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Differences in amino acid composition of stem and cap of *Morchella esculenta* from the Batak Mountain, Bulgaria

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The aim of this study was to evaluate the differences in amino acid composition between the cap and stem of *Morchella esculenta* - a wild edible mushroom from the Batak mountain, Bulgaria. The amino acid composition was determined by Q Exactive mass analyzer equipped with TurboFlow LC system and IonMax II electrospray ionization module (ThermoScientific Co, USA). Data acquisition and processing were carried out with XCalibur 4.2 software package. Twenty free amino acids, alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine were determined in mushroom's cap and stem. The total free amino acid (TAA) contents of cap was 26.17 mg kg^{-1} and that of the stem - 42.75 mg kg^{-1}. The essential to nonessential amino acids ratios of the cap and the stem were 0.18 and 0.33 respectively. The most substantial difference between *Morchella esculenta* cap and stem was established for ornithine – 263 %, followed by serine - 18.93 % and aspargine - 20.28 %. The smallest differences in the amino acid composition between cap and stem were demonstrated for proline - 102.77 %, followed by glutamine - 104.33 % and glutamic acid - 93.12 %. Of all 20 amino acids, only 6 were found in larger amounts in the cap than in the stem. The results showed the free amino acid contents of the analyzed wild edible mushroom was considerable, and that they may be important compounds contributing to the typical mushroom taste, nutritional value, and potent antioxidant properties.

Keywords: Amino acid composition, Wild edible mushroom - *Morchella esculenta,* LC/MS/MS analysis, Batak mountain, Bulgaria

INTRODUCTION

Mushrooms have long been favored as highly tasty, nutritive, and health-promoting foods. While preferred to cultivated fungi, wild growing mushrooms are collected and consumed as a delicacy worldwide for their specific aroma and texture. They are also an attractive source of food flavoring materials in soups and sauces due to their umami or palatable taste [1-6]. Moreover, a vast body of evidence indicates that wild edible mushrooms contain many biologically active compounds disclosing antioxidant, antibacterial, hepatoprotective, antiradical, antihyperglycemic, antiangiogenic, and even anti-inflammatory, antitumor, antiallergic, antiatherogenic, and hematological properties [7–10].

Amino acid composition is a reliable indicator of the nutritional value of food. Free amino acids are the main constituents of functionally essential compounds that are found in mushrooms. The most typical mushroom taste can be given by the nonvolatile compounds, such as free amino acids and soluble sugars [11, 13].

Morchella esculenta (L.) Pers. (morel) is a well known and extraordinary mushroom species. The head is distinctly conical in shape. The surface of head comprises a honeycomb of sharp ridges and deep pits and is rich brown in colour. The texture is sponge-like. The head and stem is generally hollow. It grows generally on chalky soil in grassy woodlands, field margins ad roadside verges. *Morchella esculenta* is picked up every year if the weather condition is suitable for growth in Bulgaria. It is collected especially in April and May, and marketed in abroad either fresh or dried.

In the available literature, no data are available regarding of *Morchella esculenta* in Bulgaria: Therefore the aim of this study was to determine the free amino acid compositions of Morchella esculenta from the Batak mountain.

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EXPERIMENTAL

Mushroom samples

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) (Fig. 1). The regions is industry-free and are characterized with forest, land and low buildings.

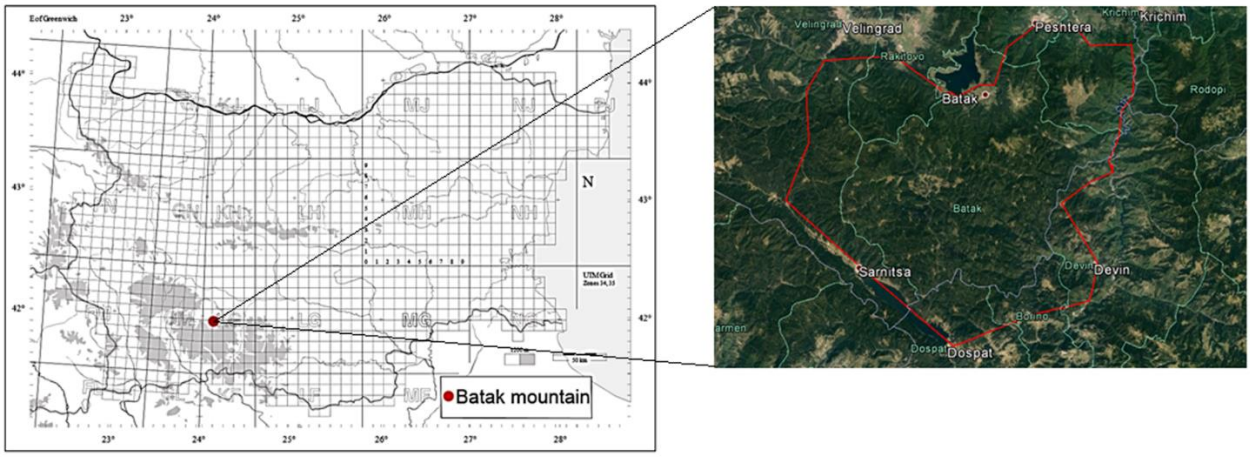

Fig. 1. Location of the sampling sites.

Mushroom samples from the species *Morchella esculenta* were collected in 2014 - 2018 from the Batak mountain by the authors themselves.

Mushroom samples were washed with distilled water and dried at 65 °C in a fan oven to constant weight. The dried samples were ground, then homogenized and stored in polyethylene bottles until analysis. The accuracy of the measurements (repeatability and reproducibility) was assessed by standard deviation for n = 3.

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 HNO_3 (1/9, v/v) and were rinsed with distilled water prior to use.

Determination of free amino acid composition. Instrumentation

Analyses were carried out on Q Exactive® mas analyzer equipped with TurboFlow® LC system and IonMax II® electrospray ionization module (ThermoScientific Co, USA). Data acquisition and processing were carried out with XCalibur® 4.2 software package.

Analysis of free amino acid concentration

Amino acid analysis was performed with a high performance amino acid analyzer. Sample equivalent to 10 mg of protein was weighed into the conical flask and mixed with 5 mL formic acid. The flask was placed in an ice bath for 16 h and sodium disulfite was added into the flask. 25 mL of HCl 6N was then added to the oxidized mixture. The flask was oven dried at 110°C for 24 h. The flask was then opened, and a Rotary evaporator was used to reduce the volume to 5–10 mL under vacuum at 60°C. Sodium citrate buffer (pH 2.20) was added to the hydrolyzed sample. Once all the soluble material was completely dissolved, the sample was ready for analysis.

Chromatographic conditions

Column: Syncronis C18, 1.7 µm (50 × 2.1 mm) (ThetmoScientific Co, USA); Mobile phase: A = 0.1 % formic acid in water; B = 0.1 % formic acid in acetonitrile; Flow rate: 300 µL min^{-1}; Gradient: 10 % B for 1 min; 10 – 90 % B for 6 min; 90 % B for 2 min; 90 – 10 % B for 1 min and 10 % B for 3 min. Injection volume: 10.0 µL.

Mass spectrometric conditions

Full-scan spectra over the m/z range 200-2000 were acquired in positive ion mode at resolution settings of 70 000. All MS parameters were optimized for sensitivity to the target analyzes using the instrument control software program (Table 1). Q Exactive parameters were - spray voltage 4.0 kV, Sheath gas flow rate 32, Auxiliary gas flow rate 10, Spare gas flow rate 3, Capillary temperature 280 ºC, Probe heater temperature 300 ºC and S-lens RF level 50. Parallel reaction monitoring (PRM) mode was used for quantitation of the amino acids, biogenic amines and polyamines. The selected ions used in PMT for quantitative analyses are presented in the Table below. Data acquisition and processing were

carried out with Xcalibur 2.4® software package (ThetmoScientific Co, USA). The calibration curves for each of analyzed compounds were constructed using external standards in range 0.1 – 1000 ng ml^{-1}.

Table 1. Detected ions and their most abundant MS2 fragments of amino acids in positive ionization mode

№	Compound	[M+H]+	MS/MS ion used for quantitation
1	Histidine	439.1431	110.0719
2	Arginine	458.1853	185.0927
3	Aspargine	416.1269	202.0717
4	Glutamine	430.1427	355.1090
5	Serine	389.1159	130.0504
6	Aspartic acid	417.1109	186.0402
7	Glutaminc acid	431.1268	218.0666
8	Threonine	403.1317	121.1017
9	Glycine	559.1050	146.0449
10	Proline	399.1367	186.0765
11	Tyrosine	465.1476	206.0819
12	Valine	401.1523	188.0920
13	Methionine	433.1246	133.0322
14	Leucine	415.1679	156.1023
15	Phenylalanine	449.1524	190.0868
16	Orn dihydrochloride	699.2285	442.1425
17	Tryptophane	488.1633	188.0711
18	Lysine	713.2444	243.0981
19	4-Hydroxyproline	415.1316	351.1149
20	GABA	387.1365	174.0766

Statistical analysis

All statistical computing, test and graphics were performed within the statistical software R version 3.4.4 (2018-03-15). The data were presented as mean value and standard deviation (SD). The results were analyzed through one-way analysis of variance (ANOVA) followed by Duncan's test with $p < 0.05$. Particular effect between stem and cap of mushroom *Morchella esculenta* and their amino acid composition were examined using a principal component analysis. To visualize individuals on the principal component map and to color individuals according to the cluster they belong to, a Factor map was used.

Percentage ratio between amino acid content in cap and stem

The percentage ratio between the amino acid content of the cap and the stem was calculated using the following formula:

$$\text{Difference } (\%) = \frac{C_{cap}}{C_{stem}} \times 100,$$

where, C represents the amino acid content of the cap and the stem.

RESULTS AND DISCUSSION

As shown in Table 2, it was possible to determine in mushroom's cap and stem 20 free amino acids: alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. The total free amino acid (TAA) contents of cap was 26.17 mg kg^{-1} and that of the stem - 42.75 mg kg^{-1}. The essential to nonessential amino acids ratios of the cap and the stem were 0.18 and 0.33 respectively. This result meets well the reference values of 0.6 recommended by FAO/WHO [14]. The most substantial difference between Morchella esculenta cap and stem was established for ornithine – 263 %, followed by serine - 18.93 % and aspargine - 20.28 %. The smallest differences in the amino acid composition between cap and stem were demonstrated for proline - 102.77 %, followed by glutamine - 104.33 % and glutamic acid - 93.12 %. Of all 20 amino acids, only 6 were found in larger amounts in the cap than in the stem. As far as we know, this is the first work in Bulgaria revealing the presence of 20 essential and nonessential free amino acids in the referred wild edible mushroom species, which is very important considering their nutritional value, typical mushroom taste, and biological properties.

Ribeiro et al. [15] reported that the total free amino acid contents in 11 wild edible mushrooms from northeastern Portugal ranged from (153.09 mg

100 g^{-1}) in F. hepatia to (2267.32 mg 100 g^{-1}) in B. edulis, whereas, data from the literature showed ca. (897 mg 100 g^{-1}) of total free amino acids in B. edulis [16]. Kıvrak et al. [17] determined free amino acid contents in Calvatia gigantean as ca. (199.6 mg 100 g^{-1}). It could be noted that up to (16.843 mg 100 g^{-1}) of total free amino acids were determined in five cultivated edible mushrooms, and the average content was (12.079 mg 100 g^{-1}) [12]. León-Guzmán et al. [18] reported that the total free amino acid range of four wild edible mushrooms from Querétaro, México was ca. (2317– 741 mg 100 g^{-1}).

The principal component analysis (PCA) carried out on the 20 free amino acids produced a two dimensional pattern for which the first principal compound explained 80.66 % of the variance, while the second principal component contributed 19.34 % of the total variance (Fig. 2). To visualize individuals on the principal component map and to color individuals according to the cluster they belong to, a Factor map was used. The Factor map visualize 3 individuals clusters. Cluster 1 consist of Asp,GABA, Gly, 4-Hyd, His, Leu, Met, Orn, Phe, Pro, Trp, Tyr, Val, cluster 2 consist of Arg, Asn, Glu, Lys, Ser, Thr and cluster 3 consist solely of Gln.

Table 2. Amino acid content in cap and stem of the dry weight (DW) mushrooms, (mg kg^{-1} DW).

			Morchella esculenta		
№			Cap	Stipe	Difference (%)
1	Histidine*	His*	0.56 ± 0.13^{ghi}	2.31 ± 0.27^{e}	24.24
2	Arginine	Arg	2.04 ± 0.34^{d}	3.58 ± 0.36^{c}	56.98
3	Aspargine	Asn	1.30 ± 0.28^{e}	6.41 ± 0.46^{b}	20.28
4	Glutamine	Gln	7.71 ± 0.59^{a}	7.39 ± 0.56^{a}	104.33
5	4-Hydroxyproline	4-Hyd	0.01 ± 0.01^{j}	0.02 ± 0.01^{j}	50.00
6	Serine	Ser	1.34 ± 0.30^{e}	7.08 ± 0.49^{a}	18.93
7	Aspartic acid	Asp	1.25 ± 0.20^{ef}	1.01 ± 0.19^{f}	123.76
8	Glutamic acid	Glu	3.52 ± 0.40^{b}	3.78 ± 0.20^{c}	93.12
9	Threonine*	Thr*	1.16 ± 0.30^{ef}	2.88 ± 0.15^{d}	40.27
10	Glycine	Gly	1.50 ± 0.28^{ghij}	0.92 ± 0.21^{fg}	163.04
11	GABA	GABA	0.97 ± 0.23^{efg}	0.64 ± 0.11^{fghi}	151.56
12	Tyrosine	Tyr	0.16 ± 0.07^{ij}	0.55 ± 0.13^{fghij}	29.09
13	Proline	Pro	0.74 ± 0.13^{fgh}	0.72 ± 0.16^{fgh}	102.77
14	Methionine*	Met*	n.d	n.d	-
15	Valine*	Val*	0.47 ± 0.11^{ghij}	0.57 ± 0.12^{fghi}	82.46
16	Leucine*	Leu*	0.30 ± 0.07^{hij}	0.46 ± 0.11^{ghij}	65.21
17	Tryptophan*	Trp*	$0.0.05 \pm 03^{ij}$	0.14 ± 0.06^{ij}	35.71
18	Phenylalanine*	Phe*	0.18 ± 0.09^{ij}	0.28 ± 0.11^{hij}	64.28
19	Ornithine	Orn	2.63 ± 0.42^{c}	n.d	263.00
20	Lysine*	Lys*	1.28 ± 0.23^{e}	4.01 ± 0.37^{c}	31.92
	Total Amino acids		26.17 ± 0.22	42.75 ± 0.23	61.22
	Essential amino acids		4.00 ± 0.14	10.65 ± 0.17	37.56
	Ratios (EAA /TAA)		0.18	0.33	54.55

Each value is expressed as mean ± SD (n = 3). Different letters in the same column indicate significant difference at $p < 0.05$ levels by Duncan's Multiple Range Test. TAA, total amino acid; EAA*, essential amino acids, were calculated as the total content of Val, Leu/ Ile, His, Lys, Thr, Met, Phe and Trp. n.d – not detected

Concerning the species described above, the differences between the results in this study and those in published reports are assumed to be caused by the diversity of extraction, derivatization, or quantification methods used in the different studies. Nevertheless, these studies suggested that, as demonstrated in our work, the free amino acid contents in mushrooms were considerably divergent between species. In addition, the different geographical origin, growth conditions, and harvesting times of the analyzed species cannot be excluded.

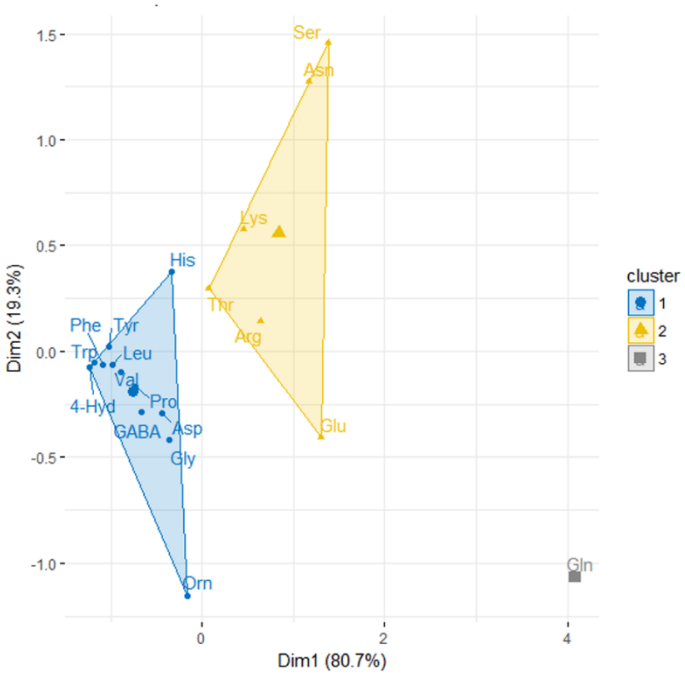

Fig. 2. Multivariate PCA and Factor map on 20 free amino acids between the cap and stem of *Morchella esculenta*.

CONCLUSIONS

The results showed the free amino acid contents of the analyzed wild edible mushroom was considerable, and that they may be important compounds contributing to the typical mushroom taste, nutritional value, and potent antioxidant properties.In general, wild edible mushrooms of Bulgaria could be a good source of essential nutrients to supplement the diet of the local people. Therefore, collected edible mushroom species are recommended in diets because of their low content of fat and energy and also can be consumed without any health risk.

REFERENCES

1. N. Rotzoll, A. Dunkel, T. Hofmann, *J. Agric. Food Chem.*, **54**, 2705 (2006).
2. P. Kalač, *Food Chem.*, **113**, 9 (2009).
3. S. Grosshauser, P. Schieberle, *J. Agric. Food Chem.*, **61**, 3804 (2013).
4. L. Wang, Z. Q. Ma, F. Du, H. X. Wang, T. B. Ng, *J. Agric. Food Chem.*, **62**, 7822 (2014).
5. M. Friedman, *J. Agric. Food Chem.*, **63**, 7108 (2015).
6. L. Dospatliev, M. Ivanova, *Bulg Chem Commun*., 49 (**4**), 787 (2017).
7. Y. T. Liu, J. Sun, Z. Y. Luo, S. Q. Rao, Y. J. Su, R. R. Xu, Y. J. Yang, *Food Chem. Toxicol.*, **50**, 1238 (2012).
8. M. Özyurek, M. Bener, K. Guclu, R. Apak, *Food Chem.*, **157**, 323 (2014).
9. S. L. Lin, T. Lai, L. Chen, H. Kwok, C. B. Lau, P. C. K. Cheung, *J. Agric. Food Chem.*, **62**, 9488 (2014).
10. L. Dospatliev, M. Ivanova, *C. R. Acad. Bulg. Sci.*, **70**, 795 (2017).
11. M. Y. Kim, L. M. Chung, S. J. Lee, J. K. Ahn, E. H. Kim, M. J. Kim, S. L. Kim, H. I. Moon, H. M. Ro, E. Y. Kang, S. H. Seo, H. K. Song, *Food Chem.*, **113**, 386 (2009).
12. J. Zhang, T. Li, Y. L. Yang, H. G. Liu, Y. Z. Wang, *Biol. Trace Elem. Res.*, **164**, 261 (2015).
13. E. K. Seow, B. Ibrahim, S. A. Muhammad, L. H. Lee, L. H. Cheng, *LWT-Food Sci. Technol.*, **65**, 428 (2016).
14. WHO: World Health Organization Evaluation of Certain Foods Additives and Contaminants (Twenty-Six Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Library Cataloguing-in-Publication Data, WHO, Geneva, Switzerland, 1982.
15. B. Ribeiro, P. B. Andrade, B. M. Silva, P. Baptista, R. M. Seabra, P. Valentão, *J. Agric. Food Chem.*, **56**, 10973 (2008).
16. S. Y. Tsai, H. L. Tsai, J. L. Mau, *Food Chem.*, **107**, 977 (2008).
17. İ. Kıvrak, Ş. Kıvrak, M. Harmandar, *Food Chem.*, **158**, 88 (2014).
18. M. F. León-Guzmán, I. Silva, M. G. López, *J. Agric. Food Chem.*, **45**, 4329 (1997).