Effects of selenium enriched *Lentinus edodes* on SELENOP and GPx activity

IVAN MILOVANOVIĆ[1*]

[1]*Innovation Centre of the Faculty of Technology and Metallurgy, Karnegijeva 4, 11000 Belgrade, Serbia*
**Corresponding author: email: imilovanovic@tmf.bg.ac.rs*

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The objective of this study was to investigate the influence of enriching the common mushroom species *Lentinus edodes* with inorganic selenium (Se) at different harvest stages, while comparing two extraction methods and evaluating their i*n vivo* effects on HepG2 liver cells. Selenoprotein (SELENOP) quantification and glutathione peroxidase (GPx) activity were used as key outcome measures. Results indicated that the first flush was more effective, increasing SELENOP levels to 72.4 μg L^{-1}. Enzymatic treatment demonstrated higher efficiency, yielding GPx activities of 62.0 mU mg^{-1} protein. When comparing treatments, GPx activity was highest during the second flush. Overall, the findings reveal subtle differences related to harvest timing, extraction method, and their impact on both toxicity and anabolic potential in promoting hepatic selenoprotein P biosynthesis. These results suggested that selenium-enriched mushrooms may represent a promising option for dietary supplementation.

Keywords: Selenium-enriched *Lentinus edodes*; HepG2 cells; Selenoprotein P; glutathione peroxidase

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1. INTRODUCTION

Selenium (Se) is an essential micronutrient, which plays an important role in human health, including proper thyroid hormone metabolism, cardiovascular health, prevention of neurodegeneration, cancer, inflammation, and immunity. Through its incorporation into selenoproteins and selenoenzymes, Se is involved in the activation, proliferation, and differentiation of cells that drive innate and adaptive immune responses (Demircan et al., 2021). In more cases, epidemiological studies have demonstrated the effects of Se-deficiency or Se-supplementation on various types of cancer and cardiovascular disease (Schomburg, 2022). Furthermore, Se chemical forms determine its biological function, metabolism, toxicity, and potential applications. In particular, inorganic forms of Se are highly toxic, whereas organic forms are less toxic and have good bioavailability in humans compared with inorganic selenium salts (Falandysz, 2013; Tsivileva and Perfileva, 2017). The nutritionally recommended dose of elemental selenium is estimated to be 50-70 micrograms per day (Institute of Medicine, 2000).

Mushrooms are capable to uptake and accumulate inorganic Se salts and metabolize it to organic Se compounds (Kalač, 2010). Nowadays, special efforts are made with cultivated mushrooms due to their Se-accumulating potential and facility to be utilized as Se-fortified food. In addition to the established link between Se deficiency and cancer prevention, adequate Se intake provides a range of other important health benefits. Particularly good selenium accumulators among others are *Agaricus bisporus*, *Lentinula edodes*, and *Pleurotus* spp. Specific Se-compounds from higher fungi have been linked to beneficial health effects, and in disease prevention due to their positive effects on the immune system and inhibitory effect on tumor cell growth, DNA, RNA and enzyme activity in transformed cells (Xu et al., 2021).

This study examines the ability of Se-exposed fruiting body of *Lentinus edodes* for supporting selenoprotein expression and GPx activity in hepatocytes.

2. MATERIALS AND METHODS

2.1. Mushroom preparation

The basidiocarp of *Lentinus edodes* (Berk.) Pegler, strain ICTMF301 used in this study, was prepared in several steps: *(1)* cooking of 100 g of wheat grain for 30 minutes and supplemented with 2 g of $Ca_3(PO_4)_2$ and 0.5 g of $CaCO_3$; *(2)* sterilization at 121 °C for 15 min; *(3)* inoculation with 25 mycelial disks (Ø 0.5 cm) obtained from 7-day-old culture; *(4)* 1 kg of plant sawdust was placed into the polypropylene bags and autoclaved at 121 ºC for 2 h; *(5)* 5 mL of sterile Na_2SeO_3 solution, at a concentration of 1.0 g Se L^{-1}, was added into bags; *(6)* bags were inoculated at 22 °C in the dark for 4 weeks; *(7)* a total of four flushes (cycles) were obtained, corresponding to four

fruiting bodies (one fruiting body per flush) over a period 95 days; *(8)* all mushrooms samples were lyophilized and ground to a fine powder.

2.2. Preparation of the mushroom extracts

The mushroom water and enzyme extracts, were prepared in several steps: *(1)* mixture of 5.0 mL of water and 20 mg of each mushroom sample were thoroughly homogenized by mechanical shaking in a water bath at 37 °C for 20 h; *(2)* mixture of 125 μL of proteinase K (10 mg mL^{-1}) and 5.0 mL of sodium phosphate buffer (100 mM, pH of 7.5) and 20 mg of each mushroom sample were thoroughly homogenized for 20 h in a water bath at 37 °C; *(3)* centrifugation at 2000 rpm for 2 min and filtration through 0.2 μm syringe filters; *(4)* enzyme sample was heated at 95 °C for 10 min in order to inhibit proteinase K.

2.3. Cell culture and cell viability assay

The human hepatocellular carcinoma cell line HepG2 (ACC 180 German Collection of Microorganisms and Cell Cultures (DSMZ)) was used in this study. Cells were prepared in several steps: *(1)* maintained in DMEM/F12 medium supplemented with 10% fetal bovine serum in tissue culture flasks at 37 °C and 5% CO_2; *(2)* culture medium was exchanged at 3-day intervals and cells were passaged on a weekly basis, using a 1:5 splitting ratio; *(3)* cells were counted and seeded at a concentration of 5×10^6 cells per T75 cell culture flask and grown to 70% confluence; *(4)* after 24 h, all medium and supernatant were removed and cells were washed twice with PBS and treated with 200 μL of water/enzyme mushroom extracts in serum-free medium; *(5)* culture media was collected and analyzed for SELENOP concentrations after 72 h of incubation; *(6)* cells were incubated in the presence of MTT (100 μL of 5 mg mL^{-1}) and analyzed for the ability to generate purple formazan crystals; *(7)* absorption was measured using a microplate reader at a wavelength of 570 nm and the ratio of viable cells was calculated.

2.4. Assessment of selenoprotein P (SELENOP) activity

Concentration of selenoprotein P (SELENOP) in cell medium was analyzed by Western Blot or dot blot analysis, and by an immunoluminometric sandwich assay. Dot blot analyses were performed based on the supernatants of HepG2 cells after seeding 1.5×10^6 cells/3 mL medium into T25 cell culture flask. After 24 h of adhesion time, cells were treated with the enzyme mushroom extracts in serum-free medium for a time period of 72 h. Afterwards, 100 μL of the conditioned culture media was blotted directly onto nitrocellulose membranes by a dot blot device (Bio-Dot SF Microfiltration Apparatus, BIO-RAD). Western blot analysis was conducted after precipitation of the proteins from cell culture supernatants. Protein pellets were resuspended in 1/10 the original volume, and equal volumes were added per lane. Proteins were separated by SDS-PAGE, blotted onto nitrocellulose membranes (Amersham Biosciences, Freiburg, Germany), and visually verified for uniform loading and complete transfer by Ponceau S staining of the membrane. Selenoprotein detection was achieved with monoclonal anti-SELENOP antibodies, as described (Hybsier et al., 2017). HPR-conjugated secondary antibodies (Sigma) were used in combination with an ECL-based detection system (Thermo Scientific Pierce, Schwerte, Germany) and X-ray films (Amersham) to visualize the Western and dot blot signals. Quantification was achieved by densitometry of the signals using the ImageJ software (National Institutes of Health). In addition, selenoprotein concentrations were directly determined from the cell culture supernatants by an immunoluminometric sandwich ELISA (selenOtest, selenOmed GmbH, Berlin, Germany).

2.5. Assessment of glutathione peroxidase (GPx) activity

The enzymatic activity of GPx was determined from HepG2 cell homogenates after stimulation with the enzymatic extracts from Se-treated mushrooms. Briefly, cells were seeded, cultivated, washed with PBS and exposed to water and enzyme aliquots in serum-free medium in cell culture flask as described above. After 72 h, cells were collected, washed, homogenized and analyzed in duplicate by a coupled photometric enzymatic test using *t*-butylhydroperoxide as substrate according to the procedure previously described by Flohé and Günzler, (1984).

3. RESULTS AND DISCUSSION

3.1. Effects on SELENOP biosynthesis

The observed SELENOP concentrations into the cell culture supernatant are shown in the Figure 1. Our data indicated positive effects of the aliquots from Se-enriched mushrooms on SELENOP expression in HepG2 cells on general selenoprotein biosynthesis. Under the experimental conditions, the SELENOP concentrations were in the range from 41.2 μg L^{-1} to 59.8 μg L^{-1} for the water extracts, and from 25.2 μg L^{-1} to 72.4 μg L^{-1} for the enzymatic extracts. The tested aliquots elicited specific extraction- and harvesting dependent effects on SELENOP concentrations. For the water aliquot, the second flush, and for the enzyme extraction, the first flush apparently showed better bioactivity and more efficiently increased SELENOP production.

SELENOP functions as the primary protein responsible for selenium transport and distribution within the human body, contributing critically to selenium metabolism and antioxidative defense mechanisms (Schomburg, 2022). Given the reason that liver is main organ for Se metabolism and SELENOP biosynthesis, the use of hepatocyte (HepG2) cells seems to be the rational choice and most suitable system for SELENOP analysis. This concept was confirmed in our previous study. Thus, Se-enriched *Ganoderma lucidum* and *Pleurotus ostreatus* mushrooms extracts significantly increased SELENOP expression, with values ranging from 13.0 - 68.1 μg L^{-1} (Milovanovic et al., 2024). Interestingly, Se-methionine (Se-Met) was the major Se constituent directly responsible for higher SELENOP yield. Therefore, the organic Se-Met form can be considered as a more promising food component, with lower toxicity and a greater capacity to support the biological potential of SELENOP biosynthesis. Similar results were obtained by Hu et al. (2010). Namely, Se-enriched yeast extracts enhanced SELENOP expression in a rodent model; Se was present mostly as Se-Met (70-80%). Com-

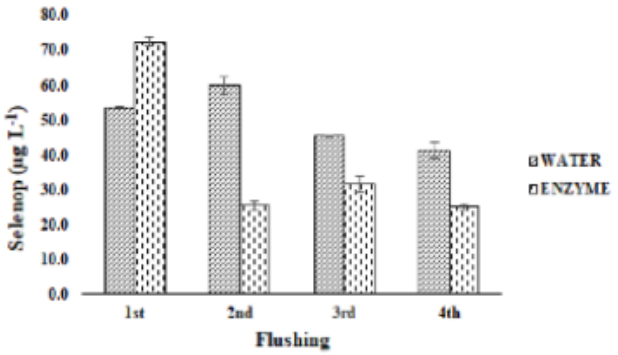

Figure 1. SELENOP expression in culture cells (indicated by concentration of Selenoprotein P) following treatment with extracts of *Lentinus edodes*. The graphs show data of four mushroom samples at different harvesting stages for comparison. The cultured cells were treated with the enzymatic and water extract of the mushroom.

pare to previous study it seems that Se enhanced *L. edodes* is suitable mushroom for SELENOP synthesis (Hu et al., 2010).

3.2. GPx activity

The effects of aliquots from the Se-exposed *L. edodes* on GPx activity are shown in the Figure 2. Following 72 hours of treatment, GPx activity in HepG2 cells exposed to extracts from Se-enriched mushrooms was significantly higher compared with control samples. Extracts from Se-enriched mushrooms showed significantly higher values compared with the control samples. In addition, enzymatic treatment of the mushroom samples yielded extracts with greater efficiency, resulting in GPx activities ranging from 31.9 to 62.0 mU mg^{-1} protein, which were slightly higher than those obtained from water-treated extracts, yielding 41.0–57.3 mU mg^{-1} protein. The direct comparison of the extracts from different harvest times, indicated that the efficiency for inducing GPx activity was highest in the 2nd flush.

Glutathione peroxidase is the selenoenzyme with peroxidase

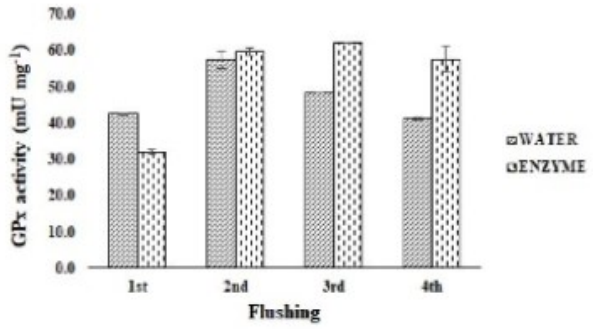

Figure 2. GPx activity in culture cells following treatment with extracts of *Lentinus edodes.* The graphs show data of four mushroom samples at different harvesting stages for comparison. The cultured cells were treated with the enzymatic and water extract of the mushroom.

activity relevant to mitigation oxidative damage caused by free radicals. Multiple analyses indicated that selenium is required for optimal GPx activity and can additionally strengthen its enzymatic function (Lubos et al., 2011). While Se concentrations in some aliquots were not sufficient to cause GPx synthesis, clinical studies in animals and humans trials revealed that organic Se intake from different Se sources influenced GPx enzyme activity differently even at the same dose (Maseko et al., 2014). According to Behne et al. (2009), intraperitoneal administration of selenized yeast resulted in elevated GPx activity in rodents, whereas supplementation with Se-Cys or selenite elicited a distinctly different response. Similar positive correlation of GPx was recorded in mouse colon trials as dietary supplementation with Se-enriched yeast (Hu et al., 2010). Significantly higher blood GPx activity in mice vs control sample was noticed after oral administration of Se-treated mushrooms *P. ostreatus* (Yan and Chang, 2012) and *A. bisporus* (Maseko et al., 2014). Similar to this study, Milovanovic et al (2024) established positive relationship between Se-enriched *G. lucidum* and *P. ostreatus* mushrooms and GPx activity. Namely, both selenium forms, selenite and selenate and both enzyme and water extracts induce GPx activities, ranging from 16.1 to 54.7 mU mg^{-1} protein. It can be assumed that Se-enriched *L. edodes* mushroom is a good choice for increasing GPx activity in *in vitro* and *in vivo* studies.

4. CONCLUSION

This study demonstrated that the application of exogenous Se significantly promoted the production of Se-biofortified edible mushroom of *L. edodes*. Among all treatments, SELENOP synthesis was most efficient in the first flush, while GPx activity reached its peak during the second flush. The supply of growing mushrooms with sodium selenite is a promising strategy for generating Se-enriched food and cell culture-based analysis of selenoprotein biosynthesis for nutritional purposes. With this study, quality and reproducibility of the fortification process can be monitored, and further attempts of improving yield and standardize production can be carried out.

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CONFLICT OF INTEREST

The authors declare that they have no financial and commercial conflicts of interest.

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