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Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Enrichment of *Pleurotus ostreatus* mushrooms with selenium in coffee husks

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ARTICLE INFO

Article history:

Received 26 April 2011

Received in revised form 3 September 2011

Accepted 8 September 2011

Available online 12 September 2011

Keywords:

Agro-industrial residues

Coffee husk

Edible mushrooms

Selenium

ABSTRACT

Pleurotus ostreatus fungus forms an edible mushroom that possesses important nutritional and medicinal properties. Selenium (Se) is essential to human diets and it is in low concentration in the soil, and consequently in food. *P. ostreatus* was grown in coffee husks enriched with various concentrations of sodium selenite. The biological efficiency of *P. ostreatus* was affected by the addition of high concentrations of Se. The highest level of Se absorption was obtained by adding 51 mg kg⁻¹ of sodium selenite. The mushrooms from first flush contained more Se than the further flushes. These results demonstrate the great potential of coffee husks in the production of Se-enriched mushrooms and show the ability of this fungus to absorb and biomagnify Se.

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1. Introduction

Mushrooms are highly appreciated for their flavour and have been well studied due to their nutritional and medicinal properties. *Pleurotus* mushrooms have high nutritional value and can be a good source of protein, carbohydrates, vitamins, calcium and iron (Schmidt, Wechsler, Nascimento, & Junior, 2003). Furthermore, these mushrooms have important medicinal properties, such as anti-tumour and immunostimulatory activity, as observed in rats (Sarangi, Ghosh, Bhutia, Mallick, & Maiti, 2006). The products derived from *Pleurotus* mycelia can promote biological responses during cancer treatment in humans and have been used as antitumorigenic drugs (Sarangi et al., 2006).

Pleurotus mushrooms have been grown in agro-industrial residues, such as banana waste (Reddy, Babu, Komaraiah, Roy, & Kothari, 2003), corn, bean and coffee (Dias, Koshikumo, Schwan, & Silva, 2003), and crop waste, such as soybean straw, cotton stalks, pigeon pea stalks and sugar cane remnants (Syed, Kadam, Mane, Patil, & Baig, 2009).

In Brazil, large amounts of agro-industrial residues are produced, including coffee husks, as Brazil is the top coffee producer in the world (~2800 million tonnes per year); coffee husks and peels comprise 50% of the grain (CONAB, 2010). This lignocellulosic residue has been used as substrate in mushroom production (Dias et al., 2003). It is important to point out that the use of these residues in the production of mushrooms prevents their direct re-

lease into the environment, increases the producer's income and leads to food product with high nutritional quality.

Mushroom yields and their chemical composition can be affected by the substrates used in their growth (Shashirekha, Rajarathnam, & Bano, 2005). For instance, yields and chemical composition are enhanced by adding essential elements, such as Se, to the substrate. Addition of sodium selenite to the substrate used for growing *Ganoderma lucidum* resulted in a proportional increase of Se content in the mushrooms (Zhao et al., 2004).

Studies have revealed that Se is incorporated into the *P. ostreatus* biomass, as this element was found to be associated with the membrane (44%) and cell wall (56%). Se incorporation into fungal proteins reveals a great potential to improve the nutritional value of the mushroom (Munoz et al., 2006). In enriched mushrooms, the Se bioavailability was verified using *in vivo* methods. The higher levels of absorption of Se in rats fed with Se enriched mushrooms were verified by Silva et al. (2010), which compared these results with the ones achieved with rats with sodium selenate in their diets.

Due to the high demand of food across the world, its enrichment with essential micronutrients, such as Se, is crucial. However, Se can also be toxic when ingested in high concentrations (Gaso et al., 2000; Hartikainen, 2005). The recommended dose for an adult, male or female, is 55 µg day⁻¹ (IOM, 2000). Selenium has several physiological functions in protein activity, enhancing immune system function, reducing cancer risk (Finley, 2006), collateral effects of chemotherapy (Sieja & Talerczyk, 2004) and functional activity of cancer metastasis (Finley, Sigrid-Keck, Robbins, & Hintze, 2005). Thus, the aim of this work was to evaluate the use

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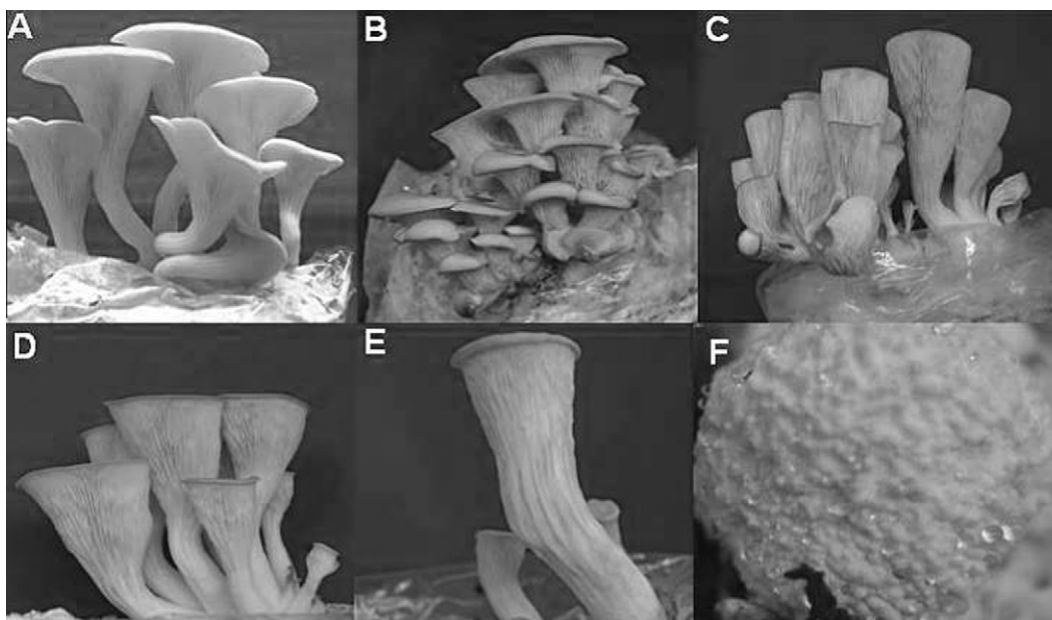


Fig. 1. Morphology of *Pleurotus ostreatus* mushrooms cultivated in coffee husk substrates in the absence (A) and presence of Se (6.4 mg kg⁻¹) (B); 12.8 mg kg⁻¹ (C); 25.4 mg kg⁻¹ (D); 51 mg kg⁻¹ (E); 102 mg kg⁻¹ (F).

Table 1

Average incubation time for *Pleurotus ostreatus* in Se-enriched coffee husk substrates, with different Se concentrations, for three consecutive flushes.

Selenium (mg kg ⁻¹)	Flushes		
	1st	2nd	3rd
<i>Days after inoculation</i>			
0	23	35	43
3.2	26	48	60
6.4	23	49	60
12.7	28	52	60
25.4	39	54	65
50.9	40	54	61
76.4	36	52	66
101.8	36	56	79

of coffee husk in the production of *Pleurotus ostreatus* mushrooms enriched with selenium.

2. Materials and methods

2.1. Enrichment of the mushrooms

The fungus used was *P. ostreatus*, and inoculation was performed in rice cooked with water for 50 min and autoclaved at 121 °C for 2 h.

The coffee husk substrate was obtained from the Incofex Coffee Corporation, in Viçosa, Minas Gerais State, Brazil. The husk was boiled in water for 2 h, in order to reduce some compounds which could inhibit fungal growth and contaminants, and centrifuged at 1800 rpm for 5 min to remove excess water. Next, 1.5 kg of each sample were placed in polypropylene bags and autoclaved for 2 h. This procedure was repeated three times at 48-h intervals. The final humidity was 80%. At the end of the procedure, once the substrate reached room temperature, the inoculation with fungus spawn was performed.

For enrichment, a 5-mL volume of sodium selenite solution at various Se concentrations (3.2; 6.4; 12.8; 25.4; 51; 76.4; 102 mg kg⁻¹) was added to packs containing coffee husks. A culture

without Se was maintained for control purposes. The inoculated packs were incubated at 25 °C for 15 days. Fungi were placed at 20 °C and 90% air humidity, until mushroom formation. Mushrooms were collected during three flushing times, over a total period of 76 days.

2.2. Biological efficiency

The biological efficiency (BE) was calculated according to Wang, Sakoda, and Suzuki (2001):

$$BE = 100 \times (\text{fresh weight of harvested mushrooms} / \text{dry weight of the substrate}).$$

2.3. Sample preparation

Acid digestion was used to prepare the samples. Mushrooms were dried at 45 °C until they reached a constant weight and then were ground in a 2-mm sieve mill. A 200-mg mass of ground mushrooms was subjected to digestion in a microwave (model Microwave 3000, Anton Paar GmbH, Graz, Austria) oven in a diluted oxidant mixture (2.0 mL HNO₃ (Merck) + 1.0 mL H₂O₂ (Merck) + 3.0 mL H₂O). The microwave heating program includes four steps (temperature/°C; ramp/min; hold/min): 1 (140, 5, 1), 2 (180, 4, 5), 3 (200, 4, 10), 4 (0, 0, 20) (Naozuka & Oliveira, 2007; Naozuka, Vieira, Nascimento, & Oliveira, 2010). The coffee husks were also submitted to acid digestion using the procedure described above.

2.4. Nutrient contents

Ca, Pb, Cu, Fe, Mg, Mn, Zn, Cd, Cr and Ni determination in the digested solutions was performed by inductively-coupled plasma optical emission spectrometry (ICP-OES) using a Perkin Elmer Optima 3.300 DV™ spectrometer (Norwalk, CT). Solutions of each element were prepared from analytical reagent-grade chemicals (Merck), using high-purity water obtained from a Milli-Q water purification system (Millipore, Bedford, MA) (Naozuka et al., 2010).

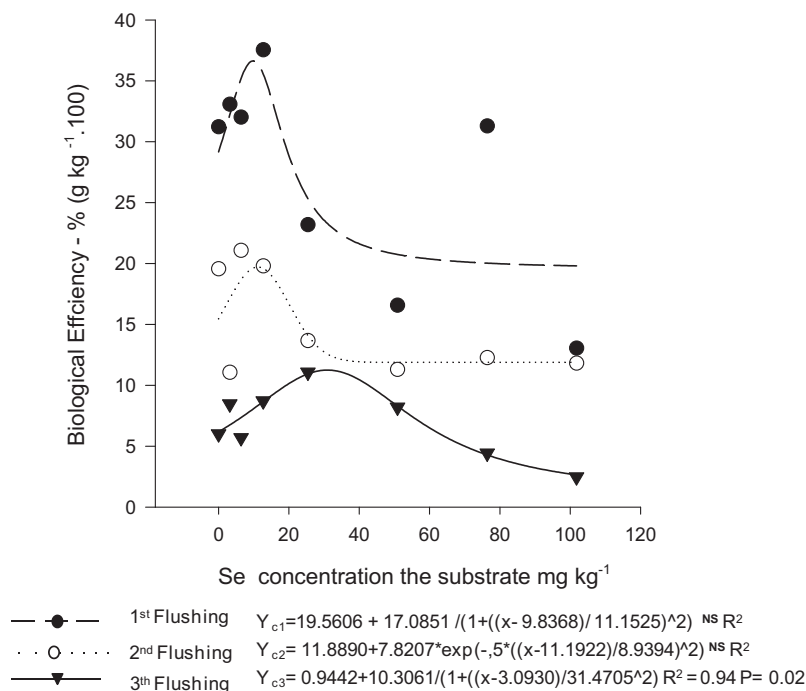


Fig. 2. Biological efficiency of the mushrooms grown in Se-enriched coffee husk substrates with different concentrations of Se for three consecutive flushes.

2.5. Se determination in mushrooms

Se was determined by GF AAS (A SIMAA-6000 graphite furnace atomic absorption spectrometer; Perkin–Elmer). Solutions were delivered into the graphite tube by means of an AS-72 autosampler. For sample analyses, a 10- μ L volume of a chemical modifier solution of 5 μ g Pd and 3 μ g Mg (solutions of 10 g L⁻¹ of Pd(NO₃) and Mg(NO₃)₂, both from Merck) was co-injected into the graphite furnace with 10 μ L of samples or analytical solutions. A Titrisol standard solution of 1000 mg L⁻¹ of Se (Merck) was used to prepare the reference analytical solutions in 0.14 M HNO₃.

2.6. Statistical analysis

This experiment was conducted using a completely randomised design. The data were subjected to Sage software, Version 9.1, for analysis of variance (ANOVA) in plots (eight doses of sodium selenite, three harvests and four replicates) and were later compared using the Tukey test or regression analysis at 5% probability.

3. Results and discussion

3.1. Characteristics of enriched mushrooms

Selenium affected mycelial growth and also the shape of the mushroom (Fig. 1). In substrates with Se concentrations greater than 12.8 mg kg⁻¹, mushrooms possessed larger stipes and smaller caps than in media without Se or with low Se concentrations (Fig. 1). In addition, an unpleasant smell was observed in mushrooms cultivated in substrates with Se concentrations higher than 25.4 mg kg⁻¹. The shape alterations of the *P. ostreatus* mushrooms differed from those observed in *Lentinula edodes*, which did not present any differences in the cap and stipe diameters and stipe length when enriched with Se (Nunes, 2005). However, those authors observed darker caps in Se-enriched mushrooms.

The time needed for incubation of *P. ostreatus* mushrooms varied according to treatment. The first harvest happened between 23

and 28 days after inoculation in the control and in samples grown in low concentrations of Se. Higher levels of Se prolonged this incubation time (Table 1), as harvesting was initiated after 36 days when grown in the substrates with 25.4, 51, 76.4 or 102 mg kg⁻¹ of Se. At this time, the second flush was beginning in the control, the substrate without Se addition (Table 1). Prolonged times for mushroom formation were also observed in *L. edodes* enriched with sodium selenite in cold water at concentrations of 0.32 and 0.64 mM, while concentrations above 0.96 mM completely inhibited mushroom formation (Nunes, 2005).

3.2. Biological efficiency (BE)

The BE was affected by both Se concentration and flushing (Fig. 2). The optimum concentration of Se which was responsible for maximum biological efficiency was different in the three flushes. High BE was observed in the first flush and for Se concen-

Table 2

Elemental concentrations in the coffee husks and in mushrooms of *Pleurotus ostreatus*.

Mineral	Coffee husk ^b	Mushroom ^c
<i>mg g⁻¹</i>		
Ca	2.21	0.11 ± 0.11
K	20.6	37.8 ± 8.63
Mg	0.79	1.17 ± 0.21
Fe	0.26	0.20 ± 0.37
Na	0.04	0.06 ± 0.04
P	0.08	9.50 ± 2.92
N	1.72	4.80 ± 0.41
Cu	0.02	0.03 ± 0.02
Mn	0.06	0.01 ± 0.01
Zn	0.01	0.07 ± 0.02
<i>μg g⁻¹</i>		
Se	0.19	a

^a See Fig. 4.

^b Only one determination was performed.

^c Values correspond to the average of all treatments (with and without Se) as statistical differences were not observed.

trations between 3 and 20 mg kg⁻¹. These results shown that the addition of small amounts of Se can stimulate mushroom production (Fig. 2).

Previous works have shown that high Se concentrations were toxic to mushroom formation, as observed by Gaso et al. (2000) and Hartikainen (2005). Se concentration higher than 25.4 mg kg⁻¹ was a good stimulus for the 3rd flush but not for the others, causing a reduction in the BE on the first and second flushes (Fig. 2). These results may be due to a reduction of Se concentration in the substrate, throughout flushes, leading to an alleviation of toxicity and enhanced mushroom formation.

The highest BE (66%) was obtained for mushrooms cultivated in substrate enriched with 12.7 mg kg⁻¹ of Se (Fig. 2). This BE was higher than that observed for *Pleurotus sajor-caju* cultivated in maize straw (51%) (Dias et al., 2003) and in cotton residues (56%) (Castro, Paiva, Dias, & Santos, 2004). However, it was lower than when this fungus was cultivated in bean residue (86%) (Dias et al., 2003). These results confirm that the choice of substrate determines the BE values of mushrooms (Curvetto, Figlas, Devalis, & Delmastro, 2002).

The extensive period of cultivation, from 43 to 79 days, favoured other saprophytic fungi and increased contamination of the incubation room. Additionally, considering the low BE values on the third flush (Fig. 2), we suggest ending mushroom production on the second flush.

3.3. Effect of Se addition on the absorption of other elements

Selenium addition to the substrate did not affect the concentrations of other elements in the mushrooms during the three consecutive flushes. Additionally, Cd, Cr and Ni concentrations were below the detection limits (1, 2, 5 µg L⁻¹) of the ICP-OES, although low concentrations of these three elements were reported in *Agaricus*, *Suillus*, and *Leccinum* mushrooms (Kalac, 2009; Tuzen, Sesli, & Soylak, 2007). Both mushrooms, cultivated in the presence and in the absence of Se, shown similar uptake behaviours for the several elements of interest (Table 2).

The concentrations of Ca, K, Mg, Mn, Cu, P and Fe (Table 2) were similar to those found in *Pleurotus* spp. (Kalac, 2009; Sturion & Ranzani, 2000). However, the Na and Zn values (Table 2) were lower than those found in *Pleurotus* spp. (Sturion & Ranzani, 2000), and *Calvatia gigantea*, *Cantharellus cibarius*, *Russula integrates*, *Gomphus floccosus*, *Lactarius quieticolor*, *Clavulina cinerea* and *Ramaria brevispora* (Agrahar-Murugkar & Subbulakshmi, 2005).

3.4. Se in *P. ostreatus*

3.4.1. Coffee husk as substrate for *P. ostreatus* mushroom production

Coffee husks have been proved to be an efficient agroindustrial residue for *Pleurotus* mushroom production. This residue is free of heavy metals and possesses very low Se content (Table 2), as observed in other agricultural Brazilian products, since its soil is Se deficient (Ferreira, 1995).

After the coffee husk analyses, Se concentration of mushrooms cultivated in the presence and absence of this element were determined. The selenium content in *P. ostreatus* mushrooms grown in coffee husk without Se enrichment ranged from 0.12 to 0.96 mg kg⁻¹ (Fig. 3); these levels can be considered low compared to other mushrooms found in natural conditions (Huerta, Sánchez, & Sanz-Medel, 2005). This result is also an evidence of the low Se concentration in coffee husk (0.19 mg kg⁻¹) (Table 2).

P. ostreatus mushrooms were able to absorb and accumulate Se when selenite was used for enrichment (Figs. 3 and 4). The Se content was proportional to the amount of sodium selenite added to the substrate (Fig. 3). The lowest concentration tested (3.2 mg of Se kg⁻¹) resulted in mushrooms with 57.6 mg kg⁻¹ of Se in the dry matter at the first flush (Fig. 3). This value is higher than that observed by Gaso et al. (2000) in 15 species of mushrooms collected in nature, which varied from 0.38 to 8.42 µg g⁻¹.

When concentrations lower than 51 mg kg⁻¹ of Se were added to the substrate, results showed a similar accumulation in mushrooms at the three flush times. However, when mushrooms were cultivated in substrate enriched with 76.4 and 102 mg kg⁻¹, these concentrations were higher in the second and third flushes (Fig. 3).

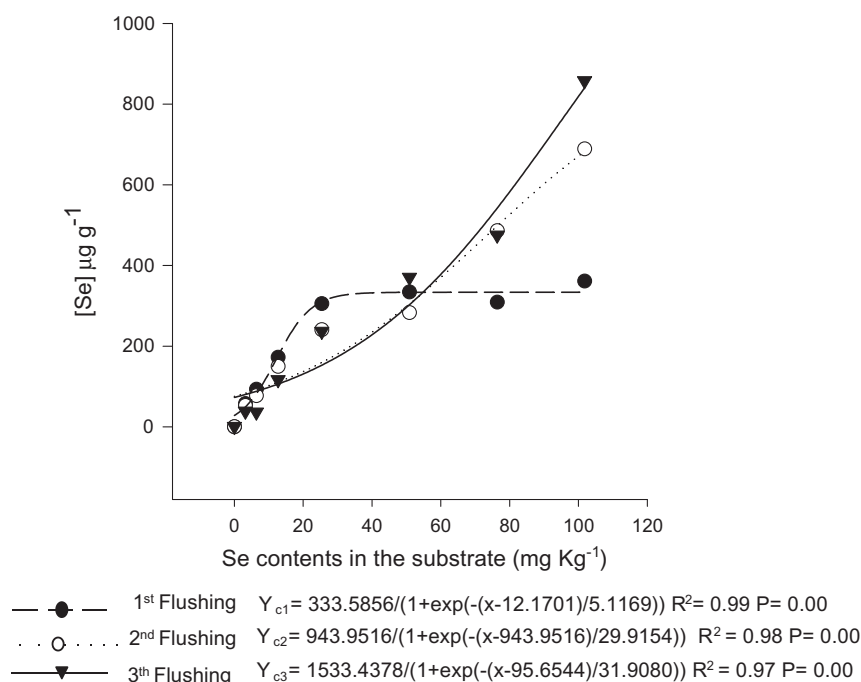


Fig. 3. Concentration of Se in the dry mass of *Pleurotus ostreatus* mushrooms produced in Se-enriched coffee husk substrate.

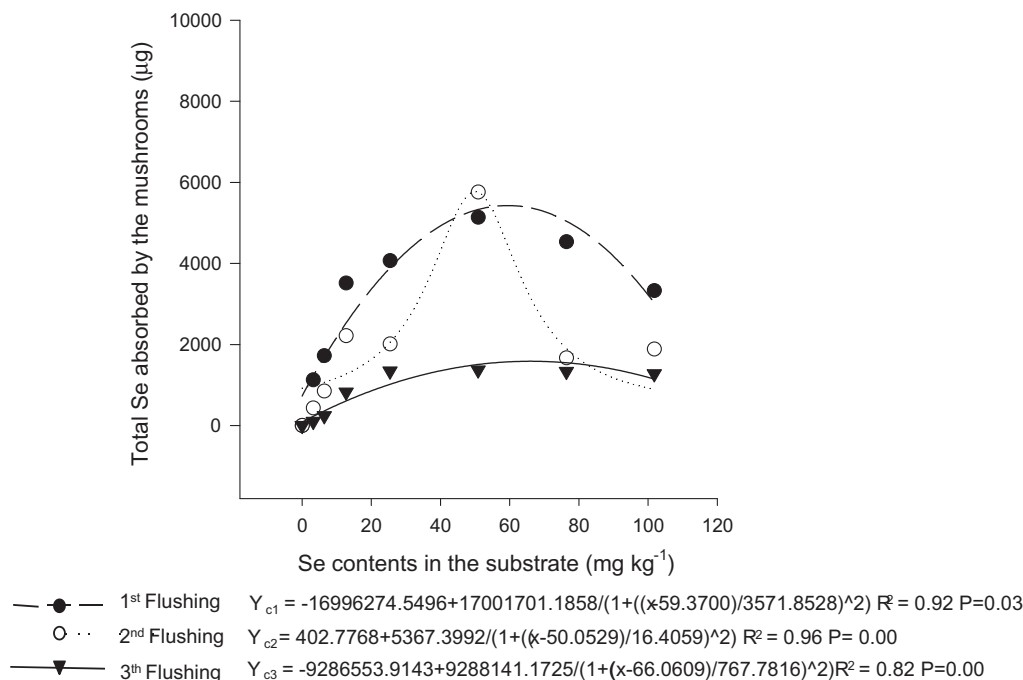


Fig. 4. Total amount of Se in the *Pleurotus ostreatus* produced in Se-enriched coffee husk substrate.

The maximum Se absorption by *P. ostreatus* mushrooms was observed when coffee husks were enriched with 51 mg kg⁻¹ of Se (Fig. 4). At higher concentrations, Se absorption was inhibited, possibly due to the excess of sodium selenite present in the substrate. At concentrations of 3.2 and 12.8 mg kg⁻¹ of Se, 34% of added Se was absorbed, while in the substrate with 51 mg kg⁻¹ only 16% was absorbed. Considering the obtained results, the consumption of 1.0 g of dried mushrooms grown on substrate with 3.2 mg kg⁻¹ of Se is enough to supply the amount of Se recommended for adults, 55 µg day⁻¹ (IOM, 2000).

P. ostreatus is a very good Se accumulator, reaching 858 mg kg⁻¹ when cultivated on substrate enriched with 102 mg kg⁻¹ of Se. The capacity to accumulate Se was verified in *Agaricus bisporus* when the mushrooms were irrigated with water plus Se, as these mushrooms absorbed 52.8 mg kg⁻¹ of Se (Spolar, Schaeffer, Beelman, & Milner, 1999). For *L. edodes*, Se concentration in the mushrooms was 356 mg kg⁻¹ (Ogra, Ishiwata, Encinar, Lobinski, & Suzuki, 2004).

The results shown that the highest BE value and Se absorption rate by *P. ostreatus* mushrooms were obtained when grown in coffee husks containing 12.8 mg kg⁻¹ of Se. Therefore, this is the optimal cultivation condition for Se enrichment. In addition, the Se present in the *P. ostreatus* mushroom has been shown to be bioavailable because it can cross the intestinal barrier and be inserted in peptides (Silva et al., 2010).

4. Conclusions

The cultivation of mushrooms enriched with Se in coffee husk substrate was effective, showing elevated biological efficiency and Se absorption.

Even the lowest Se concentration added to coffee husks, 3.2 mg kg⁻¹, resulted in *P. ostreatus* mushrooms containing sufficient quantities of Se to provide the recommended daily intake of Se for adults.

These results demonstrate the great potential of coffee husks in the production of Se-enriched mushrooms and show the ability of this fungus to absorb and biomagnify Se.

Acknowledgments

The authors are very grateful to Brazilian Agencies: CNPq, CAPES and FAPEMIG for financial support.

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