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Metallomics Approach to Trace Element Analysis in *Ustilago maydis* Using Cellular Fractionation, Atomic Absorption Spectrometry, and Size Exclusion Chromatography with ICP-MS Detection

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Huitlacoche is the ethnic name of the young fruiting bodies of *Ustilago maydis*, a common parasite of maize. In Mexico and other Latin American countries, this fungus has been traditionally appreciated as a local delicacy. In this work a metallomics approach was used with the determination of eight elements in huitlacoche by electrothermal atomic absorption spectrometry as one facet of this approach. The results obtained indicated relatively lower concentrations of commonly analyzed metals, as referred to the data reported for other mushroom types. This effect was ascribed to different accessibilities of elements, depending on fungus substrate (lower from plant than from soil). Subcellular fractionation was accomplished by centrifugation of cell homogenates suspended in Tris-HCl buffer. Recoveries of the fractionation procedure were in the range of 71−103%. For six elements (Cr, Cu, Fe, Mn, Ni, and Pb), the mean relative contributions in cytosol, cell walls, and mixed membrane fraction were 50.7, 48.2, and 1.1% respectively. To attain the molecular weight distribution of compounds containing target elements as an additional aspect of the metallomics approach, the fungus extract (1% sodium dodecyl sulfate in Tris-HCl, 30 mmol L^{-1}, pH 7.0) was analyzed by size exclusion chromatography with UV and ICP-MS detection. With spectrophotometric detection (280 nm), the elution of high molecular weight compounds was observed in the form of one peak (MW > 10 kDa), and several lower peaks appeared at higher retention times (MW < 10 kDa). On ICP-MS chromatograms, a coelution of ^{59}Co, ^{63}Cu, ^{57}Fe, ^{202}Hg, ^{60}Ni, and ^{80}Se with the first peak on the UV chromatogram was clearly observed, indicating that a fraction of each element incorporated with high molecular weight compounds (12.7, 19.8, 33.7, 100, 19.4, and 45.8%, respectively, based on the peak area measurements). From a comparison of ^{80}Se and ^{33}S chromatograms (for sulfur analysis, the extract was obtained in the absence of SDS), both elements coeluted with the first UV peak, but their lower molecular weight compounds were apparently different. These findings may contribute to a better understanding of the accumulation of elements in mushrooms.

KEYWORDS: Trace elements; *Ustilago maydis*; cellular fractionation; ETA-AAS; SEC-ICP-MS; mushrooms

INTRODUCTION

Humans long have used different wild mushrooms for food. Their nutritional value lies in relatively high protein and dietary fiber contents (respectively, up to 7 and 9 g per 100 g of the biomass). Moreover, they are low in calories (30−130 kcal/100 g) and provide vitamins and minerals (zinc, iron, copper, etc.) (*1*, *2*). Biological species that are favored in most geographical regions grow on soil-derived substrate. On the other hand, in Mexico and some other Latin America countries, a fungal delicacy is huitlacoche, which grows on corn cobs. Huitlacoche is the ethnic name of the young fruiting bodies of *Ustilago maydis*—a common maize parasite (*3*). Similarly as for other macro fungi, its food potential is due to high protein and carbohydrate contents (*3*−*5*).

The occurrence of several metals and metalloids in wild-growing and cultivated mushrooms has been extensively studied. The related research topics involve (i) possible use of the wild

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species as bioindicators of environmental pollution (*6*, *7*); (ii) optimization of the commercial cultivation processes (*8*, *9*); (iii) elucidation of the mechanisms responsible for elemental uptake, accumulation, and distribution in mushrooms (*10*−*12*); and (iv) evaluation of the edible mushrooms as possible dietary sources of bioelements (*13*−*15*). Over about 30 years, several original papers and reviews have been published on total element determination (*16*−*20*) and, only recently, the research interest has moved toward elemental speciation analysis (the identification or determination of specific elemental forms or species) (*21*−*26*), which is part of the metallomics approach wherein metals or metalloids in a cell or sample are as fully characterized as possible. Thus, different extraction procedures were studied for selenium-enriched mushrooms, and speciation was accomplished by high-performance liquid chromatography with atomic fluorescence (*23*, *27*), atomic emission (*28*), or inductively coupled plasma mass spectrometry detection (*22*). To attain selenium and cesium distribution in *Agaricus bisporus*, a radiotracer-aided study was carried out (*29*). The cytosol fraction, obtained by centrifugation of cell homogenates contained >90 and >60% of total cesium and selenium in the biomass. Cellular fractionation of commercial *A. bisporus* (stalk and caps) and *Pleurotus ostreatus* was carried out, and the relative distribution of several metals among cytosol, cell walls, and mixed membrane fraction was evaluated using electrothermal atomic absorption (*30*). Multielement speciation was carried out in the extracts of *Boletus edulis* (*24*, *25*). Size exclusion chromatography with inductively coupled plasma mass spectrometry (SEC-ICP-MS) was used in these studies to obtain the molecular weight distribution patterns of Bi, Co, Cu, Fe, I, Mo, Ni, Se, and Zn in the biomass extracts. The results indicated an important association of most of the elements to high molecular weight compounds.

Because no data concerning metals or metalloids in huitlacoche have been found in the literature, the aim of this work was to evaluate the total concentrations of typically analyzed elements and their distribution among different biomass fractions and to attain the molecular weight distribution of the compounds containing target elements. The particular interest in this fungus has arisen because of different morphology (no stalks and caps) and growth conditions (corn cob as substrate) with respect to other wild mushrooms. The results obtained in this and continuing studies could contribute to a better understanding of the mechanisms responsible for element uptake and accumulation in mushrooms.

EXPERIMENTAL PROCEDURES

Instrumentation. A model 3110 Perkin-Elmer atomic absorption spectrometer with an HGA600 graphite furnace and an AS 60 autosampler was used. The hollow cathode lamps for Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Se were Perkin-Elmer Lumina lamps. A model J2-21 Beckman centrifuge and an XL-90 Beckman ultracentrifuge were used for cellular fractionation.

Chromatographic separations were accomplished with an Agilent Technology series 1100 liquid chromatograph equipped with an autosampler, a diode array detector, and a Chemstation. The chromatographic column was a Superdex 75 HR10/300 GL.

An Agilent 7500ce inductively coupled plasma mass spectrometer with concentric nebulizer and Scott-type double-pass spray chamber was used for specific element detection. The octopole reaction system (collision cell) was used in this study.

Reagents and Samples. All chemicals were of analytical reagent grade, and deionized water was used throughout (Labconco).

For atomic absorption spectrometry, stock standard solutions containing 1000 mg L^{-1} of cadmium, chromium, copper, iron, manganese, nickel, lead, and selenium were purchased from Sigma. Working solutions corresponding to the highest calibration standard (2 μg L^{-1} Cd; 10 μg L^{-1} Cr; 50 μg L^{-1} Cu; 60 μg L^{-1} Fe; 10 μg L^{-1} Mn; 50 μg L^{-1} Ni; 50 μg L^{-1} Pb; and 100 μg L^{-1} Se) were prepared daily by appropriate dilution. The solutions of matrix modifiers, $Pd(NO_3)_2$ and $Mg(NO_3)_2$, 10000 mg L^{-1}, were from Perkin-Elmer, and the certified reference material was NIST 1572 (citrus leaves).

Calibration of the size exclusion chromatography (SEC) column was performed using standard mixtures of (1) lysozyme (14.4 kDa), aprotinin (6.5 kDa), substance P (1.35 kDa), and $(Gly)_6$ (0.36 kDa) (Sigma reagents).

The following Sigma reagents and solutions were also used: ultrapure nitric acid, hydrochloric acid, tris(hydroxymethyl)aminomethane (Tris), sodium dodecyl sulfate (SDS), hydrogen peroxide, sodium hydroxide, and protease inhibitors [phenylmethanesulfonyl fluoride (PMSF), leupeptin, antipaine.

Huitlacoche (∼1 kg) was purchased at a local market. The fungi were separated from maize, washed with deionized water, and dried at room temperature.

Procedures. The fractionation/determination scheme is presented in **Figure 1**. Fresh biomass was homogenized in liquid nitrogen. For cellular fractionation, ∼10 g of the material was suspended in 50 mL of Tris-HCl buffer (50 mmol L^{-1}, pH 7.0) containing protease inhibitors (1 μg mL^{-1}). The extract was fractionated by centrifugation as described elsewhere (*30*, *31*). In brief, two subsequent centrifugations were carried out (centrifugation I, 7300*g*, 4 °C, 10 min; centrifugation II, 147000*g*, 4 °C, 60 min) yielding pellet I (cell walls), supernatant I, pellet II (mixed membrane fraction), and supernatant II (cytosol).

For the determination of total element contents, the homogenized biomass was freeze-dried and acid digested. In parallel, the analysis of these same elements was carried out in NIST 1572. The subsample of each material (0.1−0.5 g) was placed in a glass tube, and 1 mL of concentrated nitric acid was added (triplicate analysis). The tubes were heated in the stainless steel block at 65 °C for 60 min and then at 120−140 °C for 60 min. Once cooled to room temperature, 0.2 mL of hydrogen peroxide was added to each tube, and the mixture was left for 30 min to complete reaction. The final volume was brought to 10 mL. After appropriate dilution, the determination of Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Se was carried out by electrothermal atomic absorption spectrometry (ETA-AAS). The instrumental conditions are summarized in **Table 1**, and the injected volume was 20 μL. Two matrix modifiers were used: magnesium nitrate (5 μg Mg) for Cu, Cr, Fe, Mn, and Pb and palladium nitrate (2 μg of Pd) for Ni and Se (modifier volume = 5 μL). Platform atomization and deuterium background correction were used (except for Cr). Quantification was accomplished by external calibration. As shown in **Figure 1**, supernatants I and II and pellets I and II were freeze-dried and acid-digested, and the elements were determined using the above protocol.

Another portion of the freeze-dried huitlacoche (20 mg) was used to study possible binding of elements to different molecular weight fractions. For extraction, 1 mL of 1% SDS in Tris-HCl (30 mmol L^{-1}, pH 7.0) containing 2 mmol L^{-1} PMSF was added, and the mixture was kept in a boiling water bath for 5 min. Then, it was centrifuged, and 100 μL of the supernatant was introduced to the SEC-UV-ICP-MS system. For sulfur analysis, the extraction was done without SDS. The operating conditions are given in **Table 2**.

RESULTS AND DISCUSSION

The mechanisms of metal/metalloid incorporation into the structure of fungi are not fully understood. This study was undertaken to assess total concentrations, cellular distribution, and molecular weight profile of element-containing compounds in huitlacoche. Several elements commonly determined in edible mushrooms were analyzed. The general scheme of experimental procedures is presented in **Figure 1**. All experiments were carried out on the homogenate of biomass (liquid nitrogen).

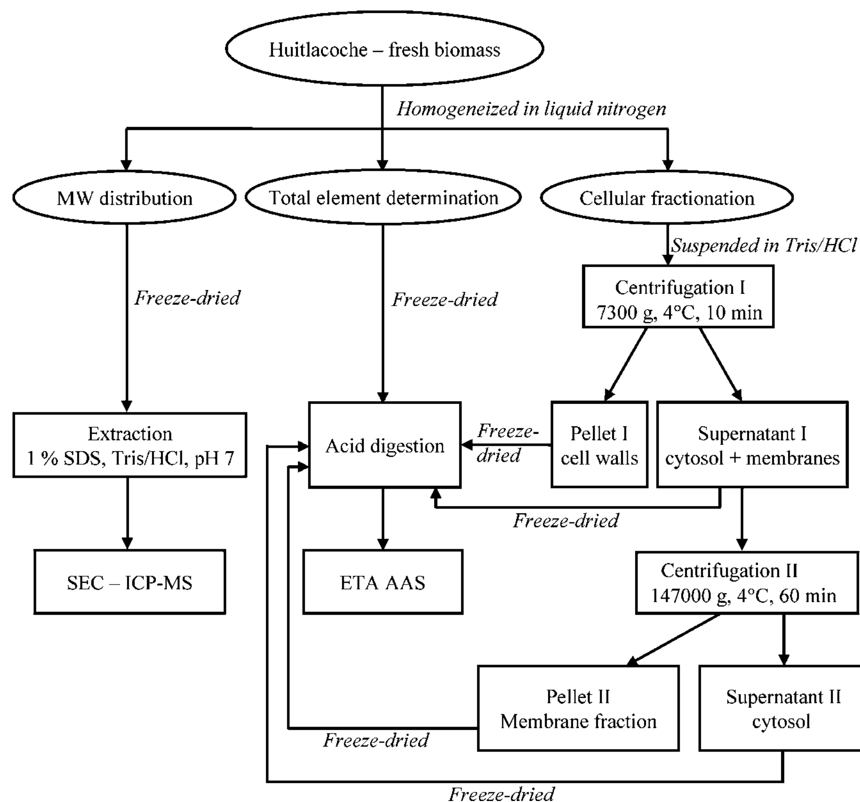

Figure 1. General scheme of the fractionation/determination procedure.

Table 1. Furnace Programs for the Determination of Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Se by ETA-AAS

element	drying T:R:H[a]	pyrolysis T:R:H	CDS[b] T:R:H	atomization T
Cd	130:25:15	800:10:10	20:1:15	1700
Cr		1200:10:10		2300
Cu		1300:10:10		2400
Fe		1200:10:10		2300
Mn		1200:10:10		2100
Ni		1300:10:10		2500
Pb		700:10:10		1800
Se		1100:10:10		2100

[a] T:R:H, temperature, °C; ramp time, s; hold time, s. [b] CDS, cool step.

Table 2. Instrument Operating Conditions Used for SEC-ICP-MS Analysis

Size Exclusion Chromatography	
column	Superdex 75 HR10/300 GL
mobile phase	Tris-HCl, 30 mmol L^{-1}, pH 7.0
flow rate	0.7 mL min^{-1}
injection volume	100 μL
UV Detection	
wavelength	280 nm
ICP-MS Detection	
forward power	1300 W
nebulizer gas flow	1.01 L min^{-1}
dwell time	100 ms
isotopes monitored	^{114}Cd, ^{59}Co, ^{51}Cr, ^{63}Cu, ^{57}Fe, ^{202}Hg, ^{60}Ni, ^{31}P, ^{112}Pb, ^{33}S, ^{80}Se
internal standardization	^{74}Ge, ^{76}Ge
collision gas flow rate	4 mL of H_2 min^{-1}

For total element determination, the homogenate of huitlacoche and the cellular fractions obtained were freeze-dried and acid-digested. ETA-AAS was used for the determination of Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Se. The limits of detection evaluated for these elements were 0.08, 0.26, 0.35, 1.8, 0.25, 1.4, 1.7, and 7.1 μg L^{-1}, respectively. For quality assurance of the analytical results, the biological certified reference material was analyzed in parallel. The results obtained for triplicate analyses of NIST 1572 (0.04 ± 0.01 μg g^{-1} Cd, 1.1 ± 0.3 μg g^{-1} Cr, 18.4 ± 1.2 μg g^{-1} Cu, 101 ± 4 μg g^{-1} Fe, 21.5 ± 0.8 μg g^{-1} Mn, 0.9 ± 0.4 μg g^{-1} Ni, and 11.8 ± 2.0 μg g^{-1} Pb) were in agreement with the certified values (0.03 ± 0.01 μg/g Cd, 0.8 ± 0.2 μg/g Cr, 16.5 ± 1.3 μg/g Cu, 90 ± 10 μg/g Fe, 23 ± 3 μg/g Mn, 0.6 ± 0.3 μg/g Ni, and 13.3 ± 2.4 μg/g Pb; no value given for Se), indicating good accuracy of the procedure.

Table 3. Results of Total Element Determination in Huitlacoche versus the Concentration Ranges Reported for Other Edible Mushrooms

element	this work: mean concn, μg/g (dry wt)	other works: range, μg/g (dry wt)	other works: refs
Cd	not found	<0.5–35	*16*, *20*, *30*, *32*, *33*, *35–38*
Cr	2.15 ± 0.04	0.1–20	*17*, *20*, *30*, *34*, *36*, *39*
Cu	2.22 ± 0.03	4.5–465	*13*, *16*, *17*, *20*, *36*, *38*, *41*
Fe	19.0 ± 0.7	33–170	*16*, *20*, *33*, *38*, *44*
Mn	0.37 ± 0.05	5–60	*16*, *20*, *33*, *38*, *30*, *39*, *40*
Ni	0.43 ± 0.05	0.4–145	*16*, *20*, *34*, *36*, *39*
Pb	1.5 ± 0.3	<0.5–10	*16*, *17*, *20*, *30*, *36*, *37*, *38*, *41*
Se	not found	1–5	*15*, *16*, *38*

The results of element determinations in the biomass of huitlacoche are presented in **Table 3** together with the concentration ranges reported in cultivated or wild (from noncontaminated areas) edible mushrooms by other authors (*13*, *15−17*, *20*, *30*, *32−41*). As can be observed, the element levels in huitlacoche tend to be lower as referred to those found in other fungus species. This effect should be ascribed to different accessibilities of metals, depending on the fungus growth conditions. As already mentioned, *U. maydis* is a corn-cob parasite and the plant seems to act as a filter, limiting the access

Table 4. Distribution of Elements in Cellular Fractions of Huitlacoche[a]

element	centrifugation I			centrifugation II		
	pellet I, μg/g	supernatant I, μg/g	recovery I, %	pellet II, μg/g	supernatant II, μg/g	recovery II, %
Cd	nf	nf		nf	nf	
Cr	0.59	1.52	98	0.01	1.39	92
Cu	0.42	1.69	95	0.02	1.62	97
Fe	12.3	3.1	81	0.23	1.98	71
Mn	0.25	0.11	97	0.05	0.09	91
Ni	0.16	0.28	102	0.01	0.28	103
Pb	0.46	0.66	76	0.01	0.52	80
Se	nf	nf		nf	nf	

[a] The values in columns pellet I, supernatant I, pellet II, and supernatant II are micrograms of element found in each fraction per gram of freeze-dried biomass (after homogenization in liquid nitrogen). Recovery I was evaluated as the ratio between element concentration in pellet I + supernatant I and total element concentration, %; recovery II was evaluated as the ratio between element concentration in pellet II + supernatant II and supernatant I, %; nf, not found.

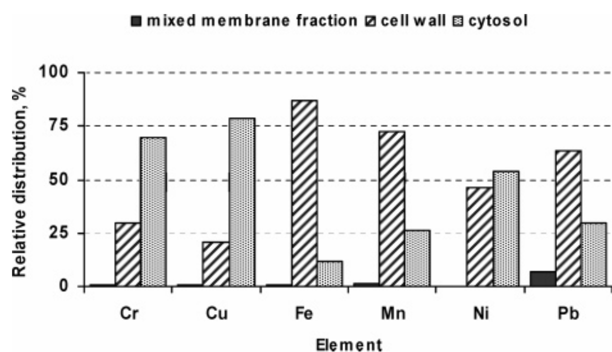

Figure 2. Relative distribution of Cr, Cu, Fe, Mn, Ni, and Pb in cellular fractions of huitlacoche.

of elements from the soil. Consequently, huitlacoche is less susceptible to accumulation of heavy metals and/or metalloids.

In **Table 4** are given the results obtained in the analyses of pellet I, supernatant I, pellet II, and supernatant II. For each element, recovery of the fractionation procedure was evaluated. For centrifugation I, recovery I was calculated by relating the sum of element contents found in pellet I and supernatant I to the total element content in the dry biomass. Recovery II was calculated as the ratio between element content in pellet II plus supernatant II and supernatant I. The values obtained for Cr, Cu, Fe, Mn, Ni, and Pb ranged from 71 to 102%. The other two elements (Cd and Se) were not found in any fraction. Similar recoveries of elements in cellular fractions after centrifugation were reported by van Elteren et al. (81−109%) (*29*) and by Serafin Muñoz et al. (70−114%) (*30*). The relative distribution of elements in huitlacoche was evaluated as the ratio between the content of element found in each cellular fraction and that recovered in the fractionation procedure (sum of three fractions). The data obtained are graphically presented in **Figure 2**. Relatively high contributions of elements occurred in cytosol and in cell wall fraction with the mean values for six elements of 50.7 and 48.2%, respectively. In cytosol, the minimum and maximum relative contributions were observed for Fe (13.6%) and Cu (78.6%). In the cell wall fraction, the relative contributions of these two elements were 84.8 and 20.5%, respectively. Only minute amounts of the elements analyzed were found in the mixed membrane fraction (mean value, 1.1%; range from 0.3% for Pb to 1.7% for Ni). These results show element-dependent distribution among cellular fractions, suggesting different incorporation mechanisms (**Figure 2**). For Cr, Cu, and Ni, water-soluble compounds were primary element forms (relative contributions in cytosol of 69.9, 78.6, and 62.8%, respectively). On the other hand, relatively high amounts of Fe and Mn were bound to the compounds contained in the cell wall fraction (84.8 and 72.7%, respectively). For lead, similar contributions were found in cytosol and in cell walls (53.5 and 46.2%, respectively). It should be mentioned that cellular distribution of elements in huitlacoche differs from that observed in *A. bisporus* and *P. ostreatus* (*30*). In the cited work, the highest accumulation rates of these elements were observed in cytosol (mean 57−74% versus 22−32% in cell walls and 4−10% in membrane fractions). Owing to apparently more efficient binding of metals to different structures in *U. maydis*, these fungi could be suitable models to study element speciation, thus contributing to a better understanding of elemental biological routes in fungi.

The molecular weight distribution of element-containing compounds in huitlacoche extract was studied by SEC with on-line UV (280 nm) and ICP-MS detection. Calibration of the Superdex 75 HR10/300 GL column was accomplished with a standard mixture of lysozyme (14.4 kDa), aprotinin (6.5 kDa), substance P (1.35 kDa), and $(Gly)_6$ (0.36 kDa), showing in this range a good linear response for the logarithm of molecular weight versus retention time ($R^2 = 0.9864$). The high sensitivity of the element-specific detector was required owing to relatively low total concentrations found in huitlacoche (**Table 3**). On the basis of the previous experience, soft extraction conditions were applied, yielding water-soluble compounds and proteins (*42*, *43*). In **Figure 3a**, the UV (280 nm) elution profile is presented and **Figures 3b** and **4** show typical ICP-MS chromatograms obtained while monitoring ^{59}Co, ^{63}Cu, ^{57}Fe, ^{202}Hg, ^{60}Ni, ^{31}P, ^{33}S, and ^{80}Se. On the UV chromatogram, the elution of high molecular weight compounds was observed in the form of one peak (MW > 10 kDa), and several less abundant peaks appeared at higher retention times (MW < 10 kDa). ICP-MS chromatograms revealed coelution of ^{59}Co,^{63}Cu, ^{57}Fe, ^{202}Hg, ^{60}Ni, and ^{80}Se, with the first peak observed with UV detection, indicating that a fraction of each element incorporated to high molecular weight compounds. On the basis of the peak area measurements, these fractions corresponded to 12.7, 19.8, 33.7, 100, 19.4, and 45.8%, respectively, of total element amount eluted from the column. For ^{59}Co, ^{63}Cu, ^{60}Ni, and ^{80}Se, one or two sharp peaks appeared in the elution region of MW < 10 kDa. The elution profile of Fe resembled that obtained with UV detection, which suggests its relatively uniform distribution among compounds of different MW. To obtain more specific information on metals/metalloid binding in huitlacoche, the isotopes ^{31}P and ^{33}S were also monitored. Only a minute amount of ^{31}P (4%) eluted in the first peak, and two peaks of this element appeared in the region of MW < 10 kDa (respectively, 77.3 and 18.7% of total eluted P). As can be seen on ^{59}Co, ^{63}Cu, ^{57}Fe, and ^{60}Ni chromatograms, there was a contribution of these metals in the molecular weight region where the most abundant phosphorus

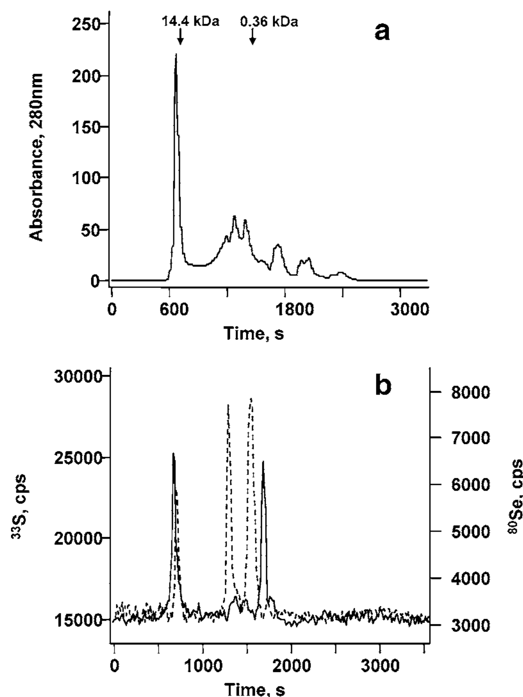

Figure 3. Typical size exclusion chromatograms of huitlacoche extract: (**a**) spectrophotometric detection (280 nm); (**b**) ICP-MS detection of ^{80}Se (—) and ^{33}S (- - -) (extraction, chromatographic, and instrument operating conditions are given in **Figure 1** and **Table 2**, respectively).

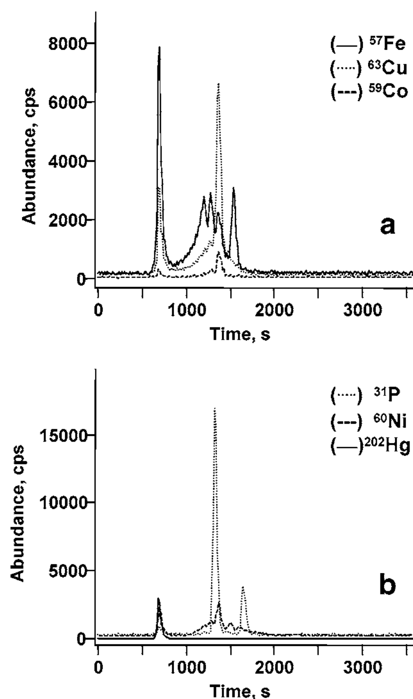

Figure 4. Typical ICP-MS chromatograms of huitlacoche extract. Isotopes monitored were (**a**) ^{57}Fe, ^{63}Cu, and ^{59}Co; and (**b**) ^{31}P, ^{60}Ni, and ^{202}Hg (extraction, chromatographic, and instrument operating conditions are given in **Figure 1** and **Table 2**, respectively).

peak appeared, which suggests possible binding of the elements to phosphorus-containing low molecular weight compounds. Due to the known affinity of selenium and sulfur, the chromatograms obtained for ^{80}Se and ^{33}S were compared (**Figure 3b**). It should be indicated that, for sulfur analysis, the extract was obtained in the absence of SDS. The two elements coeluted with the first UV peak, demonstrating their coexistence in high molecular weight compounds. However, their lower molecular weight compounds were apparently different. The results obtained in this work may contribute to a better understanding of the accumulation of elements in mushrooms.

LITERATURE CITED

(1) Manzi, P.; Aguzzi, A.; Pizzoferato, L. Nutritional value of mushrooms widely consumed in Italy. *Food Chem.* **2001**, *73*, 321−325.

(2) Manzi, P.; Gambelli, L.; Marconi, S.; Vivanti, V.; Pizzoferato, L. Nutrients in edible mushrooms: an inter-species comparative study. *Food Chem.* **1999**, *65*, 477−482.

(3) Valverde, M. E.; Paredes-Lopez, O.; Pataky, J. K.; Guevara-Lara, F. Huitlacoche (*Ustilago maydis*) as a food source—biology, composition, and production. *Crit. Rev. Food Sci. Nutr.* **1995**, *35*, 191−229.

(4) Lizarraga-Guerra, R.; Lopez Mercedes, G. Monosaccharide and alditol contents of huitlacoche (*Ustilago maydis*). *J. Food Compos. Anal.* **1998**, *11*, 333−339.

(5) Lizarraga-Guerra, R.; Lopez Mercedes, G. Content of free amino acids in huitlacoche (*Ustilago maydis*). *J. Agric. Food Chem.* **1996**, *44*, 2556−2559.

(6) Garcia, M. A.; Alonso, J.; Fernandez, M. I.; Melgar, M. J. Lead content in edible wild mushrooms in northwest Spain as indicator of environmental contamination. *Arch. Environ. Contam. Toxicol.* **1998**, *34*, 330−335.

(7) Marzano, F. N.; Bracchi, P. G.; Pizzetti, P. Radioactive and conventional pollutants accumulated by edible mushrooms (*Boletus* sp.) are useful indicators of species origin. *Environ. Res.* **2001**, *85*, 260−264.

(8) Gulser, C.; Peksen, A. Using tea waste as a new casing material in mushroom (*Agaricus bisporus* (L.) Sing.) cultivation. *Bioresour. Technol.* **2003**, *88*, 153−156.

(9) Bressa, G.; Cima, L.; Costa, P. Bioaccumulation of Hg in the mushroom *Pleurotus ostreatus*. *Ecotoxicol. Environ. Saf.* **1988**, *16*, 85−89.

(10) Falandysz, J.; Lipka, K.; Kawano, M.; Brzostowski, A.; Dadej, M., et al. Mercury content and its bioconcentration factors in wild mushrooms at Lukta and Morag, northeastern Poland. *J. Agric. Food Chem.* **2003**, *51*, 2832−2836.

(11) Ola, F. L.; Oboh, G. Nutrient distribution and zinc bioavailability. Estimation in some tropical edible mushrooms. *Nahrung* **2001**, *45*, 67−68.

(12) Demirbas, A. Heavy metal bioaccumulation by mushrooms from artificially fortified soils. *Food Chem.* **2001**, *74*, 293−301.

(13) Alonso, J.; Garcia, M. A.; Perez-Lopez, M.; Melgar, M. J. The concentrations and bioconcentration factors of copper and zinc in edible mushrooms. *Arch. Environ. Contam. Toxicol.* **2003**, *44*, 180−188.

(14) Imaeda, N.; Tokudome, Y.; Ikeda, M.; Kitagawa, I.; Fujiwara, N., et al. Foods contributing to absolute intake and variance in intake of selected vitamins, minerals and dietary fiber in middle-aged Japanese. *J. Nutr. Sci. Vitaminol. (Tokyo)* **1999**, *45*, 519−532.

(15) Piepponen, S.; Liukkonen-Lilja, H.; Kuusi, T. The selenium content of edible mushrooms in Finland. *Z. Lebensm. Unters. Forsch.* **1983**, *177*, 257−260.

(16) Kalac, P.; Svoboda, L. A review of trace elements concentrations in edible mushrooms. *Food Chem.* **2000**, *69*, 273−281.

(17) Bauer Petrovska, B. Mineral composition of some Macedonian edible mushrooms. *Acta Pharm.* **1999**, *49*, 59−64.

(18) Svoboda, L.; Kalac, P. Contamination of two edible *Agaricus* spp. mushrooms growing in a town with cadmium, lead, and mercury. *Bull. Environ. Contam. Toxicol.* **2003**, *71*, 123−130.

(19) Falandysz, J.; Jedrusiak, A.; Lipka, K.; Kannan, K.; Kawano, M., et al. Mercury in wild mushrooms and underlying soil substrate from Koszalin, North-central Poland. *Chemosphere* **2004**, *54*, 461−466.

(20) Falandysz, J.; Szymczyk, K.; Ichihashi, H.; Bielawski, L.; Gucia, M., et al. ICP/MS and ICP/AES elemental analysis (38 elements) of edible wild mushrooms growing in Poland. *Food Addit. Contam.* **2001**, *18*, 503−513.
(21) Larsen, E. H.; Hansen, M.; Goessler, W. Speciation and health risk considerations of arsenic in the edible mushroom *Laccaria amethystina* collected from contaminated and uncontaminated locations. *Appl. Organomet. Chem.* **1998**, *12*, 285−291.
(22) Ogra, Y.; Ishiwata, K.; Ruiz Encinar, J.; R, L. O.; Suzuki, K. T. Speciation of selenium in selenium-enriched shiitake mushroom, *Lentinula edodes*. *Anal. Bioanal. Chem.* **2004**.
(23) Stefanka, Z.; Ipolyi, I.; Dernovics, M.; Fodor, P. Comparison of sample preparation methods based on proteolytic enzymatic processes for Se-speciation of edible mushroom (*Agaricus bisporus*) samples. *Talanta* **2001**, *55*, 437−447.
(24) Wuilloud, R. G.; Kannamkumarath, S. S.; Caruso, J. A. Speciation of essential and toxic elements in edible mushrooms: SEC separation with on-line UV-ICP-MS detection. *Appl. Organomet. Chem.* **2004**, *18*, 156−165.
(25) Wuilloud, R. G.; Kannamkumarath, S. S.; Caruso, J. A. Multielemental speciation analysis of fungi porcini (*Boletus edulis*) mushroom by size exclusion liquid chromatography with sequential on-line UV-ICP-MS detection. *J. Agric. Food Chem.* **2004**, *52*, 1315−1322.
(26) Wilburn, R. T.; Vonderheide, A. P.; Soman, R. S.; Caruso, J. A. Speciation of selenium in the mushroom *Boletus edulis* by high-performance liquid chromatography coupled to inductively coupled plasma-mass spectrometry with a collision cell. *Appl. Spectrosc.* **2004**, *58*, 1251−1255.
(27) Slejkovec, Z.; van Elteren, J. T.; Woroniecka, U. D.; Kroon, K. J.; Falnoga, I., et al. Preliminary study on the determination of selenium compounds in some selenium-accumulating mushrooms. *Biol. Trace Elem. Res.* **2000**, *75*, 139−155.
(28) Dernovics, M.; Stefanka, Z.; Fodor, P. Improving selenium extraction by sequential enzymatic processes for Se-speciation of selenium-enriched *Agaricus bisporus*. *Anal. Bioanal. Chem.* **2002**, *372*, 473−480.
(29) van Elteren, J. T.; Woroniecka, U. D.; Kroon, K. J. Accumulation and distribution of selenium and cesium in the cultivated mushroom *Agaricus bisporus*—a radiotracer-aided study. *Chemosphere* **1998**, *36*, 1787−1798.
(30) Serafin Muñoz, A. H.; Gutierrez Corona, F.; Wrobel, K.; Martinez Soto, G.; Wrobek, K. Sub-cellular distribution of aluminum, bismuth, cadmium, chromium, copper, iron, manganese, nickel and lead in cultivated mushrooms (*Agaricus bisporus* and *Pleurotus ostreatus*). *Biol. Trace Elem. Res.* **2005**, in press.
(31) Ramirez-Salgado, P.; Caracheo-Aguilar, S.; Obregon-Herrera, L.; Sosa-Luna, L.; Amezquita-Lopez, F., et al. Copper resistance and accumulation in the zygomycete *Mucor rouxii*. *Curr. Microbiol.* **1996**, *33*, 163−166.
(32) Blanusa, M.; Kucak, A.; Varnai, V. A.; Saric, M. M. Uptake of cadmium, copper, iron, manganese, and zinc in mushrooms (*Boletaceae*) from Croatian forest soil. *J. AOAC Int.* **2001**, *84*, 1964−1971.
(33) Kucak, A.; Blanusa, M. Validation of microwave digestion method for determination of trace metals in mushrooms. *Arh. Hig. Rada Toksikol.* **1998**, *49*, 335−342.
(34) Vetter, J. Chromium and nickel contents of some common edible mushroom species. *Acta Aliment.* **1997**, *26*, 163−170.
(35) Vetter, J. Data on arsenic and cadmium contents of some common mushrooms. *Toxicon* **1994**, *32*, 11−15.
(36) Demirbas, A. Concentration of 21 metals in 18 species of mushrooms growing in the East Black Sea region. *Food Chem.* **2001**, *75*, 453−457.
(37) Cibulka, J.; Sisak, L.; Pulkrab, K.; Miholova, D.; Szakova, J., et al. Cadmium, lead, mercury and caesium levels in wild mushrooms and forest berries from different localities of the Czech Republic. *Sci. Agric. Bohemica* **1996**, *27*, 113−129.
(38) Mattila, P.; Konko, K.; Eurola, M.; Pihlava, J. M.; Astola, J., et al. Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. *J. Agric. Food Chem.* **2001**, *49*, 2343−2348.
(39) Jorhem, L.; Sundstrom, B. Levels of some trace elements in edible fungi. *Z. Lebensm. Unters. Forsch.* **1995**, *201*, 311−316.
(40) Jorhem, L.; Engman, J. Determination of lead, cadmium, zinc, copper, and iron in foods by atomic absorption spectrometry after microwave digestion: NMKL collaborative study. *J. AOAC Int.* **2000**, *83*, 1189−1203.
(41) Sturion, G. L.; de Camargo Ranzani, M. R. Mineral composition of edible mushrooms cultivated in Brazil—*Pleurotus* spp and other dehydrated species. *Arch. Latinoam. Nutr.* **2000**, *50*, 102−108.
(42) Monicou, S.; Meija, J.; Caruso, J. A. Preliminary studies on selenium-containing proteins in *Brassica juncea* by size exclusion chromatography and fast protein liquid chromatography coupled to ICP-MS. *Analyst* **2004**, *129*, 116−123.
(43) Kannamkumarath, S. S.; Wrobek, K.; Wuilloud, R. G. Studying the distribution pattern of selenium in nut proteins with information obtained from SEC-UV-ICP-MS and CE-ICP-MS. *Talanta* **2005**, in press.
(44) Alofe, F. V.; Odeyemi, O.; Oke, O. L. Three edible wild mushrooms from Nigeria: their proximate and mineral composition. *Plant Foods Hum. Nutr.* **1996**, *49*, 63−73.

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