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

M. Salihović, A. Šapčanin, E. Pehlić, A. Uzunović, S. Špirtović-Halilović, and M. Huremović

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) has positive effects on the prevention of various diseases, such as heart diseases, cancer or diabetes, 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 to a specific chemical composition. Tens of wild and cultivated mushroom species – as well as to obtain precise qualitative 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. An increase in the total antioxidative capacity has been shown in the blood plasma of individuals whose diet consisted of such plants. 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. 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.

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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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. An increase in the total antioxidative capacity has been shown in the blood plasma of individuals whose diet consisted of such plants. 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. 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.

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
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, Macropleiota 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 (Trolax) 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-lysine (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 (φ = 80%). After maceration, the extracts were centrifuged (Tehnica Železniki 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 μg 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 (φ = 96 %) and 63 μmol 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
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):

$$\text{DPPH scavenging activity} = \frac{(A_c - A_s)}{A_c} \times 100$$  \hspace{1cm} (1)

where: $A_c$ – absorbance of the control, and $A_s$ – 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.

<table>
<thead>
<tr>
<th>Mushroom species</th>
<th>L-amino acids</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Trp</td>
</tr>
<tr>
<td>Lactarius piperatus</td>
<td>−</td>
</tr>
<tr>
<td>Amanita caesarea</td>
<td>−</td>
</tr>
<tr>
<td>Agaricus bisporus</td>
<td>−</td>
</tr>
<tr>
<td>Boletus aestivalis</td>
<td>−</td>
</tr>
<tr>
<td>Cantharellus cibarius</td>
<td>−</td>
</tr>
<tr>
<td>Lactarius deliciosus</td>
<td>−</td>
</tr>
<tr>
<td>Lycoperdon pyriforme</td>
<td>−</td>
</tr>
<tr>
<td>Pleurotus ostreatus</td>
<td>−</td>
</tr>
<tr>
<td>Macrolepiota procera</td>
<td>−</td>
</tr>
<tr>
<td>Agaricus bisporus var. avellaneus</td>
<td>−</td>
</tr>
</tbody>
</table>

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. 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.
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.\textsuperscript{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.\textsuperscript{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,\textsuperscript{27} mode of expressing results (on dry or fresh mushroom basis), etc. For instance, Yildirim et al.\textsuperscript{25} used methanol to extract bioactive components from dry mushrooms. Ejelonu et al.\textsuperscript{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 \textit{Boletus aestivalis} species and the lowest value of 1.90 mg GAE/g for \textit{Amanita caesarea} species. AAc of mushroom extracts investigated by the DPPH method, presented in Fig. 5, shows that the AA of \textit{Cantharellus cibarius} extract exhibits a significant inhibition of the DPPH with 90.17\% RSA, while the lowest value determined for \textit{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 \textit{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.

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

<table>
<thead>
<tr>
<th>Mushroom species</th>
<th>Trp</th>
<th>Arg</th>
<th>Cys</th>
<th>Met</th>
<th>Ala</th>
<th>Phe</th>
<th>Lys</th>
<th>Val</th>
<th>Gly</th>
<th>Leu</th>
</tr>
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<tbody>
<tr>
<td>\textit{Lactarius piperatus}</td>
<td>0.00</td>
<td>558.00</td>
<td>40.20</td>
<td>0.00</td>
<td>26.00</td>
<td>4.87</td>
<td>2.92</td>
<td>1.80</td>
<td>46.70</td>
<td>4.40</td>
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<tr>
<td>\textit{Amanita caesarea}</td>
<td>0.00</td>
<td>30.72</td>
<td>147.00</td>
<td>0.00</td>
<td>103.30</td>
<td>48.92</td>
<td>26.00</td>
<td>16.00</td>
<td>56.30</td>
<td>44.60</td>
</tr>
<tr>
<td>\textit{Agaricus bisporus}</td>
<td>0.00</td>
<td>28.86</td>
<td>20.50</td>
<td>0.00</td>
<td>213.10</td>
<td>82.20</td>
<td>24.44</td>
<td>45.74</td>
<td>57.83</td>
<td>74.93</td>
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<tr>
<td>\textit{Boletus aestivalis}</td>
<td>0.00</td>
<td>35.55</td>
<td>132.40</td>
<td>0.00</td>
<td>47.00</td>
<td>11.66</td>
<td>85.20</td>
<td>16.60</td>
<td>51.20</td>
<td>10.60</td>
</tr>
<tr>
<td>\textit{Cantharellus cibarius}</td>
<td>0.00</td>
<td>146.70</td>
<td>55.87</td>
<td>17.67</td>
<td>168.00</td>
<td>72.10</td>
<td>65.80</td>
<td>25.30</td>
<td>83.24</td>
<td>65.74</td>
</tr>
<tr>
<td>\textit{Lactarius deliciosus}</td>
<td>0.00</td>
<td>4.77</td>
<td>57.67</td>
<td>0.00</td>
<td>69.57</td>
<td>14.04</td>
<td>7.74</td>
<td>5.99</td>
<td>14.40</td>
<td>12.79</td>
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<td>\textit{Lycomperdon pyriforme}</td>
<td>0.00</td>
<td>67.87</td>
<td>66.20</td>
<td>0.00</td>
<td>114.40</td>
<td>71.40</td>
<td>50.60</td>
<td>21.72</td>
<td>64.80</td>
<td>65.00</td>
</tr>
<tr>
<td>\textit{Pleurotus ostreatus}</td>
<td>0.00</td>
<td>0.00</td>
<td>288.00</td>
<td>0.00</td>
<td>318.70</td>
<td>196.80</td>
<td>281.20</td>
<td>111.73</td>
<td>137.72</td>
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<tr>
<td>\textit{Macrolepiota procera}</td>
<td>0.00</td>
<td>31.96</td>
<td>15.20</td>
<td>17.67</td>
<td>82.40</td>
<td>38.70</td>
<td>70.60</td>
<td>8.42</td>
<td>33.99</td>
<td>35.30</td>
</tr>
<tr>
<td>\textit{Agaricus bisporus var. avellaneus}</td>
<td>0.00</td>
<td>58.80</td>
<td>396.40</td>
<td>23.80</td>
<td>212.30</td>
<td>77.10</td>
<td>31.90</td>
<td>30.95</td>
<td>106.70</td>
<td>70.30</td>
</tr>
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</table>
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: This research was supported by the Federal Ministry of Education and Science in Bosnia and Herzegovina, and was carried out within the framework of the project “Chemical composition and an antioxidative potential of wild edible mushrooms from Bosnia and Herzegovina” (Grant no. 0101-8183-3/17, dated 01. 12. 2017).

List of abbreviations and symbols

Popis kratica o simbolu

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>AA</td>
<td>amino acids</td>
</tr>
<tr>
<td>AAc</td>
<td>antioxidant activity</td>
</tr>
<tr>
<td>FR</td>
<td>free radicals</td>
</tr>
<tr>
<td>RSA</td>
<td>radical scavenging activity</td>
</tr>
<tr>
<td>TPC</td>
<td>total phenolic content</td>
</tr>
<tr>
<td>DPPH</td>
<td>1,1-diphenyl-2-picrylhydrazyl</td>
</tr>
<tr>
<td>HPTLC</td>
<td>High Performance Thin Layer Chromatography</td>
</tr>
</tbody>
</table>

Fig. 5 – Total phenolic content and RSA of the analysed mushroom species

Slika 5 – Sadržaj ukupnih fenola i RSA analiziranih vrsta glijva

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References


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-argin (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 dobarost na starenjem.

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

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