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Optimization of submerged fermentation conditions for biosynthesis of ergothioneine and enrichment of selenium from *Pleurotus eryngii* 528

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Abstract

Ergothioneine and selenium are biologically active substances that maintain human health. In order to increase the content of ergothioneine and selenium in the submerged fermentation products of *Pleurotus eryngii*, the non-nutritive culture conditions of submerged fermentation were investigated. When the concentration of sodium selenite was 3 μg/mL, the selenum conversion efficiency (94.78%) was the highest and the dry weight was the highest. The pH value of culture medium, shaking speed, culture temperature, inoculation amount and culture time affected the dry weight of fermentation, the content of ergothionein and selenium. The optimal culture conditions were optimized by single factor experiment as follows: pH 5.5 (natural), rotation speed 180 r/min, culture temperature 26 °C, inoculation amount 10%, and culture time 7 d. Under these conditions, the dry weight of *Pleurotus eryngii* fermentation product was 38.97 g/L, the ergothioneine content was 39.42 mg/L, and the bioconversion efficiency of selenium reached 84.63%.

Keywords: *Pleurotus eryngii 528*; ergothioneine; selenium; submerged fermentation.

Practical Application: This study can provide a data basis for the production of *Pleurotus eryngii* powder rich in ergothioneine and selenium, thereby laying the foundation for the development of the third-generation functional food.

1 Introduction

Rare edible and medicinal fungi play an important role in improving human nutrition because of their nutritional value, medicinal value and unique flavor (Mwangi et al., 2022). *Pleurotus eryngii* is an edible mushroom cultivated widely in many regions of the world (Zhang et al., 2020), it can improve postprandial blood glucose, appetite, and regulate postprandial ghrelin levels (Kleftaki et al., 2022), the polysaccharide extracted from the stalk residue of *Pleurotus eryngii* have similar structure and bioactivity to fruiting bodies (Zheng et al., 2020a), the nutritional components of fruiting body and mycelia of *Pleurotus eryngii* are similar, and mycelia can become an effective substitute (Zheng et al., 2020b). The liquid fermentation products of *Pleurotus eryngii* contain rich antioxidant functional substances, and have high development and utilization value (Hou et al., 2013). However, liquid fermentation can be used for industrial continuous production, with the advantages of large scale, high yield, short fermentation period and high production efficiency (Zhao & Ma, 2017). Therefore, liquid fermentation can be used for industrial continuous production. Liquid deep fermentation will become an effective means to develop active ingredients of *Pleurotus eryngii*.

L-Ergothioneine was first discovered in *Claviceps purpurea* and has many physiological functions such as maintaining DNA biosynthesis, normal cell growth, scavenging free radicals and detoxification (Li et al., 2014; Borodina et al., 2020). Due to its unique pharmacological activity and biological function, ergothioneine has been used as a cell physiological protective agent, it has application prospects in the industries of food, health products, medicine and cosmetics (Liu et al., 2015). At present, domestic and foreign scholars have studied ergothioneine in edible fungi, Kalaras et al. (2017) pointed out that mushrooms are rich in antioxidant ergothioneine and glutathione; Liang et al. (2013b, a) studied the antioxidant properties of fruiting bodies, mycelia and fermentation products of *Pleurotus eryngii* with high ergothioneine, and pointed out that *Pleurotus eryngii* products could be used as functional food, and prolonged fermentation time could increase the content of ergothioneine in mycelia; at the same time, Chen et al. (2012) developed new edible fungi products with high ergothioneine by solid-state fermentation, and analyzed the quality of fermented products and their tastes. Selenium is one of the life elements and micronutrients for human health (Huang et al., 2019). The selenoprotein in the diet can be absorbed by the human body after being digested and hydrolyzed into selenomethionine and selenocysteine (Seale et al., 2018). Liu et al. (2022) pointed out that more than 95.5% of selenium in grains was organic selenium, and selenomethionine was dominant. Scholars have studied the Selenium enriched *Hypsizygus marmoreus* as a potential food supplement (Hu et al., 2021). Therefore, the safe and reasonable method of selenium intake and scientific control of selenium intake to achieve the best effect are worthy of consideration and research by scholars. At present, the nutritional and health care function of selenium is still a field to be cultivated. With

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the elucidation of various mechanisms of selenium, selenium will also enter the application stage as a preventive and health care medicine (Cheng & Wang, 2021). The biotransformation technology of edible fungi is to convert inorganic selenium into organic selenoprotein and selenium polysaccharide through the transformation and metabolism of matrix nutrients by mycelium cells of edible fungi, to achieve the goal of selenium biotransformation and enrichment of edible fungi (Tang et al., 2013); using edible fungi as a carrier to achieve the biological enrichment from inorganic selenium to organic selenium, so the development of selenium-rich foods by selenium-rich edible fungi as organic selenium resources has become a hot topic in scientific research (Zheng et al., 2013; Wu et al., 2011).

In order to continue the research on the liquid fermentation process of *Pleurotus eryngii* and the biological activity of its fermentation products, and to explore the ways and mechanisms of the active ingredients of *Pleurotus eryngii*, we used the directional submerged fermentation technology of biological metabolism engineering, with *Pleurotus eryngii* 528 as the fermentation strain, selenium as the directional target, and ergothioneine as the research target, in order to lay a good foundation for the further development of the third generation of functional foods. Directional submerged fermentation technology in biological metabolic engineering can achieve safe production of ergothioneine and selenium, and make the production of ergothioneine and selenium-rich edible fungi more environmentally friendly and cost-effective (Liu et al., 2013).

2 Materials and methods

2.1 Materials and reagent

Pleurotus eryngii 528 was provided by Institute of Edible Fungi, Fujian Academy of Agricultural Sciences, China. The reagents used in the experiment were purchased from professional reagent company, and the chemical reagents were analytically pure.

2.2 Determination items and methods

Submerged fermentation process for biosynthesis of ergothioneine and enrichment of selenium in Pleurotus eryngii

The fermentation process of ergothioneine biosynthesis and selenium enrichment in *Pleurotus eryngii* is shown in Figure 1.

Determination of dry weight fermentation product

The dry weight of the fermentation product was determined as the same way of the previous study (Tang et al., 2021). After the end of submerged fermentation culture, the glass beads and aerial mycelium of the triangular flask wall were removed from each bottle, and the fermentation products were into the glass plate or stainless steel tray that had been weighed. The fermentation products were pre-cooled at -40 °C for 5-10 h (DW-40L188, Qingdao Haier Co., Ltd., China), and then freeze-dried in a vacuum freeze-drying machine (SCIENTZ-30ND, Ningbo Xinzhi Biotechnology Co., Ltd., China) for 24 h. The dried fermentation products were weighed with an electronic balance and recorded, then 100 mL dry weight of fermentation products was obtained by addition and subtraction. The unit of dry weight of fermentation products was converted into g/L, which was the dry weight of fermentation products.

Determination of ergothioneine content

Determination of ergothioneine content was performed according to the procedure as reported by Tang et al. (2021). The operation was carried out by liquid chromatography (Waters e2695, Waters Technology (Shanghai) Co., Ltd., China). The mobile phase was set as acetonitrile-water (3 : 97); the flow rate was set to 1.0 mL/min; the column temperature was set at 30 °C; the detection wavelength was set to 254 nm; the injection volume was set to 10 μL. Standard diluents were prepared at different concentrations of 0.1 μg/mL, 1.0 μg/mL, 5.0 μg/mL, 10.0 μg/mL, 15.0 μg/mL and 20.0 μg/mL. After filtration with 0.45 μm membrane, the filtrate was taken for detection, and the standard curve was plotted.

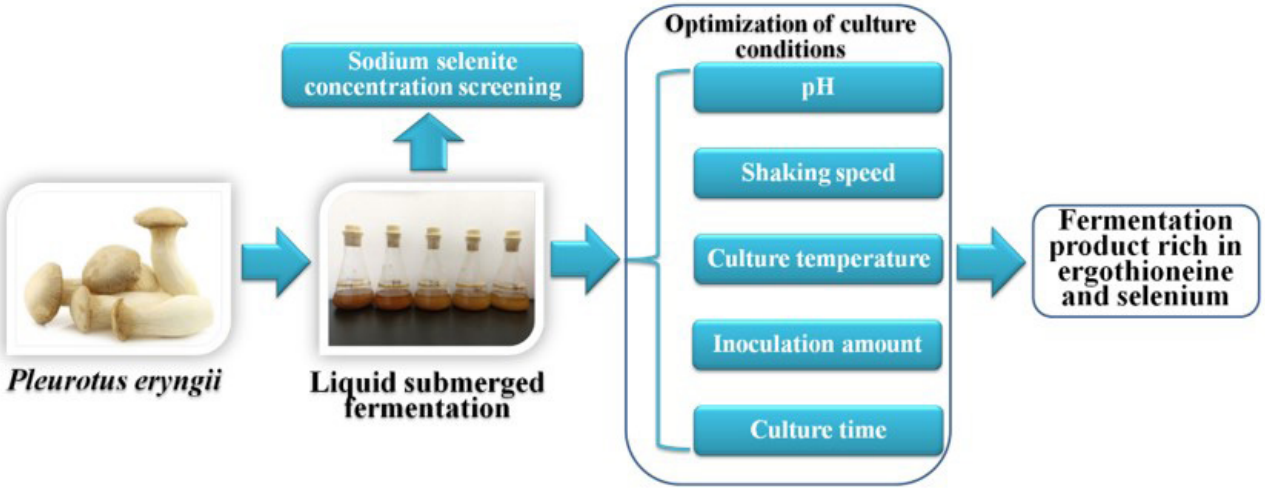

Figure 1. Diagram of submerged fermentation process of ergothioneine and selenium in *Pleurotus eryngii*.

0.5 g of the sample prepared in determination of dry weight fermentation product was weighed, added with 10 mL of methanol and stirred evenly. The sample was ultrasonically treated with a numerical control ultrasonic (KQ-600DV, Kunshan Ultrasonic Instrument Co., Ltd., China) cleaner for 20 min, and then filtered with a neutral filter paper. After that, 1 mL supernatant was taken with a syringe, and filtered with a 0.45 μm filter membrane which was used to detect the content of ergothioneine. The detected ergothioneine content was mg/g, multiplied by the dry weight of each bottle, and finally converted to mg/L.

Determination of total selenium content

The total selenium content was determined in accordance with "the first method hydride atomic fluorescence spectrometry" in GB 5009.93-2017 national food safety standard "determination of selenium in food" (China, 2017). The conversion efficiency of selenium is calculated according to the following formula (Wu et al., 2011) (Equation 1):

$$T_{Se}=\frac{Pse\times G\times 10^{-3}}{Cse\times 0.457}\times 100\% \quad (1)$$

In Equation 1: T_{Se} is the conversion rate of Se, %; P_{Se} is Se content, mg/kg; G is biomass, g/L; C_{Se} is the addition concentration of Se, μg/mL; 0.457 is the mass fraction of Se.

2.3 Experimental design

Determination of selenium concentration in submerged fermentation of Pleurotus eryngii 528

In the medium for submerged fermentation of *Pleurotus eryngii*, the addition concentrations of sodium selenite were set to be 1, 3, 5, 10, and 20 μg/mL. The selenium concentration suitable for the mycelial growth of *Pleurotus eryngii* was determined by observing the medium, mycelia color and dry weight of fermentation products. The sterilization conditions were 121 °C and 0.1 Pa for 20 min; the culture conditions were shading culture in 25 °C full temperature oscillator culture (ZWYR-2102C, ZWY-211C, Shanghai Zhicheng Analytical Instrument Manufacturing Co., Ltd., China), liquid culture was set at a 180 r/min, and each treatment was repeated three times.

It is carried out in three steps: flat plate activation culture, primary liquid culture and secondary liquid amplification culture. The plate activation medium was PDA medium; the primary liquid medium was: corn grit powder 2.0%, peptone 2.0%, wheat bran powder 1.0% (not filtered), KH_2PO_4 0.1%, $MgSO_4$ 0.1%, glutamic acid 0.1% and vitamin B_6 0.1%. The secondary liquid amplification medium was: corn grit powder 2.0%, peptone 1.5%, histidine 0.15%, wheat bran powder 1.0% (not filtered), KH_2PO_4 0.1%, $MgSO_4$ 0.1%, glutamic acid 0.1% and vitamin B_6 0.1%.

Firstly, 15-20 mL PDA medium was poured into a Φ90 mm glass culture dish. After sterilization, the medium was cooled and solidified, inoculated into *Pleurotus eryngii* mycelia for activation culture, until the mycelia grew into a full plate for primary liquid culture.

Secondly, the prepared primary liquid medium was divided into 250 mL trangular conical flasks, and the liquid volume in each bottle was 100 mL. After sterilization, the medium was cooled to room temperature, and 5 pieces of activated *Pleurotus eryngii* strains with Φ8 mm were inoculated. Shading culture was carried out for 4 ~ 6 d, and used for secondary liquid amplification culture.

Finally, the prepared secondary liquid culture medium was divided into 250 mL triangular conical flasks with 100 mL liquid volume per bottle, and different concentrations of sodium selenite solution were added to the culture medium (in order to keep the culture medium at 100 mL, before adding sodium selenite solution, the same volume of culture medium was sucked first, and then the same volume of sodium selenite solution was added). After sterilization, the culture medium was cooled to room temperature, and the aerial hyphae and glass beads in the primary liquid strains were removed, and 10 mL of the primary liquid strains were poured into the culture medium. After shading for 7 days, the aerial hyphae and glass beads were removed, and the fermentation was poured out and freeze-dried. The total selenium content of freeze-dried samples was detected, and the dry weight fermentation products and selenium conversion efficiency were used as reference factors to judge the selenium concentration suitable for the growth of *Pleurotus eryngii* mycelium.

Optimization of submerged fermentation conditions for Pleurotus eryngii 528

The pH value of the suitable medium for submerged fermentation culture of *Pleurotus eryngii* 528 in the previous study was 5.1 (Tang et al., 2021) [the optimum pH for mycelium growth of *Pleurotus eryngii* 528 was 5.5 ~ 6.0 (Cai et al., 2009)], and the mycelium growth did not reach the optimum state. Therefore, the liquid medium formula for submerged fermentation of *Pleurotus eryngii* was adjusted here. With dry weight fermentation products, ergothioneine, and selenium conversion efficiency as the main indexes, and selenium concentration determined in determination of selenium concentration in submerged fermentation of *Pleurotus eryngii* 528 as the basis, the non-nutritional conditions for directional submerged fermentation of *Pleurotus eryngii* were systematically studied, including pH value, shaking speed, culture temperature, inoculation amount, and culture time. Five treatments were designed for five different conditions, with three replicates for each treatment. The sterilization conditions were 121 °C and 0.1 Pa for 20 min; the culture conditions were shaded at 25 °C, solid culture was static, liquid culture was set at a speed 180 r/min.

Consistent with the method for determining selenium concentration in liquid fermentation, it was also divided into three levels: plate activation, primary culture and secondary amplification culture. The plate activation medium was PDA medium; the adjusted primary liquid fermentation medium formula of *Pleurotus eryngii* was: corn grit powder 2%, sucrose 1%, peptone 1%, wheat bran powder 1% (not filtered), KH_2PO_4 0.3%, $MgSO_4$ 0.15%, and the pH value was natural; the secondary liquid amplification medium was: corn grit powder 2%, sucrose 1%, peptone 1%, histidine 0.15%, vitamin $B_6$0.1%, wheat bran

powder 1% (not filtered), KH_2PO_4 0.3%, $MgSO_4$ 0.15% and selenium addition concentration 3 μg/mL, with natural pH value.

3 Results and discussion

3.1 Effects of different selenium concentrations on liquid mycelium growth of Pleurotus eryngii

As shown in Table 1, sodium selenite could affect the dry weight of fermentation products and selenium conversion efficiency of *Pleurotus eryngii*. The dry weight of the fermentation product with 3 μg/mL sodium selenite was the highest, and the selenium conversion efficiency was also the highest. The test also found that with the increase of sodium selenite addition, the color of fermentation broth and mycelium changed from light yellow to red-yellow (Figure 2). Therefore, the optimal dosage of sodium selenite in the fermentation culture of *Pleurotus eryngii* was 3 μg/mL. These results were in agreement with the previous report of Wang et al. (2021), in which the Se-enrichment rates in fermented *Pleurotus eryngii* were all more than 50%.

3.2 Optimization of submerged fermentation conditions of Pleurotus eryngii

Effect of pH on submerged fermentation of Pleurotus eryngii

The measured pH of the primary liquid fermentation medium and the natural pH of the secondary liquid fermentation medium were 5.52 and 5.45, respectively, which were suitable for the mycelial growth of *Pleurotus eryngii*. To study the effect of pH value on submerged fermentation of *Pleurotus eryngii*, the pH values were set at 5, 5.5 (natural), 6, 6.5 and 7, respectively. After the end of culture, the dry weight fermentation products and ergothioneine content in each treatment group were measured, and the bioconversion efficiency of selenium was calculated.

The effects of different pH values on dry weight fermentation products, ergothioneine content and selenium conversion efficiency were shown in Figures 3-5. Figure 3 showed that the dry weight of the fermentation product fluctuated greatly from pH 5 to pH 7. The dry weight of the fermentation product was the highest at pH 6.5, followed by pH 5.5 (natural) and pH 7, and there was no significant difference between them ($P > 0.05$). Figures 4-5 showed that the content of ergothioneine and selenium conversion efficiency reached the maximum at

Table 1. Effects of sodium selenite addition on dry weight and selenium conversion efficiency of *Pleurotus eryngii* fermentation products.

Selenium concentration (μg/mL)	Dry weight fermentation products (g)	Selenium conversion efficiency (%)
1	3.33 ± 0.08 a	82.20 ± 4.75 ab
3	3.47 ± 0.22 a	94.78 ± 11.42 a
5	3.14 ± 0.22 ab	74.20 ± 13.29 abc
10	3.16 ± 0.33 ab	61.27 ± 4.08 c
20	3.09 ± 0.01 b	71.58 ± 6.13 bc

Data are presented as the mean ± SD (n = 3). Values with different letters in the same column differ significantly ($p < 0.05$).

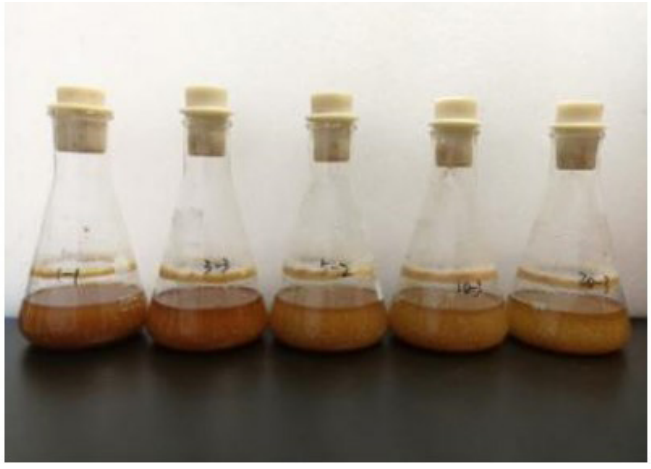

Figure 2. Effects of sodium selenite addition on color of liquid fermentation of *Pleurotus eryngii*.

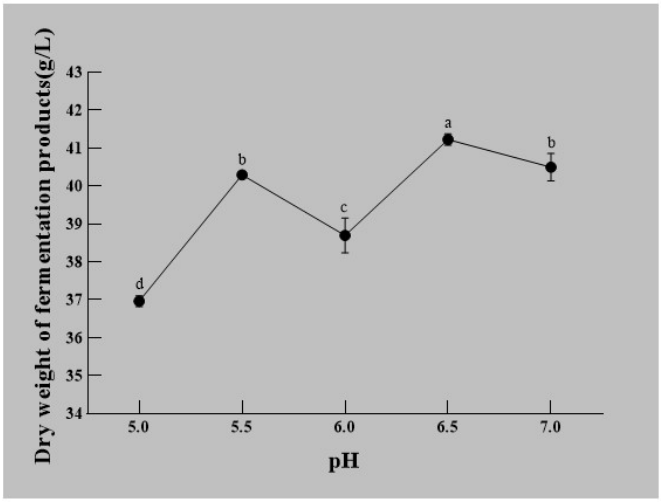

Figure 3. Effects of different pH values on dry weight fermentation products of *Pleurotus eryngii*. The letters in the figure are the differences between the treatments.

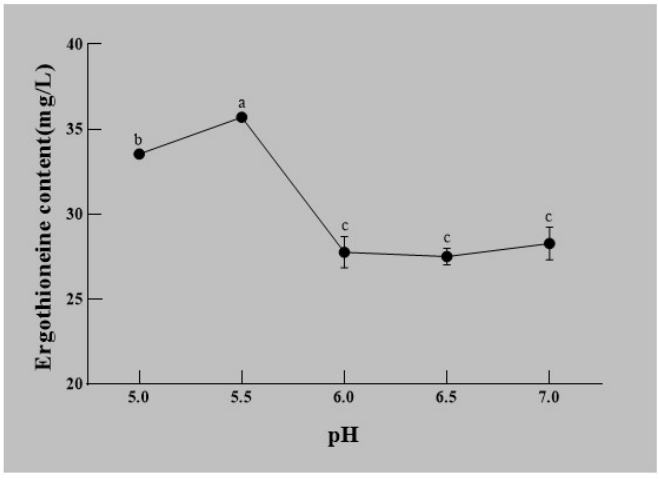

Figure 4. Effects of different pH values on ergothioneine content of *Pleurotus eryngii*. The letters in the figure are the differences between the treatments.

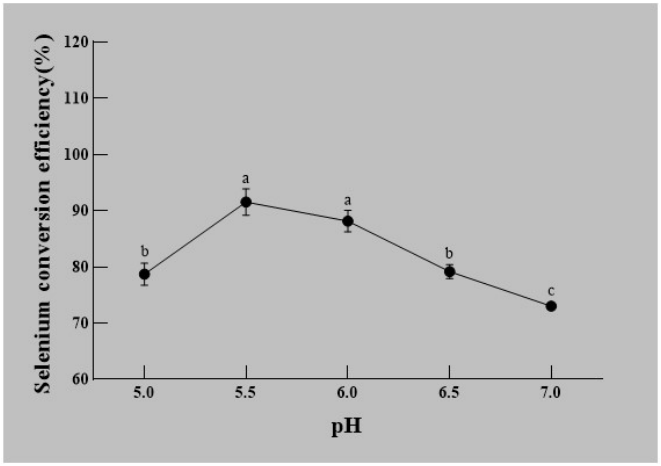

Figure 5. Effects of different pH values on selenium conversion efficiency of *Pleurotus eryngii*. The letters in the figure are the differences between the treatments.

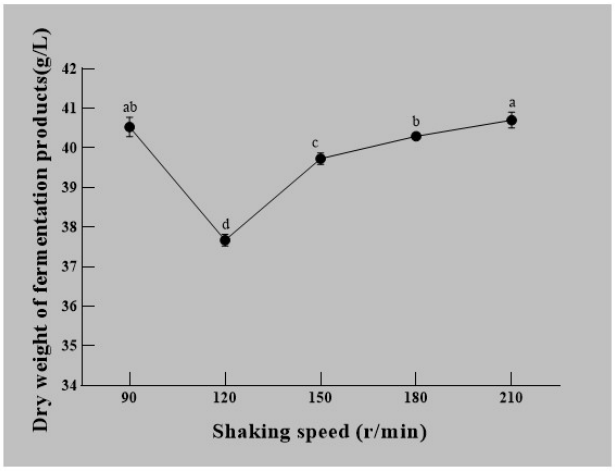

Figure 6. Effects of different shaking speeds on dry weight fermentation products of *Pleurotus eryngii*. The letters in the figure are the differences between the treatments.

pH 5.5 (natural). Therefore, the study on the effects of dry weight fermentation products, ergothioneine content and selenium conversion efficiency showed that pH 5.5 (natural) could meet the needs of directional submerged fermentation of *Pleurotus eryngii* 528. Singh et al. (2020) studied the large-scale production of *Pleurotus eryngii* mycelia under the condition of increasing mineral and vitamin D_2 content, the pH value suitable for the growth of *Pleurotus eryngii* mycelia was 6.

Effect of shaking speed on submerged fermentation of Pleurotus eryngii

To study the effect of shaking speed on submerged fermentation of *Pleurotus eryngii*, the shaking speed was set to be 90, 120, 150, 180 and 210 r/min, respectively. After the end of culture, the dry weight fermentation products and ergothioneine content in each treatment group were measured, and the bioconversion efficiency of selenium was calculated.

The effects of different shaking speeds on dry weight fermentation products, ergothioneine content and selenium conversion efficiency were shown in Figures 6-8. It can be seen from Figure 6 that the dry weight of fermentation products at the shaking speed of 210 r/min was the highest, and the significance analysis showed that there was no significant difference in the dry weight of fermentation products between the shaking speed of 90 r/min and 180 r/min or 210 r/min ($P > 0.05$). The dry weight of fermentation products between the shaking speed of 180 r/min and 210 r/min was significantly different ($P < 0.05$), and the difference in the dry weight of fermentation products at the other shaking speed was extremely significant ($P < 0.01$). In Figure 7, the ergothioneine content reached the maximum when the shaking speed was 180 r/min; and it can be seen from Figure 8 that the selenium conversion efficiency reached the maximum when the shaking speed was 90 r/min. In the study on the effects of dry weight fermentation products, ergothioneine content and selenium conversion efficiency, considering ergothioneine as the target product, although the dry weight fermentation products at 90 r/min had no significant difference with those at 180 r/min

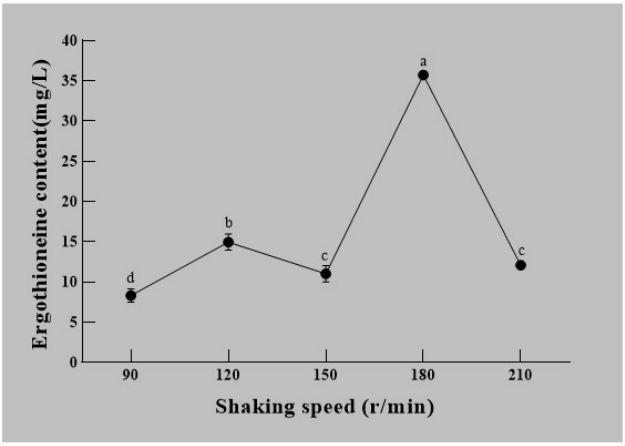

Figure 7. Effects of different shaking speeds on ergothioneine content of *Pleurotus eryngii*. The letters in the figure are the differences between the treatments.

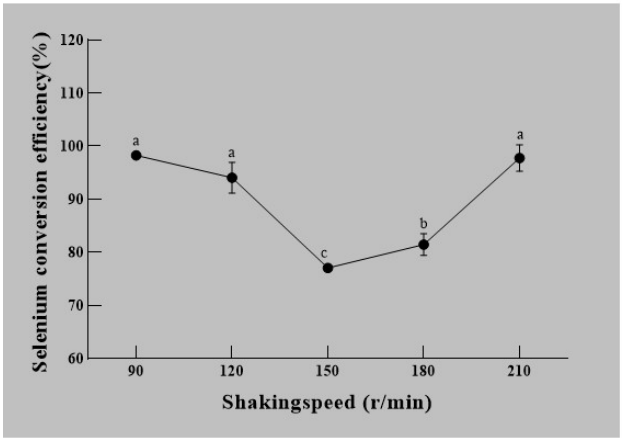

Figure 8. Effects of different shaking speeds on selenium conversion efficiency of *Pleurotus eryngii*. The letters in the figure are the differences between the treatments.

and 210 r/min, and the selenium conversion efficiency had no significant difference with those at 120 r/min and 210 r/min, the ergothioneine content at 180 r/min was the highest. Therefore, 180 r/min was selected as the shaking speed for directional submerged fermentation of *Pleurotus eryngii* 528.

Effect of culture temperature on submerged fermentation of Pleurotus eryngii

To study the effect of culture temperature on submerged fermentation of *Pleurotus eryngii*, the culture temperature was set to be 23, 24, 25, 26 and 27 °C, respectively. After the end of culture, the dry weight fermentation products and ergothioneine content in each treatment group were measured, and the bioconversion efficiency of selenium was calculated.

The effects of different culture temperatures on dry weight fermentation products, ergothioneine content and selenium conversion efficiency were shown in Figures 9-11. It can be seen from Figure 9 that the culture temperature has little effect on the dry weight fermentation products, and the dry weight fermentation products at 25 °C is the largest, which is significantly different from that of other culture temperatures ($P < 0.01$ or $P < 0.05$), there was no significant difference in dry weight between other culture temperature ($P > 0.05$). In Figure 10, the highest ergothioneine content was observed at 26 °C, and there was no significant difference between 23 °C and 24 °C, 26 °C and 27 °C ($P > 0.05$). It can be seen from Figure 11 that the difference in selenium conversion efficiency between 24 °C and 25 °C was extremely significant ($P < 0.01$), and the difference of selenium conversion efficiency among other culture temperatres was not significant ($P > 0.05$). Therefore, the study on the effects of dry weight fermentation products, ergothioneine content and selenium conversion efficiency showed that 26 °C should be selected as the culture temperature for ergothioneine as the target product.

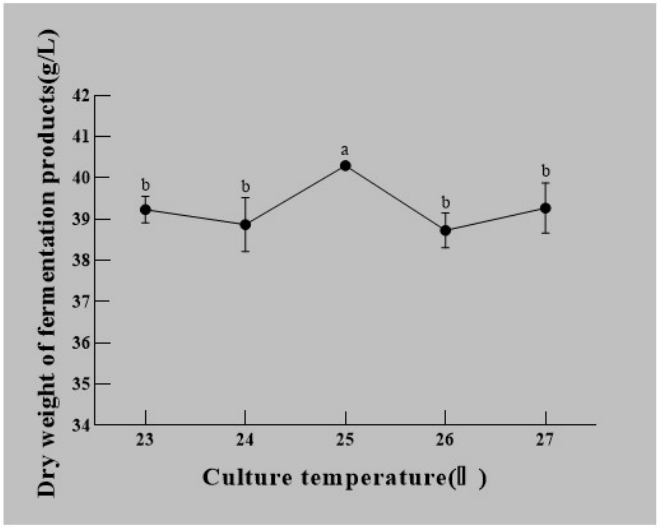

Figure 9. Effects of different culture temperature on dry weight fermentation products of *Pleurotus eryngii*. The letters in the figure are the differences between the treatments.

Effect of inoculation amount on submerged fermentation of Pleurotus eryngii

To study the effect of inoculation amount on submerged fermentation of *Pleurotus eryngii*, the inoculation amount was set to be 5%, 10%, 15%, 20% and 25%, respectively. After the end of culture, the dry weight fermentation products and ergothioneine content of each treatment group were measured, and the bioconversion efficiency of selenium was calculated.

The effects of different inoculation amount on dry weight fermentation products, ergothioneine content and selenium conversion efficiency were shown in Figures 12-14. It can be seen from Figure 12 that the dry weight fermentation products increased with the increase of inoculation amount. The dry weight of the fermentation was increased from 37.1 g/L to 42.27 g/L. In Figure 13, different inoculation amount had little effect on the ergothioneine content, and the ergothioneine content was

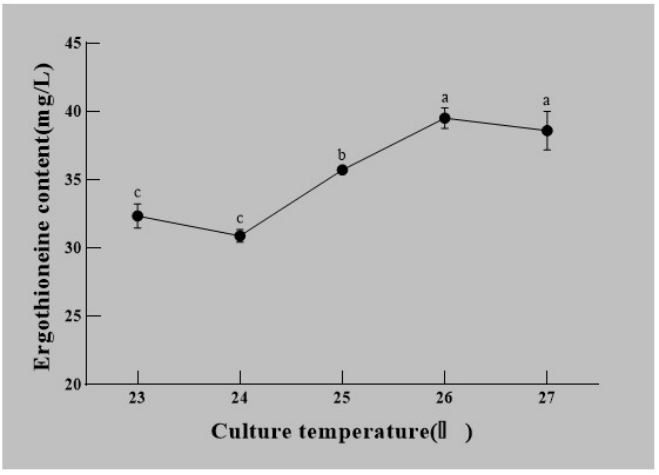

Figure 10. Effects of different culture temperature on ergothioneine content of *Pleurotus eryngii*. The letters in the figure are the differences between the treatments.

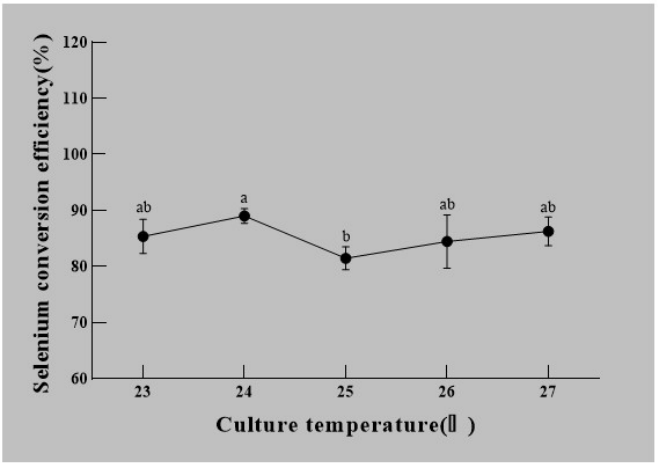

Figure 11. Effects of different culture temperature on selenium conversion efficiency of *Pleurotus eryngii*. The letters in the figure are the differences between the treatments.

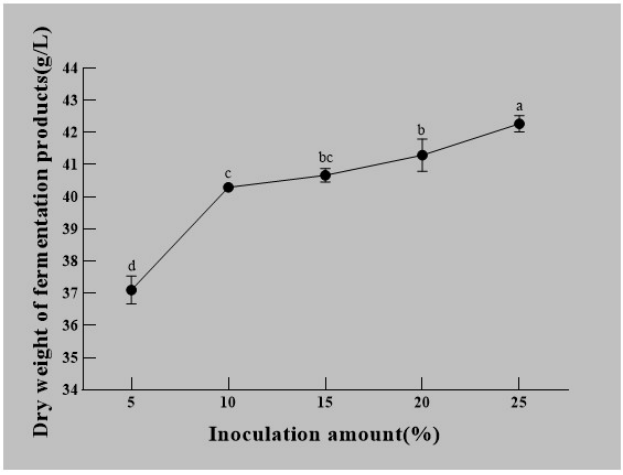

Figure 12. Effects of different inoculation amount on dry weight fermentation products of *Pleurotus eryngii*. The letters in the figure are the differences between the treatments.

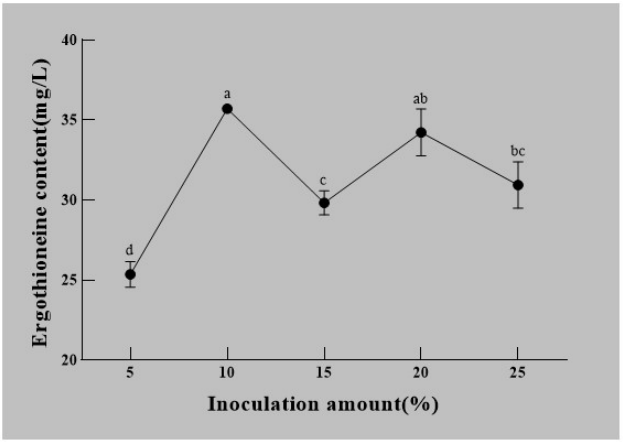

Figure 13. Effects of different inoculation amount on ergothioneine content of *Pleurotus eryngii*. The letters in the figure are the differences between the treatments.

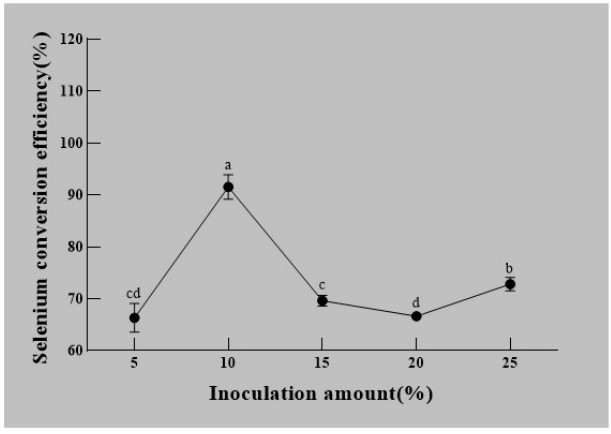

Figure 14. Effects of different inoculation amount on selenium conversion efficiency of *Pleurotus eryngii*. The letters in the figure are the differences between the treatments.

in the range of 25.35 mg/L to 35.71 mg/L. The ergothioneine content with 10% inoculation amount was the largest, and there was no significant difference between 10% and 20%, 25% and 15%, 20% inoculation amount ($P > 0.05$); It could be seen from Figure 14 that the selenium conversion efficiency reached the highest value when the inoculation amount was 10%. There was no significant difference in the selenium conversion efficiency between 5% and 15%, 20% inoculation amount ($P > 0.05$), and there were significant differences in selenium conversion efficiency of other inoculation amount ($P < 0.01$ or $P < 0.05$). In summary, it can be seen from the above analysis that from the perspective of saving economic costs, 10% inoculation amount can be used for submerged fermentation of *Pleurotus eryngii*.

Effect of culture time on submerged fermentation of Pleurotus eryngii

To study the effect of culture time on submerged fermentation of *Pleurotus eryngii*, the culture temperature was set to be 3, 5, 7, 9 and 11 d, respectively. After the end of culture, the dry weight fermentation products and ergothioneine content in each treatment group were measured, and the bioconversion efficiency of selenium was calculated.

The effects of different culture time on dry weight fermentation products, ergothioneine content and selenium conversion efficiency were shown in Figures 15-17. It can be seen from Figure 15 that the dry weight fermentation products increased with the increase of culture time in the early stage, and after 7 days, with the increase of culture time, the dry weight fermentation products began to show a downward trend, which may be due to the self-melting phenomenon of mycelium, and the dry weight fermentation products reached the maximum on the 7th day. Significant analysis showed that the dry weight fermentation products on the 7th day was significantly different from that on the 9th and 11th days ($P < 0.01$). In Figure 16, the ergothioneine content increased with the extension of culture time, and there was no significant difference in ergothioneine content between 7 and 11 days ($P > 0.05$). It can be seen from Figure 17 that the

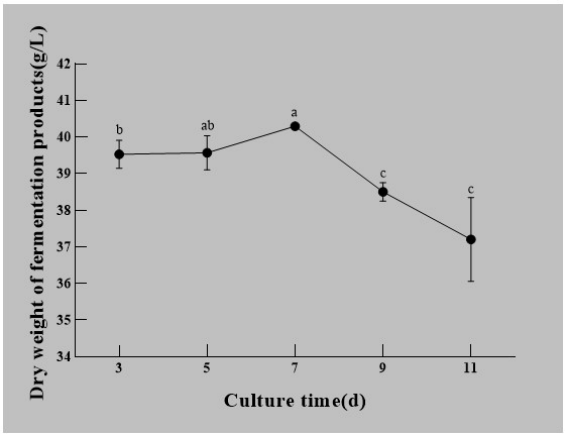

Figure 15. Effects of different culture time on dry weight fermentation products of *Pleurotus eryngii*. The letters in the figure are the differences between the treatments.

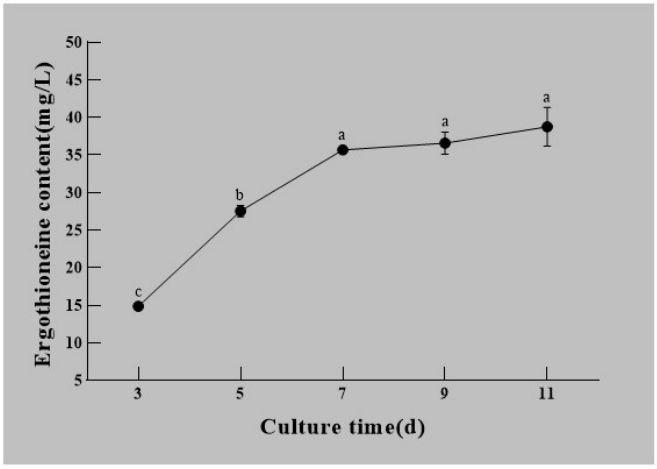

Figure 16. Effects of different culture time on ergothioneine content of *Pleurotus eryngii*. The letters in the figure are the differences between the treatments.

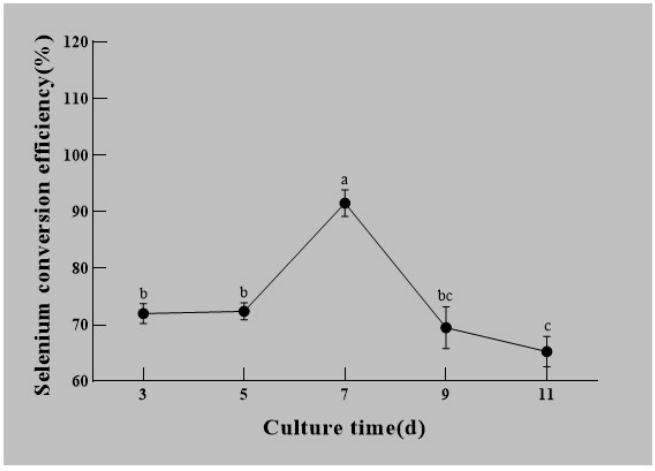

Figure 17. Effects of different culture time on selenium conversion efficiency of *Pleurotus eryngii*. The letters in the figure are the differences between the treatments.

selenium conversion efficiency also reached the highest value on the 7th day, and the selenium conversion efficiency on the 7th day was extremely significantly different from that on other incubation times ($P < 0.01$). Comprehensive analysis showed that 7 d was the best culture time for submerged fermentation of *Pleurotus eryngii*. At this time, the dry weight of fermentation product was 40.3 g/L, the ergothioneine content was 35.71 mg/L, and the selenium bioconversion efficiency was 91.52%.

4 Conclusion

In liquid culture, the dry weight of the culture was the highest and the selenium conversion efficiency was the highest when the concentration of sodium selenite was 3 μg/mL. Considering that the high concentration of sodium selenite may change the color of the medium or mycelium, and the actual needs of the study, 3 μg/mL was determined as the addition amount of *Pleurotus eryngii* ergothioneine directional fermentation.

The contents of ergothioneine and selenium in submerged fermentation metabolites of *Pleurotus eryngii* were significantly affected by non-nutritional conditions such as pH value, shaking speed, culture temperature, inoculation amount and culture time. The optimal culture conditions were optimized by single factor experiment as follows: pH value of culture medium 5.5 (natural), rotation speed of 180 r/min, culture temperature of 26 °C, inoculation amount of 10%, and culture time of 7 d. Under this optimal culture condition, the ergothioneine and selenium contents of *Pleurotus eryngii* fermentation products reached 39.42 mg/L and 29.78 mg/kg, respectively.

Ergothioneine is a natural antioxidant that can protect cells in the human body and is an important active substance in the body. Ergothioneine has a wide range of uses and market prospects in the fields of medicine, food and beverage, functional food, animal feed, cosmetics and biotechnology. Selenium is an essential trace element for the human body. In this study, edible mushroom liquid fermentation was used to prepare *Pleurotus eryngii* powder rich in ergothioneine and selenium, which can provide data reference for industrial-scale production. *Pleurotus eryngii* powder rich in ergothioneine and selenium has the potential to be developed as a third-generation functional food.

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References

Borodina, I., Kenny, L. C., McCarthy, C. M., Paramasivan, K., Pretorius, E., Roberts, T. J., van der Hoek, S. A., & Kell, D. B. (2020). The biology of ergothioneine, an antioxidant nutraceutical. Nutrition Research Reviews, 33(2), 190-217. http://dx.doi.org/10.1017/S0954422419000301. PMid:32051057.

Cai, A. Q., Xiao, J. G., Zheng, Q. H., Ke, Y., Fang, B. Y., Luo, Z. B., & Yang, X. B. (2009). Effects of temperature and pH on mycelial growth of Pleurotuseryngii strain 3 and 528. Hubei Agricultural Sciences, 48(7), 1670-1673.

Chen, S. Y., Ho, K. J., Liang, C. H., Tsai, C. H., Huang, L. Y., & Mau, J. L. (2012). Preparation of culinary-medicinal king oyster mushroom Pleurotus eryngii-fermented products with high ergothioneine content and their taste quality. International Journal of Medicinal Mushrooms, 14(1), 85-93. http://dx.doi.org/10.1615/IntJMedMushr.v14.i1.90. PMid:22339711.

Cheng, M. N., & Wang, Z. Y. (2021). Review on the relationship between selenium, organic selenium and human diseases and health. China Food Safety Magazine, 11, 44-47.

China, National Health and Family Planning Commission of the People's Republic of China, China Food and Drug Administration. (2017, October 6). National food safety standard-determination of

selenium in food- GB5009.93-2017. National Health and Family Planning Commission of the People's Republic of China/China Food and Drug Administration.

Hou, J. X., Jiang, Y. F., Zhao, X. J., Duan, S. N., & Wang, L. A. (2013). Analysis of functional components and antioxidant activity of lyophilized powder of cultured mycelia of Pleurotus eryngii. Shipin Kexue, 34(21), 329-332.

Hu, T., Li, H. F., Zhao, G. S., & Guo, Y. B. (2021). Selenium enriched Hypsizygus marmoreus, a potential food supplement with improved Se bioavailability. LWT, 140, 110819. http://dx.doi.org/10.1016/j.lwt.2020.110819.

Huang, B. X., Zhi, T. T., Zhao, Z. G., He, N., & Han, C. Y. (2019). The relationship between selenium and human health. Journal of Yichun University, 41(9), 95-101.

Kalaras, M. D., Richie, J. P., Calcagnotto, A., & Beelman, R. B. (2017). Mushrooms: a rich source of the antioxidants ergothioneine and glutathione. Food Chemistry, 233, 429-433. http://dx.doi.org/10.1016/j.foodchem.2017.04.109. PMid:28530594.

Kleftaki, S. A., Simati, S., Amerikanou, C., Gioxari, A., Tzavara, C., Zervakis, G. I., Kalogeropoulos, N., Kokkinos, A., & Kaliora, A. C. (2022). Pleurotus eryngii improves postprandial glycaemia, hunger and fullness perception, and enhances ghrelin suppression in people with metabolically unhealthy obesity. Pharmacological Research, 175, 105979. http://dx.doi.org/10.1016/j.phrs.2021.105979. PMid:34798266.

Li, R. W. S., Yang, C., Sit, A. S. M., Kwan, Y. W., Lee, S. M. Y., Hoi, M. P. M., Chan, S. W., Hausman, M., Vanhoutte, P. M., & Leung, G. P. H. (2014). Uptake and protective effects of ergothioneine in human endothelial cells. The Journal of Pharmacology and Experimental Therapeutics, 350(3), 691-700. http://dx.doi.org/10.1124/jpet.114.214049. PMid:25022513.

Liang, C. H., Ho, K. J., Huang, L. Y., Tsai, C. H., Lin, S. Y., & Mau, J. L. (2013a). Antioxidant properties of fruiting bodies, mycelia, and fermented products of the culinary-medicinal king oyster mushroom, Pleurotus eryngii (higher basidiomycetes), with high ergothioneine content. International Journal of Medicinal Mushrooms, 15(3), 267-275. http://dx.doi.org/10.1615/IntJMedMushr.v15.i3.40. PMid:23662614.

Liang, C. H., Huang, L. Y., Ho, K. J., Lin, S. Y., & Mau, J. L. (2013b). Submerged cultivation of mycelium with high ergothioneine content from the culinary-medicinal king oyster mushroom Pleurotus eryngii (higher basidiomycetes) and its composition. International Journal of Medicinal Mushrooms, 15(2), 153-164. http://dx.doi.org/10.1615/IntJMedMushr.v15.i2.40. PMid:23557367.

Liu, M. L., Cao, W. L., Gao, P., Zhao, J. H., Muhammad, U., Ni, S., Zhou, Y. Y., Wang, S., Pei, F., Zhang, Z. Z., Yuan, L. X., Wang, Z. M., Cui, A., Chen, Z. X., Feng, Z. M., Hu, K. M., Chen, H. Q., & Zuo, S. M. (2022). Effects of two different selenium fertilizers on accumulation of selenium and heavy metals in rice grains in field trials. Food Science and Technology, 42, e117521. http://dx.doi.org/10.1590/fst.117521.

Liu, Q., Zhang, W. Y., & Jiang, W. X. (2015). Biosynthesis and catabolism of L-ergothioneine. Natural Product Research and Development, 27(6), 1112-1117.

Liu, Q., Zhang, W. Y., Jiang, W. X., Mei, B. L., & Zhou, T. (2013, November 1). Research progress on the biosynthesis technology of l-ergothioneine. In China Biological Fermentation Industry Association (Ed)., Proceedings of 2013 International Amino Acid Industry Development Summit Forum (pp. 22-27). Shanghai: 2013 International Summit Forum on Amino Acid Industry Development.

Mwangi, R. W., Macharia, J. M., Wagara, I. N., & Bence, R. L. (2022). The antioxidant potential of different edible and medicinal mushrooms. Biomedicine and Pharmacotherapy, 147, 112621. http://dx.doi.org/10.1016/j.biopha.2022.112621. PMid:35026489.

Seale, L. A., Ogawa-Wong, A. N., & Berry, M. J. (2018). Sexual dimorphism in selenium metabolism and selenoproteins. Free Radical Biology & Medicine, 127, 198-205. http://dx.doi.org/10.1016/j.freeradbiomed.2018.03.036. PMid:29572096.

Singh, U., Gautam, A., Singha, T. K., Tiwari, A., Tiwari, P., Sahai, V., & Sharma, S. (2020). Mass production of Pleurotus eryngii mycelia under submerged culture conditions with improved minerals and vitamin D2. Lebensmittel-Wissenschaft + Technologie, 131, 109665. http://dx.doi.org/10.1016/j.lwt.2020.109665.

Tang, B. S., Wu, L., Chen, J. C., Shen, H. S., & Zheng, H. G. (2013). Optimizing nutritional value of wheat bran through Pleurotus geesteranus cultivation on a selenium-enriched medium. Fujian Journal of Agricultural Sciences, 28(1), 66-69.

Tang, B. S., Wu, L., Weng, M. J., Lai, P. F., & Li, Y. B. (2021). Optimization of medium for biosynthesis of ergothioneine by submerged fermentation of Pleurotus eryngii 528. Fujian Journal of Agricultural Sciences, 36(6), 728-734.

Wang, B., Zhao, N., Li, J., Xu, R., Wang, T., Guo, L., Ma, M., Fan, M., & Wei, X. (2021). Selenium-enriched Lactobacillus plantarum improves the antioxidant activity and flavor properties of fermented Pleurotus eryngii. Food Chemistry, 345, 128770. http://dx.doi.org/10.1016/j.foodchem.2020.128770. PMid:33302107.

Wu, L., Shen, H. S., Chen, W. W., Tang, B. S., & Zhang, Y. Z. (2011). Effect of culture medium with dietary fiber in selenium bioconversion by Pleurotus geesteranus. Fujian Journal of Agricultural Sciences, 26(2), 241-245.

Zhang, B., Li, Y., Zhang, F., Linhardt, R. J., Zeng, G., & Zhang, A. (2020). Extraction, structure and bioactivities of the polysaccharides from Pleurotus eryngii: a review. International Journal of Biological Macromolecules, 150, 1342-1347. http://dx.doi.org/10.1016/j.ijbiomac.2019.10.144. PMid:31759001.

Zhao, J. J., & Ma, R. X. (2017). Study on shaking flask fermentation conditions of Pleurotus eryngii liquid strain. Chinese Horticulture Abstracts, 27-28(8), 83.

Zheng, H. G., Chen, J. C., Weng, M. J., Ahmad, I., & Zhou, C. Q. (2020a). Structural characterization and bioactivities of a polysaccharide from the stalk residue of Pleurotus eryngii. Food Science and Technology, 40(Suppl. 1), 235-241. http://dx.doi.org/10.1590/fst.08619.

Zheng, S. X., Su, J., Wang, R., & Wang, G. J. (2013). Metabolism of selenium in microorganisms. Huazhong Nongye Daxue Xuebao, 32(5), 1-8.

Zheng, X., Sun, H., Wu, L., Kong, X., Song, Q., & Zhu, Z. (2020b). Structural characterization and inhibition on α-glucosidase of the polysaccharides from fruiting bodies and mycelia of Pleurotus eryngii. International Journal of Biological Macromolecules, 156, 1512-1519. http://dx.doi.org/10.1016/j.ijbiomac.2019.11.199. PMid:31783073.