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Chemical composition, antioxidant and anti-inflammatory properties for ethanolic extracts from *Pleurotus eryngii* fruiting bodies harvested at different time

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ABSTRACT

Pleurotus eryngii, a popular edible mushroom in Taiwan, is usually cultivated using sawdust medium packing bags through several procedures including culture medium confection, bagging and sterilization, spawn inoculation, fostering mycelia, full growth of mycelia, and inducing fruiting body formation. In this study, *P. eryngii* commercial products harvested at the 10th, 12th and 15th days after inducing the fruiting body formation were extracted with ethanol, individually. Through determination of chemical composition, antioxidant and anti-inflammatory properties of these extracts, the optimal harvest time of *P. eryngii* fruiting bodies with higher functional attributes was revealed. The earlier harvested sample extracts had higher effects for scavenging 2,2-diphenyl-1-picrylhydrazyl radicals, reducing power, chelating power, and β-carotene bleaching inhibition, as well as down-regulating lipopolysaccharide-stimulated nitric oxide, prostaglandin E_2, inducible nitric oxide synthase, and cyclooxygenase-2 expression in RAW264.7 macrophages. These functional responses were closely related to levels of phytochemical components including phenolic acids, flavonoids, tocopherols and carotenoids.

1. Introduction

Pleurotus eryngii, known as king oyster mushroom, is widespread in central Asia, northern Africa and southern Europe (Mau, Lin, Chen, Wu, & Peng, 1998). In Taiwan, where it is commercially available, it is successfully cultivated using sawdust medium packing bags. Reports indicated that edible mushrooms have many healthful effects such as anti-atherosclerotic, anti-cancer, anti-genotoxicity, antioxidant, anti-inflammatory, anti-thrombotic, hypocholesterolemic, hypoglycemic and immune enhancing effects (Mori, Kobayashi, Tomita, Inatomi, & Ikeda, 2008; Shamtsyan et al., 2004; Tsai, Tsai, & Mau, 2007; Wang, Hu, Liang, & Lee, 2005; Zhang et al., 1994). Because of health benefits, *P. eryngii* is popular and sold as vegetable on the market in Taiwan. *P. eryngii* cultivation should go through several procedures including culture medium confection, bagging and sterilization, spawn inoculation, fostering mycelia, full growth of mycelia, and inducing fruiting body formation. The commercial products of *P. eryngii* are those harvested at the 10th to 15th days after inducing the fruiting body formation.

Recent studies have shown that ethanolic extracts of mushroom fruiting bodies such as *Agaricus blazei*, *Agrocybe cylindracea*, *Boletus edulis*, *Pleurotus citrinopileatus*, and *Hypsizigus marmoreus* have good antioxidant capacities attributing to their β-carotene, phenolic compounds and tocopherols (Lee, Huang, Liang, & Mau, 2007; Lee, Yen, & Mau, 2007; Tsai et al., 2007). Kim et al. (2006) found that the ethanolic extract of *Phellinus linteus* fruiting body had good anti-inflammatory effects in the assay using lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages. Kim et al. (2007) reported that ethanolic extract of *Inonotus obliquus* fruiting body had higher antioxidant and anti-inflammatory activities

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than its aqueous extract. Moro et al. (2012) illustrated that phenolic compounds in alcoholic extracts of mushrooms may be responsible for the anti-inflammatory activity. In our pre-experiment, we also found that ethanolic extract of *P. eryngii* fruiting body had higher antioxidant (scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical ability, reducing power and chelating ferrous ion ability) as well as anti-inflammatory (inhibiting LPS-induced NO production in RAW264.7 cells) effects as compared with its aqueous, acetone and ethyl acetate extracts (data no shown).

There is, however, no report concerning the differences of chemical composition, and antioxidant and anti-inflammatory activities about *P. eryngii* fruiting bodies harvested at different time. In the study, the commercial *P. eryngii* products, which were harvested at the 10th, 12th and 15th days after inducing the fruiting body formation were extracted with ethanol (EtOH), individually. The compositions of carotenoids, flavonoids, phenolic acids, and tocopherols in the extracts were determined. Their antioxidant capacities for scavenging DPPH radicals, reducing power, chelating power and β-carotene bleaching inhibition, and anti-inflammatory effects for down-regulation of LPS-stimulated NO, prostaglandin E_2 (PGE_2), inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in RAW264.7 cells were also evaluated. The investigation presented more information for the proper time to harvest *P. eryngii* fruiting bodies with higher functional activities.

2. Materials and methods

2.1. P. eryngii cultivation and treatment

The spawn obtained from Q. Yo Biotechnology Farm, Chang-Hua, Taiwan was inoculated in autoclaved (121 °C for 2 h) sawdust medium packing bags, which composed of sawdust with 58% moisture content (83%), rice bran (8%), wheat bran (8%) and $CaCO_3$ (1%). The bags were then incubated in a dim lighting room at 24 °C for 27 days for full growth of the mycelia. The bags were then transferred to a growing-room (15 °C, relative humidity > 95%, carbon dioxide concentration < 0.3 mL/100 mL) to induce the fruiting body formation. The fruiting body samples used in the investigation were those harvested at the 10th (ca 1.7 cm in diameter and ca 6.6 cm in length, ca 38 g), 12th (ca 3.8 cm in diameter and ca 11.8 cm in length ca 180 g) and 15th (ca 4.4 cm in diameter and ca 13.8 cm in length, ca 220 g) days, respectively after inducing the fruiting body formation (35 fruiting bodies for each sample were gathered). Their moisture contents were 65.0, 66.3 and 68.2 g moisture/100 g fruiting body, respectively. All samples were lyophilized in a freeze-drying system (Vastech Scientific Co. Ltd., Taipei, Taiwan) and ground with the RT08 grinder (Rong-Tsong Co., Taipei, Taiwan) to 420 μm or below for sample extraction.

2.2. Chemicals

Phenolic acid standards: gallic (M.W. = 170), gentisic (M.W. = 174), chlorogenic (M.W. = 354), *p*-hydroxybezoic (M.W. = 138), vanillic (M.W. = 168), caffeic (M.W. = 180), *p*-coumaric (M.W. = 164), ferulic (M.W. = 194), sinapic (M.W. = 224), syringic (M.W. = 198), *p*-anisic (M.W. = 152) and rosmarinic acids (M.W. = 360), flavonoid standards: catechin (M.W. = 290), epicatechin (M.W. = 290), rutin (M.W. = 610), naringin (M.W. = 580), myricetin (M.W. = 318), hesperidin (M.W. = 610), quercitrin (M.W. = 448), neohesperidin (M.W. = 610), eriodictyol (M.W. = 288), diosmin (M.W. = 608), morin (M.W. = 302), daidzein (M.W. = 254), quercetin (M.W. = 302), glycitein (M.W. = 284), naringenin (M.W. = 272), luteolin (M.W. = 286), genistein (M.W. = 270), hesperetin (M.W. = 302), kaempferol (M.W. = 286), apigenin (M.W. = 270) and isorhamnetin (M.W. = 316), carotenoid standards: *trans* forms of α-carotene (M.W. = 537), β-carotene (M.W. = 537), lutein (M.W. = 569) and zeaxanthin (M.W. = 569), and tocopherol standards: α- (M.W. = 430), β- (M.W. = 416), γ- (M.W. = 416) and δ- (M.W. = 402) tocopherols were purchased from Sigma Co. (St. Louis, MO, USA). Acetonitrile (ACN), acetone, acetic acid, methanol (MeOH), *n*-hexane, methylene chloride (CH_2Cl_2) and EtOH were obtained from Merck Co. (Darmstadt, Germany). Distilled deionized water (dd H_2O) was prepared by Ultrapure™ water purification system (Lotun Co., Ltd. Taipei, Taiwan). Ascorbic acid, 2-2′-azino-bis-(3-ethylbenz-thiazoline-6-sulphonic acid) (ABTS), butylhydroxytoluene (BHT), pyrogallol, 1, 4-dioxane, ethylenediaminetetraacetic acid (EDTA), ferric chloride ($FeCl_3$), ferrozine, linoleic acid, vanillin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), horseradish peroxidase, Tween 80, trichloroacetic acid (TCA), glutamine, penicillin, streptomycin, Griess reagent (1% sulfanilamide, 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride and 2.5% phosphoric acid), *Escherichia coli* LPS, and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Co. (St. Louis, MO, USA). Hydrochloric acid (HCl), magnesium carbonate ($MgCO_3$) sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), sodium nitrite ($NaNO_2$), and disodium hydrogen phosphate (Na_2HPO_4) hydrogen peroxide (H_2O_2) were obtained from Wako Co. (Osaka, Japan). Ferrous chloride ($FeCl_2$), chloroform ($CHCl_3$) potassium hydroxide (KOH), potassium ferricyanide ($K_3Fe(CN)_6$), and sodium dihydrogen phosphate (NaH_2PO_4) were purchased from Merck Co. (Darmstadt, Germany).

2.3. HPLC system

The HPLC system consisted of a *PrimeLine*™ Gradient Model 500G HPLC pump system (Analytical Scientific Instruments, Inc., El Sobrante, CA, USA), an injection valve with a 20 μL loop (Rheodyne Inc., Cotati, CA), an S-3210 photodiode-array detector (PDA) (Schambeck SFD GmbH, Bad Honnef, Germany) and an F-1080 fluorescence detector (Hitachi Co., Tokyo, Japan).

2.4. Preparation of P. eryngii fruiting body extracts

Each dried sample powder (100 g) was extracted with 2 L of EtOH at 25 °C for 24 h. After filtration, EtOH was removed by a rotary evaporator (Panchun Scientific Co., Kaohsiung, Taiwan). The extract was then preserved in the dark under nitrogen at −80 °C before experiment.

2.5. Determination of antioxidant components

2.5.1. Flavonoids and phenolic acids

Extraction procedures and analytical conditions were carried out according to the report of Lin, Liu, Tsay, and Yang (2010). Each extract (100 mg) was dissolved in 2 mL of MeOH, diluted with 2 mL of H_2O, and passed through a Bond-Elute C_{18} cartridge (500 mg) (Varian Co. Harbor City, CA, USA). The eluate was collected for analysis.

A Hypersil GOLD C_{18} column (4.6 × 250 mm, 5 μm; Thermo Fisher Scientific Inc., San Jose, CA USA) and a gradient solvent system consisting of solvent A (MeOH) and solvent B (9 mL glacial acetic acid/100 mL dd H_2O)(conditions: 5–17% A from 0 to 5 min and kept at 17% A from 5 to 25 min; 17–31% A from 25 to 40 min and kept at 31% A from 40 to 76 min; 31–40% A from 76 to 80 min and kept at 40% A from 80 to 120 min; flow rate, 0.8 mL/min) were used for separation of phenolic compounds. UV spectra were recorded (1.00 spectrum/s) from 220 to 450 nm.

2.5.2. Carotenoids

Extraction of carotenoids was performed according to the method of Liu, Lin, and Yang (2009). Each extract (100 mg) was mixed with 30 mL of *n*-hexane/acetone/EtOH (2/1/1, v/v/v)