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Multielemental Speciation Analysis of Fungi Porcini (*Boletus edulis*) Mushroom by Size Exclusion Liquid Chromatography with Sequential On-line UV-ICP-MS Detection

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An analytical methodology to determine the molecular weight (MW) distribution patterns of several elements among different compounds present in commonly consumed edible mushrooms is presented in this work. A hyphenated technique based on size exclusion liquid chromatography (SEC) coupled on-line to UV and inductively coupled plasma mass spectrometry (ICP-MS) detection was used. The association of the elements to high and low MW fractions was confirmed with sequential detection by UV and ICP-MS. Separation of the fractions was performed by injecting a 100 μL sample volume to a Superdex 75 column. The effect of different mobile phases on the separation was evaluated. Additionally, three different extraction conditions including 0.05 mol L^{-1} NaOH, 0.05 mol L^{-1} HCl, and hot water at 60 °C were applied to extract the elemental species from the mushroom samples. Significant differences were observed in the chromatograms depending on the extraction conditions utilized. Optimization of the experimental variables involved in the SEC-UV-ICP-MS coupling was carried out. The method was applied to investigate the fractionation patterns of Bi, Co, Cu, Fe, I, Mo, Ni, Se, and Zn in fungi porcini (*Boletus edulis*) mushroom. The results obtained in this work indicate an important association of most of the elements to high MW fractions.

KEYWORDS: Multielemental speciation; edible mushrooms; metalloproteins; SEC-UV-ICP-MS

INTRODUCTION

Mushrooms are thought of as healthy foods with relatively few calories and fat but rich in vegetable proteins, chitin, vitamins, and minerals. Furthermore, it is suggested that they constitute an increasing share in the world diet (*1*). Mushrooms are important in the ecosystem because they can biodegrade the substratum, and therefore their waste byproducts can be used for agricultural production (*1*, *2*). Mushrooms are also said to be beneficial for such diseases as hypertension, hypercholesterolemia, and cancer (*3*, *4*).

The occurrence of high metal content in edible plants is of considerable importance, because there may be a possible toxicological hazard. Certain metals, when concentrated in living organisms, are regarded as human toxins. Many studies have dealt with determining metals in mushrooms, particularly in edible ones (*5*−*13*). Several types of mushrooms, especially those belonging to the genus *Agaricus*, are well-known for their high metal accumulation, which has led to governmental recommendations to restrict their dietary use (*14*, *15*). Heavy metals in mushrooms are well-documented, especially those elements of toxicological and nutritional interest such as iron (*16*), zinc (*17*), copper (*18*), manganese (*19*), selenium (*20*), cobalt (*21*), silver (*22*), mercury (*6*, *18*, *23*−*27*), chromium (*28*), and lead (*18*, *24*, *29*). Although a number of papers have been published about the presence of metals in mushrooms, speciation studies have not been extensively pursued. Mercury, arsenic, and selenium are the primary targets in these studies. Especially for mercury, efforts have been made to get more insight into uptake and accumulation mechanisms because the highly toxic methylmercury species have been found in various mushrooms (*14*, *30*). The determination of arsenic species in mushrooms has been developed using anion- and cation-exchange high-performance liquid chromatography (HPLC) systems coupled to inductively coupled plasma mass spectrometry (ICP-MS) (*31*, *32*) and separation by liquid chromatography and purge and trap gas chromatography interfaced with atomic fluorescence spectrometry (AFS) (*33*). Selenium species have been analyzed in selenized mushrooms using HPLC coupled to AFS, prior to hydride generation (HG), or inductively coupled plasma optical emission spectrometry (ICP-OES) (*34*).

Size exclusion chromatography (SEC) is a very useful technique to study the distribution of elements in different molecular weight (MW) fractions and gives information about the association of elements with the different sample compounds of various molecular weights (*35*−*38*). In the specific case of mushrooms, off-line SEC has been applied for distribution studies of ^{75}Se and ^{134}Cs, prior to their determination by using a Ge−Li γ-detector (*14*). The distribution of selenium among different fractions of the *Agaricus bisporus* genus showed that a considerable proportion of the selenium was located in low molecular weight (LMW) organic or inorganic compounds,

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Table 1. ICP-MS and SEC Chromatography Instrumental Parameters

ICP-MS Parameters	
forward power	1350 W
plasma gas flow rate	15.0 L min^{-1}
auxiliary gas flow rate	0.87 L min^{-1}
carrier gas flow rate	0.975 L min^{-1}
sampling depth	6 mm
sampling and skimmer cones	nickel
dwell time	0.1 s per isotope
isotopes monitored	^{82}Se, ^{59}Co, ^{58}Ni, ^{63}Cu, ^{54}Fe, ^{95}Mo, ^{209}Bi, ^{127}I, ^{68}Zn
SEC Chromatography Parameters	
column	Superdex 75
separation range	1.35–66 kDa
mobile phase	10 mmol L^{-1} CAPS buffer, pH 10.0
flow rate	0.7 mL min^{-1}
injection volume	100 μL
UV–vis wavelengths studied	200–500 nm (230 nm working λ)

whereas smaller proportions were distributed in high molecular weight (HMW) fractions, between lipids, proteins, and carbohydrates (*14*, *39*). Although information about the above elemental species in mushrooms has been obtained, the accumulation and transformation process of those elements are still unknown. Moreover, there are numerous other elements of nutritional and toxicological interest in mushrooms, especially edible ones, which have not been studied. The coupling of SEC to ICP-MS has been widely recognized as a very useful tool in speciation studies, and it is considered as the starting point for a more detailed evaluation of the nature of elemental species (*38*, *40*). However, data regarding on-line coupling of SEC to ICP-MS for elemental speciation analysis in mushrooms are only now appearing.

The aim of the present work is to determine the distribution patterns of several elements in different MW fractions of edible mushrooms commonly consumed by humans. The SEC technique was coupled to ICP-MS for the separation and on-line detection of the elements in mushrooms. Additionally, different procedures for the extraction of the organometallic species from the mushrooms were developed and evaluated. The study and optimization of the SEC variables involved in the separation of the elemental species present in mushrooms were performed.

EXPERIMENTAL PROCEDURES

Instrumentation. An HPLC system was used. This was a Shimadzu (Shimadzu Scientific Instrument Inc, Columbia, MD) LC-6A pump with a 100 μL loop (Rheodyne 7725 injection valve, Rheodyne, Cotati, CA), and an SPD-6A UV−vis spectrophotometric detector coupled through an R9 232 Dionex interface with a 133 MHz PC equipped with AI-450 release 3.21 software.

Elemental detection was performed by using an ICP-MS Perkin-Elmer SCIEX Elan 6000 (Ontario, Canada) with a Gem Tip cross-flow nebulizer (Perkin-Elmer) and Ryton spray chamber (Perkin-Elmer). The nebulizer gas flow and ion lens setting were optimized using on-board computer algorithms with the Elan 6000 software. The outlet of the SEC column was connected to the liquid sample inlet of the nebulizer using 0.5 mm i.d. PEEK tubing of 30 cm in length. The instrumental operating conditions are given in **Table 1**. The closed vessel microwave digestion system used was an 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.

Reagents. Optimization of the ICP-MS instrumental conditions was performed using a PE Pure Plus Elan Calibration 1 solution (Perkin-Elmer, CA) containing Ba, Cd, Ce, Cu, Ge, Mg, Pb, Rh, Sc, Tb, and Tl in a concentration of 10 μg L^{-1} in 2% HNO_3 solution.

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

All water was deionized (18 MΩ·cm) and prepared by passing through a NanoPure treatment system (Barnstead, Boston, MA). All reagents were of analytical reagent grade, and the presence of trace element contaminants was not detected in the working range of these experiments.

Sample Collection and Preparation. Mushroom samples commonly consumed in the United States were purchased from a local market and identified (*41*), and the fungus porcini (*Boletus edulis*) was selected as the primary mushroom for these studies. Mushroom quality is strongly affected by different parameters, such as stage of growth and pre- and post harvest conditions. To somewhat compensate for the sample variability, samples from local markets and larger food stores were purchased on different days. Before digestion, the samples were washed with demineralized water. Mushroom samples were then dried at 60 °C for 24 h without the addition of reagents and ground using a household coffee grinder. All of the instruments used were previously washed with a 10% (v/v) HNO_3−water solution and then with ultrapure water.

Total Elemental Analysis by Microwave-Assisted Acid Digestion. 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%, increasing to 45 and 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 1.41, 5.62, 8.43, and 11.95 kg cm^{-2}. At the end of the digestion, the samples were diluted to 200 mL with 18 MΩ·cm deionized water. A portion of this solution was filtered through a 0.45 μm surfactant-free cellulose acetate (SFCA) membrane filter. Both indium and germanium were added as internal standards. Quantitative analysis was carried out according to the standard addition method.

Extraction of Elemental Species from Edible Mushrooms. The extraction of HMW and LMW compounds was developed by weighing 0.1 g of dried and powdered mushroom sample precisely in plastic tubes. Extraction was carried out by adding 2 mL of 0.05 mol L^{-1} sodium hydroxide. After agitating (Vortex) for 30 min at room temperature, the mixture was centrifuged at 3000 rpm for 10 min, and 100 μL of the supernatant was introduced into the SEC-UV-ICP-MS system after filtration. The extraction of LMW species was carried out by the addition of 2 mL of a 0.05 mol L^{-1} hydrochloric acid solution to 0.1 g of dried mushroom sample. The mixture was then stirred (Vortex) for 30 min at room temperature and centrifuged at 3000 rpm for 10 min. A 100 μL volume of the supernatant phase was introduced into the SEC-UV-ICP-MS system. For comparative purposes a water extraction procedure was carried out by weighing 0.1 g of dried mushroom sample. After addition of 2 mL of ultrapure water, the mixture was heated at 60 °C for 30 min on a stirrer plate with constant agitation.

Speciation Analysis Using SEC-UV-ICP-MS. A Superdex 75 SEC column (Amersham Biosciences, Inc., Piscataway, NJ) was used for SEC separation of mushroom supernatants. The SEC column was calibrated with a range of MW markers according to the manufacturer's specifications. For the separation of the compounds present in the mushroom supernatants, a flow rate of 0.7 mL min^{-1} was set, and 100 μL of sample was injected. A diode array detector was connected to the outlet of the size exclusion column for the on-line detection of the compounds. An effort was made to determine the most adequate mobile phase solution to give the highest resolution possible. Percentage distribution of the individual elements among different molecular size

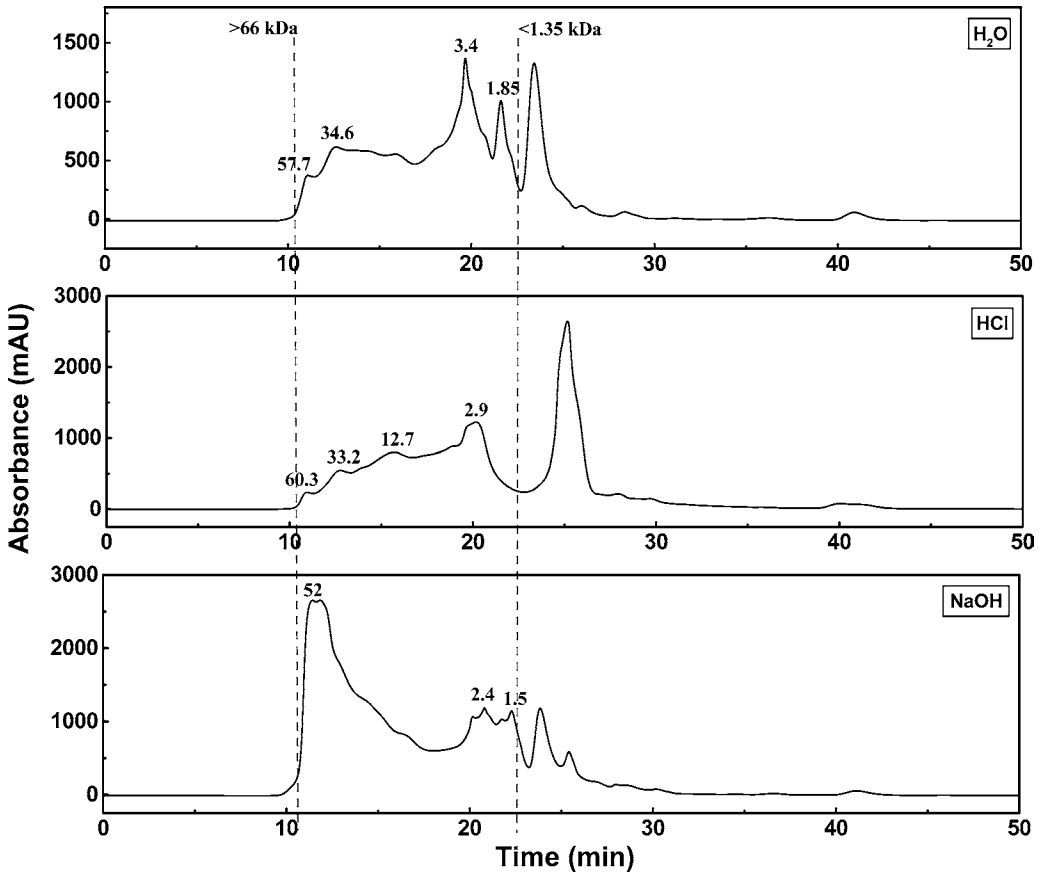

Figure 1. Chromatographic profiles obtained for the extraction of fungi porcini mushroom with 0.05 mol L^{-1} NaOH, 0.05 mol L^{-1} HCl, and hot water at 60 °C. Absorbance was monitored at a wavelength of 230 nm. Analytical conditions were as described in **Table 1**.

fractions was evaluated by relating the area of the particular peak to the total peak area under the chromatogram.

RESULTS AND DISCUSSION

Fraction Identification by UV Detection. The Superdex 75 size exclusion column was calibrated utilizing the following standard calibrants: apo-transferrin (76000−81000 Da), bovine albumin (66000 Da), myoglobin (16000 Da), aprotinin (6500 Da), substance P (1350 Da), and Gly_6 (0.36 kDa). The linearity of the retention time versus the log MW curve was evaluated using different calibrants to cover the whole MW range. It was observed that good linear response was obtained from 1.35 to 66 kDa. Compounds with MW >66 kDa eluted with the dead volume of the column, whereas for MWs <1.35 kDa a nonlinear response was observed. The calibration equation obtained for this column was

$$t_R\ (\text{min}) = 44.59 - 7.04 \log(\text{MW})$$

In the present study, UV detection and ICP-MS detection were sequentially applied to measure the SEC column separation. The absorbance of the chromatographic eluent was initially studied in the 200−500 nm wavelength range to detect the different compounds present in the extraction solutions. It was observed that all of the fractions showed good response when 230 nm was used. Typical UV chromatographic profiles resulting from fungi porcini for different extraction media (NaOH, HCl, and hot water) are shown in **Figure 1**.

Separation conditions related to the pH values of the media were also studied to obtain proper resolution of the fractions present in the mushroom extract. Different mobile phase solutions were tested including 20 mmol L^{-1} Tris buffer solution at pH 8.0, 10 mmol L^{-1} phosphate buffer solution at pH 7.5, and 10 mmol L^{-1} CAPS buffer solution at pH 10.0. The best results between minimal retention times and resolution were obtained when a 10 mmol L^{-1} CAPS buffer solution was used as the mobile phase. Additionally, it was more convenient to diminish precipitation, especially proteins, due to the high working pH value and thereby avoid possible column blocking. Under these conditions the chromatogram was complete in <40 min.

The chromatograms shown in **Figure 1** show marked differences depending on the extraction conditions utilized, specifically in the case of NaOH compared to HCl and hot water extractions. A HMW fraction at 52 kDa was observed when NaOH was utilized, whereas for HCl and water extractions this HMW fraction was not seen. This HMW fraction is likely a protein fraction. It is known that mushrooms contain a significant amount of proteins; therefore, these results are in agreement with those published previously (*42*−*46*). On the other hand, the HCl extraction showed a predominant fraction at a retention time of 25.2 min, which clearly suggests the extraction of LMW compounds. Unfortunately, evaluation of the molecular weight for this fraction was not possible as it was out of the calibration range. Extraction of different species with water is a common procedure used in speciation analysis, and it has been utilized for other sample types such as yeast, onion, and garlic (*36*, *47*). In the present work hot water extraction was studied to establish the differences compared to NaOH and HCl extractions. In **Figure 1**, it can be observed that LMW fractions were extracted when hot water was used. These fractions were in the 1.35−4 kDa MW range, and similar fractions were extracted for the NaOH solution. However, the HMW fraction of ∼50 kDa was not observed with the aqueous extraction. This is understandable

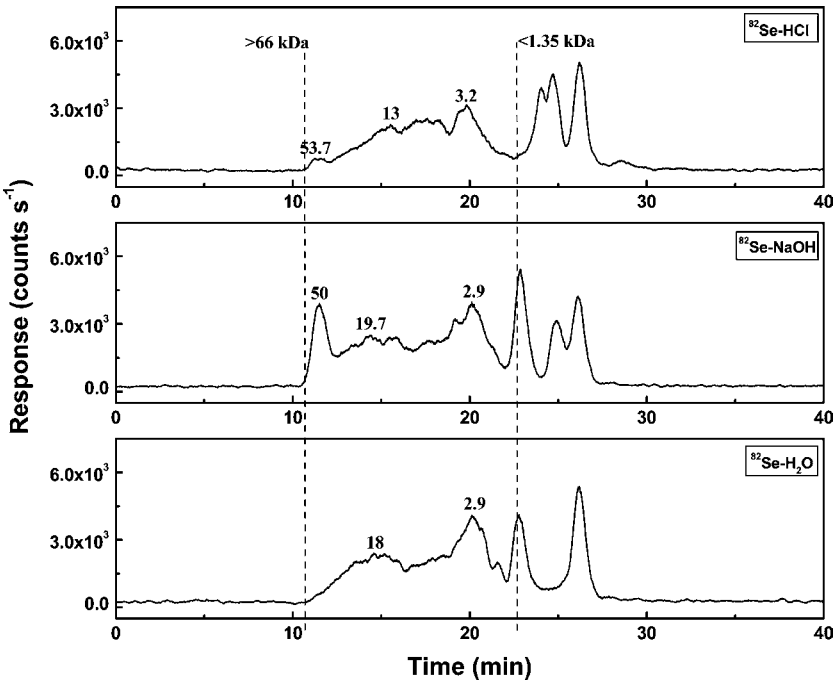

Figure 2. Typical chromatogram obtained for the fractionation of ^{82}Se compounds in different extraction media from fungi porcini mushroom. Analytical conditions were as described in **Table 1**.

Table 2. Comparison of Different Media for the Extraction of Elemental Fractions from Fungi Porcini Mushroom (95% Confidence Interval, $n = 6$)

	total content		extractability (%)		
element	concn (mg kg^{-1})	RSD(%)	NaOH	HCl	water
Bi	0.2	6.3	46.6	54.2	49.7
Co	3.8	5.7	75.2	51.5	57.6
Cu	104	5.2	97.0	32.5	23.7
Fe	5841	3.6	62.6	40.9	13.5
I	23.0	6.6	89.1	92.8	82.4
Mo	0.5	5.5	89.1	90.3	86.6
Ni	54.9	4.1	63.9	82.4	18.1
Se	16.3	5.9	74.7	62.5	65.0
Zn	186	2.9	89.9	44.2	88.8

considering the higher solubility of protein compounds in an alkaline medium compared to the neutral and acidic media provided by the hot water and HCl extractions.

Elemental Determination and SEC-UV-ICP-MS Coupling. Instrumental and medium extractability conditions were optimized to permit proper determination of elemental distribution. The ICP-MS instrument was tuned by using a multielemental standard solution containing Ba, Cd, Ce, Cu, Ge, Mg, Pb, Rh, Sc, Tb, and Tl at a concentration of 10 μg L^{-1} of each element. The presence of possible polyatomic interferences was taken into account. Therefore, the use of alternative isotopes to perform the determination by ICP-MS was considered to avoid interference effects that could arise from biological samples. The isotopes selected are shown in **Table 1**. The possibility for matrix effects was also considered; therefore, quantification of the elements present in the different extracted solutions was performed by the standard addition method. With the determination of the elemental composition in the fractions, extraction solutions, and total mushroom analysis, it was possible to estimate a mass balance for each element to calculate the recoveries, which came between 89.1 and 104%.

The detection of the different fractions was performed using an on-line sequential UV-ICP-MS detection system. The chromatographic eluent was monitored initially by UV−vis. On-line coupling of the SEC system to the ICP-MS detector was performed using PEEK tubing of 0.5 mm i.d. 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 dispersion in the chromatographic signals.

Influence of the Media on Extractability of the Elemental Fractions. Soluble elemental species were extracted from mushroom samples by using 0.05 mol L^{-1} NaOH, 0.05 mol L^{-1} HCl, and hot water (60 °C). Mushroom samples were put in contact with different media for 30 min with constant agitation. In the case of water, additional heating was applied, which is common for water extraction procedures (*20*, *48*). A solution of 0.05 mol L^{-1} NaOH was expected to extract both LMW and HMW compounds due to the alkaline medium increasing the solubilization of compounds through deprotonation. On the other hand, the HCl solution was expected to extract mainly LMW compounds due to the lower solubility of protonated species (*49*). Additionally, the extraction with hot water is a conventional approach used to extract organoelemental species. Extractabilities of elements expressed as percentages of total element content in the mushroom sample are shown in **Table 2**. Generally higher extraction efficiency was obtained with the NaOH solution compared to HCl and water solutions. The NaOH solution permitted extractability values on the order of 46.6−97%. Extractability values were also element-dependent. For example, elements such as Zn, Cu, Ni, Co, and Fe showed better extraction in an alkaline media, whereas for Bi, I, Mo, and Se, no significant differences were observed using the different media.

Molecular Weight Distribution of Elemental Species. The SEC separation was performed according to the analytical conditions given in **Table 1**. In general, good resolution was obtained for the chromatograms of all the elements determined. However, in those cases where fractions were not completely separated, deconvolution software was utilized to calculate the peak areas. The chromatographic profile obtained for selenium fractionation is given in **Figure 2**. As can be observed,

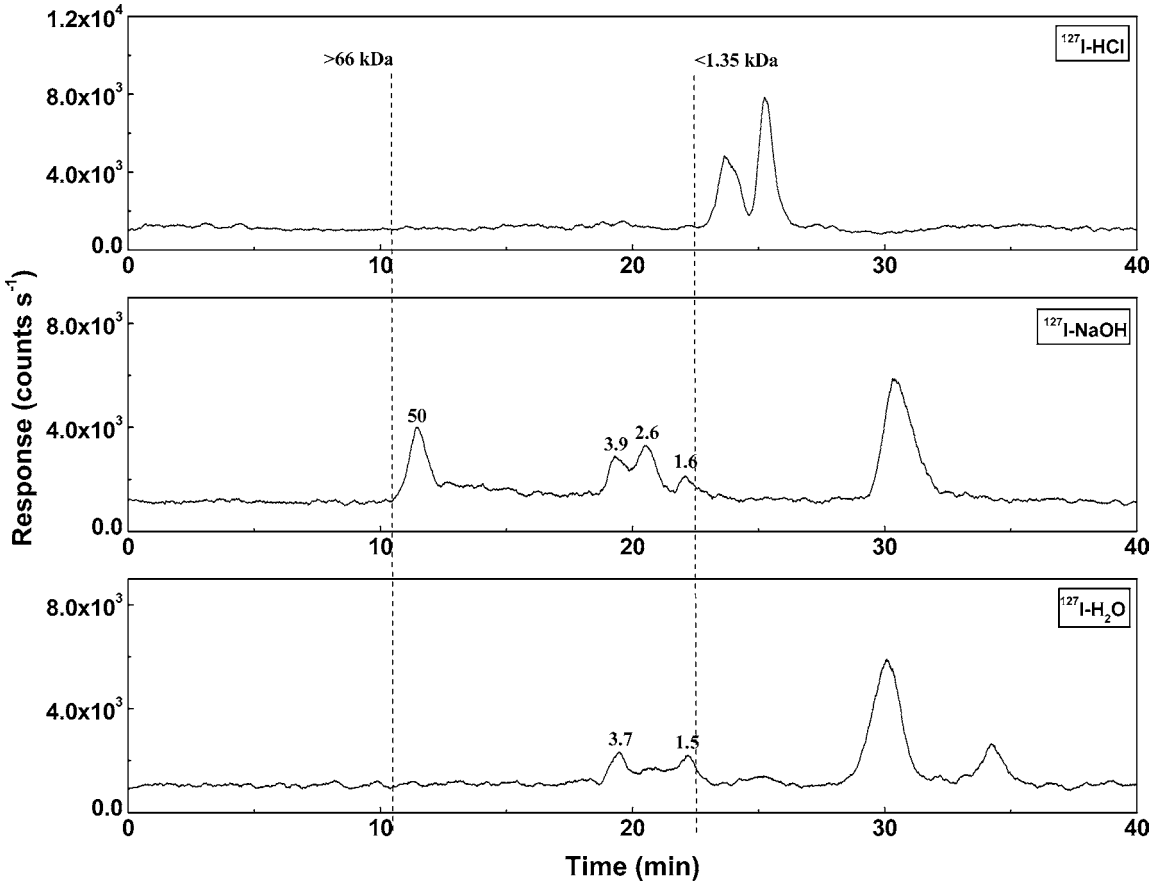

Figure 3. Chromatographic profile of ^{127}I fractionation obtained for different extraction media from fungi porcini mushroom. Analytical conditions were as described in **Table 1**.

significant differences in the fractionation patterns were obtained depending on extraction media. In the case of NaOH extraction, selenium was found to be associated with a HMW fraction at 50 kDa, which corresponds to 10.5% of the total selenium eluted from the column. However, Se was primarily associated with fractions in the LMW region (<1.35−20 kDa). The presence of a LMW fraction corresponding to 2.9−3.2 kDa was indicated for all three types of extractions. Additionally, diverse selenium fractions with different MW values were obtained for each extraction medium utilized. These findings suggest that selenium in mushroom may be present as small peptides or free selenoamino acids, specifically for those LMW fractions below 1.35 kDa. These findings support those reported by van Elteren et al. (*14*), who found selenium in LMW fractions in portabella (*Agaricus bisporus*) mushroom.

The methodology was also applied to determine the fractionation pattern of iodine. **Figure 3** shows the chromatographic profiles obtained for iodine in NaOH, HCl, and hot water solutions. Extraction of LMW fractions in the region of <1.35 kDa was observed with the 0.05 mol L^{-1} HCl solution. Additional peaks in the HCl extraction were not observed, and it may be concluded that 100% of the iodine injected into the SEC column was associated with LMW fractions. Interestingly, chromatograms of both NaOH and hot water extracts showed similar peaks in the region of <1.35−4 kDa [3.9 kDa (18.3%), 2.6 kDa (7.2%), and 1.6 kDa (3.1%) for NaOH extraction; and 3.7 kDa (28.2%) and 1.5 kDa (4.7%) for hot water extraction]. These fractions may correspond to the association of iodine to some LMW peptides, which can be easily extracted with hot water and NaOH solutions but not with HCl. The chromatogram of iodine fractionation also shows a peak in the HMW region at ∼50 kDa (25.2%), which could be due to the association of iodine with proteins at higher molecular weights. Significant differences in the chromatographic profiles were obtained in the case of ^{209}Bi (**Figure 4a**). Bismuth eluted mainly in two HMW fractions of 49.5 kDa (84.6%) and 33.2 kDa (15.4%) with the NaOH extraction. Fractions at 33.2 kDa (100%) and 13.8 kDa (100%) were observed for hot water and HCl extractions, respectively.

Due to the essential nature of molybdenum, an initial speciation study of molybdenum in mushroom was performed. Similar chromatographic profiles with each of the extraction solutions can be observed in **Figure 4b**. A single peak corresponding to a LMW fraction of 3−4.15 kDa was indicated. The amount of ^{95}Mo associated with that peak was 100% of the total Mo eluted from the SEC column. The ^{95}Mo peak obtained for the HCl solution was smaller compared with those obtained for NaOH solution and hot water, suggesting a low extraction efficiency with HCl. ^{95}Mo in fungi porcini is likely present as relatively small organic species. The appearance of peaks at a retention time of ∼20 min in the chromatograms obtained with UV detection confirms the association of this element to organic fractions of LMW.

Chromatographic profiles for the fractionation of ^{63}Cu and ^{68}Zn in fungi porcini are given in **Figure 5**. Both elements show association to a HMW fraction, 49.1 kDa (91.8%) for Cu and 51.9 kDa (38.8%) for Zn, with the NaOH extraction. In the case of zinc, two additional fractions were also observed with relative MWs of 30.5 kDa (54.8%) and 3.3 kDa (6.4%). A 3.6 kDa fraction was observed for Cu in the alkaline extraction. On the other hand, HCl and hot water extractions did not remove the molecular weight fraction of ∼50 kDa. The highest

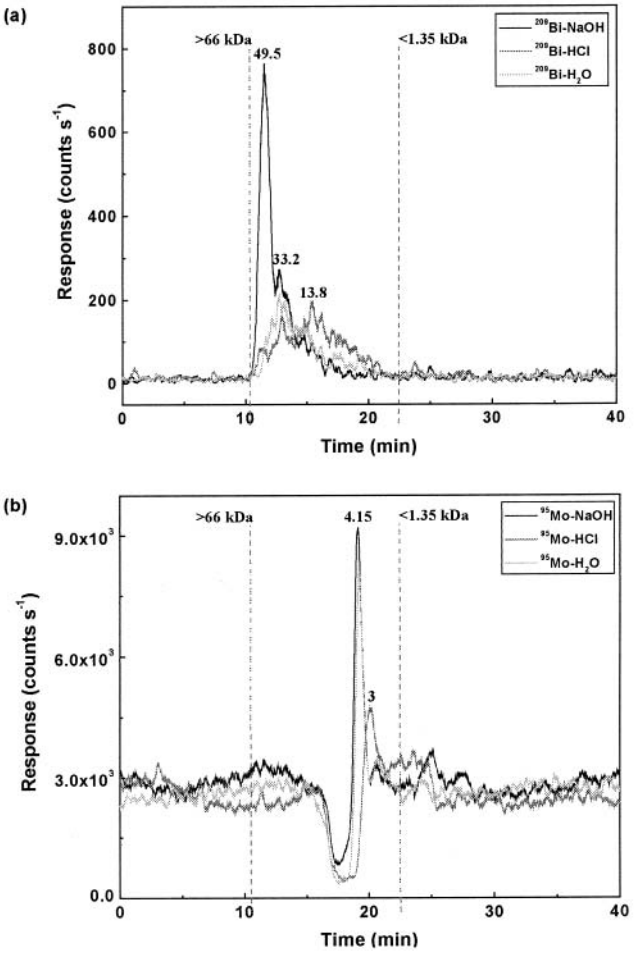

Figure 4. Fractionation profiles obtained for (**a**) ^{209}Bi and (**b**) ^{95}Mo after extraction of fungi porcini mushroom samples by NaOH, HCl, and hot water media. Analytical conditions were as described in **Table 1**.

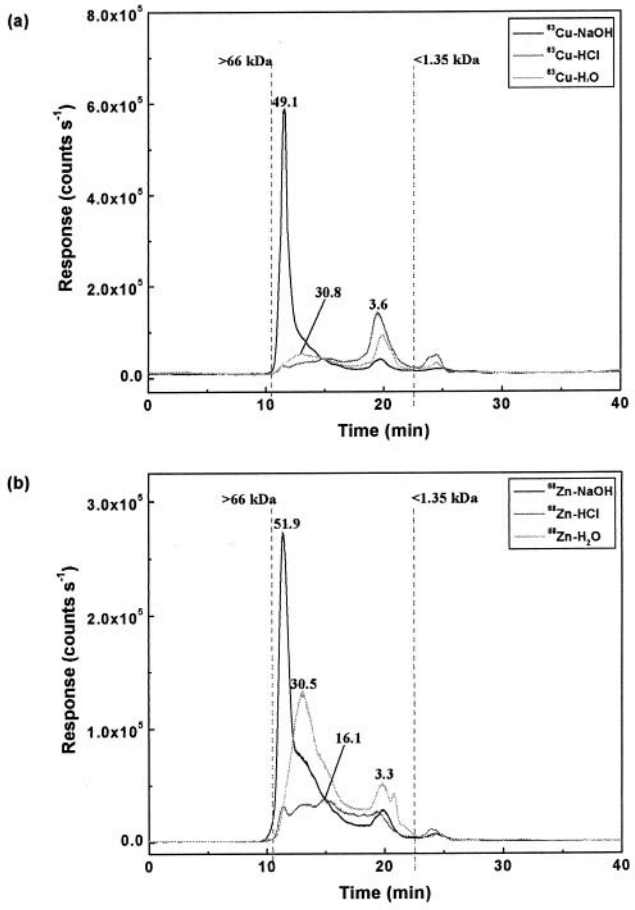

Figure 5. Fractionation profiles obtained for (**a**) ^{63}Cu and (**b**) ^{68}Zn after extraction of fungi porcini mushroom samples by NaOH, HCl, and hot water media. Analytical conditions were as described in **Table 1**.

extractability for the 30.5 kDa (84.9%) fraction for Zn was obtained with hot water. Extraction of the HMW fraction around 50 kDa with alkaline medium suggests that Cu and Zn may be present in proteins. In fact, an earlier study reported by Munger et al. (*50*) indicates bonding of Cu with HMW proteins. The isolation and chemical characterization of a copper metallothionein from *Agaricus bisporus* was presented together with the complete amino acid sequence of the protein. The copper protein had a MW of ~3 kDa, which is approximately the same as the MW found in the present study corresponding to the late eluting fraction at 3.6 kDa. Different mushroom species may not have the same metal species due to differences in the metabolic processes, and the 3.6 kDa fraction could contain additional or different species as well. The prominent HMW fraction around 50 kDa remains uncharacterized, so future studies are necessary.

Finally, the speciation by SEC for three essential transition elements was performed. The fractionation profiles of ^{54}Fe, ^{59}Co, and ^{58}Ni are shown in panels **a**, **b**, and **c**, respectively, of **Figure 6**. Different distribution patterns for these elements in fungi porcini were found. As can be observed in **Figure 6a**, Fe eluted in a fraction with a relative MW of 51.9 kDa (100%) for the NaOH extraction. The chromatogram of Fe for the hot water extraction shows the elution of a single fraction of 32.7 kDa (100%). On the other hand, Fe was distributed into two fractions of 13.2 kDa (54.4%) and 5.7 kDa (45.6%) for the acidic extraction. Distribution patterns of Co and Ni were found to be more complicated than that of Fe. In **Figure 6b** four different MW fractions can be observed for Co. Three fractions corresponding to MWs of 48.5 kDa (12.8%), 27.4 kDa (60.4%), and 3.6 kDa (21%) for the alkaline extraction were found. An additional fraction (<1.35 kDa) was observed at a retention time of 24.6 min (5.7%). The chromatogram of ^{59}Co for the acid extraction showed the presence of two LMW fractions of 3.6 kDa (80.9%) and another (<1.35 kDa) at 24 min (19.1%), whereas in the case of hot water extraction three fractions of 27.4 kDa (72.3%), 3.6 kDa (24.8%), and <1.35 kDa (24.6 min, 2.9%) were found. The HMW fraction around 50 kDa could not be extracted with either hot water or acid. The fractionation profile in **Figure 6c** shows that ^{58}Ni was clearly associated with two predominant fractions of 51.2 kDa (19.5%) and 3.9 kDa (15.1%) for the NaOH extraction. However, there is also an incompletely resolved fraction at 30.5 kDa (65.3%). In the HCl extraction two MW fractions of 16.1 kDa (28.9%) and 3.9 kDa (71.1%) were obtained. It is interesting to note that the fraction at 3.9 kDa could be extracted by all of the media and with almost the same amount of Ni. It is possible that this LMW fraction could correspond to a polar organic compound which is soluble in all media. The occurrence of a peak at about the same retention time in the chromatogram obtained with UV detection further suggests the association of Ni with LMW organic compounds.

CONCLUSION

Multielemental fractionation in fungi porcini mushroom has been studied with the application of SEC-ICP-MS for speciation

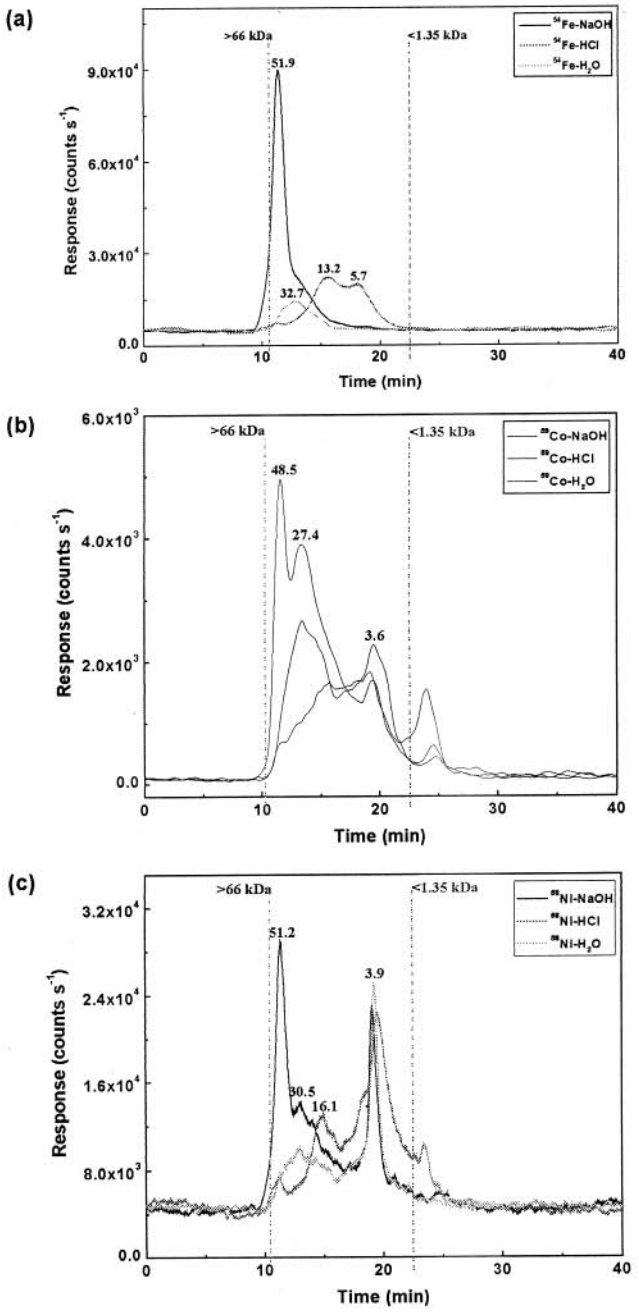

Figure 6. Fractionation profiles obtained for (**a**) ^{54}Fe, (**b**) ^{59}Co, and (**c**) ^{58}Ni after extraction of fungi porcini mushroom samples by NaOH, HCl, and hot water media. Analytical conditions were as described in **Table 1**.

studies. The extraction conditions had a strong influence on the MW distribution patterns obtained for most of the elements, which highlights the importance of selecting a proper extraction technique when elemental speciation studies are performed. Substantial differences were observed in the fractionation profiles depending on the elements. Most of the elements (Bi, Cu, Zn, Fe, Co, and Ni) were found to be associated with a HMW fraction around 50 kDa, which was most effectively extracted with the NaOH solution. On the other hand, for elements such as Se, Mo, and I, distribution patterns were primarily in the LMW fractions. SEC does not permit the separation of all species present in a sample as individual chemical species. Therefore, the application of multidimensional separation techniques and the use of identification tools such as mass spectrometry are required. Future studies will be conducted along these lines to elucidate the individual elemental species present in mushrooms.

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