



Enrichment of mushrooms: An interesting strategy for the acquisition of lithium

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ABSTRACT

The capability of *Pleurotus ostreatus* mushroom to accumulate lithium (Li) and the accessibility of this Li compared with lithium carbonate (Li_2CO_3), often used as psychiatric medicine, were investigated. Mushrooms were produced on a substrate-based on coffee husk, with different added concentrations of lithium chloride (LiCl). Biological efficiency (BE), the crude protein content, the concentration of Li and other elements present in mushrooms were determined. The sequential extraction and *in vitro* test were used to verify the accessibility and the degree of solubility of this element. Li concentration in mushrooms was directly influenced by increasing LiCl concentration in the substrate ($P < 0.05$). The BE was not affected by different concentrations of LiCl. Li present in enriched mushrooms showed greater accessibility than in Li_2CO_3 . Therefore, *P. ostreatus* mushrooms, enriched with lithium can be an alternative source of Li, as well as being a food with high nutritional value.

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1. Introduction

The edible mushroom *Pleurotus ostreatus* has a pleasant taste and nutritional properties that are beneficial to health. Daily intake of this mushroom may influence the lipid profile in hypercholesterolaemic patients and improves antioxidant status (Hossain et al., 2003; Jayakumar, Thomas, & Geraldine, 2007). This mushroom can also be a source of elements, such as iron (Fe), zinc (Zn), selenium (Se), copper (Cu) and molybdenum (Mo), which are involved in many essential biochemical processes (Zaidman, Yassin, Mahajna, & Wasser, 2005).

The bioaccumulation potential of nutrients by fungi enriched with essential elements for human health has been investigated in mycelium and also in mushroom (Munoz et al., 2006; Rabinovich, Figlas, Delmastro, & Curvetto, 2007; Silva et al., 2010, 2012). These studies are important because much of the world's population consumes cereal-based food or lives in regions where the soil has a mineral imbalance, which can frequently result in a lack of essential nutrients in their diet (Johns & Eyzaguirre, 2007).

Lithium is an alkali metal, whose dietary effects have been little investigated. The main sources of lithium are vegetables and grains (Schrauzer, 2002). This element has also been found at different concentrations in mushrooms (e.g., *P. ostreatus*, *Craterellus cornucopioides*, *Amanita strobiliformis*, *Psathyrella candolleana*;

Vetter, 2005). Li is not considered an essential mineral for vital functions because no symptoms of its deficiency in humans have been reported. However, it can influence behaviour without causing physiological changes (Schrauzer, 2002).

The mechanism by which Li acts to promote mood-stabilizing effects has been investigated. Gould et al. (2008) proposed that Li ions inactivate the enzyme activity of glycogen synthase kinase 3 β (GSK-3 β). This enzyme is involved in the pathophysiology of numerous psychiatric disorders. In rats, a decrease of serotonin is associated with aggression and seems to favour the activity of GSK-3 β ; it is possible that Li reduces aggression by inhibiting the activity of GSK-3 β (Jope, 2003). This element can thus restore normal brain function in some people. The regulation of GSK-3 β by Li can affect the circadian clock. When GSK-3 β is activated, the BMAL1 protein is unable to reset the “master clock” inside the brain, and as a result, the body's natural cycle is interrupted. When this cycle is interrupted, the routine schedules of many functions, such as metabolism, sleep and body temperature, are disturbed (McClung, 2007).

The enrichment of *P. ostreatus* mushrooms can provide a promising source of Li, since food sources rich in this mineral are limited.

2. Materials and methods

2.1. Microorganism, fungal growth conditions and inoculum production

The isolate Plo 02 of *P. ostreatus* was grown in a Petri dish containing culture medium potato dextrose agar (PDA; Merck,

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Darmstadt, Germany) at pH 5.8 and incubated at 25 °C. After seven days, the mycelium was used for inoculum production in a substrate based on rice grains that was previously boiled and autoclaved at 121 °C for 90 min.

2.2. Mushroom enrichment with Li

Coffee husks were boiled for 2 h and centrifuged for 5 min at 1500g. Next, 1.5 kg of substrate was placed in polypropylene bags and autoclaved at 121 °C for 90 min, as described by Silva et al. (2012). After cooling, 25 mL of a previously autoclaved solution containing 0, 62.5, 125, 250 or 500 mg of lithium chloride (LiCl, Sigma®) per kg of coffee husks were added to each package. Then, the packages were inoculated with 100 g of inoculum of Plo 02 and were incubated at 25 °C for about 30 days. After the incubation period, the packages were transferred to a fruiting room with controlled temperature and humidity of 20 °C and 80%, respectively. There were three packages for each concentration.

Three harvests of mushrooms were performed at intervals from the 40th to the 60th day after inoculation. The fresh weight of the mushrooms was recorded to determine the biological efficiency (Silva et al., 2012; Wang, Sakoda, & Suzuki, 2001):

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

Subsequently, the mushrooms were dried in an oven at 45 °C for the determination of their dry weight. To determine the content of minerals, crude proteins and to evaluate the accessibility of Li, the dried mushrooms were ground using a knife mill and passed through a 2-mm sieve.

2.3. Mineral content in the mushroom

Samples of 100 mg of dry mushrooms were milled and submitted to digestion with a mixture of nitric acid and perchloric acid (3:1, v:v) at 200 °C for 2 h (Tedesco, Gianello, Bissani, Bohnen, & Volkweis, 1995).

The levels of Li were determined using a flame photometer. The standard curve was prepared with the following concentrations of this element: 0.00; 0.09; 0.36; 0.72; 1.44; 1.80; 2.88; 3.60 and 9.00 mg L⁻¹. The percentage of Li was calculated according to the formula:

$$\text{Concentration of Li in dry mass } (\mu\text{g g}^{-1}) = \frac{[\text{M}] \times \text{DF}}{\text{DM}} \times 1000$$

where, [M] = mineral concentration in mg L⁻¹, DF = dilution factor = 0.025, DM = dry mass of sample.

The content of Fe, Zn, Cu, potassium (K), calcium (Ca), phosphorus (P), sulphur (S), lead (Pb), chromium (Cr), magnesium (Mg), aluminium (Al), cadmium (Cd) and nickel (Ni) contained in the mushrooms were measured by inductively-coupled plasma optical emission spectrometry (Optima 3300 DV; Perkin Elmer, Waltham, MA), using specific standards for each mineral.

2.4. Protein content in the mushrooms

The crude protein content was determined using the semimicro-Kjeldahl method (AOAC, 1996). The nitrogen content was multiplied by a factor of 4.38 to calculate the percentage of crude protein (Kalac, 2009).

2.5. Lithium accessibility

The sequential extraction and *in vitro* methods were used to evaluate the accessibility of Li. We compared mushrooms grown in substrate enriched or not with LiCl (0 and 500 mg kg⁻¹) and a

psychiatric drug containing lithium carbonate (140.9 mg of Li per g of pill, as reported by the manufacturer).

2.5.1. Solubility of lithium by sequential extraction

To evaluate the solubility of Li, 1 g of dried mushroom and also 1 g of the psychiatric drug pill were processed according to sequential extraction methodology described by Ramos, Hernandez, and Gonzalez (1994) and modified by Ma and Rao (1997). After each successive extraction, the extracts were separated by centrifugation at 1500g for 10 min, and the supernatant was collected. The sediment obtained after each extraction was resuspended and again subjected to extraction to collect a new supernatant. This procedure was repeated until six fractions were obtained. We then conducted the analysis of dissolved Li using a flame photometer.

2.5.2. *In vitro* digestibility

The second method was the *in vitro* simulation of gastrointestinal digestion, with the purpose of predicting the accessibility of Li in the digestive tract (Elless, Blaylock, Huang, & Gussman, 2000; Glahn et al., 1998). For this, 250 mg of samples of both dried mushrooms and of the psychiatric drug were crushed. Next, the samples were centrifuged at 1500g for 10 min and filtered to obtain soluble extracts. We then conducted the analysis of Li using a flame photometer.

2.6. Experimental design and statistical analysis

A factorial randomised design was used with five concentrations of LiCl, three harvests, and three replicates, to obtain the following variables: biological efficiency (BE), crude protein content and mineral contents. The data were subjected to analysis of variance (ANOVA), Tukey test or regression at 5% significance using SAS statistical software Version 9.1, licensed to Federal University of Viçosa.

3. Results

3.1. Biological efficiency (BE) of *P. ostreatus* mushrooms grown on coffee husk supplemented with LiCl

The BE of the mushrooms was affected only by the harvest ($P < 0.05$), with a higher EB at the first harvest (Table 1).

3.2. Mineral and protein content in the Li-enriched mushrooms

The minerals most abundant in the substrate, coffee husk, were Ca and K (Table 2). In the mushrooms K was also the most abundant, followed by P, S, and Mg (Table 2). Additionally Al, Cd, Cu, Cr, Ni and Pb concentrations were below the limit of detection, respectively, 3.0, 1.0, 0.4, 2.0, 5.0 and 10.0 μg L⁻¹ in the *P. ostreatus* mushrooms enriched or not with Li. The percentage of crude protein (Table 2) was not altered by the LiCl concentration in the coffee husk nor by the harvesting time ($P > 0.05$). Presence of Li was also observed in coffee husk without LiCl addition and in the non-enriched mushrooms (Table 2, Fig. 1).

3.3. Lithium accumulation in the mushrooms

Lithium added in the substrate was efficiently accumulated in the mushrooms. The concentration of Li in the mushroom increased 2–5 times by adding the mineral in the growth substrate. However, the time of harvest did not influence the accumulation of Li in the mushrooms. Fig. 1 shows the linear increase of Li concentration in the mushrooms as a function of increasing the

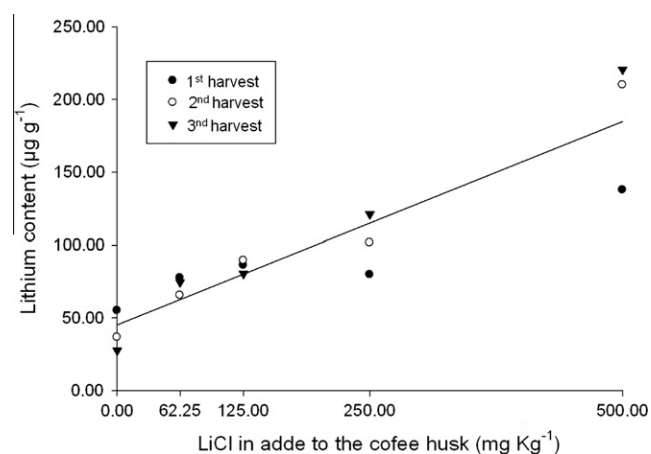
Table 1Biological efficiency (BE) of *Pleurotus ostreatus* mushrooms cultivated in non-enriched (control) or enriched substrate with various concentrations of lithium chloride.

Lithium chloride added to the substrate (mg kg ⁻¹)	Biological efficiency (%)		
	1st harvest	2nd harvest	3rd harvest
0 (Control)	23.38 ± 3.50 ^{A a}	14.51 ± 0.15 ^{A b}	6.28 ± 2.21 ^{A c}
62.5	22.03 ± 5.58 ^{A a}	12.79 ± 2.32 ^{A b}	5.99 ± 1.01 ^{A c}
125	19.57 ± 3.33 ^{A a}	11.64 ± 3.27 ^{A b}	4.66 ± 1.58 ^{A c}
250	23.27 ± 5.06 ^{A a}	16.13 ± 0.35 ^{A b}	4.17 ± 1.44 ^{A c}
500	19.43 ± 3.56 ^{A a}	13.29 ± 5.6 ^{A b}	6.77 ± 3.10 ^{A c}

Means with different superscript letters, lowercase (in row) and uppercase (in column) differ by analysis of variance and Tukey test at 5% probability.

Table 2Mineral composition and crude protein content in the coffee husk substrate and in the *Pleurotus ostreatus* mushrooms, comparing with data from the literature.

Composition	Limit of detection ^a (µg L ⁻¹)	Coffee husk ^b (mg g ⁻¹ of dry mass)	Non-enriched mushroom (control)	Enriched mushroom ^c	Data on mushroom in literature ^d
K	20.00	3.590	14.36 ± 2.79	19.48 ± 2.65	21.90
P	30.00	0.090	4.63 ± 0.97	4.74 ± 0.99	3.260
S	30.00	0.570	3.17 ± 1.28	3.28 ± 1.59	1.000–3.000
Mg	2.000	0.850	1.11 ± 0.15	1.052 ± 0.13	1.700
Ca	0.020	4.310	0.35 ± 0.10	0.47 ± 0.24	1.260
Fe	2.000	1.420	0.16 ± 0.04	0.17 ± 0.04	0.682
Zn	1.000	0.030	0.09 ± 0.01	0.10 ± 0.03	0.142
Li	30.00	0.094	See Fig. 1	See Fig. 1	0.0001
Crude protein (%)	–	nd ^e	22.82 ± 1.64	23.42 ± 1.84	18.70–52.80

^a Limit of detection for plasma emission spectrometry (K, P, S, Mg, Ca, Fe and Zn) and flame photometer (Li).^b Only one determination was performed.^c Mushrooms growth in coffee husk enriched with 500 mg kg⁻¹ of lithium chloride.^d Sturion and Ranzani (2000), Vetter (2005), Genççelep et al. (2009) and Kalac (2009).^e nd – not determined.**Fig. 1.** Lithium content in the dry mass of *Pleurotus ostreatus* mushrooms in three subsequent harvests (● 1st harvest; ○ 2nd harvest and ▲ 3rd harvest) grown on non-enriched or enriched coffee husk substrates with lithium chloride.concentrations of Li chloride added to the growth substrate ($P < 0.05$).

3.4. Accessibility of Li in the *P. ostreatus* mushrooms

3.4.1. Solubility by sequential extraction

Li found in enriched mushrooms was associated with the water-soluble fraction, followed by the reducible, exchangeable and soluble acid fraction, whereas the Li in the non-enriched mushrooms was totally from the water-soluble fraction. However, all of the recovered Li from the drug Li_2CO_3 was present in the residual fraction, which is not considered bioavailable (Fig. 2).

From the six recovered fractions after the extraction steps, only 3.81% was obtained from the non-enriched mushrooms, 45% from

the mushrooms enriched with 500 mg kg⁻¹ LiCl and only 0.02% from the drug Li_2CO_3 , which represents a very low percentage of Li compared to the enriched and non-enriched mushrooms.

3.4.2. In vitro digestibility

The percentage of digested Li that was obtained after the simulation *in vitro* gastrointestinal digestion of the mushrooms enriched with 500 mg kg⁻¹ was higher than that observed for the non-enriched mushrooms (Table 3). In this simulation no Li was detected after digestion of the psychiatric drug containing Li_2CO_3 (Table 3).

4. Discussion

Although many metals are essential for the growth and metabolism of fungi, they can be toxic when present above certain concentrations. Metals that have no known biological functions, such as Pb, Cd, Hg and Li, can also accumulate and be toxic (Gadd, 2007). The toxic effects of these metals include enzyme inhibition, the displacement or replacement of essential ions, the rupturing of membranes and the inhibition of growth and spore germination of fungi, which affect reproduction and metabolism (Gadd, 2007). However, in this work, the addition of LiCl to the coffee husk did not affect the mycelial growth nor the BE of *P. ostreatus* mushrooms ($P > 0.05$, Table 1). The fact that BE was not reduced by the addition of different concentrations of LiCl may indicate (a) a resistance or tolerance of the fungus to the metal or (b) that the amount of LiCl added to the substrate was not sufficient to cause inhibition or any toxic effect to the fungus. Some fungal mechanisms may have contributed to this tolerance, for example, a reduction of absorption or an increase in the efflux of metals through cell wall adsorption, the precipitation of minerals and polysaccharides or extracellular binding by intracellular sequestration of metallothionein (Gadd, 2007).

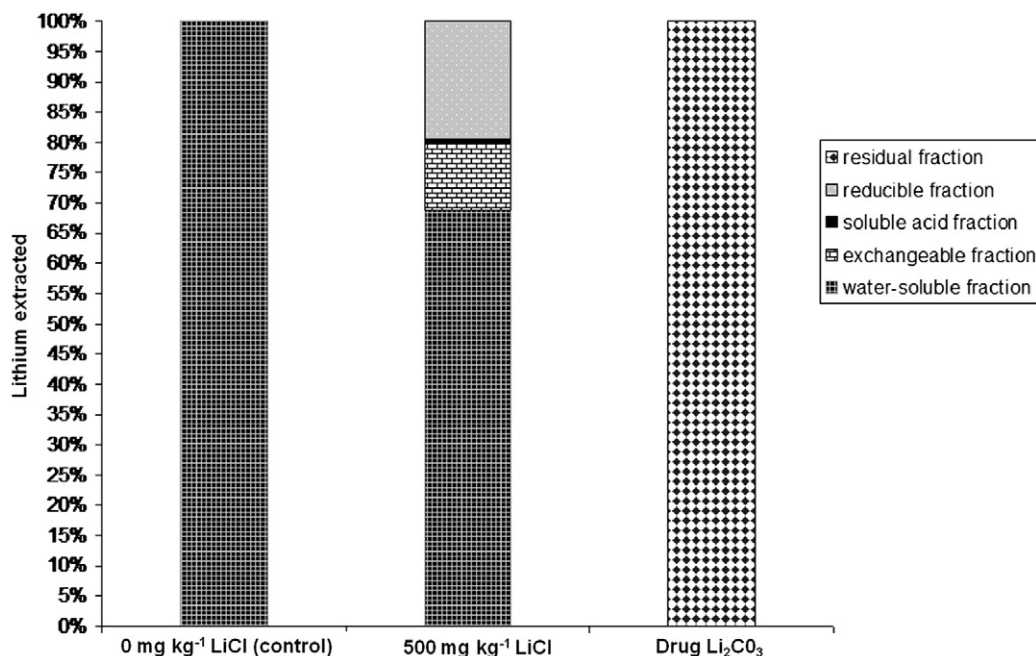


Fig. 2. Solubility of Li based on the sequential extraction of *Pleurotus ostreatus* non-enriched (control) and enriched mushrooms, compared to psychiatric drug containing Li_2CO_3 .

Table 3

In vitro lithium solubility based on the gastrointestinal digestion of *Pleurotus ostreatus* non-enriched (control) and enriched mushrooms compared to psychiatric drug containing Li_2CO_3 .

Source of Li	Digestibility (%)
Non-enriched mushroom (control)	27.46 ± 12.64 ^b
Mushroom Li-enriched	70.51 ± 7.04 ^a
Lithium carbonate	0.00 ± 0.00 ^c

Means with different superscript letters differ at the 5% level of probability by Tukey test.

The levels of minerals and the percentage of crude protein found in the mushrooms enriched with Li were consistent with data from the literature (Genççelep, Uzun, Tunçtürk, & Demirel, 2009; Kalac, 2009; Petrovska, 2001; Sturion & Ranzani, 2000) and, therefore, the enrichment of the substrate with LiCl did not affect the nutritional quality of the mushrooms according to the parameters observed in this work (Table 2).

The high concentration of Li in mushroom without enrichment (Fig. 1) can be due to the presence of Li in substrate without addition of the lithium chloride (Table 2). In different vegetables concentrations of Li greater than 200 ppm have been found (Schrauzer, 2002). Vetter (2005) observed concentrations less than those found in this work, when investigating wild mushroom growing in Hungary. This may be due to the low concentration of lithium in the soil.

As shown in Fig. 1, the increase in the accumulation of Li in *P. ostreatus* mushrooms was directly dependent on the concentration of LiCl that was added to the coffee husk. This result shows the potential to use mushrooms enriched with the desired concentration of Li to obtain a positive effect when administered to patients for psychiatric treatment.

Although accessibility of a mineral cannot be considered synonymous with bioavailability, it is an important factor that affects bioavailability. In addition, for an element to be absorbed and possibly used by an organism, it must be in an accessible form in the intestinal fluid: (a) as a free ion or (b) as a complex with other

nutrients (Elless et al., 2000). The chemical forms of highly accessible minerals are also considered more bioavailable. It can be seen, therefore, that minerals present in non-residual fractions (water-soluble, exchangeable, acid-soluble or reduced bound) are potentially more bioavailable than those present in the residual fraction (Rabinovich et al., 2007). The residual fraction is only solubilised chemically using a very aggressive extraction, which suggests that the mineral is not bioavailable. There are limitations in the sequential extraction process that provide semiquantitative evidence about the chemical forms, accessibility and, indirectly, bioavailability (Ma & Rao, 1997). In this context, it was found that the Li present in the mushroom was more accessible than the same element in the psychiatric drug containing Li_2CO_3 (Fig. 2). Similar results were also reported by Elless et al. (2000) when comparing the solubility of Fe, Zn, Mn, Se and Cr present in *Brassica juncea* enriched with different doses of minerals with multimineral supplements. They verified that all of the minerals present in plant tissue were more accessible and potentially more bioavailable than those in the supplements.

In vitro digestion using gastric and intestinal fluids was conducted independently, to confirm the results of the sequential extraction. The *in vitro* digestion is a method to quantify the accessibility of nutrients but not the bioavailability; thus, not all of the accessible material is absorbed. Therefore, this method does not utilise most of the physiological factors that are involved in the uptake and utilisation of the nutrient. However, it has a low cost and allows for an accurate control of the variables, which makes it an important model for predicting bioavailability (Glahn et al., 1998).

Both results, including the sequential extraction and *in vitro* digestion, showed that the accessibility of Li in the mushrooms was higher than the accessibility of psychiatric drug containing Li_2CO_3 (Fig. 2, Table 3). According to Elless et al. (2000), the high pH of the intestinal fluid can precipitate cationic metals; however, metals associated with the biomass of the fungus can be chelated with organic compounds, which rules out precipitation as one of the reasons for the greater accessibility of the Li was present in the mushrooms compared to that in the tested drug.

Thus, using the digestion data that were obtained in the present study and assuming that an adequate intake of Li is 1 mg d^{-1} (Aral

& Vecchio-Sadus, 2008) and that its accessibility in the gastrointestinal tract is 70.51% (Table 3), the consumption of 10 g of dried mushrooms produced from coffee husks that are enriched with 500 mg kg⁻¹ of LiCl would provide approximately 100% of the recommended intake.

5. Conclusions

P. ostreatus mushroom enriched with lithium has high potential for being used as an alternative source of high accessibility of this microelement. The high concentration of the minerals in the biomass of the fungus was associated with a higher degree of accessibility in sequential extraction and *in vitro* digestion, in relation to a psychiatric drug containing Li₂CO₃. This result supports the use of Li-enriched mushrooms as a source of Li.

Further research on the bioavailability of minerals in *P. ostreatus* mushrooms will provide important information about the effective absorption, physiological effects and influences of Li-enriched mushrooms in promoting and maintaining human health.

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References

- AOAC (1996). Association of official analytical chemists. Official methods of analysis of the association of official analytical chemists (16th ed.). Washington, D.C.
- Aral, H., & Vecchio-Sadus, A. (2008). Toxicity of lithium to humans and the environment – A literature review. *Ecotoxicology and Environment Safety*, *70*, 349–356.
- Elless, M., Blaylock, M., Huang, J., & Gussman, C. (2000). Plants as a natural source of concentrated mineral nutritional supplements. *Food Chemistry*, *71*.
- Gadd, G. M. (2007). Geomycology: Biochemical transformations of rocks, minerals, metals and radionuclides by fungi, biowithering and bioremediation. *Mycological Research*, *3*, 3–49.
- Genççelep, H., Uzun, Y., Tunçturk, Y., & Demirel, K. (2009). Determination of mineral contents of wild-grown edible mushrooms. *Food Chemistry*, *113*, 1033–1036.
- Glahn, R. P., Lai, C., Hsu, J., Thompson, J. F., Guo, M., & Van Champen, D. R. (1998). Decreased citrate improves iron availability from infant formula: Application of an *in vitro* digestion/Caco-2 cell culture model. *The Journal of Nutrition*, *128*, 257–264.
- Gould, T. D., O'Donnell, K. C., Dow, E. R., Du, J., Chen, G., & Manji, H. K. (2008). Involvement of AMPA receptors in the antidepressant-like effects of lithium in the mouse tail suspension test and forced swim test. *Neuropharmacology*, *54*, 577–587.
- Hossain, S., Hashimoto, M., Choudhury, E. K., Alam, N., Hussain, S., Hasan, M., Choudhury, S. K., & Mahmuds, I. (2003). Dietary mushroom (*Pleurotus ostreatus*) ameliorates atherogenic lipid in hypercholesterolaemic rats. *Clinical and Experimental Pharmacology and Physiology*, *30*, 470–475.
- Jayakumar, T., Thomas, P. A., & Geraldine, P. (2007). Protective effect of an extract of the oyster mushroom, *Pleurotus ostreatus*, on antioxidants of major organs of aged rats. *Experimental Gerontology*, *42*, 183–191.
- Johns, T., & Eyzaguirre, P. B. (2007). Biofortification, biodiversity and diet: A search for complementary applications against poverty and malnutrition. *Food Policy*, *32*, 1–24.
- Jope, R. S. (2003). Lithium and GSK-3 one inhibitor, two inhibitory actions, multiple outcomes. *Trends in Pharmacological Sciences*, *24*, 441–443.
- Kalac, P. (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry*, *113*, 9–16.
- Ma, L., & Rao, G. (1997). Chemical fractionation of cadmium, copper, nickel, and zinc in contaminated soils. *Journal of Environmental Quality*, *26*, 259–264.
- McClung, C. A. (2007). Role of the clock gene in bipolar disorder. *Cold Spring Harbor Symposia on Quantitative Biology*, *72*, 1–8.
- Munoz, A. H. S., Kubachka, K., Wrobel, K., Corona, J. F. G., Yathavakilla, S. K. V., Caruso, J. A., & Wrobel, K. (2006). Se-Enriched mycelia of *Pleurotus ostreatus* distribution of selenium in cell walls and cell membranes/cytosol. *Journal of Agricultural and Food Chemistry*, *54*, 3440–3444.
- Petrovska, B. B. (2001). Protein fractions in edible Macedonian mushrooms. *European Food Research and Technology*, *212*, 469–472.
- Rabinovich, M., Figlas, D., Delmastro, S., & Curvetto, N. (2007). Copper- and zinc-enriched mycelium of *Agaricus blazei* Murril: Bioaccumulation and bioavailability. *Journal of Medicinal Food*, *10*, 175–183.
- Ramos, L., Hernandez, L. M., & Gonzalez, M. J. (1994). Sequential extraction of copper, lead, cadmium, and zinc in soils from or near Donana National Park. *Journal of Environmental Quality*, *23*, 50–57.
- Schrauzer, G. N. (2002). Lithium: occurrence, dietary intakes, nutritional essentiality. *Journal of the American College of Nutrition*, *21*, 14–21.
- Silva, M. C. S., Naozuka, J., da Luz, J. M. R., Assunção, L. S., Oliveira, P. V., Vanetti, M. C. D., Bazzolli, D. M. S., & Kasuya, M. C. M. (2012). Enrichment of *Pleurotus ostreatus* mushrooms with selenium in coffee husks. *Food Chemistry*, *131*, 558–563.
- Silva, M. C. S., Naozuka, J., Oliveira, P. V., Vanetti, M. C. D., Bazzolli, D. M. S., Costa, N. M. B., & Kasuya, M. C. M. (2010). *In vivo* bioavailability of selenium in enriched *Pleurotus ostreatus* mushrooms. *Metallomics*, *2*, 162–166.
- Sturion, G. L., & Ranzani, M. R. T. C. (2000). Composição em minerais de cogumelos comestíveis cultivados no Brasil – *Pleurotus* spp. e outras espécies desidratadas, portuguesas (Composition of edible mushrooms cultivated in Brazil – *Pleurotus* spp. and other dehydrated species). *Archivos Latinos Americanos de Nutricion*, *50*, 102–108.
- Tedesco, M. J., Gianello, C., Bissani, C. A., Bohnen, H., & Volkweis, S. J. (1995). *Análises de solo, plantas e outros materiais, portuguesas (Soil analysis, plant and other minerals)* (2nd ed.). Porto Alegre, Departamento de Solos, UFRGS. 174.
- Vetter, J. (2005). Lithium content of some common edible wild-growing mushrooms. *Food Chemistry*, *90*, 31–37.
- Wang, D., Sakoda, A., & Suzuki, M. (2001). Biological efficiency and nutritional value of *Pleurotus ostreatus* cultivated on spent beer grain. *Bioresource Technology*, *78*, 293–300.
- Zaidman, B. Z., Yassin, m., Mahajna, j., & Wasser, S. P. (2005). Medicinal mushroom modulators of molecular targets as cancer therapeutics. *Applied Microbiology Biotechnology*, *67*, 453–468.