CHEMICAL COMPOSITION AND TRACE ELEMENT ANALYSIS OF *TRICHOLOMA EQUESTRE* COLLECTED FROM BATAK MOUNTAIN IN BULGARIA

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Abstract: *Tricholoma equestre* was rich in carbohydrates ($88.03 \text{ g } 100^{-1} \text{ g}^{-1} \text{ dw}$), followed by proteins ($5.65 \text{ g } 100^{-1} \text{ g}^{-1} \text{ dw}$), ash ($4.57 \text{ g } 100^{-1} \text{ g}^{-1} \text{ dw}$) and fat ($1.75 \text{ g } 100^{-1} \text{ g}^{-1} \text{ dw}$). Moisture (88.31%) and Energy ($390.49 \text{ kcal } 100^{-1} \text{ g}^{-1} \text{ dw}$) were also calculated. The concentrations of trace elements : Fe ($12.85 \pm 0.68 \text{ mg kg}^{-1}$), Zn ($8.63 \pm 0.19 \text{ mg kg}^{-1}$), Cu ($1.16 \pm 0.14 \text{ mg kg}^{-1}$) and Mn ($0.85 \pm 0.13 \text{ mg kg}^{-1}$), were assayed. Trace elements content of samples indicated that the Batak mountain was an ecologically pure region in Bulgaria, and therefore the mushrooms collected from this location could be consumed without any risk for human health. According to this study, the edible wild mushroom *Tricholoma equestre* could be used in human nutrition due to its good properties.

Keywords: Chemical Composition, Trace elements, *Tricholoma equestre*, Batak mountain, Bulgaria.

1. INTRODUCTION

Mushrooms have been exploited in human diet for centuries because of their specific taste and flavour. Nowadays, they attract attention because of their beneficial effects and possible use in the prevention or treatment of diseases [1]. Numerous reports demonstrate beneficial in vivo effects of cultivated and wild edible mushrooms. It has been proven that the polysaccharide extract of *Pleurotus pulmonarius* delays the progression of hepatocellular carcinoma [2]; polysaccharide from *Pholiota nameko* has anti-inflammatory properties in rodents [3]; *Agaricus bisporus* inhibits prostate tumor growth in mice [4]; *Pleurotus eryngii*, *Grifola frondosa*, and *Hypsizygus marmoreus* protect apolipoproteinE deficient mice from development of atherosclerosis [5]. Simultaneously, edible mushrooms are regarded as an important dietary supplement for people interested in calorie restriction, because of the low amount of fat, cholesterol, and calories in their bodies and high concentration of fiber [1, 6–8]. The therapeutic action of mushrooms is attributed to the presence of bioactive compounds such as vitamins, polysaccharides, and secondary metabolites in their fruiting bodies. Some of them have antioxidant properties which are referred repeatedly to be the key aspect of their observed beneficial effects. Polyphenols and carotenoids, abundant in the fruiting bodies of mushrooms, are antioxidants efficient in biological systems [9]. Polyphenols have been reported to interfere with the initiation and progression of cancer [10, 11], to act as antiageing [12], anti-inflammatory [13, 14], and brain-protective factors [15] and to protect against cardiovascular diseases [16, 17]. Apart from provitamin A properties, carotenoids are known as singlet oxygen quenchers [18, 19] and lipid peroxidation chain breakers [20]. They have been reported to reduce the risk of prostate cancer [21, 22], digestive tract cancers [23, 24], and chronic diseases [25–27].

Herein, we report the chemical compositions and trace element analysis (Fe, Zn, Cu and Mn) of *Tricholoma equestre* (Fig.1) wild edible mushroom collected from Batak mountain in

Bulgaria, with reference to the content (as d.w. basis) of ash, moisture, carbohydrate, fat, protein and energy. To our knowledge, no data have previously been reported on the chemical compositions and trace element analysis of *Tricholoma equestre* which are examined from a different habitat in the same region.

Figure 1. *Tricholoma equestre*

2. METHODS

2.1. Mushroom Samples

Fifteen mushroom samples were collected in 2014 and 2015 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.

2.2. Reagents

All chemicals were at least of analytical-reagent grade. Water was de-ionized in a Milli-Q system (Millipore, Bedford, MA, USA) to a resistivity of 18.2 MΩ cm.

All plastic and glassware were cleaned by soaking in diluted HNO₃ (1/9, v/v) and were rinsed with distilled water prior to use.

2.3. Sample preparation for nutritional analysis

The whole macrofungal samples were used in this study. Fresh samples, after the removal of extraneous material such as mud, bush, soil, plant, etc. by washing with demineralized water, were air-dried in between Whatman's filter papers. Approximately 5 g of each sample was taken immediately for the determination of moisture. Remaining samples were stored in deep-freezer until use. While examining the nutritional composition of mushroom samples, the maturation stage of them was not considered.

2.4. Chemical analysis

The following components were determined on airdried material: moisture, by drying in a moisture determination apparatus at 110 °C until circulation was completed; ash, from the incinerated residue obtained at 550°C after 3 h; crude protein, by the Kjeldahl method with a conversion factor of 6.25; crude fat, gravimetrically determined after Soxhlet extraction with petroleum ether. Total energy values were calculated by multiplying the amounts of protein and carbohydrate by the factor of 4 kcal/g and lipid by the factor of 9 kcal/g. In all tables, data points represent mean of three determinations.

2.5. Trace Element analysis

Fresh mushrooms, after removal of external material, were dried in an oven at 105 °C for 24 h after airdried for several days. Dried samples were homogenized, using an agate homogenizer, and stored in pre-cleaned polyethylene bottles until analysis. 1 g of sample was placed in a porcelain crucible and ashed at 450 °C for 20 h; then the ash was dissolved in 1 ml concentrated HNO₃, evaporated to dryness, heated again at 450 °C for 4 h, treated with 1 ml concentrated H₂SO₄, 1 ml HNO₃ and 1 ml H₂O₂, and then diluted with double deionized water up to a volume of 10 ml. The blank samples were treated in the same way. For the determination of metal contents, an *Varian Spectra AA 220 model Atomic Absorption Spectrometer (AAS)* was used. The determination of all metal contents was carried out in an air/acetylene flame. The maximum absorbance was obtained by adjusting the hollow cathode lamps at the operation conditions.

2.6. Statistical

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

3. EXPERIMENTAL

3.1. Chemical Composition

Chemical composition of *Tricholoma equestre* are presented in Table 1. *Tricholoma equestre* showed to be rich in carbohydrates (88.03 g 100⁻¹ g⁻¹ dw), which were the most abundant macronutrients. Proteins were present at (5.65 g 100⁻¹ g⁻¹ dw), ash (4.57 g 100⁻¹ g⁻¹ dw) and fat (1.75 g 100⁻¹ g⁻¹ dw). Moisture (88.31%) and Energy (390.49 kcal 100⁻¹ g⁻¹ dw) were also calculated.

Despite some similarities in the composition of mushroom samples, it is known that the chemical composition of mushrooms are affected by a number of factors, namely, mushroom strain/type, composition of growth media, time of harvest, management techniques, handling conditions and preparation of the substrates. This situation, they are diversity in antimicrobial activity of mushrooms at different cultivation status of same species. It can change the content and amount of active compounds according to growth media of mushroom. Therefore chemical contents and antimicrobial substances of mushroom species naturally grown in different geographic locations of world must be analyzed and comparison of this analysis is very important [28].

Table 1. Moisture ($\text{g } 100^{-1} \text{ g}^{-1}$ of fresh weight), macronutrients ($\text{g } 100^{-1} \text{ g}^{-1}$ of dry weight) and total energy ($\text{kcal } 100^{-1} \text{ g}^{-1}$ of dry weight) in the wild edible mushrooms.

Components	$\bar{X}$ mg kg^{-1}	SD mg kg^{-1}	-95% Confid.	+95% Confid.
Moisture	88.31 %	0.89	87.82	88.81
Ash	4.57	0.16	4.48	4.66
Crude protein	5.65	0.12	5.58	5.72
Crude fat	1.75	0.20	1.64	1.86
Total carbohydrates	88.03	0.26	87.89	88.18
Energy (kcal)	390.49	1.45	389.69	391.29

A Each value is expressed as mean $\pm$ SD ($n = 15$). Means with different letters within a row are significantly different ($p < 0.05$).

Table 2. Trace metal concentrations (mg kg^{-1} , dry weight basis) in mushroom samples ($n=15$).

Fungal species	Fe	Zn	Cu	Mn
<i>Tricholoma equestre</i>	12.85 ± 0.68	8.63 ± 0.19	1.16 ± 0.14	0.85 ± 0.13

4. RESULTS

In this study, Fe, Zn, Cu and Mn concentrations as micronutrients in dry matter basis of *Tricholoma equestre* were analyzed (Table 2).

The micronutrient metal composition of wild edible mushrooms used in this study were investigated [29-32]. The observation of different results can be attributed that the trace element profile of mushrooms has been affected by environmental factors such as climate, growing conditions, region and soil content.

Minimum and maximum Fe levels, in the present study, were $11.84 \text{ mg kg}^{-1} \text{ d.w.}$ and $13.85 \text{ mg kg}^{-1} \text{ d.w.}$ for *Tricholoma equestre*. Higher Fe levels were reported for *C. cornucopioides*, *A. mellea*, *S. imbricatus*, *R. flava*, *H. repandum* and *C. cibarius* [29-32].

In this study, the highest ($8.36 \text{ mg kg}^{-1} \text{ d.w.}$) and the lowest ($8.97 \text{ mg kg}^{-1} \text{ d.w.}$) Zn content was found in *Tricholoma equestre*, respectively. Zinc is widespread among living organisms due to its biological significance [30].

The Cu contents of *Tricholoma equestre* changed from $0.92 \text{ mg kg}^{-1} \text{ d.w.}$ to $1.34 \text{ mg kg}^{-1} \text{ d.w.}$. These Cu levels for the same species were in accordance with Sesli and Tüzen [30], Mendil et al. [31] and Ouzouni et al. [32]. *C. Cibarius* Cu level ($1.16 \text{ mg kg}^{-1} \text{ d.w.}$) was considerably lower than reported Sesli and Tüzen [30]. The Recommended Dietary Allowances (RDA) for adults is $0.90 \text{ mg copper/ day}$ [34]. Present concentrations of copper in mushrooms are not considered a health risk [33]. In general, copper contents in mushrooms are higher than those in green plant and vegetables and should be considered as a nutritional source of this element [35].

In this study, Mn contents of *Tricholoma equestre* were measured in the range of $0.64 - 1.06 \text{ mg kg}^{-1} \text{ d.w.}$. Higher Mn levels of *C. cornucopioides*, *L. volemus*, *A. mellea*, *C. cibarius*, *S. Imbricatus* were reported [29-32]. Whereas higher Mn levels of *L. perlatum* and *L. volemus* were found [29,30]. However, similar Mn levels for *L. perlatum* and *H. Repandum* were reported [29-32].

5. CONCLUSIONS

It can be concluded that the investigated wild edible mushroom *Tricholoma equestre* is good food sources in terms of protein, carbohydrate, crude fat, and energy values and may be cultivated. Our micronutrient values are in agreement with reports in the literature. So, it can be said that these determinations make the investigated wild edible mushroom popular and easily able to consume.

6. REFERENCES

- [1] Chang, R., 1996. Functional properties of edible mushrooms, *Nutrition Reviews*, 54(11), 91–93.
- [2] Wasonga, C., S. Okoth, J. Mukuria, and C. Omwandho, 2008. Mushroom polysaccharide extracts delay progression of carcinogenesis in mice. *Journal of Experimental Therapeutics and Oncology*, 7 (2), 147–152.
- [3] Li, H., X. Lu, S. Zhang, M. Lu, H. Liu, 2008. Anti-inflammatory activity of polysaccharide from *Pholiota nameko*. *Biochemistry*, 73(6), 669–675.
- [4] Adams, L., S. Phung, X. Wu, L. Ki, S. Chen, 2008. White button mushroom (*Agaricus bisporus*) exhibits antiproliferative and proapoptotic properties and inhibits prostate tumor growth in athymic mice. *Nutrition and Cancer*, 60(6), 744–756.
- [5] Mori, K., C. Kobayashi, T. Tomita, S. Inatomi, M. Ikeda, 2008. Antiatherosclerotic effect of the edible mushrooms *Pleurotus eryngii* (Eringi), *Grifola frondosa* (Maitake), and *Hypsizyus marmoreus* (Bunashimeji) in apolipoprotein Edeficient mice. *Nutrition Research*, 28(5), 335–342.
- [6] Sakata, T., 1995. A very-low-calorie conventional Japanese diet: its implications for prevention of obesity. *Obesity Research*, 3, 233–239.
- [7] Fukushima, M., T. Ohashi, Y. Fujiwara, K. Sonoyama, M. Nakano, 2001. Cholesterol-lowering effects of maitake (*Grifola frondosa*) fiber, shiitake (*Lentinus edodes*) fiber, and enokitake (*Flammulina velutipes*) fiber in rats. *Experimental Biology and Medicine*, 226(8), 758–765.
- [8] De Roman, M., E. Boa, S. Woodward, 2006. Wild-gathered fungi for health and rural livelihoods. *Proceedings of the Nutrition Society*, 65(2), 190–197.
- [9] Barros, L., B. A. Venturini, P. Baptista, L. M. Estevinho, I. C. F. R. Ferreira, 2008. Chemical composition and biological properties of Portuguese wild mushrooms: a comprehensive study. *Journal of Agricultural and Food Chemistry*, 56(10), 3856–3862.
- [10] Chen, D., Q. P. Dou, 2008. Tea polyphenols and their roles in cancer prevention and chemotherapy. *International Journal of Molecular Sciences*, 9(7), 1196–1206. 2008.
- [11] Lambert, J., J. Hong, G. Y. Yang, J. Liao, C. S. Yang, 2005. Inhibition of carcinogenesis by polyphenols: evidence from laboratory investigations. *The American Journal of Clinical Nutrition*, 81(1), 284–291.
- [12] Markus, M., B. J. Morris, 2008. Resveratrol in prevention and treatment of common clinical conditions of aging. *Clinical Interventions in Aging*, 3(2), 331–339.
- [13] Biesalski, H., 2007. Polyphenols and inflammation: basic interactions. *Current Opinion in Clinical Nutrition and Metabolic Care*, 10(6), 724–728.
- [14] Rahman, I., S. K. Biswas, P. A. Kirkham, 2006. Regulation of inflammation and redox signaling by dietary polyphenols. *Biochemical Pharmacology*, 72(11), 1439–1452.
- [15] Rossi, L., S. Mazzitelli, M. Arciello, C. R. Capo, G. Rotilio, 2008. Benefits from dietary polyphenols for brain aging and Alzheimer's disease. *Neurochemical Research*, 33(12), 2390–2400.
- [16] Halpern, M., A. L. Dahlgren, I. Laakso, T. Seppanen-Laakso, J. Dahlgren, P. A. Mcanulty, 1998. Red-wine polyphenols and inhibition of platelet aggregation: possible

- mechanisms, and potential use in health promotion and disease prevention. *Journal of International Medical Research*, 26(4), 171–180.
- [17] Zern, T., M. L. Fernandez, 2005. Cardioprotective effects of dietary polyphenols. *Journal of Nutrition*, 135(10), 2291–2294.
 - [18] Sies, H., 1992. Carotenoids and tocopherols as antioxidants and singlet oxygen quenchers,” *Journal of Nutritional Science and Vitaminology*, 27–33.
 - [19] Di Mascio, P., S. P. Kaiser, P. Thomas, P. Devasagayam, H. Sies, 1991. Biological significance of active oxygen species: in vitro studies on singlet oxygen-induced DNA damage and on the singlet oxygen quenching ability of carotenoids, tocopherols and thiols. *Advances in Experimental Medicine and Biology*, 283, 71–77.
 - [20] Frei, B., 1994. Reactive oxygen species and antioxidant vitamins: mechanisms of action. *American Journal of Medicine*, 97(3), 5–13.
 - [21] Dahan, K., M. Fennal, N. B. Kumar, 2008. Lycopene in the prevention of prostate cancer. *Journal of the Society for Integrative Oncology*, 6(1), 29–36.
 - [22] Kristal, A., E. A. Platz, H. L. Parnes, D. Albanes, 2004. Vitamin A, retinoids and carotenoids as chemopreventive agents for prostate cancer. *Journal of Urology*, 171(2), 54–58.
 - [23] Liu, C. and R. M. Russell, 2008. Nutrition and gastric cancer risk: an update. *Nutrition Reviews*, 66(5), 237–249.
 - [24] Lotan, R., 1997. Retinoids and chemoprevention of aerodigestive tract cancers. *Cancer and Metastasis Reviews*, 16(3- 4), 349–356.
 - [25] Rao A. and L. G. Rao, 2007. Carotenoids and human health. *Pharmacological Research*, 55(3), 207–216.
 - [26] Tapiero, H., D. M. Townsend, and K. D. Tew, 2004. The role of carotenoids in the prevention of human pathologies. *Biomedicine and Pharmacotherapy*, 58(2), 100–110.
 - [27] Riccioni, G., B. Mancini, E. Di Ilio, T. Bucciarelli, and N. D’Orazio, 2008. Protective effect of lycopene in cardiovascular disease. *European Review for Medical and Pharmacological Sciences*, 12(3), 183–190.
 - [28] Özen, T., C., Darcan, Ö. Aktop, İ. Turkecul, 2011. Screening of antioxidant, antimicrobial activities and chemical contents of edible mushrooms wildy grown in the Black Sea Region of Turkey. *Combinatorial Chem. High Throughput Screening*. 14, 72-84.
 - [29] Demirbaş, A., 2002. Metal ion uptake by mushrooms from natural and artificially enriched soils. *Food Chem.*, 78, 89-93.
 - [30] Tüzen, M., M. Özdemir, A. Demirbaş, 1997. Study of heavy metals in some cultivated and uncultivated mushrooms of Turkish origin. *Food Chem.*, 63, 247-251.
 - [31] Sesli, E., M. Tüzen, 1999. Levels of trace elements in the fruiting bodies of macrofungi growing in the East Black Sea region of Turkey. *Food Chem.*, 65, 453-460.
 - [32] Mendil, D., O.D. Uluözlü, E. Hasdemir, A. Çağlar, 2004. Determination of trace elements on some wild edible mushroom samples from Kastamonu, Turkey. *Food Chem.*, 88, 281-285.
 - [33] Ouzouni, P.K., P.G. Veltsistas, E.K. Paleologos, K.A. Riganakos, 2007. Determination of metal content in wild edible mushroom species from regions of Greece. *J Food Compos Anal.*, 20, 480-486.
 - [34] European Commission. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Copper. Health and Consumer Protection Directorate-General, Brussels, Belgium 2003.
 - [35] Isiloglu, M., F.Yilmaz, M. Merdivan, 2001. Concentrations of trace elements in wild edible mushrooms. *Food Chem.*, 73, 169-175.