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# Analytical Methods

# Nutritional value and metal content of wild edible mushrooms collected from West Macedonia and Epirus, Greece

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## ABSTRACT

Ten wild edible mushroom species (Cantharellus cibarius, Rusula delica var chloroides, Ramaria largentii, Hygrophorus russula, Amanita caesaria, Fistulina hepatica, Boletus aureus, Armillaria tabesceus, A. mellea, Lepista nuda) from West Macedonia and Epirus, regions of Northern Greece, were analysed for their basic composition (moisture, crude protein, crude fat, total carbohydrates and ash) and metal content profile (Mg, Cr, Mn, Fe, Co, Ni, Cu, Zn, Pb, Cd, Al, As and Sn). The moisture content of mushrooms varied from 8.66% (L. nuda) to 17.43% (C. cibarius). The dry matter of mushrooms contained 21.57% (C. cibarius) – 34.77% (A. caesaria) proteins, 2.10% (A. mellea) – 6.00% (H. russula) fat, 5.61% (Russula delica) – 9.44% (C. cibarius) ash and 53.33% (H. russula) – 66.87% (A. tabesceus) carbohydrates.

The metal content of mushroom samples ranged 688.7–1150.7 for Mg, 0.12–5.34 for Cr, 7.19–62.63 for Mn, 38.9–499.0 for Fe, 0.05–7.22 for Co, 0.76–9.93 for Ni, 7.38–75.06 for Cu, 34.43–98.99 for Zn, not detected–1.16 for Pb and 0.07–1.80  $\mu$ g/g for Cd. As, Sn and Al concentrations were under the detection limit of the method used. The detection limits of the method for As, Sn and Al are 0.02  $\mu$ g/g for each element.

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## 1. Introduction

Wild-growing mushrooms are a popular and favourite delicacy in many European countries, mainly in middle and south Europe. For instance, many people collect wild edible mushrooms in Greece substantially contributing to food intake. Therefore, it is important to determine the basic composition and the levels of essential and toxic elements in wild edible mushrooms.

However, data on the diversity of the mycoflora in Greece are very scarce and fragmentary, covering mainly fungi of phytophalogical importance and macrofungi. Existing figures produce a total of about 2500 recorded mushroom species [\(Zervakis. G.I., 2001\)](#page-5-0). Check list of Greek fungi reports 811 species, assigned in 214 genera and 58 families of Basidiomycotina and 77 species of macrofungi assigned in 17 families and 42 genera, belonging to Ascomycetes. If this amount is added to the number of Basidiomycetes, the total number of mushrooms species in Greece rises to over 900 ([Venturella & Zervakis, 2000\)](#page-5-0).

West Macedonia and Epirus regions located in Northwestern Greece have a mild and rainy climate, especially, in spring and autumn, providing nearly ideal conditions for fungal growth, with temperatures ranging between 8 and 25  $°C$ .

Fruiting bodies of mushrooms are appreciated, not only for texture and flavour but also for their chemical and nutritional characteristics [\(Manzi, Aguzzi, & Pizzoferrato, 2001\)](#page-5-0). Mushrooms are valuable healthy and nutritious foods, low in calories and high in vegetable proteins, vitamins, iron, zinc, selenium, sodium, chitin, fibres and minerals (Mendil, Uluözlü, Hasdemir, & Cağlar, 2004; [Ouzouni, 2004; Ouzouni & Riganakos, 2007; Ouzouni, Veltsistas,](#page-5-0) [Paleologos, & Riganakos, 2007; Racz, Papp, Prokai, & Kovacz, 1996\)](#page-5-0).

In general, the fruiting bodies of mushrooms, on dry weight basis, contain about 56.8% carbohydrate, 25.0% protein, 5.7% fats and 12.5% ash [\(Demirbas, 2002; Latiff, Daran, & Mohamed, 1996; Men](#page-5-0)[dil et al., 2004\)](#page-5-0). Compared to green plants, mushrooms can build up large concentrations of some heavy metals, such as lead, cadmium and mercury, and a great effort has been made to evaluate the possible danger to human health from the ingestion of mushrooms ([Gast, Jansen, Bierling, & Haanstra, 1988; Soylak, Saraço?lu,](#page-5-0) [Tüzen, & Mendil, 2005\)](#page-5-0). Lead, cadmium, iron, copper, manganese, zinc, cobalt, chromium, nickel, magnesium, aluminium, tin and arsenic were chosen as representative trace metals whose levels in the environment represent a reliable index of environmental pollution. Metals such as iron, copper, manganese, and zinc are essential metals since they play an important role in biological systems, whereas lead and cadmium are non-essential metals as they are toxic even in traces [\(Schroeder, 1973\)](#page-5-0). The essential metals can also produce toxic effects when the metal intake is excessively elevated.

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#### <span id="page-1-0"></span>Table 1

Certified Reference Materials (CRMs) values and determined values for the measured metals.



<sup>a</sup> Average of five digestions and duplicate measurements.

The aim of the present study was to determine the basic composition and the essential and toxic elements (Mg, Cr, Mn, Fe, Co, Ni, Cu, Zn, Pb, Cd, Al, As and Sn) using Atomic Absorption Spectroscopy (AAS) of ten wild edible mushrooms species from West Macedonia and Epirus, regions of Greece.

## 2. Materials and methods

## 2.1. Sample preparation

Fruiting bodies of ten (10) wild edible species (Cantharellus cibarius, Rusula delica var chloroides, Ramaria largentii, Hygrophorus russula, Amanita caesaria, Fistulina hepatica, Boletus aureus, Armillaria tabesceus, A. mellea, Lepista nuda), belonging to nine (9) different families, were collected from forests in West Macedonia and Epirus, during autumn 2006 and spring 2007. The areas of the study included pasturelands and forests (unpolluted areas) distant from potential pollution sources, and the species were selected in relation to edible quality, commercialisation and frequency in the areas of the study.

One hundred fifty (150) samples of the above ten wild edible species (15 subsamples from each species) were analysed for their basic composition and their metal content profile. The mushroom samples were cleaned from forest debris (without washing) with a plastic knife, transported to the laboratory within 4 h of collection and placed temporarily in glass vessels at  $-18$  °C. Samples of each species were assayed individually. They were then characterised on the basis of carpophores morphology and spore shape using a Zeiss Axiostar Plus (Germany) microscope [\(Breitenbach &](#page-5-0) [Kranzlin, 1986; Breitenbach & Kranzlin, 1991; Breitenbach &](#page-5-0) [Kranzlin, 1994](#page-5-0)).

## 2.2. Analytical methods

#### 2.2.1. Moisture

To obtain moisture contents, samples of the mushrooms were dried in an oven at 105 °C overnight for 17 h [\(Mattila, Vaananen,](#page-5-0) [Konko, Aro, & Jalava, 2002\)](#page-5-0).

## 2.2.2. Crude proteins

The crude protein content of the samples was estimated by the macroKjeldhal method [\(AOAC, 1995](#page-5-0)), in which the sample was digested with a known quantity of concentrated sulphuric acid in the Kjeltec digestion apparatus (1007 Digestion Unit, Tecator, Sweden). The digested material was distilled after the addition of alkali. The released ammonia was collected in 4% boric acid in the 1002 Kjeltec Automatic Distilling Unit (Tecator, Sweden). The resultant boric acid, now contained the ammonia released from the digested material, was then titrated against 0.1 N HCl, manually. The nitrogen content thus determined was multiplied by a factor of 6.25 to arrive at the amount of crude protein.

#### 2.2.3. Crude fat

The fat content of the samples was determined by Soxhlet, using petroleum ether as a solvent ([AOAC, 1995\)](#page-5-0).

## 2.2.4. Ash

The ash content was analysed by weighing the samples before and after burning at 500  $\degree$ C for 24 h.

#### 2.2.5. Total carbohydrates

The amount of total carbohydrates was calculated with the following formula: total carbohydrates (% fresh weight) = 100 – moisture (%) – protein content (% fresh weight) – crude fat (% fresh weight) - ash (% fresh weight) = total carbohydrates (g/ 100 g fresh weight).

# 2.2.6. Comparison of the experimental data to the certified reference material data

In order to validate the accuracy, reliability and sensitivity of the above analytical methods the certified reference material (CRM) BCR-381 (Rye Flour) was used. The CRM was supplied by the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium). The CRM was stored under specified controlled conditions to ensure its stability. Ten measurements on the CRM were performed and the results were compared with the certified values. The values for total N, fat and ash in the certified reference material BCR-381 were  $1.25 \pm 0.02$ ,  $1.1 \pm 0.1$  and  $0.86 \pm 0.03$ respectively. The analysis of the BCR-381 using the above experimental methods gave values of  $1.23 \pm 0.03$ ,  $1.2 \pm 0.2$  and

#### Table 2

Families, habitat and common name of mushroom species.



## <span id="page-2-0"></span>Table 3

Basic composition of wild edible species in a dry weight (d.w.) basis.



Data are expressed as mean ± SD.

 $n = 15$ .

Table 4 Levels of the trace metals ( $\mu$ g/g, dry weight basis) in the analysed mushroom samples.

Mushroom species	Mg	Cr	Mn	Fe	Co	Ni	Cu	Zn	Pb	Cd
Cantharellus cibarius	$866.3 \pm 11.90$	$.57 \pm 0.04$	$22.09 \pm 0.54$	$118.2 \pm 2.24$	$0.05 \pm 0.01$	$1.07 \pm 0.03$	$32.49 \pm 0.30$	$54.29 \pm 1.23$	n.d.	$0.38 \pm 0.10$
Rusula delica	$688.7 \pm 5.25$	$0.12 \pm 0.04$	$16.61 \pm 0.32$	$81.80 \pm 1.26$	$0.05 \pm 0.02$	$1.90 \pm 0.02$	$51.71 \pm 0.30$	$56.58 \pm 0.54$	n.d.	$0.22 \pm 0.03$
Ramaria largentii	$837.5 \pm 5.03$	$5.34 \pm 0.04$	$62.63 \pm 0.44$	$302.1 \pm 5.48$	$7.22 \pm 0.05$	$9.93 \pm 0.10$	$17.79 \pm 0.23$	$46.33 \pm 1.08$	$0.12 \pm 0.02$	$1.13 \pm 0.03$
Hygrophorus russula	$758.4 \pm 8.29$	$1.38 \pm 0.06$	$34.14 \pm 0.90$	$300.7 \pm 3.87$	$1.06 \pm 0.02$	$0.86 \pm 0.03$	$9.44 \pm 0.07$	$57.01 \pm 0.87$	$0.08 \pm 0.01$	$1.17 \pm 0.04$
Amanita caesaria	$833.1 \pm 4.94$	$1.23 \pm 0.03$	$47.99 \pm 0.78$	$356.9 \pm 4.64$	$0.75 \pm 0.02$	$0.76 \pm 0.02$	$19.32 \pm 0.21$	$65.65 \pm 0.48$	$0.09 \pm 0.02$	$1.30 \pm 0.06$
Fistulina hepatica	$898.3 \pm 9.38$	$4.79 \pm 0.02$	$7.19 \pm 0.04$	$38.90 \pm 1.97$	$0.18 \pm 0.02$	$1.74 \pm 0.05$	$7.38 \pm 0.05$	$34.43 \pm 0.47$	$0.14 \pm 0.03$	$0.07 \pm 0.01$
Boletus aureus	$755.1 \pm 7.33$	$0.86 \pm 0.02$	$18.31 \pm 0.46$	$112.8 \pm 5.80$	$0.18 \pm 0.02$	$1.61 \pm 0.04$	$41.47 \pm 1.67$	$89.45 \pm 2.21$	$0.09 \pm 0.02$	$0.23 \pm 0.02$
Armillaria tabesceus	$1150.7 \pm 41.45$	$4.37 \pm 0.06$	$11.18 \pm 0.27$	$60.40 \pm 3.45$	$0.14 \pm 0.03$	$4.94 \pm 0.11$	$17.47 \pm 0.43$	$64.45 \pm 0.68$	$0.79 \pm 0.03$	$1.80 \pm 0.14$
Armillaria mellea	$1063.1 \pm 7.71$	$4.20 \pm 0.05$	$55.59 \pm 1.59$	$499.0 \pm 7.82$	$0.61 \pm 0.03$	$2.58 \pm 0.05$	$17.38 \pm 0.34$	$54.12 \pm 0.92$	$0.49 \pm 0.03$	$1.67 \pm 0.03$
Lepista nuda	$949.8 \pm 13.38$	$0.59 \pm 0.02$	$33.65 \pm 0.83$	$74.6 \pm 0.63$	$0.39 \pm 0.03$	$1.39 \pm 0.03$	$75.06 \pm 1.55$	$98.99 \pm 1.10$	$1.16 \pm 0.04$	$0.25 \pm 0.04$

n.d. = Not detected.

Data are expressed as mean ± SD.

 $n = 15$ .



Fig. 1A. Mean values of nutrients versus mushroom species. Vertical bars represent the 95% confidence intervals of the means based on the pooled standard deviation of the analysis of variance.



Fig. 1B. Mean values of metals versus mushroom species. Vertical bars represent the 95% confidence intervals of the means based on the pooled standard deviation of the analysis of variance.

0.84 ± 0.04 respectively. Therefore our experimental results are in excellent agreement with the certified values.

#### 2.2.7. Mineral, trace elements and heavy metals

Mushroom samples were dried at 105  $\degree$ C for 24 h. After thorough dehydration, 2 g of each sample were digested with 8 ml of concentrated HNO<sub>3</sub> (65%, Riedel de Haën, Seelze, Germany), 2 ml  $H<sub>2</sub>SO<sub>4</sub>$  (95–97%, Fluka, Buchs, Switzerland) and 2 ml  $H<sub>2</sub>O<sub>2</sub>$  (30%, POCH, Gliwice, Poland) and heated at 70  $\degree$ C for 4 h. Upon cooling, 20 ml of double distilled water (DDW) was added and the mixture was digested again by heating with concentrated nitric acid and sulphuric acid. Subsequently concentrated nitric acid was added dropwise, until complete oxidation of the organic matter. This point was reached when no further darkening of the solution occurred on continuous heating and a clear yellow solution was finally obtained. At last, the mixture was cooled, quantitatively transferred to a volumetric flask to a final volume of 100 ml with DDW. The solutions were then transferred to suitable plastic containers, after they were filtered through Whatman No. 42 filter paper (Kent, U.K.), in order to remove any insoluble silicates and other solid materials. Resulting solutions (pH = 1.0) were used for direct spectrophotometric analysis.

The concentrations of iron, zinc and magnesium were determined in an air–acetylene flame by Atomic Absorption Spectrometry (AAS) (A Perkin–Elmer Analyst 700 model atomic absorption spectrometer, Waltham, MA, USA), using a deuterium background correction. Lead, cadmium, chromium, manganese, cobalt, nickel, copper, arsenic, tin and aluminium content in the mushroom samples were determined with HGA graphite furnace, using argon as inert gas. The wavelengths (nm) used for the determination of iron, zinc, magnesium, cadmium, lead, chromium, manganese, cobalt, nickel, copper, arsenic, tin and aluminium were: 248.30, 285.20, 213.90, 228.80, 283.30, 357.90, 279.50, 240.70, 232.00, 324.80, 193.70, 224.60 and 309.30, respectively. Standards used to construct appropriate calibration curves were purchased from Perkin–Elmer (Waltham, MA, USA).

The reliability of measurements towards several of the selected elements was assessed by analysing ERM-CE 278 certified reference material supplied by the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium). As shown in [Table 1](#page-1-0) the obtained results are in good agreement with the certified values. In order to further control the stability of measurements, the solution obtained after digestion of the CRMs was placed in the auto sampler loading list and was analysed every 10 samples. Each time its concentration deviated more than 10% from the certified value the calibration curve was reconstructed.

## 2.3. Statistical analysis

A one factor analysis of variance (ANOVA) was employed to detect potential effects of mushroom species on chemical composition ([Zar, 1984\)](#page-5-0). Statistically significant effects were further treated comparing pair-wise the species mean chemical values according to the 95% confidence intervals based on the ANOVA' s pooled standard deviation.

Particular effects between mushroom species and their chemical composition were examined using a principal component analysis ([Sharma, 1996\)](#page-5-0).

## 3. Results and discussion

The families of mushroom species used in this study, their habitat and their common names are given in [Table 2](#page-1-0).

<span id="page-4-0"></span>

Fig. 1C. Mean values of metals versus mushroom species. Vertical bars represent the 95% confidence intervals of the means based on the pooled standard deviation of the analysis of variance.

Basic chemical composition of mushrooms is depicted in [Table](#page-2-0) [3](#page-2-0), expressed in a dry weight (d.w.) basis as g/100 g. Additionally, all metal concentrations of mushroom samples were determined on a dry weight basis respectively and are given in [Table 4](#page-2-0) as  $\mu$ g/g. The conventionally adopted as toxic metals studied in these experiments are Cd, Pb, and As.

When the nutritional value of mushrooms is evaluated, perhaps the most important factor is their dry matter/moisture content, which directly affects the nutrient content of mushrooms [\(Mattila](#page-5-0) [& Vaananen et al., 2002\)](#page-5-0). As shown in [Table 3](#page-2-0) the moisture content



Fig. 2. Biplot based on principal component analysis of mushroom chemical composition and species arrangement.

of the ten mushroom species studied varied from 8.66 for L. nuda to 17.43% for C. cibarius.

Present dry matter values are in agreement with earlier published data. According to [Crisan and Sands \(1978\), Bano and Rajarathnam](#page-5-0) [\(1988\), Kurzman \(1997\)](#page-5-0) and [Manzi, Gampelli, Marconi, Vivanti,](#page-5-0) [and Pizzoferrato \(1999\)](#page-5-0) fresh mushrooms contained 5–15% dry matter. This variability is dependent on the mushroom species and other parameters such as environmental temperature, relative humidity during growth and relative amount of metabolic water that may be produced or utilised during storage [\(Crisan & Sands, 1978\)](#page-5-0).

Crude fat in mushrooms includes several classes of lipid compounds, free fatty acids, mono, di, and triglycerides, sterols, sterol esters and phospholipids [\(Crisan & Sands, 1978\)](#page-5-0). Various species are especially high in ergosterol, which is the precursor of vitamin D2 (ergocalciferol) [\(Mattila, Lampi, Ronkainen, Toivo, & Piironen, 2002\)](#page-5-0). Fat content expressed as g/100 g dry weight (d.w.), in the ten analysed mushrooms, varied from 2.10% (A. mellea) to 6.00% (H. russula).

Protein concentrations of the ten mushroom species were generally high and in the range between 21.57 (C. cibarius) and 34.77% (A. caesaria) on a dry matter basis (d.w.). Mushrooms proved to be good sources of protein compared with green vegetables.

Also, total carbohydrate concentration, calculated by difference varied from 55.33% (H. russula) to 66.87% (A. tabesceus) on a dry matter basis (d.w.).

Ash contents varied from 5.61% (R. delica) to 9.44% (C. cibarius) on a dry matter basis (d.w.). As compared with vegetables, mushrooms proved to be good sources of many mineral elements. The main constituents in the mushrooms ash are K and P [\(Mattila](#page-5-0) [et al., 2001\)](#page-5-0).

Metal concentrations in the studied ten wild-grown edible mushroom species [\(Table 4,](#page-2-0) [Figs. 1A, 1B, 1C](#page-2-0)) ranged 688.7 (R. delica)–1150.7 (A. tabesceus)  $\mu$ g/g (magnesium), 0.12 (R. delica)–5.34

<span id="page-5-0"></span> $(Ra. \text{ larger}$ ii)  $\mu$ g/g (chromium), 7.19 (F. hepatica)–62.63 (Ra. largentii)  $\mu$ g/g (manganese), 38.90 (F. hepatica)–499.0 (A. mellea)  $\mu$ g/g (iron), 0.05 (C. cibarius and R. delica)–7.22 (Ra. largentii)  $\mu$ g/g (cobalt), 0.76 (A. caesaria)–9.93 (Ra. largentii) µg/g (nickel), 7.38 (F. hepatica)–75.06 (L. nuda) µg/g (copper), 34.43 (F. hepatica)–98.99 (L. nuda) lg/g (zinc), not detected (C. cibarius, R. delica)–1.16 (L. nuda)  $\mu$ g/g (lead) and 0.07(F. hepatica)–1.80 (A. tabesceus)  $\mu$ g/g (cadmium). These results are in agreement with literature values (Sesli, Tüzen, & Soylak, 2008; Turkekul, Elmastas, & Tüzen, 2004; Tüzen, Sesli, & Soylak, 2007). As, Sn and Al concentrations were under the detection limit of the method used. The detection limits of the method for As, Sn and Al are  $0.02 \mu g/g$  for each element.

The mean metal concentrations across all the mushrooms studied were in the order: Mg > Fe > Zn > Mn > Cu > Ni > Cr > Co >  $Cd > Pb$ .

From the above results it can be seen that all collected samples from West Macedonia and Epirus, can be used as important nutrient sources due to their high protein, carbohydrate and mineral content, and their low content of toxic metals (Pb, Cd and As). Lead is especially toxic to the growing brain and can affect the behavioural development of youngsters, even at low concentrations (Demirbas, 2001). Cadmium is known as a principal toxic element, since it inhibits many life processes (Vetter, 1993).

Statistically significant correlation coefficients ( $r$  >  $\pm$ 0.514 at 0.05 probability level) were established between metal concentrations. Correlations exist between magnesium and chromium  $(r = 0.59)$ , magnesium and lead ( $r = 0.71$ ), magnesium and cadmium ( $r = 0.58$ ), chromium and nickel ( $r$  = 0.68), chromium and copper ( $r$  =  $-0.67$ ), chromium and zinc ( $r$  =  $-0.64$ ), manganese and iron ( $r$  = 0.85), manganese and cobalt ( $r = 0.67$ ), iron and cadmium ( $r = 0.62$ ), cobalt and nickel ( $r = 0.87$ ), copper and zinc ( $r = 0.77$ ), copper and cadmium ( $r$  =  $-0.55$ ), and zinc and lead ( $r$  = 0.53). Positive correlation denotes an increase or decrease in concentration between pairs of correlated variables. Negative correlation occurs when an increase in concentration of one component causes a decrease to the other.

To elucidate specific relationships between chemical composition and mushroom species a principal component analysis was used regarding only the effects of the first two principal axes. Chromium, proteins, dry matter, carbohydrates, zinc, ash and lastly copper, are the most important variables for the formation of axis 1, judging from the values of the correlation coefficients with that axis, which are greater than 0.60 (–0.82, 0.82, –0.80, –0.75, 0.71, –0.66 and 0.65, respectively). For the same reason, Mn, Co and Fe are the most important variables of axis 2 (0.87, 0.82 and 0.76, respectively). Both axes explain 58.1% of the total variation of the analysis.

[Fig. 2,](#page-4-0) gives a global view of the effect of all chemical variables based on the results of the principal component analysis. Variables with longer arrows are more important in producing effects while those with same direction show positive correlation. The intensity of this correlation increases as the angle between the variables diminishes. Species positioned close to an arrow of a variable show strong relationship. Thus, high chromium concentrations are indicative of Armilaria mellea presence, whereas Ra. largentii is the richest in Co composition. The species L. nuda, R. delica and B. aureus are important for their high Zn and Cu concentrations. These two metals correlate strongly negatively with chromium. The species Hyggrophorus russula is rich in fat. High protein values are found in A. caesaria and low ones together with high carbohydrates values are present in F. hepatica, C. cibarius and A. tabesceus.

## 4. Conclusions

The mushrooms studied were found to be a good source of proteins (mean value 27.52% d.w.), carbohydrates (mean value 61.45% d.w.) and trace functional minerals. They have a low fat content making them ideal components in several diets. Also, their low content of toxic metals (Pb, Cd and As) shows that the collection areas are not polluted. Therefore all these collected edible mushroom species can be used in well-balanced diets and also can be consumed unreservedly without any health risk.

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