ACCUMULATION OF SELENIUM FROM SELENIZED YEAST IN THE CULTIVATED MUSHROOM *AGARICUS BISPORUS*

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ABSTRACT

Agaricus bisporus (button mushrooms) are the most common mushrooms in Europe and on the world market. The nutritive value of that mushroom comes from its chemical composition. The ratio of protein, fats, minerals and amino acids is very suitable for human consumption. The purpose of our research was to increase the accumulation of selenium in mushrooms in order to meet the recommended human daily intake. Based on our previous research, it was determined that mushrooms are good accumulators of selenium from enriched substrate on which they are cultivated. In the study, Sel-Plex (Alltech) was used as a source of selenium. Sel-Plex is an organic source of selenium, selenized yeast which has the approval of the European Union. Sel-Plex application was done in three different ways, by adding compounds in freshly inoculated compost, compost casing layer and to the compost after raking, Sel-Plex was dissolved in water for irrigation, knowing that 1g of Sel-Plex contains 2 mg of selenium. The cultivation was made in the industrial conditions and the total selenium content was determined by optical emission spectrometry with induced coupled plasma, ICP-OES method. Control sample of mushrooms contained approximately 2.118 ppm of total selenium. We achieved the concentration in mushrooms which could meet the daily requirements for adults (50-200 ppm).

Keywords: *Agaricus bisporus*, ICP-OES, Selenium, Sel-Plex

INTRODUCTION

Mushrooms are often called "wild meat" because of the relatively high content of protein and essential amino acids. They have a suitable chemical composition and some medicinal properties, which are of increasing importance in human nutrition.

Agaricus bisporus have a great nutritional and culinary value for human consumption. They contain plenty of protein, less carbohydrates and negligible amounts of fat. Depending on the strain and production techniques these mushrooms contain approximately 5% of proteins which are composed of many essential amino acids. Mushrooms contain 3-4% carbohydrates. During digestion, the human body converts polysaccharides, starch and sugar to glucose known as blood sugar. It provides a much-needed energy for the brain and central nervous system [1].

Mushrooms uptake the necessary organic matter from the substrate on which they are grown through mycelium and brake down them into small components by their enzyme system [2]. Considering the fact that mushrooms contain a high percentage of protein, it is expected that they are able to incorporate high percentage of selenium into amino acids such as selenocistein

and selenomethionine. The content of selenium in mushrooms mostly ranges between 0.57 and 19.46 mg / kg, depending on the type, age and place of finding mushrooms [3, 4].

In nature, selenium exists in two main chemical forms: inorganic and organic. Inorganic forms of selenium are: selenite, selenate and selenide. In many countries, total content of selenium in soil ranges of 0.1 to 2 ppm [5, 6]. Low percent of selenium in foods is related to the endemic occurrence of cardiovascular diseases and disorders of bone and joint system, metabolic disorders and malignancies of the thyroid glands. Glutathione peroxidase containing selenium performs decomposition of hydrogen peroxide and lipid hydro peroxides in tissues and tissue fluids, and together with vitamin E protects cell membranes and other structures of peroxides, thereby inhibiting lipid per oxidation in tissues [7]. There are a few ways for selenium supplementation (selenized salt, pellet, injection), but it is thought that the best way to increase selenium content in the human body is by using plants. Some authors propose that the animals and humans absorb better organic source of selenium. Organic forms of selenium as selenium yeast, proved to be a good source of selenium. The company "Alltech" produced product called Sel-Plex, which is used in this research. The product is produced from yeast *Saccharomyces cerevisiae*, containing organically bound selenium in the form of selenomethionine. The purpose of our research was to try increase the accumulation of selenium in mushrooms in order to meet the human recommended daily intake [8].

MATERIALS AND METHODS

Materials. Sel-Plex (Alltech) was used in the experiment. One gram of Sel-Plex itself contains 1990 mg of selenium. The product is produced from yeast *Saccharomyces cerevisiae* CNCM I-3060, containing organically bound selenium in the form of selenomethionine. Sel-Plex is approved by European Commission, no 1750/2006, for the use in addition to animal feed.
The possibility of assimilation of selenium in the *Agaricus bisporus* fruit bodies was examined. The application was investigated in commercial mushroom growing farm Klraljevo, Serbia. Compost was inoculated with Sylvan spawn A 15. Content of moisture in the compost was aw= 0.68, nitrogen content=1.9% ammonia content=0.08%. The average weight of one bag was 19 kg.

Growing mushroom *Agaricus bisporus* (button mushroom). Seeded substrate was packed in plastic blocks which were sorted on shelves for the production of mushrooms. In the first stage of production (spawn running) the incubation temperature was 25 °C. The incubation period was 18-21 days. Thirteen days after spawing, the compost was covered by casing layer. Raking were done after seven days for the uniform grown of mushrooms. Fructification was at the temperature of 19° C. Mushrooms were harvested in 4 flushes.

Application of Sel-Plex. Sel-Plex was added to the substrate to reach Se concentrations of 35mg/kg of dry substrate, 70mg/kg of dry substrate and 150 mg/kg of dry substrate. Addition of selenium was carried out together with watering of the compost in three stages of mushroom production: after spawning, casing and after raking the casing layer.

Determination of the total selenium content in samples. The total selenium content was determined by using an optical emission spectrometer with induced coupled plasma, ICP-OES Thermo, ICA 6500 Duo. Samples were cut into small pieces and dried (80ºC, 24h). 300 mg of prepared sample was placed in a Teflon dish with 6 ml 65% HNO_3 and 2 ml 30% H_2O_2. The

sample was digested for 40 min (program 10). Subsequently, the sample was diluted with 25 ml of deionized water. Control sample was prepared in the same way. For the determination of total selenium, working solutions were prepared daily by appropriate dilution of 1mg/ml Se (IV) standard solution.

RESULTS AND DISCUSSION

The obtained results indicate that the fruit body of *Agaricus bisporus* mushrooms uptake the most selenium from substrate where it was added immediately after spawning nutrient substrate (see Table 1). The control sample of fruit body contained 3.9 mg/kg d.w. of total selenium. Mushroom samples which developed from substrate with addition of 35 mg/kg d.w. selenium, contained approximately 192.7 mg/kg of selenium, while the samples which were grown on substrate with 140 mg/kg d.w. selenium, contained almost twice. From these results we can conclude that fungus absorbed almost all selenium from the substrate, at the beginning of mycelia growth.

Table 1: Content of selenium in substrate and fruit body of *A.bisposrus* after supplementation of Sel-Plex at the begining of spawn running

Treatment	Se in substrate (mg/kg d.w.)	Se in fruit body (mg/kg d.w.)
Se (0ppm)a	0.4±0.1	3.9±1.1
Se(35ppm)	0.6±0.4	192.7±13.8
Se(70ppm)	6.1±2.3	275.2±3.2
Se(140ppm)	N.A.	353.2±7.5

a control
NA: not analyzed
Each value is shown as the mean ± standard deviation (n = 3).

However, in the experiment where selenium is added to the casing layer, selenium concentration in fruit body was not significantly different comparing to the control samples, except in the case when the higher concentrations were added (see Table 2). Based on these results and the results obtained from the sample in which the selenium were added to the substrate after spawning, it may be concluded that the fungus adopts a greater amount of selenium in early stage of development. So it is supposed that for higher accumulation of Se complete development of mycelium is needed

Table 2: Content of selenium in substrate and fruit body of *A.bisposrus* after supplementation of Sel-Plex in casing layer

Treatment	Se in substrate (mg/kg d.w.)	Se in fruit body (mg/kg d.w.)
Se (0ppm)a	0.1±0.1	4.2±0.8
Se(35ppm)	10.0±0.6	5.4±0.2
Se(70ppm)	13.1±1.2	13.8±1.3
Se(140ppm)	34.9±1.9	28.4±3.6

a control
Each value is shown as the mean ± standard deviation (n = 3).
NA: not analyzed

In Table 3 the results obtained by adding selenium into the casing layer after raking are presented. The control sample contained 2.1 mg/kg of selenium. Samples uptaked more selenium in fruit bodies than samples that were grown on substrate with addition of selenium into casing layer. The reason for that is still unknown and it should be discovered in the future experiments.

Table 3: Content of selenium in substrate and fruit body of *A.bisposrus* after supplementation of Sel-Plex after raking

Treatment	Se in substrate (mg/kg d.w.)	Se in fruit body (mg/kg d.w.)
Se (0ppm)a	1.3±0.1	2.1±1.2
Se(35ppm)	28.4±1.4	19.1±2.1
Se(70ppm)	32.3±0.7	28.1±2.4
Se(140ppm)	40.8±1.5	71.4±0.8

a control
Each value is shown as the mean ± standard deviation (n = 3).
NA: not analyzed

CONCLUSION

Selenium enriched mushrooms are good potential dietary supplement. The results of this study showed that it is possible to grow mushrooms on a substrate enriched with selenium from organic compounds. Negative impact on the growth of mushrooms, yield of mushrooms and time delay were not noticed. The big difference in the content of selenium in fruit bodies depending on the way and time of selenium application and should be explained by the fact that selenium retained in cover. Based on these results, it can be concluded that the product Sel-Plex can be efficiently used as a potential supplement in the production of mushrooms in order to obtain selenium enriched mushrooms. It is necessary to investigate the selenium metabolism in the fruit body. It is necessary to consider the possibilities for using mushrooms with the addition of selenium as a dietary supplement, and as potential functional food in order to correct a deficiency in the human diet. Also, results of our new experiments suggest that Sel-Plex has an inhibiting effect upon the growth of some fungi that occur in the production of mushrooms *Agaricus bisporus* as contaminant, which is another advantage of using this form of selenium (unpublished results).

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REFERENCES

[1] Zs. Stefanka et al. (2001). Comparison of sequential sample preparation methods for the Se-enriched Agaricus bisporus Second International Conference on Trace Element Speciation in Biomedical, *Nutritional and Environmental Sciences, Talanta 55*: *437*-447.

[2] Borovička J. & Randa Z. (2007). Distribution of iron, cobalt, zinc and selenium in macrofungi. *Mycol. progress* 6:249–259.

[3] Falandysz J. (2008). Selenium in edible mushrooms. *Journal of Environmental Science and Health*, Part C, 26: 256-299.

[4] Savic M. et al. (2009). Growth and fruit body formation of *Pleurotus ostreatus* on media supplemented with inorganic selenium. *Matica srpska proceesings for natural sciences*,116:7-14.
[5] Jianan T. et al. (2002). Selenium in soil and endemic diseases in China. *The science of the total environment*, 284: 227-235.
[6] Klapec, T et al. (1998). Daily dietary intake of selenium in eastern Croatia. *The science of the total environment,* 217: 127-136.
[7] Gromadzinska, J. et al. (2008): Selenium and cancer: biomarkers of selenium status and molecular action of selenium supplements. *Eur J Nutr* 47 (suppl 2):29–50
[8] Sotirov L. et al. (2007). Sel-Plex® And Sodium Selenite Dietary Supplements With Resulting Serum Lysozyme And Complement Activities In Sows And Progeny During Post Partum Periods. *Trakia Journal of Sciences*, 5: 20-27.