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ORIGINAL ARTICLE

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

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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.
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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

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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.

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