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Amino acids in edible wild mushroom from the Batak mountain, Bulgaria

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The samples were collected from the Batak mountain, Bulgaria. Three popular wild edible mushroom species *Boletus pinophilus*, *Cantharellus cibarius* and *Craterellus cornucopioides* were analyzed for their free amino acid compositions 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: histidine, arginine, aspargine, glutamine, serine, aspartic acid, glutamic acid, threonine, glycine, proline, tyrosine, valine, methionine, leucine/isoleucine, phenylalanine, ornithine dihydrochloride, tryptophane, lysine, 4-hydroxyproline and γ-amino butyric acid, were determined. The total free amino acid (TAA) contents were from 26.46 mg kg^{-1} in *Cantharellus cibarius* to 44.18 mg kg^{-1} in *Boletus pinophilus*. The ratio of EAA to TAA were from 0.04 mg kg^{-1} in *Cantharellus cibarius* to 0.14 mg kg^{-1} in *Craterellus cornucopioides*. Glutamine, arginine, ornithine, and serine were among the most abundant amino acids present in all species. The results showed that the analyzed mushrooms contained a significant amount of free amino acids which may be important compounds contributing to the typical mushroom taste, nutritional value, and potent antioxidant properties of these wild edible mushrooms. Furthermore, the principal component analysis (PCA) showed that the accumulative variance contribution rate of the first two principal components reached 90.57%.

Keywords: Amino acid composition, Wild edible mushroom, LC/MS/MS analysis

INTRODUCTION

Mushrooms have long been favored as highly tasty, nutritive, and health-promoting foods [1–5]. While preferred to cultivated fungi, wild growing mushrooms are collected and consumed as a delicacy worldwide for their specific aroma and texture [6,7]. They are also an attractive source of food flavoring materials in soups and sauces due to their umami or palatable taste [8-10]. 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 [11-15].

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 [16-18]. No literature data on wild edible mushrooms in Bulgaria have been reported, nor data on free amino acid compositions. Therefore, the aim of this study was to determine the free amino acid compositions of the species *Boletus pinophilus*, *Cantharellus cibarius* and *Craterellus cornucopioides* from the Batak mountain.

EXPERIMENTAL

Mushroom samples

The Batak mountain is located in the 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 region is industry-free and is characterised with forests, land and low buildings. Mushroom samples from the species *Boletus pinophilus*, *Cantharellus cibarius* and *Craterellus cornucopioides* were collected in 2014 and 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.

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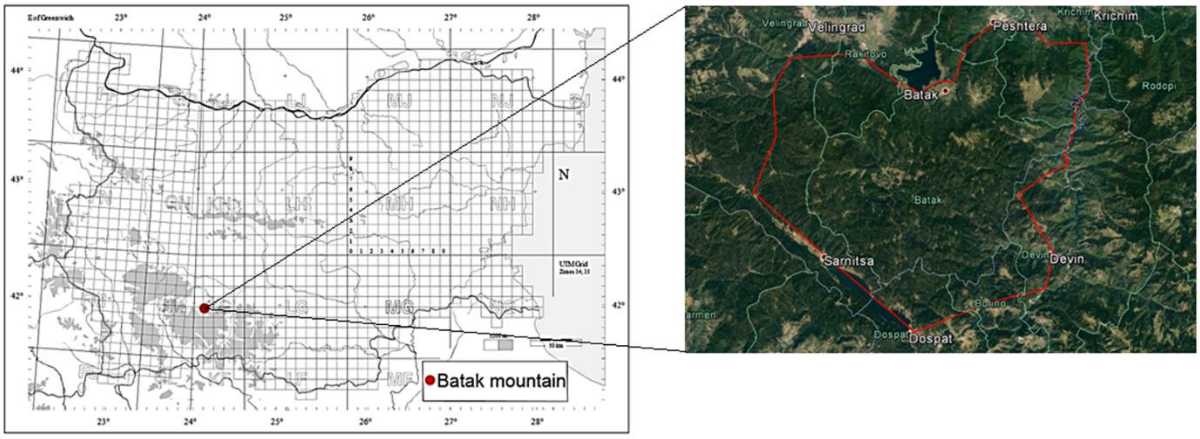

Fig. 1. Location of the sampling sites.

The dried samples were ground, then homogenized and stored in polyethylene bottles until analysis.

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 dilute 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® 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.

Chromatographic conditions

Column: Syncronis C18, 1.7 µm (50 × 2.1 mm) (ThermoScientific 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. 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 the analyzed compounds were constructed using external standards in the 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	Asparagine	416.1269	202.0717
4	Glutamine	430.1427	355.1090
5	Serine	389.1159	130.0504
6	Aspartic acid	417.1109	186.0402
7	Glutamic 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/Isoleucine	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	γ-Amino butyric acid	387.1365	174.0766

Statistical analysis

All analyses were carried out in triplicate and the data were reported as means ± standard deviation (SD). Statistical analysis and all chartings were performed within the R program version 3.4.4 (2018-03-15). The results were analyzed through one-way analysis of variance (ANOVA) followed by Duncan's test with $p < 0.05$. Particular effects between mushroom species and their amino acids were examined using a principal component analysis.

RESULTS AND DISCUSSION

The three wild edible mushroom species studied in this paper, such as *Boletus pinophilus*, *Cantharellus cibarius* and *Craterellus cornucopioides*, are considered the most delicious mushrooms by indigenous peoples for soup or fried with excess oil and salt for long-term consumption. These mushroom species are difficult for storage and transportation due to their crisp and tender texture. Therefore, they are commercially popular for the local markets. On the other hand, they are very important economic species for domestic and foreign trade.

As shown in Table 2 in almost all of the species it was possible to determine 20 free amino acids: histidine, arginine, asparagine, glutamine, serine, aspartic acid, glutamic acid, threonine, glycine, proline, tyrosine, valine, methionine, leucine/isoleucine, phenylalanine, ornithine dihydrochloride, tryptophan, lysine, 4-hydroxyproline and γ-amino butyric acid.

Of the 20 specified amino acids in the three types of mushrooms with the highest content is glutamine (from 11.91 mg kg^{-1} in *Craterellus cornucopioides* to 21.54 mg kg^{-1} in *Boletus pinophilus*), followed by arginine (from 4.06 mg kg^{-1} in *Cantharellus cibarius* to 4.45 mg kg^{-1} in *Boletus pinophilus*), ornithine (from 1.30 mg kg^{-1} in *Cantharellus cibarius* to 3.25 mg kg^{-1} in *Craterellus cornucopioides*) and serine (from 0.81 mg kg^{-1} in *Cantharellus cibarius* to 2.89 mg kg^{-1} in *Boletus pinophilus*).

The total free amino acid (TAA) contents in the analyzed samples ranged from 26.46 mg kg^{-1} in *Cantharellus cibarius* to 44.18 mg kg^{-1} in *Boletus pinophilus* (Table 2).

Table 2. Amino acid content in mushroom species per dry weight (DW), (mg kg^{-1} DW).

Amino acid	Abbreviation	*Boletus pinophilus*	*Cantharellus cibarius*	*Craterellus cornucopioides*
Histidine*	His*	0.69 ± 0.14^{ghijk}	0.13 ± 0.04^{f}	0.73 ± 0.12^{efg}
Arginine	Arg	4.45 ± 0.35^{b}	4.06 ± 0.33^{b}	4.10 ± 0.34^{b}
Asparagine	Asn	1.38 ± 0.12^{efg}	0.26 ± 0.08^{ef}	0.76 ± 0.28^{efg}
Glutamine	Gln	21.54 ± 1.34^{a}	15.90 ± 1.28^{a}	11.94 ± 1.46^{a}
Serine	Ser	2.89 ± 0.24^{c}	0.81 ± 0.16^{de}	1.15 ± 0.21^{de}
Aspartic acid	Asp	1.08 ± 0.09^{fghij}	0.58 ± 0.16^{ef}	0.73 ± 0.16^{efg}
Glutamic acid	Glu	1.14 ± 0.26^{fghi}	1.88 ± 0.25^{c}	2.77 ± 0.29^{c}
Threonine*	Thr*	1.00 ± 0.35^{fghij}	0.18 ± 0.06^{f}	0.41 ± 0.08^{fg}
Glycine	Gly	1.18 ± 0.12^{fgh}	0.38 ± 0.08^{ef}	0.26 ± 0.09^{fg}
Proline	Pro	0.79 ± 0.13^{fghijk}	0.17 ± 0.04^{f}	0.35 ± 0.08^{fg}
Tyrosine	Tyr	0.45 ± 0.12^{ijk}	0.06 ± 0.02^{f}	0.74 ± 0.06^{efg}
Valine*	Val	0.48 ± 0.17^{hijk}	0.12 ± 0.02^{f}	0.10 ± 0.04^{g}
Methionine*	Met*	0.17 ± 0.04^{k}	n.d	n.d.
Leucine/Isoleucine*	Leu/Ile*	0.43 ± 0.14^{jk}	0.10 ± 0.01^{f}	0.12 ± 0.04^{g}
Phenylalanine*	Phe*	0.41 ± 0.12^{jk}	0.06 ± 0.01^{f}	0.21 ± 0.07^{g}
Ornithine	Orn	2.33 ± 0.26^{cd}	1.30 ± 0.17^{cd}	3.25 ± 0.21^{c}
Tryptophan*	Trp*	0.37 ± 0.11^{jk}	n.d	0.99 ± 0.12^{def}
Lysine*	Lys	1.42 ± 0.29^{ef}	0.40 ± 0.05^{ef}	1.64 ± 0.12^{d}
4-Hydroxyproline	4-HYP	n.d	n.d	n.d.
γ-Amino butyric acid	GABA	1.97 ± 0.32^{de}	0.08 ± 0.01^{f}	0.18 ± 0.06^{g}
Total Amino acids		44.18	26.46	30.40
Essential amino acids		4.98	0.99	4.18
Ratios (EAA /TAA)		0.11	0.04	0.14

Each value is expressed as mean ± SD (n = 3). Means with different letters within a column are significantly different ($p < 0.05$). 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

The average total free amino acid concentration of the 3 species was 33.68 mg kg^{-1}. 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.* [19] 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* [20]. Kıvrak *et al*. [21] 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} [16]. León-Guzmán *et al*. [22] reported that the total free amino acid range of four wild edible mushrooms from Querétaro, México was ca. 2317–4741 mg 100 g^{-1}. 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 the free amino acid contents in mushrooms were considerably divergent between species, as demonstrated in our work. In addition, the different geographical origin, growth conditions, and harvesting times of the analyzed species cannot be excluded [9, 18, 23-28]. The ratios of the essential amino acids to nonessential amino acids were 0.11, 0.04 and 0.14 in *Boletus pinophilus*, *Cantharellus cibarius* and *Craterellus cornucopioides*, respectively. This result meets well the reference values of 0.6 recommended by FAO/WHO [29].

Principal component analysis (PCA), also known as Karhunen-Loéve (KL) transformation [30], is a mathematical procedure that transforms a number of (possibly) correlated variables into a (smaller) number of uncorrelated variables called principal components. The first principal component accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible. Here, PCA was used to demonstrate similarities and differences in the accumulation of 20 amino acids in 3 species of wild edible mushrooms. From Table 3 we may notice that the accumulative variance contribution rate of the first two principal components (from nine principal components) was 90.57% [31], which reflected most of the information regarding the free amino acid compositional variability in the three wild edible mushrooms. The first principal compound (PC1) explained 62.92% of the variation. The second principal component (PC2) contributed 27.65% of the total variation and positively loaded on Trp, Orn and Tyr, showing -0.432, -0.415 and -0.408 of the loading values, respectively.

Table 3. Factor loadings after normalized rotation.

Species	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
His	-0.185	-0.307	0.191	-0.262	0.263	-0.288	0.451	0.394	0.109
Arg	-0.193	0.002	-0.536	-0.658	0.228	0.170	-0.354	0.041	0.024
Asn	-0.280	-0.098	0.007	-0.008	-0.226	0.418	0.150	0.233	-0.123
Gln	-0.230	0.245	-0.185	0.026	-0.355	0.046	0.081	0.001	-0.621
Ser	-0.281	0.051	0.189	-0.112	-0.015	-0.099	-0.016	-0.148	0.085
Asp	-0.275	-0.038	-0.168	-0.203	-0.419	-0.441	0.119	-0.246	0.239
Glu	0.167	-0.330	-0.250	-0.125	-0.385	-0.066	0.260	-0.004	0.057
Thr	-0.246	0.017	0.502	-0.193	-0.315	0.390	-0.288	0.053	0.311
Gly	-0.263	0.163	0.158	-0.113	-0.104	-0.092	0.037	0.162	-0.071
Pro	-0.284	-0.017	-0.034	-0.010	0.424	0.067	0.289	-0.274	-0.205
Tyr	-0.097	-0.408	0.016	0.122	0.033	-0.010	-0.331	0.162	-0.180
Val	-0.262	0.080	-0.265	0.314	-0.053	-0.064	0.099	-0.016	0.286
Met	-0.263	0.126	0.290	-0.093	0.183	-0.173	-0.006	-0.035	-0.266
Leu/Ile	-0.266	0.061	-0.220	0.358	0.087	-0.253	-0.307	0.492	0.131
Phe	-0.271	-0.096	-0.173	0.241	0.029	0.453	0.313	0.092	0.160
Orn	-0.084	-0.415	0.064	0.075	-0.162	-0.159	-0.134	-0.044	-0.355
Trp	-0.034	-0.432	0.026	0.008	0.069	0.230	-0.009	-0.266	0.059
Lys	-0.165	-0.353	-0.012	0.164	0.056	-0.106	-0.233	-0.184	-0.017
GABA	-0.280	0.084	-0.010	0.193	0.088	-0.032	-0.095	-0.467	0.134
Eigenvalues	11.96	5.25	0.92	0.64	0.16	0.04	0.02	0.005	0.00
Variance	62.92%	27.65%	4.85%	3.38%	0.85%	0.20%	0.12	0.03%	0.00
Cumulative	62.92%	90.57%	95.42%	98.80%	99.65%	99.85%	99.97%	100.00%	100.00%

The percentages of variance explained by remainder seventh PCs were 4.85%, 3.38%, 0.85%, 0.20%, 0.12%, 0.03 and 0.06e-31%, respectively. The components with the greatest load were Arg (-0.658) in PC4 and Gln (-0.621) in PC9, respectively.

CONCLUSIONS

The edible mushrooms were found to be a good source of essential amino acids. It is also interesting to note that the majority of the wild mushrooms are consistently more nutritious than their cultivated relatives.

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.

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