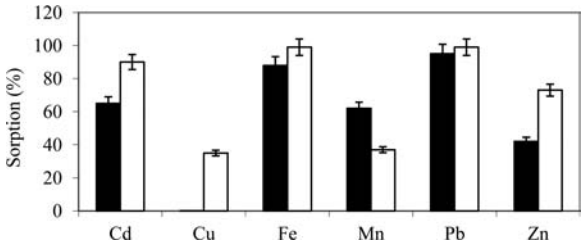

Figure 1. Solid phase sorption properties of hazelnut kernels (cultivars Ata Baba and Ran Trapezundski) before (black columns) and after (white columns) intestinal digestion.

and 2 for hazelnuts and walnuts, respectively. As can be seen, only copper remains quantitatively in the solution (without any sorption) above the raw undigested hazelnuts. In the absence of complexing agents in the solution Fe and Pb were almost quantitatively sorbed from raw and digested walnuts and hazelnuts. The sorption extent of Cu and Zn from hazelnut residues left after simulated gastrointestinal digestion (Figure 1) is definitely lower in comparison to their retention ability on walnut residues (Figure 2). This explains the higher solubility

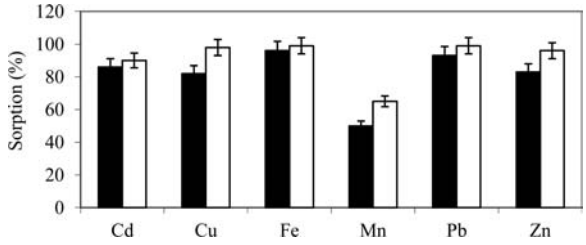

Figure 2. Solid phase sorption properties of walnut kernels (cultivars Sheynovo and Pedro) before (black columns) and after (white columns) intestinal digestion.

of these elements from hazelnuts in the simulated intestinal juice in comparison to walnuts. The solid residue of hazelnuts left after gastrointestinal digestion acts as a better sorbent than the raw nuts, that is, before digestion, particularly in the case of copper. This means that during digestion new active functional groups with higher sorption efficiency are formed on the nut surface.

The observations regarding the high sorption efficiency of the toxic elements (Cd and Pb) are very important and show the detoxication properties of walnuts and hazelnuts. Even when these toxic elements enter the body for some reasons, the consumption of nuts will detoxify the body due to sorption. According to the regulations, the maximum admissible levels of Cd and Pb in hazelnut and walnut kernels are 0.05 mg/kg Cd and 0.1 mg/kg Pb.[25] Because the very low solubility of lead and its high sorption affinity to insoluble nuts fraction under both gastric and intestinal conditions, it seems that the regulation is stricter than necessary for this element (Pb).

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Notes

The authors declare no competing financial interest.

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