Evaluation of Three Kinds of Nutshell with Respect to Utilization as Culture Media

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The material basis of nutshells was explored in this study for *Camellia oleifera* Abel, *Castanea mollissima* Blume, and *Carya cathayensis* Sarg., The authors measured the moisture content, pH, electronic conductivity (EC), seed germination index (GI), and tannin content of the fresh shells of these three species. The contents of cellulose, hemicellulose, lignin, organic extracts, ash, saponin, cellulose crystallinity, organic carbon, and mineral elements of the dried shells was also measured. The results showed that the total mass fractions of cellulose, hemicellulose, and lignin in the shells of the three species were all above 80% of dry weight; the content of organic matter was higher than 66%, and the pH values were in the range of 5.5 to 8.5. The shells of the three species are good raw materials for the growth of plants and edible fungi. There were some shortcomings if used as fertilizers or substrates. However, the C/N and C/P ratios were high, the EC values were low, and the GI was < 100%. Additionally, the shells all contained tannin, saponin, and alkaloids, which were not conducive to the growth of plants and mycelia of edible fungi. Therefore, they can be used as culture media only after being processed.

Keywords: Camellia oleifera Abel shells; Castanea mollissima Blume shells; Carya cathayensis Sarg shells; Material basis; Matrix application

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INTRODUCTION

Camellia oleifera Abel (*C. oleifera*), *Castanea mollissima* Blume (*C. mollissima*) and *Carya cathayensis* Sarg (*C. cathayensis*) are three important economic forest species in China. The *C. oleifera* is an important woody oil tree species growing in southern China, with a cultivated area of about 5.5 million hm^2 (Jiang *et al.* 2011). The annual production of *C. oleifera* fruit is about 10 million tons (Qiu *et al.* 2009; Jiang *et al.* 2010; Shen *et al.* 2010). *C. oleifera* shells (also known as *Camellia oleifera* tea bag) is the shell of the fruit, accounting for more than 60% of fresh fruit (Qiu *et al.* 2009); and the annual production of *C. oleifera* is nearly 8 million tons (Zhang *et al.* 2015). *C. mollissima* is a widely cultivated deciduous tree in both northern and southern China. It is distributed throughout 26 provinces and cities across the country. The planting area in China is about 300 thousand hectares, accounting for more than 80% of the world's total *C. mollissima* production (Qi *et al.* 2012). The *C. mollissima* shells (also known as a *Castanea mollissima* pod) are the prickly shells of *C. mollissima.* According to previous literature, *C. mollissima* shells mainly contains organic acids, phenols, phytosterols (or triterpenes), flavonoids (or saponins), lactones, coumarins (or their glycosides), sugars, polysaccharides (or glycosides), tannins, and more (Zhao Deyi *et al.* 2003). *C. cathayensis* is a member of the genus *Carya,* in the Juglandaceae family, and is mainly

distributed in the Tianmu area at the boundary between the Zhejiang and Anhui provinces; the total planting area in this region is approximately 86,700 hm^2. In the main producing areas, income from *C. cathayensis* accounts for more than 70% of the total income of local farmers, and *C. cathayensis* is one of the major poverty-reducing economic tree species for farmers (Ding *et al.* 2011). When *C. oleifera*, *C. mollissima*, and *C. cathayensis* trees are harvested and processed, a large number of processing by-products, such as *C. oleifera* shells, *C. cathayensis* shells, and *C. mollissima* shells are produced.

These shell wastes are currently disposed through discarding in landfills, or incineration, which contribute to pollution in the environment around the soil and rivers (Xiao *et al.* 1998). For example, discarding in landfills require large amounts of land resources, and the natural rotting process of the shell in the field may bring pests and diseases to re-planted crops. Furthermore, some components in the shell, such as tannins, saponins, and alkaloids, *etc.* (Zhao *et al.* 2003; Chen *et al.* 2007; Chen *et al.* 2009; Jiang *et al.* 2018), adversely affect the survival of organisms. After having entered the soil and bodies of water *via* rain, they can cause damage to the ecological environment (Ding *et al.* 2011). Incineration generates toxic gases, such as greenhouse gases and carbon dioxins, which can cause serious pollution to the atmosphere (Zhang 2015). At the same time, shells from *C. oleifera*, *C. mollissima*, and *C. cathayensis* contain large amounts of cellulose, hemicellulose, and lignin, as well as certain amounts of beneficial nutrients that make the shells an important organic resource (Liao 2016). If these abandoned shells can be utilized to develop a low-cost, high-potential cultivation medium, turning waste into treasure, the utilization of the raw materials will ease the pressure on the local ecological environment and increase the economic benefits of the forest industry. Therefore, understanding the material basis for its use as a cultivation medium is the primary and key task. However, to date, there have been few related research studies in this area.

The main components of *C. oleifera* shells, *C. mollissima* shells, and *C. cathayensis* shells are similar to the raw materials of the cultivation medium for peat and edible fungi, and thus they are very good raw materials for the cultivation of flowers, seedlings, vegetables, edible fungi, *etc.* (Zhang *et al.* 2015). In this study, by using *C. cathayensis* shells, *C. oleifera* shells, and *C. mollissima* shells as the research materials, the authors studied the material basis for their use as cultivation media, aiming to provide a scientific basis for the further development of cultivation matrix products.

EXPERIMENTAL

Materials

C. oleifera shells were obtained from the Dongfanghong Forest Farm in Jinhua City, Zhejiang Province, China. *C. mollissima* shells came from Qingyuan County, Lishui City, Zhejiang Province, China, and *C. cathayensis* shells came from Lin'an District, Hangzhou City, Zhejiang Province, China.

All three kinds of fruits were shelled by shellers (machines used for the initial husking) without any other treatment and exposed under the sunlight for removing excess water. Then, the shells were placed in an oven (DHG-9076A; Shanghai Jinghong Laboratory Instrument Co., Ltd., Shanghai, China) at 105 °C until constant weight. A broken machine (DFT-40) was used to crush the dried shells into powder. Then the

powder was sifted through an 80-mesh sieve (GB6003-88) and finally stored in a desiccator as a sample for use.

Methods

pH and electronic conductivity

The fresh shells were extracted with deionized water, in the ratio of water to sample ratio of 10:1 [V(mL):W(g)], by shaking at 200 rpm for 1 h at room temperature. Next, the pH was measured with a Model 2F pH meter (Inesa Scientific Instrument, Co., Ltd., Shanghai, China), and the electronic conductivity (EC) (ms/cm) was measured with a DDS-307 conductivity meter (Inesa Scientific Instrument, Co., Ltd., Shanghai, China).

Moisture content

The moisture content was determined according to the national standard GB/T 2677.2 (2011).

Contents of organic carbon and mineral elements

The content of organic carbon was determined according to the national standard HJ615 (2011); the contents of nitrogen and phosphorus were determined according to the national standards LY/T1271 (1999). The contents of copper, zinc, manganese, iron, sodium, and potassium were determined in accordance with the national standards, LY/T 1270 (1999). The contents of arsenic, cadmium, lead, chromium, and mercury were determined according to the national standard LY/T 1269 (1999).

Contents of cellulose, hemicellulose, lignin, and ash

The contents of organic extracts, cellulose, hemicellulose, lignin, and ash were determined in accordance with the national standards GB/T 2677.6 (1994), GB/T 2677.10 (1995), GB/T 2677.9 (1994), GB/T 2677.8 (1994), and GB/T 742 (2008). Three parallel measurements were performed for each sample and the average mass percentage of each major component was calculated based on the data obtained.

Contents of tannin and saponin

The tannin and saponin contents were determined according to the standards NY/T 1600 (2008) and SN/T 1852 (2006).

X-Ray diffraction analysis

The 80-mesh shell powder was oven-dried at 50 °C for 6 h and sheeted into thin slices at room temperature and measured using a Rigaku D/max 2550 PC type X-ray diffractometer (Rigaku Corporation, Tokyo, Japan). The experimental conditions were as follows: the X-ray tube was a Cu-target, and the CuKβ radiation was eliminated with a nickel sheet. The tube voltage was 40 kV and the tube current was 40 mA. The measurement method was a $2\theta/\theta$ linkage scan. The X-ray crystallinity index was calculated according to Eq. 1,

$$C_rI = \frac{I_{002} - I_{am}}{I_{002}} \times 100\% \quad (1)$$

where C_rI is the relative crystallinity (%), I_{002} is the (002) lattice diffraction maximum intensity angle (°), I_{am} represents the scattering intensity (°) of the 2θ angle near the amorphous background diffraction, and I_{am} is the same as the I_{002} unit.

Seed germination index

A total of 20 g of the fresh sample and 200 mL of distilled water were mixed and shaken well for 1 h. Then, the mixture was extracted by being immersed at 30 °C for 1 day and night and then it was filtered. Next, 6 mL of the filtrate was aspirated and added to a 9-cm dish covered with filter paper. Then, 20 plump cabbage seeds were sowed on each petri dish and placed in a 20 ± 1 °C incubator. The seed germination rate was measured at 24 h. Each treatment was repeated three times and distilled water was used as the control. The germination index (GI) calculation method is given in Eq. 2,

$$\text{GI (\%)} = \frac{G_t \times L_t}{G_c \times L_c} \times 100 \quad (2)$$

where G_t is the seed germination rate (%) of the extractive of raw material, L_t is the length (cm) of seed root cultivated by extractive of raw material, G_c is the seed germination rate (%) of the control, and L_c represents the length (cm) of the seed root cultivated by water.

RESULTS AND DISCUSSION

Chemical Composition

Composting, as a technology for organic waste utilization, is attracting increased attention. The cellulose, hemicellulose, and lignin content is an important factor that determines the utilization of three kinds of nutshell substrate, as shown in Table 1. The total amounts of cellulose, hemicellulose, and lignin in the shells of *C. oleifera*, *C. mollissima*, and *C. cathayensis* were 97.67%, 81.97%, and 92.89%, respectively. It has been shown that the hemicellulose in compost is degraded rapidly by microorganisms. Before cellulose begins to decompose, more hemicellulose has been decomposed but lignin is hardly degraded (Xi *et al.* 2002). Table 1 shows that the hemicellulose content (49.3%) in *C. oleifera* shells was the highest while the cellulose content (18.6%) was the lowest; the cellulose crystallinity (39.1%) was the lowest and the lignin content was 29.7%. The cellulose content (27.3%) in *C. mollissima* shells was the highest and the crystallinity was 46.4%; lignin content was 21.4%. Cellulose content of hickory shells was 20.6%, the maximum degree of cellulose crystallinity was 64.4%, the lowest hemicellulose content was 22.5%, and the highest lignin content was 49.8%. From the above analysis, it can be seen that composting of these three kinds of raw materials requires acceleration of the composting process and improvement of the quality of compost by adding microbial agents and initiating agents. Due to the high lignin content and high fiber crystallinity in *C. cathayensis* shells, proper microbial agents and primers must be applied to improve the compost quality.

Water is an indispensable condition for the decomposition of organic matter and the growth of microorganisms. Microorganisms can only take up mineral nutrients if they are dissolved. The moisture content directly affects the decomposition speed and maturity of the three kinds of shell compost. Table 1 shows that the moisture content of the fresh

fruit of the three kinds of fruit shells was 80.9% of the *C. oleifera* shells, 70.8% of *C. mollissima* shells, and 81.7% of *C. cathayensis* shells. The experimental results showed that the upper limit of the moisture content of three kinds of nutshells compost was between 55% and 60%, and when the level was over 60% anaerobic fermentation had occurred. Therefore, the three fresh fruit shells needed to be treated before composting.

Table 1. Composition and Content of the Three Kinds of Nutshell (%)

Ingredient / Species	*C. oleifera* Shells	*C. mollissima* Shells	*C. cathayensis* Shells
Moisture content of fresh fruit shell	69.60	70.80	81.70
Cellulose content	18.62 ± 0.22	27.34 ± 0.01	20.63 ± 0.07
Degree of cellulose crystallinity	39.1	46.4	64.4
Hemicellulose content	49.34 ± 0.07	33.23 + 0.02	22.48 ± 0.02
Lignin content	29.71 ± 0.14	21.40 ± 0.12	49.78 ± 0.01
Ash content	2.57 ± 0.04	3.22 ± 0.01	6.88 ± 0.02
Organic extracts	2.50	3.48	6.79
Organic matter	83.78	66.37	78.26
Tannin	2.26	5.58	3.64
Saponin	4.80	3.44	3.20

Organic matter is an important material for the survival, growth, and reproduction of microorganisms. Their contents affect the temperature of stacking materials, the ventilation, and the oxygen supply requirements. It has been shown that the most suitable organic content of the stack is 20% to 80%. The contents of organic matter in *C. oleifera* shells, *C. mollissima* shells, and *C. cathayensis* shells were 83.8%, 66.4%, and 78.3%, respectively, and anaerobic fermentation was likely to occur because the organic matter content was too high. As aerobic composting progresses, the amount of organic matter gradually decreases. Table 1 shows that the three kinds of shells have a high of organic matter content and can provide sufficient nutrients for the survival and reproduction of microorganisms.

Tannin is a secondary metabolite of plants and has a relative molecular mass of 500 to 3000 Da. It is a water-soluble polyphenol widely found in plants and has a variety of biological activities. Tannin is divided into hydrolyzed tannins and condensed tannins (He, *et al.* 2001). The content of tannin in the substrate will affect the quality of the plants or edible fungi (Harrison 1971). Table 1 shows that the tannin content in *C. oleifera* shells, *C. mollissima* shells, and *C. cathayensis* shells are 2.26%, 5.58%, and 3.64%, respectively. Saponin is widely found in the roots, leaves, flowers, and fruits of plants. The saponin content directly affects the application of three kinds of shells for medium applications. When added to the culture medium, saponin had an inhibitory effect on the growth of the mycelia of edible fungi (Fang and Huang 1994). As shown in Table 1, the saponin contents in the *C. oleifera* shells, *C. mollissima* shells, and *C. cathayensis* shells were 4.80%, 3.44%, and 3.20%, respectively. Thus, it is of great significance to study the

transformation and degradation of tannin and saponin during composting of the three kinds of shells.

Analysis of the Contents of Nutrient and Mineral Elements

Carbon and nitrogen are important nutrients required for the propagation and decomposition of microorganisms. Carbon can provide energy sources for microorganisms and makes up 50% of the components of microbial cells. Nitrogen is an important component of proteins, nucleic acids, amino acids, and enzymes for microbial growth. The C/N ratio is an important factor that reflects the nutritional needs of microorganisms. In theory, the initial C/N of the compost is 25 to 35. During composting, carbon sources are consumed and converted to carbon dioxide and humic substances, while nitrogen is lost in the form of ammonia, or becomes nitrates and nitrites, or is assimilated by organisms to facilitate the generation of humic acids. Phosphorus is an important element that constitutes biological activities. It is an important element for microorganisms to decompose organic matter. It also affects the quality of compost, and generally requires a C/P ratio between 75 and 150. As shown in Table 2, the C/N ratios of ordinary *C. oleifera* shells, *C. mollissima* shells, and *C. cathayensis* shells were 116.0, 61.1, and 63.2, respectively, and the C/P ratios were 2876, 688, and 824, respectively. The C/N ratio and C/P ratio of the three shells are all higher than their standards in compost, thus it is necessary to add nitrogen and phosphorus-containing substances to adjust the initial C/N ratio and C/P ratio of the compost. It has been shown (Qin *et al.* 2009) that because of its high lignin content, *C. cathayensis* shells cannot use urea to adjust the carbon-nitrogen ratio; any urea that is added will soon lead to ammonia volatilization.

The nutrients required for the growth of edible fungi (Zhang 1998) include carbon sources, nitrogen sources, mineral elements, and growth factors. The carbon sources available for the growth of edible fungi are extensive, such as cellulose, hemicellulose, lignin, monosaccharides, organic acids, *etc.*, and the mycelial mainly utilizes substances during its growth stage, such as starch, monosaccharides, organic acids, amongst others that are easily absorbed. The growth stage of the fruit body mainly utilizes macromolecular polysaccharides, such as cellulose, hemicellulose, and lignin. Edible fungi use inorganic nitrogen and grow slowly, while rice bran, wheat bran, bean cake powder, cottonseed cake flour, silkworm cocoon powder, and horse dung are all good nitrogen sources (Song *et al.* 2001; Huang *et al.* 2007). The C/N ratios required for the growth of different species of edible fungi are different; for example, the best C/N for the growth of *Pleurotus eryngii* is 60:1 (Gong *et al.* 2002) and *Pleurotus geesteranus* is 69:1 (Zhang 2013).

Therefore, depending on the end uses of the three kinds of shells, the shells should be treated in different ways and with different additives.

Table 2. Analysis of Nutrient Content (%)

Nutrient/Species	*C. oleifera* Shells	*C. mollissima* Shells	*C. cathayensis* Shells
C	48.60	38.50	45.40
N	0.42	0.63	0.72
C/N	115.99	61.11	63.23
P	0.017	0.056	0.055
C/P	2875.73	687.50	823.95
K	0.85	0.72	3.52
Total Nutrients	2.56	2.63	9.46
* Total nutrients [total nitrogen (in N) + total phosphorus (in P_2O_5) + total potassium (in K_2O)] (in dry basis)/%			

Mineral elements are indispensable nutrients for the biological activities of edible fungi. Their main function is to constitute the components of the microorganisms. They act as an enzyme, part of a coenzyme, or to maintain enzyme activity, to regulate osmotic pressure, hydrogen ion concentration, and redox potential. The mineral elements required for the growth and development of edible fungi include P, S, K, Mg, Ca, Fe, Mo, Mn, Zn, Co, and other similar elements. The suitable concentrations of P, S, K, Mg, and other elements in the culture medium are in the range of 100 µg/L to 500 µg/L, while the requirements for Fe, Co, Mn, Mo, Zn, and other elements are very small (a few milligrams per thousand) in the production of crops. In general, attention should be paid to the addition of such elements as P, S, K, and Mg. The addition of these elements increased production yield, while elements, such as Fe, Co, Mn, Mo, and Zn, will be present in the three shells and other culture materials, but they do not need to be added separately, but rather when the culture media are formulated.

If the heavy metals content in medium products is too high, the long-term large-scale application of such products will inevitably lead to a marked increase in heavy metals in soil and plants, resulting in environmental risks and a reduction in soil quality, as well as a decline in the quality of farm produce. Heavy metals are used in edible fungi and other agricultural products that can enrich people's health. The contents of trace elements in the three kinds of shells are shown in Table 3, and they are all far below the LY/T1970 (2011) standards for greening organic substrates, which meet the requirements of the matrix.

Physical Characteristics Analysis

Microbial activities require slightly acidic or neutral environmental conditions. At the initial stage of aerobic composting, the pH can generally drop to 5 to 6, and then it begins to rise. The process can reach a pH of 8.5 to 9.0 before the completion of the process, and the final product can reach a pH of 7.0 to 8.0. The rates of microbial growth and protein degradation were optimal at a pH of 7 to 8, and the rate of glucose degradation was optimal at a pH of 6 to 9. Generally, the pH of the compost material varied from 5.5 to 8.5, but the composting effect was better under neutral conditions. Table 4 shows that the initial pH of the three shells meets the composting requirements.

Table 3. Mineral Element Content Analysis (mg/kg)

Species	*C.oleifera* Shells	*C.mollissima* Shells	*C.cathayensis* Shells	LY/T1970 (2011) Limit (I Class)
Co	3.36	11.2	10.9	≤ 150
As	0.032	0.106	0.093	≤ 10
Cd	0.0589	0.272	0.113	≤ 1.5
Pb	0.522	0.806	0.910	≤ 120
Cr	0.389	1.12	0.899	≤ 70
Zn	5.01	20.00	32.10	≤ 300
Na	38.50	38.90	61.20	
Fe	24.2	141	185	
Mn	675	1223	328	
S	654	380	911	
Mg	609	1440	1170	
Ca	2140	5550	5290	
Mo	45.30	4.52	23.00	
Co	124	195	739	

Table 4. Analysis of Physical Characteristics

Species	*C. oleifera* Shells	*C. mollissima* Shells	*C. cathayensis* Shells	LY/T1970 (2011)		
				Improved Substrate	Cutting Seeding Substrate	Culture Substrate
pH (25 °C)	5.54	5.35	7.13	4.5 to 9.5	5.0 to 7.8	5.0 to 8.0
EC (mS/cm)	0.21	0.05	0.16	0.5 to 3.0	< 0.5	0.35 to 1.5
GI (%)	50.59	89.02	84.02	-	≥ 95	≥ 80

Note: EC- electronic conductivity

The EC is an important indicator of the amount of soluble salts in the heaped material. During the composting process, the organic macromolecules in the compost materials are decomposed by the fermenting microbial strain into countless small molecule organic acids, ammonium salts, and soluble salts. The EC value expresses the concentration of the substrate nutrient, and it has a close relationship with the growth and development of plants and edible fungi. As the time for composting is prolonged, the late rise of the EC value gradually tends to balance. Table 4 shows that the EC values of the three kinds of shells were low and needed to be fermented by composting.

Many plant seeds are inhibited from growth in compost materials and un-decomposed compost extracts. Growth is promoted in decomposed compost. The GI is calculated from the seed germination and root length. In theory, the GI < 100% indicates that the plant is poisoned and the used materials were detrimental to plant growth. In

Table 4, the GI (50.6%) of the *C. oleifera* shells was lower, which may have been related to the higher saponin content in the *C. oleifera* shells. The seed germination rate of *C. cathayensis* shells was relatively high, which was associated with a high lignin content and high degree of cellulose crystallinity, and also related to less soluble substances in aqueous solution. The three kinds of shells need to be decomposed before they can be used as a matrix for plant and edible mushroom cultivation.

CONCLUSIONS

1. The nutshells of *C. oleifera* Abel, *C. mollissima* Blume, and *C. cathayensis* Sarg. were expected to be good raw materials for the growth of plants and edible fungi. The total mass fraction of these three fruit shells were above 80% of the dry weight, of which the hemicellulose content of the *C. oleifera* shell was up to 49.3%. The highest lignin content of the shell was 49.8%, and the highest degree of cellulose crystallinity was 64.4%. They also had a high content of organic matter, providing sufficient nutrients for the survival, growth, reproduction of microorganisms and edible fungi based on the measured chemical and physical characteristics.
2. The nutshells of the three species could be used as culture media after being composted. The C/N ratio and C/P ratio of the nutshells were all high. Before composting, nitrogen fertilizer or phosphate fertilizer was needed to adjust the C/N ratio to 25/35 and the C/P ratio to 75/150. The heavy metal elements met the plant growth needs without causing secondary pollution. The initial pH values were all in accordance with the initial pH of the compost materials, 5.5 to 8.5. The EC value of conductivity was low, and the germination rate of the seeds was less than 100%. The nutshells contain tannin and saponin, which inhibit the growth of plants and the mycelia of edible fungi.

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