

Amino Acids Composition and Antioxidant Activity of Selected Mushrooms from Bosnia and Herzegovina

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Abstract

Many studies highlight the health benefits of mushrooms, which are consequently becoming more and more interesting for researchers. The content of amino acids (AA), total phenolic content (TPC), and antioxidative activity (AAC) were determined in wild as well as cultivated mushrooms. The AA included: L-tryptophan (Trp), L-arginine (Arg), L-cysteine (Cys), L-methionine (Met), L-alanine (Ala), L-phenylalanine (Phe), L-lysine (Lys), L-valine (Val), L-glycine (Gly), and L-leucine (Leu). The wild mushrooms: *Lactarius piperatus*, *Amanita caesarea*, *Lactarius deliciosus*, *Lycoperdon pyriforme*, *Macrolepiota procera*, and cultivated mushrooms: *Agaricus bisporus*, *Boletus aestivalis*, *Cantharellus cibarius*, *Pleurotus ostreatus*, and *Agaricus bisporus* var. *avellaneus* were investigated in this study. The AA was determined by HPTLC method and quantified with a Camag TLC scanner and WINCAT software by scanning the plates at 540 nm. The TPC was spectrophotometrically estimated as gallic acid equivalents/g of fresh weight according to Folin-Ciocalteu's method. The radical scavenging activity (RSA) of mushroom extracts was determined by DPPH assay. The highest content of Ala, Gly, Phe, Lys, Val, and Leu was found in *Pleurotus ostreatus*. The total phenolic content (mg GAE/g) in investigated mushrooms ranged from 1.90 to 35.56, and the % RSA ranged from 43.88 to 90.17. This study promotes the consumption of food rich in bioactive compounds, mushrooms being among such food. Further research on mushrooms from Bosnia and Herzegovina and their benefits in the overall maintenance of human health and protection from age-related diseases is necessary.

Keywords

Wild mushrooms, cultivated mushrooms, amino acids, HPTLC, antioxidant activity

1 Introduction

Empirical and scientific studies have shown that consumption of mushrooms with high content of essential amino acids (AA) and high antioxidative activity (AAC)^{1,2,3,4,5,6} has positive effects^{1,2} on the prevention of various diseases, such as heart diseases, cancer or diabetes^{7,8,9}, as well as on the extension of the human life-span in general. The positive effects of mushroom consumption on human health may be linked with the specific chemical composition of mushrooms^{10,11} and, in particular, with the content of antioxidants present in edible plant organs. Various vitamins, such as vitamins E or C, bioflavonoids, polyphenols, anthocyanin glycosides and other chemical components of plant organs all include phyto-antioxidants. It has been proven that these components have a high AAC, namely that they have the capacity to prevent oxidation of another substance when it is attacked by free radicals (FR). Since it is possible that FR take part in the pathogenesis of many diseases, then the consumption of mushrooms, which are traditionally part of the locals' diet in Bosnia and Herzegovina, may contribute to neutralization of FR *in vivo*. Therefore, it is of great importance to gain exact

data on the AAC of different edible mushrooms – both wild and cultivated species – as well as to obtain precise quantitative data regarding the chemical composition of mushroom species. Edible plants containing high quantities of flavonoids, polyphenols and anthocyanin glycosides have a high antioxidative capacity.^{12,13} An increase in the total antioxidative capacity has been shown in the blood plasma of individuals whose diet consisted of such plants.¹⁴ Chemical components of some edible plants, such as fruits, vegetables, mushrooms and herbal teas have positive effects on slowing down pathochemical processes which occur in the etiopathogenesis of neurodegeneration, tumours, cardiovascular diseases, and diabetes type II.^{7,8,9} Such positive effects of the consumption of fruit and vegetables on reduction and slowing down of pathochemical processes are linked to a specific chemical composition. Tens of wild mushroom species are widely consumed as a delicacy by European populations, including the population of Bosnia and Herzegovina. Credibility of evaluation of the mushrooms' nutritional value has so far been limited due to the fragmentary knowledge of their composition and mainly due to the poor information on the bioavailability of their varieties.^{15,16}

The aim of this research was to determine the composition of AA, the total phenolic content (TPC) and AAC of the selected wild and cultivated mushrooms. Fast, reliable, and

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simple analytical methods were applied to gain a better understanding of the total AAc of these mushroom species. Despite the fact that mushrooms have been part of the locals' diet for centuries, and particularly in rural areas, no serious research has been done so far in regards to the chemical analysis of either wild or cultivated mushrooms found in Bosnia and Herzegovina. Consequently, there is no reliable data to support the idea that mushroom consumption is beneficial for the overall human health. Thorough studies of the chemical composition of edible wild or cultivated mushroom species have never been done in our country; therefore, this study aims to collect such data which might further lead to the suggestion that the consumption of mushroom species found in these areas could provide not only nutritional but also potentially medical benefits. Taking into consideration that the population in these areas is getting older, further research on the benefits of mushrooms in the overall maintenance of health and protection from age-related diseases is necessary.

2 Experimental

2.1 Material

Wild mushrooms: *Lactarius piperatus*, *Amanita caesarea*, *Lactarius deliciosus*, *Lycoperdon pyriforme*, *Macrolepiota procera*, and cultivated mushrooms: *Agaricus bisporus*, *Boletus aestivalis*, *Cantharellus cibarius*, *Pleurotus ostreatus*, and *Agaricus bisporus* var. *avellaneus* were investigated in this study. All mushrooms were collected from Bosnia-Herzegovina markets. Identification was done by comparing their morphological, anatomical and physiological characteristics and monographs with descriptions given in the manual.^{16,17,18}

2.2 Chemicals and reagents

The following chemicals and reagents were used in this study: TLC Silica gel 60 F254 plates (obtained from Merck, Germany); Folin-Ciocalteu reagent (Kemika, Zagreb, Croatia); gallic acid (Fluka Chemicals, Switzerland); anhydrous sodium carbonate (Kemika, Zagreb, Croatia); solvents (Merck, Darmstadt, Germany); 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma Aldrich, St. Louis, USA) and (Trolox) 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Sigma Aldrich, St. Louis, USA); hydrochloric acid (Sigma Aldrich, St. Louis, USA); potassium chloride (Fluka Chemicals, Switzerland); acetic acid (Fluka Chemicals, Switzerland); sodium acetate (Sigma Aldrich, St. Louis, USA); Cyanidine-3-galactoside, amino acid standard mix was composed of: L-tryptophan (Trp), L-arginine (Arg), L-cysteine (Cys), L-methionine (Met), L-alanine (Ala), L-phenylalanine (Phe), L-lysine (Lys), L-valine (Val), L-glycine (Gly), and L-leucine (Leu) (obtained from Fluka Chemicals, Switzerland). All the chemicals were reagent grade.

2.3 Sample preparation

Extraction was performed according to a known method with some modifications. Fresh mushroom samples in

quantities of 0.5 g were cut into small pieces, crushed using a mortar and pestle, and consecutively extracted with 10 ml of ethanol ($\varphi = 80\%$). After maceration, the extracts were centrifuged (Tehnica Zelezniki LC-320) at 4000 rpm for 20 min, after which the supernatant was separated. Furthermore, ethanol was evaporated (IKA Rotary evaporator R-10). Extracts obtained by the described method were used for further investigations. Prepared extracts were stored in a refrigerator at 4 °C until the analysis was performed.

2.4 TLC amino acids identification

TLC amino acids identification method is based on the identification of AA in mushroom extracts and on the determination of their retention factor (Rf) which is compared with Rf values of standard AA solutions. Mushroom extracts and AA standard mix were dissolved in ethanol and applied on a TLC plate previously coated with silica gel. For chromatographic separation, silica gel F254 was used as a stationary stage; TLC plates were developed in the mobile stage in the ratio: 2-butanol : glacial acetic acid : distilled water (8 : 2 : 2). Detection was performed on 254 nm, and Rf values were calculated on the obtained chromatogram.²⁰

2.5 HPTLC amino acids quantification

For the HPTLC analysis of AA, 10 × 10 cm aluminum plates coated with silica gel 60 F254 (Merck, Germany) were used. The AA standard solutions and sample extracts were applied to the plates using a sample applicator (Camag Linomat V – Switzerland). The plates developed at room temperature with mobile phase consisting of 2-butanol : acetic acid glacial : water (8 : 2 : 2). The developed plates were then air dried and treated with ninhydrin solution. The AA was quantified with a Camag TLC scanner and WINCAT software by scanning the plates at 540 nm.

2.6 Determination of total phenolic content

TPC was determined spectrophotometrically with Folin-Ciocalteu reagent.²¹ 2 ml of the sample were dissolved in ethanol and mixed with 10 ml of Folin-Ciocalteu reagent. 1 ml of solution was diluted in 10 ml of distilled water. 8 ml of sodium carbonate were then added in to the solution. The solution was stored in the dark for two hours, and the absorbance was measured at 765 nm (spectrophotometer Shimadzu UV-1280). A standard curve was prepared by using gallic acid as standard with a concentration range from 100 to 500 $\mu\text{g ml}^{-1}$. Results are expressed as mg of gallic acid equivalents per gram of fresh weight (mg GAE/g f.w.).⁵

2.7 DPPH Assay

The mushroom extracts were mixed with methanol ($\varphi = 96\%$) and 63 $\mu\text{mol l}^{-1}$ solution of DPPH. After 30 min at room temperature, the absorbance was measured at 517 nm and converted into a percentage of free radical

Table 1 – TLC identification of L-amino acids in the selected mushroom species

Tablica 1 – TLC identifikacija L-aminokiselina u odabranim vrstama gljiva

Mushroom species	L-amino acids									
	Trp	Arg	Cys	Met	Ala	Phe	Lys	Val	Gly	Leu
<i>Lactarius piperatus</i>	–	+	+	–	+	+	+	+	+	+
<i>Amanita caesarea</i>	–	+	+	–	+	+	+	+	+	+
<i>Agaricus bisporus</i>	–	+	+	–	+	+	+	+	+	+
<i>Boletus aestivalis</i>	–	+	+	–	+	+	+	+	+	+
<i>Cantharellus cibarius</i>	–	+	+	+	+	+	+	+	+	+
<i>Lactarius deliciosus</i>	–	+	+	–	+	+	+	+	+	+
<i>Lycoperdon pyriforme</i>	–	+	+	–	+	+	+	+	+	+
<i>Pleurotus ostreatus</i>	–	+	+	–	+	+	+	+	+	+
<i>Macrolepiota procera</i>	–	+	+	+	+	+	+	+	+	+
<i>Agaricus bisporus</i> var. <i>avellaneus</i>	–	+	+	+	+	+	+	+	+	+

scavenging activity (% RSA).²² The comparative analysis of samples was made by calculating the DPPH scavenging activity, which stands for the relative decrease in absorbance in the analysed samples. The DPPH scavenging activity was calculated using the following equation (1):

$$\text{DPPH scavenging activity} = (\text{Ac} - \text{As}) / \text{Ac} \times 100 \quad (1)$$

where: Ac – absorbance of the control, and As – absorbance of the sample.

3 Results and discussion

Results of TLC analysis of AA are shown in Table 1, Figs. 1 and 2.

Figs. 1 and 2 show the TLC chromatograms of AA standards and investigated mushroom species.

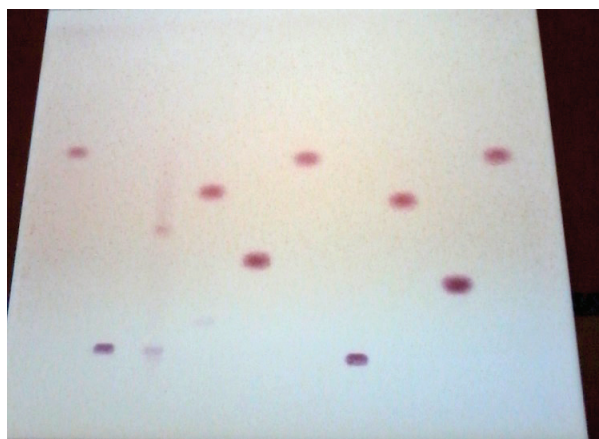


Fig. 1 – TLC chromatogram of amino acids standards
Slika 1 – TLC kromatogram standarda aminokiselina

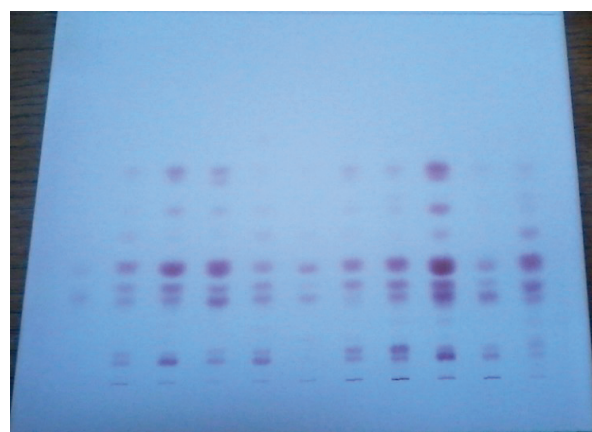


Fig. 2 – TLC chromatogram of the analysed mushroom species
Slika 2 – TLC kromatogram analiziranih vrsta gljiva

The qualitative TLC identification confirmed the presence of nine AA. Trp was not detected in the investigated mushrooms. Results of the HPTLC analysis of AA are shown in Table 2 and Figs. 3 and 4.

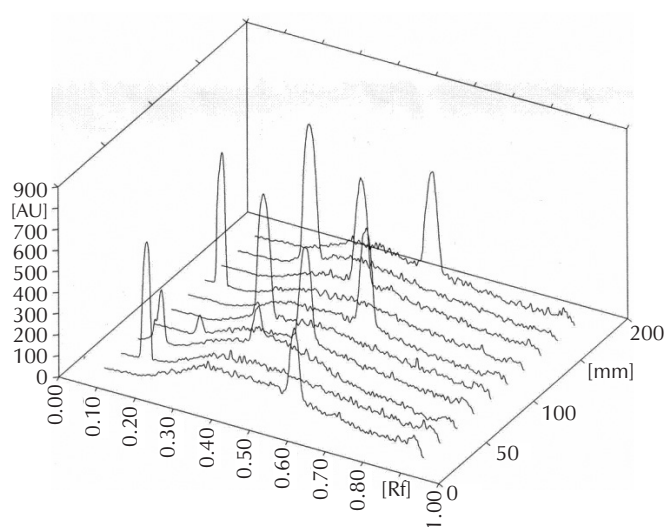
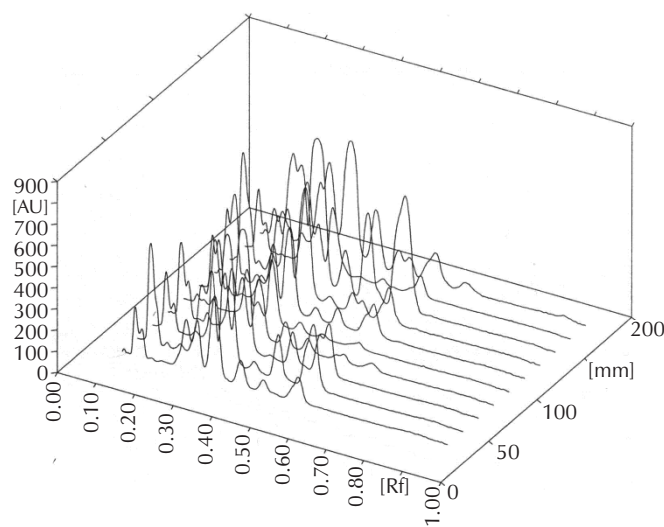
Our results show that *Lactarius piperatus* mushroom species has the highest content of Arg and the lowest content of Ala, Phe, Lys, Val, and Leu. The results show that *Pleurotus ostreatus* is the mushroom species with the highest content of Ala, Phe, Lys, Val, Gly, and Leu, which is consistent with the findings of other authors.²³ Arg was the major AA component in the *Lactarius piperatus* species.

Data regarding research on AA in different mushroom species is limited; it is believed that almost all of them contain AA, while the total AA ranges from 93.6 to 230 g kg⁻¹, and for the essential AA it ranges from 39.7 to 86.8 g kg⁻¹. Besides the essential AA, mushrooms can contain significant quantities of the following components: Lys, Ala, Arg, Gly, histidine, glutamic acid, asparaginic acid, Pro, and serine.¹⁰

Table 2 – HPTLC determination of amino acids content in the mushroom species

Tablica 2 – HPTLC određivanje sadržaja aminokiselina u vrstama gljivama

Mushroom species	L-amino acids/mg ml ⁻¹									
	Trp	Arg	Cys	Met	Ala	Phe	Lys	Val	Gly	Leu
<i>Lactarius piperatus</i>	0.00	558.00	40.20	0.00	26.00	4.87	2.92	1.80	46.70	4.40
<i>Amanita caesarea</i>	0.00	30.72	147.00	0.00	103.30	48.92	26.00	16.00	56.30	44.60
<i>Agaricus bisporus</i>	0.00	28.86	20.50	0.00	213.10	82.20	24.44	45.74	57.83	74.93
<i>Boletus aestivalis</i>	0.00	35.55	132.40	0.00	47.00	11.66	85.20	16.60	51.20	10.60
<i>Cantharellus cibarius</i>	0.00	146.70	55.87	17.67	168.00	72.10	65.80	25.30	83.24	65.74
<i>Lactarius deliciosus</i>	0.00	4.77	57.67	0.00	69.57	14.04	7.74	5.99	14.40	12.79
<i>Lycoperdon pyriforme</i>	0.00	67.87	66.20	0.00	114.40	71.40	50.60	21.72	64.80	65.00
<i>Pleurotus ostreatus</i>	0.00	0.00	288.00	0.00	280.80	318.70	196.80	281.20	111.73	137.72
<i>Macrolepiota procera</i>	0.00	31.96	15.20	17.67	82.40	38.70	70.60	8.42	33.99	35.30
<i>Agaricus bisporus var. avellaneus</i>	0.00	58.80	396.40	23.80	212.30	77.10	31.90	30.95	106.70	70.30

Fig. 3 – HPTLC chromatogram of amino acids standards
Slika 3 – HPTLC kromatogram standarda aminokiselinaFig. 4 – HPTLC chromatogram of the analysed mushroom species
Slika 4 – HPTLC kromatogram analiziranih vrsta gljiva

Results obtained for the total phenolic content and the RSA are shown in Fig. 5.

It can be seen that the total phenolic content (mg GAE/g f.w.) ranged from 1.90 to 35.56. According to a review by Mujic et al.²⁴, the content of total phenolics (mg GAE/g) in mushrooms ranges from 7.8–23.07 29.49 to 32.21, which was determined by different researchers.^{25,26} However, it is difficult to compare our results with the findings of other authors due to the differences in the applied extraction method,²⁷ mode of expressing results (on dry or fresh mushroom basis), etc. For instance, Yildirim et al.²⁵ used methanol to extract bioactive components from dry mushrooms. Ejeloni et al.²⁸ extracted bioactive components from dry mushrooms with distilled water and obtained results (mg GAE/g) in the range from 103.34 to 123.35. Our results show that the highest value of 35.56 mg GAE/g was determined for fresh *Boletus aestivalis* species and the lowest value of 1.90 mg GAE/g for *Amanita caesarea* spe-

cies. AAC of mushroom extracts investigated by the DPPH method, presented in Fig. 5, shows that the AA of *Cantharellus cibarius* extract exhibits a significant inhibition of the DPPH with 90.17 % RSA, while the lowest value determined for *Pleurotus ostreatus* was 43.88 % RSA. The results of this study indicate that the examined mushroom extracts possess a good AAC. Their rich antioxidant contents make the mushrooms an ideal dietary supplement. Further studies are needed to identify which phenolic components are responsible for the mushroom AA, and to assess the way in which the phenolic substances contribute to this activity. Additional *in vivo* antioxidant assays are necessary to confirm the potential use of mushrooms in the treatment of different diseases. Therefore, we can conclude that the analysed mushroom species represent a rich source of phenolic components, and thereby might serve as possible nutraceuticals in human diet. It can be suggested that they could assist in the reduction of oxidative damage and, consequently, in slowing down the ageing processes.

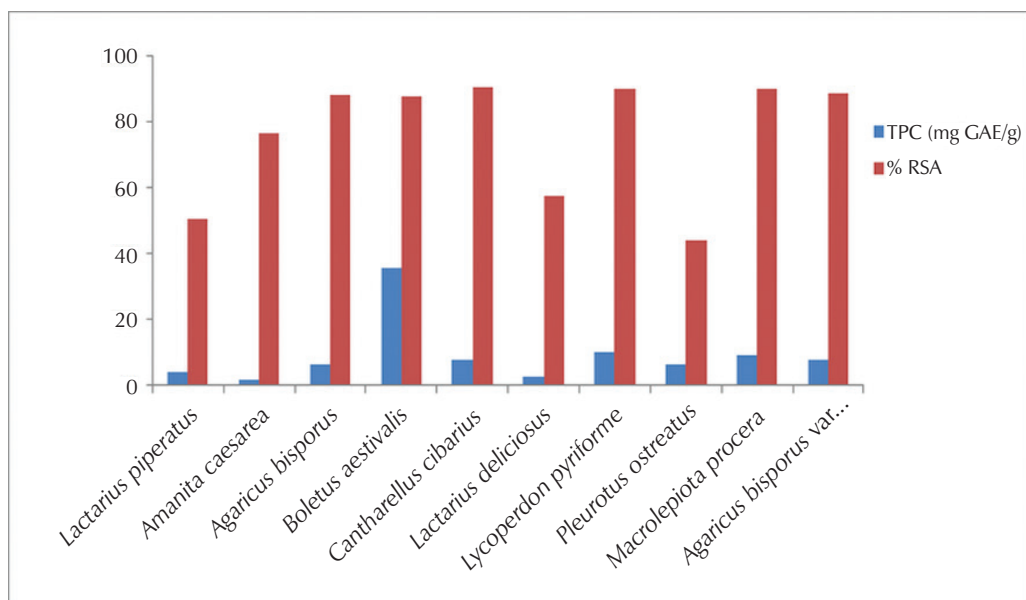


Fig. 5 – Total phenolic content and RSA of the analysed mushroom species
Slika 5 – Sadržaj ukupnih fenola i RSA analiziranih vrsta gljiva

4. Conclusions

Phenolic components have been detected in all the examined samples. The results indicate that extracts of the wild and cultivated mushrooms from Bosnia and Herzegovina contain significant amounts of essential AA, and that they possess high AAC.

This study promotes consumption of foods rich in bioactive compounds, such as selected mushrooms, especially as supplementary food for the ageing population.

The HPTLC method has proved to be a useful tool for the identification of AA in mushrooms for several reasons: it enables fast screening of investigated samples; it does not require complicated sample preparation; the analysis time is short; multiple samples can be analysed and compared simultaneously.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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List of abbreviations and symbols

Popis kratica o simbola

AA	– amino acids – aminokiseline
AAC	– antioxidant activity – antioksidacijska aktivnost
FR	– free radicals – slobodni radikali
RSA	– radical scavenging activity – antiradikalna aktivnost
TPC	– total phenolic content – ukupni sadržaj fenola
DPPH	– 1.1-diphenyl-2-picrylhydrazyl – 1.1-difenil-2-pikrilhidrazil
HPTLC	– High Performance Thin Layer Chromatography – tankoslojna kromatografija visoke djelotvornosti

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SAŽETAK

Aminokiselinski sastav i antioksidacijska aktivnost odabranih gljiva Bosne i Hercegovine

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Mnoga istraživanja ističu zdravstvene prednosti gljiva, koje stoga postaju sve zanimljivije za konzumente i istraživače. Sadržaj aminokiselina (AA) i ukupnih fenola (TPC) te antioksidacijska aktivnost (AAC) utvrđeni su u samoniklim i kultiviranim gljivama. Analizirane AA su: L-triptofan (Trp), L-arginin (Arg), L-cistein (Cys), L-metionin (Met), L-alanin (Ala), L-fenilalanin (Phe), L-lizin (Lys), L-valin (Val), L-glicin (Gly) i L-leucin (Leu). Od samoniklih gljiva analizirane su: *Lactarius piperatus*, *Amanita caesarea*, *Lactarius deliciosus*, *Lycoperdon pyriforme*, *Macrolepiota procera*, a od kultiviranih gljiva ispitivane su: *Agaricus bisporus*, *Boletus aestivalis*, *Cantharellus cibarius*, *Pleurotus ostreatus* i *Agaricus bisporus* var. *avellaneus*. Određivanje AA provedeno je metodom HPTLC, a kvantifikacija je provedena skenerom Camag TLC i softverom WINCAT skeniranjem ploča na 540 nm. TPC je spektrofotometrijski određen kao ekvivalent galne kiseline/g svježe mase metodom *Folin-Ciocalteu*. Aktivnost hvatanja radikala (RSA) ekstrakata gljiva određena je DPPH testom. Najveći sadržaj Ala, Gly, Phe, Lys, Val i Leu nađen je u *Pleurotus ostreatus*. Sadržaj ukupnih fenola (mg GAE/g) u ispitivanim gljivama kretao se u rasponu od 1,90 do 35,56, a % RSA u rasponu od 43,88 do 90,17. Ovo istraživanje promovira konzumiranje hrane bogate bioaktivnim spojevima, kojima pripadaju i gljive. Stoga je nužno nastaviti istraživanje gljiva s područja Bosne i Hercegovine radi utvrđivanja njihove dobrobiti za cjelokupno održavanje zdravlja ljudi i zaštite od oboljenja povezanih sa starenjem.

Ključne riječi

Samonikle gljive, kultivirane gljive, aminokiseline, HPTLC, antioksidacijska aktivnost

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