JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Se-Enriched Mycelia of *Pleurotus ostreatus*: Distribution of Selenium in Cell Walls and Cell Membranes/Cytosol

ALMA HORTENSIA SERAFIN MUÑOZ,[†] KEVIN KUBACHKA,[§] KAZIMIERZ WROBEL,[†] J. FELIX GUTIERREZ CORONA,[#] SANTHA K. V. YATHAVAKILLA,[§] JOSEPH A. CARUSO,[§] AND KATARZYNA WROBEL*,[†]

Instituto de Investigaciones Científicas and Instituto de Investigación en Biología Experimental, Facultad de Química, Universidad de Guanajuato, L de Retana No. 5, 36000 Guanajuato, Mexico, and Department of Chemistry, University of Cincinnati, Cincinnati, Ohio 45221-0172

The incorporation of Se to fungi has been studied, focusing on element distribution among different cellular compartments and, in particular, polysaccharide structures contained in cell walls. Se-enriched mycelia of *Pleurotus ostreatus* were obtained in submerged cultures. The incorporation of selenium from the growth medium to mycelia was observed with the relative distribution between cytosol plus cell membranes fraction (CCM) and cell walls fraction (CW) of about 44 and 56%, respectively. CCM fractions were analyzed by size exclusion chromatography with on-line UV (280 nm) and ICP-MS detection (^{80}Se). The results obtained showed selenium binding to components of different molecular masses (about 24% of total selenium coeluted with the compounds of molecular mass > 10 kDa). A polysaccharide-containing fraction of mycelia was treated alternatively with Tris-HCl at pH 7.5 or with chitinase. Better solubility and increased contribution of low molecular mass compounds were observed in chitinase extracts (UV detection), confirming the degradation of polysacharides by the enzyme. The total area under the ICP-MS chromatogram of chitinase extract was 2 times higher with respect to the area for Tris-HCl extract. Furthermore, the relative contribution of selenium in the low molecular mass fraction (molecular mass < 1 kDa) in chitinase extract was 72% as compared to 45% in Tris-HCl extract (based on peak area measurements with respect to total area under the chromatogram). The results obtained suggest selenium binding to chitin-containing polysaccharide structures in fungi cell walls.

KEYWORDS: **Seleniun; speciation; mycelium; *Pleurotus ostreatus*; ETA-AAS; LC-ICP-MS**

INTRODUCTION

Selenium is an element of fundamental importance to human health, even though it exhibits beneficial effects within a narrow tolerance band (*1*). The recommended selenium reference nutrient intakes (RNI) are 75 and 60 μg day^{-1} for adult males and females, respectively (*2*). Many geographical regions are characterized by low soil and water selenium levels, so in local populations the Se supply from food and drinking water is far below the recommended values (as low as 10−20 μg day^{-1} in Keshan, China, the United Kingdom, Finland, etc.) (*3*, *4*). Low selenium status has been related to the etiology of serious health disorders including immunity impairment, viral infections, male fertility failure, thyroid dysfunction, cardiovascular disease, and cancer (*1*, *5*). To deal with this problem, in several health conditions selenium supplementation has been recommended (*5*, *6*). Because the bioavailability, retention, and fate of the element in the human body are species-dependent, the chemical form of selenium in such supplements is important (*7*). Actually, there is consensus that selenium should be handled as relatively nonreactive organic forms that also have higher bioavailability with respect to the inorganic species (*8*−*11*). Consequently, the food products preferred are those naturally rich in selenium or vegetables and yeasts enriched during their growth. Actually, the majority of commercial supplements are based on Se-enriched yeast (*5*, *12*). The characterization of selenium species in these materials is mandatory, because it may help to link the observed beneficial (or adverse) effects of the element to its specific chemical form (*13*−*18*).

The accumulation of selenium in different mushroom types is well documented (*19*−*23*). Recently, elemental speciation analyses have been carried out, both at natural element levels (*24*−*26*) and in enriched in vivo cultures (*19*, *20*). The suitability of the hyphenated technique that couples on-line liquid chromatography with inductively coupled plasma mass spectrometry (ICP-MS) has been demonstrated (*20*, *25*−*27*). The results of various investigations show that selenium is associated with both high and low molecular mass compounds. Furthermore, sele-

* Corresponding author (e-mail katarzyn@quijote.ugto.mx).
[†] Instituto de Investigaciones Científicas, Universidad de Guanajuato.
[§] University of Cincinnati.
[#] Instituto de Investigación en Biología Experimental, Universidad de Gunajuato.

10.1021/jf052973u CCC: $33.50 © 2006 American Chemical Society
Published on Web 04/04/2006

nomethionine was the primary species found in proteinase digests (*20*, *24*). On the other hand, dietary fiber and, in particular, chitin contained in cell walls have been linked to the potential immune-stimulating properties of edible mushrooms (*28*). Relevant to this work, selenium incorporation into fungal polysaccharides has recently been reported in Se-enriched *Ganoderma lucidum* (*29*). Chitin is a nitrogen polysaccharide composed on *N*-acetylglucosamine, and its content in fruiting bodies of *Pleurotus ostreatus* was reported as 0.32 g per 100 g of biomass (as glucosamine) (*30*). The results of analytical speciation of selenium in urine enabled the identification of Se-methyl-*N*-acetylgalactosamine and Se-methyl-*N*-glucosamine (*31*−*33*), which confirms the feasibility of selenium binding to aminosugars in the living organisms.

To gain further insight into the process of selenium incorporation (as selenite) to edible mushrooms, this study has been focused on possible element binding to polysaccharide structures contained in cell walls of selenium-enriched mycelia of *P. ostreatus*. It should be mentioned that selenium incorporation to mycelia of other biological species (moulds) has already been reported (*34*). In the first approach, we evaluated the relative contribution of selenium in cell walls, by means of cellular fractionation and electrothermal atomic absorption spectrometry (ETA-AAS). Speciation analysis was then carried out by size exclusion chromatography (SEC) with UV and ICP-MS detection to provide experimental evidence of Se binding to chitin-containing structures. The original idea was to compare SEC-ICP-MS elution profiles obtained by analyzing the Tris-HCl extract (without degradation of chitin) and enzymatic extract (chitinase). The use of a double detection system (UV and ICP-MS) enabled us to observe the degradation of polysaccharide structures and the accompanying changes in the elution profile of Se.

EXPERIMENTAL PROCEDURES

Instrumentation. Total selenium concentrations were determined using a model 3110 Perkin-Elmer atomic absorption spectrometer with HGA600 graphite furnace and AS 60 autosampler. The hollow cathode lamp for Se was a Perkin-Elmer Lumina lamp. The instrumental parameters were as follows: spectral line, 196.0 nm; slit width, 0.7 nm; background correction with deuterium lamp; platform atomizatioh; and peak area measurements. The heating program applied was as follows: (1) drying (130 °C, 25 s ramp, 15 s hold), (2) pyrolysis (1100 °C, 10 s ramp, 10 s hold), (3) cooling step (20 °C, 1 s ramp, 15 s hold), (4) atomization (2100 °C, maximum power heating, 4 s hold) and cleaning (2700 °C, 1 s ramp, 3 s hold). Matrix modifier was used (2 μg of Pd + 5 μg of Mg, 5 μL), and the volume of sample was 20 μL.

A model J2-21 Beckman centrifuge and an XL-90 Beckman ultracentrifuge were used for cellular fractionation. A model Nikon Labophot optical microscope (400×) was used for sample observation during the homogeneization and cellular fractionation.

A Multi-Block heater from Barnstead/Lab-line was used for the acid digestion of samples.

Chromatographic separations were accomplished with an Agilent Technology series 1100 liquid chromatograph equipped with an autosampler, a diode array detector, and a Chemstation for taking data. 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. ICP-MS instrumental conditions were as follows: forward power, 1300 W; nebulizer gas flow, 1.01 L min^{-1}; dwell time, 100 ms. The octopole reaction system with a hydrogen flow of 4 mL min^{-1} (collision cell) was used in this study, allowing the monitoring of Se at m/z 80.

Reagents and Samples. All chemicals were of analytical reagent grade, and deionized water was used throughout (Labconco). For atomic absorption spectrometry, a stock standard solution containing 1000 mg L^{-1} of selenium was purchased from Sigma. Working solution corresponding to the highest calibration standard (100 μg L^{-1} Se) was prepared daily by appropriate dilution of the stock solution with 0.2 mol L^{-1} nitric acid. The solutions of matrix modifiers, $Pd(NO_3)_2$ and $Mg(NO_3)_2$, 10000 mg L^{-1}, were from Perkin-Elmer.

Calibration of the SEC column was performed using standard mixtures of 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: sodium selenite, ultrapure nitric acid, hydrochloric acid, tris(hydroxymethyl)-aminomethane (Tris), hydrogen peroxide, sodium hydroxide, sodium dodecyl sulfate (SDS), chitinase from *Streptomyces griseus* and protease inhibitors [phenylmethanesulfonyl fluoride (PMSF), leupeptin, antipaine]. Acetone was from J. T, Baker.

Solid growth medium was agar−potato−dextrose (APD) from Bioxon. Liquid growth medium wheat grain−potato−dextrose−yeast extract (WPDY) was obtained as follows: 400 g of wheat grain and 300 g of potato slices were suspended in 600 mL of deionized water and boiled (100 °C, 1 h), and the mixture was filtered through Whatman no. 1 paper. The filtrate was recovered and supplemented with 20 g of dextrose, 2 g of yeast extract, 0.5 g of $MgSO_4$, 0.5 g of KH_2PO_4, and 1.0 g of NaCl (Sigma reagents were used). Finally, the volume was brought to 1000 mL with deionized water (pH 5.0 ± 0.5) (*35*). Sterilization was carried out in an autoclave (121 °C, 15 min), and medium was amended with 2.5 or 5.0 mg L^{-1} of Se(IV). The solutions of Se(IV) were sterilized by filtration (0.22 μm).

In the preliminary experiments, two other liquid media were also used: (1) potato−dextrose (PD) and (2) potato−dextrose−yeast extract (PDY). In each case, 300 g of potato slices was boiled in 600 mL of deionized water, as for WPDY preparation. After filtration, 20 g of dextrose (PD), or 20 g of dextrose and 2 g of yeast extract (PDY) were added together with the inorganic salts as detailed for WPDY and the volume was brought to 1 L. Finally, the media were sterilized and amended with selenite, as described for WPDY.

The wild-type strain 005071 of *P. ostreatus* employed in this study was generously provided by Dr. Gerardo Martinez Soto from Instituto de Ciencias Agrícols, Universidad de Guanajuato.

Procedures. *Mycelium Growth.* Mycelia were first grown in solid medium: after inoculation, the cultures were incubated in Petri dishes at 28 °C during 3 weeks. A small piece (1 cm^2) of mycelium from solid medium was then transferred to the liquid medium containing selenite (several 50 mL flasks) and incubated with shaking at 28 °C for 8 days. Control mycelium was grown in medium without selenite.

Cellular Fractionation and Determination of Se by ETA-AAS and SEC. The mycelium obtained was filtered through Whatman no. 1 paper, washed with deionized water, freeze-dried, and placed in liquid nitrogen. The homogenization of cells was performed by mortar grinding, yielding a fine powder. The efficiency of cell disruption was checked by microscopic observation. About 10 g of whole-cell homogenate was suspended in 50 mL of buffer solution containing 50 mmol L^{-1} Tris-HCl, pH 7.0, 1% SDS, and the protease inhibitors (PMSF, leupeptin, and antipain, 1 μg mL^{-1} each) and centrifuged at 7300*g* (4 °C, 60 min). The supernatant corresponded to the mixture of cytosol and cell membranes (CCM fraction), and the pellet consisted of cell walls (CW fraction). The separation of CW and CCM fractions was also verified by microscopic observation. Thus, the viscous and turbid sediment (CW) contained mostly cell walls and was devoid of unbroken cells (efficient homogenization). The less viscous and milky supernatant was devoid of cell walls and unbroken cells. The CCM and CW fractions were freeze-dried. The three subsamples of each material, including freeze-dried mycelia (0.1−0.5 g), were placed in a glass tube, and 1 mL of concentrated nitric acid was added. The tubes were heated in the stainless steel heating 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, and Se was determined by ETA-AAS. Quantification was accomplished by external calibration. Additionally, the aliquot of freeze-dried CCM fraction (50 mg) was dissolved in 1 mL of the mobile phase (containing 1% SDS and 1 μg mL^{-1} PMSF). After centrifugation

Table 1. Comparison of the Mycelium Biomasses Obtained Using Three Different Liquid Growth Media, in the Absence and at Two Different Concentrations of Se(IV)[a]

Se in growth medium (mg L^{-1})	mycelium biomass after 8 days (g L^{-1}, wet wt)		
	PD	PDY	WPDY
0	9.7	44.7	97.6
2.5	5.8	60.1	122
5.0	3.4	19.0	87.8

[a] Liquid media: PD, potato–dextrose; PDY, potato–dextrose–yeast extract; WPDY, grain–potato–dextrose–yeast extract (*35*).

Table 2. ETA-AAS Results for Selenium Determination in Freeze-Dried Mycelia and in Subcellular Fractions[a]

Se in growth medium (mg L^{-1})	Se in freeze-dried biomass (μg/g)	Se in subcellular fractions ± SD (μg/g, $n = 3$)	
		CCM	CW
0	nd[b]	nd	nd
2.5	5.4 ± 0.4	1.9 ± 0.3	2.4 ± 0.4
5.0	6.6 ± 0.4	2.3 ± 0.2	2.9 ± 0.3

[a] Values presented are micrograms of element found in each fraction per gram of freeze-dried biomass (after homogenization in liquid nitrogen), obtained in the analysis of three subsamples. [b] Not detected.

(1820*g*, 4 °C, 10 min), the supernatant (100 μL) was analyzed by SEC with UV (280 nm) and ICP-MS (^{80}Se) detection. The separation was accomplished with Tris-HCl mobile phase (30 mmol L^{-1}, pH 7.0), at the flow rate of 0.7 mL min^{-1}.

Enzymatic Extraction and SEC-ICP-MS Analysis. The aliquot (100 mg) of freeze-dried and homogenized mycelium was suspended in 5 mL of 1% SDS in Tris-HCl (50 mmol L^{-1}, pH 7.5), and 1 mL of proteinase K (20 mg mL^{-1}) was added. After overnight incubation (37 °C), 24 mL of acetone was added (final concentration of 80%), and the samples were stirred (vortex, 2 min) and centrifuged (1820*g*, 4 °C, 10 min). The pellet was divided into two portions: the first one was suspended in 1 mL of Tris-HCl at pH 6.0 and kept in a boiling water bath for 3 min, and the mixture was centrifuged (1820*g*, 4 °C, 10 min). The second portion of pellet was treated with 250 μL of chitinase solution (10 mg mL^{-1}) mixed with 750 μL of Tris-HCl at pH 6.0. After incubation (25 °C, 2 h), the mixture was centrifuged (1820*g*, 4 °C, 10 min). The two supernatants were analyzed by SEC-ICP-MS. The injection volume was 100 μL, and the separation was accomplished with Tris-HCl mobile phase (30 mmol L^{-1}, pH 7.0) at the flow rate of 0.7 mL min^{-1}. Two on-line detection systems were used, namely, diode array (280 nm) and ICP-MS (^{80}Se, ^{78}Se).

RESULTS AND DISCUSSION

In contrast to the previous studies, we investigated selenium distribution and speciation in mycelium and not in the fruiting bodies (*19*, *20*, *24*−*27*). Mycelia were grown in submerged cultures, which facilitated accessibility of selenium from medium with lower risk of culture contamination. Furthermore, the morphology of mycelium is uniform, so the analytical results were representative for total biomass obtained.

To ensure efficient growth of mycelium in the presence of sodium selenite [Se(IV)], three different liquid growth media without selenium and at two concentrations of Se(IV) (2.5 and 5.0 mg L^{-1}) were examined [35]. In **Table 1**, the data on biomass produced in each case during 8 days are given. It can be observed that the mycelium growth was affected by the composition of medium and that the largest amount of biomass was obtained in WPDY medium. On the other hand, a lower concentration of Se(IV) (2.5 mg L^{-1}) stimulated noticeably the growth of mycelium as compared to medium without selenium. However, at 5 mg L^{-1} of Se the growth was clearly inhibited. The determination of selenium in mycelia obtained in the absence and in the presence of Se(IV) in medium demonstrated the incorporation of element to fungal cells (**Table 2**). Apparently, there was no significant increase of selenium level in mycelia grown in 5 mg L^{-1} Se(IV) as compared to 2.5 mg L^{-1}. For further experiments, WPDY liquid medium was amended with 2.5 mg L^{-1} of selenium.

The mycelia grown in WPDY medium in the absence and in the presence of selenium were freeze-dried, homogenized, and fractionated by low-speed centrifugation as described under Experimental Procedures. The obtained fractions were freeze-dried and acid-digested, and the determination of selenium was carried out by ETA-AAS. The recovery of selenium in this fractionation scheme (evaluated as the ratio between the sum of element concentration in CCM and CW fractions and total element concentration found in mycelium) was ∼80%, in agreement with earlier results (*23*, *26*). The results obtained are presented in **Table 2**. As can be observed, selenium added to the growth medium was incorporated to the biomass with relative distribution between CCM and CW fractions of about 44 and 56%, respectively. Apparently, this distribution was not affected by the concentration of sodium selenite in medium. Worth mentioning is that the relative contribution of other elements in cytosol obtained from fruiting bodies of *P. ostreatus* was higher (73.6%, mean value for Al, Bi, Cd, Cr, Cu, Fe, Mn, Ni, and Pb at their natural levels) (*36*). This could be ascribed to biochemical differences between mycelium and fruiting bodies, with regard to qualitative and/or quantitative differences in metal-binding molecules. In particular, and of relevance to this study, significant differences in chemical composition and structure of wall polysaccharides were found in *Agaricus bisporus* at different morphological stages of its growth (mycelium versus fruiting bodies) (*37*). Furthermore, in *Ustilago maydis* (a common parasite of maize) the contribution of elements in cytosol was generally lower than in *P. ostreatus* or *A. bisporus* (mean of 50.7% for Cr, Cu, Fe, Mn, Ni, and Pb) (*26*), which also indicates that metal-binding molecules and/or metal uptake capabilities might be different among basidiomycetes.

SEC is a suitable separation technique to attain the molecular mass distribution of compounds, so the CCM fractions obtained in cellular fractionation were analyzed by SEC-UV-ICP-MS. 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 mass versus retention time (R^2 = 0.9864). Typical chromatograms of CCM obtained from control mycelium and from mycelium grown in the presence of 2.5 mg L^{-1} Se(IV) are shown in **Figure 1**. The results obtained indicate the incorporation of selenium to the compounds of different molecular masses. On the basis of peak area measurements, in CCM from selenized mycelium, ∼24% of the element coeluted with the compounds of molecular mass $>$ 10 kDa (proteins). The binding of the element to low molecular mass CCM components can also be observed (∼60% of selenium eluted in the elution region corresponding to molecular mass $<$ 1 kDa).

Speciation studies carried out in proteolytic digests indicated the incorporation of selenium (in the form of selenomethionine) to fungal proteins (*20*). On the other hand, mushrooms contain high polysaccharide levels, mainly in the form of chitin in cell walls (*30*, *38*). Because selenoaminosugars have already been

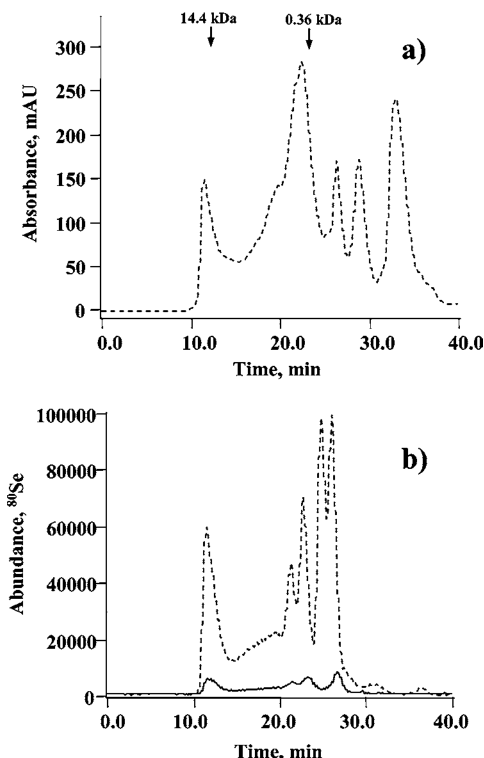

Figure 1. Typical SEC chromatograms of cytosol and cell membrane fractions (CCM) obtained from mycelium grown in the absence (solid line) and in the presence of 2.5 mg L^{-1} selenite in medium (dashed line): (**a**) UV detection (280 nm); (**b**) ICP-MS detection (^{80}Se).

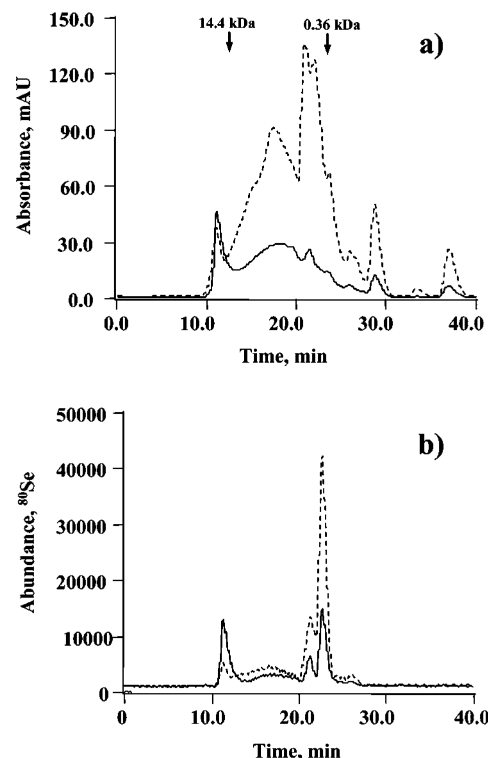

Figure 2. Typical chromatograms of the polysaccharide-containing fractions of mycelium grown in the presence of 2.5 mg L^{-1} selenite: (—) after proteinase K treatment and extraction with Tris-HCl; (- - -) after proteinase K and chitinase treatment; (**a**) UV detection (280 nm); (**b**) ICP-MS detection (^{80}Se).

identified in living organisms (*33*), we focused on possible element binding to polysaccharide structures. To eliminate proteins, the homogenized and freeze-dried mycelium was extracted with 1% SDS in Tris-HCl buffer and proteins were hydrolyzed with proteinase K. Acetone was used for precipitation of polysaccharides and, after centrifugation, the supernatant was eliminated. The new pellet, containing polysaccharide structures, was collected: one portion was extracted with Tris-HCl buffer at pH 7.5, and another portion was treated with chitinase prior to SEC-UV-ICP-MS analysis. The SEC-UV-ICP-MS chromatograms obtained (**Figure 2**) showed important differences in the elution profile, depending on the extraction procedure applied. The area under the UV chromatogram of chitinase extract was significantly larger (~3 times larger than that of the Tris-HCl extract), and it showed higher contribution of compounds eluting later (in the elution region of low molecular mass compounds) with respect to the chromatogram of Tris-HCl extract. Better solubility and increased contribution of low molecular mass compounds confirm the degradation of polysacharides by chitinase. Similar differences can be observed on ICP-MS selenium chromatograms (**Figure 2b**). The total area under the chromatogram of chitinase extract was ~2 times larger with respect to the area for the Tris-HCl extract. Furthermore, the relative contribution of selenium in the low molecular mass fraction (<1 kDa) in chitinase extract was 72% as compared to 45% in the Tris-HCl extract (based on peak area measurements with respect to total area under the chromatogram). These results suggest selenium binding to polysaccharides and specifically to chitin in the cell walls of *P. ostreatus*.

The results obtained in this work confirmed selenium incorporation into fungal proteins (*20*, *29*), which could be interesting in the view of possible enhanced nutritional value of Se-enriched edible mushrooms. On the other hand, selenium also seems to be associated with chitin-containing structures in cell walls. Because chitin is not bioavailable to humans, the evaluation of Se-enriched mushrooms as a possible dietary source of the element requires further studies. Within this context, experiments focusing on the structural characterization of selenium species in polysaccharide extracts are in progress.

ACKNOWLEDGMENT

We gratefully acknowledge Dr. Gerardo Martínez Soto for providing the *Pleurotus ostreatus* strain.

LITERATURE CITED

(1) Rayman, N. P. The importance of selenium to human health. *Lancet* **2000**, *356*, 233−241.
(2) Barelay, M. N. I.; MacPherson, A.; Dixon, J. *J. Food Compos. Anal.* **1995**, *8*, 307−314.
(3) Foster, L. H.; Sumar, S. Selenium in the environment, food and health. *Nutr. Food Sci.* **1995**, *5*, 17−23.
(4) Reilly, C. Selenium: a new entrant into the functional food arena. *Trends Food Sci. Technol.* **1998**, *9*, 114−118.
(5) Whanger, P. D. Selenium and its relationship to cancer: an update dagger. *Br. J. Nutr.* **2004**, *91*, 11−28.
(6) Schrauzer, G. N. The nutritional significance, metabolism and toxicology of selenomethionine. *Adv. Food Nutr. Res.* **2003**, *47*, 73−112.
(7) Larsen, E. H.; Hansen, M.; Paulin, H.; Moesgaard, S.; Reid, M.: Rayman, M. Speciation and bioavailability of selenium in yeast-based intervention agents used in cancer chemoprevention studies. *J. AOAC Int.* **2004**, *87*, 225−232.
(8) Arthur, J. R. Micronutrient group symposium on "micronutrient supplementation: when and why?" *Proc. Nutr. Soc.* **2003**, *62*, 393−397.
(9) Sanz Alaejos, M.; Díaz Romero, F. J.; Díaz Romero, C. Selenium and cancer: some nutritional aspects. *Nutrition* **2000**, *16*, 376−383.

(10) Kim, Y. Y.; Mahan, D. C. Comparative effects of high dietary levels of organic and inorganic selenium on selenium toxicity of growing-finishing pigs. *J. Anim. Sci.* **2001**, *79*, 942−948.
(11) Rayman, M. P. The use of high-selenium yeast to raise selenium status: how does it measure up? *Br. J. Nutr.* **2004**, *92*, 557−573.
(12) B'Hymer, C.; Caruso, J. A. Evaluation of yeast-based selenium food supplements using high-performance liquid chromatography and inductively coupled plasma mass spectrometry. *J. Anal. At. Spectrom.* **2000**, *15*, 1531−1539.
(13) Cubadda, F. Inductively coupled plasma-mass spectrometry for the determination of elements and elemental species in food: a review. *J. AOAC Int.* **2004**, *87*, 173−204.
(14) Whanger, P. D.; Ip, C.; Polan, C. E.; Uden, P. C.; Welbaum, G. Tumorgenesis, metabolism, speciation, bioavailability, and tissue deposition of selenium in selenium-enriched ramps (*Allium tricoccum*). *J. Agric. Food Chem.* **2000**, *48*, 5723−5730.
(15) Ip, C.; Birringer, M.; Block, E.; Kotrebai, M.; Tyson, J. F.; Uden, P. C.; Lisk, D. J. Chemical speciation influences comparative activity of selenium-enriched garlic and yeast in mammary cancer prevention. *J. Agric. Food Chem.* **2000**, *48*, 2062−2070.
(16) Ip, C.; Lisk, D. J. Enrichment of selenium in allium vegetables for cancer prevention. *Carcinogenesis* **1994**, *15*, 1881−1885.
(17) Kannamkumarath, S. S.; Wrobel, K.; Wrobel, K.; Vonderheide, A.; Caruso, J. A. HPLC-ICP-MS determination of selenium distribution and speciation in different types of nut. *Anal. Bioanal. Chem.* **2002**, *373*, 454−460.
(18) Wrobel, K.; Wrobel, K.; Kannamkumarath, S. S.; Caruso, J. A.; Wysocka, A.; Bulska, E.; Swiatek, J.; Wierzbicka, M. HPLC-ICP-MS speciation of selenium in enriched onion leaves—a potential dietary source of Se-methylselenocysteine. *Food Chem.* **2004**, *86*, 617−623.
(19) 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.
(20) 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**, *379*, 861−866.
(21) Slejkovec, Z.; van Elteren, J. T.; Woroniecka, U. D.; Kroon, K. J.; Falnoga, I.; Byrne, A. R. Preliminary study on the determination of selenium compounds in some selenium-accumulating mushrooms. *Biol. Trace Elem. Res.* **2000**, *75*, 139−155.
(22) Spolar, M. R.; Schaffer, E. M.; Beelman, R. B.; Milner, J. A. Selenium-enriched *Agaricus bisporus* mushrooms suppress 7,12-dimethlybenz[*a*]anthracene bioactivation in mammary tissue. *Cancer Lett.* **1999**, *138*, 145−150.
(23) 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.
(24) Díaz Huerta, V.; Fernández Sanchez, M. L.; Sanz-Medel, A. Qualitative and quantitative speciation analysis of water soluble selenium in three edible wild mushrooms species by liquid chromatography using post-column isotope dilution ICP-MS. *Anal. Chim. Acta* **2005**, *538*, 99−105.
(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) Serafin Muñoz, A. H.; Kubachka, K.; Wrobel, K.; Yathavakilla, S. K. V.; Gutierrez Corona, F.; Caruso, J. A.; Wrobel, K. A metallomics approach to trace element analysis in *Ustilago maydis* using cellular fractionation, atomic absorption spectrometry and size exclusion chromatography with ICP-MS detecion. *J. Agric. Food Chem.* **2005**, *53*, 5138−5143.
(27) 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.
(28) Bobek, P.; Galbavy, S. Hypocholesterolemic and antiatherogenic effect of oyster mushroom (*Pleurotus ostreatus*) in rabbit. *Nahrung* **1999**, *43*, 339−342.
(29) Zhao, L.; Zhao, G.; Zhao, Z.; Chen, P.; Tong, J.; Hu, X. Selenium distribution in a Se-enriched mushroom species of the genus *Ganoderma*. *J. Agric. Food Chem.* **2004**, *52*, 3954−3959.
(30) Manzi, P.; Aguzzi, A.; Pizzoferato, L. Nutritional value of mushrooms widely consumed in Italy. *Food Chem.* **2001**, *73*, 321−325.
(31) Ogra, Y.; Suzuki, K. T. Speciation of selenocompounds by capillary HPLC coupled with ICP-MS using multi-mode gel filtration columns. *J. Anal. At. Spectrom.* **2005**, *20*, 35−39.
(32) Suzuki, K. T.; Kurasaki, K.; Okazaki, N.; Ogra, Y. Selenosugar and trimethylselenonium among urinary Se metabolites: dose- and age-related changes. *Toxicol. Appl. Pharmacol.* **2005**, *206*, 1−8.
(33) Gammelgaard, B.; Bendahl, L. Selenium speciation in human urine samples by LC- and CE-ICP-MS-separation and identification of selenosugars. *J. Anal. At. Spectrom.* **2004**, *19*, 135−142.
(34) Prange, A.; Birzele, B.; Hormes, J.; Modrow, H. Investigation of different human pathogenic and food contaminating bacteria and moulds grown on selenite/selenate and tellurite/tellurate by X-ray absorption spectroscopy. *Food Control* **2005**, *16*, 723−728.
(35) Stamets, P.; Chilton, J. S. *The Mushroom Cultivator*; Agarikon Press: Olympia, WA, 1983.
(36) 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**, *106*, 265−278.
(37) Calonje, M.; Garcia Mendoza, C.; Novaes-Ledieu, M. New contributions to the wall polysaccharide structure of vegetative mycelium and fruit body cell walls of *Agaricus bisporus*. *Microbiologia* **1996**, *12*, 599−606.
(38) Bauer Petrovska, B.; Kulevanova, S.; Stefov, V. Infrared analysis of Macedonian mushroom dietary fibre. *Nahrung/Food* **2002**, *46*, 238−239.

Received for review November 28, 2005. Revised manuscript received March 8, 2006. Accepted March 11, 2006. The U.S. National Institute of Environmental Health Sciences provided partial support through Grant ES04908. We thank CEM and Agilent Technologies for continuing support of our studies. A.H.S.M. gratefully acknowledges the fellowship from Consejo Nacional de Ciencia y Tecnología (CONACYT), Mexico.

JF052973U