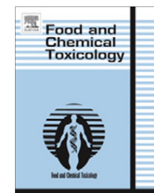
Food and Chemical Toxicology 55 (2013) 378–385

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

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



Bioactive microconstituents and antioxidant properties of wild edible mushrooms from the island of Lesvos, Greece

Nick Kalogeropoulos [a,*], Amalia E. Yanni [a], Georgios Koutrotsios [b], Maria Aloupi [c]

[a] Laboratory of Chemistry, Biochemistry, Physical Chemistry of Foods, Department of Nutrition and Dietetics, Harokopio University, Athens, Greece
[b] Laboratory of General and Agricultural Microbiology, Department of Agricultural Biotechnology, Agricultural University of Athens, Athens, Greece
[c] Water and Air Analysis Laboratory, Department of Environment, University of the Aegean, Mytilene, Greece

ARTICLE INFO

Article history:
Received 1 December 2012
Accepted 9 January 2013
Available online 24 January 2013

Keywords:
Mushrooms
Ergosterol
Polyphenols
Terpenic acids
Antioxidant activity
Metal chelating

ABSTRACT

Crude composition, fatty acids, sterols, total phenolic content (TPC), individual polyphenols and terpenic acids were determined in five wild edible mushrooms species (*Lactarius deliciosus*, *Lactarius sanguifluus*, *Lactarius semisanguifluus*, *Russula delica*, *Suillus bellinii*) from Lesvos Island, Greece. In addition, the DPPH scavenging capacity, the ferric ion reducing power (FRAP) and the ferrous ion chelating activity of mushroom methanolic extracts were assessed. Among sterols, ergosterol predominated at concentrations 9.2–18.0 mg/100 g fw. Total phenolic content of mushroom extracts ranged from 6.0 to 20.8 mg GAE/100 g fw. Up to 19 simple polyphenols were determined in mushrooms extracts, the more abundant being *p*-OH-benzoic acid, *p*-OH-phenylacetic acid, *o*-coumaric acid, ferulic acid and chrysin. In addition, the triterpenic acids oleanolic and ursolic were detected for the first time in mushrooms. All species exerted antioxidant activity and ferrous ion chelating capacity. Principal component analysis revealed good correlations between TPC, DPPH and FRAP but not with metal chelating activity. It seems that mushrooms polyphenols exert antiradical and reducing activities, but they are not strong metal chelators, the observed chelating ability being probably due to other classes of compounds.

To our knowledge, this is the first report on the bioactive microconstituents and antioxidant activity of wild Greek edible mushrooms.

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1. Introduction

Mushrooms have been a perennial component of the human diet, consumed since antiquity not only as part of the normal diet but also as a delicacy, because of their texture and highly desirable taste and aroma. Wild edible mushrooms are consumed with sustainable popularity in many countries of central and Eastern Europe. In Greece, wild mushrooms comprise an important ingredient for the traditional cuisine and gastronomy.

Research conducted during the last decades has indicated that mushrooms exert a number of nutritional and nutraceutical properties and they are source of beneficial bioactive compounds (Ferreira et al., 2009; Yaltirak et al., 2009; Jayakumar et al., 2009). Mushrooms are quite rich in protein, providing all the essential amino acids, they have a low fat content, and they contain relatively high amounts of carbohydrates and fiber (Kalač, 2009). They are considered as low-energy functional foods, which could notably contribute to the design of healthy dietary patterns. Besides macronutrients, mushrooms contain significant amounts of bioactive substances such as vitamins and vitamin precursors, minerals and trace elements (Kalăc, 2009), specific b-glucans, and exert antioxidant properties which are mainly attributed to their phenolic content (Ferreira et al., 2009; Yaltirak et al., 2009). A large body of evidence supports the implication of oxidative stress in the pathogenesis of several chronic and degenerative diseases such as atherosclerosis, diabetes mellitus, chronic inflammation, cancer and aging (Halliwell, 1996; Valko et al., 2007). Therefore, the enhancement of the antioxidant systems for the prevention of cellular oxidative damage via the consumption of antioxidant rich foods is of great interest.

Among the bioactive mushrooms' constituents are sterols, with the predominance of ergosterol, the precursor of vitamin D. In mushrooms, ergosterol is converted to vitamin D_2 (ergocalciferol) when exposed to UV radiation. Vitamin D_2 from fungi and mushrooms serves as the only available dietary source of vitamin D for those who eat no animal products. The crucial role of vitamin D for bone health is well established, while during the last decade its role in immune system modulation (Cantorna et al., 2004) and cancer prevention (Zhao and Feldman, 2001; Mezawa et al., 2010) has been recognized.

* Corresponding author. Tel.: +30 2109549251; fax: +30 2109577050.
E-mail addresses: nickal@hua.gr (N. Kalogeropoulos), ayanni@hua.gr (A.E. Yanni), georgioskoutrotsios@gmail.com (G. Koutrotsios), malou@env.aegean.gr (M. Aloupi).

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http://dx.doi.org/10.1016/j.fct.2013.01.010

Since food composition in bioactive or potentially bioactive compounds is recognized as critical for throwing light upon the association between diet and health, in the present work we studied the crude composition, fatty acids, sterols, total and individual phenolics, terpenic acids, and antioxidant activity – radical scavenging activity, reducing potential and metal binding capacity – in five wild edible mushrooms, from the island of Lesvos, Greece. To our knowledge rather few studies on sterols and individual polyphenols and no studies on the terpenic acids content of the species studied are found in the literature.

2. Materials and methods

2.1. Reagents and chemicals

Butylated hydroxytoluene (BHT), boron trifluoride in methanol (14% BF_3/MeOH), 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), 5-α-cholestane, ergosterol, β-sitosterol, stigmasterol, campesterol, squalene, *p*-hydroxybenzoic acid, vanillin, *p*-coumaric acid, syringic acid, gallic acid, *p*-hydroxyphenylacetic acid, resveratrol, luteolin, chlorogenic acid, ferulic acid, catechin, tyrosol and ursolic acid were obtained from Sigma. Folin–Ciocalteu reagent, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®), bis-(trimethylsilyl)-trifluoroacetamide (BSTFA), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH·), ferrozine, homovanillic alcohol, phloretic acid, cinnamic acid, quercetin, oleanolic acid and 3-(4-hydroxyphenyl)-1-propanol were obtained from Aldrich. Caffeic acid, protocatechuic acid, and sinapic acid were from Fluka. 3,4-dihydroxyphenylacetic acid, chrysin and genistein were from Alfa Aesar; hydroxytyrosol, kaempferol, and naringenin from Extrasynthèse; vanillic acid from Serva. A standard mixture of 37 fatty acid methyl esters was purchased from Supelco; conjugated linoleic acid (CLA) methyl ester was obtained by Sigma–Aldrich. All the solvents used were of HPLC grade and were purchased from Merck or Aldrich.

2.2. Mushroom samples

Specimens of five wild edible mushroom species, namely *Lactarius deliciosus*, *Lactarius sanguifluus*, *Lactarius semisanguifluus*, *Russula delica*, and *Suillus bellinii* were collected from several sites in the island of Lesvos, Northeast Aegean, Greece, as described by Aloupi et al. (2012) (Fig. 1). For the species studied, 5–15 specimens of similar size were obtained from each sampling location (Table 1). Taxonomic identification was based on visual inspection of specimens and observation of their spores with optical miscroscopy, combined with the use of keys from reference books (Konstantinidis, 2009; Phillips, 1981). Immediately after collection, the specimens were transferred to the laboratory. Their fruiting bodies were thoroughly cleaned from soil and forest debris with a soft tissue without washing, cut into small pieces with a plastic knife and weighed. The samples were pre-frozen at −50 °C overnight and freeze-dried in a Labconco FreeZone 4.5 apparatus. Freeze dried samples were ground to a fine powder and specimens from each sampling site belonging to the same species were pooled, wrapped in plastic bags, and stored in the dark at room temperature prior to analysis. In this way 13 composite samples from 139 specimens were obtained.

2.3. Crude composition and energy content

Proximate analysis of the composite samples including moisture, ash, protein, fat, carbohydrates and total energy was performed in triplicate. Moisture was calculated from the weight loss during freeze-drying, as the water content of lyophilised samples was found to be less than 3%. The ash content of freeze dried samples was determined using a programmable muffle furnace. Crude protein was calculated from Kjeldahl nitrogen, which was determined with a Kjeltec 2300 analyser unit (Foss Tecator AB, Hoganas, Sweden), by employing the converting factor 4.38, instead of the commonly employed 6.25, as mushrooms contain significant amounts of non-protein nitrogen, originating mainly from chitin (Kalač, 2009). Fat was determined gravimetrically after extraction of lipids according to Bligh and Dyer (1959). Carbohydrate content was calculated by difference: carbohydrates = 100 − (water − protein − fat − ash). Energy content (kcal/100 g fw) was calculated according to the following equation: Energy = 4 × (g protein + g carbohydrate) + 9 × (g fat).

2.4. Fatty acids

Fatty acids were determined in the form of their methyl esters (FAME) in aliquots of Bligh Dyer extracts. FAME were prepared in screw-capped tubes containing 5–10 mg of lipids after hot saponification with 2 mL 0.5 M KOH in methanol, for 15 min at 90 °C, followed by methylation of fatty acids with 1.5 mL 14% BF3 in methanol for 2 min at 90 °C. The resulting FAME were analysed by GC–MS using an HP 6890 GC equipped with an MSD-5972 mass selective detector, and were separated on a BPX 70 capillary column, as previously described (Kalogeropoulos et al., 2010). Peak identification was based on the known standard mixture of 37 FAME and was confirmed by means of NIST05 mass spectra library.

2.5. Sterols

Sterols were determined in 100 mg of freeze-dried powdered samples after hot saponification with 2 mL 0.5 M KOH in methanol, followed by derivatisation to trimethylsilyl ethers with BSTFA for 20 min at 70 °C and analysis by GC–MS, running in splitless mode. Chromatographic conditions were those described by Kalogeropoulos et al. (2010). Ergosterol, β-sitosterol, campesterol, and stigmasterol identification was based on the retention times and the presence of the expected ion fragments in pure substances. The other sterols were tentatively identified by employing NIST 98 (NIST MS search v6.1d) and Wiley 275 (Wiley, New York, NY) mass spectra libraries, combined with AMDIS-32 deconvolution software. We must add here, that among the minor sterols detected, only those exhibiting matching qualities higher than 80% were concerned and included in the respective Table 4. As no β-sitosterol, campesterol, and stigmasterol were observed in the mushrooms studied, the detected sterols were quantitated by constructing a calibration curve of ergosterol covering the range 0–120 μg, and employing 5-a-cholestane as internal standard.

2.6. Polyphenols extraction

Freeze-dried mushroom samples (2 g) were extracted in screw-capped tubes with 40 mL methanol at room temperature by shaking in a tube roller (Stewart Scientific) for 48 h in the dark. The extracts were separated by centrifugation at 2500 rpm for 10 min and the residues were re-extracted with 10 mL methanol for 2 h. Extracts were combined, methanol was evaporated to dryness at 40 °C under Speed Vac (Labconco Corp.) and the extracts were weighed and then redissolved in 1 mL of methanol. The methanolic solutions were sealed in GC vials and kept at 4 °C until analysis, for no more than 1 week.

2.7. Individual polyphenols and triterpenic acids determination by GC–MS

Individual phenolic compounds and triterpenic acids were determined in mushrooms extracts after derivatisation to their trimethylsilylethers by GC–MS, as previously described (Kalogeropoulos et al., 2009). A selective ion monitoring (SIM) GC–MS method was applied for the detection of 19 target polyphenols, 1 stilbene and 2 triterpenic acids, based on the ±0.05 RT presence of target and qualifier ions of com-

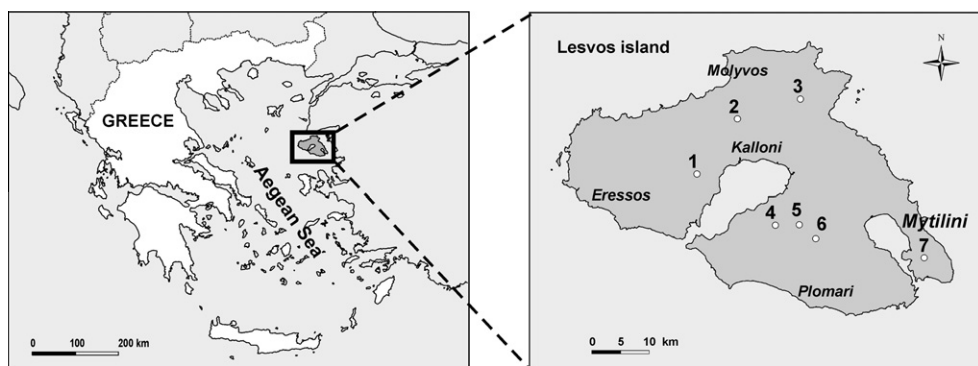

Fig. 1. Mushrooms sampling sites.

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