Towards the antioxidant and chemical characterization of mycorrhizal mushrooms from Northeast Portugal

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Running head: Chemical characterization of mushrooms

ABSTRACT

Mushrooms are widely appreciated all over the world for their nutritional properties and pharmacological value as sources of important bioactive compounds. Mycorrhizal macrofungi associate with plant roots constituting a symbiotic relationship. This symbiosis could influence the production of secondary metabolites, including bioactive compounds. We focused on the evaluation of antioxidant potential and chemical composition of mycorrhizal mushrooms species from Northeast Portugal: *Amanita caesarea*, *Amanita muscaria*, *Amanita pantherina*, *Chroogomphus fulmineus*, *Cortinarius anomalus*, *Cortinarius collinitus*, *Cortinarius violaceus*, *Lactarius quietus*, *Lactarius volemus*, *Russula sardonia*, *Suillus luteus* and *Tricholoma ustale*. A similar profile of metabolites was observed in the studied species with the order sugars > fat > ascorbic acid > phenolic compounds > tocopherols. Nevertheless, the samples revealed different compositions: prevalence of sugars in *L. volemus*, fat and ascorbic acid in *A. muscaria*, phenolic compounds in *C. anomalus* and tocopherols and antioxidant activity in *S. luteus*.

Practical Applications

Chemical characterization of twelve mycorrhizal mushrooms was achieved. They are sources of nutraceuticals such as sugars and fatty acids, and contain bioactive compounds such as vitamins and phenolic acids.

Edible species can be incorporated in diets as sources of antioxidants, while non-edible species can be explored as sources of bioactive metabolites.

Keywords: Mycorrhizal mushrooms; Antioxidant activity; Nutrients; Bioactive compounds

Introduction

Mushrooms are widely appreciated all over the world not only for their nutritional properties (Kalač 2009) but also for their pharmacological value as sources of important bioactive compounds (Ferreira and others 2009; Ferreira and others 2010). Different studies in samples from Spain (Díez and Alvarez 2001), Finland (Mattila and others 2002), Greece (Ouzouni and others 2009), Italy (Manzi and others 2004), India (Kavishree and others 2008), Mexico (Léon-Guzmán and others 1997), Nigeria (Aletor 1995), Portugal (Heleno and others 2010), Taiwan (Tsai and others 2008), Tanzania (Mdachi and others 2004) and Turkey (Yilmaz and others 2006) described them as rich in water, minerals, proteins and carbohydrates, with low levels of fat being suitable for inclusion in low caloric diets.

Various biological activities of mushrooms have been studied, which include antibacterial, antifungal, antiviral, antitumour, cytostatic, immunosuppressive, antiallergic, antiatherogenic hypoglycemic, anti-inflammatory and hepatoprotective activities (Ferreira and others 2010; Lindequist and others 2005). Particularly antioxidant properties of samples from Brasil, China, Korea, Spain, India, Portugal, Taiwan and Turkey have been reported (Ferreira and others 2009). Our research group has performed studies of radical scavenging capacity, reducing power and inhibition of lipid peroxidation in more than thirty different species (Barros and others 2007; Barros and others 2008a; Barros and others 2008b; Ferreira and others 2007; Heleno and others 2010). In most of these studies it was possible to observe significant correlations between phenolics concentration and antioxidant activity, proved by a QCAR (Quantitative Composition-Activity Relationships) model (Froufe and others 2009).

The number of macrofungi species on the earth is estimated around 140,000, and only 10% are known (Lindequist and others 2005). Considering the high biodiversity of mushrooms all over the world, and particularly in Northeast Portugal, our research group has been interested in the chemical characterization of the highest number of species as possible. Here, we focused on mycorrhizal species.

Mycorrhizal macrofungi associate with plant roots constituting a symbiotic relationship, the ectomycorrhizas, beneficial for both plant and fungus (Smith and Read 1997). This symbiosis could influence the production of secondary metabolites, including bioactive compounds. Nevertheless, some mycorrhizal mushrooms are known for their toxicity, such as *A. muscaria* (fly agaric) and *A. pantherina* (panther). The main toxic substances in *A. muscaria* include isoxazoles, such as muscimol responsible for narcotic-intoxicant effects, ibotenic acid, inducer of lassitude and sleep, and a furan derivative muscarine, with insecticidal properties. The effects of muscarine poisoning are the result of its agonist activity on a subset of acetylcholine receptors that are designated as muscarinic receptors. Muscarine causes profound activation of the peripheral parasympathetic nervous system (Shibamoto and Bjeldanes 2009). *A. muscaria* and *A. pantherina* are the species mainly involved in the "pantherina-muscaria" poisoning syndrome. Poisoning cases are sometimes accidental, mainly those caused by *A. pantherina*, because this mushroom might be mistaken with other species; but, in some cases, intentional consumption of *A. muscaria* occurs for recreational purposes. Prognosis of the poisoning is generally minor; although, very seldom lethal cases are mentioned (Michelot and Melendez-Howell 2003). Despite these considerations, edible and non-edible mushrooms can be powerful sources of bioactive compounds, making important their chemical characterization.

In the present work twelve mycorrhizal species (*A. caesarea, A. muscaria, A. pantherina, C. fulmineus, C. anomalus, C. collinitus, C. violaceus, L. quietus, L. volemus, R. sardonia, S. luteus* and *T. ustale*) have been studied for their antioxidant properties and chemical composition in bioactive compounds such as phenolic compounds, vitamins, sugars and fatty acids. As far as we know, among the mentioned species, there are only reports on antioxidant activity (Sarikurkcu and others 2010) and fatty acids composition (Ribeiro and others 2009) of *A. caesarea*, and on phenolic compounds (Ribeiro and others 2009) and fatty acids (Ribeiro and others 2009) composition of *S. luteus.*

Material and methods

Mushroom species

The wild mycorrhizal species were collected in the area of Bragança (Northeast Portugal), in autumn 2009. Information about these species is provided in **Table 1**. Taxonomic identification of sporocarps was made according to several authors (Courtecuisse and Duhem 2005; Kirk and others 2001), and representative voucher specimens were deposited at the herbarium of Escola Superior Agrária of Instituto Politécnico de Bragança.

All the samples were lyophilised (Ly-8-FM-ULE, Snijders, Holland) and reduced to a fine dried powder (20 mesh) for further analyses. The moisture was determined according to the AOAC (1995) procedure.

Standards and Reagents

Acetonitrile 99.9%, n-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Lab-Scan (Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also other individual fatty acid isomers, ascorbic acid, tocopherols, sugars and phenolic standards, and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). Racemic tocol, 50 mg/ml, was purchased from Matreya (PA, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). All other chemicals and solvents were of analytical grade and purchased from common sources. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

Evaluation of antioxidant potential

Extracts preparation. The lyophilized powder (1.5 g) was extracted by stirring with 40 ml of methanol (25ºC at 150 rpm) for 2 h and subsequently filtered through Whatman No. 4 paper. The residue was then extracted with 20 ml of methanol (25ºC at 150 rpm) for 2 h. The combined methanolic extracts were evaporated at 40ºC (rotary evaporator Büchi R-210) to dryness redissolved in methanol at a concentration of 50 mg/ml, and stored at 4 ºC until analysis. Three *in vitro* assays (DPPH radical scavenging capacity, reducing power and inhibition of lipid peroxidation using β-carotene-linoleate model system) already described by the authors (Heleno and others 2010) were used to evaluate the antioxidant activity of the samples, according to mechanisms proposed by Amarowicz and others (2004), Berker and others (2007), and Prior and others (2005).

DPPH radical-scavenging activity. $X^{\bullet} + AH \rightarrow XH + A^{\bullet}$, where $X^{\bullet}$ represents DPPH radical and AH represents antioxidants present in the wild mushrooms. Antioxidants donate a hydrogen atom to DPPH radical decreasing its absorbance at 515 nm. This assay was performed in 96-well microtiter plates using an ELX800 Microplate Reader (Bio-Tek Instruments, Inc). The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the equation: $\% RSA = [(A_{DPPH}-A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution. The extract concentration providing 50% of radicals scavenging activity (EC_{50}) was calculated from the graph of RSA percentage against extract concentration. Trolox was used as standard.

Reducing power. $Fe(CN)_6^{3-} + AH \rightarrow Fe(CN)_6^{4-} + AH^{+}$; $Fe(CN)_6^{4-} + Fe^{3+} \rightarrow Fe[Fe(CN)_6]^{-}$, where $Fe(CN)_6^{3-}$ is the compound with the ferric form, and $Fe(CN)_6^{4-}$ is the compound with the ferrous form. Antioxidants present in the wild mushrooms transfer an electron to ferricyanide complex, reducing Fe^{3+} to Fe^{2+}. The second reaction is to measure the absorbance at 690 nm; a higher absorbance corresponds to a higher reducing power. This assay was also performed using microtiter plates and the Microplate Reader described above. The extract concentration providing 0.5 of absorbance ($EC_{0.5}$) was calculated from the graph of absorbance at 690 nm against extract concentration. Trolox was used as standard.

Inhibition of β-carotene bleaching. β-carotene – H (orange) + $LOO^{\bullet} \rightarrow$ β-carotene• (bleached) + LOOH; β-carotene – H (orange) + $LOO^{\bullet}$ + AH → β-carotene – H (orange) + LOOH + $A^{\bullet}$, where $LOO^{\bullet}$ represents the linoleate free radical. Antioxidants present in

the wild mushrooms donate a hydrogen atom neutralizing the linoleate free radical formed in the system avoiding its attack to the highly unsaturated β-carotene and therefore inhibiting β-carotene bleaching. The assay was conducted spectrophotometrically (Analytikjena 200) at 470 nm. β-Carotene bleaching inhibition was calculated using the following equation: (β-carotene content after 2h of assay/initial β-carotene content) × 100. The extract concentration providing 50% antioxidant activity (EC_{50}) was calculated by interpolation from the graph of β-carotene bleaching inhibition percentage against extract concentration. Trolox was used as standard.

Evaluation of Chemical Composition

Phenolic compounds. The lyophilised powder (~3 g) was extracted with acetone:water (80:20; 30 ml) at -20 °C for 6h, following a procedure previously described (Barros and others 2009). The phenolic extracts were analysed using a Hewlett-Packard 1100 series liquid chromatograph (Agilent Technologies). The phenolic compounds were quantified by comparison of the area of their peaks recorded at 280 nm with calibration curves obtained from commercial standards of each compound (protocatechuic acid, *p*-hydroxybenzoic acid, *trans-p*-coumaric acid, cinnamic acid, 5.0-80.0 μg/ml). The results were expressed as mg per 100 g of dry weight (dw).

Tocopherols. Tocopherols content was determined following a procedure previously optimized and described by the authors (Heleno and others 2010). Analysis was performed by an HPLC system consisted of an integrated system with a pump (Knauer, Smartline system 1000), degasser system (Smartline manager 5000), auto-sampler (AS-2057 Jasco) and a fluorescence detector (FP-2020; Jasco) programmed for excitation at

290 nm and emission at 330 nm. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response of each standard, using the IS method and by using calibration curves obtained from commercial standards of each compound (α-, β-, γ- and δ-tocopherol, 0.04-1.00 μg/ml). Tocopherol contents in the samples were expressed in μg per 100 g of dw.

Ascorbic acid. The lyophilised powder (150 mg) was extracted with metaphosphoric acid (1%, 10 ml) for 45 min at room temperature and filtered through a Whatman Nº 4 filter paper. The filtrate (1 ml) was mixed with 2,6-dichloroindophenol (9 ml) and the absorbance was measured within 30 min at 515 nm. Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (0.006-0.1 mg/ml), and the results were expressed as mg per 100 g of dw.

Sugars. Free sugars were determined by high performance liquid chromatography (equipment described above) coupled to a refraction index detector (RI detector Knauer Smartline 2300) as previously described by the authors (Heleno and others 2009). Sugar identification was made by comparing the relative retention times of sample peaks with standards. Data were analysed using Clarity 2.4 Software (DataApex). Quantification was based on the RI signal response of each standard, using the IS method and by using calibration curves obtained from commercial standards of each compound (mannitol and trehalose, 0.2-20 mg/ml). The results are expressed in g per 100 g of dw.

Fatty Acids. Fat was determined by extracting a known weight of the lyophilised power with petroleum ether, using a Soxhlet apparatus. Fatty acids were determined after a transesterification procedure as described previously by the authors (Heleno and others 2009). The fatty acid profile was analyzed with a DANI 1000 gas chromatographer (GC) equipped with a split/splitless injector and a flame ionization detector (FID). Fatty acid identification was made by comparing the relative retention times of FAME (fatty acids methyl esters) peaks from samples with standards. The results were recorded and processed using CSW 1.7 software (DataApex 1.7) and expressed as a weight percentage of the crude fat.

Statistical analysis

For each one of the mushroom species three samples were used and all the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD). The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $\alpha = 0.05$. This analysis was carried out using SPSS v. 16.0 program.

Results and discussion

The edible *S. luteus* and the poisonous *A. muscaria* and *A. pantherina* had the highest antioxidant activities (**Table 2**). A species of unknown edibility, *C. collinitus*, and a sample of questionable edibility, *L. quietus*, showed the lowest antioxidant properties.

The studied sample of *A. caesarea* revealed higher DPPH scavenging activity (50% at 7.41 mg/ml *versus* 43% at 8 mg/ml), higher reducing power (absorbance 0.5 at 1.85 mg/ml *versus* 0.5 at 4 mg/ml), but lower β-carotene bleaching inhibition (50% at 7.47

mg/ml *versus* 70% at 2.5 mg/ml) than *A. caesarea* sample from Turkey (Sarikurkcu and others 2010).

Different metabolites were analysed in order to achieve chemical characterization of the studied species. All the results were expressed in a dry weight basis; nevertheless, moisture contents are given in **Table 2** to make possible the conversion to a fresh weight basis.

Three phenolic acids (protocatechuic, *p*-hydroxybenzoic and *p*-coumaric acids) and a related compound (cinnamic acid) were identified and quantified in the different samples by HPLC-DAD (**Table 3**). *C. anomalus* showed the highest concentration of phenolic acids, due to the contribution of *p*-hydroxybenzoic acid (8.68 mg/100 g), and cinnamic acid (1.92 mg/100 g). *A. pantherina* sample revealed the highest concentration of protocatechuic (1.18 mg/100g) and *p*-coumaric (0.36 mg/100 g) acids. Although the highest concentration of phenolic acids found in *C. anomalus*, this species did not show the highest antioxidant activity. Therefore, other antioxidant compounds besides phenolic acids are contributing to the observed antioxidant activities.

Similarly to the present study, Ribeiro and others (2006) could not find *p*-hydroxybenzoic acid in a sample of *S. luteus* from Portugal also collected in mixed stands habitat in 2005. However, it was possible to quantify protocatechuic acid in the studied sample collected in 2009, but not quercetin as it was described by those authors, which seems logical as, indeed, no flavonoids are expected to occur in fungi (Iwashina 2000).

Vitamins (tocopherols and ascorbic acid) contents in the studied wild mychorrhizal mushrooms are given in **Table 4**. γ-Tocopherol was the major compound in *A. muscaria*, *A. pantherina*, *C. fulmineus*, *C. violaceus*, *L. quietus*, *R. sardonia*, *S. luteus*

and *T. ustale*, while δ-tocopherol was the major compound in *A. caesarea*, *C. anomalus*, *C. collinitus* and *L. volemus*.

The edible *S. luteus* showed the highest content of total tocopherols (449.76 μg/100 g) with the highest levels of γ-tocopherol (336.77 μg/100 g), while the *C. collinitus* revealed the lowest contents. The samples of *L. quietus* and *C. violaceus* showed the highest levels of α-tocopherol (47.98 μg/100 g) and β-tocopherol (26.44 μg/100 g), respectively.

The poisonous *A. muscaria* and *C. collinitus*, with unknown edibility, gave the highest levels of ascorbic acid (865.21 mg/100 g and 898.61 mg/100 g, respectively) without significant statistical differences ($p<0.05$).

In relation to sugar composition, determined by HPLC-RI (**Table 5**), the sample of *L. volemus* gave the highest total sugars content (28.22 g/100 g), with the highest levels of mannitol (27.29 g/100 g). The highest concentration of trehalose was found in *C. anomalus* sample (9.92 g/100 g). *A. caesarea*, *A. pantherina*, *C. anomalus*, *C. violaceus* and *S. luteus* gave trehalose as the main sugar, while mannitol predominated in *A. muscaria*, *C. fulmineus*, *C. collinitus*, *L. quietus*, *L. volemus*, *R. sardonia* and *T. ustale*. A third sugar, arabinose, was only detected in *C. fulmineus* (3.29 g/100 g).

The results for fatty acid composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) of the studied wild mycorrhizal mushrooms are given in **Table 6**. Twenty-seven fatty acids were detected by GC-FID. The major fatty acid found was oleic acid (C18:1n9) for *A. caesarea*, *A. muscaria*, *A. pantherina*, *C. fulmineus* and *R. sardonia* samples, and linoleic acid (C18:2n6) for *C. anomalus*, *C. collinitus*, *C. violaceus*, *L. quietus*, *L. volemus*, *S. luteus* and *T. ustale* contributing to the prevalence of PUFA in these species.

The studied samples also revealed palmitic acid (C16:0) as a major fatty acid, and in some cases stearic acid (C18:0), particularly in *L. quietus* sample. In fact, this sample gave the highest SFA contents (33.68%), but the lowest total fat content (1.22 g/100 g). *R. sordida* gave the highest levels of MUFA (65.12%) with the highest levels of oleic acid; *C. violaceus* showed the highest levels of PUFA (66.58) with the highest levels of linoleic acid. *A. muscaria* revealed the highest total fat content (4.07 g/100 g).

The UFA oleic and linoleic acids have also been reported as main fatty acids in a sample of *A. caesarea* collected in 2006 and studied by Ribeiro and others (2009). Otherwise, the studied sample of *S. luteus* revealed C18:2>C18:1 with prevalence of PUFA, while a sample collected in 2005 gave C18:1>C18:2 with prevalence of MUFA (Ribeiro and others 2009).

Some inedible and poisonous mushrooms were included in the present study in order to clarify if they present a profile in vitamins, sugars, fatty acids and phenolic compounds similar to the edible species. Despite numerous studies of the substances present in the *A. muscaria* and *A. pantherina*, the correlation between the physicochemical properties of the active components and the mode of consumption is still unclear. Ibotenic acid (pantherine, agarine) and muscimol, which are readily soluble in cold water, are their main active substances. Another substance in *A. muscaria* is muscazone, which is a lactame isomer of muscimol. The substances have effects on the central nervous system (tiredness, confusion), but they act with a longer latency period. Other substances found include choline, acetylcholine, betaine, muscaridine; minor amounts of tropane alkaloids: atropine, hyoscyamine, scopolamine and bufotenine. The possible occurrence of amatoxins and phallotoxins – the typical toxins of *Amanita phalloides* – is taken into consideration (Michelot and Melendez-Howell 2003).

Conclusions

A similar profile of metabolites was observed in the studied mushroom species with the order sugars > fat > ascorbic acid > phenolic compounds > tocopherols. Nevertheless, the samples revealed different compositions: prevalence of sugars in *L. volemus*, fat and ascorbic acid in *A. muscaria*, phenolic compounds in *C. anomalus* and tocopherols and antioxidant activity in *S. luteus*. Furthermore, it was possible to conclude that other than the analysed antioxidant compounds are contributing to the antioxidant properties of the studied mushrooms, since the species with the greatest antioxidant activity (*S. Luteus*), did not revealed the highest content of bioactive compounds such as phenolic acids or sugars.

This systematic study aimed to contribute to the chemical characterization of wild mushrooms, particularly mycorrhizal species, from Northeast Portugal, one of the European regions with higher biodiversity. Mycorrhization is related to metabolites production, such as phenolic compounds, and the control of their synthesis and the role of these compounds on the symbiotic process are far from being completely understood.

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References

Aletor VA. 1995. Compositional studies on edible tropical species of mushrooms. Food Chem 54:265–268.

Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B, Weil JA. 2004. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chem 84:551-562.

AOAC. 1995. *Official methods of analysis* (16th Ed.). Arlington VA, USA: Association of Official Analytical Chemists.

Barros L, Dueñas M, Ferreira ICFR, Baptista P, Santos-Buelga C. 2009. Phenolic acids determination by HPLC-DAD-ESI/MS in sixteen different Portuguese wild mushrooms species. Food Chem Toxicol 47:1076–1079.

Barros L, Falcão S, Baptista P, Freire C, Vilas-Boas M, Ferreira ICFR. 2008a. Antioxidant activity of *Agaricus* sp. Mushrooms by chemical, biochemical and electrochemical assays. Food Chem 111:61-66.

Barros L, Ferreira M-J, Queirós B, Ferreira ICFR, Baptista P. 2007. Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. Food Chem 103:413-419.

Barros L, Venturini BA, Baptista P, Estevinho LM, Ferreira ICFR. (2008b). Chemical composition and biological properties of Portuguese wild mushrooms: A comprehensive study. J Agric Food Chem 56:3856–3862.

Berker KI, Güçlü K, Tor I, Apak R. 2007. Comparative evaluation of Fe(III) reducing power-based antioxidant capacity assays in the presence of phenanthroline, batho-phenanthroline, tripyridyltriazine (FRAP), and ferricyanide reagents. Talanta 72:1157-1165.

Courtecuisse R, Duhem B. 2005. *Guía de los hongos de la Península Ibérica*, Europa y Norte de África. Ediciones Omega, Barcelona.

Díez VA, Alvarez A. 2001. Compositional and nutritional studies on two wild edible mushrooms from northwest Spain. Food Chem 75:417–422.

Ferreira ICFR, Baptista L, Vilas-Boas M, Barros L. 2007. Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. Food Chem 100:1511-1516.

Ferreira ICFR, Barros L, Abreu RMV. 2009. Antioxidants in wild mushrooms. Cur Med Chem 16:1543-1560.

Ferreira ICFR, Vaz JA, Vasconcelos MH, Martins A. 2010. Compounds from wild mushrooms with antitumor potential. Anti-cancer Agents Med Chem 10:424-436.

Froufe HJC, Abreu RMV, Ferreira ICFR. 2009. A QCAR model for predicting antioxidant activity of wild mushrooms. SAR QSAR Environ Res 20:579-590.

Heleno SA, Barros L, Sousa MJ, Martins A, Ferreira ICFR. 2009. Study and characterization of selected nutrients in wild mushrooms from Portugal by gas chromatography and high performance liquid chromatography. Microchem J 93:195–199.

Heleno SA, Barros L, Sousa MJ, Martins A, Ferreira ICFR. 2010. Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity. Food Chem 119:1443-1450.

Iwashina T. The structure and distribution of the flavonoids in plants. 2000. J Plant Res 13:287-299.

Kalač P. 2009. Chemical composition and nutritional value of European species of wild growing mushrooms: A review. Food Chem 113:9–16.

Kavishree S, Hemavathy J, Lokesh BR, Shashirekha MN, Rajarathnam S. 2008. Fat and fatty acids of Indian edible mushrooms. Food Chem 106:597–602.

Kirk PM, Cannon PF, David JC, Stalpers JA. 2001. Dictionary of the fungi, 9th ed. CABI Bioscience, Wallinford, UK.

Léon-Guzmán MF, Silva I, López MG. 1997. Proximate chemical composition, free amino acid contents, and free fatty acids contents of some wild edible mushrooms from Querétaro, México. J Agric Food Chem 45:4329–4332.

Lindequist U, Niedermeyer THJ, Julich WD. 2005. The pharmacological potential of mushrooms. eCAM 2:285-299.

Manzi P, Marconi S, Aguzzi A, Pizzoferrato L. 2004. Commercial mushrooms: nutritional quality and effect of cooking. Food Chem 84:201-206.

Mattila P, Salo-Väänänen P, Könkö K, Aro H, Jalava T. 2002. Basic composition and amino acid contents of mushrooms cultivated in Finland. J Agric Food Chem 50:6419-6422.

Mdachi SJM, Nkunya MHH, Nyigo VA, Urasa IT. 2004. Amino acid composition of some Tanzanian wild mushrooms. Food Chem 86:179–182.

Michelot D, Melendez-Howell LM. 2003. Amanita muscaria: chemistry, biology, toxicology, and ethnomycology. Micol Res 107:131-146.

Ouzouni PK, Petridis D, Koller W-D, Riganakos KA. 2009. Nutritional value and metal content of wild edible mushrooms collected from West Macedonia and Epirus, Greece. Food Chem 115:1575–1580.

Prior RL, Wu XL, Schaich K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem 53:4290-4302.

Ribeiro B, Pinho PG, Andrade PB, Baptista P, Valentão P. 2009. Fatty acid composition of wild edible mushrooms species: A comparative study. Microchem J 93:29-35.

Ribeiro B, Valentão P, Baptista P, Seabra RM, Andrade PB. 2006. Contents of carboxilic acids and two phenolics and antioxidant activity of dried Portuguese wild edible mushrooms. J Agric Food Chem 54:8530-8537.

Sarikurkcu C, Tepe B, Semiz DK, Solak MH. 2010. Evaluation of metal concentration and antioxidant activity of three edible mushrooms from Mugla, Turkey. Food Chem Toxicol 48:1230-1233.

Shibamoto T, Bjeldanes LF. 2009. Introduction to food toxicology. Academic press.

Smith SE, Read DJ. 1997. Mycorrhizal Symbiosis. 2nd ed. Academic Press, London.

Tsai S-Y, Tsai H-L, Mau J-L. 2008. Non-volatile taste components of *Agaricus blazei*, *Agrocybe cylindracea* and *Boletus edulis*. Food Chem 107:977-983.

Yilmaz N, Solmaz M, Türkekul I, Elmastas M. 2006. Fatty acid composition in some wild edible mushrooms growing in the middle Black Sea region of Turkey. Food Chem 99:168-174.

Table 1. Information about the wild mycorrhizal species analysed.

Scientific name	English name	Edibility	Habitat	Date of collection	Water content (g/100 g fw)
Amanita caesarea (Scop.) Pers.	Caesar's Mushroom	Edible	*Castanea sativa*	2009-11-08	90.88 ± 1.21
Amanita muscaria (L.:Fr.) Lam.	Fly agaric/fly Amanita	Poisonous	*Castanea sativa*	2009-10-29	94.25 ± 1.44
Amanita pantherina (DC. ex Fr.) Krombh.	Panther/False Blusher	Poisonous	*Castanea sativa*	2009-10-29	90.40 ± 2.64
Chroogomphus fulmineus (R. Heim) Courtec.	Unknown	Inedible	*Castanea sativa*	2009-11-08	90.14 ± 0.88
Cortinarius anomalus (Fr.) Fr.	Unknown	Edible	Mixed stands	2009-11-16	90.58 ± 0.26
Cortinarius collinitus (Pers.) Fr.	Belted slimy cort/Smeared cort	Unknown	Mixed stands	2009-11-16	93.26 ± 3.42
Cortinarius violaceus (L.: Fr.) Gray	Unknown	Edible	*Quercus pyrenaica*	2009-11-16	88.38 ± 2.32
Lactarius quietus (Fr. ex Fr.) Fr.	Oak milkcap/Oakbug milkcap/Southern milkcap	Questionable	Mixed stands	2009-11-16	91.14 ± 3.06
Lactarius volemus (Fr.) Fr.	Weeping milk cap/Voluminous-latex milky	Edible	*Quercus pyrenaica*	2009-11-08	90.58 ± 0.42
Russula sardonia Fr.	Primrose brittlegill	Inedible	Mixed stands	2009-11-16	96.49 ± 1.47
Suillus luteus (L.: Fries) Gray	Slippery Jack/Sticky bun	Edible	Mixed stands	2009-11-16	90.79 ± 2.73
Tricholoma ustale (Fr.) P. Kumm	Burnt knight	Inedible	*Quercus pyrenaica*	2009-11-04	85.72 ± 0.35

Table 2. Antioxidant activity of wild mycorrhizal mushrooms. In each row different letters mean significant differences ($p<0.05$).

	Amanita caesarea	*Amanita muscaria*	*Amanita pantherina*	*Chroogomphus fulmineus*	*Cortinarius anomalus*	*Cortinarius collinitus*	*Cortinarius violaceus*	*Lactarius quietus*	*Lactarius volemus*	*Russula sardonia*	*Suillus luteus*	*Tricholoma ustale*	Trolox
DPPH scavenging activity EC_{50} (mg/ml)	7.41 ± 0.58 e	2.87 ± 0.08 ji	3.89 ± 0.03 hi	6.88 ± 0.95 fe	6.49 ± 0.65 fe	36.73 ± 3.90 a	15.70 ± 0.76 d	34.14 ± 0.08 b	21.68 ± 0.52 c	5.70 ± 0.38 fg	1.92 ± 0.08 j	4.89 ± 0.45 hg	0.04 ± 0.00
Reducing power $EC_{0.5}$ (mg/ml)	1.85 ± 0.17 c	0.77 ± 0.00 f	0.78 ± 0.02 f	1.04 ± 0.04 e	1.52 ± 0.02 d	3.12 ± 0.13 a	2.36 ± 0.09 b	3.01 ± 0.05 a	2.42 ± 0.20 b	1.31 ± 0.32 d	0.75 ± 0.03 f	0.99 ± 0.05 fe	0.03 ± 0.00
β-carotene bleaching inhibition EC_{50} (mg/ml)	7.47 ± 0.04 d	0.62 ± 0.02 j	0.65 ± 0.00 j	4.06 ± 0.28 g	7.96 ± 0.10 e	13.93 ± 0.9 b	5.73 ± 0.28 f	25.00 ± 0.52 a	9.08 ± 0.04 c	2.00 ± 0.18 i	0.61 ± 0.02 j	3.43 ± 0.03 h	0.003 ± 0.00

Table 3. Phenolic acids and related compound in wild mycorrhizal mushrooms. In each row different letters mean significant differences ($p<0.05$).

	Amanita caesarea	*Amanita muscaria*	*Amanita pantherina*	*Chroogomphus fulmineus*	*Cortinarius anomalus*	*Cortinarius collinitus*	*Cortinarius violaceus*	*Lactarius quietus*	*Lactarius volemus*	*Russula sardonia*	*Suillus luteus*	*Tricholoma ustale*
Protocatechuic acid	0.67 ± 0.07 b	nd	1.18 ± 0.12 a	0.36 ± 0.01 c	nd	nd	nd	nd	nd	nd	0.47 ± 0.01 c	nd
p-Hydroxybenzoic acid	0.72 ± 0.03 e	3.41 ± 0.11 b	0.67 ± 0.10 e	0.22 ± 0.00 g	8.68 ± 0.38 a	1.04 ± 0.00 d	1.30 ± 0.12 c	nd	0.46 ± 0.09 fe	nd	nd	nd
p-Coumaric acid	nd	0.07 ± 0.00 d	0.36 ± 0.03 a	0.12 ± 0.00 c	nd	0.25 ± 0.01 b	nd	nd	nd	nd	nd	nd
Total phenolic acids (mg/100 g dw)	1.39 ± 0.10 d	3.48 ± 0.11 b	2.21 ± 0.25 c	0.70 ± 0.01 e	8.68 ± 0.38 a	1.29 ± 0.01 d	1.30 ± 0.12 d	nd	0.46 ± 0.09 e	nd	0.47 ± 0.01 e	nd
Cinnamic acid (mg/100 g dw)	0.03 ± 0.00 i	1.41 ± 0.07 b	1.86 ± 0.11 a	1.19 ± 0.00 c	1.92 ± 0.11 a	0.99 ± 0.01 d	0.56 ± 0.02 f	0.70 ± 0.03 e	0.32 ± 0.00 hg	0.27 ± 0.03 h	0.41 ± 0.04 g	0.27 ± 0.04 h

nd- not detected.

Table 4. Vitamins in wild mycorrhizal mushrooms. In each row different letters mean significant differences ($p<0.05$).

	Amanita caesarea	*Amanita muscaria*	*Amanita pantherina*	*Chroogomphus fulmineus*	*Cortinarius anomalus*	*Cortinarius collinitus*	*Cortinarius violaceus*	*Lactarius quietus*	*Lactarius volemus*	*Russula sardonia*	*Suillus luteus*	*Tricholoma ustale*
α-tocopherol	7.01 ± 0.30 d	49.63 ± 1.58 a	7.61 ± 0.62 d	4.03 ± 0.92 d	20.63 ± 1.05 c	7.69 ± 0.08 d	8.28 ± 0.98 d	47.98 ± 4.96 a	7.46 ± 0.95 d	8.44 ± 0.23 d	19.14 ± 2.44 c	38.54 ± 3.25 b
β-tocopherol	nd	nd	nd	nd	nd	7.78 ± 0.68 c	26.44 ± 2.83 a	nd	nd	nd	15.34 ± 1.87 b	nd
γ-tocopherol	45.99 ± 1.61 ef	185.02 ± 13.37 c	44.04 ± 0.94 ef	92.47 ± 3.07 d	nd	nd	258.07 ± 21.45 b	42.07 ± 1.36 egf	31.46 ± 2.11 gf	52.85 ± 2.62 edf	336.77 ± 44.56 a	78.31 ± 5.02 ed
δ-tocopherol	74.27 ± 0.42 cb	82.29 ± 5.13 b	nd	nd	122.28 ± 1.67 a	13.02 ± 1.35 f	55.24 ± 3.57 d	26.32 ± 5.22 e	69.43 ± 3.52 c	23.61 ± 0.61 e	78.51 ± 4.09 b	46.97 ± 0.38 d
Total tocopherols (μg/100 g dw)	127.27 ± 1.44 dce	316.94 ± 20.08 b	51.65 ± 1.57 gf	95.50 ± 2.15 dfe	142.91 ± 2.72 dc	28.49 ± 1.94 g	348.03 ± 28.82 b	116.37 ± 1.61 dce	108.35 ± 4.67 de	84.90 ± 1.78 fe	449.76 ± 52.96 a	163.82 ± 8.66 c
Ascorbic acid (mg/100 g dw)	175.22 ± 11.98 d	865.21 ± 85.31 a	182.19 ± 4.10 d	462.17 ± 57.36 b	343.96 ± 30.98 c	898.61 ± 85.04 a	70.10 ± 8.46 f	48.97 ± 3.46 f	83.50 ± 3.45 f	505.21 ± 81.75 b	87.21 ± 10.34 ef	157.12 ± 22.12 ed

nd- not detected.

Table 5. Sugars in wild mycorrhizal mushrooms. In each row different letters mean significant differences ($p<0.05$).

	Amanita caesarea	*Amanita muscaria*	*Amanita pantherina*	*Chroogomphus fulmineus*	*Cortinarius anomalus*	*Cortinarius collinitus*	*Cortinarius violaceus*	*Lactarius quietus*	*Lactarius volemus*	*Russula sardonia*	*Suillus luteus*	*Tricholoma ustale*
Mannitol	2.10 ± 0.09 g	1.02 ± 0.02 g	4.41 ± 0.10 f	7.21 ± 1.18 e	1.85 ± 0.03 g	8.12 ± 0.12 ed	6.35 ± 0.04 e	9.84 ± 0.30 d	27.29 ± 1.81 a	20.14 ± 0.32 b	1.29 ± 0.03 g	12.53 ± 0.51 c
Trehalose	3.15 ± 0.01 e	0.94 ± 0.03 f	5.31 ± 0.33 dc	5.61 ± 0.84 c	9.92 ± 0.11 a	2.82 ± 0.06 e	7.90 ± 0.22 b	4.73 ± 0.01 d	0.93 ± 0.00 f	nd	1.35 ± 0.04 f	1.67 ± 0.07 f
Arabinose	nd	nd	nd	3.29 ± 0.65	nd	nd	nd	nd	nd	nd	nd	nd
Total sugars (g/100 g dw)	5.24 ± 0.08 f	1.97 ± 0.04 g	9.74 ± 0.43 e	16.11 ± 2.67 c	11.78 ± 0.14 de	10.95 ± 0.06 e	14.26 ± 0.18 dc	14.57 ± 0.31 dc	28.22 ± 1.81 a	20.14 ± 0.32 b	2.64 ± 0.07 gf	14.20 ± 0.44 dc

nd- not detected.

Table 6. Fatty acids in wild mycorrhizal mushrooms. In each row different letters mean significant differences ($p<0.05$).

	Amanita Caesarea	*Amanita muscaria*	*Amanita pantherina*	*Chroogomphus fulmineus*	*Cortinarius anomalus*	*Cortinarius collinitus*	*Cortinarius violaceus*	*Lactarius quietus*	*Lactarius volemus*	*Russula sardonia*	*Suillus luteus*	*Tricholoma ustale*
C6:0	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.03 ± 0.00	0.25 ± 0.02	0.06 ± 0.00	0.77 ± 0.07	0.18 ± 0.03	0.07 ± 0.00	0.01 ± 0.00	0.03 ± 0.00
C8:0	0.14 ± 0.01	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.14 ± 0.03	0.03 ± 0.00	0.14 ± 0.02	0.06 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.06 ± 0.00
C10:0	0.06 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	2.38 ± 0.54	1.43 ± 0.07	0.02 ± 0.00	0.01 ± 0.00	0.14 ± 0.01
C12:0	0.03 ±0.00	0.03 ± 0.00	0.05 ± 0.00	0.11 ± 0.00	0.03 ± 0.00	0.18 ± 0.01	0.04 ± 0.00	0.29 ± 0.03	0.25 ± 0.04	0.05 ± 0.00	0.03 ± 0.00	0.15 ± 0.02
C14:0	0.12 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	0.12 ± 0.01	0.12 ± 0.00	0.30 ± 0.03	0.19 ± 0.02	0.25 ± 0.01	0.38 ± 0.02	0.15 ± 0.01	0.11 ± 0.00	0.28 ± 0.02
C15:0	0.23 ± 0.02	0.42 ± 0.01	0.30 ± 0.01	0.05 ± 0.00	0.13 ± 0.00	0.21 ± 0.01	0.19 ± 0.01	1.06 ± 0.22	0.93 ± 0.03	0.28 ± 0.02	0.40 ± 0.01	0.57 ± 0.02
C16:0	12.42 ± 0.37	7.88 ± 0.03	9.41 ± 0.83	12.59 ± 0.38	12.12 ± 0.06	19.03 ± 0.30	14.02 ± 0.04	13.71 ± 1.13	12.19 ± 0.44	7.84 ± 0.47	10.57 ± 0.05	9.72 ± 0.21
C16:1	0.64 ± 0.02	0.45 ± 0.01	0.29 ± 0.01	0.65 ± 0.00	0.41 ± 0.01	1.79 ± 0.06	0.61 ± 0.04	0.65 ± 0.07	0.65 ± 0.01	0.65 ± 0.02	0.53 ± 0.01	0.18 ± 0.01
C17:0	0.08 ± 0.00	0.12 ± 0.01	0.09 ± 0.01	0.18 ± 0.00	0.08 ± 0.00	0.15 ± 0.01	0.11 ± 0.00	0.19 ± 0.03	0.15 ± 0.01	0.08 ± 0.00	0.12 ± 0.00	0.38 ± 0.01
C17:1	0.01 ± 0.00	nd	nd	0.05 ± 0.00	0.01 ± 0.00	0.28 ± 0.02	0.02 ± 0.00	0.38 ± 0.08	0.03 ± 0.00	0.07 ± 0.00	0.02 ± 0.00	nd
C18:0	4.88 ± 0.24	6.13 ± 0.02	10.41 ± 0.12	2.47 ± 0.06	2.23 ± 0.02	2.94 ± 0.10	1.67 ± 0.01	12.76 ± 0.49	6.44 ± 0.59	6.56 ± 0.25	2.06 ± 0.07	3.46 ± 0.21
C18:1n9c	53.78 ± 1.22	60.08 ± 1.03	53.06 ± 0.36	47.09 ± 0.94	37.74 ± 0.38	35.21 ± 0.13	14.59 ± 1.26	24.55 ± 0.70	39.27 ± 0.15	63.19 ± 1.72	31.24 ± 0.26	23.46 ± 0.57
C18:2n6c	25.74 ± 1.66	21.87 ± 1.07	21.72 ± 0.46	31.54 ± 1.47	44.39 ± 0.39	35.89 ± 0.79	66.22 ± 1.12	35.15 ± 2.98	34.38 ± 0.13	16.93 ± 1.25	52.31 ± 0.68	59.29 ± 0.35
C18:3n6	0.16 ± 0.00	0.05 ± 0.00	0.03 ± 0.00	nd	0.04 ± 0.00	0.10 ± 0.00	0.02 ± 0.00	nd	0.57 ± 0.03	0.43 ± 0.03	0.02 ± 0.00	nd
C18:3n3	0.02 ± 0.00	0.06 ± 0.00	0.43 ± 0.01	1.47 ± 0.03	0.05 ± 0.00	0.16 ± 0.07	0.15 ± 0.00	4.42 ± 0.65	0.01 ± 0.00	1.71 ± 0.05	0.29 ± 0.03	0.07 ± 0.00
C20:0	0.27 ± 0.03	0.69 ± 0.01	0.87 ± 0.01	0.22 ± 0.01	0.26 ± 0.02	0.43 ± 0.04	0.23 ± 0.02	0.80 ± 0.09	0.38 ± 0.02	0.25 ± 0.02	0.22 ± 0.03	0.23 ± 0.02
C20:1	0.06 ± 0.00	0.12 ± 0.00	0.19 ± 0.00	0.39 ± 0.03	0.20 ± 0.02	0.19 ± 0.03	0.02 ± 0.00	0.06 ± 0.00	0.15 ± 0.01	0.37 ± 0.02	0.14 ± 0.01	0.14 ± 0.01
C20:2	0.02 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.27 ± 0.00	0.08 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.05 ± 0.00	0.10 ± 0.00	0.04 ± 0.00
C20:3n6	nd	0.02 ± 0.00	0.01 ± 0.00	nd	0.02 ± 0.00	0.08 ± 0.00	0.02 ± 0.00	nd	0.03 ± 0.00	nd	nd	nd
C20:4n6	nd	0.07 ± 0.01	0.04 ± 0.00	nd	0.06 ± 0.00	0.29 ± 0.01	0.09 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.01 ± 0.00	nd	nd
C20:3n3+C21:0	0.09 ± 0.01	0.03 ± 0.00	0.02 ± 0.00	0.05 ± 0.00	0.02 ± 0.00	0.18 ± 0.00	0.02 ± 0.00	0.18 ± 0.01	0.17 ± 0.03	0.05 ± 0.01	0.04 ± 0.00	0.02 ± 0.00
C20:5n3	0.01 ± 0.00	0.10 ± 0.00	0.09 ± 0.00	0.04 ± 0.00	0.08 ± 0.00	0.31 ± 0.03	0.08 ± 0.00	0.16 ± 0.03	0.18 ± 0.01	0.05 ± 0.00	nd	0.13 ± 0.01
C22:0	0.31 ± 0.00	0.66 ± 0.01	0.56 ± 0.00	0.34 ± 0.02	0.71 ± 0.03	0.54 ± 0.02	0.59 ± 0.03	0.68 ± 0.06	0.66 ± 0.05	0.80 ± 0.06	0.33 ± 0.00	0.51 ± 0.07
C22:1n9	0.05 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.64 ± 0.00	0.06 ± 0.00	0.04 ± 0.00	0.12 ± 0.01	0.02 ± 0.00	0.08 ± 0.00	0.02 ± 0.00	0.45 ± 0.04	nd
C23:0	0.09 ± 0.00	0.15 ± 0.00	0.16 ± 0.00	0.06 ± 0.00	0.14 ± 0.05	0.07 ± 0.00	0.15 ± 0.02	0.15 ± 0.01	0.05 ± 0.00	0.03 ± 0.00	0.08 ± 0.01	0.12 ± 0.01
C24:0	0.75 ± 0.06	0.71 ± 0.02	1.91 ± 0.10	0.36 ± 0.00	0.58 ± 0.02	0.63 ± 0.04	0.53 ± 0.07	1.02 ± 0.10	1.23 ± 0.01	0.29 ± 0.01	0.23 ± 0.07	0.76 ± 0.07
C24:1	0.01 ± 0.00	0.07 ± 0.00	0.08 ± 0.00	1.26 ± 0.05	0.35 ± 0.02	0.54 ± 0.03	0.13 ± 0.02	0.16 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.68 ± 0.13	0.12 ± 0.01
SFA (%)	19.16 ± 0.46d	16.46 ± 0.05 ef	23.51 ± 0.11 c	16.89 ± 0.52 ed	15.93 ± 0.04 ef	24.58 ± 0.58 b	17.46 ± 0.16 ed	33.68 ± 2.71 a	23.92 ± 0.18 cb	15.71 ± 0.29 ef	14.32 ± 0.19 f	16.22 ± 0.28 ef

MUFA (%)	54.81 ± 1.20 c	61.38 ± 1.02 ba	54.17 ± 0.37 b	49.77 ± 1.00d	39.40 ± 0.35 e	38.54 ± 0.16 e	15.96 ± 1.30 h	26.47 ± 0.93 g	40.80 ± 0.12 e	65.12 ± 1.62 a	32.93 ± 0.45 f	24.37 ± 0.64 g
PUFA (%)	26.03 ± 1.66 g	22.16 ± 1.07 h	22.31 ± 0.49 h	33.33 ± 1.52 f	44.68 ± 0.39 d	36.87 ± 0.74 fe	66.58 ± 1.13 a	39.86 ± 3.64 e	35.28 ± 0.06 f	19.17 ± 1.33 h	52.75 ± 0.64 c	59.41 ± 0.35 b
Total fat (g/100 g dw)	2.87 ± 0.31 bdc	4.07 ± 0.33 a	3.04 ± 0.17 bdc	3.51 ± 0.22 ba	2.60 ± 0.06 ed	2.71 ± 0.69 edc	1.27 ± 0.03 gf	1.22 ± 0.18 g	2.32 ± 0.16 ed	2.02 ± 0.21 ef	3.45 ± 0.65 bac	1.43 ± 0.52 gf

Caproic acid (C6:0); Caprylic acid (C8:0); Capric acid (C10:0); Lauric acid (C12:0); Myristic acid (C14:0); Pentadecanoic acid (C15:0); Palmitic acid (C16:0); Palmitoleic acid (C16:1); Heptadecanoic acid (C17:0); *cis*-10-Heptadecenoic acid (C17:1); Stearic acid (C18:0); Oleic acid (C18:1n9c); Linoleic acid (C18:2n6c); γ-Linolenic acid (C18:3n6); α-Linolenic acid (C18:3n3); Arachidic acid (C20:0); Eicosenoic acid (C20:1c); *cis*-11,14-Eicosadienoic acid (C20:2c); Arachidonic acid (C20:4n6); *cis*-11,14,17-Eicosatrienoic acid and Heneicosanoic acid (C20:3n3 + C21:0); *cis*-5,8,11,14,17-Eicosapentaenoic acid (C20:5n3); Behenic acid (C22:0); Behenic acid (C22:1n9); Tricosanoic acid (C23:0); Lignoceric acid (C24:0); Nervonic acid (C24:1). nd- not detected.