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

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Received 23 May 2015; revised 1 October 2015; accepted 1 October 2015
Available online 8 October 2015

KEYWORDS
Pleurotus ostreatus; Perilla stalk; Physical characteristics; Nutritional value; Antioxidant activity

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.

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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...
(Ruiz-Rodríguez 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, 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 measuring the 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 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 hydrolysate solution was diluted with 10% lanthanum chloride for the determination of calcium content.
Scavenging activity of a dry basis. This was expressed as mg of gallic acid equivalent (GAE)/g of sample on previously (Zhang et al., 2012b). Samples were extracted with distilled water according to the method described by Patrı´cia et al. (2013). Results were obtained by the pyrogallic acid method, as described previously. The time for complete substrate colonization (TCSC) and time from inoculation to harvest (TIH) were considered as physical parameters of oyster mushrooms (10 g) 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 was determined using the following equation: 

\[
\text{Total phenolic content} = \frac{\text{Absorbance at } 517 \text{ nm} - \text{Absorbance of control}}{\text{Absorbance of standard}} \times \text{Concentration of standard}
\]

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% H₂O₂ (1 mL), and 360 lg mL⁻¹ crocus. The absorbance at 520 nm was measured 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 H₂O₂ was substituted with sodium phosphate buffer. Hydroxyl radical-scavenging activity was calculated using the following equation: 

\[
\text{Scavenging effect} (%) = \left(1 - \frac{A_1}{A_2}\right) \times 100
\]

Differences between the means of individual groups were assessed by a one-way analysis of variance with Duncan's multiple range test. Means with the different letters in the same line are significantly different (P ≤ 0.05) by Duncan's multiple range test.

### 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 bug⁻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   |
| Physical characteristics    |               |                       |                       |                       |                 |
| PT/cm                       | 76.33 ± 6.66b | 92.33 ± 5.31a         | 96.17 ± 8.97a         | 93.00 ± 3.95a         | 83.00 ± 2.94b   |
| 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 ≤ 0.05) by Duncan’s multiple range test.
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 PS50 and PS25 substrates, respectively.

Environmental conditions and substrate supplements improve the growth, yield, and quality of mushrooms (Sanchez, 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 Pyracantha ongoleubis, Ceiba pentandra, and Canarium 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 CS 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., 2009). The crude protein levels in P. ostreatus were similar to those reported previously (24.83–27.23%) (Ashraf et al., 2009). The protein as well as ash contents of 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.

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
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 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 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 | 1.655 ± 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 ≤ 0.05) by Duncan’s multiple range test.

### 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 | 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 | 0.6 ± 0.02b | 0.42 ± 0.03d | 0.49 ± 0.02c | 0.61 ± 0.01b | 0.69 ± 0.01a |
| Met | 0.16 ± 0.00b | 0.16 ± 0.02b | 0.16 ± 0.01b | 0.16 ± 0.02b | 0.28 ± 0.01a |
| Ile | 0.51 ± 0.01b | 0.37 ± 0.02d | 0.43 ± 0.01c | 0.52 ± 0.01b | 0.58 ± 0.01a |
| Leu | 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 | 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 | 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 ± 0.12b | 14.22 ± 0.18b | 17.57 ± 0.17a |

**Note:** means with the different letters in the same row are significantly different (P ≤ 0.05) by Duncan’s multiple range test.

*The essential amino acids.
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 (Kalac¸ 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) (Patrı́cia 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 Patrı́cia et al. (2013).

Antioxidant activities in the *P. ostreatus* extracts were evaluated by determining the IC₅₀ values of DPPH, superoxide, and hydroxyl radicals (Table 5); lower IC₅₀ values represent higher antioxidant activity. IC₅₀ 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 peroxo 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

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**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* ≤ 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) | IC₅₀(g/L) | DPPH radical | Superoxide radical | Hydroxyl radical |
|------------|-------------------------------------|----------|--------------|-------------------|-----------------|
| CSH        | 3.761 ± 0.035c                      | 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* ≤ 0.05) by Duncan’s multiple range test.
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 promoted higher antioxidant activity in oyster mushrooms. 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 effectiveness 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.

Acknowledgments

This work was supported by the International S&T Cooperation Program of Shanxi Province, China (2013081004) and the Scientific and technological project in Shanxi Province (201530311009-4).

References

Alam, N., Amin, R., Khan, A., Ara, I., Shim, M.J., Lee, M.W., Lee, T.C., 2006. Nutritional analysis of cultivated mushrooms in Bangladesh. - Pleurotus ostreatus, Pleurotus sajor-caju, Pleurotus flavidus and Calocybe indica. Mycobiology 36 (4), 228–232.
Ashraf, J., Ali, M.A., Ahmad, W., Ayyub, C.M., Shafi, J., 2013. Effect of different substrate supplements on oyster mushroom (Pleurotus spp.) production. Food Sci. Technol. 1 (3), 44–51.
Cheung, P., 1997. Chemical evaluation of some lesser known edible mushroom mycelia produced in submerged culture from soy milk waste. Food Chem. 60 (1), 61–65.
da Silva, M.A., Arruda, M.A., 2006. Mechanization of the Bradford reaction for the spectrophotometric determination of total proteins. Anal. Biochem. 351 (1), 155–157.
Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substance. Anal. Chem. 28 (3), 350–356.
Fernandes, A., Barreira, J.M., Amilcar, L.A., Oliveira, M.B., Martins, A., Ferreira, I.C., 2014. Effects of gamma irradiation on chemical composition and antioxidant potential of processed samples of the wild mushroom Macrolepiota procera. Food Chem. 149 (15), 91–98.
Ferreira, I.C.F.R., Barros, L., Abreu, R., 2009. Antioxidants in wild mushrooms. Curr. Med. Chem. 16 (12), 1543–1560.
Franziska, M.W., Judith, S., Willfried, H.S., Grassmann, J., 2010. Determination of toxic perilla ketone secondary plant metabolites and antioxidative capacity in five Perilla frutescens L. varieties. Food Chem. Toxicol. 48 (1), 264–270.
Gast, C.H., Jansen, E., Bierling, J., Haanstra, L., 1988. Heavy metals and other contaminants in wild mushrooms and their relationship with soil characteristics. Chemosphere 17 (4), 789–799.
Guo, X.F., Yue, Y.D., Tang, F., Wang, J., Yao, X., 2008. Detection of antioxidative capacity of bamboo leaf extract by scavenging superoxide anion free radical. Spectrosc. Spectral Anal. 28 (8), 1823–1826 (In Chinese with English abstract).
Hilal, A., Dandur, A., Yildiz, A., 2012. Effect of using different lignocellulosic wastes for cultivation of Pleurotus ostreatus (Jacq.) P. Kumm. on mushroom yield, chemical composition and nutritional value. Afr. J. Biotechnol. 8 (4), 662–666.
Jayakumar, T., Thomas, P.A., Geraldine, P., 2009. In-vitro antioxidative properties of an ethanolic extract of the oyster mushroom, Pleurotus ostreatus. Innovative Food Sci. Emerg. Technol. 10 (2), 228–234.
Josen, J.K., 2000. Antioxidant and antitumour activity of Pleurotus flavidus. Curr. Sci. 79 (7), 941–943.
Kalaq, P., Svoboda, L., 2000. A review of trace element concentrations in edible mushrooms. Food Chem. 69 (3), 273–281.
Kalmis, E., Azhar, N., Yildiz, H., Kalyoncu, F., 2008. Feasibility of using olive mill effluent (OME) as a wetting agent during the cultivation of oyster mushroom. Bioresearch. Technol. 99 (1), 164–169.
Khan, M.R., Rahman, M.M., Islam, M.S., Begum, S.A., 2006. A simple UV-spectrophotometric method for the determination of vitamin C content in various fruits and vegetables at Sylhet area in Bangladesh. J. Biol. Sci. 6 (2), 388–392.
Khan, M.A., Amin, S., Uddin, M.N., Tania, M., Alam, N., 2008. Comparative study of the nutritional composition of oyster mushrooms cultivated in Bangladesh. Bangladesh J. Mushroom 2 (1), 9–14.
Khata, N., Mahtab, H., Sayeed, P.A., Sayeed, M.A., Khan, K.A., 2007. Oyster mushroom reduced blood glucose and cholesterol in diabetic subjects. Myrmecological News 16 (1), 94–99.
Lee, J.H., Park, K.H., Lee, M.H., Kim, H.-T., Seo, W.D., Kim, J.Y., Baek, I.-Y., Jang, D.S., Ha, T.G., 2013. Identification, characterisation, and quantification of phenolic compounds in the antioxidant-activity-containing fraction from the seeds of Korean perilla (Perilla frutescens) cultivars. Food Chem. 136 (2), 843–852.
Manjunathan, J., Kaviyarasan, V., 2011. Nutrient composition in wild and cultivated edible mushroom, Lentinus tuberregium (Fr.) Tamil Nadu, India. Int. Food Res. J. 18 (1), 809–811.
Mateus, D.N., Rodrigues, M.J., Paes, S.A., Ribeiro, J.J.O., da Silva, M.C.S., Kasuya, M.C.M., 2012. Nitrogen supplementation on the productivity and the chemical composition of oyster mushroom. J. Food Res. 1 (2), 113–119.
Mendez, L.A., Castro, C.S., Casso, R.B., Leal, C.L., 2005. Effect of substrate and harvest on the amino acid profile of oyster mushroom (Pleurotus ostreatus). J. Food Compos. Anal. 18 (5), 447–450.
Mintesnot, B., Ayalew, A., Kebede, A., 2013. Evaluation of biomass of some invasive weed species as substrate for oyster mushroom (Pleurotus spp.) cultivation. Pak. J. Biol. Sci. 17 (2), 1–7.
Murcia, A.M., Martinez, T.M., Jimenez, A.M., Vera, A.M., Honrubia, M., Parras, P., 2002. Antioxidant activity of edible fungi (truffles and mushrooms): losses during industrial processing. J. Food Prot. 65 (10), 1614–1622.
Narain, R., Sahu, R.K., Kumar, S., Garg, S.K., Singh, C.S., Kanaujaet, R.S., 2009. Influence of different nitrogen rich supplements during cultivation of Pleurotus flavidus on maize cobs substrate. Environmentalist 29 (1), 1–7.
Oyetayo, V.O., Olatomiwa, O.A., 2013. Micro and macronutrient properties of pleurotus ostreatus (Jacq Fries) cultivated on different wood substrate. Jordan J. Biol. Sci. 6 (3), 223–226.
Patricia, A.V., Douglas, C.G., Vieira, B.C., Breno, C., Fontes, E.A., Assunção, L.S., Leite, J.P., Oliveira, M.G., Kasuya, M.C., 2013. Antioxidant activities, total phenolics and metal contents in Pleurotus ostreatus mushrooms enriched with iron, zinc or lithium. LWT Food Sci. Technol. 54 (2), 421–425.
Reddy, G.V., Babu, P.R., Komaraiah, P., Roy, K.R., Kothari, I.L., 2003. Utilization of banana waste for the production of lignolytic and cellulolytic enzymes by solid substrate fermentation using two Pleurotus species (P. ostreatus and P. sajor-caju). Process Biochem. 38 (10), 1457–1462.
Rodrigues, M.J., Paes, S.A., Torres, D.P., Nunes, M.D., da Silva, M.C., Mantovani, H.C., Kasuya, M.C., 2013. Production of edible mushroom and degradation of antinutritional factors in jatropha biodiesel residues. Food Sci. Technol. 50 (2), 575–580.
Ruiz-Rodriguez, A., Soler-Rivasb, C., Polonia, I., Wichers, H.J., 2010. Effect of olive mill waste (OMW) supplementation to oyster mushrooms substrates on the cultivation parameters and fruiting bodies quality. Int. Biodeterior. Biodegradation 64 (7), 638–645.
Sánchez, C., 2004. Modern aspects of mushroom culture technology. Appl. Microbiol. Biotechnol. 64 (6), 756–762.
Sarangi, I., Ghosh, D., Bhutia, S.K., Mallick, S.K., Maiti, T.K., 2006. Anti-tumor and immunomodulating effects of Pleurotus ostreatus mycelia-derived proteoglycans. Int. Immunopharmacol. 6 (8), 1287–1297.
Tendekayi, H.G., Martinus, J.N., Eddy, J.S., Smid, E.J., Boekhout, T., Zwietering, M.H., 2011. Nutritive value of masau (Ziziphus mauritiana) fruits from Zambezi Valley in Zimbabwe. Food Chem. 138 (1), 168–172.
Vasudewa, N.S., Abeytunga, D., Ratnasooriya, W.D., 2008. Activity directed fractionation of Pleurotus ostreatus in search of analgesics. Pharm. Biol. 46 (5), 295–301.
Xia, F.G., Fan, J.H., Zhu, M., Tong, H., 2011. Antioxidant effects of a water-soluble proteoglycan isolated from the fruting bodies of Pleurotus ostreatus. J. Taiwan Inst. Chem. Eng. 42 (3), 402–407.
Yang, W.J., Guo, F.L., Wan, Z.J., 2013. Yield and size of oyster mushroom grown on rice/wheat straw basal substrate supplemented with cotton seed hull. Saudi J. Biol. Sci. 20 (4), 333–338.
Zhang, T., Wang, X.Y., Cao, Z.Y., 2005. Plant regeneration in vitro directly from cotyledon and hypocotyl explants of Perilla frutescens and their morphological aspects. Biol. Plant. 49 (3), 423–426.
Zhang, Z.J., Li, H.Z., Qiao, S.J., Zhang, X., Liu, P., Liu, X., 2012a. Effect of salinity on seed germination, seedling growth, and physiological characteristics of Perilla frutescens. Plant Biosyst. 146 (4), 246–251.
Zhang, Y.X., Dai, L., Kong, X.W., Chen, L., 2012b. Characterization and in vitro antioxidant antioxidant activities of polysaccharides from Pleurotus ostreatus. Int. J. Biol. Macromol. 51 (3), 259–265.