

Nutritional and amino acid content of stem and cap of *Agaricus bisporus*, Bulgaria

Nikolai Valchev

Trakia University, Faculty of Agriculture, Department of Plant Production, 6000 Stara Zagora, Bulgaria
E-mail: neya.plovdiv@gmail.com

Abstract

Valchev, N. (2020) Nutritional and amino acid content of stem and cap of *Agaricus bisporus*, Bulgaria. *Bulg. J. Agric. Sci.*, 26 (Suppl. 1), 192-201

The purpose of this research was to evaluate nutritional value and the differences in the free amino acid content between the cap and stem of main cultivated species *Agaricus bisporus* - marketed in Bulgaria. The amino acid composition was determined by AccQ-Fluor amino acid derivatives were separated on ELITE LaChrom HPLC system (VWR™ Hitachi, Tokyo, Japan). The amino acid peaks were acquired using EZChrom Elite™ software and were calculated based on amino acid calibration standard (amino acid standard H, Thermo Fisher Scientific). *Agaricus bisporus* is rich in carbohydrates (6.76 g/100 g fw), followed by proteins (1.28 g/100 g fw), ash (0.88 g/100 g fw) and fat (0.20 g/100 g fw). Also moisture (90.88% fw), and total energy (123.6 kJ/100 g fw) were calculated. The total phenolic and total flavonoid contents of *Agaricus bisporus* species were 28.74 mg/g and 9.56 mg/g respectively. The content of saturated fatty acids (SFA) was (45.64%). The content of unsaturated fatty acids (UFA) in the oil from mushroom was (54.36%) and that of monounsaturated fatty acids (MUFA) consisted of (21.75%). On the other hand, the amount of monounsaturated fatty acids (PUFA) was higher (32.61%). On the grounds of the obtained data, it can be seen that in the phospholipid fraction from mushrooms, there predominated phosphatidic acids (29.60%) as a major component, followed by lysophosphatidylcholine (13.70%). The quantities of diphosphatidylglycerol and phosphatidylinositol in the phospholipid fraction were from (6.30% to 8.10%). Seventeen free amino acids, aspartic acid, serine, glutamic acid, glycine, histidine, arginine, threonine, alanine, proline, glycine, tyrosine, valine, methionine, lysine, isoleucine, leucine and phenylalanine acid were determined in mushroom's cap and stem. The total amino acid (TAA) content of cap was (139.22 mg/kg dw) and that of the stem (47.80 mg/kg dw). The essential amino acids (EAA) content of cap was (54.59 mg/kg dw) and that of the stem (39.49 mg/kg dw). The essential to total amino acids ratios of the cap and the stem were (0.39 and 0.83) respectively. The principal component analysis (PCA) carried out on the 17 free amino acids produced a two dimensional pattern for which the first principal compound explained (90.71%) of the variance, while the second principal component contributed (9.29%) of the total variance. The results showed the free amino acid content 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: chemical composition; amino acid composition; edible mushroom - *Agaricus bisporus*; ELITE LaChrom HPLC analysis

Introduction

Agaricus bisporus (*A. bisporus*) white button mushroom (WBM) is the most popular mushroom in the world. *A. bisporus* is the most extensively cultivated edible mushroom with yields accounting for 70% of total edible fungi (Rotzoll et al., 2006; Jagadish et al., 2009; Kalač, 2009; Liu et al., 2012; Grosshauser & Schieberle, 2013; Wang et al., 2014; Friedman, 2015).

Edible mushrooms are a good source of protein, several essential amino acids, vitamins (B₂, niacin, and folate), polyphenols and mineral elements (potassium, phospho-

rus, selenium, zinc, cobalt and copper) (Manzi et al., 2001; Kim et al., 2009; Lin et al., 2014; Özyurek et al., 2014; Dospatliev & Ivanova, 2017). In addition, *A. bisporus* contains several nonvolatile taste compounds, including soluble sugars and polyols, free amino acids and 50-nucleotides. Tsai et al. (2007) reported that 1 g of *A. bisporus* contained 48.8–64.2 mg of free amino acids and 6.59–8.14 mg of 50-nucleotides. However, *A. bisporus* has a short shelf life of only 1–3 days, and the commercial value decreases completely within a few days, largely due to its high water content (approximately 90%), high level of enzyme activity and the presence of microflora (Lau et al., 2014; Seow et al., 2016; Ivanova et al., 2019b).

Edible mushrooms are low in fat, and is known for having zero cholesterol but it contains some essential fatty acids such as linoleic acid (Tsai et al., 2008; Li et al., 2011; Atila et al., 2017; Borthakur et al., 2019; Rathore et al., 2020), caprylic, palmitic, stearic, oleic, eicosanoic and erucic acids and is a good source of vitamins (Dospatliev et al., 2019a). In general, the mushroom is a good source of K, Fe, Zn, Cu, Na, Se, Co and Mn (Adriano, 2001; Akinyele & Shokunbi, 2015; Ivanova et al., 2019a; Owaid et al., 2020). In particular, the main constituents in mushroom fruiting bodies are potassium and phosphorus and are usually followed by Ca, Mg, Na and Fe, Zn (Dospatliev et al., 2019b; El Sebaaly et al., 2019).

A. bisporus have a very good history of use in many traditional therapies and recent investigations have shown that nutraceutical therapy is a promising source of new therapeutics against many life-threatening diseases (Atila et al., 2017; Mahfuz et al., 2019). In addition, the high levels of dietary fibers, vitamin C, D, and B₁₂; folates and polyphenols contained in *A. bisporus* may provide beneficial effects on cardiovascular and diabetic diseases (Gasecka et al., 2018; Dospatliev et al., 2019c; Ramos et al., 2019; Samsudin & Abdullah, 2019; Singhal et al., 2019).

The purpose of this research was to evaluate nutritional value and the differences in the free amino acid content between the cap and stem of main cultivated species *Agaricus bisporus* - marketed in Bulgaria.

Materials and Methods

Mushroom samples

The edible mushrooms *Agaricus bisporus* used as raw material in this study were purchased from a large retail chain in Plovdiv, Bulgaria. Mushroom samples were washed with distilled water and dried in a fan oven to constant weight. The dried samples were ground, then homogenized and stored in polyethylene bottles until analysis.

Analytical Methods

Proximate composition analysis

For determination of proximate value, the following parameters were studied by using the mushroom material.

Moisture content

The fresh weight of each mushroom sample was taken using a chemical balance. These samples were then oven-dried separately at 105°C for 24 h. The loss in weight obtained after drying was regarded as the moisture content (AOAC, 2016).

Determination of crude protein

Protein content was determined using folin phenol reagent. 0.5 g of the powdered mushroom sample was extracted with 50 mL of 2% NaCl in a water bath at 60°C for 1 h. The extract was filtered out and 50 mL of 3% copper acetate monohydrate was added to the filtrate to precipitate the protein. The precipitated protein was then centrifuged out and dissolves in 50 mL (Kadiri & Fasidi, 1990).

Determination of crude fat

Crude fat was determined by extracting 2 g moisture-free samples with petroleum ether in a soxhlet extractor, heating the flask on a sand bath for about 6 h till a drop taken from the drippings left no greasy stain on the filter paper. After boiling with petroleum ether, the residual petroleum ether was filtered using Whatman No 40 filter paper and the filtrate was evaporated in a preweighed beaker. Increase in weight of beaker gave the crude fat (Sheikh et al., 2015).

Determination of ash content

The powdered mushroom sample (3 g) was ashed in a previously ignited and cooled crucible of known weight in a Gallenkamp furnace at 55°C for 6 h. The fairly cooled crucibles were put in desiccators and weighed (Manzi et al., 2001).

Determination of carbohydrates

Total carbohydrate was determined by adding 2 g of each sample in 50 mL distilled water, 0.2 mL of which was ten fold diluted. To 1 mL of the resulting solution and serial dilutions of glucose stock (10 mg/100 mL) solution, 4 mL of anthrone reagent was added and the absorbance of the solutions was measured by a spectrophotometer at 620 nm against a reagent blank (Plummer, 1971).

Energy

Total energy was calculated according to the following equations: Total energy (kJ/100g) = 17 (g protein + g carbohydrate) + 37 (g lipid). The weight of individual nutrients is g/100 g dw sample (Dir. 90/496/EEC, 2006).

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.

Total flavonoid content

To determine total flavonoid content by spectrophotometrically, 10 mg of the powdered mushroom sample was diluted to 1 mL by methanol and extracted. Then, 100 µL mushroom extract was mixed with 100 µL of 20% aluminium trichloride (AlCl₃) and a drop of acetic acid was added to this mixture. Total volume was completed to 5 mL with ethanol. After 40 min incubation at room temperature, absorbance of pink colour in the samples was measured spectrophotometrically at 415 nm and calculated using quercetin as standard. For calibration curve, quercetin was used with 5 different concentrations range of 0.03–0.5 mg/mL. The results were calculated using regression equation of the curve obtained and expressed as mg of quercetin equivalents per g of dry mushroom sample (Kumaran & Karunakaran, 2006).

Total phenolic content

The total phenolic content of mushrooms was determined pectrophotometrically using the Folin-Ciocalteu method (Singleton et al., 1999). 1 g of the powdered mushroom sample was diluted to 1 mL with methanol and extracted.

A 2.5 ml Folin-Ciocalteu reagent (10%) was added to extract (0.5 mL) and mixed. Afterwards, 2.5 mL of 7.5% saturated sodium carbonate solution was added to this mixture and mixed thoroughly. The mixture was incubated at 45°C for 45 min in the dark. At the end of incubation, formation of blue colour was observed. Finally, absorbance of blue colour in the samples was measured at 765 nm using a spectrophotometer. For total phenolic analysis, a calibration curve was obtained by using 5 different concentrations of gallic acid ranged from 0.007 to 0.125 mg/mL as standard. The total phenolic content were calculated using regression equation of the curve obtained and the results were expressed as mg of gallic acid equivalents per g of dry mushroom sample.

Analysis of fatty acids

The fatty acid composition was determined by gas chromatography (GC) after transmethylation of the sample with 2% H₂SO₄ in CH₃OH at 50°C (ISO 12966-2, 2011). Fatty acid methyl esters (FAME) were purified by thin-layer chromatography (TLC) on 20 × 20 cm plates covered with 0.2 mm silica gel 60 G (Merck) layer with mobile phase hexane:diethyl ether (97:3, v/v). GC was performed on a HP 5890 series II (Hewlett Packard GesmbH, Vienna, Austria) gas chromatograph equipped with a 75 m × 0.18 mm (I.D.) × 25 µm (film thickness) capillary column Supelco and a flame ionization detector. The column temperature was programmed from 140°C (5 min), at 4°C/min 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; split ratio was 1:50. 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 (ISO 12966-1, 2014).

Analysis of phospholipids

Air-dried mushrooms (10 g) were subjected to Folch extraction (Folch et al., 1957). The phospholipid classes were isolated by a variety of two-dimensional TLC on 20 cm × 20 cm glass plates with 0.2 mm Silica gel 60 G layer impregnated with aqueous (NH₄)₂SO₄ (1 g in 100 mL water). In the first direction the plate was developed with chloroform:methanol:ammonia, 65:25:5 (v/v/v) and in the second – with chloroform: acetone:methanol:acetic acid:water, 50:20:10:10:5 (v/v/v/v/v)) (Schneiter & Daum,

2014). The identification of the individual phospholipids 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 (v/v) (ISO 10540-1, 2014).

Determination of free amino acid composition

Amino acid analysis was performed with a high performance amino acid analyser. 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 were 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 hydrolysed sample. Once all the soluble material was completely dissolved, the sample was ready for analysis. The filtered sample was collected and processed to derivatisation.

Derivatisation of amino acids

The sample (20 µL) was derivatized with AccQ-Fluor reagent kit WAT052880 (Waters, Dublin, Ireland). AccQ-Fluor bo-rate buffer (60 µL) was added by micropipette to the sample and vortexed. Then, 20 µL of AccQ-Fluor reagent were added and the sample was additionally vortexed for 30 s. The sample was heated in a waterbath MLW W3 (Labexchange, Burladingen, Germany) at 55°C for 10 min before separation of amino acids using HPLC system.

High-performance liquid chromatography separation of amino acids

The AccQ-Fluor amino acid derivatives were separated on ELITE LaChrom HPLC system (VWR™ Hitachi, Tokyo, Japan). Sample of 20 µL was injected into an HPLC reversed phase AccQ-Tag™ silica-bonded amino acid column C18, 3.9 mm × 150 mm (Waters). The elution of the amino acids was performed by gradient system with mobile phase, eluent A, buffer WAT052890 (Waters) and mobile phase, eluent B, 60% acetonitrile (Sigma-Aldrich, Merck), in a separation gradient with a flow rate of 1.0 mL/min (Table 1).

Table 1. Gradient conditions of high-performance liquid chromatography separation of amino acids

t/min	Flow rate (mL/min)	Phase A (%)	Phase B (%)
0	1.0	100	0
0.5	1.0	98	2
15.0	1.0	93	7
19.0	1.0	90	10
32.0	1.0	67	33
33.0	1.0	67	33
34.0	1.0	0	100
37.0	1.0	0	100
38.0	1.0	100	0
64.0	1.0	100	0
65.0	1.0	0	100

The amino acids were detected using a diode array detector (DAD) at 254 nm with the column condition set at 37°C for 40 min. The amino acid peaks were acquired using EZChrom Elite™ software and were calculated based on amino acid calibration standard (amino acid standard H, Thermo Fisher Scientific). The proportional molar concentration for each amino acid was calculated based on the concentration of standard amino acids.

Statistical analysis

Statistical analysis and all chartings were performed within the R program version 3.5.3. The data were presented as mean value and standard deviation (SD). The particular effect between the parts of mushroom (cap and stem) and their nutritional composition were examined using a principal component analysis.

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

The main components of the chemical composition of *Agaricus bisporus* are presented in Table 2.

Agaricus bisporus is rich in carbohydrates (6.76 g/100 g fw), followed by proteins (1.28 g/100 g fw), ash (0.88 g/100 g fw) and fat (0.20 g/100 g fw). Also moisture (90.88% fw), and total energy (123.6 kJ/100 g fw) were calculated. A sample of *Agaricus bisporus* growing in Portugal was also reported to have carbohydrates as the most abundant macronutrient (Heleno et al., 2011). The carbohydrates in mushrooms comprise various compounds: sugars (monosaccharides, their derivatives and oligosaccharides) and both reserve and structural polysaccharides (Stojkovic et al., 2014).

Table 2. Moisture (g/100 g of fresh weight), macronutrients (g/100 g of fresh weight) and total energy (kJ/100 g of fresh weight) in the edible mushrooms

Components	\bar{X}	SD	-95% Confid. level	+95% Confid. level
Moisture	90.88	± 0.32	90.09	91.66
Ash	0.88	± 0.11	0.61	1.15
Crude protein	1.28	± 0.04	1.20	1.37
Crude fat	0.20	± 0.02	0.17	0.24
Total carbohydrates	6.76	± 0.52	5.47	8.05
Total energy	386.80	± 28.34	316.40	457.19

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

Total phenolic and flavanoids contents of *Agaricus bisporus*

The total phenolic and flavanoids contents in the mushroom samples were 28.74 mg/g and 9.56 mg/g respectively. Phenolic compounds have been reported to be of great interest due to their possible use as dietary supplements or food preservatives (Orhan & Ustun, 2011). Phenolic compounds have been reported in mushrooms previously and linked to various biological functions including antioxidant activity. Flavanoids in mushrooms on the other hand have been reported to possess a number of beneficial effects on human health, including antioxidant, anti-

inflammatory, antiallergic, antiviral, and anticarcinogenic activities. Phenol and flavonoids have been reported to scavenge free radicals such as peroxide, hydroperoxide of lipid hydroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Jayakumar et al., 2009). Phenolic compounds have also been associated with antioxidant activity and stabilization of lipid peroxidation. Previous studies have shown that food consumption with high phenolic content can reduce the risk of heart disease (Singla et al., 2010). From this study, the high levels of phenols and flavanoids make these mushrooms favourable for nutritional and therapeutic application.

Palmitoleic acid is an important fatty acid for pharmaceutical applications. It is postulated to have anti-thrombotic effects, which can help prevent stroke. At present, it is mainly obtained from Macadamia oil (*Macadamia integrifolia*), which contains 17% palmitoleic acid. Palmitoleic acid is produced by desaturation of palmitic acid. It

is envisaged that $\Delta 9$ -stearoyl-ACP desaturase, which acts mainly on stearic acid, can also use palmitic acid as substrate to produce palmitoleic acid. (Mattila et al., 2002; Keen et al., 2004; Teichmann et al., 2007; Phillips et al., 2011; De Silva et al., 2013; Stojkovic et al., 2014).

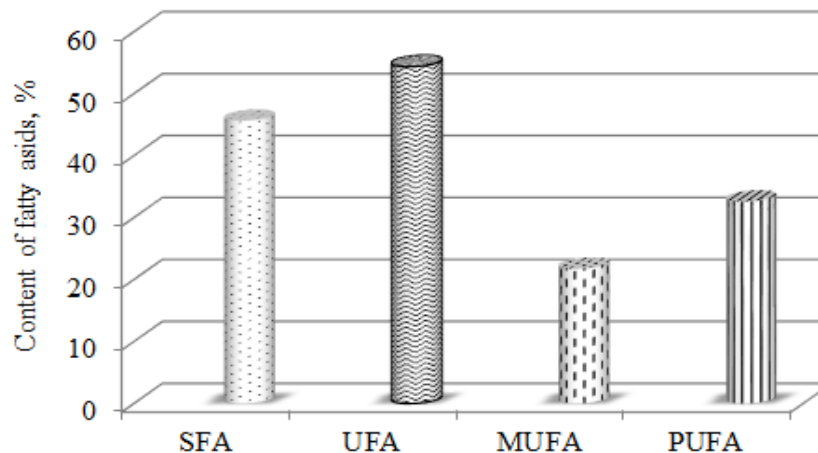


Fig. 1. Content of saturated (SFA), unsaturated fatty acids (UFA), monounsaturated (MUFA), monounsaturated (PUFA) fatty acids in *Agaricus bisporus* (n = 3)

Phospholipid composition of *Agaricus bisporus*

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

phosphatidic acids (29.60%) as a major component, followed by lysophosphatidylcholine (13.70%). The quantities of diphosphatidylglycerol and phosphatidylinositol in the phospholipid fraction were from (6.30%) to (8.10%). The results obtained in this study are consistent with the previously reported results in the literature (Keen et al., 2004; Kolundzić et al., 2017; Dospatliev & Ivanova, 2020)

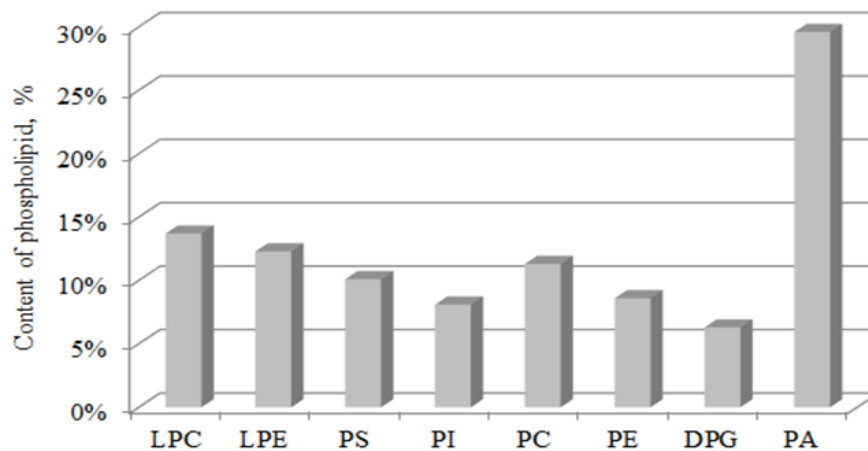


Fig. 2. Individual composition of phospholipid fraction of mushroom *Agaricus bisporus* (n = 3)

Legend: LPC – Lysophosphatidylcholine; LPE – Lysophosphatidylethanolamine; PS – Phosphatidylserine; PI – Phosphatidylinositol; PC – Phosphatidylcholine; PE – Phosphatidylethanolamine; DPG – Diphosphatidylglycerol; PA – Phosphatidic acids

As shown in Table 3, it was possible to determine in mushroom's cap and stem 17 free amino acids: Aspartic acid, Serine, Glutamic acid, Glycine, Histidine, Arginine, Threonine, Alanine, Proline, Glycine, Tyrosine, Valine, Methionine, Lysine, Isoleucine, Leucine and Phenylalanine

The total amino acid (TAA) content of cap was 139.22 mg/kg and that of the stem - 47.80 mg/kg. The essential amino acids (EAA) content of cap was 54.59 mg/kg and that of the stem - 39.49 mg/kg. The essential to total amino acids ratios of the cap and the stem were 0.39 and 0.83 respectively. This result meets well the reference values of 0.6 recommended by WHO (1982). The results obtained by me are also consistent with those obtained by

Dospatliev et al., (2019b) for species *Morchella esculenta* (the cap and the stem were 0.15 and 0.25), *Boletus pinophilus* (0.11), *Cantharellus cibarius* (0.04) and *Craterellus cornucopioides* (0.14).

The most substantial difference between *A. bisporus* cap and stem was established for aspartic acid – 444.62%, followed by glycine - 332.65% and arginine - 177.81%. The smallest differences in the amino acid composition between cap and stem were demonstrated for phenylalanine - 101.36%, followed by alanine - 114.53% and valine acid - 118.49%. Of all 17 amino acids, only 1 was found in larger amounts in the stem than in the cap.

Table 3. Amino acid content in cap and stem of the dry weight (DW) mushrooms, (mg/kg DW)

<i>Agaricus bisporus</i>					
№	Amino acid	Abbreviation	Cap	Stem	Difference (%)
1	Aspartic acid	Asp	16.94	3.81	444.62
2	Serine	Ser	11.40	8.29	137.52
3	Glutamic acid	Glu	0.99	4.85	20.41
4	Glycine	Gly	1.63	0.49	332.65
5	Histidine	His*	14.53	11.23	129.39
6	Arginine	Arg	6.97	3.92	177.81
7	Threonine	Thr*	7.93	5.14	154.28
8	Alanine	Ala	16.87	14.73	114.53
9	Proline	Pro	8.21	7.46	110.05
10	Glycine	Cys	0.21	0.12	175.00
11	Tyrosine	Tyr	6.39	4.13	154.72
12	Valine	Val*	5.83	4.92	118.49
13	Methionine	Met*	0.95	0.68	139.71
14	Lysine	Lys*	9.16	5.93	154.47
15	Isoleucine	Ile*	6.93	4.85	142.89
16	Leucine	Leu*	1.15	0.79	145.57
17	Phenylalanine	Phe*	8.11	5.95	101.36
Total Amino acids			124.20	87.29	2753.47
Essential amino acids			54.59	39.49	1086.16
Ratios (EAA /TAA)			0.44	0.45	0.39

Each value is expressed as mean (n = 3). TAA, total amino acid; EAA*, essential amino acids, were calculated as the total content of Val, Leu, Ile, His, Lys, Thr, Met and Phe

Ribeiro et al. (2008) reported that the total free amino acid content in 11 wild edible mushrooms from northeastern Portugal ranged from (153.09 mg/100 g) in *F. hepatica* to (2267.32 mg/100 g) in *B. edulis*, whereas, data from the literature showed ca. (897 mg/100 g) of total free amino acids in *B. edulis* (León-Guzmán et al., 1997). Kivrak et al. (2014) determined free amino acid content in *Calvatia gigantea* as ca. (199.6 mg/100 g). It could be noted that up to (16.843 mg/100 g) of total free amino acids were determined in five cultivated edible mushrooms, and

the average content was (12.079 mg/100 g) (WHO, 1982; Zhang et al., 2015). León-Guzmán et al. (1997) reported that the total free amino acid range of four wild edible mushrooms from Querétaro, México was ca. (2317 – 4741 mg/100 g).

The principal component analysis (PCA) carried out on the 17 free amino acids produced a two dimensional pattern for which the first principal compound explained 90.71% of the variance, while the second principal component contributed 9.29% of the total variance (Fig. 3).

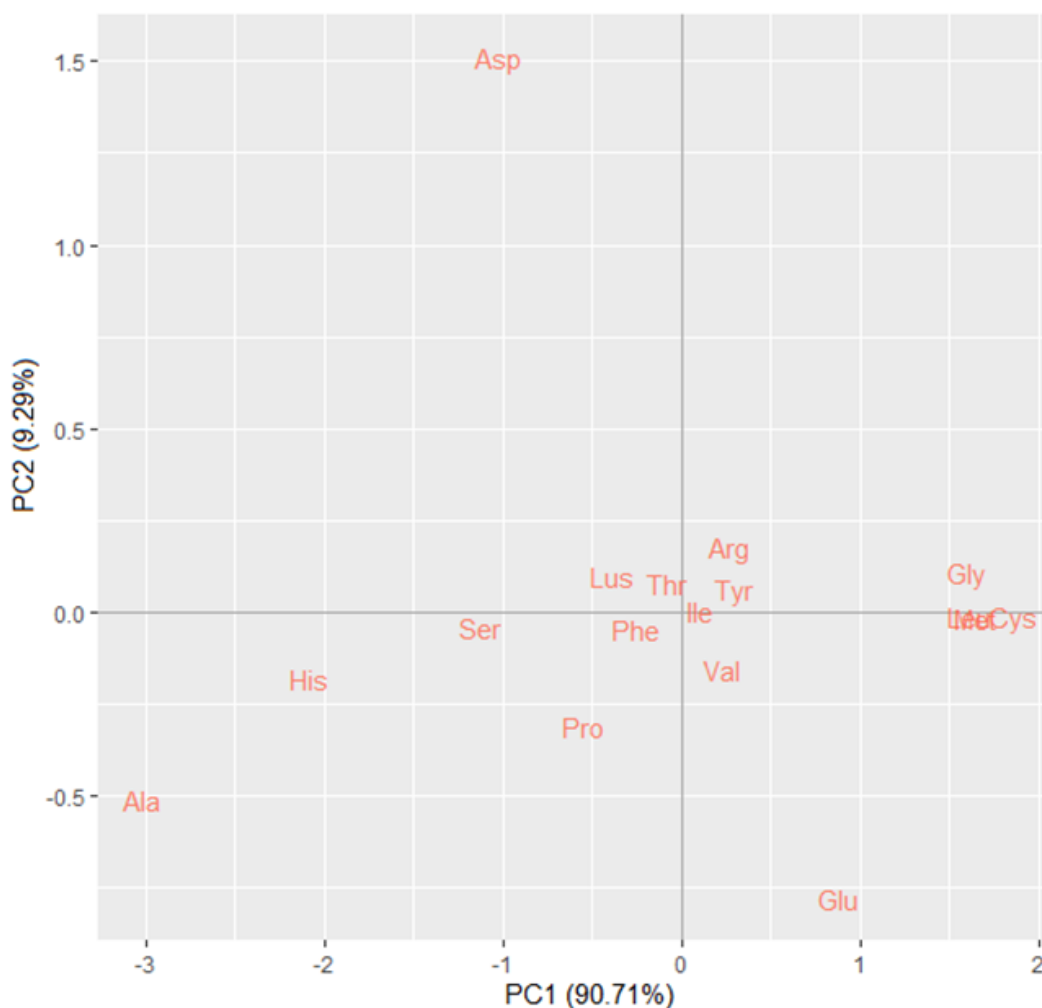


Fig. 3. PCA on 17 free amino acids between the cap and stem of *Agaricus bisporus*

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 content 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.

Conclusions

In general, cultivated edible mushroom of Bulgaria could be a good source of essential nutrients to supplement the diet of the local people. Therefore, collected edible mushroom species *Agaricus bisporus* recommended in diets because of their low content of fat and energy and also can be consumed without any health risk.

References

- Adriano, D. C.** (2001). Trace elements in the terrestrial environment. Springer-Verlag, Berlin.
- Akinyele, I. & Shokunbi, O.** (2015). Comparative analysis of dry ashing and wet digestion methods for the determination of trace and heavy metals in food samples. *Food Chemistry*, 173, 682–684.
- AOAC** (2016). Association of Official Analytical Chemist. Official methods of analysis, 20th ed., Washington, DC.
- Atila, F., Tüzel, Y., Cano, A. F. & Fernandez, J. A.** (2017). Effect of different lignocellulosic wastes on *Hericium americanum* yield and nutritional characteristics. *Journal of the Science of Food and Agriculture*, 97, 606–612.
- Borthakur, M. & Joshi, S. R.** (2019). Chapter 1 - Wild Mushrooms as functional foods: The significance of inherent perilous metabolites. In: *New and future developments in microbial biotechnology and bioengineering. microbial secondary metabolites biochemistry and applications*, Gupta, V. K., Pandey, A. (eds.) Elsevier, Amsterdam, The Netherlands, 1–12.
- De Silva, D. D., Rapior, S., Sudarman, E., Stadler, M., Xu, J., Alias, S. A. & Hyde, K. D.** (2013). Bioactive metabolites from macrofungi: ethnopharmacology, biological activities and chemistry. *Fungal Diversity*, 62, 1–40.
- Directive 90496/EEC** (2006). Nutritional Labelling for Foodstuffs: Discussion Paper on Revision of Technical Issues. Brussels: European Commission.
- Dospatliev, L. & Ivanova, M.** (2017). Spectrophotometric investigations on liquid-liquid extraction systems containing cobalt and tetrazolium salts. Application of the developed method for analysis of the cobalt content of biological samples (mushrooms and tobaccos). *Bulgarian Chemical Communications*, 49(4), 787–791.
- Dospatliev, L. & Ivanova, M.** (2020). Fatty acids and phospholipids of edible wild mushroom (*Amanita caesarea*) from the Batak mountain, Bulgaria. *Bulgarian Chemical Communications*, 52, 59–64.
- Dospatliev, L., Ivanova, M. & Papazov, P.** (2019a). Fatty acids, phospholipids, health risk index and daily intake of metals in edible wild mushroom (*Tricholoma equestre*) from the Batak mountain, Bulgaria. *Bulgarian Chemical Communications*, 51(D), 229–234.
- Dospatliev, L., Lozanov, V., Ivanova, M., Papazov, P. & Sugareva, P.** (2019b). Amino acids in edible wild mushroom from the Batak mountain, Bulgaria. *Bulgarian Chemical Communications*, 51(D), 92–96.
- Dospatliev, L., Lozanov, V., Ivanova, M., Papazov, P., Sugareva, P., Petkova, Z. & Bojilov, D.** (2018). Comparison of free amino acid compositions of stem and cap in wild edible mushrooms, Bulgaria. *Oxidation Communications*, 41, 542–549.
- Dospatliev, L., Petkova, Zh., Bojilov, G., Ivanova, M., Antova, G. & Angelova-Romova, M.** (2019c). A comparative study on the methods of antioxidant activity in wild edible mushrooms from the Batak Mountain, Bulgaria. *Bulgarian Chemical Communications*, 51(A), 245–250.
- El Sebaaly, Z., Assadi, F., Sassine, Y. N. & Shaban, N.** (2019). Substrate types effect on nutritional composition of button mushroom (*Agaricus bisporus*). *Agriculture & Forestry*, 65, 73–80.
- Folch, J., Lees, M. & Sloane-Stanley, G. H.** (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry*, 226, 497–509.
- Friedman, M.** (2015). Chemistry, nutrition, and health-promoting properties of *Hericium erinaceus* (Lion's Mane) mushroom fruiting bodies and mycelia and their bioactive compounds. *Journal of Agricultural and Food Chemistry*, 63, 7108–7123.
- Gasecka, M., Magdziak, Z., Siwulski, M. & Mleczek, M.** (2018). Profile of phenolic and organic acids, antioxidant properties and ergosterol content in cultivated and wild growing species of *Agaricus*. *European Food Research and Technology*, 244, 259–268.
- Grosshauser, S. & Schieberle, P.** (2013). Characterization of the key odorants in pan-fried white mushrooms (*Agaricus bisporus* L.) by means of molecular sensory science: Comparison with the raw mushroom tissue. *Journal of Agricultural and Food Chemistry*, 61, 3804–3813.
- Hanuš, L. O., Shkrob, I. & Dembitsky, V. M.** (2008). Lipids and fatty acids of wild edible mushrooms of the Genus *Boletus*. *Journal of Food Lipids*, 15, 370–383.
- Heleno, A., Barrosa, L., Sousa, M., Martins, A., Santos-Buelga & C., Ferreira, I.** (2011). Targeted metabolites analysis in wild *Boletus* species. *LWT Food Science and Technology*, 44, 1343–1348.
- Heleno, S. A., Stojkovic, D., Barros, L., Glamoclija, J., Sokovic, M., Martins, A., Queiroz, M. J. R. P. & Ferreira, I. C. F. R.** (2013). A comparative study of chemical composition, antioxidant and antimicrobial properties of *Morchella esculenta* (L.) Pers. from Portugal and Serbia. *Food Research International*, 51, 236–243.
- ISO 10540-1** (2014). Animal and Vegetable Fats and Oils. Determination of Phosphorus Content. Part 1: Colorimetric Method.
- ISO 12966-1** (2014). 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.
- ISO 12966-2** (2011). Animal and Vegetable Fat and Oils. Gas Chromatography of Fatty Acid Methyl Esters – Part 2: Preparation of Methyl Esters of Fatty Acids.
- Ivanova, M., Dospatliev, L. & Papazov, P.** (2019a). Application of ICP-OES method of determination of K, P, Mg, Na and Ca in nine wild edible mushrooms with a chemometric approach. *Comptes rendus de l'Academie bulgare des Sciences*, 72(2), 182–190.
- Ivanova, M., Katrandzhiev, N., Dospatliev, L. & Papazov, P.** (2019b). Comparison of mathematical models describing mushroom (*Amanita caesarea*) drying. *Journal of Chemical Technology and Metallurgy*, 54(5), 920–925.
- Jagadish, L. K., Krishnan, V. V., Shembhagaraman, R. & Kaviyaran, V.** (2009). Comparative study on the antioxidant, anticancer and antimicrobial property of *Agaricus bisporus* (J. E. Lange) Imbach before and after boiling. *African Journal of Biotechnology*, 8, 654–661.
- Jayakumar, T., Thomas, P. A. & Geraldine, P.** (2009). *In-vitro* antioxidant activities of an ethanolic extract of the oyster mushroom, *Pleurotus ostreatus*. *Innovative Food Science & Emerging Technologies*, 10, 228–234.
- Kadiri, M. & Fasidi, I. O.** (1990). Variations in chemical composition of *Chlorophyllum molybdites* (Mayer Ex. Fr.) Massee and *Pleurotus tuber-regium* (Fries) during fruit body development. *Nigerian Journal of Science*, 24 (1,2), 86–89.
- Kalač, P.** (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry*, 113, 9–16.
- Kavishree, S., Hemavathy, J., Lokesh, B. R., Shashirekha, M. N. & Rajarathnam, S.** (2008). Fat and fatty acids of Indian edible mushrooms. *Food Chemistry*, 106, 597–602

- .Keen, C. L., Uriu-Adams, J. Y., Ensuma, J. L. & Gershwin, M. E. (2004). Trace elements/minerals and immunity. In: *Handbook of Nutrition and Immunity*. Humana Press, Totowa, NJ, 117-140.
- Kim, M. Y., Chung, L. M., Lee, S. J., Ahn, J. K., Kim, E. H., Kim, M. J., Kim, S. L., Moon, H. I., Ro, H. M., Kang, E. Y., Seo, S. H. & Song, H. K. (2009). Comparison of free amino acid, carbohydrates concentrations in Korean edible and medicinal mushrooms. *Food Chemistry*, 113, 386-393.
- Kivrak, İ., Kivrak, Ş. & Harmandar, M. (2014). Free amino acid profiling in the giant puffball mushroom (*Calvatia gigantea*) using UPLC-MS/MS. *Food Chemistry*, 158, 88-92.
- Kolundzić, M., Stanojković, T., Radović, J., Tačić, A., Dođevska, M., Milenković, M., Kumaran, A. & Karunakaran, R. J. (2006). Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. *Food Chemistry*, 97, 109-114.
- Lau, C. C., Abdullah, N., Shuib, A. S. & Aminudin, N. (2014). Novel angiotensin I-converting enzyme inhibitory peptides derived from edible mushroom *Agaricus bisporus* (J.E. Lange) Imbach identified by LC-MS/MS. *Food Chemistry*, 148, 396-401.
- León-Guzmán, M. F., Silva, I. & López, M. G. (1997). Proximate chemical composition, free amino acid content, and free fatty acid content of some wild edible mushrooms from Querétaro, México. *Journal of Agricultural and Food Chemistry*, 45, 4329-4332.
- Li, Q., Zhang, H. H., Claver, I. P., Zhu, K. X., Peng, W. & Zhou, H. M. (2011). Effect of different cooking methods on the flavour constituents of mushroom (*Agaricus bisporus* (Lange) Sing) soup. *International Journal of Food Science Technology*, 46, 1100-1108.
- Lin, S. L., Lai, T., Chen, L., Kwok, H., Lau, C. B. & Cheung, P. C. K. (2014). Antioxidant and antiangiogenic properties of phenolic extract from *Pleurotus tuber-regium*. *Journal of Agricultural and Food Chemistry*, 62, 9488-9498.
- Liu, Y. T., Sun, J., Luo, Z. Y., Rao, S. Q., Su, Y. J., Xu, R. R. & Yang, Y. J. (2012). Chemical composition of five wild edible mushrooms collected from Southwest China and their antihyperglycemic and antioxidant activity. *Food Chemical Toxicology*, 50, 1238-1244.
- Mahfuz, S., Song, H., Miao, Y. & Liu, Z. (2019). Dietary inclusion of mushroom (*Flammulina velutipes*) stem waste on growth performance and immune responses in growing layer hens. *Journal of the Science of Food and Agriculture*, 99, 703-710.
- Manzi, P., Aguzzi, A. & Pizzoferrato, L. (2001). Nutritional value of mushrooms widely consumed in Italy. *Food Chemistry*, 73, 321-325.
- Mattila, P., Lampi, A.-M., Ronkainen, R., Toivo, J. & Piironen, V. (2002). Sterol and vitamin D₂ contents in some wild and cultivated mushrooms. *Food Chemistry*, 76, 293-298.
- Orhan, I. & Ustun, O. (2011). Determination of total phenol content, antioxidant activity and acetylcholinesterase inhibition in selected mushrooms from Turkey. *Journal of Food Composition and Analysis*, 24, 386-390.
- Owaid, M. N., Naeem, G. A., Muslim, R. F. & Olewi, R. S. (2020). Synthesis, characterization and antitumor efficacy of silver nanoparticle from *Agaricus bisporus* Pileus, Basidiomycota. *Walailak Journal of Science and Technology*, 17, 75-87.
- Özyurek, M., Bener, M., Guclu, K. & Apak, R. (2014). Antioxidant/antiradical properties of microwave-assisted extracts of three wild edible mushrooms. *Food Chemistry*, 157, 323-331.
- Phillips, K. M., Ruggio, D. M., Horst, R. L., Minor, B., Simon, R. R., Feeney, M. J., William, C. B. & Haytowitz, D. B. (2011). Vitamin D and sterol composition of 10 types of mushrooms from retail suppliers in the United States. *Journal of Agricultural and Food Chemistry*, 59, 7841-7853.
- Plummer, D. (1971). An introduction to practical biochemistry, McGraw Hill, 112.
- Ramos, M., Burgos, N., Barnard, A., Evans, G., Preece, J., Graz, M., Ruthes, A. C., Jiménez-Quero, A., Martínez-Abad, A., Vilaplana, F., Ngoc, L. P., Brouwer, A., van der Burg, B., Garrigós, M. C. & Jiménez, A. (2019). *Agaricus bisporus* and its by-products as a source of valuable extracts and bioactive compounds. *Food Chemistry*, 292, 176-187.
- Rathore, H., Prasad, S., Schwag, S. & Sharma, S. (2020). Vitamin D₂ fortification of *Calocybe indica* mushroom by natural and artificial UVB radiations and their potential effects on nutraceutical properties. *3 Biotech*, 10. <https://doi.org/10.1007/s13205-019-2024-x>
- Ribeiro, B., Andrade, P. B., Silva, B. M., Baptista, P., Seabra, R. M. & Valentão, P. (2008). Comparative study on free amino acid composition of wild edible mushroom species. *Journal of Agricultural and Food Chemistry*, 56, 10973-10979.
- Rotzoll, N., Dunkel, A. & Hofmann, T. (2006). Quantitative studies, taste reconstitution, and omission experiments on the key taste compounds in morel mushrooms (*Morchella deliciosa* Fr.). *Journal of Agricultural and Food Chemistry*, 54, 2705-2711.
- Samsudin, N. I. P. & Abdullah, N. (2019). Edible mushrooms from Malaysia; a literature review on their nutritional and medicinal properties. *International Food Research Journal*, 26, 11-31.
- Schneiter, R. & Daum G. (2006). Analysis of yeast lipids. In: *Yeast protocol. Methods in molecular biology*. Humana Press Inc., Totowa, NJ, 75-84.
- Seow, E. K., Ibrahim, B., Muhammad, S. A., Lee, L. H. & Cheng, L. H. (2016). Differentiation between house and cave edible bird's nests by chemometric analysis of amino acid composition data. *LWT- Food Science and Technology*, 65, 428-435.
- Sheikh, P. A., Dar, G. H., Dar, W. A., Shah, S., Bhat, K. A. & Kousar, S. (2015). Chemical composition and antioxidant activities of some edible mushrooms of Western Himalayas of India. *Vegetos - An International Journal of Plant Research & Biotechnology*, 28(2), 124-133.
- Singhal, S., Rasane, P., Kaur, S., Garba, U., Singh, J., Raj, N. & Gupta, N. (2019). Mushroom cultivation, processing and value-added products: A patent based review. *Recent Patents on Food, Nutrition & Agriculture*, 10, 3-19.
- Singla, R., Ganguli, A. & Ghosh, M. (2010). Antioxidant activities and polyphenolic properties of raw and osmotically dehydrated dried mushroom (*Agaricus bisporus*) snack food. *International Journal of Food Properties*, 13, 1290-1299.
- Singleton, V. L., Orthofer, R. & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymology*, 299, 152-178.
- Sisto, F., Masia, C., Farronato, G., Nikolić, V. & Kunda-ković, T. (2017). Cytotoxic and antimicrobial activities of *Cantharellus cibarius* Fr. (Cantarellaceae). *Journal of Medicinal Food*, 20, 790-797.

- Stojkovic, D., Reis, F., Glamoclija, J., Ćirić, A., Barros, L., Van Griensven, L., Ferreira, I. & Soković, M.** (2014). Cultivated strains of *Agaricus bisporus* and *A. brasiliensis*: chemical characterization and evaluation of antioxidant and antimicrobial properties for the final healthy product – natural preservatives in yoghurt. *Food & Function*, 5, 1602-1612.
- Teichmann, A., Dutta, P. C., Staffas, A. & Jägerstad, M.** (2007). Sterol and vitamin D₂ concentrations in cultivated and wild grown mushrooms: Effects of UV irradiation. *LWT - Food Science Technology*, 40, 815-822.
- Tsai, S. Y., Tsai, H. L. & Mau, J. L.** (2008). Non-volatile taste components of *Agaricus blazei*, *Agrocybe cylindracea* and *Boletus edulis*. *Food Chemistry*, 107, 977-983.
- Tsai, S. Y., Wu, T. P., Huang, S. J. & Mau, J. L.** (2007). Nonvolatile taste components of *Agaricus bisporus* harvested at different stages of maturity. *Food Chemistry*, 103, 1457-1464.
- Wang, L., Ma, Z. Q., Du, F., Wang, H. X. & Ng, T. B.** (2014). Feruloyl esterase from the edible mushroom *Panus giganteus*: A potential dietary supplement. *Journal of Agricultural and Food Chemistry*, 62, 7822-7827.
- WHO** (1982). 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.
- Zhang, J., Li, T., Yang, Y. L., Liu, H. G. & Wang, Y. Z.** (2015). Arsenic concentrations and associated health risks in *Laccaria* mushrooms from Yunnan (SW China). *Biological Trace Element Research*, 164, 261-266.

Received: May, 18, 2020; Accepted: October, 13, 2020; Published: December, 31, 2020