



Research Status and Application Prospects of Selenium-Enriched Shiitake Mushrooms

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Abstract

Shiitake mushrooms possess extremely high edible and medicinal value. Selenium is of great importance to human health, and research on selenium-enriched shiitake mushrooms has broad application prospects. The research status of selenium-enriched shiitake mushrooms was elaborated with respect to the metabolism of selenium in shiitake mushroom bodies, the selenium-enrichment approaches and capacity of shiitake mushrooms, the strain breeding of selenium-enriched shiitake mushrooms, cultivation techniques and product development, and the existing problems, future development directions and application prospects were also discussed.

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1. Introduction

Selenium is regarded as one of the essential trace elements for the human body and can prevent and treat tumors, cancers and other diseases. The selenium content in the diet is markedly influenced by geographical and environmental factors, and a large proportion of the Chinese population is in a selenium-deficient state. Shiitake mushrooms occupy an important position in the human dietary structure; they can convert inorganic selenium into the organic selenium required by the human body, thereby promoting human absorption and utilization. Consequently, the production of selenium-enriched shiitake mushrooms by biotransformation has become a research hotspot both at home and abroad. This paper analyzes and summarizes the strain breeding and preparation, cultivation techniques and application status of selenium-enriched shiitake mushrooms in recent years, with a view to providing a reference for future research, development and utilization.

2. Metabolism of Selenium in Shiitake Mushrooms

2.1. Metabolic Mechanism of Selenium in Shiitake Mushroom Cells

The cell wall of shiitake mushrooms contains a variety of ionic groups such as sulfhydryl groups and amino groups, making adsorption one of the ways for shiitake mushrooms to enrich selenium. The possible metabolic mechanism of selenium in shiitake mushroom cells is as follows. The shiitake mushroom mycelia transport SeO_3^{2-} or SeO_4^{2-} into the cells through active transport or facilitated diffusion, and convert the inorganic selenium salts into H-Se-H through redox reactions, which is further converted into selenocysteine and selenomethionine; selenocysteine can replace cysteine and be inserted into proteins mediated by the specific codon (UGA), constituting the active centers of enzymes, and selenomethionine can replace methionine and be incorporated into proteins to form selenoproteins.

2.2. The Role of Selenium in Shiitake Mushrooms

With the development of selenium-compound separation and analysis techniques, the speciation analysis of selenium in edible fungi has become more accurate. Studies have shown that the main form of selenium in edible fungi is selenomethionine (Se-Met), followed by selenocysteine, in addition to small amounts of methylselenium, dimethylselenium, trimethylselenium and methylselenocysteine, among others. Sun *et al.* carried out submerged liquid fermentation of the shiitake mushroom strain

L13 with Na_2SeO_3 , and the selenium content in the mycelia was 225 $\mu\text{g/g}$, with the degree of organic selenium conversion reaching 72.9%, of which 68.2% existed in the form of selenoproteins^[1]. Studies found that selenium promotes the synthesis of amino acids and proteins, whose contents increased by 15.96% and 18.3%, respectively, compared with the control group. At present, domestic and foreign scholars have conducted considerable research on the effects of selenium on the activity and content of functional enzymes and other substances in shiitake mushrooms. Jiang *et al.* added Na_2SeO_3 to the cultivation substrate to study the effects of selenium on the soluble protein content, superoxide dismutase (SOD) activity, peroxidase (POD) activity, polyphenol oxidase (PPO), catalase (CAT) activity and malondialdehyde (MDA) content in the fruiting bodies of big-cup shiitake mushrooms^[2]. The results showed that, under the same substrate conditions, when the Na_2SeO_3 addition was 30 mg/kg, the soluble protein content in the fruiting bodies of big-cup shiitake mushrooms increased by 4.75% on average compared with the control group, showing a significant difference ($p < 0.05$); the SOD, POD, CAT and PPO activities increased by 53.05%, 89.21%, 6.67% and 176.58%, respectively, compared with the control group, showing extremely significant differences ($p < 0.01$); and with the increase of selenium level, the glutathione peroxidase (GSH-Px) and SOD activities showed a decreasing trend, whereas the MDA content increased significantly ($p < 0.05$). Nunes *et al.* conducted cultivation experiments on selenium-enriched shiitake mushrooms, and the results showed that Na_2SeO_3 did not inhibit the activity of the hydrolytic enzymes (cellulase and xylanase) in shiitake mushrooms, but increased the activity of the oxidase (laccase)^[3]. The respiratory activity of shiitake mushrooms was negatively correlated with the Na_2SeO_3 addition in the cultivation substrate. Turlo *et al.* found that selenium-enriched culture of shiitake mushroom mycelia could increase the antioxidant activity of the mycelial extracts, and the antioxidant effect was 100%~400% higher than that of the control group^[4].

3. Selenium-Enrichment Approaches and Capacity of Shiitake Mushrooms

In the natural environment, selenium mainly exists in an inorganic form, and plants can convert the inorganic selenium element into organic selenium through absorption. Shiitake mushrooms can utilize both inorganic selenium and the organic selenium present in plants. Therefore, the selenium-enrichment approaches of shiitake mushrooms mainly include the enrichment of inorganic selenium and the enrichment of natural selenium-rich raw materials.

3.1. Inorganic Selenium

The main inorganic selenium sources for selenium-enriched shiitake mushrooms include Na_2SeO_3 , Na_2SeO_4 and selenium ore powder, among others. Lin *et al.* found that shiitake mushroom mycelia possess strong selenium tolerance and strong selenium-enrichment capacity. When the selenium addition in the liquid medium was 10 $\mu\text{g/mL}$, the selenium content of the cultured mycelia reached 230 $\mu\text{g/g}$, 114 times higher than that of the control. Shao *et al.* domesticated shiitake mushroom strains with selenium addition and then performed submerged liquid fermentation^[5]. The results showed that the domesticated shiitake mushroom strains grew well on solid medium with a Na_2SeO_3 concentration of 60 mg/kg, in a liquid seed medium containing Na_2SeO_3 at 80

mg/kg, and in a liquid fermentation medium with Na_2SeO_3 at 130 mg/kg, and could enrich selenium within the mycelia. When the selenium concentration in the medium is too high, the shiitake mushroom mycelia are also inhibited. Owing to differences in strains and culture conditions, the degree of inhibition of shiitake mushroom mycelia varies considerably. Hu *et al.* carried out solid-state selenium-enrichment research on the shiitake mushroom Huangshi No. 1 strain^[6]. The results showed that when the selenium content was 1~8 mg/kg, mycelial growth was slightly inhibited. When the selenium content was 20 mg/kg, the mycelial growth rate decreased significantly, and at this time the colony diameter was only 64% of that of the control; and when the selenium content was 100 mg/kg, the mycelia almost stopped growing, with only slight germination, and the colonization time was delayed. Peng Hao *et al.* conducted selenium-enriched culture of the shiitake mushroom “Nanshan No. 1” strain, and the results showed that the optimal Na_2SeO_3 concentration in both the solid and liquid media was 60 mg/L. Liang *et al.* conducted selenium-enriched cultivation research on the shiitake mushroom 905 strain; the results showed that when the selenium concentration was lower than 80 mg/kg, the mycelia grew rapidly, with short spawn-running time, thick and white mycelia, short fruiting time and improved uniformity; when the selenium concentration was higher than 80 mg/kg, the mycelia grew slowly, with more spawn-running days, delayed and prolonged fruiting time and poor uniformity; and when the selenium concentration was 60 mg/kg, the mycelia grew best.

3.2. Natural Selenium-Rich Raw Materials

The main selenium-rich regions in China are Enshi Prefecture in Hubei Province, Ziyang County in Shaanxi Province, Longyou County in Zhejiang Province, and the southern Anhui region. These regions possess abundant selenium-rich soils and selenium-rich water resources, and natural selenium-rich raw materials are formed through the absorption and accumulation of selenium by plants. In recent years, the use of natural selenium-rich raw materials for selenium-enriched shiitake mushroom cultivation has become one of the effective ways to increase the organic selenium content in shiitake mushrooms and reduce the production cost of selenium-enriched shiitake mushrooms. Cheng used hard hardwood from the selenium-rich region of Ankang as the main cultivation substrate to carry out cultivation experiments on the shiitake mushroom “Weixiang No. 1” strain and the oyster mushroom “Nanjing No. 1” strain, among others. The results showed that the higher the selenium content in the cultivation substrate, the higher the selenium content of the cultivated edible fungi, with the two increasing in direct proportion; compared with other edible-fungus cultivars, shiitake mushrooms had the strongest selenium-enrichment capacity, reaching 1.2 mg/kg. Cheng also used hard hardwood with a selenium content of 15 mg/kg as the main cultivation substrate and detected the selenium content of the Tian-bai-hua mushroom trial samples; it was found that different parts of the same mushroom body contained different selenium contents, but overall the values ranged between 0.7~3.8 mg/kg.

4. Strain Breeding of Selenium-Enriched Shiitake Mushrooms

Breeding excellent strains is one of the keys to the production technology of selenium-enriched shiitake mushrooms,

because whether the strains are excellent affects the yield of selenium-enriched shiitake mushrooms and the extraction and research of their bioactive substances. At present, the traditional selenium-enrichment domestication breeding technology has been reported most frequently. Studies have shown that, in order to improve the selenium tolerance of shiitake mushrooms, it is necessary to conduct selenium-enrichment domestication of the original strains. Liang *et al.* conducted selenium-enriched cultivation research on the shiitake mushroom 905 strain. The results showed that the spawn domesticated with selenium was more conducive to the absorption of selenium by the shiitake mushroom fruiting bodies, and the selenium content in the fruiting bodies could reach up to 129.8 mg/kg, 308 times higher than that of ordinary shiitake mushrooms^[7]. Shao *et al.* used the shiitake mushroom SD03 as the original strain, prepared protoplasts, induced mutation of the protoplasts with ultraviolet (UV) radiation, and screened the mutant strains to obtain a selenium-enriched shiitake mushroom strain SD73 with stable inherited traits. After shake-flask culture and cultivation experiments, the selenium content in the fruiting bodies was determined to reach 38.64 µg/g. In recent years, domestic scholars have used techniques such as UV mutagenesis and protoplast fusion to study the breeding of excellent strains of selenium-enriched *Cordyceps sinensis* and selenium-enriched *Ganoderma lucidum*, achieving good results; the innovations in breeding methods provide theoretical references for the strain breeding research of selenium-enriched shiitake mushrooms. At present, the traditional cross-breeding technology, the emerging genetic-engineering breeding technology and the molecular marker-assisted breeding technology have not yet been applied to the breeding of selenium-enriched shiitake mushrooms.

5. Cultivation Techniques of Selenium-Enriched Shiitake Mushrooms

5.1. Conventional Solid-State Cultivation Techniques

The solid-state cultivation methods of shiitake mushrooms include log cultivation and bag cultivation. At present, most of the cultivation research on selenium-enriched shiitake mushrooms concerns bag cultivation, and there is no literature report on log cultivation. Cheng used hard hardwood with a selenium content of 15 mg/kg as the main cultivation substrate and screened out the optimal cultivation medium for Tian-bai-hua mushroom through comparative experiments: 76%~80% miscellaneous wood sawdust, 18%~22% bran and 1%~2% gypsum. Li used a medium (78% miscellaneous wood sawdust, 20% wheat bran, 1% brown sugar, 1% gypsum powder, 60% water, natural pH) to conduct selenium-enriched cultivation experiments on Qingyuan flower mushrooms. The results showed that the selenium content in the flower mushroom fruiting bodies was linearly and positively correlated with the Na₂SeO₃ addition; when the Na₂SeO₃ addition was 2.85 µg/g, the selenium content in the first-flush flower mushroom fruiting bodies reached 12.2 µg/g, which, calculated on a per-capita daily intake of 85 g, accounted for 109.1% of the per-capita daily selenium intake stipulated by the US Food and Drug Administration (FDA). Compared with the cultivation of ordinary shiitake mushrooms, selenium-enriched shiitake mushroom cultivation has many advantages. Ye conducted selenium-enriched cultivation experiments on four shiitake mushroom varieties in the Lishui region using a medium (78% miscellaneous wood sawdust, 20% wheat bran, 1.2%

gypsum, 0.5% sugar, 0.3% magnesium sulfate). The results showed that, after adding selenium nutrients to the cultivation substrate, the cultivated selenium-enriched shiitake mushrooms exhibited fast substrate consumption, thick mycelia, good luster, strong stress resistance, early primordium formation, tender mushroom flesh, strong aroma and fast flush turnover; in particular, the yield increased by 10%~20%, and the selenium content in the shiitake mushroom fruiting bodies was higher than the national standard. Chen used a medium (78% miscellaneous wood sawdust, 16% wheat bran, 4.0% corn flour, 1.0% gypsum powder, 0.5% sucrose, 0.5% calcium superphosphate, Na₂SeO₃ 60 mg/kg) to conduct selenium-enriched cultivation experiments on shiitake mushrooms. The results showed that selenium-enriched shiitake mushrooms exhibited fast mycelial growth, short spawn-running time, thick and white mycelia, short fruiting time and high uniformity.

5.2. Liquid-State Cultivation Techniques

In recent years, the submerged liquid fermentation technology of edible fungi has attracted increasing attention, because this technology can obtain large quantities of mycelia or spawn in a short period. When liquid spawn is inoculated into solid culture substrates, it has the characteristics of fast flow, easy dispersion, rapid germination and many spawning points, thereby effectively controlling the contamination of bag-grown edible fungi during inoculation. At present, there is little research on the selenium-enriched liquid fermentation of shiitake mushrooms, and there is no literature report on the cultivation using selenium-enriched shiitake mushroom liquid spawn. In the preparation process of selenium-enriched shiitake mushroom liquid spawn, the existing reports only use shake-flask fermentation experiments, and the fermentation-tank spawn-preparation process conditions for selenium-enriched shiitake mushrooms still require extensive research and exploration. Huo *et al.* used distillers' grains waste liquor as the raw material and determined the submerged fermentation process for selenium-enriched shiitake mushrooms: 100 g/L distillers' grains waste liquor, 10 g/L corn flour, Na₂SeO₃ concentration of 100×10⁻⁶ mg/mL, initial pH 5.0, inoculation volume 10%, fermentation temperature 28 °C, shaker speed 150 r/min, and 250 mL of liquid in a 500 mL Erlenmeyer flask. Peng *et al.* used an L9(3⁴) orthogonal experiment to screen the optimal selenium-enriched liquid medium for the Nanshan No. 1 shiitake mushroom strain: 30.0 g/L corn flour, 6.0 g/L soybean, 1.5 g/L KH₂PO₄, 0.5 g/L MgSO₄·7H₂O, Na₂SeO₃ concentration of 60 mg/L, and natural pH.

6. Existing Problems and Prospects

6.1. The Strain Breeding of Selenium-Enriched Shiitake Mushrooms and The Production Process of Liquid Spawn Require Further In-Depth Research

In recent years, domestic and foreign scholars have conducted extensive research on the forms of selenium in shiitake mushrooms and on the effects of selenium on the activity and content of functional enzymes and other substances in shiitake mushrooms; however, the research on the metabolic mechanism of selenium in shiitake mushroom mycelia is not yet in-depth. Because the previous strain breeding technology for selenium-enriched shiitake mushrooms was relatively low-tech and single-method, the existing strains cannot meet production requirements. Therefore, the use of genetic-engineering breeding and other

technologies to breed excellent selenium-enriched shiitake mushroom strains is the future development direction. At present, there is no literature report on the production process of selenium-enriched shiitake mushroom liquid spawn. The application of shiitake mushroom liquid spawn can promote the development of the shiitake mushroom cultivation industry toward standardization, scale and mechanization. Hence, exploring the production process of selenium-enriched shiitake mushroom liquid spawn has extremely high research value. It should be noted that, in the production of selenium-enriched shiitake mushroom liquid spawn, issues such as the diversity of selenium sources, the environmental pollution caused by selenium and the comprehensive utilization of selenium need to be considered.

6.2. The Industrial Production and Quality Safety Standards of Selenium-Enriched Shiitake Mushroom Products Need to Be Strengthened and Improved

The extremely high edible and medicinal value of shiitake mushrooms and the importance of selenium nutrition have been recognized globally. Therefore, by using the submerged liquid culture and cultivation techniques of selenium-enriched shiitake mushrooms to produce selenium-enriched shiitake mushroom mycelia or fruiting bodies that contain essential amino acids (including selenomethionine), selenoproteins, selenium nucleic acids, selenium polysaccharides and other nutrients, and to develop high-yield, high-quality selenium-enriched shiitake mushroom products, is of far-reaching significance for human health. With the deepening of research on selenium and the advancement of science and technology, selenium-enriched shiitake mushroom products have emerged one after another, and their technological content has continuously increased. In China, related products such as selenium protein powder and selenium polysaccharide injections developed from selenium-enriched shiitake mushrooms have appeared. Sun Zhongtao *et al.* used submerged fermentation technology to enrich and convert the inorganic element selenium in shiitake mushrooms, obtaining shiitake mushroom mycelia with a selenium content of 225 µg/g; the shiitake mushroom mycelia converted inorganic selenium into organic selenium with high rates of absorption and utilization by the human body, and the contents of various amino acids were greatly increased, thereby strengthening the nutritional value; the functional milk powder formulated with selenium-enriched shiitake mushroom mycelia as the base material not only contains the nutrients of ordinary milk powder, but also contains abundant shiitake mushroom polysaccharides and organic selenium, and possesses special health-care functions. However, such products are expensive and difficult for ordinary people to afford. Therefore, the industrialized production of selenium-enriched shiitake mushroom products and the reduction of the price of related selenium-enriched products are future research tasks. In addition, the safety standard range for selenium intake is narrow, and the selenium contents in related selenium-enriched shiitake mushroom products also differ from one another. A standard and management system that is connected with domestic, international and industry standards has not yet been formed. Consequently, improving the quality safety and guarantee system related to selenium enrichment is particularly important.

Since the vast majority of countries and regions are selenium-poor or selenium-deficient areas, improving the selenium

content through food fortification is the main way to develop selenium-enriched foods in the future. China is the world's largest producer of shiitake mushrooms, and its selenium reserves account for one third of the world's total. Moreover, the production process of selenium-enriched shiitake mushrooms is not much different from that of ordinary shiitake mushrooms. Therefore, combining traditional shiitake mushroom production technology with selenium-enriched shiitake mushroom production is of great significance for the development of selenium-enriched shiitake mushroom products in China.

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