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ANTIOXIDANT ACTIVITY OF WATER EXTRACTS FROM FRUIT BODY OF *LENTINUS EDODES* ENRICHED WITH SELENIUM

ABSTRACT: Shiitake *(Lentinus edodes)* belongs to medically important and delicious fungi. It is recognizable for its healing properties, excellent taste and rich aroma. According to the traditional Japanese and Chinese medicine, shiitake mushroom significantly increases the strength and vitality of the body. Shiitake contains immunostimulants, compounds that lower cholesterol, prevents clogging of blood vessels, regulates the pressure, balances blood sugar levels, regulates digestion, and improves the performance of respiratory organs by its antirheumatic and antiallergic activities. Shiitake is recommended to use as food, prevention and cure, usually in a form of a spice (dried and ground) or tea. It can be consumed fresh, too.

The objective of this study was to test the effect of enrichment in selenium on antioxidant, reducing and free radical scavenging activity of water extracts from fruit body of *Lentinus edodes*. The fungus was enhanced by adding organic selenium, zinc (II) complex with the ligand 2.6-bis diacetylpyridine (selenosemicarbazon) and inorganic compounds (Na_2SeO_3) of selenium in nutritional substrate where the fungus was grown. The total selenium content in fruit body was around 50 ppm for the sample enriched with selenium originating from organic sources, and 80 ppm for the sample enriched with selenium from inorganic sources. Samples were prepared by extraction of fruiting bodies in heated water. The results indicated that water extracts of whole fruit bodies, from both control and mushrooms supplemented with selenium, had quite good antioxidant activity. However, there was no significant difference between the samples supplemented with selenium content and those that were not.

KEY WORDS: *Antioxidant activity, Extract, Selenium, Shiitake*

INTRODUCTION

Selenium is essential micronutrient for mammals and birds. It is essential antioxidant, necessary for the proper functioning of hormones and immune system. The content of selenium in plants that are known as the source of this compound has been reduced due to the poverty of the land on which they grow (Jian' an et. al., 2002; Klapec et. al., 1998; Sanjiv et. al., 2005). Sele-

nium deficiency can cause many disorders in the body (G r o m a d z i n s k a et. al., 2008). Based on previous studies, it is known that the fungi are good accumulators of selenium (B o r o v i č k a and R a n d, 2007; S a v i ć et. al., 2009). Selenium content in dry mass of fungi is between 0.57 and 19.46 mg/kg, depending on the type, age and location of fungi (F a l a n d y s z , 2008). The aim of the study was to compare the possibility of adoption of selenium in fruit body of industrial mushroom *Lentinus edodes* from organic and inorganic selenium sources. *L.edodes* is medicinal mushroom originating from Asian countries. Fruit body of the fungus is used as food, but also as medicine. It builds up the immune system, lowers cholesterol, helps blood coagulation and relieves symptoms in the cancer treatment. Mentioned fungi can be consumed as freshly prepared, concentrated extracts, or dietary supplements (DS). Several types of DS are derived from mushrooms *L.edodes*: dried and pulverized fruiting bodies, extracts in hot water and alcohol extracts, biomass or extract of mycelium. Commercial products are available on the market in the form of tablets, capsules and teas.

The total selenium content in enriched mushrooms is determined by the optical emission spectrometer with inductively coupled plasma, ICP-OES. Antioxidant potential, scavenging effect, as well as the reduction potential of fungi with and without addition of selenium in the form of extract of fruit bodies in hot water was determined in the experiment.

MATERIAL

Possibility of accumulation of selenium from nutrient rich substrate in mushroom fruit bodies of *Lentinus edodes* (commercial designation L-31) was examined. This strain was grown at Department of Microbiology, Faculty of Agriculture, University of Belgrade. Sodium selenite, Na_2SO_3 was used as inorganic source of selenium, while the organic compound used in the work was newly synthesized organic complex of Zn (II) with the ligand 2,6-bis-diacetylpyridine (selenosemicarbazon) (H2dapsesc) – [Zn (dapsesc)] (T o d o r o v i ć et. al., 2007). The compounds were added in the nutrient substrate where the mushrooms were grown in the concentrations of 50 mg/kg selenium and 15 mg/kg selenium, in the form of inorganic salts and organic complexes. The total selenium content was determined in dry mass of the sample by ICP-OES. The average content of selenium in the substrate without the addition of supplements was about 0.2 mg/kg, while this value in the fruit bodies of the control fungi was 0.4-0.6 mg/kg. This confirms the initial statement that the total selenium content in the substrate, and fungi that grow on it, was low. The average content of selenium in the fruit body of fungi that grew on media supplemented with selenium from organic sources (15 mg/kg Se) was around 50.4 mg/kg. The content of selenium in fruit body of the fungi that grew on the substrate with the addition of inorganic salt (52.3 mg/kg Se) was about 81.0 mg/kg.

METHODS

The antioxidant activity was determined by the conjugated diene method with slight modification (T u r l e y et al., 2010; Y u – H s i u et. al., 2008). The negative control was the solution with all reagents but without extract. The antioxidant activity was calculated as follows: antioxidant activity (%) = [(A_0 – A_1)/ A_0] x 100, where A_0 was the absorbance of the control reaction and A_1 the absorbance in the presence of sample. Ascorbic acid and α-tocopherol were used as the positive control. Value of 100% indicated the strongest inhibitory ability.

Test for determination of the potential neutralization of 1.1-diphenyl-2-picrylhydrazyl (DPPH) radical was prepared in accordance with the modified method by B i l o s (P r a s h a n i et. al., 2005). Binding capacity of DPPH free radical method was calculated based on the following equation: % scavenging = [1-(A_i-A_j)/Ac] x100, where Ai was the absorbance of 2 mL extract mixed with 1 mL DPPH solution; Aj was the absorbance of 2 mL extract mixed with 1 mL DMSO solution, and Ac was the absorbance of blank-2 mL of DMSO mixed with 1 mL of DPPH solution. Ascorbic acid, BHT and α-tocopherol dissolved in DMSO were used as the positive control.

The reducing power was determined according to the method of O y a i z u (T u r l e y et al., 2010). The blank was the solution with all reagents but without extract. Higher absorbance indicated higher reducing power. Ascorbic acid was used as the positive control.

Results were expressed as mean ± standard deviation of three parallel measurements. Tests were performed using computer program Microsoft Excel 2007. The data were analyzed using one-way analysis of variance (ANOVA) and Student's *t* test at significance level 0.05. The lowest effective concentration (EC_{50}) was obtained by interpolation from linear regression analysis.

RESULTS AND DISCUSSION

Antioxidant activity

Using a modified method of conjugated diene, water extracts of whole mushrooms showed strong antioxidant activity at concentrations of 10 mg/ml (Figure 1). The potential values of fungi *L. edodes* at the concentration of 10 mg/ml for control samples and of mushrooms enriched with selenium from selenite and selenium from organic sources were 16.41%, 26.33% and 49.27%, respectively. The antioxidant activity of ascorbic acid was most pronounced at concentrations of 2.5 mg/ml and amounted to 71.7%, while for α-tocopherol this activity was at concentrations of 0.1 mg/ml, and it was 79.7%.

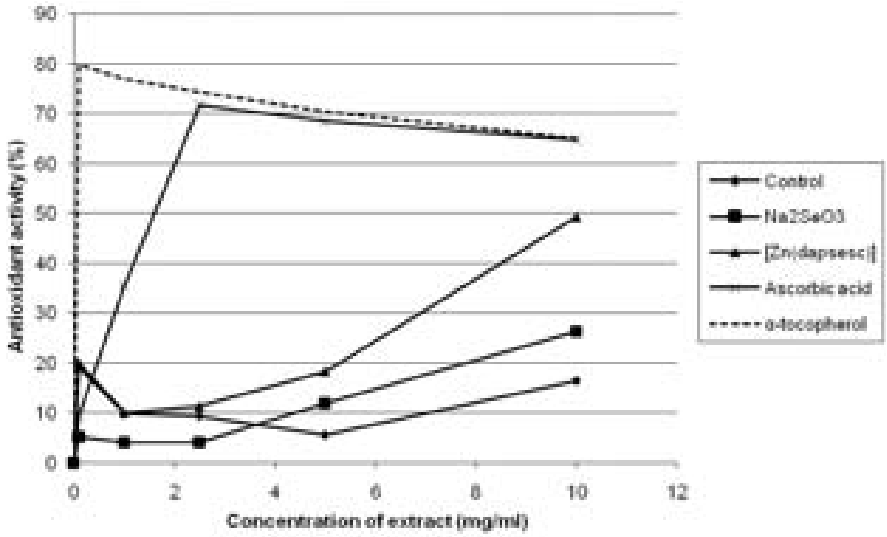

Fig. 1 – The antioxidant activity of hot water extracts of *Lentinus edodes* L31. Values are expressed as mean ± standard deviation (n = 3).

Reducing power

Reducing power of hot water extracts from fruit bodies of *L. edodes* increases with higher concentration. At concentrations of 20 mg/ml, the reducing power of control mushrooms *L.edodes* and samples with selenium from inorganic and organic compounds was 1566, 1645, 1156, respectively (Figure 2). Reducing power of ascorbic acid was significantly higher in comparison to the samples and it amounted to 3.956 at the concentration of 1 mg/ml.

Scavenging activity

Absorbance of DPPH radical binding is shown in Figure 3. Hot water extract of fruit body showed a strong ability to bind DPPH radicals. The ability of radical binding of ascorbic acid, BHT and α-tocopherol at concentrations of 0.1-10 mg/ml, was 80.6-87.7%, 1.13-55.23% and 79.9-78.4%. Scavenging activity of *L. edodes* at concentrations 0.1-10 mg/ml without addition of selenium, with the addition of inorganic and organic selenium was 83.21-108.39%, 89.11-108.31% and 79.01-106.8%. EC_{50}

The antioxidant activity of hot water extract from whole mushroom is summerized in Table 1. Results are expressed as EC_{50} values for easier comparison of the results. EC_{50} is the lowest effective concentration related with antioxidant activity.

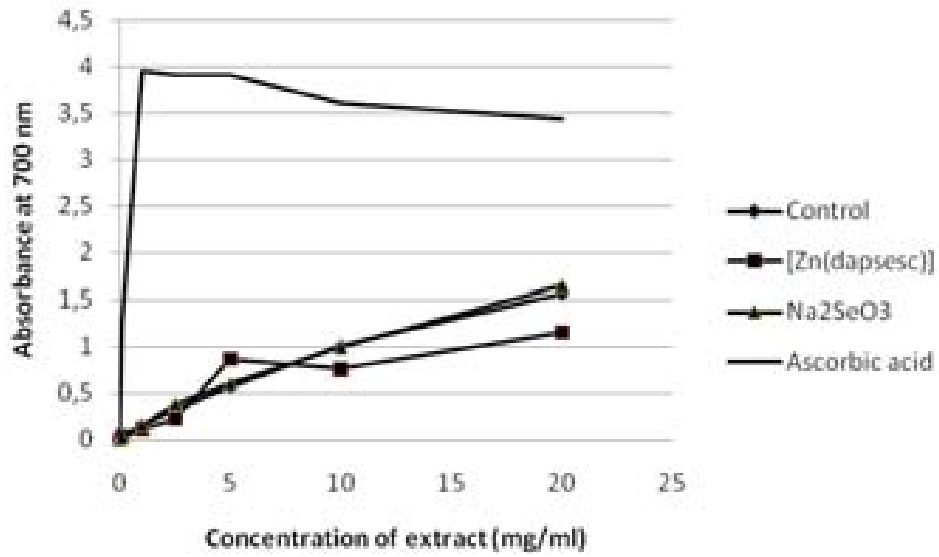

Fig. 2 – Reducing power of hot water extracts of whole mushrooms *L.edodes* L31. Each value is expressed as the mean ± standard deviation (n = 3).

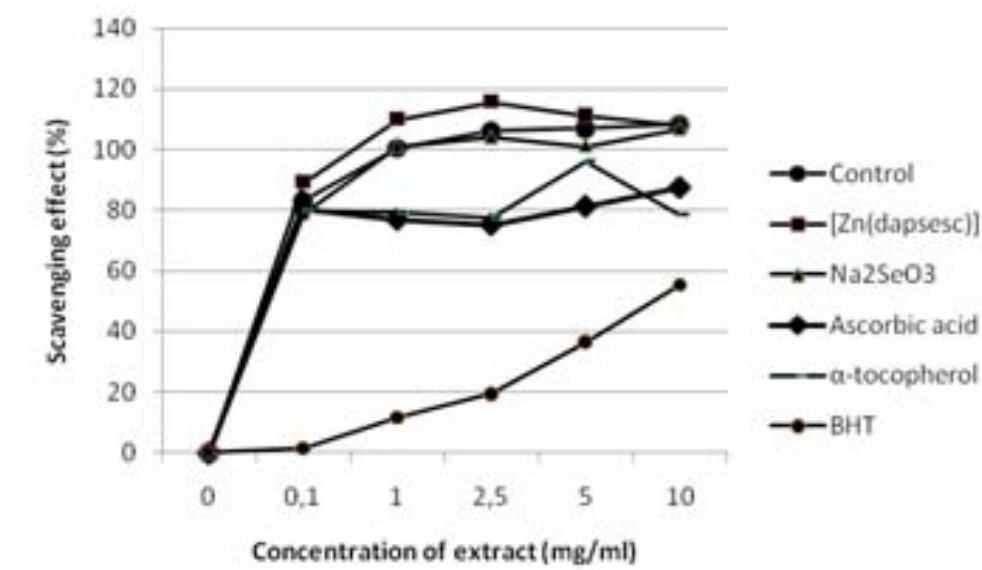

Fig. 3 – The possibility of binding DPPH radicals of hot water extract from whole mushrooms *L.edodes*. Each value is expressed as mean ± standard deviation (n = 3)

Table 1- EC_{50} values for the antioxidant activity of hot water extract from whole mushroom enriched with selenium

	EC_{50}[a] (mg/ml)		
	Antioxidant activity	Reducing power	Scavenging effect
Ascorbic acid	1.63±0.25[b]	<0.1	<0.1
α-tocopherol	< 0.1	NA	<0.1
BHT	NA[c]	NA	8.49±0.03
Lentinus edodes L31, control	12.02±0.09	>20	<0.1
Lentinus edodes L31 with inorganic selenium (50mg/kg Se)	5.1±0.06	>20	<0.1
Lentinus edodes L31 with organic selenium (15mg/kg Se)	3.79±0.04	>20	<0.1

a EC_{50} value: The effective concentration at which the antioxidant potential was 50%, the absorbance was 0.5 for reduction power, the power of neutralizing the DPPH radical was 50%. EC_{50} value was obtained by interpolation from linear regression analysis.
b Each value is expressed as the mean ± standard deviation (n = 3).
c NA: not analyzed

CONCLUSION

Mushrooms enriched with selenium are potential dietary supplements. Samples enriched with selenium from the organic sources showed significantly higher antioxidant activity than samples enriched with selenium from the inorganic sources. The results indicated that hot water extracts of whole mushrooms enriched with selenium showed good antioxidant activity at higher concentrations (10 mg/ml), regardless of the presence of molecules in the aqueous extract. The results of other studies indicate that the samples that have undergone dialysis showed higher antioxidant potential than extracts of whole mushrooms (Yu – Hsiu, 2008). From the results presented in the previous chapter, it is obvious that the reducing power of the control sample was 20-50% higher than the power of the enriched samples. Values of the samples enriched with inorganic selenium were slightly higher than those obtained from the samples enriched with organic selenium. Results of the previous studies show significant reducing capability of the aqueous extract from whole mushroom compared to the polysaccharide extracts (Yu – Hsiu, 2008). It is assumed that this is due to the presence of small molecules in the extract of whole mushrooms. Scavenging ability of both control and enriched fungus compared with positive tests was higher. Ascorbic acid and α-tocopherol showed the scavenging activity similar to that of fungi samples, while the BHT showed significantly lower activity, which differs from the results of the previous studies (Turley et al., 2010). It can be concluded that the hot water extracts have very good ability to bond DPPH radicals, similar to samples that passed the dialysis (Yu – Hsiu, 2008). Further research should include dialysis of the samples in order to remove small molecules from the extract. It is assumed that the hot

water extraction degrades polysaccharide molecules to smaller molecules that can later cause problems during sample analyses.

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АНТИОКСИДАТИВНА АКТИВНОСТ ВОДЕНОГ ЕКСТРАКТА ГЉИВЕ *LENTINUS EDODES* ОБОГАЋЕНЕ СЕЛЕНОМ

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Резиме

Shiitake (*Lentinus edodes*) припада групи медицински значајних и деликатесних гљива. Препознатљива је по својој лековитости, изванредном укусу и богатој ароми. Према традиционалној јапанској и кинеској медицини, гљива shiitake значајно повећава снагу и виталност организма. Shiitake садрже имуностимулансе, састојке који снижавају холестерол, спречавају зачепљење крвних судова, регулишу притисак, уравнотежују ниво шећера у крви, регулишу пробаву, побољшавају рад дисајних органа, делују антиреуматски и антиалергијски. Препорука је да се shiitake користе као укусна храна, превентива и лек, најчешће као зачин (сушене и млевене) или чај. Могу се конзумирати и као свеже припремљене.

Циљ рада био је да се разјасни да ли селен додат у супстрат за гајење гљиве *Lentinus edodes* утиче на редукциона својства екстракта, антиоксидативну активност екстракта, као и процена реактивности екстраката према радикалским врстама. Гљива је обогаћена селеном додавањем органских, Zn (II) комплекс са лигандом 2,6-диацетилпиридин бис (селеносемицарбазон), и неорганских једињења (Na_2SeO_3) селена у хранљиви супстрат на којем је гљива узгајана. Укупан садржај селена у плодоносном телу кретао се око 50 ppm за узорак обогаћен селеном из органског извора и 80 ppm за узорак обогаћен селеном из неорганског извора. Узорци су припремљени екстракцијом плодоносних тела у загрејаној води. Добијени резултати указују на то да водени екстракти целих гљива, како контролних тако и са додатком селена, имају добру антиоксидативну активност. Међутим, није примећена значајна разлика између узорака са и без садржаја селена.