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Effects of the *Castanea mollissima Blume* Shell Cultivation Substrate on the Yield and Nutritional Composition of *Pleurotus geesteranus*

Jinping Zhang, Xuebin Li, Yue Ying, Xiaohua Yao

Research Institute of Subtropical Forestry, Chinese Academy of Forestry, Hangzhou, China
Email: jinpingzhang@126.com

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Abstract

Cottonseed hull substrates blended with different ratios of *Castanea mollissima Blume* shell were prepared and used for the cultivation of *Pleurotus geesteranus*. The effects of the chestnut shell content on the mycelial growth rate, yield, nutritional composition and contents of heavy metals of the cultivated *Pleurotus geesteranus* were investigated. The results suggest that the *Castanea mollissima Blume* shell in substrate can increase the mycelial growth rate, yield, biological efficiency and the contents of protein, crude fiber, amino acids and essential amino acids of *Pleurotus geesteranus*. Further investigation suggests that the tannin and saponin in *Castanea mollissima Blume* shell and the C/N ratio of substrate significantly influence the mycelial growth rate. The crude fat content of *Pleurotus geesteranus* decreased, while the contents of heavy metals including mercury (Hg), arsenic (As), cadmium (Cd) and lead (Pb) increased with the increase of the *Castanea mollissima Blume* shell content in substrate. Based on these results, the content of *Castanea mollissima Blume* shell was optimized to be less than 30% for the cultivation of *Pleurotus geesteranus*.

Keywords

Castanea mollissima Blume Shell, *Pleurotus geesteranus*, Yield, Nutrients, Heavy Metals

1. Introduction

Castanea mollissima Blume (*C. mollissima*) is a member of the family Fagaceae and an important species of Castanea cultivated in the north and south of China.

It is cultivated in 26 provinces and cities across the country with the cultivation area of ~300 thousand hectares, accounting for more than 80% of the total production of the world [1]. *Castanea mollissima Blume* shell is the thorny shell of the fruit accounting for 10% of the weight of the fruit. It is rich in cellulose, hemicellulose, lignin, phenols, organic acids, polysaccharides, flavonoids, phytosterols, lactones, coumarins and tannins [2] [3]. With the development of the production and deep processing of *Castanea mollissima Blume* nut, more and more *Castanea mollissima Blume* shells have also produced. At present, *Castanea mollissima Blume* shell has been mainly used for the tannin production, chemical extractions and activated carbon preparation [4] [5]. Due to the lack of cost-effective treatment methods, most of the *Castanea mollissima Blume* shell products are still discarded or incinerated on site, imposing serious threats on land, water and atmospheres. To reduce environmental pollution and avoid wasting resources, it is imperative to develop suitable waste disposal methods of *Castanea mollissima Blume* shells. Lai *et al.* used *Castanea mollissima Blume* shells as the substrate to cultivate shiitake mushroom, and found that the mycelial growth rate, yield and quality of the shiitake mushroom cultivated on the substrate containing 30% *Castanea mollissima Blume* shell were comparable with those of the control group [6]. In addition, it was reported that the biology efficiency of the Pleurotusostreatus cultivated on *Castanea mollissima Blume* shell substrate reached up to 100% - 120% [7].

Pleurotus geesteranus, also known as pocket-sized oyster or pleurotusostreatus, belongs to the genus Pleurotaceae, order Agaricales, phylum basidiomycetes, domain Enkarya. It is rich in protein, fat, polysaccharides, vitamins, mironutrients, and 8 essential amino acids. *Pleurotus geesteranus* is very popular in Asia because of its crispy and delicious taste [8] [9] [10]. However, the sustainable development of the *Pleurotus geesteranus* cultivation has been limited due to the shortage of cultivation substrates, high costs and unstable market prices. In the current study, *Castanea mollissima Blume* shell was used to partially substitute the cottonseed hull substrate for the cultivation of *Pleurotus geesteranus*. The effects of the content of *Castanea mollissima Blume* shell on the mycelial growth rate, yield, biological efficiency, nutritional composition and contents of heavy metals of the *Pleurotus geesteranus* were evaluated, aimed to establish the optimal cultivation substrate for the cultivation of *Pleurotus geesteranus* and to provide a scientific foundation and technical support for the cultivation of *Pleurotus geesteranus* on *Castanea mollissima Blume* shell substrates.

2. Materials and Methods

2.1. Materials

Castanea mollissima Blume shell was collected from Xindeng Town, Hangzhou City, Zhejiang Province, China, and was dried and grinded for further use. *Pleurotus geesteranus* spawn and cottonseed hull were supplied by Wuhan SuiSuiFeng Agricultural Technology Development Co., Ltd., China. Rice bran, lime

and gypsum were collected from the Fuyang District, Hangzhou, Zhejiang, China as shown in Table 1.

2.2. Formulation of Substrates

Four cottonseed hull substrates (CK, A, B and C) containing different contents of *Castanea mollissima Blume* shell were prepared, among which the pure cottonseed hull substrate (CK) was used as the control, as shown in Table 2.

2.3. Experimental Methods

The ingredients of each substrate were weighted and homogenously blended. The water content of each substrate was adjusted to 55% - 60% with water. The substrate was mixed well and bagged into polypropylene plastic bags (17 cm × 33 cm × 0.5 cm). The number of bags and the dry weight of each bag were recorded. The bagged substrates were autoclaved for sterilization at 121˚C under 103.4 KPa for 2 h, cooled to room temperature, inoculated and cultured in a dark culture chamber at 20˚C - 23˚C and the humidity of 50% - 70%. The collar and lid were removed after the mycelia completely covered the bag. The inoculated substrates were then moved to the mushroom cultivation room of the temperature of 22˚C - 24˚C and the humidity of above 90%. The cultivation room was consistently ventilated to avoid the accumulation of carbon dioxide that might cause the malformation of *Pleurotus geesteranus*. The *Pleurotus geesteranus* were harvest as the mushroom caps became 2 - 3 cm big.

2.4. Measurement of Mycelial Growth Rate

For each substrate, 10 bags were randomly selected for the measurement of mycelial growth rate. The lengths of mycelia were measured every 5 days and the mycelial growth rate at the measuring time was calculated. The last measurement was conducted when the mycelia completely covered the bag. The average growth rate on each substrate was then calculated and analyzed [11].

Table 1. Chemical compositions of *Castaneamollissim ablume* shell and cottonseed hull (%).

	Cellulose	Hemicellulose	Lignin	Ash	TOC	TN	C/N	Tannin	Saponin
Castaneamollissim ablume shell	27.34	33.23	21.40	3.22	38.5	0.63	61.11	5.58	3.44
Cottonseed hull	24.83	24.83	31.68	2.41	56	2.03	27.6	-	-

-, not detected.

Table 2. Cottonseed hull substrates containing different ratios of *Castanea mollissima Blume* shell (%) and the corresponding C/N ratios.

Formula	Cottonseed hull	*Castanea mollissima Blume*shell	Rice bran	Lime	Gypsum	C/N
CK	78	0	20	1	1	25.94
A	68	10	20	1	1	27.01
B	48	30	20	1	1	29.54
C	28	50	20	1	1	33.21

A-C, *Castanea mollissima Blume* shell containing substrate; CK, conventional cottonseed hull substrate.

2.5. Biological Efficiency [12]

Biological efficiency (%) = yield per bag (g)/dry weight per bag (g) × 100%.

2.6. Determination of Nutrients

Fresh *Pleurotus geesteranus* were harvested and the residues of stipe were removed. The mushrooms were dried in an oven at 60˚C and pulverized for the measurements. The contents of protein, fat, crude fiber and amino acids were determined according to the protocol of GB 5009.5-2016, GB 5009.6-2016, GB/T 5009.10-2003 and GB 5009.124-2016 respectively. The contents of heavy metals including mercury (Hg), cadmium (Cd), arsenic (As) and lead (Pb) were determined according to the method of GB 5009.268-2016. Each measurement was repeated three times and the mean value was reported.

2.7. Data Analysis

All data analyses and processing were conducted in EXCEL.

3. Results and Discussion

3.1. Effects of *Castanea mollissima Blume* Shell Content on the Mycelial Growth Rate of *Pleurotus geesteranus*

Figure 1 shows the mycelial growth rates of *Pleurotus geesteranus* on substrates CK and A-C. It is clear that the mycelial growth rates on the substrates containing *Castanea mollissima Blume* shell are higher than that on substrate CK, following the order of A > B > C > CK. The mycelial growth rate on substrate A containing 10% *Castanea mollissima Blume* shell is the highest 0.49 ± 0.025 cm/d,

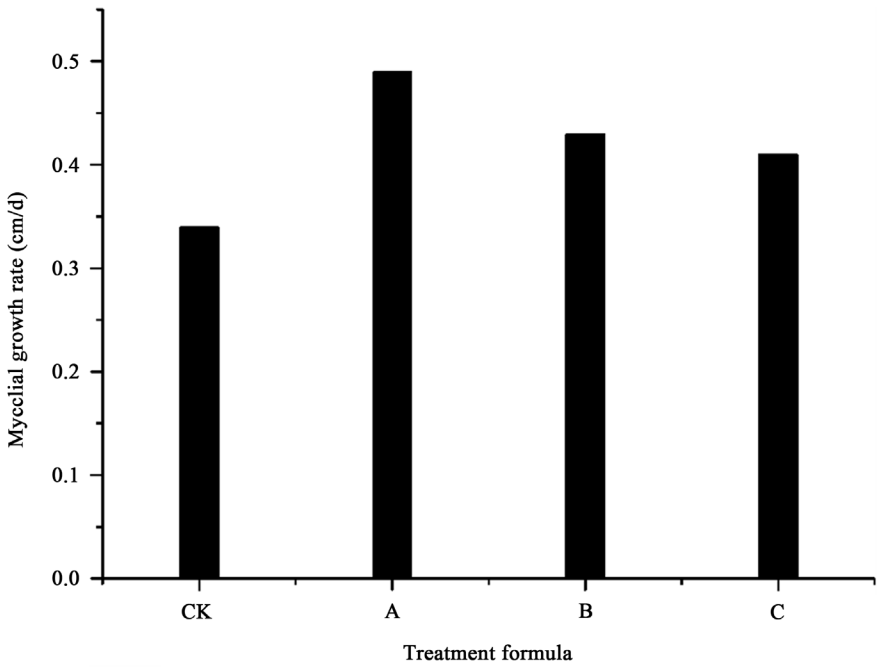

Figure 1. Effects of different substrates on the growth rate of *Pleurotus geesteranus.*

0.15 ± 0.005 cm/d higher than that on the control substrate CK. Both of the contents of tannin and saponin, as well as C/N, increase with the increase of the shell content in substrate, which causes the decrease of mycelial growth rate. These results indicate that partially substituting cottonseed hull substrate with *Castanea mollissima Blume* shell can accelerate the growth of *Pleurotus geesteranus*. However, the mycelial growth rate of *Pleurotus geesteranus* decreases with the increase of the shell content. The suitable C/N of substrate for the cultivation of *Pleurotus geesteranus* is 27. Higher or lower C/N ratios slow the mycelial growth of *Pleurotus geesteranus*.

3.2. Effects of *Castanea mollissima Blume* Shell on the Yield and Biological Efficiency of *Pleurotus geesteranus*

The *Pleurotus geesteranus* cultivated on the 4 substrates exhibited significantly different yields and biological efficiencies (Table 3) with the order of B > C > CK > A. Therefore, both of the yield and biological efficiency of *Pleurotus geesteranus* increase first and then decrease with the increase of the shell content. The highest yield and biological efficiency were obtained on substrate B containing 30% *Castanea mollissima Blume* shell with the C/N of 29.54. Increasing the *Castanea mollissima Blume* shell content of substrate increases the C/N ratio and decreases the yield and biological efficiency of the cultivated *Pleurotus geesteranus*.

3.3. Protein Content

The protein in edible fungi is considered as the "plant meat" and is a very good source of protein. In general, edible fungi contain usually 15% - 60% proteins [13]. The protein content of *Pleurotus geesteranus* cultivated on the *Castanea mollissima Blume* shell containing substrate increased with the increases of the shell content and the C/N ratio of substrate (Figure 2). The protein content obtained on substrate C reached up to 28.3 ± 0.83 g/100 g, 4.5 ± 0.08 g/100 g higher than that obtained on substrate CK. Therefore, it can be concluded that the protein content of *Pleurotus geesteranus* can be improved by blending certain amounts of *Castanea mollissima Blume* shell to the substrate and is increased with the increase of the C/N of substrate.

Table 3. Yields and biological efficiencies of the *Pleurotus geesteranus* cultivated on different substrates.

Formula	Yield (g/bag)	BE (%)
CK	180.70 ± 13.55	46.94
A	179.71 ± 11.14	46.68
B	237.86 ± 14.74	61.78
C	198.11 ± 12.28	51.46

Note: 385 g dry substrate per bag.

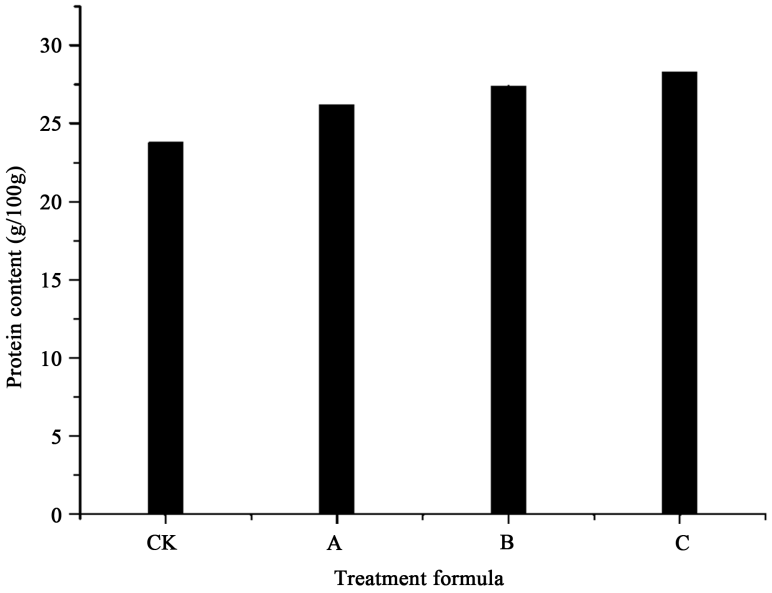

Figure 2. Comparison of protein content in different substrates of *Pleurotus geesteranus.*

3.4. Fat Content

The fat contents of edible fungi are low, usually lower than 10% [13]. The *Pleurotus geesteranus* cultivated on the substrates exhibited the fat contents in the order of CK > A > B > C (**Figure 3**), among which, the fat content of substrate C was lower 0.8 ± 0.03 g/100g, which was 1.1 ± 0.04 g/100g lower than that of group CK. Suggesting that adding *Castanea mollissima Blume* shell substrate could reduce the fat content of the mushroom.

3.5. Crude Fiber Content

Crude fiber refers to the insoluble dietary fibers mainly including cellulose, hemicellulose and lignin. As shown in **Figure 4**, the crude fiber content of *Pleurotus geesteranus* increased with the increases of the shell content and the C/N of substrate. The crude fiber content reached up to 10.63% ± 0.89% on substrate B containing 30% shell, which was 8.43% ± 0.71% higher than that obtained on substrate CK. Further increasing the shell content to 50% decreased the crude fiber content, yet the crude fiber content was still 6.92% ± 0.59% higher than that obtained on substrate CK. These results suggest that a certain amount of *Castanea mollissima Blume* shell in cottonseed hull substrate can increase the crude fiber content of the cultivated *Pleurotus geesteranus.*

3.6. Composition and Contents of Amino Acids

Edible fungi contain 17 - 18 of the 20 amino acids required by the human body, and can provide almost all 8 essential amino acids, especially lysine, methionine and threonine that are lacked in cereals [13]. **Table 4** lists the contents of amino acids, the total content of amino acids, the total content of essential amino acids, the total content of non-essential amino acids and the total content of umami amino acids of the *Pleurotus geesteranus* cultivated on different substrates. It is

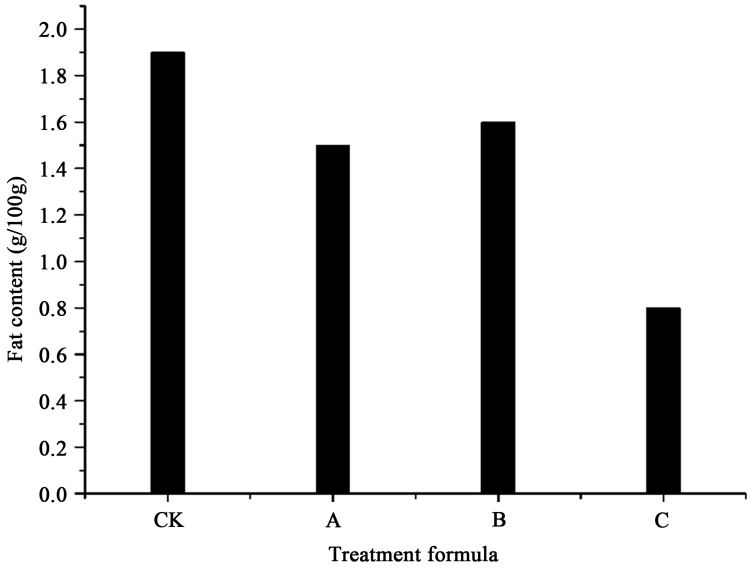

Figure 3. Comparison of fat content in different substrates of *Pleurotus geesteranus.*

Table 4. Composition and contents of amino acids in *Pleurotus geesteranus* cultivated on different substrates (%).

	CK	A	B	C
Aspartic acid#	1.10	1.84	1.75	2.01
Threonine*	0.52	0.98	0.94	1.02
Serine	0.60	1.05	0.98	1.06
Glutamic acid#	4.16	3.83	4.44	4.52
Glycine	0.19	0.94	0.91	0.96
Alanine	1.30	1.19	1.16	1.28
Valine*	0.68	1.93	2.05	1.98
Cystine	0.14	0.14	0.15	0.14
Methionine*	0.11	0.32	0.31	0.32
Isoleucine*	0.40	0.84	0.85	0.88
Leucine*	0.78	1.45	1.42	1.50
Tyrosine	0.50	0.55	0.64	0.61
Phenylalanine*	0.89	0.70	0.72	0.92
Lysine*	0.84	1.15	1.08	1.2
Histidine	0.18	0.36	0.34	0.33
Arginine	0.65	0.92	0.82	1.02
Proline	0.32	0.93	0.91	0.95
Total amino acids (T)	13.35	19.12	19.47	20.7
Total essential amino acids (E)	4.22	7.37	7.37	7.82
Total non-essential amino acids (N)	9.13	11.75	12.1	12.88
Total umami amino acids (W)	5.26	5.67	6.19	6.53
E/N (%)	46.19	62.72	60.91	60.71
E/T (%)	31.59	38.54	37.85	37.78

*essential amino acid; #umami amino acid.

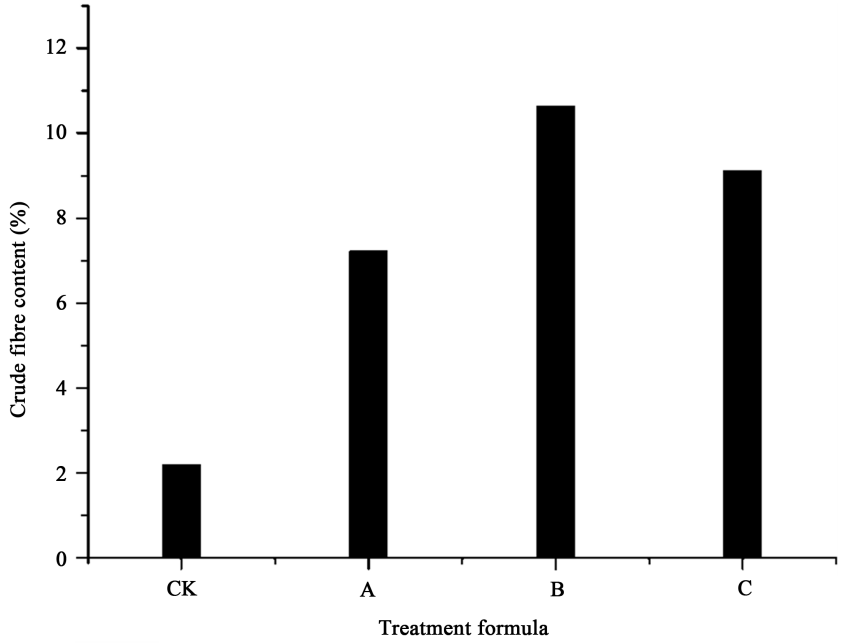

Figure 4. Comparison of crude fiber content in different substrates of *Pleurotus geesteranus.*

clear all of them are in the order of C > B > A > CK. In addition, the total essential amino acids account for over 37.78% of the total amino acids and are more than 60% of the total non-essential amino acids in the *Pleurotus geesteranus* cultivated on the *Castanea mollissima Blume* shell containing substrates. In contrast, in the *Pleurotus geesteranus* cultivated on substrate CK, the total essential amino acid content accounts for 31.59% of the total amino acid content and is only 46.19% of the total non-essential amino acid content. In all, the total contents of all kinds of amino acids increased with the increases of the shell content and the C/N of substrate. Therefore, *Castanea mollissima Blume* shell can be used as a substrate material to increase the total amino acid content and essential amino acid content of *Pleurotus geesteranus*.

3.7. Contents of Heavy Metals

The national standard of China GB 7096-2003 Hygienic standard for edible fungi requires total arsenic (As) ≤ 1.0 mg/kg, total mercury (Hg) ≤ 0.2 mg/kg and total lead (pb) ≤ 2.0 mg/kg in edible fungi. China National Food Safety Standard: Limits of Contaminants in Foods GB 2762-2012 stipulates that the Pb in foods is no more than 1.0 mg/kg and that of Cd is no more than 0.5 mg/kg. Green Food-Edible FungiGB 2762-2012 NY 749-2012 states the limits of Pb and Cd of no more than 1.0 mg/kg and 0.5 mg/kg, respectively. All contents of Hg, As, Cd and Pb of *Pleurotus geesteranus* increased with the increase of the *Castanea mollissima Blume* shell content in substrate and the Cd content became higher than the limit as the shell content increased to 50%. Therefore, *Castanea mollissima Blume* shell content in substrate for the cultivation of *Pleurotus geesteranus* should be no more than 30% (Table 5).

Table 5. Contents of heavy metals the *Pleurotus geesteranus* cultivated on different substrates (mg/kg).

	Hg	As	Cd	Pb
CK	0.013	0.152	0.440	0.042
A	0.156	0.465	0.124	0.036
B	0.029	0.556	0.192	0.034
C	0.056	0.763	0.520	0.058

4. Conclusion

Partially substituting the cottonseed hull cultivation substrate with *Castanea mollissima Blume* shell accelerated the mycelial growth and effectively increased the yield, biological efficiency, and the contents of protein, crude fiber, amino acid and essential amino acids of *Pleurotus geesteranus*. The mycelial growth rate of *Pleurotus geesteranus* decreased with the increase of the *Castanea mollissima Blume* shell content in substrate. The suitable C/N of substrate for the cultivation of *Pleurotus geesteranus* was determined to be 27. Higher or lower C/N ratios affected the mycelial growth rate of *Pleurotus geesteranus*. The fat content of *Pleurotus geesteranus* decreased and the contents of heavy metals increased with the increase of the shell content. The Cd content of *Pleurotus geesteranus* exceeded the limits defined in multiple national standards as the *Castanea mollissima Blume* shell content in substrate was increased to 50%. Therefore, the *Castanea mollissima Blume* shell content in substrate should be no more than 30% for the cultivation of *Pleurotus geesteranus*.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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