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Phenolic profiles, antioxidant capacities and metal chelating ability of edible mushrooms commonly consumed in China

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Chemical compounds studied in this article:
2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (PubChem CID: 9570474)
DPPH (PubChem CID: 2735032)
EDTA (PubChem CID: 6049)
Trolox (PubChem CID: 40634)
Condensed tannin (PubChem CID: 108065)
(+)-catechin (PubChem CID: 9064)
Gallic acid (PubChem CID: 370)
2,4,6-tri (2-pyridyl) -s-triazine (PubChem CID: 113228)
3,4-dihydroxybenzoic acid (PubChem CID: 1491)
Gentisic acid (PubChem CID: 3469)

ABSTRACT

Antioxidant activities of 43 commonly consumed mushrooms in China were evaluated using ABTS free radical scavenging (ABTS) assay, DPPH free radical scavenging (DPPH) assay, ferric reducing antioxidant power (FRAP) assay, and metal chelating ability (MCA) assay. Phenolic profiles in total phenol content (TPC) and total flavonoid contents (TFC) of mushrooms were also determined by colorimetric methods. The contents of free phenolic acids in mushrooms were determined by HPLC. The mushroom samples exhibited diverse antioxidant activity in different assays. The highest antioxidant ability was found in porcino nero in DPPH value, mulberry yellow in FRAP value, stone ear in ABTS value, and maitake in MCA value. Total phenolic and flavonoid content determination showed that all mushrooms are rich in phenolics and flavonoids. Stone ear and pine-spike had the highest phenolic and flavonoid content. Mushrooms exhibited a positive linear correlation between TPC and ABTS antioxidant capacities at the level of 0.01. Mushrooms have different phenolic acid profiles. Gallic acids were detected with high quantity in most of the mushrooms. Other phenolic acids were detected with low content, and some of phenolic acids were not detected in mushrooms.

1. Introduction

Mushrooms have been used for many years in oriental culture because of their special desirable flavor and favorable texture (Manzi, Gambelli, Marconi, Vivanti, & Pizzoferrato, 1999). Edible mushroom is becoming the most popular nutritious, delicious new resources food in the world, and edible mushrooms are rich in proteins, vitamins, fibers and minerals while the content of fat is low (Guillamon et al., 2010). Edible mushroom production is continuously increasing in the world; China has becoming the biggest producer worldwide (Aida, Shuhaimi, Yazid, & Maaruf, 2009). Mushroom is consumed as a traditional food in China. For some regions of China, the mushroom consumption is relatively high, e.g. up to 20–24 kg fresh product per capita annually, which is a substantially higher figure than the people is living in many other countries (Zhang et al., 2010).

Edible mushrooms usually contain various bioactive molecules, such as phenolic compounds, terpenes and steroids (Barros, Baptista, & Ferreira, 2007; Barros et al., 2008). These secondary metabolites in mushrooms are recognized as excellent antioxidants due to their ability to scavenge free radicals (Manzi, Aguzzi, & Pizzoferrato, 2001; Ejelonu, Akinmoladun, Elekofehinti, & Olaleye, 2013). Free radicals are involved in the pathogenic of various

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diseases and the radicals may disturb the normal function of human organism due to the damage of cellular lipids, protein and DNA. Most of the foods and beverage are from plant origin. They are capable of removing free radicals, chelating metal catalysts, reducing radicals and inhibiting oxides (Ejelonu et al., 2013). It is well-known that antioxidants prevent oxidative damage related to aging and diseases, such as atherosclerosis, diabetes, cancer and cirrhosis (Adebayo, Oloke, Ayandele, & Adegunlola, 2012). Mushroom species had been shown to possess antioxidant capacity in *in vitro* system (Ribeiro et al., 2006). Phenolics are an important group of secondary metabolites, which are biosynthesized by fungi adoption to biotic and abiotic stress condition such as infection, water stress, and cold stress, these phenolics can protect the human body from free radicals, whose formation is associated with the normal natural metabolism of aerobic cells (Oboh & Rocha, 2007). Phenolics are considered to contribute to the prevention of various degeneration of human diseases, such as Alzheimer's diseases (Akinmoladun, Obuotor, & Farombi, 2010; Amic, Davidovic-Amic, Beslo, & Trinajstic, 2003; Elekofehinti & Kade, 2012). Flavonoids have been reported to protect against cancer and heart diseases (Filippos et al., 2007).

The main objectives of the current study were to evaluate the phenolic profile and antioxidant properties of the most popular edible mushroom species marketed in China, and to explore the correlation between the antioxidant capacities and phenolic profiles, and to provide scientific information on the *in vitro* antioxidant capacities of edible mushrooms to consumers and food manufactures.

2. Materials and methods

2.1. Mushroom samples and moisture content analysis

The mushroom samples (fruiting bodies) were purchased from different provinces in China. All samples were dry mushroom and stored in a cool place. The moisture content of mushroom samples was measured by a moisture analyzer (MA-150, Sartorius, Germany) by referring the operation protocol of moisture testing. The scientific name and moisture contents of mushroom samples are listed in Table 1.

2.2. Chemicals and reagents

2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), EDTA, Folin-Ciocalteu reagent, 2-diphenyl-1-picryhydrazyl (DPPH), (+)-catechin, 2,4,6-tri (2-pyridyl) -s-triazine (TPTZ), potassium persulphate ($K_2S_2O_8$), sodium carbonate, gallic acid, sodium hydroxide, sodium nitrite, sodium acetate, acetic acid, ferric chloride, ferrous sulfate, acetone, aluminum chloride hexahydrate, iron(II) chloride, methanol, butylated hydroxytoluene (BHT), trifluoroacetic acid (TFA), and 14 phenolic acids were purchased from Shanghai Yuanye Biological Technology Co., Ltd. (Shanghai, China). Ferrozine, 6-hydroxy-2,5,7,8-tetramethlchroman-2-carboxylic acid (Trolox), and high-performance liquid chromatography (HPLC) - grade acetonitrile was obtained from Sigma–Aldrich Co. (Shanghai, China). Absolute ethanol was purchased from Tianjin Fuyu Fine Chemical Co., Ltd. (Tianjin, China). Other chemical reagents were supplied by Tianjin Damao Chemical Reagent Co., Ltd. (Tianjin, China). All chemicals were of analytical grade unless specially mentioned.

2.3. Extraction of mushroom sample

The mushroom sample extraction procedure was described by Xu and Chang (2007). Briefly, 0.5 g of dry mushroom samples (in triplicate) were extracted for 3 h at room temperature with 5 mL extraction solvent of acetone/water/acetic acid (70:29.5:0.5), extraction was repeated again. The combined extracts from two times extraction were used for determination of total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities.

2.4. Determination of total phenolic content (TPC)

TPC was determined using a colorimetric method as described by Xu and Chang (2007). The absorbance was measured by an UV–visible spectrophotometer at 765 nm. The TPC was expressed as gallic acid equivalents (mg GAE/g sample) in accordance to standard calibration curve of gallic acid with linear range of 50–500 μg/mL ($R^2 > 0.99$).

2.5. Determination of total flavonoids content (TFC)

TFC was determined using a colorimetric method as described by Xu and Chang (2007). The absorbance was measured by the UV–visible spectrophotometer at 510 nm. The TFC was expressed as catechin equivalents (mg CAE/g sample) in accordance to standard calibration curve of catechin with linear range from 10 to 250 μg/mL ($R^2 > 0.99$).

2.6. Determination of DPPH free radical scavenging capacity

DPPH was determined using a colorimetric method as described by Xu and Chang (2007). The absorbance was measured by the UV–visible spectrophotometer at 517 nm using extraction solvent to replace the sample as blank. The DPPH was expressed as Trolox equivalents (μmol TE/g sample) according to standard calibration curve of Trolox with a linear range from 100 to 750 μM ($R^2 > 0.99$).

2.7. Determination of ferric reducing antioxidant capacity (FRAP)

FRAP was determined using a colorimetric method as described by Xu and Chang (2007). The absorbance was measured by the UV–visible spectrophotometer at 593 nm using extraction solvent to replace the sample as blank. The FRAP value was expressed as mmol of Fe^{2+} equivalents per 100 g of sample (mmol Fe^{2+} E/100 g sample) according to standard calibration curve of Fe^{2+} with linear range from 0.1 to 1.00 mM ($R^2 > 0.99$).

2.8. Determination of radical scavenging assay of foods extracts (ABTS)

ABTS was determined using a colorimetric method as described by Miller, Rice-Evans, Davies, Copinathan, and Milner (1993) and Re et al. (1999). The absorbance was measured by the UV–visible spectrophotometer at 734 nm after 6 min reaction in spectrophotometer set at 30 °C, extraction solvent used as blank. The ABTS was expressed as Trolox equivalents (μmol TE/g sample) in accordance to standard calibration curve of Trolox with linear range from 50 to 1000 μM ($R^2 > 0.99$).

2.9. Determination of metal chelating ability (MCA)

The metal chelating ability of mushroom was determined using a colorimetric method described by Dinis, Madeira, and Almeida (1994) with slight modification. Briefly, 20 μL of extract sample was mixed with 50 μL $FeCl_2$. $4H_2O$ (2.00 mM), and then 200 μL of ferrozine (5 mM) was added into the each tube. 1230 μL of 70% acetone solvent was added into the mixture, the total volume was 1.5 mL. Extraction solvent was used as blank. The absorbance was