Food Chemistry 175 (2015) 549–555

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Metal concentration and antioxidant activity of edible mushrooms from Turkey

Cengiz Sarikurkcu [a,*], Bektas Tepe [b], Mehmet Sefa Kocak [c], Mehmet Cemil Uren [c]

[a] Suleyman Demirel University, Faculty of Pharmacy, Department of Analytical Chemistry, Isparta, Turkey
[b] Kilis 7 Aralık University, Faculty of Science and Literature, Department of Molecular Biology and Genetics, Kilis, Turkey
[c] Süleyman Demirel University, Atabey Vocational School, Department of Medicinal and Aromatic Plants, Isparta, Turkey

ARTICLE INFO

Article history:
Received 24 March 2013
Received in revised form 1 December 2014
Accepted 7 December 2014
Available online 13 December 2014

Keywords:
Polyporus sulphureus
Macrolepiota procera
Lycoperdon perlatum
Gomphus clavatus
Antioxidant activity
Heavy metal content

ABSTRACT

This study presents information on the antioxidant activity and heavy metal concentrations of *Polyporus sulphureus*, *Macrolepiota procera*, *Lycoperdon perlatum* and *Gomphus clavatus* mushrooms collected from the province of Mugla in the South-Aegean Region of Turkey. Antioxidant activities of mushroom samples were evaluated by four complementary tests. All tests showed *L. perlatum* and *G. clavatus* to possess extremely high antioxidant potential. Antioxidant activity of the samples was strongly correlated with total phenolic-flavonoid content. In terms of heavy metal content, *L. perlatum* exceeded the legal limits for daily intake of Pb, Fe, Mn, Cr, Ni and Co contents (0.461, 738.00, 14.52, 1.27, 1.65, 0.417 mg/day, respectively) by a 60-kg consumer. Co contents of *M. procera* (0.026 mg/day) and *P. sulphureus* (0.030 mg/day) and Cd contents of *G. clavatus* (0.071 mg/day) were also above the legal limits. According to these results, *L. perlatum* should not be consumed, despite the potentially beneficial antioxidant activity. Additionally, *M. procera* and *G. clavatus* should not be consumed daily due to their high levels of Cd and Co.

1. Introduction

Wild edible mushrooms are prized for their desirable taste, with their annual consumption exceeding 10 kg per individual in some countries (Kalac & Svaboda, 2000). In addition to their flavor and texture, the nutritional value of the fruiting bodies is much appreciated (Manzi, Aguzzi, Vivanti, Paci, & Pizzoferrato, 1999). Mushrooms have also been proven to have therapeutic properties, counteracting diseases such as hypertension, hypercholesterolemia and cancer (Gast, Jansen, Bierling, & Haanstra, 1988). Because the fruiting bodies of mushrooms accumulate high levels of heavy metals, analyzing mushrooms for heavy metal content can also offer an indication of the degree of industrial pollution from various sources, including cement factories, gold mines and thermal power plants, in the location where they are found (Bargagli & Baldi, 1984; Laaksovirta & Alakuijala, 1978; McCreight & Schroeder, 1977). For example, one study found mushrooms collected from areas adjacent to smelters contained high amounts of lead (43.0 mg/kg dry weight), cadmium (6.2 mg/kg dry weight), zinc (120.0 mg/kg dry weight) and iron (3750.0 mg/kg dry weight) (Isiloglu, Merdivan, & Yilmaz, 2001; Kalac, Burda, & Staskova, 1991; Kalac, Niznanska, Bevilaqua, & Staskova, 1996). It should be noted that some studies have suggested that heavy metal accumulation may be species-specific (Falandysz & Chwir, 1977; Falandysz, Kawano, Swieczkowski, Brzostowski, & Dadej, 2003), and that while metal levels in mushrooms may be an important indicator of environmental pollution, some wild mushroom species can exceed legal limits for heavy metals even in non-polluted areas where heavy metal concentrations in soils are low (FAO, 1985; Greger, 1998; FAO/WHO, 1999; European Union, 2002).

This study focused on the antioxidant potential and heavy metal concentrations of edible wild mushrooms growing along the Mediterranean coastline of Turkey in the Aegean and Mediterranean regions. Not only does this provide the opportunity for creating a detailed map of pollution along the Turkish coastline from the Aegean to the Eastern Mediterranean, but because mushrooms are a preferred food source of local residents as well as many middle-income families in other regions, evaluation of the nutritional contribution of various mushrooms species also represents a measure towards the protection of public health.

This study analysed the heavy metal content, antioxidant activity and phenolic and flavonoid contents of four edible wild mushroom species, namely *Polyporus sulphureus* (Bull.: Fr.) Fr., *Macrolepiota procera* (Scop.) Sing., *Lycoperdon perlatum* Pers. and *Gomphus clavatus* S.F. Gray. Heavy metal concentrations of

* Corresponding author. Tel.: +90 505 645 30 51 (mobile).
E-mail address: sarikurkcu@gmail.com (C. Sarikurkcu).

http://dx.doi.org/10.1016/j.foodchem.2014.12.019
0308-8146/

mushroom samples were measured using inductively coupled plasma optical emission spectrometry (ICP-OES) and were evaluated in terms of limits on daily intake recommended by authorized institutions (FAO, 1985; FAO/WHO, 1999; European Union, 2002). Antioxidant activity was evaluated by four complementary test systems, namely ferric thiocyanate testing, chelating activity, DPPH free-radical-scavenging potential and reducing power.

2. Materials and methods

2.1. Mushrooms

Fruiting bodies of the edible mushrooms (*P. sulphureus*, *M. procera*, *L. perlatum* and *G. clavatus*) were collected from Mugla-Turkey in 2011. They were authenticated based on their microscopic and macroscopic characteristics by Prof. M. Halil SOLAK of the Fungi Program at the Mugla University Ali Kocman Vocational School in Ula (Mugla), Turkey. The fruiting bodies of the mushroom samples were divided into several parts and then air-dried in an oven for 48 h at 40 °C before analysis.

2.2. Preparation of the solvent extracts

Petroleum ether, ethyl acetate, methanol and water were used to fractionate the soluble compounds from the mushrooms species.

The dried fruiting bodies of the mushroom samples (10 g) were extracted using a Soxhlet extractor with 250 ml of petroleum ether (ACS reagent, bp 40–60 °C, Sigma–Aldrich Chemical Co., St. Louis, USA), ethyl acetate (HPLC grade, ⩾99.7%, Sigma–Aldrich Chemical Co., St. Louis, USA) and methanol (ACS reagent, ⩾99.8%, Sigma–Aldrich Chemical Co., St. Louis, USA) under reflux conditions. The residues were then extracted with boiling deionized water (18.2 MΩ/cm) (300 ml). Any remaining organic solvent was removed with a rotary evaporator. The water extract was freeze-dried and all extracts were stored at +4 °C until analyzed. Extract yields of *P. sulphureus*, *M. procera*, *L. perlatum* and *G. clavatus* were determined as 1.28%, 1.71%, 0.48% and 1.01% (w/w) for petroleum ether; 0.67%, 0.98%, 0.55% and 0.66% (w/w) for ethyl acetate; 19.88%, 14.19%, 8.37% and 22.11% (w/w) for methanol; 8.72%, 22.03%, 7.76% and 35.28% (w/w) for water, respectively.

Yields of the petroleum ether and ethyl acetate extracts were found inadequate (less than 2.0% dry weight of the mushrooms). Therefore, only the methanol and water extracts were analyzed for antioxidant activities.

2.3. Determination of metal contents

The samples were cleaned, cut and finally dried at 105 °C for 24 h. Dried samples were homogenized using an agate homogenizer and stored at +4 °C in pre-cleaned polyethylene bottles until analyzed. Deionized water (18.2 MΩ/cm) from a Milli-Q system (Human Power I Plus, Korea) was used to prepare all aqueous solutions. All mineral acids and oxidants (HNO_3 and H_2O_2) were of the highest quality (Merck, Darmstadt, Germany). All of the plastic equipment and glassware were cleaned by soaking overnight in a 10% nitric acid solution and then rinsed with deionized water. For the elemental analysis, a Perkin-Elmer Optima 2000 ICP-OES was used.

For digestion, a CEM Mars 5 microwave closed system was used. Samples (0.25 g) were digested with 9 ml of HNO_3 (65%) and 1 ml of H_2O_2 (30%) for 7 min, and finally diluted to 50 ml with deionized water. A blank digestion was carried out in the same way. For the digestion procedure, the heat was run up to 180 °C in 5 min and kept constant for 2 min. This process was repeated once more (Sarikurkcu, Tepe, Solak, & Cetinkaya, 2012).

2.4. Total antioxidant activity determination by ferric thiocyanate method

Antioxidant activities of the mushroom extracts were determined using the thiocyanate method (Sarikurkcu, 2011). Extracts (1 ml at 4 mg/ml) were mixed with 2.5 ml linoleic acid emulsion, (155 μl linoleic acid, 175 μg Tween-20 made up to 50 ml with potassium phosphate buffer (0.04 mol/l, pH 7.0) and phosphate buffer (1.5 ml, 0.04 mol/l, pH 7.0). The mixed solution was then incubated at 37 °C. Sample solution (0.1 ml) was added to ethanol (4.7 ml, 75%), ferrous chloride (0.1 ml, 20 mmol/l in 3.5% HCl) and ammonium thiocyanate (0.1 ml, 30 mmol/l). The peroxide value was determined, after stirring for 3 min, by measuring the absorbance at 500 nm. The percent inhibition of lipid peroxidation was calculated by the following equation:

$$I\% = 100 \times (A_{\text{Control}} - A_{\text{Sample}})/A_{\text{Control}}$$

where, A_{Control} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{Sample} is the absorbance of the test compound.

2.5. Chelating effects on ferrous ions

The metal chelating activity on ferrous ions was determined using the method of Aktumsek, Zengin, Guler, Cakmak, and Duran (2013). Briefly, the extract solution (1 ml) was added to $FeCl_2$ solution (0.05 ml, 2 mmol/l). The reaction was initiated by the addition of ferrozine (0.2 ml, 5 mmol/l) and total volume was adjusted to 5 ml with methanol. In the case of control experiment, methanol and/or water were used instead of the mushroom samples. Then, the mixture was shaken vigorously and left at room temperature for 10 min. Absorbance of the solution was measured at 562 nm using a spectrophotometer. The metal chelating activity was expressed as equivalents of quercetin according to the equation obtained from the graph for quercetin standards (0.5–3.0 mg/ml).

2.6. Scavenging effect on 1.1-diphenyl-2-picrylhydrazyl

The effect of samples on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was estimated using the method of Sarikurkcu (2011). Extract solution (1 ml) was added to a 4 ml of a 0.004% methanol solution of DPPH. The sample absorbance was read at 517 nm after 30 min incubation at room temperature in dark.

2.7. Reducing power

The reducing power was determined according to the method of Oyaizu (1986). Extracts (1 ml at 4–20 mg/ml) were mixed with sodium phosphate buffer (2.5 ml, 200 mmol/l, pH 6.6) and potassium ferric cyanide (2.5 ml, 1%), and the mixture was incubated at 50 °C for 20 min. Then trichloroacetic acid solution (2.5 ml, 10%) was added. The mixture was centrifuged at 3000 rpm (MSE Mistral 2000, London, UK) for 10 min. The upper layer (2.5 ml) was mixed with deionised water (2.5 ml) and ferric chloride (0.5 ml, 0.1%), and the absorbance was measured at 700 nm against a blank. BHT, ascorbic acid and α-tocopherol were used as control agents.

2.8. Determination of total phenolic content

The total phenolic content was determined by employing the methods given in the literature (Sarikurkcu, 2011) with slight modification. Extract solution (0.25 ml) was mixed with diluted Folin–Ciocalteu reagent (1 ml, 1:9) and shaken vigorously. After 3 min, Na_2CO_3 solution (0.75 ml, 1%) was added and the sample absorbance