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Speciation of essential and toxic elements in edible mushrooms: size-exclusion chromatography separation with on-line UV–inductively coupled plasma mass spectrometry detection

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Size-exclusion liquid chromatography was coupled to UV and inductively coupled plasma mass spectrometry (ICP-MS) for detection to perform elemental speciation studies on different edible mushrooms. Molecular weight (MW) distribution patterns of several elements among different fractions present in various edible mushrooms are presented. The association of the elements with the high and low MW fractions was observed using sequential detection by UV and ICP-MS. Separation was performed using a Superdex 75 column. Variability of the fractionation patterns with three different extraction media (0.05 mol l^{-1} NaOH; 0.05 mol l^{-1} HCl; hot water at 60 °C) was evaluated for mushroom species. A comparative elemental speciation study was performed in order to determine the differences in the fractionation patterns of silver, arsenic, cadmium, mercury, lead, and tin in *Boletus edulis*, *Agaricus bisporus*, and *Lentinus edodes*. Differences in the fractionation patterns of the elements were found to depend on the mushroom species and the extraction medium. Most of the elements were associated with high mw fractions. It was not possible to assess the trace metal contributions from the mushroom growth media. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: edible mushrooms; molecular weight fractionation; elemental speciation; metalloproteins; SEC; UV–ICP-MS

INTRODUCTION

Edible mushrooms are considered healthful, low in calories, and abundant in vegetable proteins, vitamins and minerals. The possible medicinal value of mushrooms in traditional Chinese medicine has been well known for a long time. Among the several effects of mushrooms, their role in promoting good health, vitality and increasing the body's adaptive response have been documented.[1] Other applications of mushrooms have involved food therapy and the suggestions that they may be used in preventing diseases such as hypertension, hypercholesterolemia and cancer.[2,3]

Since the early 1970s, studies have been performed to determine the occurrence of trace elements in mushrooms.[4] Most of these studies were developed to evaluate the possible use of mushrooms as bioindicators of environmental pollution and identifying those edible mushrooms that could accumulate elements in high concentrations. The presence of high concentrations of many different elements of toxicological and nutritional interest in mushroom have been reported. Concentration levels on the order of milligrams per kilogram have been found for iron,[5] zinc,[6] copper,[7] manganese,[8] selenium,[9] cobalt,[10] silver,[11] mercury,[7,12–17] chromium,[18] and lead.[7,14,19] Although several papers reporting total element concentration have been published, elemental speciation studies are scarce. In fact, mercury,[20] arsenic[21,22] and selenium[23] have been the only elements studied by speciation analysis in mushrooms. However, information is incomplete, with the speciation of other elements of toxicological or nutritional interest being unreported.

Hyphenated techniques represent the most common approach to performing elemental speciation in biological samples. In general, efficient separation techniques such

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as high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), and gas chromatography (GC) are coupled to element-specific detectors such as atomic absorption spectrometry (AAS) or inductively coupled plasma mass spectrometry (ICP-MS). Size-exclusion chromatography (SEC) is a very useful technique for performing elemental speciation studies for determining the elemental distribution in different molecular weight (MW) fractions. The resulting fractionation profiles provide information on the association of elements with the types of compound present in the sample and are normally considered as initial steps in elemental speciation studies. The main advantages of SEC are simplicity of application, compatibility of mobile-phase composition with the specific demands of certain biological samples and the possibility of estimating the MW of the associated elemental species. SEC may be generally employed as the initial method for fraction separation of element-containing biomolecules of interest, with subsequent application of another separation step with element-specific or species-specific detection and finally mass spectrometric identification.[24–27]

The purpose of this study was to determine the MW distribution of several elements in different edible mushrooms. Different extraction procedures were designed in order to evaluate the extractability of the elemental species from the mushroom samples. SEC was coupled on-line to a UV–ICP-MS detection system for fractionation and monitoring of the elemental fractions in mushrooms. Optimization of the chromatographic and detection conditions was undertaken for the best analytical performance. A comparison among different mushrooms in terms of elemental MW distributions was considered for evaluating the possible use of elemental speciation analysis as a means of identifying mushroom species.

MATERIALS AND METHODS

Reagents

Optimization of the ICP-MS instrumental conditions was performed using a PE Pure Plus Elan Calibration 1 Solution (Perkin–Elmer, CA, USA) containing barium, cadmium, cerium, copper, germanium, magnesium, lead, rhodium, scandium, terbium, and thallium in a concentration of 10 μg l^{-1} in 2% HNO_3 solution.

A 0.1 mol l^{-1} 3-cyclohexylamino-1-propane-sulfonic acid (CAPS; Aldrich, Milwaukee, WI, USA) mobile-phase solution was prepared by dissolution of the reagent in ultrapure water and adjusting to pH 10 with NaOH (Merck, Darmstadt, Germany) solution. A 2 mol l^{-1} tris(hydroxymethyl)aminomethane (Tris; Fisher Scientific, Fairlawn, NJ, USA) mobile-phase solution was prepared by dissolving 242.28 g Tris in 1000 ml of water and adjusting to pH 8.0 with HCl (Merck, Darmstadt, Germany) solution. A 1.0 mol l^{-1} sodium phosphate (Aldrich, Milwaukee, WI, USA) mobile-phase solution was prepared by dissolution of the reagent in ultrapure water and adjusting to pH 9.0 with NaOH (Merck, Darmstadt, Germany) solution. Lower concentrations were prepared by serial dilution with ultrapure water.

Prior to the injection of the extracted solutions into the chromatographic system, the Superdex 75 SEC column was calibrated. Calibration standards included apo-Transferrin (76 000–81 000 Da), albumin bovine (66 000 Da), myoglobin (16 000 Da), aprotinin (6500 Da), substance P (1350 Da), and Gly_6 (360 Da). A linear response between retention time and the logarithm of MW was obtained in the MW range of 1.35 to 66 kDa. Compounds with an MW higher than 66 kDa eluted in the dead volume of the column. For MWs lower than 1.35 kDa, a nonlinear response was observed. All the calibration standards were from Sigma (Sigma Chemical Co., St Louis, MO).

All water was deionized (18 MΩ cm) and prepared by passing through a NanoPure treatment system (Barnstead, Boston, MA). The commercial standard solutions containing 1000 μg ml^{-1} of silver, arsenic, cadmium, mercury, lead and tin were from SPEX CertiPrep, Inc. (Metuchen, NJ, USA). All reagents were of analytical reagent grade and the presence of trace elements was not detected in the working range.

Instrumentation

An HPLC system was used, consisting of a Shimadzu LC-6A pump (Shimadzu Scientific Instrument Inc, Columbia, MD, USA) with a 100 μl loop (Rheodyne 7725 injection valve; Rheodyne, Coati, CA, USA), and an SPD-6A UV–Vis spectrophotometric detector coupled through an R9 232 Dionex Interface with 133 MHz PC equipped with AI-450 release 3.21 software.

Elemental detection was performed using a Perkin–Elmer SCIEX Elan 6000 (Ontario, Canada) ICP mass spectrometer with a Gem Tip cross-flow nebulizer (Perkin–Elmer) and a Ryton spray chamber (Perkin–Elmer). The nebulizer gas flow and ion lens settings were optimized using on-board computer algorithms with the Elan 6000 software. The outlet of the UV detector was connected to the liquid-sample inlet of the nebulizer using 0.25 mm i.d. polyether ether ketone (PEEK) tubing of 30 cm in length. The instrument operating conditions are given in Table 1. A closed-vessel microwave digestion system was used (MES 1000; CEM Corp., Matthews, NC). A model RC5C centrifuge (Sorvall Instruments, DuPont) was used to accelerate the phase separation process in the extraction of the compounds.

Mushroom collection and preparation

Different mushroom samples commonly were acquired from different local markets and supermarkets. The mushroom samples were identified as being *Boletus edulis*, *Agaricus bisporus*, and *Lentinus edodes*. Some of these were of international origin. Prior to the sample digestion step, the mushroom samples were washed with demineralized water, dried at 60 °C for 24 h without addition of other reagents, then finely ground using a household coffee grinder. All the

Table 1. ICP-MS and SEC instrumental parameters

ICP-MS parameters	
Forward power (W)	1350
Plasma gas flow rate (l min^{-1})	15.0
Auxiliary gas flow rate (l min^{-1})	0.87
Carrier gas flow rate (l min^{-1})	0.975
Sampling depth (mm)	6
Sampling and skimmer cones	Nickel
Dwell time per isotopes	0.1
Isotopes monitored	^{107}Ag, ^{75}As, ^{114}Cd, ^{202}Hg, ^{208}Pb, ^{120}Sn
SEC parameters	
Column	Superdex 75
Separation range (kDa)	1.35–66
Mobile phase	10 mmol l^{-1} CAPS buffer, pH 10.0
Flow rate (ml min^{-1})	0.7
Injection volume (μl)	100
UV–Vis wavelengths studied (nm)	200–500 (230 nm working line)

instruments used were previously washed with a 10% (v/v) aqueous HNO_3 solution and then with ultrapure water.

Determination of total elemental concentration

A 10 ml volume of 50% nitric acid solution was added to 0.5 g of ground mushroom sample in a microwave vessel. Microwave power was increased over four steps at 5 min intervals, starting at 25%, 45%, 55%, and ending at 65%, where 100% power was 1000 W. Temperature limits of 120, 140, 150, and 160 °C were set for each of the four steps. Pressure limits for the four steps were 20, 80, 120 and 170 psi. At the end of the digestion, the samples were diluted to 20 ml with 18 MΩ cm deionized water. A portion of this solution was filtered through a 0.2 μm surfactant-free cellulose acetate (SFCA) membrane filter. Both indium and germanium were added as internal standards. The measurements were performed using ICP-MS, and quantitative analysis was carried out by the method of standard additions. The same analytical procedure was followed for each type of mushroom.

Extraction procedures for elemental speciation in mushrooms

Different procedures were designed to permit the selective extraction of high and low MW compounds from the mushroom samples. The dried and powdered samples (0.1 g) were weighed in plastic tubes. Alkaline and acidic extractions were performed by adding 2 ml of 0.05 mol l^{-1} NaoH and 0.05 mol l^{-1} HCl respectively. After agitation (Vortex) for 30 min at room temperature, the mixture was centrifuged at 3000 rpm for 10 min and filtered using a 0.45 μm filter. A solution volume of 100 μl of the supernatant phase was introduced into the SEC–UV–ICP-MS system for the fractionation analysis. The only difference for the hot-water extraction procedure was heating the mixture at 60 °C for 30 min with constant agitation on a stirrer plate after adding 2 ml of ultrapure water.

Elemental fractionation and detection by SEC–UV–ICP-MS

The samples were injected onto the Superdex column with a Rheodyne 9025 injector utilizing a 100 μl PEEK sample loop. Separation was performed at a mobile-phase flow rate of 0.7 ml min^{-1} and by injecting 100 μl of sample onto the column. The chromatographic eluent coming from the SEC column was monitored initially by UV detection (230 nm) with a diode array detector and then connected to the ICP mass spectrometer. On-line coupling of the SEC system to the ICP mass spectrometer detector was performed using 0.25 mm i.d. PEEK tubing from the outlet of the UV detector directly to the inlet of the cross-flow nebulizer. The on-line use of the UV–Vis detector prior to ICP-MS did not produce significant dispersion of the analyte signal. The percentage distribution of the individual elements among different molecular size fractions was evaluated by relating the area of the particular peak to the total peak area.

RESULTS AND DISCUSSION

Elemental determination by ICP-MS

The ICP mass spectrometer was tuned with a multielemental standard solution (10 μg l^{-1} barium, cadmium, cerium, copper, germanium, magnesium, lead, rhodium, scandium, terbium, and thallium of each element) and the final conditions are given in Table 1. Possible polyatomic interferences in the analytical solutions were considered, and so the use of alternative isotopes to monitor the determination was considered. The m/z values selected are shown in Table 1. The possibility of matrix effects was also considered; therefore, quantification of the elements present in the different extract solutions was done by standard additions. With the determination of elemental concentrations in the fractions, extraction solutions and totals, it was possible to estimate a mass balance for each element to calculate the recovery values for different types of extraction. The recoveries were between 90.1 and 104.9%.

The results obtained for the total elemental analysis in the different mushroom species are shown in Table 2. The highest concentration levels were obtained for *B. edulis*. These results are in agreement with values reported by other researchers, showing that *Boletus* may be a good elemental accumulator.[5,28–31] Interestingly, high concentrations were found for mercury and silver mainly in *B. edulis* and *A. bisporus*. This bioaccumulation may be affected by environmental factors such as the influence of atmospheric mercury on the accumulation of this element in mushroom species,[12,13,32] although growing conditions and environment

Table 2. Total elemental concentration in different edible mushroom species (95% confidence interval, $n = 6$)

Element	*Boletus edulis* Concentration[a] (μg g^{-1} DW)	*Boletus edulis* RSD (%)	*Agaricus bisporus* Concentration[a] (μg g^{-1} DW)	*Agaricus bisporus* RSD (%)	*Lentinus edodes* Concentration[a] (μg g^{-1} DW)	*Lentinus edodes* RSD (%)
Ag	11	2.2	2.4	4.1	0.9	2.4
As	1.5	5.5	1.7	5.3	1.3	3.7
Cd	3.1	6.5	0.8	3.5	<0.002	—
Hg	13	1.9	11	2.2	3.8	4.6
Pb	2.4	4.8	1.5	3.6	<0.01	—
Sn	5.0	6.3	4.0	3.9	5.1	4.4

Dry weight.

were not studied in this work. We were unable to address the possibly differing metal concentrations arising from different collection sites with the likely different soil metal concentrations.[5,31] Considering the limited number of mushroom samples obtained for this study, the elemental concentration values obtained could not be generalized to concentrations commonly found in these mushroom species. A further study would need to be done with broader sampling or under controlled growing conditions.

Extraction efficiencies of different media

Elemental species were extracted from mushroom samples by using 0.05 mol l^{-1} NaOH, 0.05 mol l^{-1} HCl, or hot water (60 °C) as extraction solutions. Mushroom samples were put in contact with the different media for 30 min with constant agitation. Additional heating was applied in the case of water as is commonly used in water-extraction procedures.[9,33] Extraction with water at room temperature (~25 °C) was also performed in this work. However, no significant extraction was obtained. The extractability of elemental species associated with different compounds present in the mushroom samples was evaluated for these solutions. It was expected that the 0.05 mol l^{-1} NaOH solution would extract both low and high MW compounds. On the other hand, the HCl solution was expected to extract mainly low MW compounds, owing to the lower solubility of protonated compounds.[34] The presence of precipitates was not observed in the extractions performed with HCl solution. This could suggest that elements such as silver and lead are predominantly associated in the sample and are not precipitated with chloride ions. Additionally, extraction with hot water is a conventional approach used to extract organoelemental species of a wider MW range.[35,36] Generally, higher extraction efficiency was obtained with the NaOH solution than with either the HCl solution or hot water. The extraction values were in the range of 88 to 98.5%. For certain elements, such as silver, mercury, cadmium, and lead, the use of NaOH resulted in an extraction of about 96% of the total amount of these elements in the mushroom samples. On the other hand, neither HCl or hot water showed such efficiency, and only around 10–20% extraction was obtained with these solutions. Arsenic extraction was not influenced by the nature of the extraction medium, and similar extraction efficiencies (~89%) were obtained for all the media. Tin was different from the rest of the elements, with the best extractions (~90%) obtained when HCl solution was employed. The use of NaOH or hot water yielded tin extractions of 10–30%.

Chromatographic separation and spectrophotometric detection of fractions

The retention times and resolution of the different elemental fractions were evaluated for three different mobile phases. The mobile-phase solutions studied were Tris buffer solution at pH 8.0, phosphate buffer solution at pH 7.5, and CAPS buffer solution at pH 10.0. An adequate resolution was obtained when a 10 mmol l^{-1} CAPS buffer solution was used. It was more convenient to use CAPS buffer in order to avoid protein precipitation, considering the high pH value at which the separation was done. Additionally, the effect of the mobile-phase flow rate on the separation was also studied in this work. The flow rate was evaluated in the 0.3 to 1.0 ml min^{-1} range. The resolution deteriorated when the highest flow rate was employed. On the other hand, 0.3 ml min^{-1} did not produce any enhancement in the resolution and deteriorated the sensitivity. A mobile-phase flow rate of 0.7 ml min^{-1} yielded adequate resolution. Under these conditions, the complete chromatogram was obtained in 40 min. Typical UV chromatographic profiles obtained from the study of *A. bisporus* for different extraction media (NaOH, HCl, hot water) are shown in Fig. 1. The chromatographic profiles of different mushroom samples for the three extraction media were similar, and for this reason only the chromatograms obtained for *A. bisporus* are shown.

Figure 1 indicates the significant differences in the fractionation patterns as a function of the extraction medium employed. In the case of NaOH extraction, a high MW fraction around 51 kDa was observed as a major fraction. In contrast, a low MW fraction at 2.9 kDa was observed primarily for the HCl extraction. Another low MW fraction (25.5 min) was observed for HCl; however, it could not be characterized in terms of relative MW because the peak was outside the calibration range. The extraction of the MW

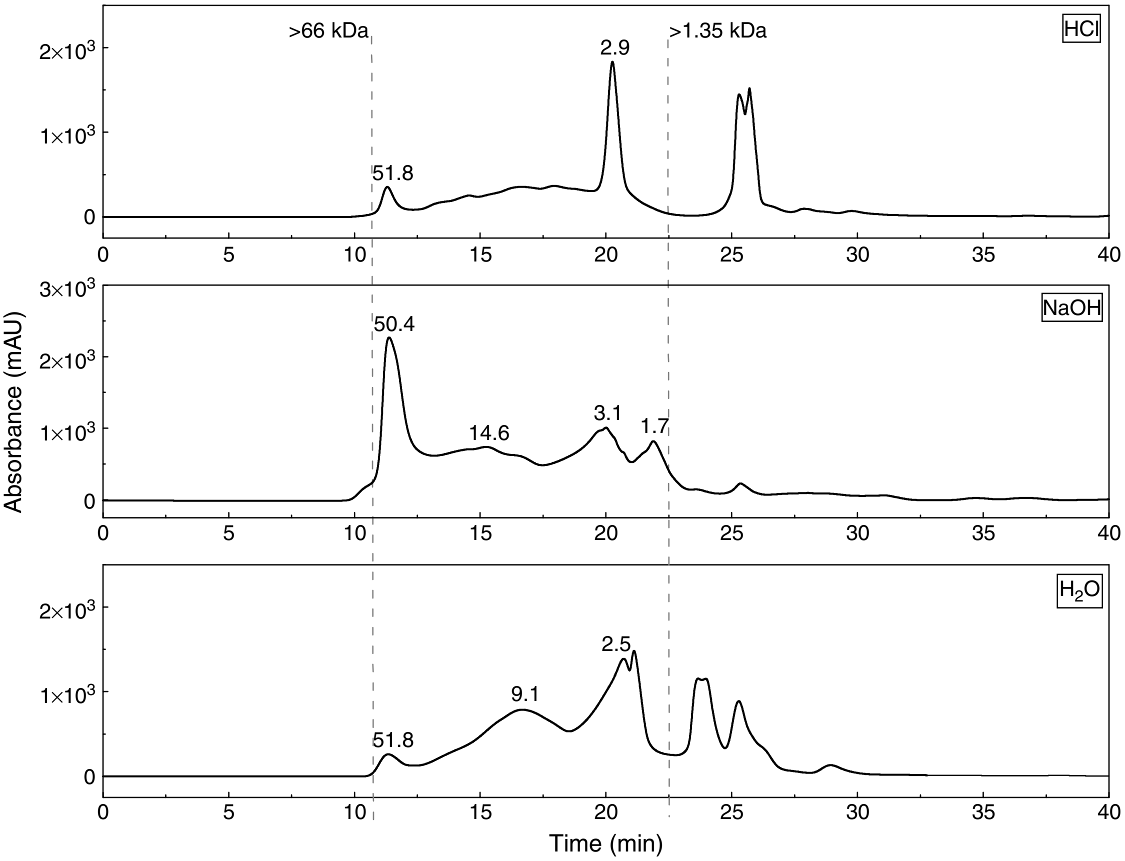

Figure 1. Fractionation profiles obtained for the extraction of *A. bisporus* with 0.05 mol l^{-1} of NaOH, 0.05 mol l^{-1} of HCl, or hot water at 60 °C. On-line UV–Vis monitoring of the absorbance was at 230 nm. Other conditions were as described in Table 1.

fraction at 51.8 kDa with HCl was less efficient than that for NaOH. Extraction of different species with water is a common procedure used in speciation analysis, and has also been utilized for other types of sample, such as yeast, onion, and garlic.[36,37] In this study, hot-water extraction was used to establish differences relative to NaOH and HCl extractions. In Fig. 1, it can be observed that low MW fractions were extracted when hot water was used. Hot-water extraction at 60 °C for 30 min allowed solubilization of primarily low MW compounds and a minimal high MW fraction at 51.8 kDa. A predominant low MW fraction of 2.5 kDa was obtained. The more efficient extraction of the high MW fraction around 50 kDa with NaOH could be explained by considering the higher solubility of certain compounds, such as proteins, in this solvent. This high MW fraction is likely attributed to the presence of large amounts of protein. It is known that mushrooms contain significant amounts of vegetable protein.[38–42]

Elemental fractionation patterns in different mushroom types

Complete information on the different MW distribution patterns of elements in the mushroom species studied is given in Tables 3 and 4. The different fractions found in the samples for NaOH, HCl and hot-water extractions are expressed in terms of the retention times and the area percentage of each peak with respect to the total peak area. The SEC separation of the extraction solutions was performed according to the analytical conditions given in Table 1. Generally, appropriate chromatographic resolution was obtained. However, in those cases where resolution was incomplete, deconvolution software was used.

The MW distribution patterns for silver in different extraction media and different mushroom types are shown in Fig. 2. It can be seen that, independent of the mushroom species, ^{107}Ag was associated exclusively with a high MW fraction around 49.1–49.8 kDa, which represents 100% of the silver present in the NaOH extraction solution. On the other hand, HCl solution permitted the extraction of low MW fractions with relative MWs in the range of 16–19 kDa. Additionally, a high MW fraction was also observed corresponding to MW around 50 kDa, but this was not appreciable compared with the high MW fraction obtained with the NaOH extraction. The use of hot water helped in obtaining fractions of diverse MWs, mainly for *A. bisporus*. For *B. edulis* and *L. edodes*, hot water did not extract the elemental fractions efficiently. These findings indicate the possible association of silver with proteins, which

Table 3. Comparison of different media for the extraction of elemental fractions from *B. edulis* and *L. edodes*

	Boletus edulis						*Lentinus edodes*					
Element	t_{R} NaOH	NaOH (%)	t_{R} HCl	HCl (%)	t_{R} water	Water (%)	t_{R} NaOH	NaOH (%)	t_{R} HCl	HCl (%)	t_{R} water	Water (%)
Ag	11.5	100	11.2	32	11.5	21.1	11.4	100	10.8	40.6	11.2	100
			14.7	68	13.1	78.9			14.7	59.4		
As	11.9	5.6	19.5	49.5	18.9	12.9	18.8	66.9	19.0	33.1	18.7	62.6
	19.1	25.2	24.4	34.3	20.0	30.0	19.8	33.1	23.9	66.9	19.8	10.8
	20.1	16.2	55.3	16.2	22.5	6.9					21.9	26.6
	24.4	32.7			24.3	34.1						
	39.0	9.8			41.1	9.9						
	55.1	10.5			54.7	6.2						
Cd	11.4	29.0	11.2	93.0	13.2	85.7	11.5	100	—	—	—	—
	13.1	53.4	19.1	7.0	19.0	14.3						
	18.8	17.6										
Hg	11.5	100	11.2	65.6	11.5	100	11.4	13.5	15.0	100	20.2	100.0
			14.9	34.4			20.6	86.5				
Pb	11.6	36.2	14.7	67.7	13.6	73.7	11.3	76.0	—	—	—	—
	13.5	56.3	19.1	32.3	19.0	26.3	18.8	24.0				
	19.0	7.5										
Sn	11.3	100	11.2	4.2	19.2	75.6	11.4	72.1	23.4	65.6	19.3	91.5
			14.8	11.8	24.7	24.4	24.9	27.9	24.8	34.4	25.1	8.5
			19.0	32.5								
			22.3	39.1								
			24.7	12.4								

Table 4. Comparison of different media for the extraction of elemental fractions from *A. bisporus*

Element	t_{R} NaOH	NaOH (%)	t_{R} HCl	HCl (%)	t_{R} water	Water (%)
Ag	11.5	100.0	11.2	2.3	11.4	19.6
			13.1	19.5	13.4	77.9
			14.5	32.5	23.7	2.5
			24.3	45.7		
As	18.7	74.6	18.7	43.6	18.8	53.1
	20.1	16.4	22.9	56.4	19.9	39.2
	24.2	9.0			22.1	7.7
Cd	11.4	23.3	16.1	100.0	13.0	52.5
	13.3	76.7			15.9	47.5
Hg	11.5	100.0	11.2	0.5	11.4	62.7
			14.9	2.7	37.8	37.3
			39.7	96.8		
Sn	11.2	100.0	22.5	84.3	18.7	76.2
			24.7	15.7	24.9	23.8

is known to solubilize easily in alkaline media compared with neutral or acidic conditions. In fact, in a study by Byrne and Tusekznidaric[20] with *A. bisporus* utilizing gel chromatography, silver was found to be associated with mainly two fractions, a high MW fraction and another fraction with intermediate MW. The results obtained in this work are in agreement with those described above, and it was found that 100% of the silver in the NaOH extraction solution was associated exclusively with a high MW fraction. Considering the possible different growth conditions used, e.g. soil (or compost) composition, nutrients, atmospheric factors, etc., this could have induced changes in the metabolism of the fungi.[5,42,43] Since the mushrooms used in this study were purchased locally from diverse sources, we had no control over those conditions. However, we are beginning a controlled growth study.

The chromatographic profiles for ^{75}As fractionation in the mushrooms are shown in Fig. 3. Arsenic was found to be associated mainly with a MW fraction of 4.4–4.9 kDa in *L. edodes* (Fig. 3b) and *A. bisporus* (Fig. 3c) for all the extraction media. However, a more diverse distribution was obtained in the case of *B. edulis*, with a high MW fraction of 51.5 kDa, an intermediate MW fraction around 2.9–4.5 kDa, and a predominant peak in the low MW region smaller than 1.3 kDa. Similar extraction efficiencies obtained independently on the extraction media indicate that the arsenic species in the mushrooms studied are mainly in a form that is not associated with proteins or other high MW compounds.

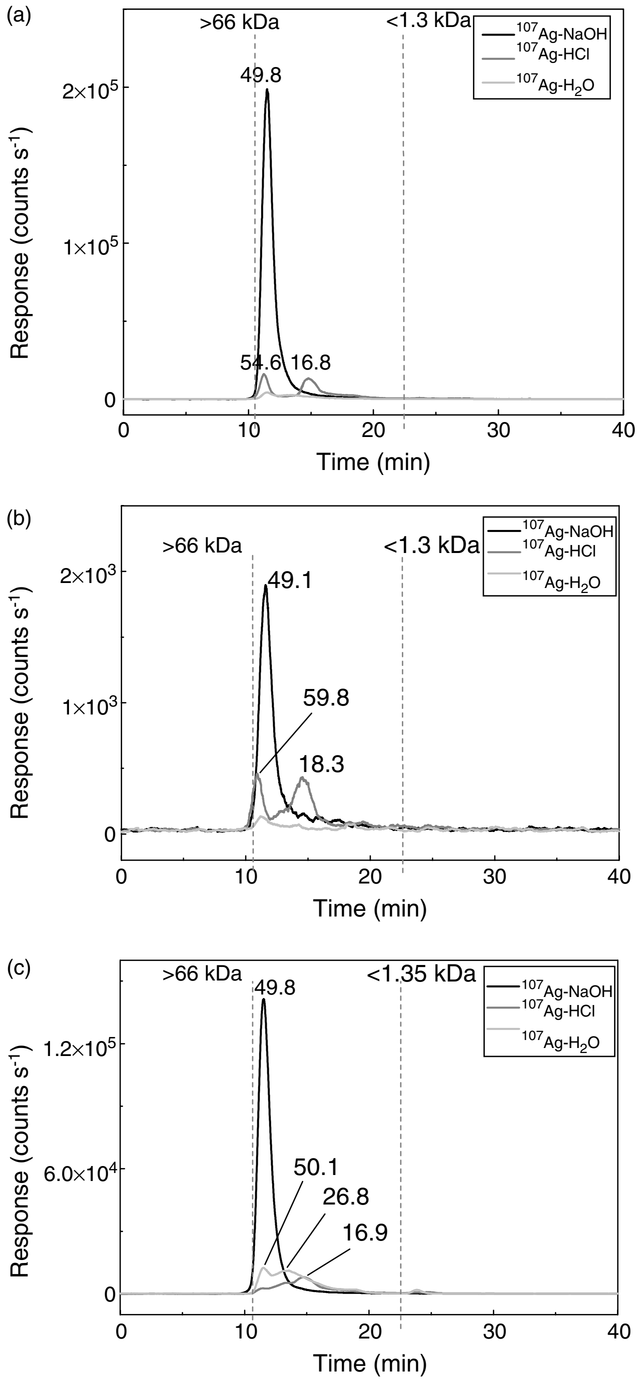

Figure 2. Chromatographic profiles of ^{107}Ag in different extraction media: (a) *B. edulis*; (b) *L. edodes*; (c) *A. bisporus*. Other conditions were as described in Table 1.

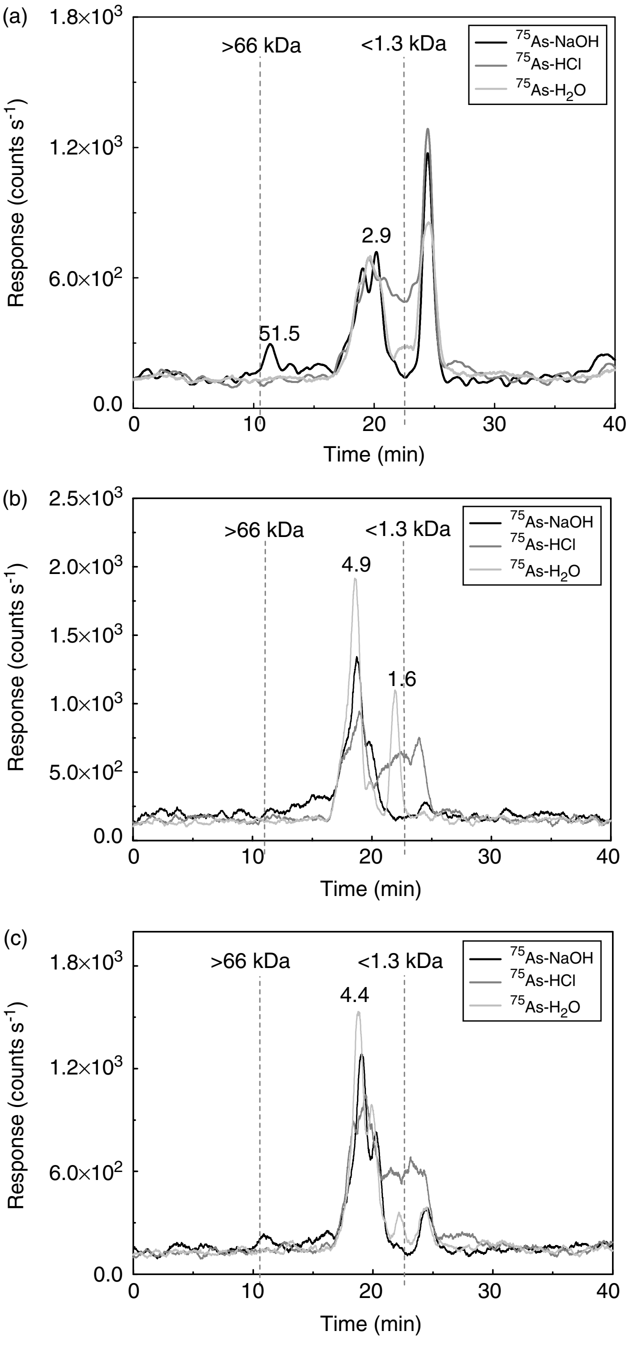

Figure 3. Chromatographic profiles of ^{75}As in different extraction media: (a) *B. edulis*; (b) *L. edodes*; (c) *A. bisporus*. Other conditions were as described in Table 1.

These results are in agreement with those found by others for *Laccaria amethystina* species.[21,44] In those cases, arsenobetaine, dimethylarsenic acid, methylarsonic acid, trimethylarsine oxide and arsenic acid were the arsenic species identified.

Fractionation of ^{120}Sn in the different mushroom species showed a more complex distribution pattern than for the rest of the elements (Fig. 4). This was mainly in cases where HCl solution was used as an extraction solution. Marked changes in the chromatographic profiles were obtained when different extraction media were employed. For *B. edulis*, tin was associated with a high MW fraction of 51.5 kDa and another one of 3.9 kDa. The high MW fraction around 50 kDa was also observed for *L. edodes* and *A. bisporus* when NaOH solution was used as the extraction medium. Interestingly, similar extraction of this high MW fraction in different extraction media indicates is independent of pH. This could be explained if tin is associated with a more soluble protein. The best results for tin extraction were obtained when the HCl solution was used. In this case, the high MW fraction around 50 kDa was obtained for all the mushroom species. Additional MW fractions of 15.4–17.5 kDa, 3.9 kDa, 1.4 kDa and another lower than 1.3 kDa were observed for *B. edulis* (Fig. 4a) and *A. bisporus* (Fig. 4b). However, the chromatographic profile for *L. edodes* (Fig. 4c) was significantly different from the other two mushroom species and the fraction around 1.3–1.4 kDa was not predominant. Instead, tin was associated mainly with a low MW fraction that was out of the calibration range (<1.3 kDa) or unassociated beyond solvation. Similar results were obtained for hot-water extractions with the different mushrooms.

Mercury was studied in this work because of the high toxicity of certain mercury species for many biological systems. The chromatographic profiles of ^{202}Hg in different extraction media and for different mushroom species are illustrated in Fig. 5. Mercury was found to be associated mainly with a high MW fraction of 49.2–49.8 kDa, particularly for the *B. edulis* (Fig. 5a) and *A. bisporus* (Fig. 5c) species in the NaOH extractions. However, the fractionation pattern of mercury for *L. edodes* (Fig. 5b) was significantly different than for the other two mushroom species examined. In this case, mercury was predominantly associated with a MW fraction at 2.4 kDa; however, a high MW fraction was also observed. The low MW fraction here might correspond to mercury bound to metallothioneins, as suggested in previous studies for other kinds of mushroom species.[20] From Fig. 5 it is also clear that the use of HCl solution or hot water did not result in an efficient extraction of the mercury-containing fractions.

Finally, two other elements of toxicological interest were studied. Typical fractionation results for ^{208}Pb and ^{114}Cd in *B. edulis* are shown in Fig. 6a and b. Chromatographic profiles for *A. bisporus* were similar to those obtained for *B. edulis* and for this reason are not shown: here; however, detailed information can be found in Tables 3 and 4. Lead was eluted bound to a high MW fraction around 50 kDa, an intermediate MW fraction of 24.2 kDa, and a 4.3 kDa fraction when NaOH extraction was used. Extractions with HCl or hot water did not allow the solubilization of the high MW fraction of around 50 kDa. These results indicate possible association of lead with high MW proteins. The two fractions corresponding to 24.2 and 4.3 kDa were also present in the HCl and the hot-water extractions. The distribution of ^{114}Cd in different MW fractions (Fig. 6b) was similar to that obtained for lead. Cadmium was eluted mainly in a high MW fraction of 49.2 kDa for the NaOH extraction. On the other hand, the HCl solution and hot water led to extraction of low WM fractions and high MW fraction with lower efficiency than with the alkaline media. The chromatographic profiles of cadmium for the different mushroom samples did not show significant differences, and for this reason the chromatogram obtained for *B. edulis* is shown. Some research has indicated an association of cadmium with compounds present in edible mushrooms.[45] Poor correlation between the cadmium accumulated in *Agaricus* and the content of sulfhydryl groups was found in those studies. Therefore, the association of cadmium with high MW fractions such as metallothionein-like proteins seems unrealistic.

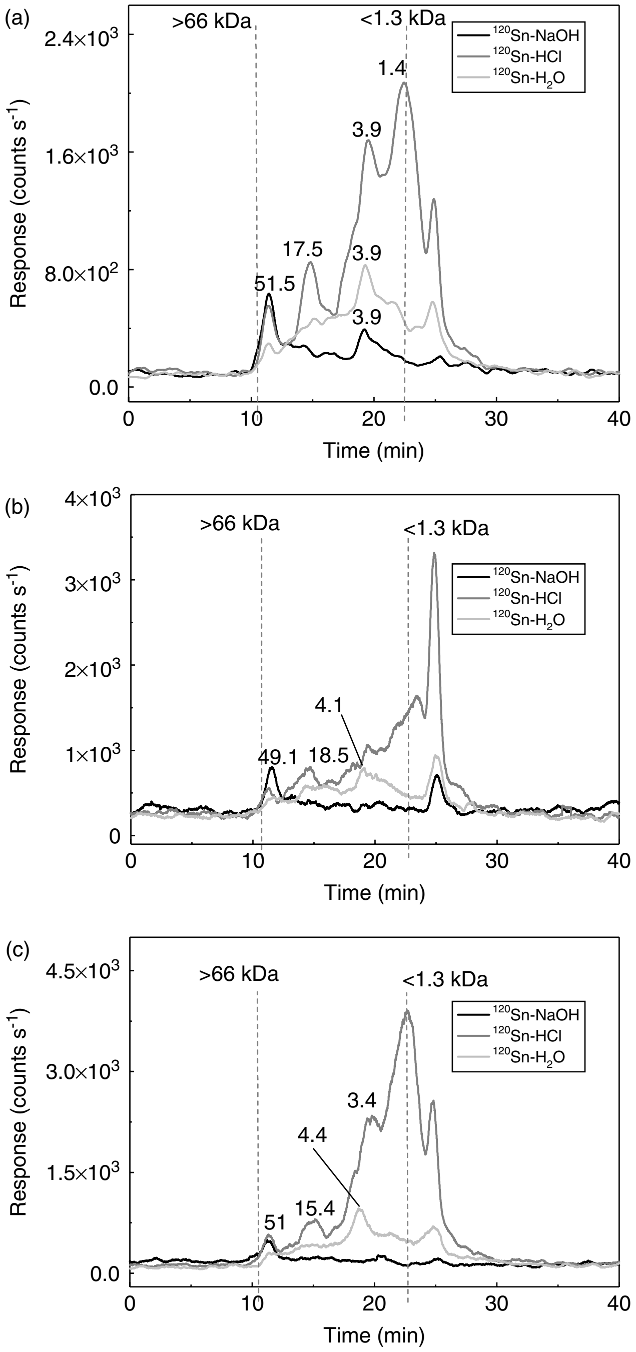

Figure 4. Chromatographic profiles of ^{120}Sn in different extract media: (a) *B. edulis*; (b) *L. edodes*; (c) *A. bisporus*. Other conditions were as described in Table 1.

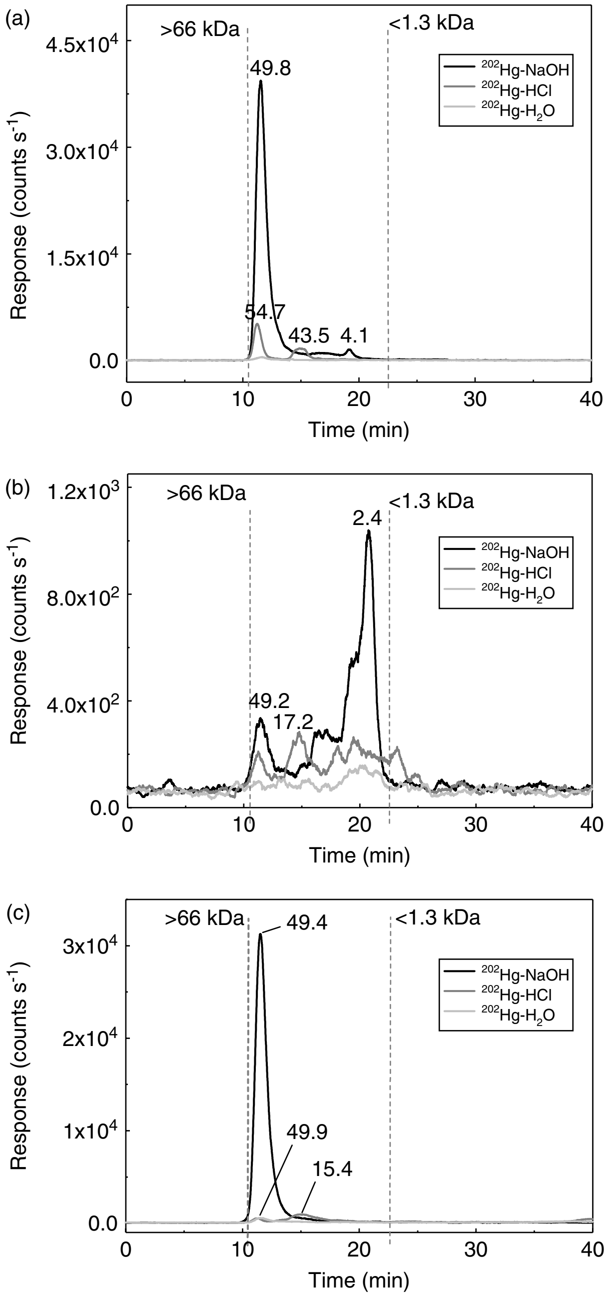

Figure 5. Chromatographic profiles of ^{202}Hg in different extraction media: (a) *B. edulis*; (b) *L. edodes*; (c) *A. bisporus*. Other conditions were as described in Table 1.

CONCLUSIONS

A multielemental speciation study in the mushroom species *B. edulis*, *L. edodes*, and *A. bisporus* has been performed using SEC–UV–ICP-MS. The chromatographic profiles were dependent on the extraction conditions. This highlights the importance of the extraction steps in speciation analysis. The fractionation patterns for silver, arsenic, cadmium, and lead were similar for the different mushrooms species. However, different fractionation patterns were obtained for mercury and tin in *L. edodes* compared with the other two mushroom species. Although SEC allows elemental fractionation, the application of multidimensional separation techniques and the use of identification tools such as biological MS are required to identify the unknown species. Future studies will be undertaken to determine the individual elemental species present in the mushrooms analyzed in this work.

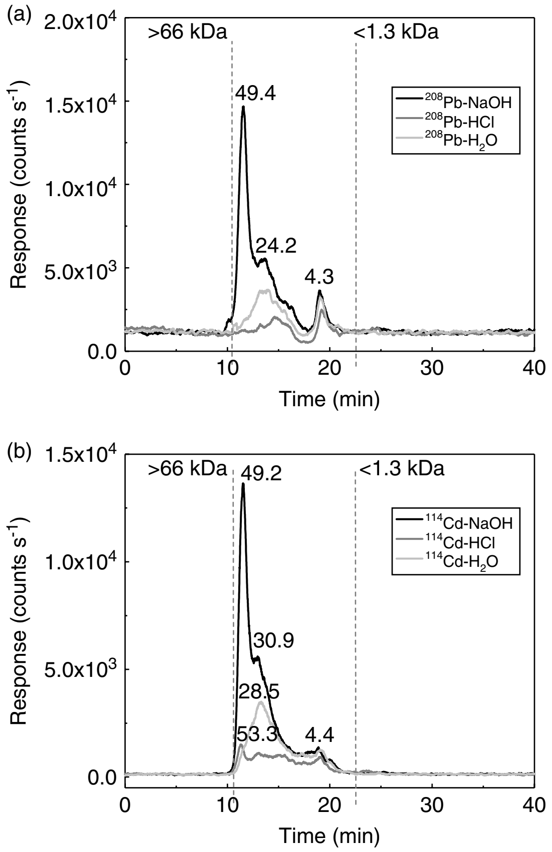

Figure 6. Typical fractionation patterns of (a) ^{208}Pb and (b) ^{114}Cd in *B. edulis* utilizing 0.05 mol l^{-1} NaOH, 0.05 mol l^{-1} HCl, or hot water as an extraction medium. Other conditions were as described in Table 1.

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