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Fatty acids, phospholipids, health risk index and daily intake of metals in edible wild mushroom (*Tricholoma equestre*) from the Batak mountain, Bulgaria

L. Dospatliev[1*], M. Ivanova[2], P. Papazov[3]

[1]*Department of Pharmacology, Animal Physiology and Physiological Chemistry, Trakia University, 6000 Stara Zagora, Bulgaria*

[2]*Department of Informatics and Mathematics, Trakia University, 6000 Stara Zagora, Bulgaria*

[3]*Department of Organic Chemistry and Inorganic Chemistry, University of Food Technology, 4000 Plovdiv, Bulgaria*

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Samples were collected from the Batak Mountain, Bulgaria. The aim of this study was to perform analysis of the content of Pb, Cd, Ni, Cr, Mn, Co, Cu and Zn, fatty acid and phospholipids, in wild edible mushroom *Tricholoma equestre*. The average content of studied elements: Pb, Cd, Ni, Cr, Mn, Co, Cu and Zn in *Tricholoma equestre* samples was: 1.16 mg kg^{-1}, 1.09 mg kg^{-1}, 0.74 mg kg^{-1}, 0.08 mg kg^{-1}, 0.85 mg kg^{-1}, 0.15 mg kg^{-1}, 1.16 mg kg^{-1} and 8.63 mg kg^{-1}. The content of Saturated fatty acids (SFA) consisted of 50.5 %. Unsaturated fatty acids (UFA) in the oil from mushroom (49.5 %) and the content of monounsaturated fatty acids (MUFA) consisted of 43.9 %. On the other hand, the amount of monounsaturated fatty acids (PUFA) was lower (5.6 %).

In the phospholipid fraction from mushrooms, there predominated phosphatidylcholine (PC) (37.90 %) as a major component, followed by diphosphatidylglycerol DPG (16.50 %). The quantities of monophosphatidylglycerol (MPG) and lysophosphatidylcholine (LPC) in the phospholipid fraction were from 1.50 % to 3.20%.

Keywords*: Health risk index, Fatty acid, Phospholipids, Mushroom (Tricholoma equestre), Bulgaria*

INTRODUCTION

Mushrooms have been viewed as gourmet food over the globe since vestige for their unique taste and inconspicuous flavor. As of late, it has been found that many mushroom species are miniature pharmaceutical factories producing thousands of novel constituents with exceptionally helpful biologic properties. They have a long history of utilization in Oriental prescription, however their incredible impacts in advancement of good health and imperativeness are being upheld by contemporary reviews. Recently, mushrooms have developed as great wellspring of nutraceuticals, anti-oxidants, anticancer, prebiotic, immune modulating, anti-inflammatory, cardiovascular, anti-microbial and anti-diabetic [1-9].

The known essential micronutrient minerals are iron, zinc, selenium, manganese, cobalt and copper. These microminerals play an important role in the catalytic processes within the enzyme system that includes a wide range of enzyme activities associated with metabolic, endocrine and immune system [10-19].

The aims of this study were to determine Pb, Cd, Ni, Cr, Mn, Co, Cu and Zn, phospholipids and fatty acid in edible wild mushroom (*Tricholoma equestre*) growing in the Batak Mountain, Bulgaria and thus to assess the health risk index arisen from the long-term consumption of them.

EXPERIMENTAL

Analytical procedure

Quantitative determination of the concentration of the studied trace elements (Pb, Cd, Ni, Cr, Mn, Co, Cu and Zn) was carried out in the mineralized samples by Perkin Elmer AAnalyst 800 atomic absorption spectrometer with deuterium background corrector.

Mushroom Samples

Mushroom samples were collected in 2014 and 2018 from the Batak Mountain by the authors themselves.

The Batak Mountain is located in western Rhodopes. Its western border is defined by the Chepinska river, the southern border – by Dospatska river and Dospat dam, the eastern border – by Vacha river and the northern border – by the Thracian Plane (GPS41°46'02.6"N 24°08'48.4"E). The regions is industry-free and is characterised with forests, land and low buildings.

Reagents and Moisture content

Reagents are qualified "AR" (pa Merck & Fluka). The fresh weight of each mushroom sample was taken using chemical balance. These samples were then oven dried separately at 105 ˚C for 24 h. The

* To whom all correspondence should be sent.
E-mail: lkd@abv.bg

loss in weight obtained after drying was regarded as the moisture content.

Digestion procedures

Multiwave 3000 closed vessel microwave system (maximum power was 1400 W, and the maximum pressure in Teflon vessels - 40 bar) was used in this study mushroom samples (0.25 g) were digested with 6 ml of HNO_3 (65 %) and 1 mL of H_2O_2 (30 %) in microwave digestion system for 23 min and diluted to 25 ml with deionized water. A blank digest was carried out in the same way. All sample solutions were clear. Digestion conditions for the microwave system are given in Table 1.

Table 1. Microwave acid digestion programme

Step	Ramp time, (min)	Hold time, (min)	Cooling period, (min)	Pressure (MPa)	Temperature (°C)
1	10	10	5	0.758	110
2	10	10	5	1.023	150
3	20	10	5	0.758	190

Accuracy and precision

In order to validate the method for accuracy and precision the certified reference materials (CRM) - Virginia Tobacco Leaves (CTA-VTL-2) was analysed for the corresponding elements. The results are shown in Table 3. For evaluation of the correctness of the results, three generally accepted criteria are used as follows:

$$D = X - X_{CRM}, \quad (1)$$

where X is the measured value and X_{CRM} is the certified value. When D is within the borders of $\pm 2\sigma$, where σ is the standard deviation from the certified value, the result is considered to be good; when it is $-3\sigma \leq D \leq 3\sigma$ - satisfactory, and beyond these limits the result is unsatisfactory.

$$D\% = D / X_{CRM} \cdot 100 \text{ - percentage difference.} \quad (2)$$

When the values of D % are in the limits $\pm 200\sigma / X_{CRM}$, the result is considered to be good; when the value is in the limits $\pm 200\sigma / X_{CRM}$ and $\pm 300\sigma / X_{CRM}$ - satisfactory; and when it is out of the limits $\pm 300\sigma / X_{CRM}$, the result is unsatisfactory.

$$Z = X - X_{CRM} / \sigma. \quad (3)$$

When $Z \leq 2$, the result is considered to be good; when $2 \leq Z \leq 3$ - satisfactory; when $Z > 3$ - unsatisfactory.

For evaluation of the accuracy of the digestion and measuring procedures, we have used R criterion showing the extent of extraction of the element in percent from the certified value. When the measured value X is within the borders of $X_{CRM} \pm U_{CRM}$, where U_{CRM} is the indefiniteness of the certified value, we accept an extent of extraction to be 100 %. In all the remaining cases, the extent of extraction is equal to $X / X_{CRM} \cdot 100$. As can be seen from the tables, the results obtained for all certified materials yield a recovery of 100 % for both elements.

Daily intake of metals

The daily intake of metals (DIM) was determined by the following equation

$$DIM = \frac{C_{metal} \times C_{factor} \times D_{food\ intake}}{BW},$$

where C_{metal}, C_{factor}, $D_{food\ intake}$ and BW represent the heavy metal concentrations in the mushroom given for dry weight (mg kg^{-1}), conversion factor, daily intake of mushrooms and average body weight (kg), respectively [20, 21].

Health risk index

The health risk index (HRI) for the locals through the consumption of contaminated mushrooms was assessed based on the food chain and the reference oral dose (R_fD_0) for each metal (Table 2) [22].

The health risk index (HRI) for the local population through the consumption of mushrooms was assessed using the followimg formula [21-23]

$$HRI = \frac{DIM}{R_f D_0}.$$

HRI of < 1 means the exposed population is assumed to be safe [22-24].

The total HRI (THRI) formula prescribed in Saha et al. [25] is a summation of the individual HRI value, as shown below

$$THRI = HRI_{Pb} + HRI_{Cd} + HRI_{Ni} + HRI_{Cr} + HRI_{Mn} + HRI_{Co} + HRI_{Cu} + HRI_{Zn}.$$

Table 2. R_fD_0 value, mg kg^{-1} bw day

Elements	Pb	Cd	Ni	Cr	Mn	Co	Cu	Zn
R_fD_0	0.004	0.001	0.02	0.003	0.14	0.043	0.04	0.30

Fatty acids

The fatty acid composition was determined by gas chromatography (GC) after transmethylation of the sample with 2 % H_2SO_4 in CH_3OH at 50 °C [26]. GC was performed on a HP 5890 series II gas chromatograph equipped with a 75 m × 0.18 mm × 25 μm capillary column Supelco and a flame ionization detector. The column temperature was programmed from 140 °C (5 min), at 4 °C min^{-1} to 240 °C (3 min); injector and detector temperatures were kept at 250 °C. Hydrogen was the carrier gas at a flow rate 0.8 mL min^{-1}. Identification of fatty acids was performed by comparison of retention times with those of a standard mixture of fatty acids subjected to GC under identical experimental conditions [27].

Phospholipids

Air-dried mushrooms (10 g) were subjected to Folch [28] extraction. Polar lipids were isolated from the total lipids by column chromatography according to Christie [29]. The phospholipid classes were isolated by a variety of two-dimensional thin-layer chromatography (TLC). In the first direction the plate was developed with chloroform methanol:ammonia, 65:25:5 (by volume) and in the second – with chloroform:acetone:methanol:acetic acid:water, 50:20:10:10:5 (by volume). The identification was performed by comparing the respective R_f values with those of authentic commercial standards subjected to Silica gel TLC under identical experimental conditions. The quantification was carried out spectrophotometrically at 700 nm after scrapping the respective phospholipid spot and mineralization of the substance with a mixture of perchloric acid and sulphuric acid, 1:1 (by volume) [30].

Statistical

Statistical Package for Social Science (SPSS) program for Windows was used for statistical data processing.

RESULTS AND DISCUSSION

In order to validate the method for accuracy and precision the certified reference material (CRM) - Virginia Tobacco Leaves (CTA-VTL-2) was analysed for the corresponding elements. The results are shown in Table 3.

In this study, Pb, Cd, Ni, Cr, Mn, Co, Cu and Zn concentrations in dry matter basis of wild edible mushrooms were analyzed (Table 4).

The daily intake of heavy metals was estimated according to the average mushroom consumption. The estimated DIM through the food chain is given in Table 5, for both adults and children.

Table 3. Effectiveness of microwave mineralization in the determination of Fe, Ni, Cr, Cu, Co, Zn, Mn, Pb and Cd in Virginia Tobacco-CTA-VTA-2 certified reference material (mg/kg dry matter) (n = 15)

Elements	Certified value	Observed value Microwave digestion	Recovery (%)
Pb	22.1 ± 1.2	23.0 ± 0.8	104
Cd	1.52 ± 0.17	1.50 ± 0.05	98.7
Ni	1.98 ± 0.21	1.92 ± 0.17	98.1
Cr	1.87 ± 0.16	1.83 ± 0.14	93.6
Mn	79.7 ± 2.6	77.5 ± 2.1	97.2
Co	0.429 ± 1.4	0.420 ± 0.02	98.0
Cu	18.2 ± 0.8	18.1 ± 0.7	99.4
Zn	43.3 ± 2.1	44.1 ± 1.6	101.8

Table 4. Concentration of trace elements in mushroom samples (*Tricholoma equestre*) collected from Batak Mountain, Bulgaria (mg kg^{-1} dry matter) (n = 15)

Elements	Pb	Cd	Ni	Cr	Mn	Co	Cu	Zn
Mean	1.16	1.09	0.74	0.08	0.85	0.15	1.16	8.63
SD	0.14	0.20	0.12	0.01	0.13	0.05	0.14	0.19

Table 5. DIM for individual heavy metals caused by the consumption of mushrooms *Tricholoma equestre* grown in Batak Mountain, Bulgaria.

Average body weight (kg)	12	23	43	61	70
Age-groups	1 - 3	3 - 10	10 - 14	14 - 18	adult
DIM_{Pb}	0.000618	0.000322	0.000172	0.000122	0.000213
DIM_{Cd}	0.000580	0.000303	0.000162	0.000114	0.000200
DIM_{Ni}	0.000394	0.000206	0.000110	0.000078	0.000136
DIM_{Cr}	0.000043	0.000022	0.000012	0.000008	0.000015
DIM_{Mn}	0.000453	0.000236	0.000126	0.000089	0.000156
DIM_{Co}	0.000080	0.000042	0.000022	0.000016	0.000028
DIM_{Cu}	0.000618	0.000322	0.000172	0.000122	0.000213
DIM_{Zn}	0.004595	0.002398	0.001282	0.000904	0.001587

Table 6. Health risk index values for mushrooms *Tricholoma equestre* grown in the Batak Mountain

Average body weight (kg)	12	23	43	61	70
Age-groups	1 - 3	3 - 10	10 - 14	14 - 18	adult
HRI_{Pb}	0.154425	0.080570	0.043095	0.030379	0.053319
HRI_{Cd}	0.580425	0.302830	0.161979	0.114182	0.200404
HRI_{Ni}	0.019703	0.010280	0.005498	0.003876	0.006803
HRI_{Cr}	0.014200	0.007409	0.003963	0.002793	0.004903
HRI_{Mn}	0.003233	0.001687	0.000902	0.000636	0.001116
HRI_{Co}	0.001858	0.000969	0.000518	0.000365	0.000641
HRI_{Cu}	0.015443	0.008057	0.004310	0.003038	0.005332
HRI_{Zn}	0.015318	0.007992	0.004275	0.003013	0.005289
THRI	0.804604	0.419793	0.224541	0.158283	0.277807

Health risk index

In order to assess the contribution of some heavy metals to the HRI of mushroom consumption, we calculated the hazard quotients. We evaluated the health risk of mushroom consumption concerning different age-groups relying on the HRI (Table 6).

According to the above results, all calculated HRI values of heavy metals were within the safe limits for children and adults (HRI < 1). Furthermore, the THRI values, which varied from 0.158283 to 0.804604 for children and 0.277807 for adult, were also in the safe limit (THRI < 1). Therefore, we can conclude that people might have no potential significant health risk through only consuming mushrooms from the studied area.

The values HRI_{Cd} (72.14 %) and HRI_{Pb} (19.19 %) have the highest percentage content from THRI, followed by HRI_{Ni} (2.45 %), HRI_{Cu} (1.92 %), HRI_{Zn} (1.90 %), HRI_{Cr} (1.76 %), HRI_{Mn} (0.40 %) and HRI_{Co} (0.23 %).

The element Cd (72.14 %) have the highest percentage content from THRI for the mushroom type Tricholoma equestre, the concentration of which is 1.09 mg kg^{-1} dry weight at 90 % moisture. This concentration in relation to Directive No 1881 [31] is below the permissible weight of 0.2 mg kg^{-1} wet weight (from 10 kg of wet mushrooms 1 kg of dried mushrooms is obtained).

Fatty acid composition

The content of Saturated fatty acids (SFA) consisted of 50.5 %. Unsaturated fatty acids (UFA) in the oil from mushroom (49.5 %) and the content of monounsaturated fatty acids (MUFA) consisted of 43.9 %. On the other hand, the amount of monounsaturated fatty acids (PUFA) was lower (5.6 %).

The data of fatty acid composition are shown in Fig. 1. In general, the major fatty acid found in the studied species was oleic acid (42 %), followed by palmitic acid (39.8 %), stearic acid (7.9 %) and linoleic acid (3.4 %). Besides the four main fatty

acids already described, eleven more were identified and quantified. The results obtained in this study are consistent with the previously reported results in the literature [11,32].

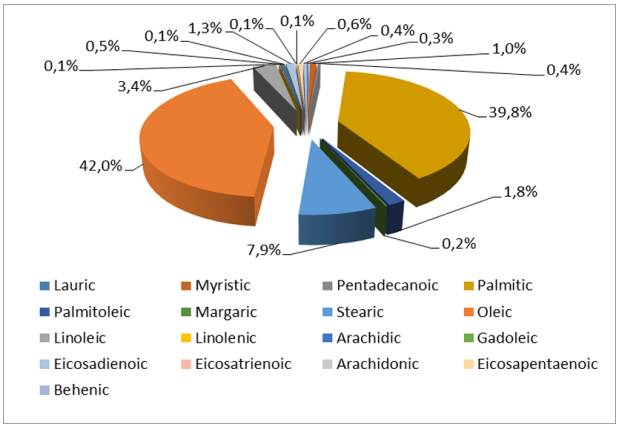

Fig. 1. The data of fatty acid composition

Phospholipid composition

The composition of the phospholipid fraction of the mushrooms oils is presented in Fig. 2. In the phospholipid fraction of the mushrooms oils from different varieties, there were identified all major classes of phospholipids. On the grounds of the obtained data, it can be seen that in the phospholipid fraction from mushrooms, there predominated phosphatidylcholine (37.90 %) as a major component, followed by diphosphatidylglycerol (16.50 %). The quantities of monophosphatidyl-glycerol and lysophosphatidylcholine in the phospholipid fraction were from 1.50 % to 3.20%.

The results obtained in this study are consistent with the previously reported results in the literature [31,34].

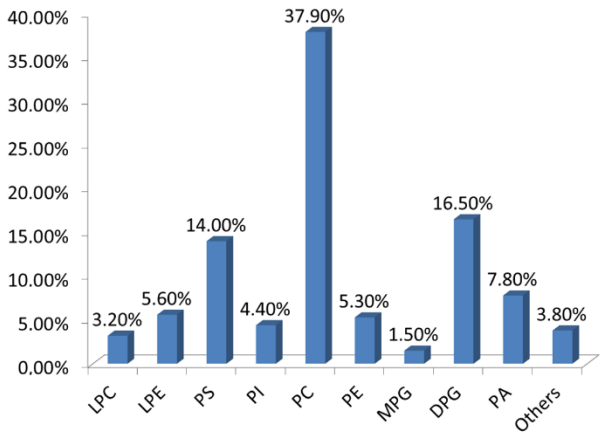

Fig. 2. Individual composition of phospholipid fraction of mushrooms
Legend: LPC – Lysophosphatidylcholine; LPE – Lysophosphatidylethanolamine; PS – Phosphatidylserine; PI – Phosphatidylinositol; PC – Phosphatidylcholine; PE – Phosphatidylethanolamine; MPG – Monophosphatidylglycerol; DPG – Diphosphatidylglycerol; PA – Phosphatidic acids; Others

CONCLUSIONS

Mushrooms consumption data collected are used for several purposes: to monitor nutrient and food intakes in the population as well as to carry out mushrooms-based risk–benefit assessments and policy making within the European Union. It is therefore important that the data collected meet the requirements set out both on a national level as well as by the European Food Safety Agency [35] and are as accurate as possible.

REFERENCES

1. G. Kanagasabapathy, S. N. A. Malek, U. R. Kuppusamy, S. Vikineswary, *J. Agric. Food Chem.*, **59**, 2618 (2011).
2. M. A. Ebrahimzadeh, Y. Safdari, M. Khalili, *Int. J. Med. Mushrooms*, **17**, 557 (2015).
3. E. Jabłornska-Ryrs, A. Sławirnska, D. Szwajgier, *Food Sci. Biotechnol.*, **25**, 439 (2016).
4. M. Kosanić, B. Ranković, A. Rančić, T. Stanojković, *JFDA*, **24**, 477 (2016).
5. L. Figueiredo, W. C. B. Régis, *Nutrire*, **42**, 1 (2017).
6. M. Gąsecka, P. Rzymski, M. Mleczek, M. Siwulski, S. Budzyńska, Z. Magdziak, P. Niedzielski, K. Sobieralski, *J. Environ. Sci. Heal B*, **52**, 171 (2017).
7. S. Kathiravan, S. Krishnakumari, *Int. J. Recent Sci. Res.*, **8**, 21362 (2017).
8. S. P. Wasser, *Int. J. Med. Mushrooms*, **19**, 279 (2017).
9. L. Dospatliev, M. Ivanova, *Cr. Acad. Bulg. Sci.*, **70**, 795 (2017).
10. I. Ivanov, N. Petkova, I. Tumbarski, I. Dincheva, I. Badjakov, P. Denev, A. Pavlov, *Zeitschrift für Naturforschung C*, 73(**1-2**), 41 (2018).
11. C. L. Keen, J. Y. Uriu-Adams, J. L. Ensuma, M. E. Gershwin, in: Handbook of nutrition and immunity, M. E. Gershin, P. Nestel, C. L Keen (eds), Humana Press: Totowa, NJ, 2004, p. 117.
12. M. Kuka, I. Cakste, R. Galoburda, in: Food for Consumer Well-Being (Proc. 9th Baltic Conference on Food Science and Technology, Foodbalt, Jelgava, Latvia, 2014) E. Straumite (ed.), Jelgava, Latvia, 2014, p. 248.
13. J. Falandysz, M. Drewnowska, *J. Environ. Sci. Heal B*, **50**, 374 (2015).
14. M. Khalili, M. A. Ebrahimzadeh, M. Kosaryan, A. Abbasi, M. Azadbakht, *RSC Advances*, **5**, 4804 (2015).
15. S. Sumaira, M. Ghulam, M. Hira, M. W. Connie, J. Yasir, S. Muhammad, *JFNR*, **4**, 703 (2016).
16. L. Dospatliev, M. Ivanova, *Bulg. Chem. Commun.*, **49**, 5 (2017).
17. X. Wang, H. Liu, J. Zhang, T. Li, Y. Wang, *J. Environ. Sci. Heal B*, **52**, 178 (2017).
18. A. M. Massadeh, A. T. Al-Massaedh, *Environ. Sci. Pollut. Res.*, **25**, 1914 (2018).

19. I. Ivanov, I. Dincheva, I. Badjakov, N. Petkova, P. Denev, A. Pavlov, *International Food Research Journal*, 25(**1**), 1 (2018).
20. Q. M. Ru, Q. Feng, J. Z. He, *Food Chem. Toxicol.*, **53**, 256 (2013).
21. J. Guo, T. Yue, X. Li, Y. Yuan, *Environ. Sci. Pollut. Res. Int.*, **23**, 14560 (2016).
22. USEPA, Risk Based Concentration Table. United States Environmental Protection Agency, Philadelphia, Washington, 2016.
23. M. Harmanescu, L. M. Alda, D. M. Bordean, I. Gogoasa, I. Gergen, *Chem. Cent. J.*, **5**, 64 (2011).
24. Q. Liang, Z. J. Xue, F. Wang, Z. M. Sun, Z. X. Yang, S. Q. Liu, *Environ. Monit. Assess.*, **187**, 754 (2015).
25. N. Saha, M. Zaman, *Environ. Monit. Assess.*, **18595**, 3867 (2013).
26. ISO 12966-2. Animal and vegetable fat and oils. Gas chromatography of fatty acid methyl esters – Part 2: Preparation of methyl esters of fatty acids, 2017.
27. ISO 12966-1. Animal and vegetable fats and oils. Gas chromatography of fatty acid methyl esters – Part 1: Guidelines on modern gas chromatography of fatty acid methyl esters, 2014.
28. J. Folch, M. Lees, G. Sloane-Stanley, *J. Biol. Chem.*, **226**, 497 (1957).
29. W. Christie, Lipid Analysis, The Oily Press: Bridgwater, U.K., 2003.
30. ISO 10540-1. Animal and vegetable fats and oils. Determination of phosphorus content. Part 1, 2014.
31. Commision, EC. Commission Regulation (EC) No 1881/2006 of 19 December 2006 Setting Maximum Levels for Certain Contaminants in Foodstuff, 2006.
32. L. Barros, T. Cruz, P. Baptista, L. M. Estevinho, I. C. F. R. Ferreira, *Food Chem. Toxicol.*, **46**, 2742 (2008).
33. D. D. De Silva, S. Rapior, E. Sudarman, M. Stadler, J. Xu, S.A. Alias, K. D. Hyde, *Fungal Divers.*, **62**, 1 (2013).
34. M. Kolundzić, T. Stanojković, J. Radović, A. Tačić, M. Dodevska, M. Milenković, F. Sisto, C. Masia, G. Farronato, V. Nikolić, T. Kundaković, *J. Med. Food*, **20**, 790 (2017).
35. European Food Safety Authority (2009) General principles for the collection of national food consumption data in the view of a pan-European dietary survey. EFSA J 7, 1435, 2009.