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Effect of *Carya cathayensis Sarg* Shell Substrate on Yield and Nutrient Amount of *Pleurotus geesteranus*

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Abstract

Using cottonseed hull as the control substrate, different proportions of the untreated and treated *Carya cathayensis Sarg* shells were added to cultivation of *Pleurotus geesteranus*. The mycelial growth rate, yield, nutritional composition and contents of heavy metals of the *Pleurotus geesteranus* cultivated on these substrates were determined. The results suggest that added to treated *Carya cathayensis Sarg* shell promoted the mycelial growth and increased the yield, biological efficiency and the contents of crude fiber, ash, amino acids and essential amino acids of *Pleurotus geesteranus*. In addition, the fat content and soluble sugar contents of *Pleurotus geesteranus* were decreased with the increase of the *Carya cathayensis Sarg* shell content in substrate, but their contents of heavy metals including mercury (Hg), arsenic (As), cadmium (Cd) and lead (Pb) were higher than that obtained on control substrate.

Keywords

Carya cathayensis sarg Shell, *Pleurotus geesteranus*, Mycelial Growth Rate, Yield, Nutrients

1. Introduction

Carya cathayensis Sarg (*C. cathayensis*) belongs to the genus Carya Nutt. of the Juglandaceae family with a history of 40 - 25 million years. It is mainly distributed in the Tianmu Mountain area (29˚ - 30˚N, 118˚ - 118˚E) along the border of Zhejiang and Fujian provinces in China, centered at Lin'an, Zhejiang Province (Dong et al., 2018). The *C. cathayensis* fruit is composed of the husk and the dry seed. The shell includes the outer and middle peels of the fruit. The hard-inner shell and nut are called the dry seed. The mass ratio between the

husk and dry seed is about 5:1 (Jiang, 2008). The *C. cathayensis* shell produced from the processing of hickory nut is usually discarded onsite or burned as a fuel, which imposes serious pollutions on the environment. Researchers have explored the application of *C. cathayensis* shell as the substrate for the cultivation of *Pleurotus geesteranus* (Wang, 2012). However, only the nutrients in the *Pleurotus geesteranus* cultivated on the *C. cathayensis* shell substrate were analyzed. The nutritional composition and heavy metals in the *Pleurotus geesteranus* cultivated on *C. cathayensis* shell containing substrates, as well as the effects of the saponin and tannin in *C. cathayensis* shell on the mycelial growth rate and nutritional composition of *Pleurotus geesteranus* have not yet reported.

Pleurotus geesteranus, also known as the pocket-sized mushroom and Pleurotus ostreatus, belongs to genus Pleurotaceae of the order Agaricales of the phylum basidiomycetes of the domain Enkarya. It contains abundant proteins, fats, fungal polysaccharides, vitamins and micronutrients, and 8 essential amino acids. *Pleurotus geesteranus* is very popular in China due to its crispy and delicious taste (Chen et al., 2018; Lu et al., 2001). In the present work, the conventional cottonseed hull substrate was partially substituted with the treated or untreated *C. cathayensis* shell for the cultivation of *Pleurotus geesteranus*. The formula of substrate was optimized by evaluating the mycelial growth rates, yields, biological efficiencies, nutritional compositions and contents of heavy metals of *Pleurotus geesteranus* cultivated on 9 designed substrates, aimed to provide a scientific foundation and technical supports for the cultivation of *Pleurotus geesteranus* on *C. cathayensis* shell containing substrates.

2. Materials and Methods

2.1. Materials

C. cathayensis shell was collected from the Lin'an District, Hangzhou City, Zhejiang Province, China. *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. EM microbial agent mainly composed of Bacillus, Lactobacillus, Bifidobacterium, Yeast, Photosynthetic Bacteria, Acetic Acid Bacteria, Actinobacillus, and other original species were purchased from Henan Nanhua Qianmu Biotechnology Co, Ltd, China.

2.2. Treatment and Sampling of *C. cathayensis* Shell

The composting of *C. cathayensis* shell was conducted in the greenhouse of Chinese Academy of Forestry Institute of Subtropical Forestry, Fuyang District, Hangzhou, Zhejiang Province, China, from March to April 2017. A certain amount of *C. cathayensis* shell was blended with 2% dry weight of brown sugar and 3% dry weight of the EM agent. The C/N ratio of the blend was adjusted to 30 with urea and the moisture content was adjusted to 55% with water. The blend was mixed well again and piled up for composting for one month. The

composting pile was turned every 5 days. The compost temperature increased to 40˚C on day 5, and the high temperature phase (>40˚C) lasted for 10 days with the highest temperature of 42˚C. The compost temperature decreased to room temperature on day 30. The compost pile was sampled by a five-point sampling method (Zhao et al., 2013). A portion of the fresh sample was stored at −20˚C, and the remaining sample was dried at 65˚C and pulverized for further analysis. Table 1 lists the physical and chemical properties of the *C. cathayensis* shell before and after the treatment determined by the method reported previously (Zhang et al., 2018) and those of the cottonseed hull reported in literature (Tian et al., 2013).

2.3. Formation of Substrates

Nine substrates including the pure cottonseed hull as the control substrate and the cottonseed hull partially substituted with different ratios of *C. cathayensis* shell were designed and prepared as shown in Table 2.

2.4. Experimental Methods

The mushroom test was conducted in January 2019 in the laboratory of the institute of subtropical forestry, Chinese academy of forestry, fuyang district, hangzhou city, zhejiang province, China. The ingredients of each substrate were weighed and well mixed. The moisture content of each substrate was adjusted to

Table 1. Physicochemical parameters of raw materials.

	Cellulose	Hemicellulose	Lignin	Ash	TOC	TN	C/N	Tannin	Saponin
Untreated *C. cathayensis* shell	20.63	22.48	49.78	6.88	45.4	0.72	63.23	3.64	4.86
Treated *C. cathayensis* shell	23.12	11.29	60.27	7.85	36.4	1.74	20.9	1.32	1.28
Cottonseed hull	24.83	24.83	31.68	2.41	56	2.03	27.6	-	-

-: not detected.

Table 2. Different substrate formulations (%) and C/N.

Formula	Cottonseed hull	*C. cathayensis* shell	Rice bran	Lime	Gypsum	C/N
A1	73	5	20	1	1	26.58
A2	68	10	20	1	1	27.07
A3	48	30	20	1	1	30.38
A4	28	50	20	1	1	34.62
B1	73	5	20	1	1	25.68
B2	68	10	20	1	1	25.22
B3	48	30	20	1	1	24.11
B4	28	50	20	1	1	22.68
CK	78	0	20	1	1	25.94

Note: A1 - A4: untreated *C. cathayensis* shell; B1 - B4: treated *C. cathayensis* shell; CK conventional formula.

55% - 60% with water. Each substrate was then mixed well and bagged with polypropylene plastic bags (17 cm × 33 cm × 0.5 cm). The number of bags of each substrate and the dry weight of each bag were recorded. The bagged substrates were autoclaved 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 under the humidity of 50% - 70%. The collar and lid were removed after the mycelia covered the bag completely, and the bags were moved to a mushroom cultivation room with the temperature of 22˚C - 24˚C and the humidity of over 90%. The cultivation room was kept ventilated to avoid the accumulation of carbon dioxide that might cause the malformation of the mushroom. The *Pleurotus geesteranus* were harvest as the mushroom caps became 2 - 3 cm big.

2.5. Determination of Mycelial Growth Rate

For each substrate, 10 bags were randomly selected. The lengths of mycelia were measured every 5 days and the mycelial growth rate at every measurement was calculated. The last measurement was conducted as the mycelia completely covered the bag. The average mycelial growth rate on each substrate was calculated and analyzed (Song et al., 2017).

2.6. Biological Efficiency

Biological efficiency (%) = yield per bag (g)/dry weight per bag (g) × 100% (Lin et al., 2010).

2.7. Determination of Nutritional Composition

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 ash, fat, crude fiber and amino acids were determined according to the protocol defined in GB 5009.4-2016, GB 5009.6-2016, GB/T 5009.10-2003 and GB 5009.124-2016. The contents of heavy metals including Hg, Cd, As and Pb were determined according to the method of GB 5009.268-2016. Determination of soluble sugar by anthrone colorimetry. Each measurement was repeated three times and the mean value was reported.

2.8. Data Analysis

All data analyses and processing were conducted in EXCEL.

3. Results and Analysis

3.1. Effects of *C. cathayensis* Shell Content on the Mycelial Growth of *Pleurotus geesteranus*

Figure 1 shows the different mycelial growth rates obtained on the substrates containing different amounts of untreated or treated *C. cathayensis* shell. The mycelial growth rates on substrates A1 - A4 and B1 - B4 are higher than that on the control substrate CK. For the substrates A1 - A4, the contents of tannin and

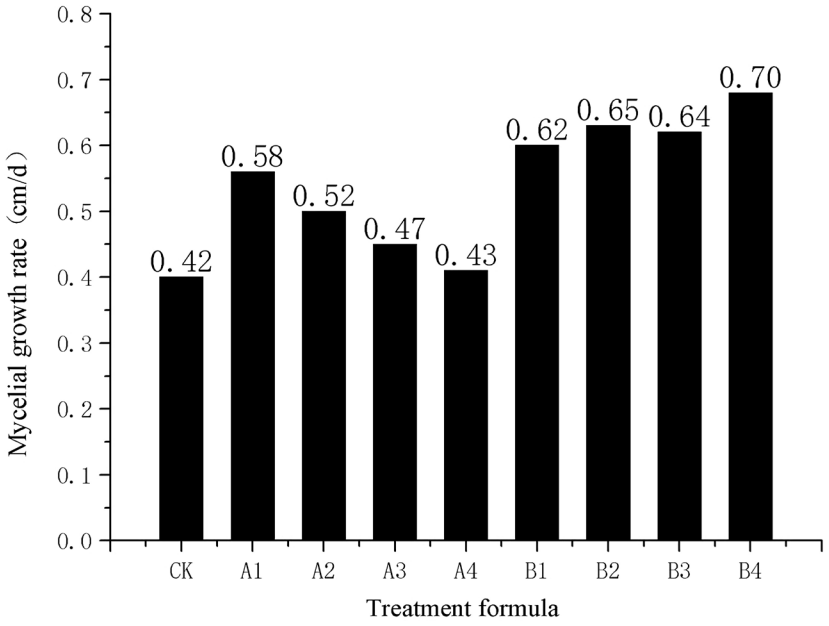

Figure 1. Effects of different substrates on the growth rate of *Pleurotus geesteranus*.

saponin and the C/N of substrate increase with the increase of the untreated *C. cathayensis* shell content. The mycelial growth rate more slowly, following the order of A1 > A2 > A3 > A4 > CK. On the substrates B1-B4, the mycelial growth rate increased with the increase of the treated *C. cathayensis* shell with the order of B4 > B2 > B3 > B1 > CK. In addition, the overall mycelial growth rate on substrates B1-B4 is higher than that on substrates A1-A4. These results suggest that the *C. cathayensis* shell added in cottonseed hull substrate can accelerate the mycelial growth of *Pleurotus geesteranus*. High concentrations of Tannin and saponin inhibit the mycelial growth of *Pleurotus geesteranus*, and low concentrations of tannin and saponin promote the growth of *Pleurotus geesteranus*. The C/N ratios of substrate greater than 30 inhibit the mycelial growth of *Pleurotus geesteranus*.

3.2. Effects of *C. cathayensis* Shell on the Yield and Biological Efficiency of *Pleurotus geesteranus*

Table 3 lists the yields and biological efficiencies of *Pleurotus geesteranus* cultivated on different substrates. It is clear that both of them follow the order of B > A > CK. For the substrates A1 - A4, the contents of tannin and saponin, as well as the C/N of substrate increase with the increase of the untreated *C. cathayensis* shell content. The highest yield and biological efficiency of *Pleurotus geesteranus* were obtained on substrate A2, and those obtained on substrate A4 were the lowest, yet 3.44 g and 1.34% higher than those obtained on substrate CK. Among the substrates B1 - B4, substrate B3 gave the *Pleurotus geesteranus* of the highest yield and biological efficiency, which were 101.86 g and 26.45% higher than those obtained on substrate CK, and 83.34 g and 21.64% higher than those on substrate A3. When the addition amount of *C. cathayensis* shell was higher than

Table 3. Yield and Biological efficiency of different formulas of *Pleurotus geesteranus.*

Formula	Yield (g/bag)	BE (%)
CK	180.7	46.49
A1	262.43	68.16
A2	271.88	70.62
A3	199.22	51.75
A4	184.14	47.83
B1	267.74	69.54
B2	270.81	70.34
B3	282.56	73.39
B4	223.64	58.09

Each bag contain 385 g dry weight of substrate.

30%, the yield and biological efficiency of *Pleurotus geesteranus* began to decrease, but both were higher than CK group. These results suggest that certain amounts of *C. cathayensis* shell in cottonseed hull substrate can improve the yield and biological efficiency of *Pleurotus geesteranus.*

3.3. Ash Content

The ash content reflects the mineral content of an edible fungus. The higher the ash content, the richer the mineral elements in the edible fungus. In general, the mineral content in edible fungi is 3% - 12% with the average value of 7%, among which K, P, Na, Ca and Mg account for 56% - 80% of the total mineral content. These minerals play important roles in regulating the body fluids and maintaining normal metabolism of cells (Tong et al., 2006). **Figure 2** shows the ash contents of the *Pleurotus geesteranus* cultivated on different substrates. Because the ash content of the untreated *C. cathayensis* shell is very low (**Table 1**), the ash content of *Pleurotus geesteranus* decreases first and then increases with the increase of the untreated husk content in substrate. In addition, the ash contents of the *Pleurotus geesteranus* cultivated on substrates A1 - A4 are higher than that of the control group. The treatment significantly increased the ash content of *C. cathayensis* shell. Therefore, the ash content of *Pleurotus geesteranus* increased with the increase of the treated husk content in substrate. The highest ash content of *Pleurotus geesteranus* was obtained on substrate B4 with the value of 8.60 g/100 g, 1.40 g/100 g higher than that obtained on CK and 0.60 g/100 g higher than that obtained on A4. The *Pleurotus geesteranus* cultivated on substrate B1 gave the lowest ash content of 7.70 g/100 g, but which was still 0.50 g/100 g higher than that obtained on substrate CK. Based on these results, it can be concluded that adding *C. cathayensis* shell to the cultivation substrate can increase the ash content of the cultivated *Pleurotus geesteranus.*

3.4. Fat Content

The fat contents of edible fungi are usually low, generally below 10% (Tong et

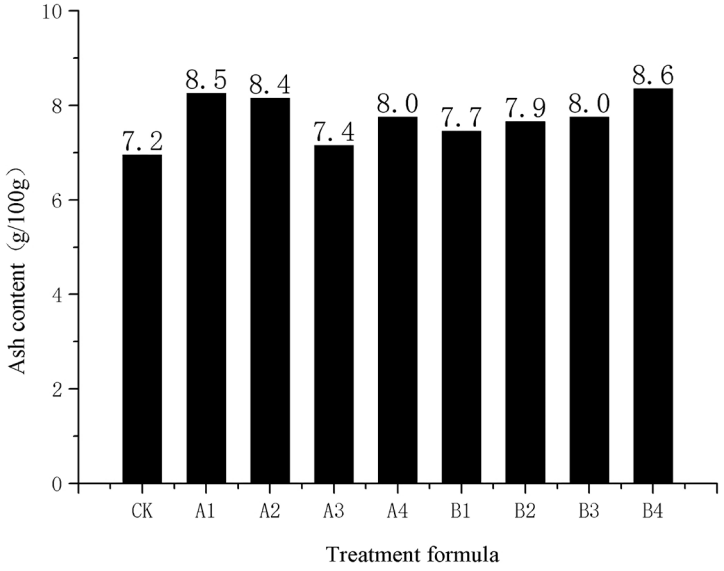

Figure 2. Comparison of ash content in different substrates of *Pleurotus geesteranus*.

al., 2006). As shown in **Figure 3**, the fat contents of *Pleurotus geesteranus* cultivated on the substrates containing untreated or treated *C. cathayensis* shell are lower than that of the control group. For the *Pleurotus geesteranus* cultivated on substrates containing untreated *C. cathayensis* shell, the fat content follows the order of A1 > A2 > A3 > A4 due to the low crude fat content of the untreated *C. cathayensis* shell. The *Pleurotus geesteranus* cultivated on substrates B1 - B4 containing treated *C. cathayensis* shell exhibited the fat contents in the order of B1 > B2 > B3 > B4. The overall fat contents of *Pleurotus geesteranus* cultivated on different substrates are in the order of CK > A1 > A2 = B1 > B2 > A3 > B3 > A4 > B4, e.g. the fat content of *Pleurotus geesteranus* decreases with the increase of the *C. cathayensis* shell content in substrate. These results indicate that *C. cathayensis* shell can be used as a substrate material to reduce the fat content of cultivated *Pleurotus geesteranus*.

3.5. Content of Soluble Sugar

Figure 4 shows the contents of soluble sugar of the *Pleurotus geesteranus* cultivated on different substrates, with the overall order of CK > B > A. Either on substrates A1 - A4 containing untreated *C. cathayensis* shell or on substrates B1 - B4 containing treated *C. cathayensis* shell, the soluble sugar content of *Pleurotus geesteranus* increases first and then decrease with the increase of the husk content in substrate, but is lower than that of the control group. It can be explained with the low contents of soluble sugar and hemicellulose and high lignin contents in the *C. cathayensis* shell containing substrates.

3.6. Crude Fiber Content

Crude fiber refers to insoluble dietary fibers mainly including cellulose, hemicellulose and lignin. As shown in **Figure 5**, the crude fiber contents of the *Pleurotus*

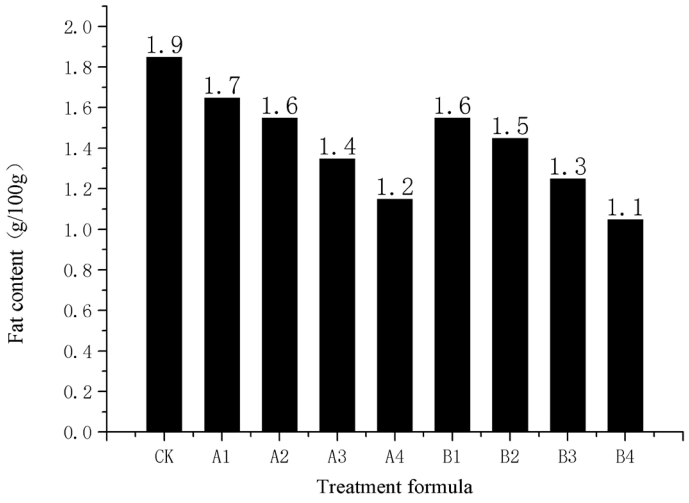

Figure 3. Comparison of fat content in different substrates of *Pleurotus geesteranus*.

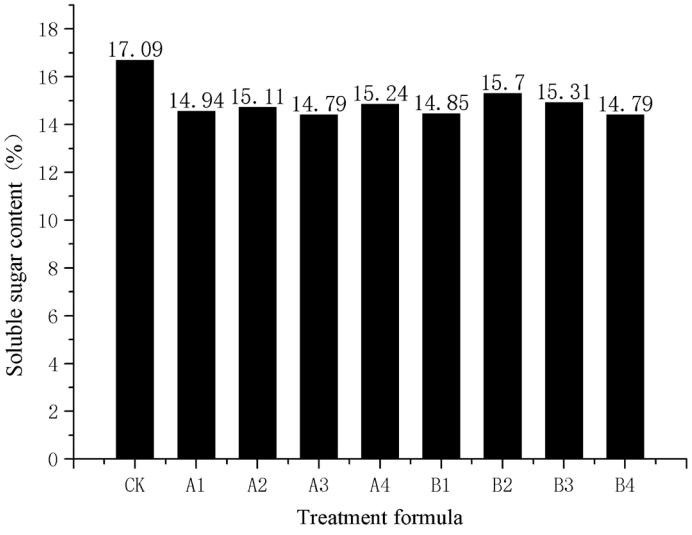

Figure 4. Comparison of soluble sugar content in different substrates of *Pleurotus geesteranus*.

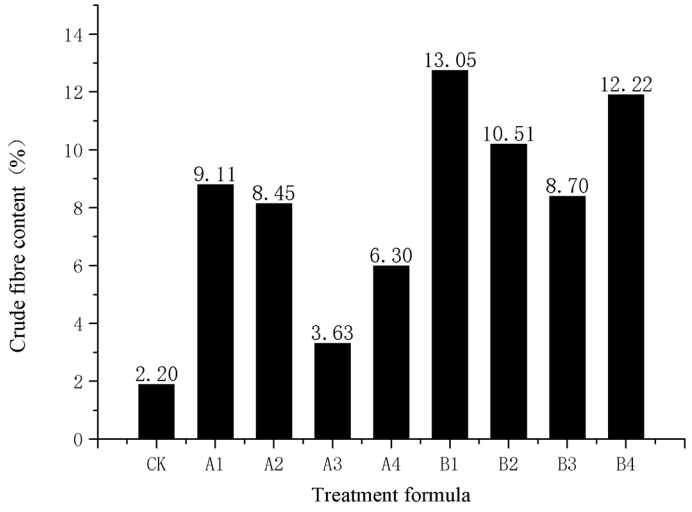

Figure 5. Comparison of crude fiber content in different substrates of *Pleurotus geesteranus*.

geesteranus cultivated on different substrates are significantly different. The crude fiber content of *Pleurotus geesteranus* cultivated on the treated *C. cathayensis* shell containing substrate is highest, followed by that obtained on the untreated *C. cathayensis* shell substrate. The control group exhibited the lowest crude fiber content. On the substrates A1 - A4 containing untreated *C. cathayensis* shell, the crude fiber content of *Pleurotus geesteranus* decreased first and then increased with the increase of the untreated husk content. The crude fiber content of the *Pleurotus geesteranus* cultivated on substrate A1 is the highest with the value of 9.11%, 6.91% higher than that of the control group. That of the *Pleurotus geesteranus* cultivated on substrate A3 is the lowest with the value of 3.63%, but it is still 1.43% higher than that of the control group. The crude fiber content of *Pleurotus geesteranus* cultivated on the treated *C. cathayensis* shell containing substrate decreased first and then increased with the increase of the husk content. However, the overall crude fiber contents are higher than those obtained on substrates A1 - A4. The *Pleurotus geesteranus* cultivated on substrate B1 exhibited highest crude fiber content of up to 13.05%, which was 10.85% higher than that of the control group and 3.94% higher than that obtained on A1. The cultivation on substrate B3 resulted in the lowest crude fiber content of 8.70%, but it was still 6.50% higher than that of the control group and 5.07% higher than that obtained on substrate A3. It can be explained that the cellulose and lignin in the untreated *C. cathayensis* shell are macromolecules and thus cannot be effectively utilized by the edible fungus. In contrast, the structures of cellulose and lignin in the treated *C. cathayensis* shell have been destroyed during composting, which makes them easily utilized by the *Pleurotus geesteranus*. Therefore, the crude fiber content of the *Pleurotus geesteranus* cultivated on the substrate containing treated *C. cathayensis* shell is higher than those obtained with substrate containing the untreated *C. cathayensis* shell and the control substrate. The cultivation on the substrate containing the untreated *C. cathayensis* shell resulted in the *Pleurotus geesteranus* with higher crude fiber contents than that of the control. Based on these results, it can be concluded that blending *C. cathayensis* shell into the conventional cottonseed hull substrate can increase the crude fiber content of *Pleurotus geesteranus*.

3.7. Composition and Contents of Amino Acids

Edible fungi contain 17 - 18 of amino acids required by human body and almost all 8 essential amino acids, especially lysine, methionine and threonine that are lacked in cereals (Tong et al., 2006). **Table 4** lists the contents of amino acids in the *Pleurotus geesteranus* cultivated on different substrates. It is clear that partially substituting cottonseed hull substrate with *C. cathayensis* shell increased the total amino acid content of *Pleurotus geesteranus* in the order of B > A > CK. On the substrates A1 - A4, the total amount of amino acids, the total amount of essential amino acids, the total amount of non-essential amino acids and the total amount of umami amino acids of *Pleurotus geesteranus* decreased

Table 4. Amino acid composition and content of *Pleurotus geesteranus* cultivated in different substrates.

	CK	A1	A2	A3	A4	B1	B2	B3	B4
Aspartic acid (Asp)#	1.10	1.74	1.77	1.63	1.73	1.76	1.63	1.47	1.99
Threonine (Thr)*	0.52	0.95	0.95	0.87	0.98	0.96	0.96	0.82	1.07
Serine (Ser)	0.60	1.00	1.00	0.93	1.00	1.00	0.95	0.84	1.11
Glutamic acid (Glu)#	4.16	4.39	3.90	3.50	4.56	4.38	4.30	3.72	4.62
Glycine (Gly)	0.19	0.87	0.88	0.80	0.88	0.86	0.91	0.79	1.00
Alanine (Ala)	1.30	1.33	1.24	1.14	1.26	1.28	1.24	1.07	1.37
Valine (Val)*	0.68	1.93	1.88	1.93	2.01	1.91	2.06	1.80	2.00
Cystine (Cys)	0.14	0.12	0.12	0.12	0.13	0.14	0.12	0.11	0.14
Methionine (Met)*	0.11	0.28	0.31	0.28	0.31	0.31	0.28	0.25	0.34
Isoleucine (Ile)*	0.40	0.83	0.84	0.74	0.83	0.80	0.86	0.72	0.93
Leucine (Leu)*	0.78	1.41	1.44	1.28	1.44	1.37	1.44	1.19	1.59
Tyrosine (Tyr)	0.50	0.54	0.62	0.49	0.65	0.63	0.58	0.54	0.65
Phenylalanine (Phe)*	0.89	0.87	0.88	0.78	0.92	0.92	0.83	0.66	0.93
Lysine (Lys)*	0.84	1.13	1.16	1.02	1.19	1.14	1.14	0.96	1.28
Histidine (His)	0.18	0.32	0.34	0.28	0.31	0.33	0.30	0.26	0.36
Arginine (Arg)	0.65	0.84	0.90	0.84	0.87	0.91	0.77	0.75	1.04
Proline (Pro)	0.32	0.94	0.89	0.81	0.93	0.92	0.91	0.76	1.01
Total amino acids (T)	13.35	19.49	19.12	17.44	20.00	19.62	19.28	16.71	21.43
Total essential amino acids (E)	4.22	7.40	7.46	6.90	7.68	7.41	7.57	6.40	8.14
Total non-essential amino acids (N)	9.13	12.09	11.66	10.54	12.32	12.21	11.71	10.31	13.29
Total umami amino acids (W)	5.26	6.13	5.67	5.13	6.29	6.14	5.93	5.19	6.61
E/N (%)	46.19	61.21	63.98	65.46	62.34	60.69	64.65	62.08	61.25
E/T (%)	31.60	37.97	39.02	39.56	38.40	37.77	39.26	38.30	37.98

*essential amino acid; #umami amino acid.

first and then increased with the increases of the untreated *C. cathayensis* shell content and the C/N of the substrate. The *Pleurotus geesteranus* cultivated on substrate A4 exhibited the highest total amount of amino acids, of which the essential amino acids accounted for 38.40% of the total amino acids and are 62.34% of the non-essential amino acids. For the *Pleurotus geesteranus* cultivated on substrates B1 - B4, the total amounts of various amino acids decreased first and then increased with the increase of the treated *C. cathayensis* shell con-

tent, but the overall amounts are higher than those obtained with the untreated *C. cathayensis* shell and those of the control group. The highest total amino acid content was obtained on substrate B4, with the essential amino acids accounting for 37.98% of total amino acids. The total essential amino acid content is 61.25% that of the non-essential amino. It is clear that the total amount of each amino acid of *Pleurotus geesteranus* decreases first and then increases with the increase of the *C. cathayensis* shell content and is higher than that of the control group. In addition, the ratios of the essential amino acids in the *Pleurotus geesteranus* cultivated on the *C. cathayensis* shell containing substrates meet the ideal E/T of protein (40%) and E/N (60%) of edible fungi proposed by WHO/FAO.

3.8. Contents of Heavy Metals

The national standard of China *Hygienic standard for edible fungi*, GB 7096-2003, 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. *Green Food-Edible Fungi*, GB 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. Table 5 lists the contents of As, Cd, Pb and Hg detected in the *Pleurotus geesteranus* cultivated on different substrates. The heavy metal contents of *Pleurotus geesteranus* cultivated on the substrate CK are within the limits defined in the national standards. As the untreated *C. cathayensis* shell added into the substrate, the heavy metal contents of *Pleurotus geesteranus* increased with the increase of the husk content, but all heavy metal contents were within the limits defined above. Similarly, on substrates B1 - B4, the heavy metal contents of *Pleurotus geesteranus* increased with the increase of the treated husk content, and the As content became higher than the limits mentioned above as the husk content increased to 30%. It has been reported that edible fungi have strong abilities to accumulate heavy metals. It is well accepted that inorganic arsenic is highly toxic, and organic arsenic is less toxic or non-toxic. Therefore, it is recommended that limit of As refers to the inorganic As (Lin et al., 2012).

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

	Hg	As	Cd	Pb
A1	0.023	0.844	0.117	0.031
A2	0.018	0.829	0.091	0.022
A3	0.014	0.609	0.065	0.013
A4	0.022	0.915	0.113	0.035
B1	0.017	0.676	0.039	0.018
B2	0.017	0.801	0.07	0.033
B3	0.024	1.24	0.067	0.053
B4	0.003	0.95	0.091	0.039
CK	0.013	0.152	0.440	0.042

4. Conclusion

C. cathayensis shell was added to the cottonseed hull substrate for the cultivation of *Pleurotus geesteranus*. It was found that the treated *C. cathayensis* shell in substrate accelerated the mycelial growth and effectively increased the yield, biological efficiency and the contents of crude fiber, amino acids and essential amino acids of *Pleurotus geesteranus*. The content of tannin and saponin and the C/N ratio of substrate affected the mycelial growth rate of *Pleurotus geesteranus*. Adding *C. cathayensis* shell to the substrate also reduced the fat and soluble sugar contents of the cultivated *Pleurotus geesteranus*. The ash content of the *C. cathayensis* shell in substrate directly affects the ash content of cultivated *Pleurotus geesteranus*. The contents of heavy metals including Hg, As, Cd and Pb increase with the increase of the *C. cathayensis* shell content in substrate, but are within the limits defined in national standards, except for the As content as the *C. cathayensis* shell content increased to 30%.

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Conflicts of Interest

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

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