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Effects of the *Camellia oleifera* Shell 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

Pleurotus geesteranus was cultivated on the substrates blended with different ratios of treated and untreated *Camellia oleifera* shells using cottonseed hull as the control substrate. The mycelial growth rate, yield, nutritional composition, ash and heavy metals of the *Pleurotus geesteranus* cultivated on these substrates were compared. The results suggest that the *Camellia oleifera* shell in substrate can accelerate the mycelial growth and increase the yield, nutrients and the contents of protein, ash, crude fiber and amino acid of *Pleurotus geesteranus*. It was found that the contents of tannin and saponin in *Camellia oleifera* shell affected the mycelial growth rate. The optimal C/N of the substrate for the growth of *Pleurotus geesteranus* was determined to be 27 ± 0.7. The C/N ratios higher than 30 reduced the protein, fat and soluble sugar contents of the cultivated *Pleurotus geesteranus*. The contents of heavy metals including Hg, As, Cd and Pb were found in *Pleurotus geesteranus* cultivated on the substrates containing *Camellia oleifera* shell complex.

Keywords

Camellia oleifera Shell, *Pleurotus geesteranus*, Saponin, Nutrient, Yield

1. Introduction

Camellia oleifera is an important woody oil crop in China. It belongs to the genus *Camellia* in the family Theaceae. *Camellia oleifera* is widely distributed in the provinces of Hunan, Jiangxi and Guangxi in China with the cultivation area of ~55 million mu. The oil content of *Camellia oleifera* seed is as high as ~55%. *Camellia* seed oil is rich in unsaturated fatty acids, such as oleic acid and linoleic acid, and thus is known as the "Oriental Olive Oil" [1]. *Camellia oleifera* shell

(*C. oleifera* shell) is a by-product of *Camellia* seed oil processing, which generally accounts for more than 60% of the whole fresh fruit. It is an excellent renewable source containing 18.62% of cellulose, 49.34% of hemicellulose, 29.71% of lignin, 2.57% of ash, 2.26% of tannin and 4.8% of saponin [2]. However, *Camellia oleifera* shell is usually discarded or burned, which not only wastes the resource, but also causes environmental pollutions. *Camellia oleifera* shell has been used as a substitute substrate of cottonseed hull for the cultivation of edible mushrooms, such as enokitake mushroom [3], shiitake mushroom [4], *Auricularia auricula-judae* [5], *Hericium erinaceus* [6] and so on, to improve their yields. However, the effects of *Camellia oleifera* shell substrate on the nutritional composition and heavy metal contents of the cultivated edible mushrooms have not been investigated. Whether the saponin and tannin in *Camellia oleifera* shell inhibit the mycelial growth and affect the nutritional composition of the cultivated mushrooms are not yet studied.

Pleurotus geesteranus, also known as pocket-sized oyster, belongs to domain Enkarya, phylum basidiomycetes, order Agaricales, genus Pleurotaceae. It is rich in nutrients, proteins, polysaccharides, vitamins, micronutrients and the 8 essential amino acids for the human body. The crispy and delicious taste of *Pleurotus geesteranus* makes it very popular in China [7] [8]. In the present study, *Pleurotus geesteranus* was cultivated on the cottonseed hull substrates partially substituted with treated and untreated *Camellia oleifera* shells. The composition of the substrate was optimized based on the mycelial growth rate, yield, biological efficiency, nutrient contents and heavy metal contents of the *Pleurotus geesteranus* cultivated on different substrates, aiming to provide a scientific foundation and technical support for the mushroom cultivation on *Camellia oleifera* shell based substrates.

2. Materials and Methods

2.1. Materials

Camellia oleifera shell was collected from Dongfanghong Forest Farm in Jinhua 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. Tannin and saponin-degrading bacteria, *Bacillus amyloliquefaciens* S301 (CGMCC No. 15834), *Meyerozyma guilliermondii* S302 (CGMCC No. 15835) and *Aspergillus awamori* T301 (CGMCC No. 15862) were screened by the Chinese Academy of Forestry Institute of Subtropical Forestry and stored at the China General Microbiological Culture Collection Center (CGMCC).

2.2. Treatment and Sampling of *Camellia oleifera* Shell

Composting of *Camellia oleifera* shell was conducted in the greenhouse of Chinese Academy of Forestry Institute of Subtropical Forestry, Fuyang District,

Hangzhou, Zhejiang Province, China, from February to April 2018. *Camellia oleifera* shells were blended with 1% dry weight of brown sugar and 3% dry weight of the mixture of tannin and saponin-degrading bacteria. The C/N ratio of the blend was adjusted to 27 with urea and the moisture content was adjusted to 55% with water. The blend was stirred homogenously and piled up for composting for one month. The compost pile was turned every 5 days. The pile temperature rapidly increased to 58˚C on day 2 and reached as high as 65˚C in the 20-day high temperature phase (>55˚C) and started to decrease on day 30. The compost pile was sampled by a five-point sampling method [9] A portion of the fresh sample was stored at −20˚C, and the remaining sample was dried at 65˚C and pulverized for further use. Table 1 lists the physical and chemical parameters of the *Camellia oleifera* shell before and after the treatment and the main composition of the cottonseed hull reported in literature [2] [10].

2.3. Formulation of Substrates

The conventional cottonseed hull mushroom cultivation substrate was used as a control substrate. Cottonseed hull substrates were respectively blended with different proportions of *Camellia oleifera* shell to formulate 9 substrates as shown in Table 2.

2.4. Methods

The ingredients of each substrate were weighed and well mixed. The moisture content of each substrate was adjusted to 55% - 60% and mixed homogeneously for further use. The substrates were respectively bagged into polypropylene plastic bags (17 cm × 33 cm × 0.5 cm). The numbers of bags of each substrate was recorded, and dry weight of each bag was calculated. The substrates in bags were autoclaved at 121˚C under 103.4 KPa for 2 h, cooled for 2 h, 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 malformation of mushrooms caused by high concentrations of carbon dioxide. The *Pleurotus geesteranus* were harvest as the mushroom cap became 2 - 3 cm big.

Table 1. Physicochemical parameters of raw materials (%).

Camellia shell	Cellulose	Hemicellulose	Lignin	Ash	TOC	TN	C/N	TK	Tannin	Saponin
Untreated	18.62	49.34	29.71	2.57	48.6	0.42	116	0.85	2.26	4.86
Treated	20.87	22.16	44.94	5.03	43.6	1.21	36	0.80	0.81	0.78
Cottonseed hull	24.85	34.55	31.68	2.41	56.0	2.03	27.6	0.18	-	-

Note: -, not detected.

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

Formula	Cottonseed hull	*Camellia oleifera* shell	Rice bran	Lime	Gypsum	C/N
A1	73	5	20	1	1	26.82
A2	68	10	20	1	1	27.79
A3	58	20	20	1	1	30.06
A4	48	30	20	1	1	32.74
B1	73	5	20	1	1	26.15
B2	68	10	20	1	1	26.40
B3	58	20	20	1	1	26.88
B4	48	30	20	1	1	27.52
CK	78	0	20	1	1	25.94

Note: A1-A4 with untreated *Camellia oleifera* shell; B1-B4 with treated *Camellia oleifera* shell; CK, regular cottonseed hull formula.

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 of each substrate was calculated and analyzed [11].

2.6. Biological Efficiency

Biological efficiency (%) = yield per bag (g)/dry weight per bag (g) × 100% [12]

2.7. Determination of Nutritional Composition

Fresh *Pleurotus geesteranus* were harvested and the residues of stipe were removed. The mushroom was dried at 60˚C to the constant weight and crushed for further use. The contents of protein, ash, fat, crude fiber and amino acids were measured by the protocols of GB5009.5-2016, 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 measured by the protocol of GB 5009.268-2016. The soluble sugar content was determined by the anthrone colorimetric method. Each measurement was repeated three times and the mean value was reported.

2.8. Data Analysis

All data were processed and analyzed using EXCEL.

3. Results and Discussion

3.1. Effects of *Camellia oleifera* Shell on the Mycelial Growth of *Pleurotus geesteranus*

Figure 1 shows the mycelial growth rates of *Pleurotus geesteranus* on different substrates. It is clear that the mycelial growth rates on all of the substrates A1-A4

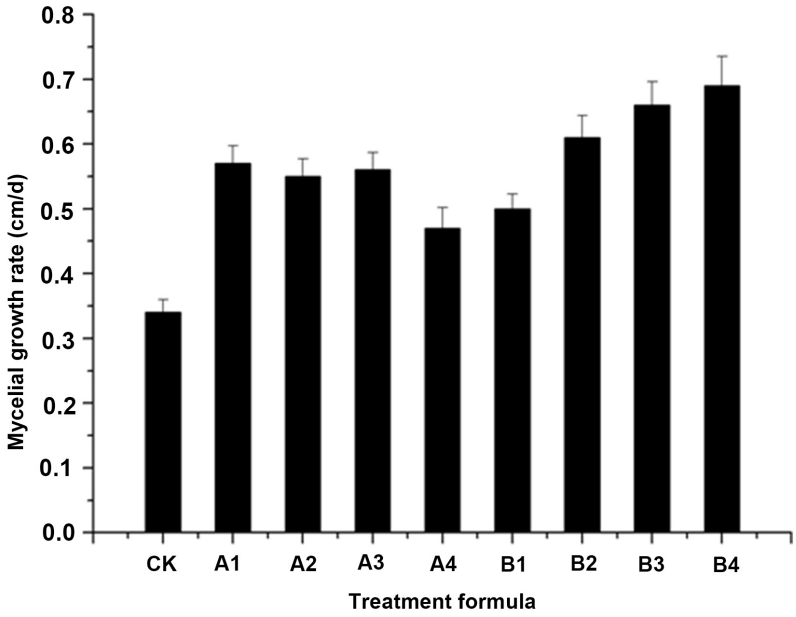

Figure 1. Mycelial growth rates of *Pleurotus geesteranus* on different substrates.

and B1-B4 containing *Camellia oleifera* shells are higher than that of the control group CK. On the substrates A1-A4, the mycelial growth rate decreased with the increase of the amount of untreated *Camellia oleifera* shell, the increase of tannin and saponin contents and the increase of C/N, e.g. followed the order of A1 > A2 > A3 > A4 > CK. In contrast, the mycelial growth rate increased with the increase of the amount of treated *Camellia oleifera* shell, following the order of B4 > B3 > B2 > B1 > CK. The mycelial growth rates on the substrates B1-B4 are higher than those on the substrates A1-A4. These results suggest that *Camellia oleifera* shell can promote the mycelial growth. The mycelial growth rate of *Pleurotus geesteranus* is correlated to the contents of tannin and saponin in the *Camellia oleifera* shell (Table 1). The overall mycelial growth rate obtained with treated *Camellia oleifera* shell is higher than that obtained with the untreated *Camellia oleifera* shell. The optimal C/N of the substrate for the cultivation of *Pleurotus geesteranus* was found to be 27 ± 0.7. Higher and lower C/N ratios decreased the growth rate of *Pleurotus geesteranus.*

3.2. Effects of *Camellia oleifera* Shell on the Yield and Biological Efficiency of *Pleurotus geesteranus*

Table 3 lists the yields and biological efficiencies of *Pleurotus geesteranus* on different substrates. It is clear that both of the yield and biological efficiency are in the order of B > A > CK. Among the substrates A1-A4, substrate A2 gave the highest yield and biological efficiency and those obtained on substrate A1 are the lowest, but still 22.15 g and 5.75% higher than those obtained on the control substrate CK. Among the substrates B1-B4, substrate B1 gave the highest yield and biological efficiency, which were respectively 117.57 g and 30.53% higher than those obtained on CK and 45.82 g and 11.9% higher than those obtained on

Table 3. Yield and biological efficiency of *Pleurotus geesteranus* cultivated on different substrates.

Formula	Yield (g/bag)	Biological Efficiency (%)
CK	180.7	46.94
A1	202.85	52.69
A2	252.45	65.57
A3	226.51	58.83
A4	249.46	64.79
B1	298.27	77.47
B2	239.35	62.17
B3	270	70.13
B4	253.85	65.94

Each bag contains 385 g dry weight of substrate.

substrate A2. The yield and biological efficiency decreased as the content of treated *Camellia oleifera* shell increased to 30%, but both of them were higher than those obtained on the control substrate CK and the substrates containing untreated *Camellia oleifera* shell. These results suggest that a certain amount of *Camellia oleifera* shell in the cultivation substrate can effectively increase the yield and biological efficiency of *Pleurotus geesteranus*.

3.3. Protein Content Analysis

The protein edible fungus is considered as the "plant meat" and is well recognized as a very good source of protein worldwide. The protein content of edible fungi can be up to 15% - 60% [13]. **Figure 2** shows the protein contents of the *Pleurotus geesteranus* cultivated on different substrates. Among the substrates A1-A4, the protein content increased first and then decreased with the increases of the untreated *Camellia oleifera* shell content and the C/N of the substrate (**Table 2**). The protein content of *Pleurotus geesteranus* cultivated on substrate A3 is the highest with the value of 30.4 ± 0.54 g/100 g, which is 6.6 ± 0.21 g/100 g higher than that obtained on substrate CK. The *Pleurotus geesteranus* cultivated on substrate A4 exhibited the lowest protein content, and was 6.6 ± 0.05 g/100 g lower than that obtained on CK. Among the substrates B1-B4, the protein content decreased with the increase of the treated *Camellia oleifera* shell content. The *Pleurotus geesteranus* cultivated on substrate B1 exhibited the highest protein content with the values of 32.4 ± 0.56 g/100 g, which was 8.6 ± 0.19 g/100 g higher than that obtained on substrate CK. The protein content of the *Pleurotus geesteranus* cultivated on substrate B4 is the lowest with the value of 27.1 ± 0.96 g/100 g, but still 3.3 ± 0.21 g/100 g higher than that obtained on substrate CK. These results suggest that 5% - 20% untreated *Camellia oleifera* shell or 5% - 30% treated *Camellia oleifera* shell in substrate can significantly increase the crude protein content of the cultivated *Pleurotus geesteranus*. The C/N ratios of

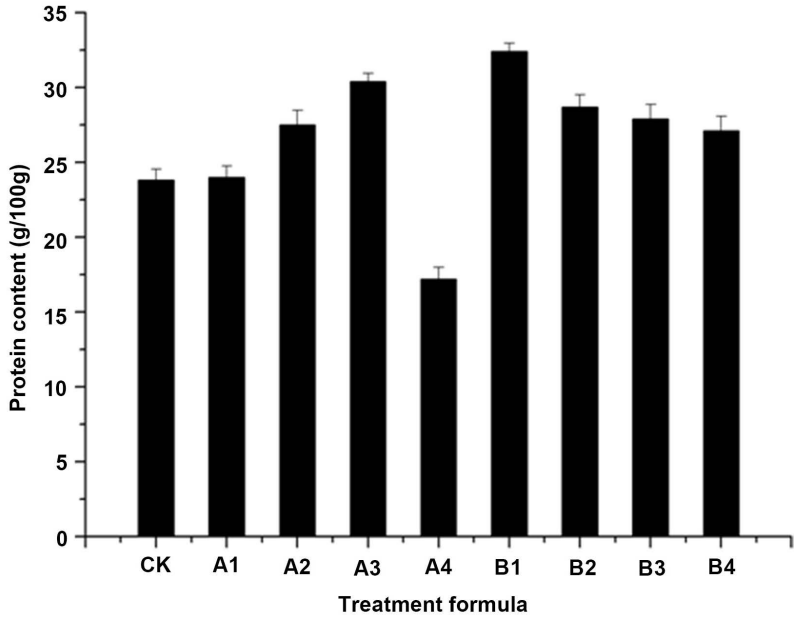

Figure 2. Protein contents of the *Pleurotus geesteranus* cultivated on different substrates.

substrate greater than 30 significantly decrease the protein contents of the cultivated *Pleurotus geesteranus*.

3.4. Ash Content Analysis

The ash content reflects the mineral content of an edible fungus. The higher the ash content is, 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 can regulate body fluids and maintain normal metabolism of cells [13]. **Figure 3** shows the ash contents of the *Pleurotus geesteranus* cultivated on different substrates. Because the ash content of the untreated *Camellia oleifera* shell is very low, the ash content of the *Pleurotus geesteranus* is not significantly affected by the its content in the substrate. The ash contents of *Pleurotus geesteranus* cultivated on the substrates A1-A4 are lower than that of obtained on substrate CK. Among the substrates B1-B4, the treated *Camellia oleifera* shell contains a higher amount of ash. Therefore, the ash content of the *Pleurotus geesteranus* increased with the increase of the treated *Camellia oleifera* shell content. The cultivation on substrate B3 yielded the *Pleurotus geesteranus* of the highest ash content with the value of 8.3 ± 0.13 g/100 g, 1.1 ± 0.02 g/100 g higher than that obtained on substrate CK and 1.7 ± 0.12 g/100 g higher than that obtained on substrate A3. The *Pleurotus geesteranus* cultivated on substrate B1 exhibited the lowest ash content of 7.2 ± 0.15 g/100 g, which was same as that obtained on the control substrate CK, yet 1.1 ± 0.08 g/100 g higher than that obtained on substrate A1. These results indicate that the ash content of *Pleurotus*

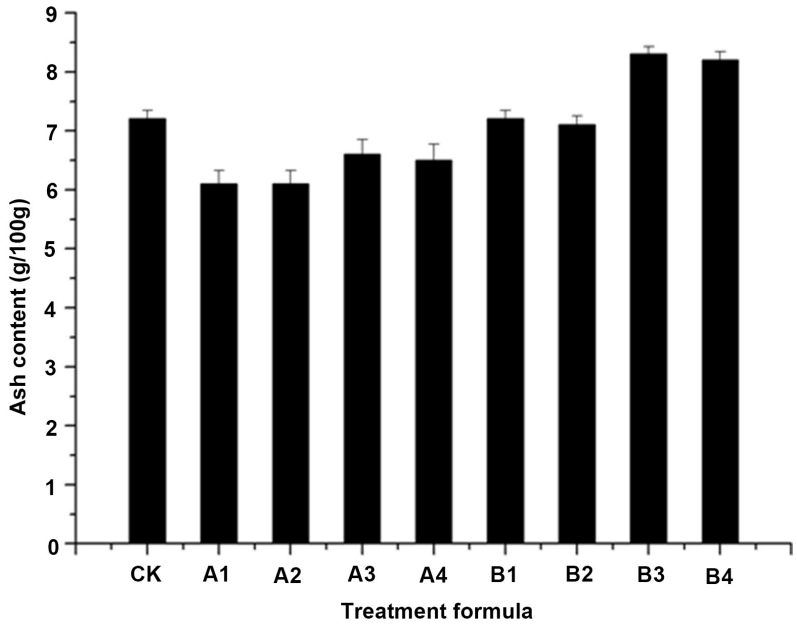

Figure 3. Ash contents of the *Pleurotus geesteranus* cultivated on the substrates of different formulas.

geesteranus is affected by the ash content of its substrate. The ash content of *Pleurotus geesteranus* cultivated on the substrate containing untreated *Camellia oleifera* shell is low and ash content of *Pleurotus geesteranus* cultivated on substrate containing treated *Camellia oleifera* shell is high, which was 7% higher than the average ash content of edible fungi.

3.5. Fat Content Analysis

The fat contents of edible fungi are generally below 10% [13]. The *Pleurotus geesteranus* cultivated on the substrates of different formulas exhibited different fat contents (**Figure 4**). The fat contents of the *Pleurotus geesteranus* cultivated on the substrates formulated with untreated *Camellia oleifera* shell are in the order of A1 > A2 > A3 > A4, which can be explained with the low crude fat content of the untreated *Camellia oleifera* shell (<1%) [14]. He fat contents of the *Pleurotus geesteranus* cultivated on the treated *Camellia oleifera* shell containing substrates follow the order of B1 > B2 > B3 > B4. The overall fat contents of the *Pleurotus geesteranus* are ordered as CK > B1 > B2 = A1 > A2 > A3 = B3 > B4 > A4. It is clear that the fat contents of all of the *Pleurotus geesteranus* cultivated on the substrates containing untreated or treated *Camellia oleifera* shells are lower than that yielded on the control substrate CK and the fat content decreases with the increase of the amount of *Camellia oleifera* shell. However, the C/N becomes more than 30 as the content of *Camellia oleifera* shell is increased to 20% and the decreasing trend of the fat content of *Pleurotus geesteranus* becomes slower. In all, the addition of *Camellia oleifera* shell to the substrate can decrease the fat content of cultivated *Pleurotus geesteranus*.

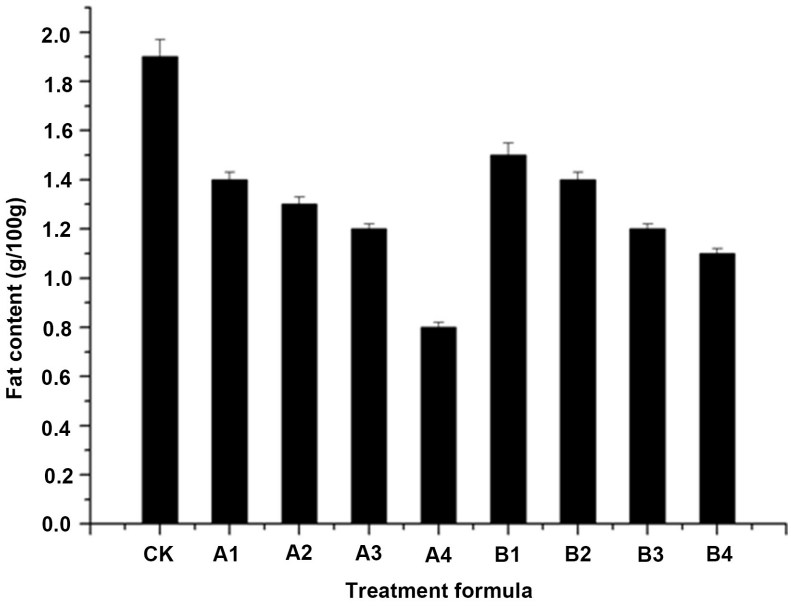

Figure 4. Fat contents of the *Pleurotus geesteranus* cultivated on the substrates of different formulas.

3.6. Content of Soluble Sugar Analysis

The soluble sugar contents of the *Pleurotus geesteranus* cultivated on the substrates containing no *Camellia oleifera* shell, untreated *Camellia oleifera* shell and treated *Camellia oleifera* shell are significantly different (**Figure 5**). The content of hemicellulose increases and those of cellulose and lignin decrease in the substrate with the increase of the untreated *Camellia oleifera* shell content. The soluble sugar content in *Pleurotus geesteranus* increases first and then decreases with the increase of the untreated *Camellia oleifera* shell content. The highest soluble sugar content of 17.67% ± 0.72% was obtained with 20% untreated *Camellia oleifera* shell in the substrate, which was 0.58% ± 0.04% higher than that obtained on substrate CK. Among the substrates B1-B4, the hemicellulose content decreases and the cellulose and lignin contents increase with the increase of the content of treated *Camellia oleifera* shell. The soluble sugar content of *Pleurotus geesteranus* cultivated on the corresponding substrate first increases and then decreases. The highest soluble sugar content was obtained with 20% treated *Camellia oleifera* shell with the values of 17.15% ± 0.69%, 0.06% ± 0.07% higher than that obtained on CK and 0.51% ± 0.06% higher than that obtained on A2. These results suggest that the total soluble sugar content of *Pleurotus geesteranus* cultivated on the substrates blended with untreated *Camellia oleifera* shell is higher than that obtained with the treated *Camellia oleifera* shell substrates and CK. It can be explained that the hemicellulose content of the untreated *Camellia oleifera* shell is higher than that of the treated shell. So, adding untreated *Camellia oleifera* shell can improve the soluble sugar content of *Pleurotus geesteranus*. The soluble sugar content increases with the increase of the C/N of substrate, but decreases as the C/N becomes over 30.

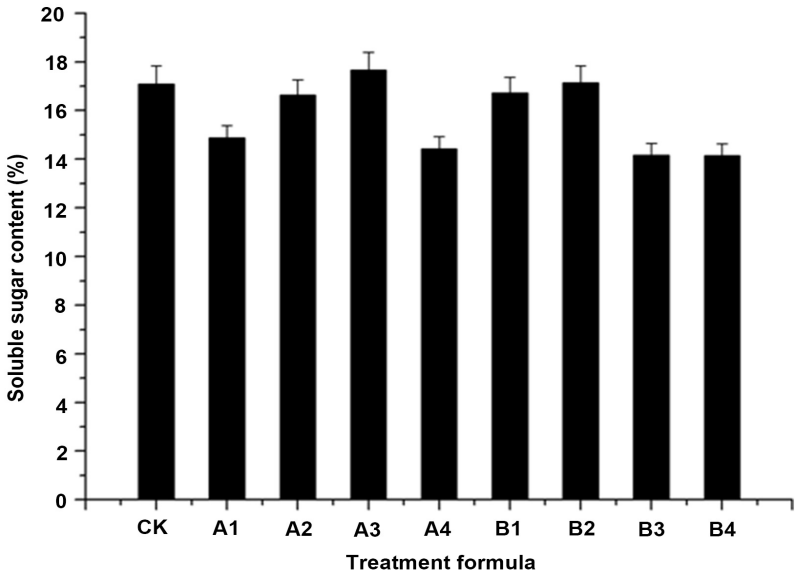

Figure 5. Soluble sugar contents of the *Pleurotus geesteranus* cultivated on the substrates of different formulas.

3.7. Crude Fiber Content Analysis

Crude fiber is mainly composed of insoluble dietary fibers including cellulose, hemicellulose and lignin. As shown in **Figure 6**, the crude fiber contents of the *Pleurotus geesteranus* cultivated on different substrates are significantly different. The *Pleurotus geesteranus* cultivated on the substrates B1-B4 shell exhibited the highest crude fiber content, followed by those cultivated on the substrates A1-A4. The crude fiber content of the *Pleurotus geesteranus* of the control group is the lowest. For the cultivations on the substrates A1-A4, the crude fiber content of *Pleurotus geesteranus* increased with the increase of the untreated *Camellia oleifera* shell content. The highest crude fiber content was obtained on substrate A4 with the value of 7.05% ± 0.56%, which was 4.85% ± 0.38% higher than that obtained on CK. The substrate A1 lowest crude fiber content was obtained with the value of 4.20% ± 0.25%, yet 2.00% ± 0.07% higher than that obtained on substrate CK. For the *Pleurotus geesteranus* cultivated B1-B4, the crude fiber content also increased with the increase of the treated *Camellia oleifera* shell content. The crude fiber content obtained on the substrate B4 is the highest with the value of 7.47% ± 0.57%, which is 5.27% ± 0.39% higher than that obtained on CK. The lowest crude fiber content with the value of 4.62% ± 0.28% was obtained on substrate B1, but it was 2.42% ± 0.1% higher than that obtained on CK. Based on these results, it can be concluded that the crude fiber content of *Pleurotus geesteranus* increases with the increase of C/N in its cultivation substrate. The *Camellia oleifera* shell in substrate can increase the crude fiber content of the cultivated *Pleurotus geesteranus*. The cellulose and lignin structure in *Camellia oleifera* shell are usually destroyed during composting and thus are more easily used by edible fungi, which explains the higher crude fiber content of *Pleurotus geesteranus* obtained on the substrates containing treated *Camellia oleifera* shell.

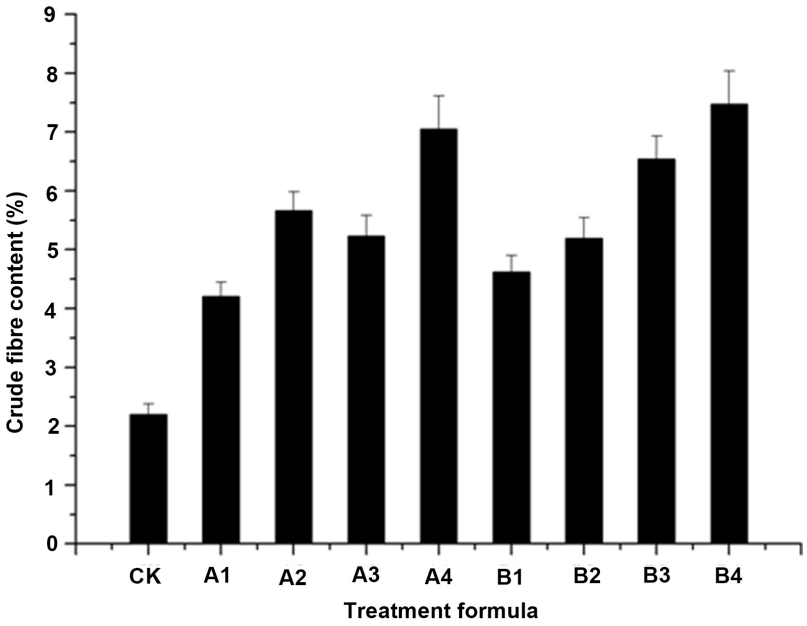

Figure 6. Crude fiber contents of the *Pleurotus geesteranus* cultivated on the substrates of different formulas.

3.8. Composition and Contents of Amino Acids Analysis

Edible fungi contain 17 - 18 of the 20 essential amino acids, and almost all 8 amino acids required by the human body, especially lysine, methionine and threonine which are lacking in cereals [13]. The total amino acid contents of the *Pleurotus geesteranus* cultivated on different substrates follow the order of B > A > CK (**Table 4**). For the *Pleurotus geesteranus* cultivated on the substrates A1-A4, the total amino acid content, total essential amino acid content, total non-essential amino acid content and total umami amino acid content increased first, peaked on substrate A3 and then decreased with the increase of the untreated *Camellia oleifera* shell content, e.g. the increases of N content and C/N ratio. The essential amino acids accounted for 38.97% of total amino acids and 63.85% of total umami amino acids. For the *Pleurotus geesteranus* cultivated on B1-B4, the total amount of various amino acids decreased with the increase of the shell content, but the overall values were higher than those obtained on substrate A1-A4 and substrate CK. These results, along with the results listed in **Table 2** suggest that the total contents of amino acids, essential amino acids, non-essential amino acids and umami amino acids in *Pleurotus geesteranus* increase with the increase of the C/N ratio of the substrate containing untreated *Camellia oleifera* shell, peaked at C/N = 32.74 and total amount of various amino acids decreases.. In contrast, the total contents of amino acids, essential amino acids, non-essential amino acids and umami amino acids decrease with the increase of the C/N in the substrate blended with treated *Camellia oleifera* shell. In all, *Camellia oleifera* shell can significantly increase the amino acid content of *Pleurotus geesteranus* as a substitute substrate, and the N content and C/N in the

Table 4. Effects of the *Camellia oleifera* shell content in substrate on the composition and contents of amino acids in *Pleurotus geesteranus* (%).

	CK	A1	A2	A3	A4	B1	B2	B3	B4
Aspartic acid (Asp)#	1.10	1.68	1.92	2.06	1.65	2.18	1.83	1.78	1.78
Threonine (Thr)*	0.52	0.89	1.02	1.08	0.89	1.22	1	0.98	0.96
Serine (Ser)	0.60	0.92	1.06	1.13	0.93	1.28	1.05	1.02	0.99
Glutamic acid (Glu)#	4.16	3.7	4.22	4.42	4.19	5.82	4.56	4.56	4.42
Glycine (Gly)	0.19	0.82	0.93	0.99	0.84	1.16	0.92	0.88	0.87
Alanine (Ala)	1.30	1.11	1.25	1.34	1.19	1.65	1.38	1.36	1.3
Valine (Val)*	0.68	1.78	1.98	2.08	1.91	2.39	2.02	2.06	1.99
Cystine (Cys)	0.14	0.13	0.15	0.13	0.11	0.14	0.13	0.14	0.14
Methionine (Met)*	0.11	0.31	0.35	0.35	0.27	0.36	0.3	0.28	0.29
Isoleucine (Ile)*	0.4	0.77	0.89	0.93	0.79	1.04	0.86	0.84	0.85
Leucine (Leu)*	0.78	1.31	1.5	1.6	1.36	1.74	1.46	1.43	1.46
Tyrosine (Tyr)	0.50	0.56	0.72	0.63	0.55	0.76	0.63	0.59	0.64
Phenylalanine (Phe)*	0.89	0.81	0.94	1.01	0.86	0.95	0.93	0.9	0.91
Lysine (Lys)*	0.84	1.07	1.34	1.34	1.11	1.46	1.19	1.09	1.13
Histidine (His)	0.18	0.27	0.32	0.36	0.34	0.45	0.37	0.3	0.3
Arginine (Arg)	0.65	0.85	0.97	1.07	0.8	1.11	0.91	0.88	0.89
Proline (Pro)	0.32	0.81	0.94	1.01	0.84	1.13	0.92	0.94	0.92
Total amino acids (T)	13.35	17.79	20.5	21.53	18.63	24.84	20.46	20.03	19.84
Total essential amino acids (E)	4.22	6.94	8.02	8.39	7.19	9.16	7.76	7.58	7.59
Total non-essential amino acids (N)	9.13	10.85	12.48	13.14	11.44	15.68	12.7	12.45	12.25
Total umami amino acids (W)	5.26	5.38	6.14	6.48	5.84	8	6.39	6.34	6.2
E/N (%)	46.19	63.96	64.26	63.85	62.85	58.42	61.10	60.88	61.96
E/T (%)	31.60	39.01	39.12	38.97	38.59	36.88	37.84	37.84	38.26

*essential amino acid; #umami amino acid.

substrate directly affect the amino acid content of the cultivated *Pleurotus geesteranus*. According to WHO/FAO, it is required that E/T is about 40% and E/N is above 60% in the ideal protein. Except for formula B1 and control group, the ratio of essential amino acids in the cultivated *Pleurotus geesteranus* under the other formulas is close to the standard of ideal protein.

3.9. Heavy Metal Contents

The GB 7096-2003 "Hygienic standard for edible fungi" requires the 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. As shown in Table 5, the Hg, As, Cd and Pb contents in

Table 5. Heave metal contents of the *Pleurotus geesteranus* cultivated on different substrates (mg/kg).

	Hg	As	Cd	Pb
A1	0.014	0.499	0.092	0.035
A2	0.017	0.496	0.091	0.033
A3	0.020	0.551	0.106	0.022
A4	0.019	1.00	0.092	0.19
B1	0.018	0.617	0.075	0.019
B2	0.020	0.569	0.052	0.010
B3	0.021	0.65	0.027	0.047
B4	0.017	0.78	0.022	0.033
CK	0.013	0.152	0.440	0.042

the *Pleurotus geesteranus* cultivated on all substrates are within the limits defined in GB7096-2003. It is worth noting that the total As content of *Pleurotus geesteranus* of each group is higher than that of the substrate. It has been reported that edible fungi can strongly accumulate heavy metals. Inorganic As is highly toxic and organic As is less toxic or non-toxic. Therefore, it is suggested that the As limit should mainly refer to the inorganic As [15].

4. Conclusion

Partially substituting the conventional cottonseed hull substrate with treated *Camellia oleifera* shell can increase the mycelial growth rate, yield, biological efficiency, protein content, crude fiber content and amino acid content of the *Pleurotus geesteranus*. The tannin and saponin in *Camellia oleifera* shell are responsible for the increased mycelial growth rate. The suitable C/N of substrate for the cultivation of *Pleurotus geesteranus* was determined to be 27 ± 0.7. The C/N ratios greater than 30 can cause low contents of protein and soluble sugar in *Pleurotus geesteranus*. *Camellia oleifera* shell in cottonseed hull substrate can also reduce the fat content of *Pleurotus geesteranus* and affects its ash content. The total contents of Hg, Cd, Pb and As of the *Pleurotus geesteranus* cultivated on the substrates containing *Camellia oleifera* shells are within the limits defined by the national regulation.

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Conflicts of Interest

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

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