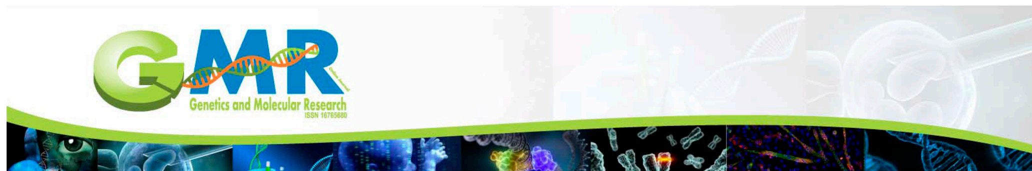

Iron translocation in *Pleurotus ostreatus* basidiocarps: production, bioavailability, and antioxidant activity

M.E. Yokota[1], P.S. Frison[1], R.C. Marcante[1], L.F. Jorge[1], J.S. Valle[1], D.C. Dragunski[2], N.B. Colauto[1] and G.A. Linde[1]

[1]Laboratório de Biologia Molecular, Universidade Paranaense, Umuarama, PR, Brasil
[2]Centro de Engenharia e Ciências Exatas,
Universidade Estadual do Oeste do Paraná, Toledo, PR, Brasil

Corresponding author: J.S. Valle
E-mail: jsvalle@unipar.br

Genet. Mol. Res. 15 (1): gmr.15017888
Received October 22, 2015
Accepted December 4, 2015
Published February 19, 2016
DOI http://dx.doi.org/10.4238/gmr.15017888

ABSTRACT. Translocation of minerals from substrate to mushrooms can change the medicinal characteristics, commercial value, and biological efficiency of mushroom. In the present study, we demonstrated that addition of iron to the substrate reduces the yield of *Pleurotus ostreatus* mushroom. The biological efficiency of the mushroom varied from 36.53% on the unsupplemented substrate to 2.08% for the substrate with 500 mg/kg iron added. The maximum iron concentration obtained for mushroom was 478.66 mg/kg (dry basis) and the maximum solubility *in vitro* was 293.70 mg/kg (dry basis). Iron translocation increased the ash and protein content, reduced antioxidant activity, and enhanced the aroma and flavor characteristics of the mushroom. However mushroom has higher amounts of iron than vegetables like collard greens, it is not feasible to use mushrooms as the only dietary source of iron. The study also indicated that because of more bioaccumulation of iron in mycelium than in the mushroom, mycelium and not mushroom, could be a better alternative as a non-animal iron source.

Key words: Mushroom; DPPH; β-carotene; Sugarcane bagasse; Anemia; Soybean fiber

 ©FUNPEC-RP www.funpecrp.com.br

INTRODUCTION

Several food products with specific bioactive ingredients, for prevention and management of diet-related chronic diseases, have emerged (Stratton et al., 2015). These include functional foods like mushrooms that improve the biological function of the human body. *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm. is a mushroom, known for its antimicrobial, antiviral, immune-modulatory, antitumor, anti-inflammatory, anti-cholesterolemic, antioxidant, and hemagglutinating activities (Cohen et al., 2002).

China is the biggest mushroom producer, consumer, and exporter in the world, with a production of over 20 million tons in 2010. *P. ostreatus* is the most cultivated mushroom in China, where almost five million tons were produced in 2010 (Li, 2012).This basidiomycete produces several agro-industrial residues (Reddy et al., 2003). It has an efficient enzymatic system that includes peroxidases and oxidases, which act on decomposition of lignocellulosic materials (Sánchez et al., 2002). Few studies have been conducted with mineral-enriched mushrooms, to improve the medicinal characteristics, commercial value, and biological efficiency (BE) of mushrooms. de Assunção et al. (2012) obtained lithium-enriched *P. ostreatus* without changes in the BE. However, for *Ganoderma lucidum* (Curtis) P. Karst, the addition of 50 and 100 mg/kg of Cu to the substrate increased mushroom productivity (Matute et al., 2011).

Iron deficiency is reportedly the most prevalent nutritional deficiency worldwide, affecting more than two billion people, most of them young children and women of reproductive age living in developing countries (WHO, 2001). Iron has been less exploited for mushroom production (Vieira et al., 2013). There is only one report of bioaccumulated iron in mycelium (Almeida et al., 2015) and another in which just 0.8 mg/kg iron added to the substrate produced mushrooms without any increase in the iron concentration (Vieiraet al., 2013). Iron-enrichment of *P. ostreatus* could supplement the therapeutic actions of mushroom (Cohen et al., 2002) with iron availability, providing a specific functional food particularly beneficial to those dependent on diets lacking foods of animal origin that are poor in heme iron concentration. Animal iron sources are mainly heminic (Fe^{2+}), with 25% of such iron being absorbed in the human intestine, whereas the non-animal iron sources are non-heminic (Fe^{3+}) and only 5% of this form of iron gets absorbed (Schümann et al., 2007).

Iron, although essential for health and normal growth, is toxic. Ferrous iron, in particular, is a strong pro-oxidant (Gutteridge and Halliwell, 1989). Vieira et al. (2013) reported a reduction in the antioxidant activity of *P. ostreatus* enriched with iron. Thus, the evaluation of the antioxidant activity becomes fundamental to establish the real impact of iron enrichment in mushrooms. Despite the potential use of iron-enriched *P. ostreatus*, there are no reports on the productivity, chemical and sensorial characteristics, and iron bioavailability in mushrooms. Therefore, the objective of the present research was to study iron supplementation to the culture media for assessing the production of iron-enriched *P. ostreatus* and to determine the *in vitro* iron bioavailability, antioxidant activity, total phenolic compounds, and the sensorial characteristics, with an aim to develop functional foods for iron complementation in diets.

MATERIAL AND METHODS

Mycelium culture

P. ostreatus U2-9, obtained from the Culture Collection of the Laboratory of Molecular Biology at Universidade Paranaense, Umuarama, Brazil, was grown in dark at 27 ± 1°C on 39 g/L

potato dextrose agar (PDA) autoclaved at 121°C for 20 min. The mycelium with homogeneous growth and without sectioning was selected as inoculum. The strain was deposited in the Culture Collection of Filamentous Fungi, registered in the World Data Centre for Microorganisms (collection No. 720).

Mycelial growth curve

The substrate used for mycelial growth consisted of sugarcane bagasse (*Saccharum officinarum* L.) from the industrial production of ethanol and soybean fiber [*Glycine max* (L.) Merr.] and was donated from Solae Company, Esteio, RS, Brazil. The substrate was analyzed, in triplicate, for moisture content at 105°C, total nitrogen content by Kjeldahl's method, and ash content at 550°C (Pregnolatto and Pregnolatto, 1985). Carbon/nitrogen ratios were calculated according to the method of Gerrits (1988), considering the carbon content as 50% of the organic matter (total dry mass minus ash content multiplied by 0.5). Subsequently, a substrate formulation was prepared with the carbon/nitrogen ratio of 30 (proportion of 20 g sugarcane bagasse to 8.16 g soybean fiber), which was ideal for the cultivation of *P. ostreatus* (Wu et al., 2004). A solution of $FeSO_4$ (50,000 mg/L) was added to the mixture, to obtain final iron concentrations 0, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1250, 1500, 1750, 2000, 3000, 4000, and 5000 mg/kg (wet basis) and the final moisture was adjusted to 77%. The substrate was transferred to borosilicate tubes (300 x 30 mm) and autoclaved at 121°C for 90 min. After cooling, the substrates were inoculated with PDA disks (30 mm diameter), making sure that the mycelium was in contact with the substrate. Tube ends were closed with cotton caps. Mycelia growth occurred at 25°C with 80% moisture, in dark for 21 days. The longitudinal mycelial growth was measured at four equidistant points on the tube. All treatments were performed in decaplicates. Substrates in which the iron concentration did not inhibit the mycelial growth were selected for mushroom production.

Mushroom production

Wheat grains (*Triticum aestivum*) were used to prepare the inoculum. They were washed and submersed in water and kept at 90°C for 40 min. Thereafter the excess of water was removed, the grains were transferred to polypropylene bags with bacteriological filter (15 x 50 cm) and autoclaved at 121°C for 60 min. After cooling, they were inoculated with six disks of PDA containing mycelium. The growth occurred for 21 days in the dark at 28 ± 1°C.

For mushroom production, a substrate formulation of sugarcane bagasse and soybean fiber with carbon/nitrogen ratio 30 was used. $FeSO_4$ (20,000 mg/L) was added to this mixture, to obtain different iron concentrations. Final substrate moisture was adjusted to 77% and the mixture was transferred to polypropylene bags with bacteriological filter (30 x 45 x 10 cm) utilizing two-third of the bag volume (approximately 9000 cm^3). The substrate in the bag was compressed to obtain a density of about 0.5 kg/L and was autoclaved at 121°C for 180 min. After cooling, it was inoculated with 1% (wet basis) of wheat grains colonized by the fungus. The growth occurred for 21 days in the dark at 28 ± 1°C.

After complete substrate colonization, the chamber temperature was reduced from 28 ± 1°C to 19 ± 1°C. After five days, the temperature was restored to 28 ± 1°C and maintained throughout the production; the relative humidity was kept at 80% and the place ventilated. The bags were perforated to expose 1% of the total bag surface for induction of fructification. The mushrooms that presented curvature loss in the pileus end-indicating mushroom growth interruption-were

harvested three times a day (morning, afternoon, and night). The mushroom fresh mass was recorded to determine BE as in Equation 1 (Miles and Chang, 1997).

$$BE = \frac{M}{S} x100 \qquad \text{(Equation 1)}$$

where BE is the biological efficiency; M is the mass of fresh mushrooms; and S is the dry mass of the substrate.

The mushrooms were dried at 60°C for 48 h in air circulation and their moisture content was evaluated at 105°C until a constant mass was achieved; proteins were determined by Kjeldahl's method using 4.38 as the factor (Furlani and Godoy, 2005); ash content was determined after incineration at 550°C (Pregnolatto and Pregnolatto, 1985).

Sensorial analysis

For sensorial analysis, the method described by ASTM (1996) and Meilgaard et al. (2006) was used and carried out by a team of six selected tasters whose preset sensory capacity was according to the standardized method (ISO, 1993). The dehydrated mushrooms were added to boiling mineral water and cooked for 4 min. After 30 min at room temperature, they were sliced. The samples were presented in random order in a sequential monadic way on disposable plates, identified by random three-number digit codes. Water was used before and between each sample tasting. Three evaluation sessions were done with six samples per session. Each sensorial evaluation was made on a seven-point scale: 0 = none; 1 = detectable; 2 = very light; 3 = light; 4 = light and moderate; 5 = moderate; 6 = moderate-strong; and 7 = strong. The global intensity of aroma and flavor, and the intensity of perceptible aroma notes and flavor and their intensities were evaluated in the order in which they were perceived.

In vitro iron bioavailability

Samples (250 mg), with granulometry smaller than 354 μm, were homogenized with 15 mL ultrapure water. The pH of the homogenate was adjusted to 2 using 5 M HCl. For hydrolysis, 0.75 mL pepsin (20 g/L prepared in 0.1 M HCl) was added. The mixture was kept at 37°C at 200 rpm for 1 h. After the digestion with pepsin, the pH was adjusted to 6 using 1 M $NaHCO_3$, and 3.75 mL of the digestive mixture (8.6 g/L biliary extract, 1.4 g/L pancreatine prepared with 0.1 M $NaHCO_3$) was added. The pH was adjusted again to 7 using 1 M NaOH, and finally 5 mL 120 mM NaCl and 5 mL 5 mM KCl were added. The samples were kept at 37°C at 200 rpm for 1 h. After digestion, the precipitate was separated at 1700 *g* for 10 min at 4°C. The precipitate and the supernatant were dried under air circulation at 60°C until a constant mass was achieved and the iron concentration was determined. For comparison of results, bioavailability was verified in conventional iron sources like collard greens (*Brassica oleracea* L. var. *acephala* DC.), beef, bovine liver, and ferrous sulfate. These materials were obtained from the local market and evaluated under the same conditions used for the mushroom samples.

Iron quantification

For iron quantification, samples previously dehydrated at 60°C were mixed with 1:12

(m/v) 1.06 M HNO_3 and kept at room temperature for 48 h. The mixture was heated at 100°C and then mixed with 1:6 (m/v) 0.88 M H_2O_2 until complete solubilization. The final volume was adjusted to 50 mL with ultrapure water, and the iron concentration was determined by an atomic flame absorption spectrophotometer (GBC, model 932 plus, Braeside, Australia) as described by Korn et al.(2008). A standard curve was prepared with analytical reagent-grade chemicals (Merck®) using ultrapure water.

Antioxidant activity

A raw extract was prepared with 1 g mushroom ground in 10 mL solvent. For hydrogen peroxide and superoxide anion measurements, water was the solvent and fordi(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH) and β-carotene, methanol was the solvent. The mixtures were incubated at 60°C for 60 min and were manually agitated every 10 min (Mourão et al., 2011). The mixture was centrifuged at 4400 *g* for 20 min at 4°C and the supernatant was lyophilized to determine the antioxidant activity.

The capacity to scavenge free radicals at different concentrations was determined. The free radical solutions were prepared with: 1) 40 mM H_2O_2 in 50 mM potassium phosphate buffer, pH 7.4, for the hydrogen peroxide method (Ruch et al., 1989); 2) 0.3 mM nitroblue tetrazolium and 0.936 mM nicotinamide adenine dinucleotide in16 mM Tris-HCl buffer, pH 8.0, with reaction initiation using 0.12 mM phenazine methosulfate solution, for the superoxide anion method (Meyer and Isaksen, 1995); 3) 78 μM DPPH in methanol for the DPPH method (Blois, 1958); and 4) β-carotene/linoleic acid co-oxidation system (Marco, 1968). After the reaction at different concentrations of the extract, the change in absorbance was measured at 230, 560, 515, and 560 nm, respectively, for each method. From a correlation between the free radical concentrations versus sample concentration, the extract concentration needed to reduce 50% free radicals (IC_{50}) was determined.

RESULTS

The mycelial growth in the substrate containing up to 600 mg/kg iron was similar to the control (without iron addition). When 700 to 1500 mg/kg iron was added to the substrate there was a decrease ($P \leq 0.05$) in the mycelial growth, and the complete mycelial inhibition occurred after 1750 mg/kg iron addition (data not shown). Only the substrates on which the mycelial growth was observed were selected for mushroom production. Therefore, addition of 0-1500 mg/kg iron to the substrate was tested for mushroom production.

Mushroom production occurred only on the substrates with iron concentration less than 500 mg/kg. The highest BE ($P \leq 0.05$) was 36.5% for the control (substrate without iron addition) and the lowest ($P \leq 0.05$) was 2.1% for the substrate added with 500 mg/kg iron (Table 1). Although the addition from 100 to 1000 mg/kg did not reduce the mycelial growth for mushroom production, the addition of only 100 or 200 mg/kg iron to the substrate reduced BE ($P \leq 0.05$) by 18.8 and 47.4%, respectively, when compared to the control. The first flush occurred at 28 days (7 days after reducing the temperature) and the second was at 37 days after spawning. The mushroom production flush was not affected by iron addition to the substrate (data not shown).

Table 1. Biological efficiency, iron, solubilized iron, iron bioavailability *in vitro*, content of ashes (dry base) and proteins (dry base) in *Pleurotus ostreatus* mushrooms cultivated on substrate consisting of sugarcane bagasse and soybean fiber with addition of various concentrations of iron and similar observations in other biological samples used as natural iron source in diet.

Added Fe (mg/kg)	Biological efficiency (%)	Fe in mushroom (mg/kg)	Solubilized Fe (mg/kg)	Bioavailability*** *in vitro* (%)	Ashes (%)	Protein (%)
0*	36.53 ± 2.54[a**]	107.80 ± 5.28[e]	69.86 ± 7.67	64.81 ± 7.12[a]	4.90 ± 0.10[b]	21.61 ± 0.29[b]
100	29.66 ± 4.08[b]	264.15 ± 2.43[d]	161.23 ± 34.62	61.04 ± 13.11[a]	5.55 ± 0.13[a]	23.85 ± 0.16[a]
200	19.22 ± 1.15[c]	262.22 ± 5.81[d]	159.37 ± 35.24	60.78 ± 8.17[a]	5.62 ± 0.04[a]	23.03 ± 0.57[a]
300	9.13 ± 4.42[d]	357.74 ± 3.08[c]	232.96 ± 32.84	65.12 ± 9.18[a]	5.67 ± 0.11[a]	23.66 ± 0.17[a]
400	3.00 ± 4.87[e]	370.17 ± 3.41[b]	230.24 ± 16.43	62.20 ± 4.44[a]	na	na
500	2.08± 5.67[e]	478.66 ± 3.47[a]	293.70 ± 40.11	61.36 ± 8.38[a]	na	na
Other samples						
Collard green	na	na	29.38 ± 1.85	44.74 ± 3.96	na	na
Beef	na	na	34.43 ± 2.54	47.59 ± 2.97	na	na
Bovine liver	na	na	65.63 ± 5.84	48.05 ± 2.68	na	na
Ferrous sulfate	na	na	6966.97 ± 154.03	41.57 ± 2.76	na	na

*Substrate naturally contains 22 mg/kg iron besides the added amounts; **Different letters on the same column indicate statistical differences among the treatments according to the Tukey test ($P \leq 0.05$) using Assistat 7.7; na: not analyzed due to lack of mushrooms produced or not applied to the sample.

Addition of 100 and 500 mg/kg iron to the substrate increased its concentration in the mushroom by 2.4 and 4.4 times ($P \leq 0.05$) and reduced the BE by 18.8 and 94.3%, respectively, when compared to the control (Table 1). Although, the increase in iron concentration on the substrate resulted in a high concentration of this element in the mushroom, there was a drastic reduction ($P \leq 0.05$) in mushroom production. Data obtained in our laboratory indicate that addition of iron in the concentration range of 30-60 mg/kg increased the BE of *P. ostreatus* up to 15%, but this was not enough to increase the iron translocation in mushroom (Linde GA and Colauto NB, unpublished data).

The amount of solubilized iron in mushrooms was between 69.86 and 293.70 mg/kg (Table 1). For the other foods that were assessed, like collard greens, beef, and bovine liver, the solubilized iron varied between 29.38 and 65.63 mg/kg (Table 1). Solubilized ferrous sulfate salt, which is the cheapest, most toxic, and most used iron supplement was solubilized (6966.97 mg/kg) (Table 1). Therefore, when compared to the major iron-providing foods, *P. ostreatus* provides approximately 2.4-to 4.5-times more iron, and therefore, could be an excellent non-animal iron source. The iron bioavailability in mushrooms ranged from 60.78 to 65.12% with no observed differences ($P \leq 0.05$) among the treatments. Thus, although the progressive iron addition to the substrate increased iron translocation in mushrooms, it did not affect ($P \leq 0.05$) iron bioavailability, *in vitro*.

The chemical composition analysis demonstrated that the mushroom ash and protein contents increased with iron addition to the substrate. In the control, the addition 100 and 200 mg/kg iron increased the ash content by 13.3 and 14.7% and the protein content by 10.4 and 6.6%, respectively (Table1). Iron translocation to the mushroom increased ($P \leq 0.05$) the IC_{50}value for all the antioxidant methods employed and reduced the polyphenol content approximately by 40% when compared to the control (Table 2).

All the mushrooms presented characteristic aroma and taste of cooked mushroom. Higher the iron concentrations in the mushroom, greater were the aroma notes for sulfur and umami, and bitter and metallic flavors (Table 3). It fits with the increase in protein content for the mushrooms produced in the iron-added substrate (Table1). Similarly, the enhanced metallic flavor was observed in mushrooms with higher concentrations of ashes and iron (Table 1). Iron-enriched mushrooms

might have reduced its acceptance as a food. Therefore, these factors should also be evaluated prior to large-scale production of this fungus.

Table 2. Antioxidant activity of *Pleurotus ostreatus* mushrooms cultivated on substrate consisting of sugarcane bagasse and soybean fiber with different iron concentrations.

Added Fe (mg/kg)*	IC_{50} H_2O_2 ($\mu g_{basidiocarp}$/mL)***	IC_{50} RAS ($\mu g_{basidiocarp}$/mL)	IC_{50} DPPH ($\mu g_{basidiocarp}$/mL)	IC_{50} β-CAROTENE ($\mu g_{basidiocarp}$/mL)	Polyphenol ($mg/g_{basidiocarp}$)
0	132.15 ± 9.2[a]**	22.0 ± 1.2[a]	3.83 ± 0.2[a]	22.67 ± 5.5[a]	17.2 ± 0.7[a]
100	189.44 ± 8.7[b]	35.3 ± 3.8[b]	4.15 ± 0.1[b]	69.24 ± 7.8[b]	11.7 ± 0.6[b]
200	193.66 ± 5.1[b]	32.5 ± 3.2[b]	4.28 ± 0.3[b]	61.66 ± 6.8[b]	11.9 ± 0.9[b]
300	198.23 ± 6.8[b]	38.2 ± 2.7[b]	5.50 ± 0.6[c]	66.57 ± 5.7[b]	10.2 ± 0.8[b]
400	231.20 ± 5.2[c]	35.4 ± 5.8[b]	5.17 ± 0.2[c]	82.89 ± 10.5[c]	11.5 ± 1.2[b]
500	236.33 ± 6.4[c]	39.3 ± 4.8[b]	6.68 ± 0.1[e]	89.16 ± 8.7[c]	10.4 ± 1.5[b]

*Substrate naturally contains 22 mg/kg iron besides the added amounts; **Different letters on the same column indicate statistical differences among the treatments according to the Tukey test ($P \leq 0.05$) using Assistat 7.7; ***IC_{50}: concentration of the same sample needed to reduce 50% concentration of free radicals utilizing the methodology to scavenge free radicals of: oxygen peroxide (H_2O_2), superoxide of anions (RAS), di(phenyl)-(2,4,6-trinitrophenyl) iminoazanium(DPPH) or by the system of co-oxidation of β-carotene/linoleic acid (β-CAROTENE).

Table 3. Aroma and flavor characteristics of *Pleurotus ostreatus* mushrooms cultivated on sugarcane bagasse and soybean fiber supplemented with different iron concentrations.

Added Fe (mg/kg)*	Characteristics**					
	Aroma		Flavor			
	Global	Sulfur	Global	Umami	Bitter	Metallic
0	6	0	3	0	0	0
100	6	2	5	2	0	0
200	6	4	4	2	3	0
300	6	5	4	3	3	2
400	4	6	4	3	2	3
500	5	6	3	4	3	3

*The substrate naturally contains 22 mg/kg of iron besides the added amounts; **The seven-point scale represents: 0 = none; 1 = detectable 2 = very light; 3 = light; 4 = light to moderate; 5 = moderate; 6 = moderate-strong; 7 = strong.

DISCUSSION

The decrease of BE on substrates with more than 100 mg/kg of iron addition could be associated with the toxicity of this ion. Although iron is essential to fungal metabolism, it can release free radicals through Fenton's reaction, when present in high amounts. These radicals can cause enzyme inhibition, dislocation or substitution of essential ions, and membrane rupture that affects the fungal metabolism and reproduction (Gadd, 2007; Oghenekaro et al., 2008).

In the present study, the BE of *P. ostreatus* was 36.5% for the substrate without iron addition and 29.6% for the one in which 100 mg/kg iron was added. Other studies reported a 66% BE in *P. ostreatus* on coffee husks supplemented with 12.7 mg/kg selenium, 44.2% on that without the supplementation (da Silva et al., 2012), and 39.5% when 500 mg/kg lithium was added to the

substrate (de Assunção et al., 2012). In *G. lucidum*, cultivated on sunflower seed hulls and barley, there was an increase of BE with the addition of 100 mg/kg copper (Matute et al., 2011).

Our results showed that the iron bioaccumulation in the mushroom reduce ($P \leq 0.05$) the mushroom production. Vieira et al. (2013) reported that the addition of ferrous sulfate (0.8 mg/kg) to the coffee husk did not affect ($P \leq 0.05$) the iron concentration in *P. ostreatus*. It indicates that the iron concentration that promotes the ion translocation in mushroom also causes a reduction in BE. Therefore, it would be relevant to analyze the economic viability of iron translocation in mushroom and whether its commercialization as an iron-rich functional food, economically off sets the reduction in mushroom production.

de Assunção et al. (2012), reported that lithium bioavailability in *P. ostreatus* increased from 27.5% (in the control) to 70.5% in the substrate supplemented with 500 mg/kg lithium. Matute et al. (2011) obtained bioavailability of 31.5 and 30.7% copper and zinc, respectively, in *G. lucidum* cultivated on substrate supplemented with 100 mg/kg copper and 25 mg/kg zinc. Thus, a great variability in the bioavailability was observed depending upon the supplemented mineral and the mushroom variety.

The dietary iron intake recommended for all age groups of men and postmenopausal women is 8 mg/day and for premenopausal women is 18 mg/day (Schümann et al., 2007). Assuming that the maximum possible iron is solubilized in mushroom (293.7 mg/kg), we speculate that an adult man and a premenopausal woman could meet the daily iron intake with 27.23 and 61.28 g dried iron-enriched mushroom, respectively, which amounts to around 270 and 613 g fresh or rehydrated mushrooms. Although *P. ostreatus* mushrooms are an alternative iron source, its daily intake in the amounts needed to attain the required iron would not be feasible. Moreover, in animal iron sources like beef and liver, most of the iron is heminic (Fe^{2+}) and 25% is absorbed in the human intestine, in this form whereas, in non-animal iron sources like collard greens and mushroom, iron is non-heminic (Fe^{3+}) and only 5% of this form is absorbed (Schümann et al., 2007). The lower amount of iron absorption from non-animal sources *in vivo* would imply an even greater amount of mushroom intake needed to obtain the recommended dietary intake.

The ferrous sulfate toxicity has been reported mainly in children and adults having prolonged intake of iron supplement (Pestaner et al., 1999). Although iron-enriched *P. ostreatus* are an alternative to non-animal iron source, more promising results were obtained with the mycelium in comparison to the mushroom. Iron bioaccumulation in the mycelium was from 507 to 3616 mg/kg in culture medium enriched with 25 to 250 mg/L iron, respectively (Almeida et al., 2015). Thus, iron bioaccumulation in the mycelia was about seven-fold higher (Almeida et al., 2015) than in *P. ostreatus*. Moreover, iron-enriched mycelia could be incorporated in capsules, infusions, as well as in dietary supplements. The use of mushrooms and/or mycelium enriched with iron can be particularly advantageous in vegetarian diets. Despite the health benefits that vegetarian and vegan-life style and dietary habits provide, such diets have been associated with iron deficiencies (Craig and Mangels, 2009). Waldmann et al. (2004) investigated the iron deficiency in German vegan females and concluded that, although their average iron intake was above the recommended level, 40% of them were considered iron-deficient because of its poor absorption, and therefore, iron supplements were prescribed to them.

Reduction of the mushroom antioxidant activity might be related to the electron transferring capacity of iron, which characterizes it as a potent pro-oxidant. Iron can stimulate lipid peroxidation by Fenton reaction, and can also accelerate peroxidation by decomposing lipid hydro-peroxides into peroxyl and alkoxyl radicals, which themselves generate hydrogen and perpetuate the chain reaction of lipid peroxidation (Halliwell, 1991; Chang et al., 2002). This suggests that iron-enriched

©FUNPEC-RP www.funpecrp.com.br

mushrooms could have an inhibiting effect on the therapeutic activity of the fungus or could even affect other biological activities.

Progressive iron addition to the substrate reduces the biological efficiency and increases the concentration of iron, ashes, and proteins in the mushrooms. The iron-enriched mushroom shows reduced antioxidant activity and enhanced taste and aroma characteristics, but the increase in iron concentration in mushroom does not affect the iron bioavailability *in vitro*. The present study indicates that iron-enriched mushrooms could be an alternative non-animal iron source despite being apparently economically unfeasible.

Conflicts of Interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

The authors thank the Universidade Paranaense, the Postgraduate Program in Biotechnology Applied to Agriculture of Universidade Paranaense, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the financial support and fellowship.

REFERENCES

Almeida SM, Umeo SH, Marcante RC, Yokota ME, et al. (2015). Iron bioaccumulation in mycelium of *Pleurotus ostreatus*. *Braz. J. Microbiol.* 46: 195-200. http://dx.doi.org/10.1590/S1517-838246120130695

American Society for Testing and Materials (ASTM) (1996). Sensory testing methods.2nd edn.ASTM Manual Series: MNL 26. American Society for Testing and Materials, Philadelphia.

Blois MS (1958). Antioxidant determinations by the use of a stable free radical. *Nature* 181: 1199-1200. http://dx.doi.org/10.1038/1811199a0

Chang LW, Yen WJ, Huang SC and Duh PD (2002). Antioxidant activity of sesame coat. *Food Chem.* 78: 347-354. http://dx.doi.org/10.1016/S0308-8146(02)00119-X

Cohen R, Persky L and Hadar Y (2002). Biotechnological applications and potential of wood-degrading mushrooms of the genus *Pleurotus*. *Appl. Microbiol. Biotechnol.* 58: 582-594. http://dx.doi.org/10.1007/s00253-002-0930-y

Craig WJ and Mangels AR; American Dietetic Association (2009). Position of the American Dietetic Association: vegetarian diets. *J. Am. Diet. Assoc.* 109: 1266-1282. http://dx.doi.org/10.1016/j.jada.2009.05.027

da Silva MCS, Naozuka J, da Luz JMR, de Assunção LS, et al. (2012). Enrichment of *Pleurotus ostreatus* mushrooms with selenium in coffee husks. *Food Chem.* 131: 558-563. http://dx.doi.org/10.1016/j.foodchem.2011.09.023

de Assunção LS, da Luz JM, da Silva MdeC, Vieira PAF, et al. (2012). Enrichment of mushrooms: an interesting strategy for the acquisition of lithium. *Food Chem.* 134: 1123-1127. http://dx.doi.org/10.1016/j.foodchem.2012.03.044

Furlani RPZ and Godoy HT (2005). Nutritional value of edible mushrooms: a revision. *Rev. Inst. Adolfo Lutz* 64: 149-154.

Gadd GM (2007). Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. *Mycol. Res.* 111: 3-49. http://dx.doi.org/10.1016/j.mycres.2006.12.001

Gerrits JPG (1988). Nutritional and compost. In: The cultivation of mushrooms (vanGriensven LJLD, ed.) Darlington Mushroom Laboratories Ltd. Rustington, 29-38.

Gutteridge JM and Halliwell B (1989). Iron toxicity and oxygen radicals. *Baillieres Clin. Haematol.* 2: 195-256. http://dx.doi.org/10.1016/S0950-3536(89)80017-4

Halliwell B (1991). Reactive oxygen species in living systems: source, biochemistry, and role in human disease. *Am. J. Med.* 91 (3C): 14S-22S. http://dx.doi.org/10.1016/0002-9343(91)90279-7

International Organization for Standardization (ISO) (1993). ISO 8586-1 Sensory analysis - General guidelines for the selection, training and monitoring of assessors - Part 1: Selected assessors. Geneva: International Organization for Standardization.

Korn MDGA, da Boa Morte ES, Batista dos Santos DCM, Castro JT, et al. (2008). Sample preparation for the determination of metals in food samples using spectroanalytical methods - a review. *Appl. Spectrosc. Rev.* 43: 67-92. http://dx.doi.org/10.1080/05704920701723980

Li Y (2012). Present development situation and tendency of edible mushroom industry in China. In: Proceedings of the Congress of the International Society for Mushroom Science, Beijing.

Marco GJ (1968). A rapid method for evaluation of antioxidants. *J. Am. Oil Chem. Soc.* 45: 594-598. http://dx.doi.org/10.1007/BF02668958

Matute RG, Serra A, Figlas D and Curvetto N (2011). Copper and zinc bioaccumulation and bioavailability of *Ganoderma lucidum. J. Med. Food* 14: 1273-1279. http://dx.doi.org/10.1089/jmf.2010.0206

Meilgaard MC, Carr BT and Civille GV (2006). Sensory evaluation techniques. (4th ed.). Boca Raton, CRC press.

Meyer AS and Isaksen A (1995). Application of enzymes as food antioxidants. *Trends Food Sci. Technol.* 6: 300-304. http://dx.doi.org/10.1016/S0924-2244(00)89140-2

Miles PG and Chang ST (1997). Mushroom biology: concise basics and current developments. Singapore: World Scientific.

Mourão F, Harue Umeo S, Seiko Takemura O, Andrea Linde G, et al. (2011). Antioxidant activity of *Agaricus brasiliensis* basidiocarps on different maturation phases. *Braz. J. Microbiol.* 42: 197-202. http://dx.doi.org/10.1590/S1517-83822011000100024

Oghenekaro AO, Okhuoya JA and Akpaja EO (2008). Growth of *Pleurotus tuberregium* (Fr) Singer on some heavy metal-supplemented substrates. *Afr. J. Microbiol. Res.* 2: 268-271.

Pestaner JP, Ishak KG, Mullick FG and Centeno JA (1999). Ferrous sulfate toxicity: a review of autopsy findings. *Biol. Trace Elem. Res.* 69: 191-198. http://dx.doi.org/10.1007/BF02783871

Pregnolatto W and Pregnolatto NP (1985). Normas analíticas do instituto Adolfo Lutz. São Paulo: Instituto Adolfo Lutz.

Reddy GV, Babu PR, Komaraiah P, Roy KRRM, et al. (2003). Utilization of banana waste for the production of lignolytic and cellulolytic enzymes by solid substrate fermentation using two *Pleurotus* species (*P. ostreatus* and *P. sajor-caju*). *Process Biochem.* 38: 1457-1462. http://dx.doi.org/10.1016/S0032-9592(03)00025-6

Ruch RJ, Cheng SJ and Klaunig JE (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis* 10: 1003-1008. http://dx.doi.org/10.1093/carcin/10.6.1003

Sánchez A, Ysunza F, Beltrán-García MJ and Esqueda M (2002). Biodegradation of viticulture wastes by *Pleurotus*: a source of microbial and human food and its potential use in animal feeding. *J. Agric. Food Chem.* 50: 2537-2542. http://dx.doi.org/10.1021/jf011308s

Schümann K, Ettle T, Szegner B, Elsenhans B, et al. (2007). On risks and benefits of iron supplementation recommendations for iron intake revisited. *J. Trace Elem. Med. Biol.* 21: 147-168. http://dx.doi.org/10.1016/j.jtemb.2007.06.002

Stratton LM, Vella MN, Sheeshka J and Duncan AM (2015). Food neophobia is related to factors associated with functional food consumption in older adults. *Food Qual. Prefer.* 41: 133-140. http://dx.doi.org/10.1016/j.foodqual.2014.11.008

Vieira PAF, Gontijo DC, Vieira BC, Fontes EA, et al. (2013). Antioxidant activities, total phenolics and metal contents in *Pleurotus ostreatus* mushrooms enriched with iron, zinc or lithium. *LWT-. Food Sci. Technol. (Campinas)* 54: 421-425.

Waldmann A, Koschizke JW, Leitzmann C and Hahn A (2004). Dietary iron intake and iron status of German female vegans: results of the German vegan study. *Ann. Nutr. Metab.* 48: 103-108. http://dx.doi.org/10.1159/000077045

World Health Organization (WHO) (2001). Iron deficiency anaemia: assessment, prevention and control: a guide for programme managers. World Health Organization.

Wu JZ, Cheung PC, Wong KH and Huang NL (2004). Studies on submerged fermentation of *Pleurotus tuber-regium* (Fr.) Singer. Part 2: effect of carbon-to-nitrogen ratio of the culture medium on the content and composition of the mycelial dietary fiber. *Food Chem.* 85: 101-105. http://dx.doi.org/10.1016/j.foodchem.2003.06.009

 ©FUNPEC-RP www.funpecrp.com.br