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Research Article

Reutilization of Different Recycled Agricultural Wastes to Culture *Pleurotus eryngii* and Comparison of Their Ingredients

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Abstract

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In this study, bamboo crumb, paddy straw crumb, sugarcane crumb, spent *Pleurotus eryngii* substrate, or spent *Flammulina velutipes* substrate was used to completely or partially replace fresh sawdust to cultivate *P. eryngii*. The nutrients and functional components were compared. The fingerprints and the contents of ergosterol and ergothioneine analyzed using high-performance liquid chromatography (HPLC) were compared. The results revealed that the samples cultivated using spent *P. eryngii* substrate or spent *F. velutipes* substrate had higher crude protein content. The water-soluble extract contents of *P. eryngii* cultured with spent *P. eryngii* substrate were higher than that cultured with sawdust. The total polysaccharide content of *P. eryngii* cultured with sugarcane substrate was significantly higher than that of *P. eryngii* cultured with sawdust, while the total polysaccharide content of *P. eryngii* cultured with bamboo was lower. The high-performance liquid chromatography fingerprint findings revealed the compositions of *P. eryngii* cultured from any agricultural waste substrate were the same. The ergosterol content was between 0.015 and 0.067 mg g^{-1}, and the ergothioneine content was between 0.890 and 2.542 mg g^{-1}. These results indicated that using recycled materials instead of sawdust to cultivate *P. eryngii* to reduce agricultural waste could be a safe and worthwhile solution.

Keywords: *Pleurotus eryngii*, Agricultural waste, Nutrient, Ergosterol, Ergothioneine.

INTRODUCTION

In recent years, because of the increasing awareness regarding environmental protection, global warming, and water and air pollution, people have gradually discovered that the past lifestyle of mass production and consumption has resulted in the production of large amounts of waste that have exceeded the environmental load (Barshteyn & Krupodorova 2016). Taiwan's agriculture is considerably developed, and it generates a large amount of agricultural waste. In the past, farmers mostly used incineration or composting to manage this waste; these techniques adversely affect air quality and the quality of life of people residing in neighboring areas. Taiwan's mushroom cultivation bring us at least 300,000 metric tons per year of spent sawdust waste (spent mushroom substrate) (Wu *et al*. 2020), the non-governmental organization

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(Citizen of the Earth, Taiwan) claims that results in the equivalent of 88 Daan Forest Parks must be cut down, which is harmful to forest environments.

Most agricultural waste is rich in lignocellulosic compounds, the disposal of which is often a problem because they do not decompose easily. In the past, agricultural waste was often treated through combustion (Udayasimha & Vijayalakshimi 2012). However, recent studies have found that agricultural waste can be effectively used for mushroom cultivation, thus benefits the economy and reduces environmental pollution (Kamthan & Tiwari 2017). If rice straw, bagasse, or wastes produced after cultivating edible mushrooms can be recycled to cultivate mushrooms, the deforestation and pollution caused by the disposal of agricultural waste can be reduced, and the mushroom industry can become a completely sustainable and environmentally friendly industry that decomposes agricultural surplus materials. Rice and banana straw can be used to cultivate *Pleurotus ostreatus* and *P. sajor-caju* (Bonatti *et al*. 2004). In addition, many studies reported the use of agricultural waste for the cultivation of *P. eryngii*, also known as the King oyster mushroom. Sawdust, rice straw, rice bran, barley straw, wheat straw, and many other agricultural waste products have been used for the cultivation of *P. eryngii* (Kirbag & Akyüz 2008; Barshteyn & Krupodorova 2016; Jeznabadi *et al*. 2017). The use of agricultural waste to cultivate mushrooms has become a trend.

P. eryngii has excellent flavor, nutritional value, and health benefits (Jeznabadi *et al*. 2016). *P. eryngii* exerts antioxidative (He *et al*. 2016), anti-inflammatory (Lin *et al*. 2014; Chien *et al*. 2016), anti-aging (Zhang *et al*. 2021), hypoglycemic (Li *et al*. 2014), and immunopotentiation (Mariga *et al*. 2014) effects. *P. eryngii*, commonly grown in Taiwan, is a crucial economic crop. Although related studies have used different agricultural waste products to cultivate *P. eryngii*, no systematic study has examined the nutritional and functional benefits of using agricultural waste to cultivate *P. eryngii*. If scientific evidence proves that the quality of mushroom cultivated with agricultural wastes does not differ from that with sawdust, then the use of agrlicultual waste can be easily promoted.

Paddy straw and sugarcane bagasse are already used to grow King Oyster Musuroom years ago (Zhou *et al*. 2023). Accoring to the agricultural statistics of Ministry of Agriculture (https://agrstat.moa.gov.tw/sdweb/public/common/Download.aspx), the average surplus of paddy straw form 2014–2023 are 1,674,452 tons, and the average surplus of spent mushroom substrates from 2014–2023 are 167,771 tons. Paddy straw is the largest amount agricultural waste of agiculture. Spent mushroom substrates are also a big amount agricultural waste. *Phyllostachys makinoi* is the major bamboo species in Taiwan. There are approximately 1,245 million culms of bamboo in Taiwan, and 80% of them are *Phy. makinoi*. The utilization of bamboo forest are less than 1% recently, bamboo needs explore a new way to be used (Chen *et al*. 2024).

This study examined differences between *P. eryngii* cultivated using general sawdust and those cultivated using different agricultural materials such as paddy straw, sugarcane bagasse, bamboo crumb, spent *P. eryngii*, or *Flammulina velutipes* mushroom substrates, and other agricultural-derived products. In addition, this study analyzed the nutrients, extract content, total triterpenoids, total polysaccharides, ergosterol, and ergothioneine of *P. eryngii* cultivated in different formulas. The results of this study can be used as reference and evidence for promoting agricultural wastes to replace sawdust in the cultivation of *P. eryngii*.

MATERIALS AND METHODS

Chemical materials

Oleanolic acid, ergosterol and ergothioneine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol and acetonitrile were purchased from Honeywell Burdick & Jackson

(Muskegon, MI, USA). All of the other chemicals used in this study were of analytical grades and were obtained commercially.

Spawn preparation

The strain of *P. eryngii*, provided by the Mushroom Laboratory of Taiwan Agricultural Research Institute, was cultured on potato dextrose agar (PDA; Difco, Sparks, MD, USA) medium at 24℃ without light, and was subcultured every 2 wk. The spawn was prepared with 85% sawdust, 14% rice bran, and 1% calcium carbonate (w/w, dry weight) followed by adjusting the water content of the mixture to about 65%. The mixture was filled to polypropylene plastic bottles (about 500 grams per bottle) before the bottles being autoclaved (121℃, 1.2 kg cm^{-2}) for 1 h. The bottles were then cooled at 20 ℃ till the center temperature of mixture in the bottle was below 30℃ before each bottle was inoculated with 1 dish of *P. erymgii* mycelia agar. The spawns were incubated at 24℃ till the mixture substrate was colonized fully by mycelium.

Sawdust and spent agricultural wastes collection

Six materials were collected. Sawdust was obtained from You-Cheng Lai (Guoxing, Nantou, Taiwan). Bamboo (*Phy. makinoi*) crumb was obtained from Chingsui Bamboo and Wood Shop (Zhushan, Nantou, Taiwan). Paddy straw and sugarcane crumb were obtained from Chin Yi Yang Development CO., Ltd. (Huwei, Yunlin, Taiwan). Spent *F. velutipes* substrate was obtained from Dewang Enoki Mushroom Farm (Wufeng, Taichung, Taiwan). Spent *P. eryngii* substrate was obtained from Huang's Farm (Wufeng, Taichung, Taiwan). Before substrate preparation, all sawdust and spent agricultural wastes were stacked and rinsed to adjust the water content of them to about 50%.

Substrate preparation, inoculation, incubation and harvest

The substrates were prepared as shown in Table 1 before the water content of the mixture

Table 1. The material contents of each formula used in culturing *Pleurotus eryngii*.

Formulas[z]	Material contents[y] (%)					
	Sawdust	Bamboo crumb	Paddy straw crumb	Sugarcane crumb	Spent *P. eryngii* substrate	Spent *Flammulina velutipes* substrate
P_{SA} (Sawdust)	59.0	0	0	0	0	0
P_{SA+BC} (Sawdust + bamboo crumb)	29.5	29.5	0	0	0	0
P_{SA+PSC} (Sawdust + paddy straw crumb)	29.5	0	29.5	0	0	0
P_{SA+SC} (Sawdust + sugarcane crumb)	29.5	0	0	29.5	0	0
P_{SA+PE} (Sawdust + spent *P. eryngii* crumb)	29.5	0	0	0	29.5	0
P_{SA+FV} (Sawdust + spent *F. veltipes* substrate)	29.5	0	0	0	0	29.5
P_{BC} (Bamboo crumb)	0	59.0	0	0	0	0
P_{PSC} (Paddy straw crumb)	0	0	59.0	0	0	0
P_{SC} (Sugarcane crumb)	0	0	0	59.0	0	0
P_{PE} (Spent *P. eryngii* substrate)	0	0	0	0	59.0	0
P_{FV} (Spent *F. velutipes* substrate)	0	0	0	0	0	59.0

[z] All formulas were amended using 15% rice bran, 25% wheat bran, and 1% $CaCo_3$ based on dry weight.
[y] The contents were based on dry weight.

was adjusted to about 65%. The mixture was filled to polypropylene plastic bags (about 1,000 grams per bag) which were then autoclaved (121℃, 1.2 kg cm^{-2}) for 1 h. The autoclaved bags were cooled at 20℃ till the center temperature of mixture in the bag below 30℃ before two spoonful of *P. erymgii* mycelia sawdust spawn were inoculated into each bag. The inoculated bags were incubated at 22℃ under dark till the mixture substrate was colonized fully by the mycelium.

Once the substrates were totally covered with the mycelium, bags were moved to the growth room followed by opening the top of each bag for exposing the surface of top-sided substrates. The bags were maintained at 13℃ under a relative humidity of 90–95% for 2 d (with light 8 h d^{-1}). Two days later, the room temperature was adjusted to 18℃ with the relative humidity adjusted to 88–90% till the fruiting bodies of *P. eryngii* formed. Once the fruiting bodies started to form, the room temperature were adjusted to 16℃ and the relative humidity adjusted to 85–88% throughout the harvest of the fruiting bodies.

The fruiting bodies were ready for harvest when the gills of fruiting body were formed and the diameter of caps were close to that of stipes. After harvest the fruiting bodies were immediately frozen and prepared for freeze-drying.

The mushrooms generated according to the above-mentioned eleven substrate cultivations were abbreviated as follows: (1) *P. eryngii* cultivated with only sawdust (P_{SA}), (2) *P. eryngii* cultivated with sawdust + bamboo crumb (P_{SA+BC}), (3) *P. eryngii* cultivated with sawdust + paddy straw crumb (P_{SA+PSC}), (4) *P. eryngii* cultivated with sawdust + sugarcane crumb (P_{SA+SC}), (5) *P. eryngii* cultivated with sawdust + spent *P. eryngii* substrate (P_{SA+PE}), (6) *P. eryngii* cultivated with sawdust + spent *F. velutipes* substrate (P_{SA+FV}), (7) *P. eryngii* cultivated with only bamboo crumb (P_{BC}), (8) *P. eryngii* cultivated with only paddy straw crumb (P_{PSC}), (9) *P. eryngii* cultivated with only sugarcane crumb (P_{SC}), (10) *P. eryngii* cultivated with only spent *P. eryngii* substrate (P_{PE}), and (11) *P. eryngii* cultivated with only spent *F. velutipes* substrate (P_{FV}).

Nutritional content analysis

The moisture was determined according to National Standards of the Republic of China (CNS) 5033 (Methods of Test for Moisture in Food). The ash content was determined according to CNS 5034 (Method of Test for Ash in Food). The crude protein content was determined according to CNS 5035 (Methods of Test for Crude Protein in Food). The crude fat content was determined according to CNS 5036 (Methods of Test for Crude Fat in Food). The carbohydrate content was calculated using the following formula: Carbohydrates (%) = 100 – (moisture + ash + crude lipid + crude protein).

Water-soluble extract and dilute ethanol-soluble extract contents

The water extract content was determined according to Taiwan Herbal Pharmacopeia 3rd Edition. Two grams of the dried product of a *P. eryngii* fruiting body was weighed and placed in an Erlenmeyer flask and 70 mL of water was added in it. The solution was shaken and soaked over 5 h (alternating shake and stand 30 min each for 5 h continuously); it was then allowed to stand for 16 h. The solution was filtered and the filtrate was diluted with water to 100 mL. Fifty milliliters of the filtrate was accurately measured and poured into an evaporating dish to evaporate and dry in a water bath. Then the evaporating dish was placed in an oven at 105℃ for 4 h before being cooled in a desiccator. The water extract content (%) was calculated as follows: Water extract (%) = (weight of the evaporating dish after drying − weight of the empty evaporating pan)/weight of the examined sample × [1 − weight loss value of dry sample (%)] × 2 × 100%.

Total polysaccharide detection

The determination of water-soluble crude polysaccharide content was performed using the phenol-sulfuric acid method, which was modified according to the method of Dubois *et al.*

(1956). A total of 0.5 g of *P. eryngii* sample was added 3 times its volume of 80% ethanol and then the solution was heated at 75℃ for 6 h to remove the fat. The residue was added with water to obtain a solid-liquid ratio of 1 : 20. The mixture was heated at 95℃ and extracted for 150 min. Then, 80% ethanol was added, and the solution was filtered after precipitating at 4℃ for 24 h. The precipitate was dried and weighed to obtain the total water-soluble crude polysaccharide. Polysaccharide sample solution was prepared at a concentration of 50 ppm. Two milliliters of the sample solution was mixed evenly in 1 mL of 5% phenol solution before 5 mL of concentrated sulfuric acid was quickly added. The mixed solution was shaken for 30 s, stood at room temperature for 10 min to allow the reaction to proceed fully and then placed in a water bath for 20 min. The absorbance of 1 mL of the reaction solution was measured at 490 nm by using a spectrophotometer. Glucose was used for the construction of the standard curve.

Total triterpenoid content detection

A total of 2 g of *P. eryngii* sample powder was accurately weighed. Then, 100 mL of ethyl acetate was added and the mixture was ultrasonically shaken for 30 min. The filtrate was filtered and quantified with ethyl acetate to 100 mL as the test liquid. A total of 0.3 mL of the test solution was placed in test tubes and evaporated to dry in a 70℃ water bath (only the solute was retained). After the test tubes were removed and cooled, 0.3 mL of 5% vanillin-glacial acetic acid (w/v) and 1.0 mL of perchloric acid were added to each test tube in sequence. After the test tubes were sealed, they were placed in a 70℃ water bath and heated for 25 min. After the reaction completed, the mixture was immediately cooled with ice water for 3 min. Finally, 10.0 mL of glacial acetic acid was added; the test tubes were placed on a shaker at room temperature, and their absorbance was measured at 550 nm by using a spectrophotometer. Oleanolic acid was used for the construction of the standard curve (Cai *et al*. 2019).

High-performance liquid chromatography (HPLC) analysis of *P. eryngii* samples

The ergosterol standard solution was prepared by dissolving 0.5 mg of ergosterol standard in 1 mL of methanol. The ergothioneine standard solution was prepared by dissolving 1 mg of ergothioneine standard in 1 mL of 50% methanol. To prepare the betulinic acid standard solution, 0.5 mg betulinic acid standard was dissolved in 1 mL of methanol. The *P. eryngii* test product solution was prepared as follows: 2 g of *P. eryngii* test product powder was accurately weighed, and 30 mL of 50% methanol was added. The mixture was ultrasonically shaken for 30 min and filtered. The remaining residue was extracted again by repeating the previous steps. The filtrates were combined, concentrated, redissolved to 10 mL with 50% methanol, and filtered using a 0.45-μm microporous membrane. The sample was analyzed using HPLC.

The chromatography fingerprint was analyzed using HPLC equipment (10AVP HPLC System, Shimadzu, Kyoto, Japan), and its chromatography column was a COSMOSIL 5C18-AR-II column (5 μm, 4.6 mm inside diameter (ID) × 250 mm). The mobile phase consisted of methanol (A) and 0.1% aqueous formic acid (B) using a gradient elution of 1% A at 0–10 min, 1–30% A at 10–20 min, 30–40% A at 20–30 min, and 40–95% A at 30–60 min. The flow rate was 1.0 mL per minute, and the detection wavelength was 280 nm for ergosterol, 254 nm for ergothioneine, and 210 nm for betulinic acid to observe the fingerprint spectrum and peaks of specific components. The analysis was performed at room temperature. The injection volume was 20 μL and analysis time was 60 min.

Ergosterol was analyzed using a COSMOSIL 5C18-AR-II column (5 μm, 4.6 mm ID × 250 mm) as the chromatography column and methanol as the mobile phase at a flow rate 1.0 mL per minute. The detection wavelength was 280 nm and the temperature was room temperature. The test sample injection volume

was 20 μL and the analysis time was 20 min. Ergothioneine was analyzed by a COSMOSIL HILIC column (5 μm, 4.6 mm ID × 250 mm). The mobile phase was acetonitrile and water (90 : 10). The flow rate was 1.0 mL per minute, and the detection wavelength was 254 nm. The temperature was at room temperature, the test product injection volume was 10 μL, and the analysis time was 30 min.

Statistical analysis

All the assays were carried out in triplicate and all experimental data are presented as mean ± standard deviation (*SD*). Data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test (IBM SPSS Statistics 25 software). The criterion for statistical significance in this study was $P < 0.05$. Histograms were drawn with Sigmaplot 14 software.

RESULTS

Yields and biological efficiency

Table 2 showed the yields and biological efficiency of *P. eryngii* cultured with different recycled agricultural wastes. The results indicated that the yield of P_{SC} was the highest (323.79 ± 27.64 g bag^{-1}), fallowed by $P_{SA + SC}$, P_{SA}, $P_{SA + PSC}$, $P_{SA + BC}$, P_{BC}, $P_{SA + PE}$, P_{PSC}, $P_{SA + FV}$, P_{FV}, and P_{PE}. The biological efficiency was defined as the numbers of substrates that could efficiently be transformed to produce fruiting bodies of *P. eryngii*. The biological efficiency of $P_{SA + PSC}$ was the highest (100.60 ± 7.61%), fallowed by P_{SC}, $P_{SA + SC}$, P_{SA}, P_{PSC}, $P_{SA + BC}$, P_{BC}, $P_{SA + PE}$, $P_{SA + FV}$, P_{FV}, and P_{PE}. The biological efficiency of formula $P_{SA + BC}$, $P_{SA + PSC}$, $P_{SA + PSC}$, P_{PSC}, and P_{SC} were all higher than 60%. The major substrates in these formulas were potential to be used well by *P. eryngii*.

Table 2. Comparisons of *Pleurotus eryngii* growth based on yield and biological efficiency.

	P. eryngii	
Formulas[z]	Yield (g bag^{-1})	Biological efficiency (%)
P_{SA}	275.94 ± 21.48 b[y]	69.89 ± 5.22 d
$P_{SA + BC}$	252.69 ± 21.79 d	62.53 ± 5.46 e
$P_{SA + PSC}$	262.17 ± 18.65 c	100.60 ± 7.61 a
$P_{SA + SC}$	282.85 ± 17.49 b	74.83 ± 4.95 c
$P_{SA + PE}$	210.98 ± 13.89 e	55.24 ± 3.58 f
$P_{SA + FV}$	191.02 ± 20.08 f	51.16 ± 5.30 g
P_{BC}	219.10 ± 19.28 e	56.18 ± 4.89 f
P_{PSC}	211.13 ± 24.82 e	67.81 ± 8.06 d
P_{SC}	323.79 ± 27.64 a	90.91 ± 7.84 b
P_{PE}	120.75 ± 22.22 g	30.57 ± 5.66 i
P_{FV}	146.02 ± 11.21 h	41.08 ± 3.44 h

[z] All formulas were amended using 15% rice bran, 25% wheat bran, and 1% $CaCO_3$ based on dry weight. Biological efficiency (%) = the weight of fresh mushroom harvested/dry matter content of substrate before inoculation × 100%.

[y] Data were presented as the mean ± *SD* ($n = 48$, one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test). Different letters represented significant differences at $P < 0.05$.

Nutritional analysis

As shown in Table 3, in each group of *P. eryngii*, the moisture content was between 87.1% and 90.4%, the crude ash content was between 0.65% and 0.91%, the crude protein content was between 1.93% and 4.00%, the crude fat content was not detected, and the carbohydrate content was between 6.94% and 8.21%. Whether bamboo crumb, paddy straw crumb, sugarcane crumb, spent *P. eryngii* substrate, or spent *F. velutipes* substrate was used to completely or partially replace sawdust, no significant difference was observed in terms of moisture, crude fat, or carbohydrate content of the cultivated *P. eryngii*. The crude ash contents of P_{PE} or P_{FV} were significantly higher than that of P_{SC}, but there was no difference compared with that of P_{SA}.

Contents of the diluted ethanol-soluble extract and the water-soluble extract

The contents of the diluted ethanol-soluble and water-soluble extracts were determined to examine the content of components that can be dissolved in diluted ethanol (50% ethanol) or water. This method is a standard for quality control in traditional Chinese medicine and is used to compare samples cultivated in differ-

Table 3. Nutrient content of *Pleurotus eryngii* cultivated in sawdust or different agricultural surplus materials.

Formulas	Nutrient contents[z]				
	Moisture (%)	Ash (%)	Protein (%)	Lipid (%)	Carbohydrate (%)
P_{SA}	89.5 ± 1.61 a[y]	0.84 ± 0.03 ab	2.41 ± 0.18 bc	N.D.	7.33 ± 0.55 a
P_{SA+BC}	88.6 ± 2.83 a	0.74 ± 0.12 ab	2.52 ± 0.13 b	N.D.	8.21 ± 0.49 a
P_{SA+PSC}	90.4 ± 1.97 a	0.72 ± 0.04 b	1.93 ± 0.11 c	N.D.	7.02 ± 0.71 a
P_{SA+SC}	89.7 ± 1.84 a	0.74 ± 0.13 ab	2.22 ± 0.24 bc	N.D.	7.44 ± 0.73 a
P_{SA+PE}	88.6 ± 1.46 a	0.72 ± 0.07 ab	2.69 ± 0.13 b	N.D.	8.03 ± 0.19 a
P_{SA+FV}	89.1 ± 2.97 a	0.82 ± 0.07 ab	2.91 ± 0.31 b	N.D.	7.17 ± 0.22 a
P_{BC}	89.9 ± 1.41 a	0.72 ± 0.01 b	2.54 ± 0.12 b	N.D.	6.94 ± 0.52 a
P_{PSC}	89.4 ± 1.80 a	0.71 ± 0.03 b	2.61 ± 0.07 b	N.D.	7.28 ± 0.63 a
P_{SC}	90.0 ± 2.21 a	0.65 ± 0.04 b	2.29 ± 0.13 bc	N.D.	7.10 ± 0.45 a
P_{PE}	87.1 ± 2.09 a	0.91 ± 0.08 a	3.92 ± 0.09 a	N.D.	8.08 ± 0.33 a
P_{FV}	87.6 ± 2.93 a	0.81 ± 0.09 ab	4.00 ± 0.15 a	N.D.	7.62 ± 0.37 a

[z] All examined products were in fresh form.
[y] Data were presented as the mean ± *SD* (n = 8, one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test). N.D. means not detected. Different letters represented significant differences at $P < 0.05$.

ent media and examine differences between products cultivated using different substrates. As shown in Table 4, the contents of diluted ethanol-soluble and water-soluble extracts in *P. eryngii* samples were 38.2–42.8% and 43.6–57.5%, respectively. From the results, it could be seen that no significant differences were observed in the contents of diluted ethanol-soluble extracts between P_{SA} and the other products. However, the water-soluble extracts of P_{PE} were higher than those of P_{SA}. Many factors such as temperature, humidity, and climate as well as the cultivation method used can cause changes in the content of these extracts.

Determination of total triterpenoids and total polysaccharides

The total triterpenoid content was based on the content of oleanolic acid per gram (mg oleanolic acid g^{-1}), and the content of total polysaccharides was mostly based on the content of glucose per gram (mg glucose g^{-1}). As shown in Table 5, the content of total triterpenoids in *P. eryngii* samples was between 1.85 and 2.30 mg oleanolic acid g^{-1}, and that of total polysaccharides was between 1.07 and 4.30 mg glucose g^{-1}. No significant difference in the total triterpenoid content was observed between P_{SA} and the other products. Whereas, the total polysaccharide content was higher in P_{SC} and lower in P_{SA+BC}, P_{SA+FV}, P_{BC}, and P_{PE} than P_{SA}.

Table 4. Contents of diluted ethanol-soluble and water-soluble extracts of mushrooms cultivated using sawdust or different agricultural surplus materials

Formulas	Contents[z]	
	Diluted ethanol-soluble extract (%)	Water-soluble extract (%)
P_{SA}	39.8 ± 0.2 a[y]	45.3 ± 0.9 bc
P_{SA+BC}	42.2 ± 0.2 a	47.8 ± 0.5 bc
P_{SA+PSC}	42.2 ± 2.1 a	46.2 ± 1.6 bc
P_{SA+SC}	39.8 ± 0.5 a	46.5 ± 2.2 bc
P_{SA+PE}	39.5 ± 1.3 a	43.6 ± 2.1 c
P_{SA+FV}	40.8 ± 0.9 a	50.9 ± 2.1 b
P_{BC}	40.9 ± 1.4 a	43.7 ± 1.7 c
P_{PSC}	40.2 ± 1.1 a	47.1 ± 2.5 bc
P_{SC}	38.2 ± 0.9 a	46.3 ± 0.8 b
P_{PE}	42.8 ± 0.8 a	57.5 ± 2.3 a
P_{FV}	41.6 ± 0.8 a	50.6 ± 2.2 b

[z] Results presented as grams of liquid extract per 100 g. All examined products were in powder form.
[y] Data were presented as the mean ± *SD* (n = 8, one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test). Different letters represented significant differences at $P < 0.05$.

Table 5. Total triterpenoid and total polysaccharide contents in mushrooms cultivated using sawdust or different agricultural surplus materials.

Formulas	Contents[z]	
	Total triterpenoids (mg g^{-1})	Total polysaccharides (mg g^{-1})
P_{SA}	1.94 ± 0.08 a[y]	2.70 ± 0.08 b
P_{SA+BC}	1.87 ± 0.13 a	1.07 ± 0.08 d
P_{SA+PSC}	1.85 ± 0.12 a	2.19 ± 0.18 bc
P_{SA+SC}	2.30 ± 0.33 a	2.04 ± 0.25 bc
P_{SA+PE}	2.16 ± 0.14 a	2.28 ± 0.05 bc
P_{SA+FV}	1.96 ± 0.12 a	1.28 ± 0.28 d
P_{BC}	2.19 ± 0.64 a	1.38 ± 0.07 d
P_{PSC}	2.14 ± 0.19 a	2.30 ± 0.34 bc
P_{SC}	1.93 ± 0.06 a	4.30 ± 0.32 a
P_{PE}	2.03 ± 0.18 a	1.95 ± 0.11 c
P_{FV}	2.11 ± 0.24 a	2.41 ± 0.03 b

[z] Total triterpenoids in milligrams of oleanolic acid per gram. Total polysaccharides in milligrams of glucose per gram. All examined products were in powder form.

[y] Data were presented as the mean ± *SD* (n = 8, one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test). Different letters represented significant differences at $P < 0.05$.

Analysis and comparison of the HPLC fingerprint of *P. eryngii*

As shown in Fig. 1A, the retention times of the three index components of ergothioneine, betulinic acid, and ergosterol were 3.15, 16.16, and 42.26 min, respectively. The presence of ergothioneine, ergosterol, and betulinic acid was observed in fingerprint chromographs by comparing the results of mushrooms cultivated in sawdust and in other agricultural surplus materials. In addition, the peak patterns of the fingerprints were found to be consistent (Figs. 1B, 1C, and 1D). The composition of *P. eryngii* grown using bamboo crumb, paddy straw crumb, sugarcane crumb, spent *P. eryngii* substrate, and spent *F. velutipes* substrate formulations was the same as that of *P. eryngii* grown using sawdust.

Analysis of ergosterol and ergothioneine

HPLC was used to analyze the content of ergosterol and ergothioneine in *P. eryngii* samples cultivated using sawdust or different agricultural surplus materials in this study. At a wavelength of 280 nm, the retention time of ergosterol was approximately 13.63 min. The retention time of ergothioneine at a wavelength of 254 nm was approximately 24.25 min (Fig. 2). The regression curve equation of the calibration curves revealed a strong linear relationship at a concentration of 3.1–200 μg mL^{-1} for ergosterol and 5.0–50 μg mL^{-1} for ergothioneine (data not shown). As shown in Table 6 and Fig. 3, the ergosterol content was between 0.015 and 0.067 mg g^{-1} and the ergothioneine content was between 0.890 and 2.542 mg g^{-1}. The contents of ergosterol were higher in P_{SA+PSC} and P_{PE} than in P_{SA}, but that was lower in P_{SA+PE} than in P_{SA} (Table 6 and Fig. 3). On the other hand, the content of ergothioneine in P_{SA+BC}, P_{SA+FV} and P_{FV} were higher than in P_{SA}, but that in P_{SA+PSC}, P_{SA+SC}, P_{SA+PE}, P_{PSC}, P_{SC} and P_{PE} was lower than that in P_{SA} (Table 6 and Fig. 3).

DISCUSSIONS

Generally, sawdust from freshly cut trees is used as the medium to cultivate commercially available *P. eryngii* (Ohga 2000). The cultivation of mushrooms with mediums other than sawdust such as recycled agricultural wastes is an environmentally friendly approach; however, scientific evidence must be obtained to prove that their production capacity and quality. In this study, we compared the differences between *P. eryngii* cultivated using general sawdust and that cultivated using different agricultural materials.

As showed in Table 2, the yield and biological efficiency of formula P_{SC} and P_{SA+SC} were higher than those of P_{SA}. Even if the sawdust contents of formula was replaced a half percent by sugarcane crumb, the yield and biological efficiency of P_{SA+SC} were also higher than that of P_{SA}. The data showed that sugarcane crumb was better than sawdust for *P. eryngii* cultivation. The yields of formula P_{SA+BC} and P_{SA+PSC} were a little less than that of P_{SA}. The yields of formula P_{BC} and P_{PSC} were much less than that of P_{SA}. It

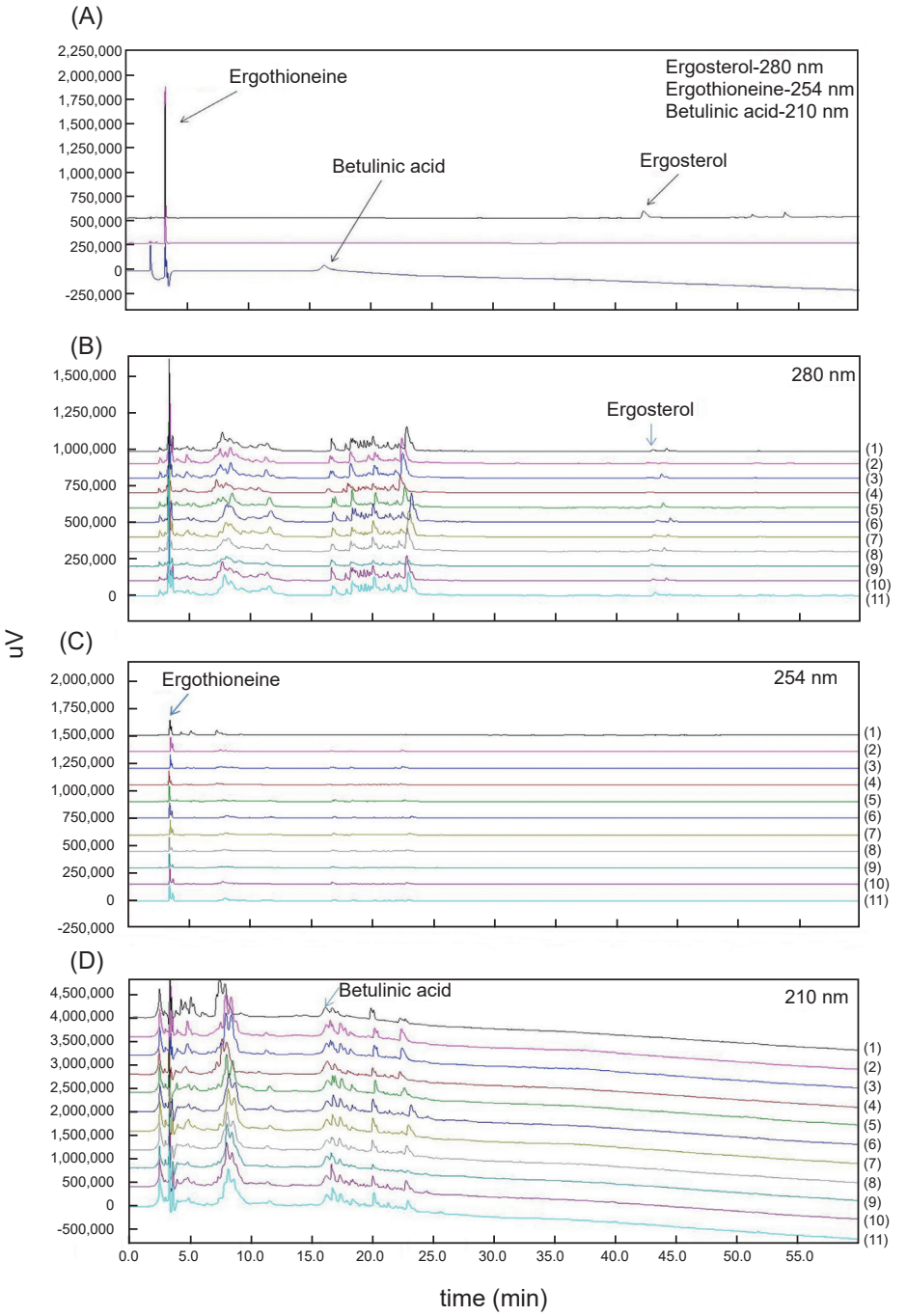

Fig. 1. Comparison of HPLC fingerprints of king oyster mushrooms cultivated using sawdust or agricultural surplus materials at different wavelengths. (A) Standards of ergosterol, ergothioneine, and betulinic acid at (B) 280 nm, (C) 254 nm, and (D) 210 nm. (1) P_{SA}: *Pleurotus eryngii* cultivated with sawdust; (2) P_{SA+BC}: *P. eryngii* cultivated with sawdust + bamboo crumb; (3) P_{SA+PSC}: *P. eryngii* cultivated with sawdust + paddy straw crumb; (4) P_{SA+SC}: *P. eryngii* cultivated with sawdust + sugarcane crumb; (5) P_{SA+PE}: *P. eryngii* cultivated with sawdust + spent *P. eryngii* substrate; (6) P_{SA+FV}: *P. eryngii* cultivated with sawdust + spent *F. velutipes* substrate; (7) P_{BC}: *P. eryngii* cultivated with bamboo crumb; (8) P_{PSC}: *P. eryngii* cultivated with paddy straw crumb; (9) P_{SC}: *P. eryngii* cultivated with sugarcane crumb; (10) P_{PE}: *P. eryngii* cultivated with spent *P. eryngii* substrate; and (11) P_{FV}: *P. eryngii* cultivated with spent *F. velutipes* substrate.

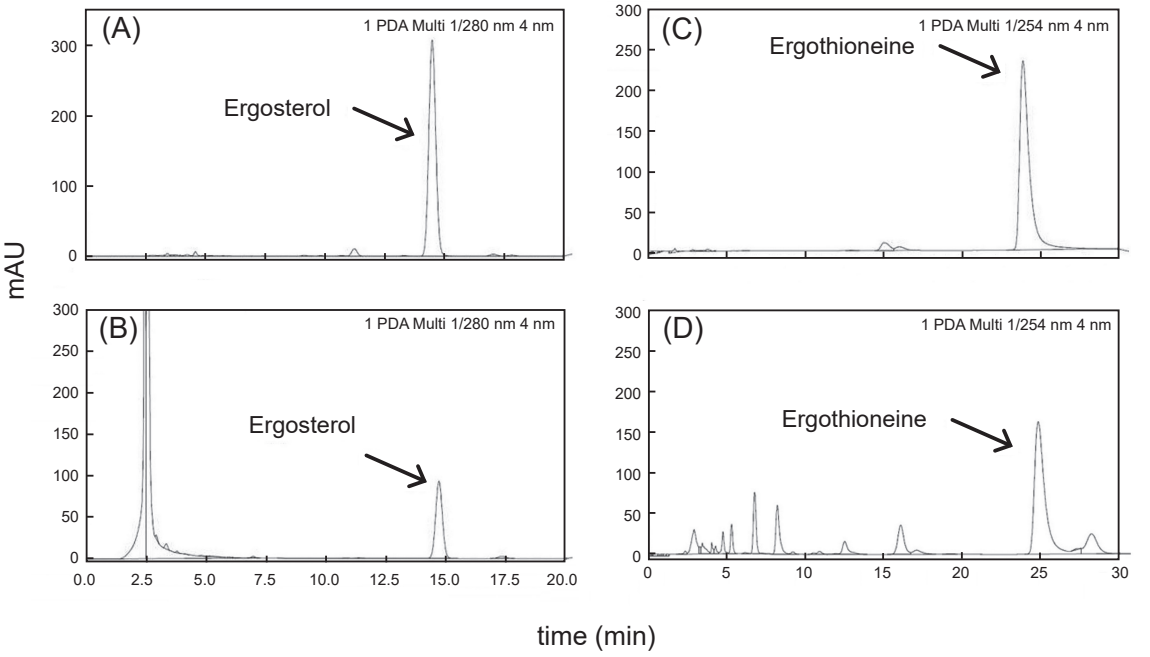

Fig. 2. HPLC chromatograms of ergosterol, ergothioneine and *Pleurotus eryngii*. (A) standard (ergosterol) and (B) *P. eryngii* at a wavelength of 280 nm; (C) standard (ergothioneine) and (D) *P. eryngii* at a wavelength of 254 nm.

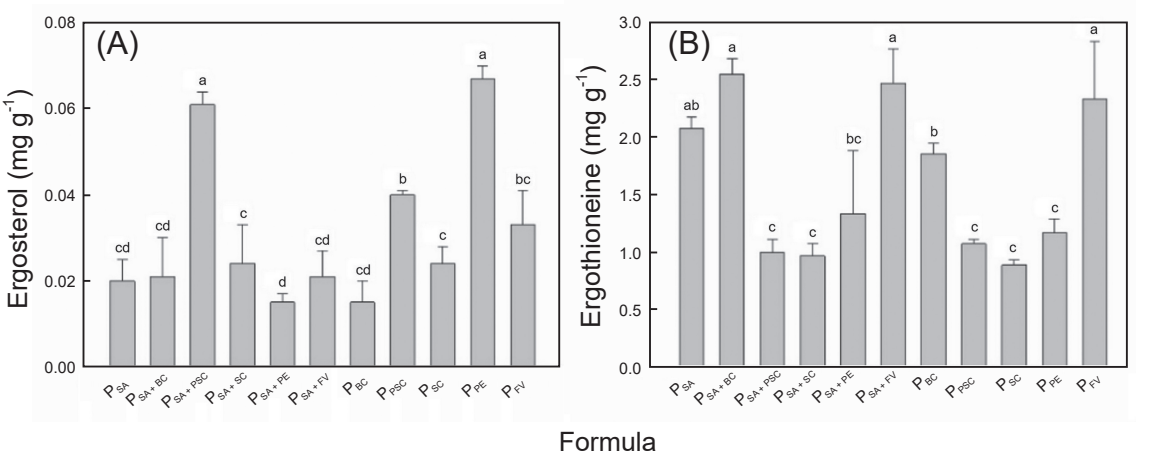

Fig. 3. Comparisons of ergosterol (A) and ergothioneine (B) contents of *Pleurotus eryngii* cultivated using sawdust or agricultural surplus materials. All values were expressed as the mean ± *SD* (n = 8, one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test). Different letters represented significant differences at $P < 0.05$. P_{SA}: *P. eryngii* cultivated with sawdust; P_{SA+BC}: *P. eryngii* cultivated with sawdust + bamboo crumb; P_{SA+PSC}: *P. eryngii* cultivated with sawdust + paddy straw crumb; P_{SA+SC}: *P. eryngii* cultivated with sawdust + sugarcane crumb; P_{SA+PE}: *P. eryngii* cultivated with sawdust + spent *P. eryngii* substrate; P_{SA+FV}: *P. eryngii* cultivated with sawdust + spent *Flammulina velutipes* substrate; P_{BC}: *P. eryngii* cultivated with bamboo crumb; P_{PSC}: *P. eryngii* cultivated with paddy straw crumb; P_{SC}: *P. eryngii* cultivated with sugarcane crumb; P_{PE}: *P. eryngii* cultivated with spent *P. eryngii* substrate; and P_{FV}: *P. eryngii* cultivated with spent *F. velutipes* substrate.

Table 6. Ergosterol and ergothioneine contents of mushrooms cultivated using sawdust or agricultural surplus materials.

Formulas	Contents[z]	
	Ergosterol (mg g^{-1})	Ergothioneine (mg g^{-1})
P_{SA}	0.020 ± 0.005 cd[y]	2.074 ± 0.099 b
P_{SA+BC}	0.021 ± 0.009 cd	2.542 ± 0.131 a
P_{SA+PSC}	0.061 ± 0.003 a	0.998 ± 0.116 d
P_{SA+SC}	0.024 ± 0.009 c	0.968 ± 0.108 d
P_{SA+PE}	0.015 ± 0.002 d	1.333 ± 0.545 c
P_{SA+FV}	0.021 ± 0.006 cd	2.462 ± 0.297 a
P_{BC}	0.015 ± 0.005 cd	1.854 ± 0.088 b
P_{PSC}	0.040 ± 0.001 b	1.074 ± 0.041 cd
P_{SC}	0.024 ± 0.004 c	0.890 ± 0.047 d
P_{PE}	0.067 ± 0.003 a	1.166 ± 0.123 cd
P_{FV}	0.033 ± 0.008 bc	2.327 ± 0.497 a

[z] All examined products were in powder form.

[y] Data were presented as the mean ± *SD* (n = 8, one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test). Different letters represented significant differences at $P < 0.05$.

showed that bamboo crumbs and paddy straw crumbs were not as good as sawdust for *P. eryngii* cultivation. Although Sardar *et al*. (2017) suggested that cellulose improve yield of *P. eryngii*, cellulose in sawdust was not as rich as that in bamboo and paddy straw, the yield of formula P_{SA} in this study was much higher than that of formula P_{BC} and P_{PSC}. Table 2 showed that adding sawdust to the formulas could improve the yields when the bamboo crumbs or paddy straw crumbs were used for cultivating of *P. eryngii*. On the contrary, a result of Hassan *et al*. (2010) showed that the yield from sawdust formula was much higher than that from sugarcane or paddy straw formula. Previous studies suggested that cellulose and lignin contents are not related to the yield of fruiting bodies of *P. eryngii* (Philippoussis *et al*. 2001; Atila 2017). The results in this research coincided with reports by Philippoussis *et al*. (2001) and Atila (2017) that the yield of *P. eryngii* was not correlated with the content of lignin, cellulose, or hemicellulose in the cultivation formula. Maybe different strains of *P. eryngii* prefer different contents of lignin or cellulose constructs or some special compounds in different formula. And *P. eryngii* strain used in this study prefer sawdust to bamboo and paddy straw. So the biological efficiency of formula P_{BC} and P_{PSC} were a little lower than that of P_{SA}. But adding sawdust to the formulas could improve the biological efficiency when the bamboo crumbs or paddy straw crumbs were used for cultivating of *P. eryngii*.

The yields of formula P_{PE} and P_{FV} were much lower than that of P_{SA}. It showed that spent *P. eryngii* substrate and spent *F. velutipes* substrate were not as good as fresh sawdust for *P. eryngii* cultivation. However adding fresh sawdust to the formulas could improve the yields when the spent *P. eryngii* substrate and spent *F. velutipes* substrate were used for cultivating of *P. eryngii*. Spent *P. eryngii* substrate and spent *F. velutipes* substrate in this test were all composted over 2 mo. In this period, perhaps the nutrient suitable for *P. eryngii* could have been broked down that led to perhaps produced some bad secondary metabolites unsuitable for *P. eryngii*. Philippoussis *et al*. (2001) showed that high yield of *P. eryngii* was the results of high carbon/nitrogen ratio formula (Philippoussis *et al*. 2001) and that could explain why the yield of the formula P_{SA+PE} was much higher than that of the formula P_{PE}, and the yield of the formula P_{SA+FV} was much higher than that of the formula P_{FV}.

The crude protein contents of P_{PE} and P_{FV} were higher than that of P_{SA}. The precise study described that *P. eryngii* and *F. velutipes* are rich in protein (Reis *et al*. 2012). There were also literatures pointing out that the nitrogen content of spent *P. eryngii* substrate was higher than that of fresh sawdust (Chen *et al*. 2013). The high nitrogen content of spent *P. eryngii* substrate or spent *F. velutipes* substrate may cause the protein content of P_{PE} or P_{FV} to be higher than that of P_{SA}. However, the type of protein in *P. eryngii* cultivated in spent mushroom substrate and whether its nutritional value has any effect should be studied in the

future. Taken together, the nutrient contents of *P. eryngii* cultivated from other matrix was close to that of from fresh sawdust, therefore using agricultural waste to completely or partially replace fresh sawdust to cultivate *P. eryngii* is a feasible method for environmental protection and economical growth.

Triterpenoids and polysaccharides are the functional components of *P. eryngii*. They exhibit antioxidative activity (Xue *et al*. 2015) and prevent hyperlipidemia and metabolic syndrome associated with obesity (Zhao *et al*. 2020); thus, they can be used as the standard for the content of functional components. The results showed that the total polysaccharide content was higher in P_{SC} and lower in P_{SA+BC}, P_{SA+FV}, P_{BC}, and P_{PE} than P_{SA}. The high sugar content may be attributable to the high sugar content in the substrate that led to higher polysaccharides in P_{sc}. But, according to the report of Abd El-Zaher *et al*. (2022), polysaccharides contents produce form *P. eryngii* cultivated of wheat straw, rice straw and sugarcane bagasse are so close (0.6–0.7 mg mL^{-1}). This is an interesting issue could be further study later.

In this study, HPLC fingerprints were used to compare the differences in mushrooms cultivated in different materials. The results indicated that the peak patterns of the fingerprints were found to be consistent. There had no different components and the difference only observed in the content of the components. Therefore, the use of agricultural waste to cultivate *P. eryngii* should be considered as a safe and feasible method. On the other hasnd, ergosterol and ergothioneine are crucial active components of *P. eryngii* that exert antioxidative and anti-inflammatory effects (Liang *et al*. 2013; Kawai *et al*. 2014). Ergosterol in mushrooms was a new focus for its medical potential in recent years. Ergosterol, a plant sterol known to have hypolipidemic and antitumor functions (Yazawa *et al*. 2000; Takaku *et al*. 2001; Hu *et al*. 2006) is well-recognized in *Pleurotus* mushrooms. It is a precursor of vitamin D2 (provitamin) which is converted to vitamin D2 by ergosterol under UV irradiation (Kalač 2013). Ergothioneine was synthesized only from fungi and mycobacterium (Rodriguez Estrada *et al*. 2009). Liang *et al*. (2013) reported that *P. eryngii* were rich in ergothioneine. Ergothioneine is similar in many respects to glutathione (GSH). Many literatures have confirmed that it has antioxidant properties such as inhibiting lipid oxidation, scavenging free radicals, and peroxynitriting (Aruoma *et al*. 1999). Previous studies also indicated that ergothioneine can significantly increase superoxide dismutase (SOD) activity, glutathione/glutathione disulfide (GSH/GSSG) ratio, and reduce thiobarbituric acid reactive substances (TBARS) content in the brain that significantly reduces the damage of chemotherapy drug-induced brain tissue associted with learning and memory (Song *et al*. 2010). The ergosterol and ergothioneine could be measured in all tested products and could be used as indicators in *P. eryngii* quality control. In this study, the contents of ergosterol and ergothioneine in *P. eryngii* cultivated from different agricultural wastes were detected. As shown in Table 6, the contents of ergosterol extracted from *P. eryngii* cultivated with formula P_{SA+PSC} and P_{PE} were significant higher than those with others, but the data did not show any correlation to the formula. The contents of ergothioneine extracted from *P. eryngii* cultivated with formula P_{SA+BC}, P_{SA+FV} and P_{FV} were significant higher than those from others. The contents of ergothioneine extracted from *P. eryngii* cultivated with formula P_{SA} and P_{BC} were the second highest in concentration (Table 6). The data indicated the contents of ergothioneine extracted from *P. eryngii* were promoted with the additon of sawdust. The content of ergothioneine was correlated to the growth substrates of *P. eryngii*. Herein, the spent *F. velutipes* substrate exhibited itself as the best to promote ergothioneine in *P. eryngii* whereas the sawdust and bamboo crumb were the second best.

CONCLUSIONS

The results of this study indicated the sam-

ples grown using spent *P. eryngii* substrate or spent *F. velutipes* substrate have higher crude protein content that need further researches. The water-soluble extract contents of *P. eryngii* cultured with spent *P. eryngii* substrate was higher than that of *P. eryngii* cultured with sawdust, but there was no difference in the diluted ethanol extract contents. The total polysaccharides content of *P. eryngii* cultured with sugarcane substrate was significantly higher, while that cultured with bamboo crumb was lower, and there had no difference in the content of total triterpenoids. From the observable range of the fingerprint spectrum, it was seen that the composition types of *P. eryngii* cultured from any agricultural waste substrate were the same. The content of health ingredients ergosterol and ergothinione was different due to different cultivation materials. Overall, recycled materials such as bamboo crumb, paddy straw crumb, sugarcane crumb, spent *P. eryngii* substrate, and spent *F. velutipes* substrate can be used to completely or partially replace fresh sawdust to cultivate *P. eryngii* to effectively reduce agricultural waste. Especially sugarcane, paddy straw and bamboo crumb are potential substrates for King Oyster Mushroom cultivation.

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不同農業廢棄物回收再利用培養杏鮑菇及其成分比較

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摘要

李瑋崧、鄭閔謙、彭文煌、蔡仁傑。2024。不同農業廢棄物回收再利用培養杏鮑菇及其成分比較。台灣農業研究 73(4):235–250。

本研究採用竹屑、稻草屑、甘蔗屑、杏鮑菇廢基質或金針菇廢基質全部或部分取代新鮮木屑來培養杏鮑菇。比較了營養成分與功能成分。比較指紋圖譜以及高效液相層析法 (high-performance liquid chromatography; HPLC) 分析的麥角固醇與麥角硫因的含量。結果表明，使用杏鮑菇廢基質或金針菇廢基質培養的樣本具有較高的粗蛋白含量。用杏鮑菇廢基質培養的杏鮑菇水溶性浸出物含量高於用新鮮木屑培養的杏鮑菇水溶性浸出物含量。甘蔗屑基質培養的杏鮑菇總多醣含量顯著高於新鮮木屑培養者，而竹屑培養的杏鮑菇總多醣含量較低。HPLC 指紋圖譜結果表明，從本試驗所用之任何農業廢棄物基質培養的杏鮑菇成分都是相同的。麥角固醇含量為 0.015–0.067 mg g^{-1}，麥角硫因含量為 0.890–2.542 mg g^{-1}。這些結果表明，使用回收農業廢棄資材代替木屑來種植杏鮑菇可能是一種安全且有價值的解決方案。

關鍵詞：杏鮑菇、農業廢棄物、營養、麥角固醇、麥角硫因。

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