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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.

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 hypercholesteraemic 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 3β (GSK-3β). 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-3β; it is possible that Li reduces aggression by inhibiting the activity of GSK-3β (Jope, 2003). This element can thus restore normal brain function in some people. The regulation of GSK-3β by Li can affect the circadian clock. When GSK-3β 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 1500*g*. 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):

(BE = fresh weight of mushrooms/dry weight of substrate) × 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:

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

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 1500*g* 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 1500*g* 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