

Review Article

Selenium-Fortified Mushrooms - Candidates for Nutraceuticals?

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***Corresponding author:** Witkowska AM, Department of Food Commodities Science and Technology, Medical University of Białystok, ul. Szpitalna 37, 15-295 Białystok, Poland**Received:** August 04, 2014; **Accepted:** November 07, 2014; **Published:** November 11, 2014**Abstract**

Nutraceuticals represent products developed from food sources, which offer health benefits including the prevention and treatment of chronic diseases. Cultivated mushroom fruit bodies or mycelia allow to derive various forms of products which can be used in medicinal preparations or as nutraceuticals. In recent years fungi attracted much attention due to their selenium-accumulating potential and facility to be utilized as Se-fortified food. They can absorb inorganic selenium from the substrate and convert it to organic selenium compounds, which are less toxic and better bioavailable in humans than the inorganic selenium salts. Cultivated mushrooms are usually poor in selenium, while these grown on substrates enriched in selenium may incorporate even up to 1300 µg Se/g dw under experimental conditions. Particularly good selenium accumulators among others are *Agaricus bisporus*, *Lentinula edodes*, *Pleurotus ostreatus*. Considering the recommended daily selenium intake of 55 µg Se/d, the consumption of high selenium mushrooms should be limited to very small amounts. In view of the data regarding adverse effects observed in clinical trials during selenium supplementation, preferably selenium inadequate populations may benefit from selenized fungi and mushroom products.

Keywords: Selenium; Mushrooms; Nutraceuticals**Abbreviations**

GPx: Glutathione Peroxidase; HDL: High Density Lipoprotein; IDD: Iodothyronine Deiodinases; LDL: Low Density Lipoprotein; PCa: Prostate Cancer; RDA: Recommended Dietary Allowance; TrxR: Thioredoxin Reductase.

Introduction

The term 'nutraceutical' is a combination of two words: 'nutrition' and 'pharmaceutical'. This means that nutraceuticals represent products developed from food sources, which offer health benefits including the prevention and treatment of chronic diseases [1]. Nutraceuticals are marketed mostly in medicinal forms as pure nutrients (vitamins, minerals), herbal products, food ingredients or food. Cultivated mushroom fruit bodies or mycelia allow deriving various forms of products which can be used in medicinal preparations or as Nutraceuticals. These forms include fresh or pulverized dried mushrooms, mycelial biomass, extracts of mycelium or culture broth.

Controversies over selenium supplementation

Selenium is an essential element for humans and animals. It exerts its physiological effects through a number of metabolic pathways, which in humans involve 25 selenoproteins [2]. Glutathione Peroxidases (GPx), Thioredoxin Reductase (TrxR) and Iodothyronine Deiodinases (IDD) are the main antioxidant selenoproteins. Severe selenium deficiency has been connected to congestive cardiomyopathy (Keshan disease) [3] and chronic endemic osteochondropathy (Kashin-Beck disease) [4]. Recently, increased incidence of colorectal cancer has been attributed to low selenium status in European populations [5]. Dietary recommendations for selenium intake vary between countries, yet the US and EU

recommendations are consistent. The US Institute of Medicine, Food and Nutrition Board and the European Scientific Committee on Food of the European Commission recommend 55 µg Se/d [6,7]. This value was derived from Chinese studies, which demonstrated that a 52 µg/d selenium dose was able to maximize plasma Glutathione Peroxidase (GPx). According to experts the Recommended Dietary Allowance (RDA) for selenium should not be exceeded, because it may be deleterious for human health. In the US population high serum selenium concentrations were associated with increased total and LDL-cholesterol, while increased HDL-cholesterol was found only at low selenium level, up to 120 ng/ml [8]. Serum selenium levels in excess of 130 ng/ml may possibly be associated with increased overall mortality [9]. What is more, results of clinical trials suggest adverse effects of selenium supplementation on incidence of cardiovascular disease, diabetes and cancer. The Selenium and Vitamin E Cancer Prevention Trial (SELECT) showed no preventive effect of 200 µg/d selenoamino acid L-selenomethionine alone or in combination with vitamin E on the incidence of prostate cancer (PCa) [10]. But another report which used toenail clippings of SELECT volunteers as a measure of long-term selenium status established that selenium supplementation had no effect in men with low selenium status, but raised the risk of high-grade PCa in those with high baseline selenium status [11]. The conclusions drawn from this latter survey emphasize unfavorable effects of selenium supplementation in men at doses exceeding recommended daily intakes. With respect to primary prevention of cardiovascular disease and type 2 diabetes, a long-term supplementation with high-selenium baker's yeast providing 200 µg Se/d was unsuccessful to demonstrate any beneficial effect [12,13]. It was also suggested that selenium supplementation may increase incidence of diabetes in selenium-adequate population

(adequacy established by the author on the basis of serum selenium concentration). Animal studies have thrown light on the mechanisms behind selenium supplementation and diabetes. In these studies high intakes of selenium caused depletion in chromium levels and contributed to lipolysis in adipose tissue, which caused an influx of fatty acids in the rat liver [14]. These processes initiated metabolic reactions leading to increased mitochondrial Reactive Oxidative Species (ROS) generation and as a result weakened insulin signaling. Recently, the excess dietary selenium and increased mushroom consumption were suggested to be independent factors associated with an elevation in blood glucose, while high intakes of both were linked to increased risk of diabetes [15]. These findings raise concerns about advising on selenized mushrooms consumption or taking supplements of selenium-enriched fungi in the populations which are selenium-adequate. Selenium is present in soil, water and food sources. Some geographical regions, however, are selenium-deficient, including certain parts of Europe, New Zealand, some areas of China, what translates into reduced selenium in food. In these terms use of selenium-containing products may possibly be an option for selenium-depleted populations to improve selenium status.

Selenium-accumulating potential of mushrooms

For a long time mushrooms were considered valuable sources of nutrients, especially protein, some vitamins and minerals. They are low-fat what can be a major advantage in terms of formulation of dietary supplements. Similarly to yeast, mushrooms can absorb inorganic selenium from the substrate and convert it to organic selenium compounds, which are less toxic and better bioavailable in humans than the inorganic selenium salts [8,16,17]. Yeast has been widely cultivated for nutraceutical purposes. In recent years, however, fungi attracted more attention due to their selenium-accumulating potential and facility to be utilized as Se-fortified food. Fungi can absorb selenium with different accumulating efficiencies. Wild-growing edible mushrooms from unpolluted areas have a potential to accumulate from <0.5 to >20 µg Se/g, and members of *Boletaceae* family are particularly remarkable Se-accumulators [18]. Cultivated mushrooms are usually poor in selenium (from 0.01 to about 4 µg Se/g dw) [19-21], while these grown on substrates enriched in selenium may incorporate even up to 1300 µg Se/g dw under experimental conditions [22] (Table 1). Particularly good selenium

accumulators among others are *Agaricus bisporus*, *Lentinula edodes*, *Pleurotus ostreatus*. By contrast, fruit bodies of *Ganoderma lucidum* have a low, ~30% potential to incorporate Se from a substrate [23]. Accumulation of selenium from selenized substrates is dose-dependent [24]. Depending on a dose, selenium added to a substrate stimulates growth of mushrooms or mycelia. High doses of selenium, however, may be toxic to mushrooms and have the opposite effect. Da Silva et al. [25] established that selenium added to culture media of *Pleurotus ostreatus* and *P. eryngii* caused macro- and microscopic changes in mushrooms' morphology, mainly diminished fungal growth rate, diameter of hyphae and distance of septum, and it also modified the color of colony. Malinowska et al. [26] demonstrated that low concentration of sodium selenite in growth media at a dose of 25 mg/L had no influence on white-rot fungus *Hericium erinaceum*, while higher doses caused a significant decrease in mycelial growth. In oyster mushroom *P. ostreatus* high selenium dosage at concentrations above 12.8 mg/kg substrate was found not only to reduce mycelial growth, but it also influenced mushroom's shape (larger stipes and smaller caps) and selenium concentrations [24]. In contrast, *A. bisporus* can be cultivated at high selenium concentrations (approx. 1.26 M sodium selenite) not showing negative effects in mushroom quality and crop yield [27].

Considering the recommended daily selenium intake of 55 µg Se/d, the consumption of high selenium mushrooms should be limited to very small amounts. Only ~30g of fresh shiitake mushrooms *L. edodes* or 0.32 g dried mushrooms can provide daily dose of selenium [24]. For *P. ostreatus* this amount equalled to 1 g dried mushrooms [28], and for white button mushroom *A. bisporus* – 180 g fresh mushrooms [29]. Selenium-enriched mushroom polysaccharide may also be used as a source of bioselenium. Intra- and extracellular polysaccharide from submerged cultures of *H. erinaceum* were found to supply 4.89 and 4.69 mg Se/g respectively [26]. By converting these amounts to the RDA for selenium, approximately 10 mg of Se-containing polysaccharide would be sufficient to provide the optimal selenium intake.

Cultivation of selenized mushrooms and mycelia

Saprobic mushrooms can be cultivated on various growth substrates. In Brazil, which is the leading coffee-producing country, attempts had been made to utilize coffee husks as a substrate for oyster

Table 1: Examples of mushroom fruit bodies or mycelia enriched in selenium.

Mushroom species	Form	Method of cultivation and Se supplementation to mushrooms	Selenium accumulation	Reference
<i>Agaricus bisporus</i>	fungus	compost, Na ₂ SeO ₃ at concentrations 30-300 µg Se/g dw	max. 1300 µg Se/g dw	[22]
<i>Agaricus bisporus</i>	fungus	compost, selenized yeast, 10 mg Se/kg compost	770 µg Se/g dw	[37]
<i>Hericium erinaceum</i>	mycelium	submerged cultivation, Selol (selenitetriglycerides) containing 50-500 µg/mL	1,560-12,170 µg Se/g dw	[26]
<i>Lentinula edodes</i>	fungus	compost, selenized yeast, 10 mg Se/kg compost	46 µg Se/g dw	[37]
<i>Lentinula edodes</i>	fungus	substrate of 78% eucalyptus sawdust; cold shock in water containing Na ₂ SeO ₃ at concentrations 0.08-0.64 mM	max. 170 µg Se/g dw	[24]
<i>Lentinula edodes</i>	mycelium	submerged culture enriched in Na ₂ SeO ₃ at concentrations 0-20 µg/mL	Se content in mycelia 23-1,800 µg/g dw, selenomethionine content in cultivated biomass 23-289 µg/g dw	[45]
<i>Pleurotus florida</i>	fungus	selenium-rich wheat straw containing approx. 28 µg Se/g dw	141 µg Se/g dw	[46]
<i>Pleurotus ostreatus</i>	fungus	coffee husk substrate, Na ₂ SeO ₃ at concentrations 3.2-102 µg/g	58-858 µg Se/g dw	[28]
<i>Pleurotus ostreatus</i>	mycelium	synthetic medium, Na ₂ SeO ₃ at concentrations 5-20 µg/mL	251-939 µg Se/g dw	[47]
<i>Stropharia rugoso-annulata</i>	mycelium	submerged culture enriched in Na ₂ SeO ₃ at concentrations 10-150 µg/mL	max. 4,728 µg Se/g dw	[33]

mushroom *P. ostreatus* [28]. Selenium-rich agricultural residues from seleniferous areas offer appropriate substrates to obtain selenium-fortified mushrooms. In a study by Bhatia et al. [30] five strains of *Pleurotus* genus cultivated on the selenium-hyperaccumulated wheat straw absorbed selenium in a range from about 20 to 140 µg Se/g dw. In this study oyster mushroom *P. ostreatus*, one of the most commonly cultivated mushrooms, accumulated the 13-fold selenium content of control mushrooms (44.3 µg Se/g dw).

Typical selenium chemical forms which have been used for mushroom fortification were sodium selenite, selenomethionine and selenized yeast. One study used selenitriglycerides containing 2-20% selenium to observe biosynthesis of selenium-containing intra- and extracellular polysaccharides [26]. These selenitriglycerides, semisynthetic Selol, is sunflower oil estrified with selenous acid (H₂SeO₃). In contrast to selenite, selenitriglycerides do not present cumulative toxicity or mutagenicity [31].

In contrast to mushrooms cultivated on solid substrates, submerged cultivation of mycelium offers higher percentages of selenium accumulation and shorter periods of production. Though particular mycelia can absorb selenium from cultivation media at different rates. Selenium content of eight mushroom species subjected to submerged cultivation in sodium selenite-enriched medium, resulted in Se concentrations from 1.4 µg Se/g dw in *P. eryngii* to 20.3 µg Se/g dw in *P. ostreatus* [32]. This latter species was characterized by a more than 60% potential to absorb Se from the medium. Mycelia can absorb much more selenium than it was mentioned for fungi. This can be incorporated not only into selenoproteins, but it may also be found in polysaccharide fraction [33].

Chemical forms of selenium found in mushrooms

Various chemical forms of selenium can be found in mushrooms. In mycelia of *Stropharia rugoso-annulata*, more than 60% of selenium provided proteins, 18% - polysaccharides, 1% - nucleic acids, and the remaining 19% - other compounds [33]. Like in mycelia of *S. rugoso-annulata*, selenium content in fungus *G. lucidum* was generally bound to proteins - 56-61% and polysaccharides - 11-18% [23]. Protein speciation of wild edible mushroom water extracts showed that selenomethionine was the major compound that was accompanied by a number of unknown low molecular weight selenocompounds [34]. While selenium-enriched mushrooms contain mostly selenocystine, selenite, selenomethionine, methyl-selenocysteine and several unidentified selenocompounds [35-37]. The main selenoamino acid of water-soluble proteins of button mushroom *A. bisporus* cultivated in growth compost irrigated with 40 mg Se/l as sodium selenite was found selenocystine [38]. Other selenoamino acids such as protein-bound selenomethionine and non-protein methyl-selenocysteine were detected in small concentrations. Selenomethionine in commercial mushrooms has been found in its free form, while in selenized mushrooms it was incorporated into proteins [29].

Selenium bioavailability from mushrooms

The amount of data available from selenium bioavailability studies is very sparse for fungi. Earlier reports documented low selenium bioavailability from mushrooms to rats and humans [39,40]. Recently, animal studies demonstrated increased GPx-1 and GPx-2 gene expression and colon GPx-1 enzyme activity in

rats fed selenium-enriched *A. bisporus* [41]. Whereas other study established that rats fed selenium-fortified *P. ostreatus* mushrooms showed higher plasma selenium concentrations than these fed with sodium selenate [42]. Generally, selenium bioavailability is higher from organic sources, but in mushrooms it depends not only on the chemical forms of selenocompounds, but it is also dependent on mushrooms' digestibility. Like it was previously mentioned, part of selenium in mushrooms is bound to polysaccharides. A structural polysaccharide of cell walls in fungi is chitin. Chemically chitin is aminopolysaccharide, N-acetyl glucosamine, which is not digested by gastrointestinal enzymes. For that reason selenium content bound to chitin may possibly be not available to humans [43].

Conclusion

In summary, cultivated mushrooms and mycelia can be successfully enriched to meet selenium RDA. Higher Se doses than the recommended, however, appear to be unsafe, therefore should be avoided. In addition to this, there are indications from only very few, but recent studies, that selenium bioavailability from mushroom sources is not as low as it was claimed before and should be carefully examined in more detailed researches. Some authors emphasize that selenized mushrooms supplying at least 20% RDA could be marketed as an excellent source of selenium, while doses exceeding the RDA are not recommended [44]. In view of the data regarding adverse effects observed in clinical trials during selenium supplementation, preferably selenium inadequate populations may benefit from selenized fungi and mushroom products.

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