Selenium Bioaccumulation in Shiitake Mushrooms: A Nutritional Alternative Source of this Element

Regiane Gonçalves Feitosa Leal Nunes, Jose Maria R. da Luz, Rodrigo de B. Freitas, Angela Higuchi, Maria Catarina M. Kasuya, and Maria Cristina D. Vanetti

Abstract: Mushrooms have effective mechanisms to absorb and accumulate trace elements from substrates and, therefore, could be used as a strategy to produce mineral-enriched food and nutritional supplements. This study aimed to enrich shiitake mushrooms with selenium (Se), an important dietary element in human health. Strains of *Lentinula edodes* (Berk.) were grown on artificial logs composed of eucalyptus sawdust, and were subjected to cold shock in water containing sodium selenite (Na$_2$SeO$_3$) at concentrations of up to 1.28 mM. The content of Se in the mushrooms increased linearly with increasing amounts of Na$_2$SeO$_3$ added to the cold water although above 0.96 mM, mushroom formation was inhibited. Concentrations greater than 17 mg Se 100/g of dried mushrooms were observed after treatment with 0.64 mM Na$_2$SeO$_3$. Shiitake mushroom had a demonstrate potential to offer an effective and economical way to produce Se-enriched products and, the strategy of adding selenite in cold water, used in this study, showed promising once it does not interfere with mycelial growth.

Keywords: food microbiology, fortification, minerals, mushroom, nutrients, selenium

Practical Application: Selenium is an essential trace element for both human and animals and is required for the 21st amino acid, selenocysteine, which is used for the synthesis of about a dozen selenoenzymes. In this study, it is demonstrated that shiitake mushroom is a good Se accumulator and only one step during fructification was necessary to obtained enriched mushroom. Se enriched shiitake mushroom can be considered to be an excellent source of this element and used to consumption in different ways.

Introduction

Selenium (Se) is classified as a metalloid and, is an essential trace element for animals, including humans. The importance of dietary Se in human health has received considerable attention in the last several years. Dietary Se has been recognized as an antioxidant, and the deficiency of this element has been associated with numerous chronic degenerative diseases, including multiple types of cancer (Clark and others 1996; Spolar and others 1998; Abdullah and others 2005; Pedrero and Madrid 2009), cardiomyopathy and endemic osteoarthropathy (Chen and others 1999). In addition, deficiencies have also caused problems with the maintenance of fertility (Rayman 2000). This element has many physiological functions, but is most often recognized for its role as a cofactor for the enzyme glutathione peroxidase, which is responsible for the removal of free radicals that reduce oxidative damage in cells (Werner and Beelman 2001). In several regions of the world, the content of Se in the general diet has been estimated to be insufficient to maintain the proper level of activity of protective selenoenzymes (Pedrero and Madrid 2009). These findings have driven the growth in interest regarding the production of Se-enriched food and nutritional supplements.

The specific type of Se that is ingested is an important factor for the determination of toxicity, nutritional importance and metabolic factors (Shiobara and others 1998). Se bioavailability varies according to the source of Se and nutritional status of the subject, and is significantly higher for organic forms of Se (Navarro-Alarcon and Cabrera-Vique 2008). Selenomethionine (SeMet) is the major selenocompound in cereal grains and enriched yeast whereas Se-methylselenocysteine (SeMCys) is the major selenocompound in Se-accumulator plants and some plants of economic importance such as garlic and broccoli exposed to excess Se (Whanger 2004).

High Se levels, between 10 and 20 mg/kg dry matter, have been observed in some widely consumed species of mushrooms including *Boletus edulis*, *B. pinicola*, *B. aestivus*, and *Xerocomus badius* (Kalač and Svoboda 2000). In commercial mushrooms, selenomethionine can be detected in its free form, and it can also incorporated into proteins in selenized mushrooms, together with a number of unknown selenocompounds (Huerta and others 2006; Silva and others 2010; Cremades and others 2012).

Shiitake is one of the most popular consumed mushrooms in the world, and is appreciated for its delicacy, specific aroma and texture. The enrichment of the shiitake mushroom with Se may provide an alternative source for nutritional Se enrichment. Thus, objective of this present study is to verify Se accumulation by shiitake mushroom when sodium selenite is added to the cold shock water used to induce primordial formation in artificial logs.
Selenium in shiitake mushrooms

Materials and Methods

**Lentinula edodes** strains

*L. edodes* (Berk.) Pegler strains UFV16 and UFV52, which were used in this study, belong to collection of the Department of Microbiology of Federal University of Viçosa, MG, Brazil. The stock cultures were maintained on potato dextrose agar (PDA, Merck, Darmstadt, Germany). Mycelium of each stock culture was obtained at 25 °C on the surface PDA agar in Petri dishes. After 15 d, mycelial disks were punched out with a 7 mm diameter cork borer and used to inoculate the substrates for subsequent mushroom production.

Substrates and mushroom production

Shiitake mushrooms were produced in a substrate containing 78% eucalyptus sawdust, 20% rice meal, 0.4% CaCO₃ and 1.6% CaSO₄. This mixture was moistened with tap water to a concentration of 65%. Approximately 700 g of the substrate was packed into polypropylene bags, and sealed with paper with the aid of two concentric polyvinyl chloride rings. The bags were then autoclaved for 120 min at 121 °C, and inoculated with shiitake mycelium in aseptic conditions. The incubation was at 25 °C, with 3 h of light a day for approximately 90 d. After 45 d of growth, gas exchange was permitted. This was allowed by making 25 cuts of about 5 mm across the entire length of the bags. This procedure was repeated at 60th day. Fruiting induction was performed by submitting the artificial logs to cold shock in water at 15 ± 2 °C, for 24 h. The water contained sodium selenite (Na₂SeO₃) at varying concentrations (0.08; 0.16; 0.32; 0.64; 0.96; or 1.28 mM). A control treatment with no Se added to water was also carried out. After the cold shock, the artificial logs were kept in a room with a temperature of 23 °C and 85% humidity for successful mushroom formation. The mushrooms were collected approximately 5 d after fruiting induction, and the veil and slides were fully exposed.

Substrate analysis

The moisture content of the substrate was determined after drying samples in an oven with forced air circulation at 105 °C for 48 h. The pH was determined using a pH meter (DM 20 Digimed, São Paulo, SP, Brazil) and the water activity (a_w) using an automatic analyzer (Aqualab Decagon Inc., Pullman, Wash., U.S.A.). After an initial digestion, with a mixture of nitric acid and perchloric acid (Mattila and others 2001), 0.5 g of dried mushroom samples ground with a Wiley mill, (model EDB-5, Arthur H. Thomas Co., Philadelphia, Pa., U.S.A.) (Bataglia and others 1983), the concentration of Se was measured using Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) (Optima 3300 Perkin Elmer, Norwalk, Conn., U.S.A.). The Se content was also analyzed in the water used for substrate preparation and substrate using the same procedure described above.

Mushroom analysis

The cap and stem diameters, and the length of the stem of the mushrooms were measured using a paquimeter in two representative positions. The surface color of the mushrooms was measured with a colorimeter (ColorQuest II; Sphere HunterLab, Reston, Va., U.S.A.) and was expressed as a luminance (L) value on the CIELAB scale, in relation to illuminant D65 at a 10° angle of observation.

The moisture content of the mushrooms was determined by drying the samples at 80 °C, in an oven, until a constant mass was obtained. The content of soluble protein was then determined using the Bradford method (Bradford 1976).

The content of calcium (Ca), magnesium (Mg), phosphorus (P), and Se was determined using ICP-AES, after nitric-perchloric digestion (Mattila and others 2001). The potassium (K) content was determined by flame photometry in the same extract (Bataglia and others 1983).

Mushroom productivity and biological efficiency (EB) were calculated using the following formulas (Chang and others 1981):

\[
\text{Productivity} = \frac{(\text{dry mass of the mushrooms})}{(\text{dry mass of the substrate})} \times 100.
\]

\[
\text{EB} = \frac{(\text{mushrooms fresh mass})}{(\text{substrate dry mass})} \times 100.
\]

Statistical analysis

A factorial design with two mushroom strains (UFV16 and UFV52), and five concentration levels of Se, in three replicates, was adopted. The data were submitted to analysis of variance and regression analysis, using the SAS statistical program.

Results and Discussion

After autoclaving, the moisture content of the substrate was 41%, the pH 4.6, and the water activity (a_w) 0.988. Se was not detected in the ligninocellulosic substrate or in the water used to humidify the substrate.

Mushrooms from *L. edodes* UFV16 and UFV52 produced in the artificial logs treated with concentrations of 0.32 mM and 0.64 mM of sodium selenite presented a veil formation delay compared with those treated with only water or with the lower concentrations of sodium selenite (0.08 and 0.16 mM). However, diameters of mushroom caps and stems, as well as the lengths of stems were not affected by Se. Furthermore, no mushrooms were produced after treatment in cold water when 0.96 or 1.28 mM of sodium selenite was added. Enrichment of *Pleurotus ostreatus* with Se added into coffee husks substrate delayed mycelia growth, the shape of the mushroom and Se concentrations above 12.8 mg/kg, produces mushrooms with the larger stipes and smaller caps than that produced without addition of Se (Silva and others 2012).

Cold shock with water added of sodium selenite rendered mushroom with L values lower than controls (Table 1). The lower L values indicated darkening, though no change of color was visually observed. The effect of Se on the color of shiitake mushrooms differs from results obtained with *Agaricus bisporus* fortified with sodium selenite where no significant differences were observed in the L values when compared to treatments containing no added Se (Spolar and others 1998; Werner and Beelman 2001).

Table 1—Color (Hunter L values) and moisture of non- and Se-enriched shiitake mushrooms strains UFV16 and UFV52 produced in artificial logs and submitted to cold shock in water contained sodium selenite.

<table>
<thead>
<tr>
<th>Selenium concentration in water (mM)</th>
<th>L valueab</th>
<th>Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>52.04A</td>
<td>89.2</td>
</tr>
<tr>
<td>0.08</td>
<td>40.77B</td>
<td>89.4</td>
</tr>
<tr>
<td>0.16</td>
<td>40.65B</td>
<td>90.5</td>
</tr>
<tr>
<td>0.32</td>
<td>42.81B</td>
<td>90.6</td>
</tr>
<tr>
<td>0.64</td>
<td>41.92B</td>
<td>91.0</td>
</tr>
</tbody>
</table>

*a* Hunter Lab Color System where “L value” = 1 lightness coordinate. 
*b* Values followed by different letters in the same column are significantly different (P < 0.05) by Tukey test.
The moisture content of fresh mushrooms ranged from 89% to 91% (Table 1) and did not vary among strains and treatments with Se. These values were within the range of moisture found in fresh mushrooms (Manzi and others 2004).

The content of soluble protein in strains UFV16 and UFVS2 was not affected by the presence of Se (P ≥ 0.05), and the medium values of 22.3 mg/g dry weight were similar to the value of 22.3 mg/g reported by Longyah and Deosthale (1998) in L. edodes.

No conclusion about bioaccumulation of Ca, Mg, P, and K in presence of Se could be done and this may be due to high coefficient of variance observed (Table 2). Moreover, the values of these minerals in these strains also varied within the range generally found in shiitake mushrooms (Longyah and Deosthale 1998; Mattila and others 2001; Casaril and others 2011). Silva and others (2012) showed that addition of sodium selenite in substrate does not affect the concentration of some element, as Ca, Mg, P, and K, in P. ostreatus mushroom.

The total Se concentration in the mushrooms positively correlated with increasing doses of sodium selenite, which were added to the water used for cold shock treatment. In addition, Se values above 17 mg/100 g of dry mass were observed in mushrooms treated with 0.64 mM of sodium selenite (Figure 1). Se concentrations were measured at 1.0, 3.0, and 5.0 mg/100 g of dry mass in mushrooms treated with 0.08, 0.16, and 0.32 mM of sodium selenite, respectively. Spolar and others (1998), Werner and Beelman (2001), and Cremaides and others (2012) related that the addition of sodium selenite in the water used for irrigation of A. bisporus mushrooms caused an increase in Se in these mushrooms.

The consumption of 17 mg of Se/100 g, detected in dried shiitake mushrooms treated with 0.64 mM of sodium selenite is around 3 fold greater than the Recommended Dietary Allowances (RDA) provided in the Dietary Reference Intakes developed by the Institute of Medicine of USA. This level is currently 55 μg for adults of both sexes (IOM 2000; Trumbo and Samakawa 2009). The consumption of only 0.32 g of dried selenized shiitake mushrooms or 29.56 g of fresh mushrooms, considering the average moisture of 91% in the mushrooms (Table 1), meets the daily needs for this element, indicating that the Se enriched shiitake mushroom can be considered to be an excellent source of this element. Silva and others (2012) also found, in P. ostreatus enriched with sodium selenite, concentrations of Se much greater than the RDA and they recommended the consumption of only 1.0 g dried mushrooms for supply the RDA. Cremaides and others (2012) also enriched white button mushrooms (A. bisporus) with Se and found that the consumption of around 180 g of fresh mushroom is necessary to reach the recommended daily dose. These authors suggested another alternative to use the Se-enriched mushroom and prepared a mushroom aqueous enzymatic extracts that could be incorporated into any type of solid or liquid food without modifying its organoleptic properties. Werner and Beelman (2001) argue mushrooms containing at least 20% of the U.S. RDA could be marketed as an excellent source of dietary Se, but enriching to levels above that for direct human consumption is not currently advisable. Indiscriminate use of Se supplementation could generate an increased risk of Se toxicity. According, Zhang and others (2009) questions remain to be answered more accurately in identifying the Se species, which may assist in understanding their role, if any, in cancer chemopreventive effects. Nowadays, the researches of Se-enriched microorganisms relate to many aspects such as enrich condition and the form of organic Se present.

The major productivity of shiitake mushroom enriched occurred when sodium selenite were added to the cold shock water in concentration of 0.16 and 0.32 mM (Table 3). A. bisporus

<table>
<thead>
<tr>
<th>Selenium concentration in water (mM)</th>
<th>Calcium (mg 100/g)</th>
<th>Magnesium (mg 100/g)</th>
<th>Phosphorus (mg 100/g)</th>
<th>Potassium (mg 100/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>16.98</td>
<td>163.35</td>
<td>742.18</td>
<td>914.94</td>
</tr>
<tr>
<td>0.08</td>
<td>22.86</td>
<td>182.80</td>
<td>828.34</td>
<td>1436.70</td>
</tr>
<tr>
<td>0.16</td>
<td>19.15</td>
<td>176.80</td>
<td>812.74</td>
<td>1323.90</td>
</tr>
<tr>
<td>0.32</td>
<td>18.41</td>
<td>146.74</td>
<td>686.70</td>
<td>872.70</td>
</tr>
<tr>
<td>0.64</td>
<td>34.00</td>
<td>223.16</td>
<td>971.70</td>
<td>1140.60</td>
</tr>
</tbody>
</table>

Table 2–Average of mineral composition of non- and Se-enriched shiitake mushrooms strains UFV16 and UFVS2 produced in artificial logs and submitted to cold shock in water contained sodium selenite.

Table 3–Productivity of shiitake mushrooms strains UFV16 and UFVS2 produced in artificial logs and submitted to cold shock in water contained sodium selenite.

<table>
<thead>
<tr>
<th>Selenium concentration in water (mM)</th>
<th>Productivity (%)</th>
<th>Biological Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UFV16</td>
<td>UFVS2</td>
</tr>
<tr>
<td>0</td>
<td>1.25Aa</td>
<td>0.96Aa</td>
</tr>
<tr>
<td>0.08</td>
<td>0.69Ab</td>
<td>0.80Ab</td>
</tr>
<tr>
<td>0.16</td>
<td>1.26Aa</td>
<td>1.32Aa</td>
</tr>
<tr>
<td>0.32</td>
<td>1.68Aa</td>
<td>0.99Bb</td>
</tr>
<tr>
<td>0.64</td>
<td>0.87Ab</td>
<td>0.95Aa</td>
</tr>
</tbody>
</table>

*Means with different superscript letters, uppercase (in line) and lowercase (in column) differ by Tukey test at 5% probability.
mushrooms, in contrast, have the capacity to grow in media containing approximately 1.26 M of sodium selenite and, do not show negative effects in quality and productivity at this concentration (Sporal and others 1998; Hartman and others 2000). The low productivity, around 1%, of shiitake mushrooms registered (Table 3) may be related to the fact that these experiments worked only with the first flow. It has been demonstrated that the production of shiitake can involve up to four harvests, resulting in good productivity and the first flow of harvesting of mushrooms was negligible (Queiroz and others 2004).

Similar to productivity, the UVF16 showed greater EB in doses of 0.16 and 0.32 mM, while isolate UFVF52 increased the EB in function of the doses of Se up to 0.16 mM (Table 3). Silva and others (2012) also showed significant increase of EB in doses of Se less than 12.8 mg/kg added in substrate. This different profile of productivity and EB between the strains, UVF16 and UFVF52, show the importance of the selection of fungal strains to best adaptation and also to major productivity of mushrooms enriched with Se.

Currently, there is a great interest in developing Se-enriched nutritional supplements as an alternative to increase its dietary intake. The results presented in this study showed that shiitake mushrooms can be easily enriched with Se via its addition to the water that is used for cold shock. Cold shock is applied in artificial water that is used for cold shock. Cold shock is applied in artificial water used for cold shock. SeMet, which is the most bioavailable form for human absorption (Ogra and others 2004; Yoshida and others 2005).

Conclusions

_L. edodes_ absorbs and accumulates Se in your mushrooms, and this accumulation not affect the color, moisture, and the protein content of mushroom. Furthermore, the Se concentration in mushroom enhance in function of the increase of the dose of sodium selenite that was added in water used to cold shock. Thus, the shiitake mushroom enriched with Se can be a source nutritional alternative of this element.

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