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ORIGINAL ARTICLE

Yield, size, nutritional value, and antioxidant activity of oyster mushrooms grown on perilla stalks

Huizhen Li, Zhijun Zhang *, Mengxue Li, Xiaojun Li, Ziwen Sun

College of Chemical & Environmental Engineering, North University of China, Taiyuan 030051, PR China

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Abstract Perilla is an edible medical plant with rapidly increasing acreage in China. In this study, we investigated the potential of perilla stalks (PSs) as an alternative substrate for the cultivation of oyster mushrooms (*Pleurotus ostreatus*). *P. ostreatus* was cultivated on cottonseed hulls (CSH) alone or mixed with PSs in different ratios. The production parameters, physical characteristics, nutritional values, and antioxidant activity of mushrooms cultivated on different substrate mixtures were determined. The addition of PSs to CSH significantly improved the growth rate, yield, biological efficiency, and proximate composition and shortened the cultivation cycle. Cultivation on PSs alone increased the amino acid content in *P. ostreatus* fruiting bodies and the antioxidant activity of mushroom extracts. The PS75 (25% CSH + 75% PS) substrate was deduced to be the most effective substrate on the basis of yield and biological efficiency obtained in a large area where perilla had been planted. The results demonstrate that mixtures of PS with CSHs could be used as novel, practical, and easily accessible alternative substrates for *P. ostreatus* cultivation.

1. Introduction

Perilla frutescens (L) Britt. (Lamiaceae) is an edible medical plant that is frequently used in Asian countries such as China, Korea, and Japan (Zhang et al., 2005). The outstanding medicinal value and unique nutritive qualities make *P. frutescens* a promising candidate for agricultural production.

Oyster mushroom (*Pleurotus ostreatus*) is a popular edible mushroom that is commercially cultivated worldwide (Zhang et al., 2012a). Oyster mushroom has high nutritional value as an important source of protein, carbohydrates, vitamins, calcium, and iron (Hilal et al., 2012). Its extract can lower cholesterol as effectively as dietary supplements (Khatun et al., 2007). Furthermore, *P. ostreatus* has potent antinociceptive, antitumor, antioxidant, and immunological activities (Jayakumar et al., 2009; Vasudewa et al., 2008; Sarangi et al., 2006). *P. ostreatus* has been cultivated using agroindustrial residues from banana (Reddy et al., 2003), olive

* Corresponding author. Tel.: +86 351 3945368.
E-mail address: zjzhang@nuc.edu.cn (Z. Zhang).
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(Ruiz-Rodriguez et al., 2010; Kalmis et al., 2008), and weed plants (Khatun et al., 2007), as well as crop waste, including soybean, cotton, and wheat stalk and sugar cane remnants (Khatun et al., 2007). Growers typically select the most effective, economical, and locally available substrate materials for mushroom production.

Cottonseed hulls (CSHs) are the most popular substrates for producing *P. ostreatus* in Shanxi, China. However, in recent years, damage by cotton bollworm has caused a sharp decline in cotton production, resulting in increased CSH prices and a subsequent rise in mushroom production costs, which continue to increase. In contrast, perilla is emerging as an industrial crop with rapidly increasing acreage. However, only a small part of perilla stalks (PSs) is used to extract biologically active compounds, and the rest is put back into the field as an organic fertilizer or even burned, leading to environmental pollution.

The objective of this study was to investigate the feasibility of using PSs as an alternative substrate for the cultivation of *P. ostreatus*. We assessed the production parameters, physical characteristics, nutritional value, and antioxidant activity of *P. ostreatus* cultivated on various mixtures of CSH and PSs.

2. Materials and methods

2.1. Microorganism and growth substrates

P. ostreatus strain DSM 1833 was obtained from the Microbiology Laboratory, Department of Life Science, North University of China, Taiyuan, China. Stock cultures were maintained at 4 °C on wheat dextrose agar plates. Commercial CSH was available as raw material; PSs were collected from the experimental fields of the North University of China, Taiyuan. After complete drying under the sun, the PSs were crushed into 0.5–1.0-cm pieces using a stalk-grinding miller (9F, Si-Fang Precision Machinery Co., Ltd., Suzhou, Anhui, China).

2.2. Experimental design

To determine the effectiveness of PSs as an alternative to CSH for oyster mushroom cultivation, various combinations of PS and CSH were used, and the carbon-to-nitrogen (C/N) ratio was calculated after measuring the carbon and nitrogen contents (Table 1). Homogeneous substrate mixtures were humidified with water to 75% of their retention capacity, and 1 kg of each substrate was placed in separate polypropylene bags and autoclaved at 121 °C for 2 h. After sterilization, the substrates were inoculated with 70 g of spawn and incubated at 25 °C for 100 days. Then, the growth index and selected physical characteristics of *P. ostreatus* grown in each mixture were determined. The mushrooms were cleaned, dried at 60 °C, and ground into powder prior to assessment of nutritional value and antioxidant activity.

2.3. Production parameters

2.3.1. Mycelium growth and cultivation cycle

Mycelium growth rate (hyphal length for a given number of days from germination to mycelium), time for complete substrate colonization (the time required for mycelia to spread on the whole surface of the substrate), and time from inoculation to harvest were determined.

2.3.2. Yield and biological efficiency

The yield of each mushroom harvest and total mushroom yield of three flushes in a harvest period of 100 days were determined. Biological efficiency (BE) was calculated as follows: BE (%) = (weight of freshly harvested mushrooms/weight of substrate dry matter) × 100.

2.3.3. Physical characteristics

Pileus thickness and diameter, stipe length, and fruiting body diameter were measured. Bulk density was calculated as the ratio of the weight of a single fresh mushroom (g) to its volume (mL).

2.4. Nutritional value

2.4.1. Proximate composition

Moisture content was determined as described previously (Alam et al., 2008). Briefly, the mushrooms were dried in the oven at 100 °C and cooled in a desiccator. Crude protein content was determined by using the Coomassie blue staining method (da Silva and Arruda, 2006) and measuringthe absorbance at 595 nm with a UV spectrophotometer (UV9600, Beifen-Ruili Analytical Instruments Co., Ltd., Beijing, China); crude fat content was determined by extraction with petroleum ether for 8 h using a Soxhlet apparatus (Fernandes et al., 2014). Total carbohydrate content was determined by measuring the absorbance of phenol and concentrated sulfuric acid extracts at 490 nm (Dubois et al., 1956); crude dietary fiber content was determined by the acid-detergent method (Tendekayi et al., 2011), which involved treating the dried sample (1 g) with acid-detergent solution for 1 h to digest non-fiber components. Ash content was determined by incinerating the dried sample in a muffle furnace (SX-2.5-10, Shanghai Hongji Instruments Co., Ltd., Shanghai, China) at 565 °C for 3 h (Khan et al., 2008); vitamin C content was assessed by coupling to 2,4-dinitrophenyl hydrazine dye and measuring the absorbance of the complex by spectrophotometry (Khan et al., 2006).

2.4.2. Amino acids

The amino acids in the acid hydrolysate of the dried fruiting bodies were analyzed by using an amino acid auto-analyzer (S433D, Sykam Company, Munich, Germany); the mushroom samples were hydrolyzed in sealed ampoules for 24 h at 110 °C using 10 mL of 6 mol/L HCl containing 5 mg mL^{-1} phenol. The resolved peaks were identified and compared with a standard run under similar conditions (Cheung, 1997).

2.4.3. Minerals

Iron, manganese, chromium, copper, lead, zinc, and calcium contents were determined by atomic absorption spectroscopy (AA-7000, Shimadzu, Japan) after mineralization with 65% hydrogen nitrate (Tendekayi et al., 2011). In order to avoid phosphate and ionization interference, the hydrolysis solution was diluted with 10% lanthanum chloride for the determination of calcium content.

Table 1 Mycelial growth, yields and physical characteristics of the *P. ostreatus* cultivated on substrates of CSH and its mixtures with PS in different ratios.

Treatments		CSH100% (CSH)	CSH75% + PS25% (PS25)	CSH50% + PS50% (PS25)	CSH25% + PS75% (PS75)	PS100% (PS100)
C/N		38.6	34.53	31.42	28.65	26.46
Mycelial growth	GR/mm d^{-1}	3.33 ± 0.01d	3.41 ± 0.03c	3.67 ± 0.01b	3.95 ± 0.02a	3.91 ± 0.01a
	TCSC/d	28.3 ± 1.5a	26.7 ± 1.5ab	26.0 ± 1.0b	26.3 ± 0.6ab	26.7 ± 0.6ab
	TIH/d	37.7 ± 0.6a	37.0 ± 1.0a	33.3 ± 1.2b	34.7 ± 1.5b	35.0 ± 1.1b
Yield /kg·bag^{-1}	1st harvest	0.397 ± 0.05b	0.465 ± 0.05a	0.483 ± 0.08a	0.443 ± 0.03ab	0.373 ± 0.02b
	2nd harvest	0.253 ± 0.02c	0.277 ± 0.05bc	0.299 ± 0.13ab	0.330 ± 0.02a	0.280 ± 0.01bc
	3rd harvest	0.113 ± 0.01b	0.153 ± 0.02a	0.168 ± 0.05a	0.150 ± 0.03a	0.172 ± 0.02a
	Total	0.763 ± 0.07b	0.923 ± 0.05a	0.962 ± 0.09a	0.930 ± 0.04a	0.830 ± 0.03b
	BE/%	76.33 ± 6.66b	92.33 ± 5.31a	96.17 ± 8.97a	93.00 ± 3.95a	83.00 ± 2.94b
Physical characteristics	PT/cm	0.55 ± 0.02d	0.63 ± 0.01c	0.68 ± 0.03b	0.85 ± 0.02a	0.51 ± 0.02e
	PD/cm	6.31 ± 0.13a	6.64 ± 0.08a	6.55 ± 0.26a	6.55 ± 0.30a	6.38 ± 0.07a
	SL/cm	6.06 ± 0.13a	5.94 ± 0.15a	6.09 ± 0.02a	6.07 ± 0.19a	5.80 ± 0.44b
	SD/cm	1.04 ± 0.08a	1.05 ± 0.09a	1.05 ± 0.05a	1.18 ± 0.07a	1.05 ± 0.10a
	BD/g mL^{-1}	0.75 ± 0.04a	0.79 ± 0.03a	0.79 ± 0.02a	0.77 ± 0.02a	0.71 ± 0.03b

Note: CSH (Cotton seed hull), PS (Perilla stalk), GR (Growth rate of mycelium), TCSC (Time for complete substrate colonization), TIH (Time for inoculation to harvest), PT (Pileus thickness/cm), PD (Pileus diameter), SL (Stipe length), SD (Stipe diameter/cm), BD (Bulk density). Means with the different letters in the same line are significantly different ($P \leqslant 0.05$) by Duncan's multiple range test.

2.4.4. Total phenolic content

Mushrooms (10 g) were added to 100 mL of distilled water and heated for 30 min. The resulting mixture was filtered through qualitative Whatman® No.1 paper, and the resulting filtrate was transferred into 50-mL volumetric flasks and diluted with distilled water until the volumetric flask was full. Total phenolic content of the aqueous extracts was determined according to the method described by Patrícia et al. (2013). Results were expressed as mg of gallic acid equivalent (GAE)/g of sample on a dry basis.

2.4.5. Antioxidant activity

The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical-scavenging activity of *P. ostreatus* was determined as described previously (Zhang et al., 2012b). Samples were extracted with 50% ethanol, and 2 mL of the extract was added to 2 mL of freshly prepared DPPH solution in a screw-capped 10-mL test tube. The tube was kept in complete darkness for 30 min to allow the reaction to reach the steady state, and then, the absorption was measured at 517 nm; control samples contained 2 mL of ethanol with 2 mL of DPPH solution and 2 mL of ethanol with 2 mL of extract. Scavenging activity was determined using the following equation: S_A (%) = 1 − $(A_i - A_j)/A_0 \times 100$, where A_0 is the absorbance of the control without the extract, A_i is the absorbance of the extract with DPPH, and A_j is the absorbance of the extract without DPPH.

The capacity of *P. ostreatus* to scavenge superoxide anions was determined by the pyrogallic acid method, as described previously (Guo et al., 2008). The absorbance at 319.5 nm was measured in a 10-mL reaction mixture containing 0.3 mL of 3 mmol L^{-1} pyrogallic acid and buffer (pH 8.2), which was incubated for 9 min.

Hydroxyl radical-scavenging activity was measured using a previously described method (Xia et al., 2011) with some modifications. The reaction mixture, containing 0.34–3.46 mg mL^{-1} of the sample, was incubated with 2 mM EDTA-Fe (0.5 mL), 3% H_2O_2 (1 mL), and 360 lg mL^{-1} crocus in 4.5 mL of sodium phosphate buffer (150 mM, pH 7.4) for 30 min at 37 °C, and hydroxyl radicals were detected by monitoring the absorbance at 520 nm. In the control reaction, the sample was substituted with distilled water, while H_2O_2 was substituted with sodium phosphate buffer. Hydroxyl radical-scavenging activity was calculated by using the following equation: scavenging effect (%) = $(1 - A_{sample520}/A_{control520}) \times 100$. Additionally, the IC_{50} value, which was defined as the concentration that caused 50% radical inhibition, was calculated.

2.4.6. Statistical analysis

Differences between the means of individual groups were assessed by a one-way analysis of variance with Duncan's multiple range tests.

3. Results and discussion

3.1. Effects of PSs on P. ostreatus mycelial growth and cultivation cycle

Mycelial growth rate, time for complete substrate colonization, and time from inoculation to harvest were considerably different among *P. ostreatus* groups cultivated on different substrate mixtures (Table 1). The highest mycelial growth rate was observed on PS75 substrate, followed by PS100 and PS50. The time for complete substrate colonization and time from

inoculation to harvest were significantly lower for mushrooms grown with the addition of PS substrate than for those cultivated on CSH alone. The earliest mushroom harvest was obtained on PS50 substrate.

Increasing the PS ratio in the mixtures significantly improved mycelial growth and shortened the cultivation cycle. Narain et al. (2009) reported that mushroom mycelial growth and primordial development depends on the properties of lignocellulosic materials, particularly on the C/N ratio, which is optimal at 30. The slower rate of spawn running on the CSH substrate may be due to its high C/N ratio, because nitrogen deficiency is known to inhibit mycelial growth, whereas slow growth on PS100 substrate may be caused by an excess of nitrogen, which is known to delay the formation of the fruiting body (Yang et al., 2013).

3.2. Effects of PSs on P. ostreatus production

Total yield and BE were significantly different among the *P. ostreatus* cultures (Table 1). The highest yield was obtained on PS50 substrate (26.08% higher than that obtained for the control), followed by PS75. The highest BE value of 96.17% was also obtained with the PS50 substrate, followed by PS75 (93.00%) and PS25 (92.33%). Mushroom yield on the same substrate was significantly different among the harvests. More than 50% of the total fruiting bodies were obtained in the first harvest, while the second and third mushroom harvests were of lower quality as well as yield.

Oyster mushrooms cultivated on the mixed substrates demonstrated higher yield and BE, which is consistent with previous results (Mateus et al., 2012) showing that nitrogen supplementation, irrespective of the agroindustrial source, increases the BE of *P. ostreatus* growing on eucalyptus sawdust, corncob, eucalyptus bark, coffee husk, or sugarcane bagasse. Our results may be attributed to the physical properties and C/N ratio of the PS/CSH mixtures, which stimulated substrate bioconversion, thereby presenting more suitable substrates for the cultivation of oyster mushrooms (Rodrigues et al., 2013).

3.3. Effects of PSs on the physical characteristics of P. ostreatus

The physical characteristics of *P. ostreatus* cultivated on different substrates are shown in Table 1. The diameters of the pileus and stipe were not significantly different between mushrooms cultured on PS-containing substrates and those grown on CSH. Mushrooms grown on PS mixtures had a higher pileus thickness than those grown on PS or CSH alone. The PS100 group demonstrated significantly decreased stipe length and bulk density compared with the CSH control. The longest stipes and highest bulk density of fruiting bodies were obtained on PS75 and PS25 substrates, respectively.

Environmental conditions and substrate supplements improve the growth, yield, and quality of mushrooms (Sánchez, 2004). Mushrooms with relatively bigger pileus, higher thickness, and shorter but wider stipes are preferred for their marketable quality. Higher bulk density and denser fruiting bodies are quality indicators of oyster mushrooms. The addition of PSs to CSH substrate did not cause significant differences in pileus diameter, stipe size, and bulk density, in comparison with CSH alone. However, PS addition significantly increased *P. ostreatus* pileus thickness, suggesting that supplementation of CSH with PS increases the marketable quality of *P. ostreatus*.

3.4. Effects of PSs on P. ostreatus proximate composition

The proximate compositions of *P. ostreatus* fruiting bodies are listed in Table 2. Considerable differences were observed in moisture, dietary fiber, fat, vitamin C, reducing sugar, crude protein, and ash content in the fruiting bodies, depending on the substrate, whereas the total sugar content remained unchanged. Moisture, crude fiber, and vitamin C contents were highest for *P. ostreatus* grown on PS50 mixture, while the highest reducing sugar content was observed with PS75. Both PS75 and PS100 caused a significant increase in crude protein content compared with the CSH control, while the highest ash content was observed for the P25 group. However, a further increase in the PS content of the substrate mixtures significantly decreased the ash content compared to that in the control. Correlation coefficient (R value) analysis further confirmed that the contents of reducing sugar, crude protein, and ash in the mushrooms were markedly correlated with the PS content (0–100%), while the contents of moisture, crude fiber, fat, total sugar and Vc exhibited lower correlation.

The moisture content (91.427–94.208%) of the mushrooms in this study was higher than that reported previously (Khan et al., 2008; Fernandes et al., 2014). Crude fiber content ranged between 19.730% and 23.628%, which is higher than that reported for *P. ostreatus* cultivated on *Pycnanthus ongoleubis*, *Ceiba pentandra,* and *Cananium* sp. (17.35–17.51%) (Oyetayo and Olatomiwa, 2013). This result is expected, considering that PS is a highly fibrous substrate.

Our results are in agreement with earlier findings (Manjunathan and Kaviyarasan, 2011) that mushrooms have a low fat content. Addition of the substrate mixtures (PS25, PS50, and PS75) decreased the fat content of the fruiting bodies, in comparison with that of mushrooms grown on CSH and PSs alone, suggesting that these mixed substrates can be useful in weight-restriction diets. The total sugar content in the fruiting bodies did not depend on the growth substrate, which is in agreement with data obtained previously for *P. ostreatus* cultivated on different lignocellulosic wastes (Hilal et al., 2012). The vitamin C content *P. ostreatus* grown on the PS substrates was lower than that of wild edible mushrooms (Ferreira et al., 2009). The crude protein levels in *P. ostreatus* were similar to those reported previously (24.83–27.23%) (Ashraf et al., 2013). The protein as well as ash contents of mushrooms depend largely on the growth substrates, as has been shown for *P. ostreatus* grown on invasive weed (Mintesnot et al., 2013).

3.5. Effects of PSs on P. ostreatus amino acid composition

The amino acid profile and content in mushrooms grown on different substrates are shown in Table 3. In all substrate groups, 17 amino acids were detected. The contents of total and essential amino acids were 10.81–17.57% and 2.82–4.64% of dry matter, respectively. The main components were non-essential amino acids (glutamate, histidine, and aspartate acid), followed by essential amino acids (leucine, lysine, and valine). Essential amino acids, except tryptophan, which were

Table 2 Proximate compositions of the *P. ostreatus* cultivated on substrates of CSH (cotton seed hull) and its mixtures with PS (perilla stalk) in different ratios.

Parameters	CSH	PS25	PS50	PS75	PS100	*R* value
Moisture/%	91.449 ± 0.488c	92.285 ± 0.810bc	94.208 ± 0.618a	93.083 ± 0.157b	91.427 ± 0.498c	0.1010
Crude fiber/%	19.730 ± 0.565c	23.226 ± 0.713a	23.628 ± 0.702a	22.885 ± 1.781ab	21.225 ± 0.652bc	0.2577
Fat/%	2.768 ± 0.146a	2.281 ± 0.162b	2.488 ± 0.154b	2.279 ± 0.050b	2.981 ± 0.132a	0.2236
Total sugar/%	38.756 ± 0.420a	40.043 ± 1.085a	40.299 ± 0.833a	38.906 ± 1.624a	38.884 ± 1.299a	0.1903
Vc/mg·100 g^{-1}	1.655 ± 0.098c	2.076 ± 0.028b	2.337 ± 0.035a	2.150 ± 0.093ab	1.613 ± 0.212c	0.2168
Reducing sugar/%	6.310 ± 0.289c	8.590 ± 0.044b	10.093 ± 0.584a	11.153 ± 1.002a	7.843 ± 0.628b	0.4697
Crude protein/%	23.800 ± 0.147b	20.457 ± 0.114d	22.514 ± 0.129c	24.314 ± 0.134b	26.129 ± 0.057a	0.6368
Ash/%	9.598 ± 0.623a	9.915 ± 1.058a	7.960 ± 0.154b	7.916 ± 0.322b	8.202 ± 0.299b	0.7885*

Note: means with the different letters in the same row are significantly different ($P \leqslant 0.05$) by Duncan's multiple range test.
R value: correlation coefficient, *mean significant correlation.

Table 3 Amino acid contents (%) and compositions of the *P. ostreatus* cultivated on substrates of CSH (Cotton seed hull) and its mixtures with PS (Perilla stalk) in different ratios.

Amino acids	CSH	PS25	PS50	PS75	PS100
Asp	1.08 ± 0.02b	0.86 ± 0.01d	0.97 ± 0.00c	1.07 ± 0.03b	1.32 ± 0.01a
Thr[e]	0.59 ± 0.01b	0.42 ± 0.02d	0.48 ± 0.03c	0.61 ± 0.02b	0.67 ± 0.02a
Ser	0.65 ± 0.01b	0.47 ± 0.02d	0.54 ± 0.02c	0.66 ± 0.03b	0.71 ± 0.01a
Glu	4.23 ± 0.02b	3.43 ± 0.05d	4.01 ± 0.02c	4.88 ± 0.01b	5.96 ± 0.03a
Gly	0.59 ± 0.02c	0.48 ± 0.01e	0.55 ± 0.01d	0.62 ± 0.01b	0.79 ± 0.02a
Ala	0.81 ± 0.02b	0.56 ± 0.01d	0.63 ± 0.02c	0.83 ± 0.03b	0.9 ± 0.01a
Cys	0.02 ± 0.01a	0.01 ± 0.00a	0.02 ± 0.01a	0.03 ± 0.01a	0.01 ± 0.01a
Val[e]	0.6 ± 0.02b	0.42 ± 0.03d	0.49 ± 0.02c	0.61 ± 0.01b	0.69 ± 0.01a
Met[e]	0.16 ± 0.00b	0.16 ± 0.02b	0.16 ± 0.01b	0.16 ± 0.02b	0.28 ± 0.01a
Ile[e]	0.51 ± 0.01b	0.37 ± 0.02d	0.43 ± 0.01c	0.52 ± 0.01b	0.58 ± 0.01a
Leu[e]	0.77 ± 0.02b	0.6 ± 0.01d	0.68 ± 0.01c	0.77 ± 0.01b	0.95 ± 0.02a
Tyr	0.22 ± 0.01e	0.27 ± 0.01d	0.31 ± 0.01b	0.25 ± 0.01c	0.48 ± 0.02a
Phe[e]	0.45 ± 0.01b	0.37 ± 0.02d	0.41 ± 0.01c	0.45 ± 0.01b	0.57 ± 0.03a
His	1.16 ± 0.01d	1.34 ± 0.02b	1.29 ± 0.02c	1.31 ± 0.01c	1.78 ± 0.02a
Lys[e]	0.57 ± 0.02b	0.48 ± 0.02c	0.58 ± 0.01b	0.61 ± 0.03b	0.9 ± 0.02a
Arg	0.55 ± 0.01c	0.43 ± 0.03e	0.52 ± 0.01d	0.6 ± 0.02b	0.74 ± 0.02a
Pro	0.22 ± 0.02a	0.14 ± 0.01c	0.17 ± 0.02b	0.24 ± 0.01a	0.24 ± 0.02a
Total essential amino acids	3.65 ± 0.04b	2.82 ± 0.06d	3.23 ± 0.04c	3.70 ± 0.05b	4.64 ± 0.06a
Total amino acids	13.18 ± 0.12c	10.81 ± 0.16e	12.24 ± 0.12d	14.22 ± 0.18b	17.57 ± 0.17a

Note: means with the different letters in the same row are significantly different ($P \leqslant 0.05$) by Duncan's multiple range test.
[e] The essential amino acids.

not measured, constituted 26.02–27.69% of the total amino acid content. The total amino acid content was highest in the P100 group (approximately 33.308% higher than that in the control group). The content of each identified amino acid was higher in mushrooms cultivated on PS100 substrate than in the other groups. The methionine, lysine, and arginine contents were 75.00%, 57.90%, and 53.44%, respectively, higher in the PS100 group than in the GSH mushrooms. The lowest content of each amino acid was found in the PS25 group.

In this study, *P. ostreatus* demonstrated an identical amino acid profile containing all the 17 amino acids tested (except cysteine) for all growth substrates. However, the content of individual amino acids was affected by different substrates, which is in agreement with previous results (Mendez et al., 2005). The main amino acids in that study were glutamate, aspartate, and lysine (in that order), but were glutamate, histidine, and aspartate in our study (in that order). This difference may be attributed to genetic variability in commercial *P. ostreatus* strains and to the differences in the growth substrates. The contents of all tested amino acids were higher in mushrooms cultivated on PS100 than in the other substrate groups, especially in the case of methionine, lysine, and arginine, whose content was 50% higher than that in the control. These results indicate that PS100 promotes protein biosynthesis in *P. ostreatus*; however, the underlying mechanisms remain to be elucidated. To our knowledge, currently, no commercial cultivation method for *P. ostreatus* focuses on protein quality. Therefore, further studies aimed at increasing the nutritional value of *P. ostreatus* need to be performed.

3.6. Effects of PSs on the mineral composition of P. ostreatus

Levels of seven minerals evaluated in *P. ostreatus* (Table 4) were significantly affected by substrate composition. Levels of most of the tested minerals, including Fe, Mn, Cd, and Cu, decreased markedly with the addition of PSs to the growth substrate, while Pb and Zn showed a slight increase in the P75 group, in comparison with the control group. Ca content

Table 4 Mineral compositions of the *P. ostreatus* cultivated on substrates of CSH (cotton seed hull) and its mixtures with PS (perilla stalk) in different ratios.

Minerals	CSH	PS25	PS50	PS75	PS100
Fe (mg/g)	0.136 ± 0.021a	0.132 ± 0.002a	0.089 ± 0.015b	0.071 ± 0.014bc	0.051 ± 0.001c
Mn (μg/g)	8.500 ± 0.750a	6.500 ± 0.500b	7.125 ± 0.125b	5.125 ± 0.125c	2.083 ± 0.764d
Cd (μg/g)	0.128 ± 0.018a	0.111 ± 0.016ab	0.091 ± 0.006bc	0.079 ± 0.004c	0.084 ± 0.020c
Cu (mg/g)	0.048 ± 0.005a	0.021 ± 0.003b	0.025 ± 0.003b	0.025 ± 0.002b	0.013 ± 0.001c
Pb (μg/g)	0.651 ± 0.048a	0.618 ± 0.024a	0.622 ± 0.098a	0.709 ± 0.056a	0.340 ± 0.028b
Zn (mg/g)	0.046 ± 0.010a	0.042 ± 0.005a	0.048 ± 0.005a	0.055 ± 0.013a	0.024 ± 0.003b
Ca (mg/g)	0.300 ± 0.019b	0.349 ± 0.025b	0.385 ± 0.024b	0.570 ± 0.004a	0.316 ± 0.014b

Note: means with the different letters in the same row are significantly different ($P \leqslant 0.05$) by Duncan's multiple range test.

Table 5 Total phenolic content and antioxidant activities of the extract of *P. ostreatus* cultivated on substrates of CSH (cotton seed hull) and its mixtures with PS (perilla stalk) in different ratios.

Substrates	Total phenolic content (mg EAG g^{-1})	IC_{50}(g/L)		
		DPPH radical	Superoxide radical	Hydroxyl radical
CSH	3.761 ± 0.035e	1.106 ± 0.013a	0.751 ± 0.023a	0.200 ± 0.004a
PS25	3.935 ± 0.065d	0.957 ± 0.014b	0.722 ± 0.020b	0.125 ± 0.004b
PS50	4.342 ± 0.031c	0.934 ± 0.069bc	0.393 ± 0.005c	0.074 ± 0.004c
PS75	4.455 ± 0.050b	0.868 ± 0.041c	0.369 ± 0.010c	0.064 ± 0.002d
PS100	4.686 ± 0.020a	0.735 ± 0.060d	0.378 ± 0.002c	0.026 ± 0.003e

Note: means with the different letters in the same column are significantly different ($P \leqslant 0.05$) by Duncan's multiple range test.

increased steadily with the addition of PSs up to 75%, but returned to the level in the control sample in the case of PS100.

Substrate composition is an important factor that affects the mineral spectrum of mushroom fruiting bodies, and significant differences exist in the uptake of individual elements (Gast et al., 1988). Cd and Cu are accumulated in the fruiting bodies, while levels of Zn and Mn are similar in the fruiting bodies and the substrate; Pb and Fe concentrations are lower in the fruiting bodies (Kalaç and Svoboda, 2000). In this study, Ca was the predominant mineral detected in *P. ostreatus*, with the concentration ranging from 0.300 mg g^{-1} in the control to 0.570 mg g^{-1} in the PS75 group; this value is higher than that observed previously (0.28–0.38 mg g^{-1}) (Patrícia et al., 2013), suggesting that mixed substrates promote Ca accumulation in oyster mushrooms.

3.7. Effects of PSs on the total phenolic content and antioxidant activity of P. ostreatus extract

The total phenolic content was determined for *P. ostreatus* cultivated on CSH and its mixtures with PS in different ratios (Table 5). The contents were different among different substrates, and the total phenolic content increased as the proportion of PS in substrates was increased from 0 (control) to 100. The values (3.45–3.71 mg GAE/g in aqueous extracts) were higher than those obtained by Patrícia et al. (2013).

Antioxidant activities in the *P. ostreatus* extracts were evaluated by determining the IC_{50} values of DPPH, superoxide, and hydroxyl radicals (Table 5); lower IC_{50} values represent higher antioxidant activity. IC_{50} values of DPPH, superoxide, and hydroxyl radicals were lower for mushrooms grown on PS-containing substrates than for the control group, and this effect increased in a PS concentration-dependent manner. The highest radical scavenging effect against DPPH and hydroxyl was observed in the extracts of mushrooms cultivated on PS100, whereas superoxide radical scavenging was highest in the PS75 group.

Mushrooms have a relatively high concentration of antioxidants such as vitamins A and C and β-carotene (Murcia et al., 2002). Mushrooms also contain various phenols, which are highly efficient scavengers of peroxy radicals (Murcia et al., 2002). Ethyl acetate and methanol extracts of *Pleurotus florida* scavenge hydroxyl radicals and inhibit lipid peroxidation activity (Josen, 2000). The addition of PSs to the *P. ostreatus* substrate significantly promoted the antioxidant activity of its extracts against DPPH, superoxide anion, and hydroxyl radicals, suggesting that the antioxidant activity of *P. ostreatus* is associated with the growth substrate. Perilla stalks and leaves contain several functional components such as terpenoids and flavonoids, and their glycosides, lipids, anthocyanin, and polysaccharides (Franziska et al., 2010; Lee et al., 2013). The accumulation of PS functional components may have contributed to the increase in the antioxidant activity of oyster mushrooms; however, the underlying mechanisms, including the relative conversion efficiency, require further investigation.

4. Conclusions

Our results suggest that mixtures of PSs and CSH could be used as novel, practical, and easily accessible alternative substrates for *P. ostreatus* cultivation. PSs significantly promoted mycelial growth, yield, and BE, as well as improved mushroom market quality and nutritional parameters, including dietary fiber, and Ca content. Increasing the PS content of the

substrate not only significantly elevated protein content, including that of total and essential amino acids, but also promoted higher antioxidant activity in oyster mushrooms. The growth substrate PS75 (25% CSH + 75% PS) was the most effective substrate for increasing the yield and BE of harvested mushrooms, while 100% PS was more suitable for the production of mushrooms with better medicinal/dietary qualities.

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