

# Chemical Ingredients of Fresh and Dry Wild Mushrooms from Bosnia and Herzegovina

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## Abstract

Many species of wild mushrooms are used as a delicacy in the diet, but data on their nutritional value and the effects of their storage on nutritional values are rare. The aim of this study was to determine the content of Free Amino Acids (FAAs), total carbohydrates, vitamin C, and total anthocyanins in six wild mushroom species collected in Bosnia and Herzegovina. Results showed that the drying of mushrooms does not have much influence on the presence of essential and non-essential amino acids. Mushrooms are an excellent source of amino acids whether they are fresh or dry. The total carbohydrate content varied between 12.25-62.75 mg g<sup>-1</sup> for fresh mushroom extracts and 40.98-167.24 mg g<sup>-1</sup> for dry mushroom extracts. The total carbohydrate content in dry mushrooms is significantly higher than in extracts of fresh mushrooms. The vitamin C content of mushrooms varied between 0.02-1.95 mg g<sup>-1</sup> for fresh mushrooms and 0.0-0.63 mg g<sup>-1</sup> for dry mushrooms. A lower vitamin C content was found in dry mushrooms, which can be affected by the method of drying mushrooms. The total anthocyanins content varied between 0.39-0.66 mg CGE mL<sup>-1</sup> for fresh mushroom extracts and 0.10-0.19 mg CGE mL<sup>-1</sup> for dry mushroom extracts. Lower total anthocyanins content was found in dry mushroom extracts, probably due to the destruction of anthocyanins by drying. Our research shows that selected wild edible mushrooms, fresh and dry, have considerable nutritional potential. However, further research is needed on both other nutrients and anti-nutrients in these mushrooms to support their nutritional dominance.

**Keywords:** Free Amino Acids (FAAs), Total Anthocyanins, Total Carbohydrates, Vitamin C, Wild Mushrooms

## 1. Introduction

Since the early beginnings of human civilization, mushrooms have been a very interesting botanical group, and their edibility and therapeutic abilities attracted even the ancient cultures of the Asians, Greeks, Romans, and Egyptians<sup>1,2</sup>. Increasing people's awareness of healthy foods attracted edible mushroom utilization since they provide important macro and micronutrients<sup>3-5</sup>. Many

species of wild edible mushrooms are rich in essential nutrients such as vitamins, minerals, carbohydrates, proteins, various amino acids, fats, and fiber. Many people consume them as a delicacy, and particularly for their specific aroma and consistency. Mushrooms generally contain many essential nutrients in good quantities<sup>6,7</sup>. Therefore, mushrooms represent a balanced nutrient composition and are considered an attractive and substitute source of high-quality protein with essential and

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non-essential amino acids. Due to the content of essential amino acid mushrooms are very useful for a vegetarian diet since they contain some essential amino acids which are found in animal proteins<sup>8</sup>. Also, edible mushrooms contain significant amounts of vitamins such as riboflavin, thiamine, niacin, ascorbic acid, pantothenic acid, and the minerals contained in mushrooms are: iron, manganese, magnesium, zinc, and selenium<sup>9,10</sup>. Carbohydrates are found in large amounts in edible mushrooms, including glycogen, chitin, mannitol, and trehalose. Furthermore, they contain fiber, b-glucans, hemicellulose, and special ingredients. The broad spectrum of carbohydrates in mushrooms ranges from homopolymers to very complex heteropolymers with anti-tumor activity. Certain carbohydrates have been found in various mushrooms that have been shown to have immunomodulatory properties: glucose, fructose, mannose, sucrose, maltose, arabinose, rhamnose, and xylose<sup>11</sup>. Although fruits are known to be the main source of anthocyanins, they are also found in mushrooms. Anthocyanins are a very complex and interesting group due to the great diversity of their presence in nature. They are nutritious bioactive components with a dual significance: firstly, technologically due to their influence on the sensory properties of foods, and secondly biologically due to their numerous health benefits, the most important of which is the cardio protective effect<sup>12,13</sup>. However, there are few studies to evaluate anthocyanins in relation to the antioxidant activity of wild edible mushrooms. One of the studies showed that high amounts of anthocyanins are found in some mushrooms. The high amounts of anthocyanins found in cultivated and wild mushrooms in this study indicate that anthocyanins are important for the conferring of antioxidant activity<sup>14</sup>. Due to the high content of anthocyanins and phenolic compounds but and nutritional values, extracts of many mushrooms are used in the pharmaceutical industry as raw materials for the production of various preparations<sup>15</sup>.

This paper analyzed wild edible mushrooms, which are commonly used in diets and occur in natural habitats in our areas. Given the limited knowledge of the nutritional values of wild edible mushrooms and the effects of their storage on nutritional values, the aim of this paper is to determine the content of free amino acids, carbohydrates, ascorbic acid, and anthocyanins in fresh and dry selected wild edible mushrooms from Bosnia and Herzegovina.

## 2. Experimental

### 2.1 Plant Material (Mushroom Samples)

Mushroom samples: *Lactarius piperatus*, *Boletus edulis*, *Craterellus cibarius*, *Hydnum repandum*, *Cantharellus cibarius*, and *Cantharellus tubiformis* used for the analysis, were collected in Vlastic (44° 16' 60.00" N; 17° 39' 59.99" E). Mount Vlastic is located in the center of Bosnia and Herzegovina. After the collection, the mushroom samples were delivered to the laboratory within 12 h and to the drying plant. The mushroom samples were first cleaned of mechanical impurities and damaged parts were removed. A part of the mushrooms was cut into slices of uniform thickness of 5-7 mm and dried in a universal tunnel dryer (type MTS, Progres Cacak) at 70°C for 4-5 h, and the second part was kept in the fridge at 4°C until analysis. Their identification was based on a comparison of their morphological, anatomical, and physiological characteristics with the monographs mentioned in the relevant literature<sup>16,17</sup>.

### 2.2 Chemicals and Reagents

Ascorbic acid, glucose, bromine, 2,4-dinitrophenylhydrazine (DNPH), acetic acid, sulfuric acid, ethanol, anthrone, potassium chloride, hydrochloric acid 37%, sodium acetate trihydrate were purchased from Sigma-Aldrich (St. Louis, USA); meta-phosphoric acid, and 10% thiourea were purchased from Kemika, Zagreb, Croatia. Amino acid: *L*-valine (Val), *L*-leucine (Leu), *L*-phenylalanine (Phe), *L*-tryptophan (Trp), *L*-methionine (Met), *L*-arginine (Arg), *L*-glycine (Gly), *L*-tyrosine (Tyr), *L*-alanine (Ala), and *L*-cysteine (Cys) were purchased from Fluka Chemicals, Switzerland. The chemicals and solvents used are of analytical quality.

### 2.3 Preparation Solutions

*Metaphosphoric acid - acetic acid*: 8.15 g of solid metaphosphoric acid was dissolved in a mixture of 20 mL acetic acid and 220 mL distilled water in a flask with a volume of 250 mL and the solution produced was filtered.

*2, 4-dinitrophenylhydrazine solution (DNPH)*: 1 g DNPH was dissolved in 100 mL 4.5 M sulfuric acid.

*Anthron*: 100 mg anthron was dissolved in a 50 mL flask in 95% sulfuric acid.

*Standard amino acid solutions:* 1 mg/mL amino acids stock solution is prepared by dissolving 10 mg amino acid in a 10 mL solution of ethanol.

*Standard ascorbic acid solutions:* 1 mg/mL ascorbic acid stock solution is prepared by dissolving 10 mg AA in a 10 mL solution of *meta*-phosphoric acid-acetic acid.

*Standard glucose solution:* 1 mg/mL glucose stock solution is prepared by dissolving 100 mg glucose in 100 mL distilled water.

## 2.4 Sample Preparation for Determination of Ascorbic Acid

The maceration was performed of 2 g of fresh and dry mushrooms in a mortar with 10 mL of a solution of *meta*-phosphoric acid - acetic acid. After maceration, the samples were centrifuged for 20 min. at 4000rpm. The supernatant was decanted and filtered after centrifugation. The filtrate is transferred into a 10 mL flask and then fills the flask volume with a solution of *meta*-phosphoric acid-acetic acid. Samples should be diluted 10 times before measurement.

## 2.5 Sample Preparation for the Determination of Free Amino Acids (FAAs), Total Carbohydrates and Total Anthocyanine

The fresh and dry edible wild mushrooms (1g) were cut into smaller pieces and macerated in a mortar with a pestle, gradually adding 15 mL of ethanol. The mixture was stirred for about 24 h at 20-24 °C. After centrifugation, the supernatants were separated by means of a micropipette and evaporated to dryness under reduced pressure at 40°C. The extracts obtained were stored in glass vials at 4°C. To determine the total carbohydrates, a solution of fresh and dry mushrooms extract in a concentration of 1 mg/mL is first prepared and then diluted with distilled water at a ratio of 1:1000.

## 2.6 Identification and Quantification of Amino Acids

*Amino acids identification:* FAAs in fresh and dry wild mushroom extracts were identified by determining their retention factor  $R_f$ , which is compared to the  $R_f$  values of standard amino acid solutions. Ethanolic extracts of fresh and dry mushrooms and amino acid standard were applied

to a TLC plate previously coated with silica gel (F254). The mobile phase for the development of chromatogram was: 2-butanol: glacial acetic acid: distilled water (8:2:2). Detection took place at 254 nm, and the  $R_f$  values were calculated using the obtained chromatogram<sup>18</sup>.

*HPTLC amino acids quantification:* For the HPTLC analysis of amino acids, 10x10 cm Al plates coated with silica gel 60 F254 were used. Standard amino acid solutions and ethanolic extracts of fresh and dry mushrooms were applied to the Al plates with a sample applicator (Camag Linomat V – Switzerland). Mobile phase: 2-butanol: glacial acetic acid: water 8: 2: 2 was used and a ninhydrin solution was used to visualize the developed plates. Quantification of FAAs performed by scanning plates at 540nm using TLC scanner “Camag” and software “WINCAT”<sup>18</sup>.

## 2.7 Procedure for Total Carbohydrates Determination

The anthron method was used to determine the total carbohydrates in extracts of fresh and dry wild mushrooms<sup>19</sup>. Extracts of fresh and dry mushrooms (0.5 mL) were added to 2 mL. Anthrone solution and heated in a warm water bath for 10 min. When heated, a green color develops, the intensity of which depends on the mass concentration of total carbohydrates. After cooling on ice is performed and the absorption is measured at 630 nm. (spectrophotometer model UV-1280 – Shimadzu). Dilutions of 0.02, 0.04, 0.06, 0.08, and 0.10 mg/mL were prepared from glucose stock solution. Repeat the same procedure as when measuring the extracts of dry and fresh mushrooms, except that the diluted glucose solution is added instead of the mushroom extracts.

## 2.8 Procedure for Ascorbic Acid Determination

Extracts of dry and fresh mushrooms (2mL) were transferred into 10 mL vials, then 115 µL of 3% bromine water was added and mixed well. 65 µL of 10% thiourea and 500 µL of DNPH solution were added. Solutions obtained were heated at 37°C for 3 h. After heating, the solutions were put on ice for 30 min. Chilled 2,5 mL 85% H<sub>2</sub>SO<sub>4</sub> was added to the cold solutions and the absorbency was measured at 521.5 nm. The blank was prepared in the same way, except that instead of the sample solution 2 mL of *meta*-phosphoric acid-acetic acid solution were

added<sup>20</sup>. Standard solutions of ascorbic acid 0.005, 0.010, 0.020, 0.040, 0.080 and 0.100 mg/mL were prepared from a stock solution of 1 mg/mL ascorbic acid by proper dilution. Before the absorption measurement at 521.5 nm (spectrophotometer model UV-1280 – Shimadzu), samples of standard solutions were prepared in the same way as extracts of dry and fresh mushroom samples.

## 2.9 Procedure for Total Anthocyanins Determination

The method of pH differential spectrophotometry was used to determine the total anthocyanins in extracts of dry and fresh wild mushroom<sup>21,22</sup>. Two test tubes were prepared to measure one sample. 1 mL of mushrooms sample solution was pipette into each tube. 2 mL pH 1.0 buffer was then put into one tube, and 2 mL pH 4.5 buffers into the other tube. After 20 min., absorptions at 520 nm and 700 nm (spectrophotometer model UV-1280 – Shimadzu) were measured with the prepared reaction solutions. Buffer pH 1.0 and buffer pH 4.5 were used as blank. The method is based on the knowledge that anthocyanins as organic substances are subject to structural changes when the pH value of the solution changes. At pH = 1.0, anthocyanins are colored, and at pH = 4.5, they are uncolored, which is measured spectrophotometrically at different wavelengths, and absorptions are read. The proportion of total anthocyanins in the extracts of dry and fresh mushroom is calculated using the absorption difference according to the following equation<sup>23</sup>:

$$\text{mg CGE/g} = \frac{A \times MW \times DF \times 1000}{\epsilon \times L}$$

**Table 2.** Free amino acids content (mg g<sup>-1</sup>) of fresh and dry wild mushrooms extracts

Amino acids	L. piperatus		B. edulis		C. cibarius	
	fresh	dry	fresh	dry	fresh	dry
Trp*	0.02±0.09	0.03±0.06	0.02±0.02	0.02±0.01	0.02±0.01	nd
Arg	34.80±0.05	23.90±0.04	3.58±0.04	2.50±0.02	1.01±0.02	0.99±0.01
Cys	2.51±0.10	4.02±0.09	13.20±0.09	nd	1.89±0.02	3.43±0.02
Met*	0.01±0.03	nd	0.02±0.05	1.03±0.02	0.69±0.04	1.10±0.02
Ala	1.63±0.05	2.10±0.04	4.70±0.03	2.93±0.02	6.87±0.03	10.5±0.01
Val*	0.18±0.09	0.11±0.06	1.66±0.02	1.08±0.04	0.23±0.02	1.58±0.02
Gly	4.92±0.03	2.90±0.04	5.12±0.02	3.21±0.03	0.72±0.03	0.51±0.02
Leu*	0.30±0.05	0.28±0.03	0.70±0.03	1.06±0.02	0.28±0.04	0.65±0.04
Phe*	0.31±0.09	0.30±0.04	0.14±0.04	1.16±0.02	nd	0.04±0.02
Tyr	0.14±0.04	1.15±0.02	nd	nd	nd	nd

where:

$A = (A_{520 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH1, 0}} - (A_{520 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH4, 5}}$

**MW**- molecular weight of cyaniding-3-glucoside

**DF**- dilution factor,

$\epsilon$  - molar absorbance

**L** – path length

## 3. Results

The content of ethanolic extracts per 1 g of fresh and dry mushroom samples is shown in Table 1.

The obtained results of HPTLC analysis of FAAs of fresh and dry mushrooms are shown in Table 2.

The results of total carbohydrates, vitamin C, and total anthocyanins of fresh and dry mushroom extracts are shown in Table 3.

**Table 1.** Content of extract per 1g fresh and dry wild mushroom samples

Mushroom species	Content (mg g <sup>-1</sup> ) ± SD	
	fresh	dry
<i>Lactarius piperatus</i>	100 ± 0.98	160 ± 0.99
<i>Boletus edulis</i>	10 ± 1.51	40 ± 1.70
<i>Craterellus cibarius</i>	70 ± 0.85	150 ± 0.98
<i>Hydnum repandum</i>	150 ± 2.20	250 ± 1.45
<i>Cantharellus cibarius</i>	50 ± 0.69	140 ± 0.77
<i>Cantharellus tubiformis</i>	20 ± 0.99	60 ± 0.56

mean ± SD; n = 3

Table 2 Contd...

Amino acids	H. repandum		Cantharellus cibarius		C.tubiformis	
	fresh	dry	fresh	dry	fresh	dry
Trp*	nd	nd	nd	nd	0.04±0.06	0.02±0.04
Arg	8.60±0.02	7.32±0.01	10.4±0.03	9.75±0.01	4.59±0.04	5.07±0.02
Cys	2.01±0.01	3.45±0.02	0.98±0.02	1.14±0.02	2.97±0.02	1.98±0.01
Met*	nd	nd	1.10±0.01	1.31±0.01	nd	0.01±0.04
Ala	1.91±0.02	2.73±0.01	5.81±0.04	7.09±0.02	4.01±0.05	3.66±0.02
Val*	8.67±0.02	7.98±0.02	3.22±0.02	2.78±0.02	2.92±0.04	2.09±0.04
Gly	3.14±0.01	2.99±0.03	4.91±0.02	6.01±0.04	4.01±0.03	4.73±0.03
Leu*	6.01±0.02	5.21±0.01	0.91±0.01	0.99±0.02	5.09±0.04	4.99±0.02
Phe*	0.98±0.02	1.09±0.02	1.78±0.04	2.11±0.01	2.01±0.02	1.73±0.02
Tyr	0.47±0.03	0.58±0.04	nd	nd	2.97±0.04	3.01±0.02

\* - Essential amino acid; **nd**- not detected; -mean ± SD; n = 3

**Table 3.** Total carbohydrates, vitamin C, and total anthocyanins contents in extracts of fresh and dry wild mushrooms

Mushroom species	Total carbohydrates (mg g <sup>-1</sup> )		Vitamin C (mg g <sup>-1</sup> )		Total anthocyanin mg CGE mL <sup>-1</sup>	
	fresh	dry	fresh	dry	fresh	dry
<i>L. piperatus</i>	33.0±0.14	167.24±0.18	0.22±0.09	nd	0.46±0.01	0.19±0.02
<i>B. edulis</i>	24.38±0.20	40.98±0.41	0.04±0.10	nd	0.39±0.02	0.12±0.01
<i>C. cibarius</i>	12.25±0.17	58.67±0.20	1.57±0.05	0.63±0.22	0.66±0.01	0.18±0.04
<i>H. repandum</i>	60.57±0.20	101.93±0.14	0.02±0.02	nd	0.50±0.04	0.10±0.02
<i>Cantharellus cibarius</i>	62.75±0.18	144.38±0.19	1.95±0.04	0.52±0.01	nd	nd
<i>C. tubiformis</i>	33.63±0.22	49.99±0.15	1.25±0.02	0.48±0.01	nd	nd

mean ± SD; n = 3; **nd**- not detected

## 4. Discussion

Content of ethanol extracts of fresh and dry wild mushrooms. According to the literature, ethanol is most commonly used in the extraction of various types of mushrooms, whereby the extracts obtained have beneficial properties in terms of composition and content of nutritional values and pharmacologically important compounds<sup>23,24</sup>. Due to the advantage of ethanol over other solvents and its non-toxicity and possible further use of the extracts in the food and pharmaceutical industries, ethanol extraction of selected wild mushrooms was carried out. *Hydnum repandum* has the highest content of ethanol extract of all analyzed fresh and dry mushrooms and the lowest has *Boletus edulis*.

### 4.1 Amino Acid Compositions

Qualitative TLC identification confirmed the presence of ten FAAs. The results of HPTLC analysis of FAAs of fresh and dry mushrooms are shown in Table 2. The need for proteins in the human diet actually boils down to the need for essential amino acids. The content of amino acids is one of the most important criteria used to determine the quality of proteins. Proteins that contain all essential amino acids in the proportions required by the body are often classified as high-quality biological proteins<sup>25</sup>. In this study, five essentials, and five non-essential amino acids were analyzed in six species of wild mushrooms.

Non-essential acids such as arginine, alanine, and glycine were detected in all six types of fresh and dry

mushrooms. These non-essential acids are known to improve the immune system. Val and Leu, essential amino acids, promote mental strength<sup>26</sup>. These amino acids have been found in all fresh and dry mushrooms. A total of 10 FAAs were found in fresh *L. piperatus* and dry *C. tubiformis*. Met, one of the essential amino acids for humans, was not detected in dry *L. piperatus* and fresh *C. tubiformis*, and fresh and dry *H. repandum*. Cys, a non-essential acid, antioxidant, helps the body protect against radiation and pollution, inhibits aging, helps in protein synthesis and cellular changes<sup>27,28</sup>. This amino acid has not only been found in dry *B. edulis*. Tyr, a non-essential acid, helps to overcome depression, improves memory, increases mental alertness, promotes healthy thyroid, adrenal and pituitary function<sup>29</sup>. Tyr has been found in fresh and dried *L. piperatus*, *H. repandum* and *C. tubiformis*. Phe, an essential amino acid produced by norepinephrine, acts as an antidepressant and improves memory. The highest content of this amino acid is found in dry *C. tubiformis* and not found, only in fresh *C. cibarius*. Trp, an essential amino acid, promotes relaxation, helps with insomnia, and reduces anxiety and depression. This amino acid has not been found in dry *C. cibarius*, fresh and dry *H. repandum* and *Cantharellus cibarius*. Our results for *B. edulis*, *C. cibarius*, and *H. repandum* are similar to those reported by Bakir *et al.* (2018)<sup>29</sup>, for *L. piperatus* and *Cantharellus cibarius* Salihović *et al.* (2019)<sup>30</sup>. Based on the concentrations of FAAs determined by HPTLC, it can be concluded that the method of fungal drying mentioned does not have much influence on the presence of essential and non-essential amino acids.

## 4.2 Total Carbohydrates

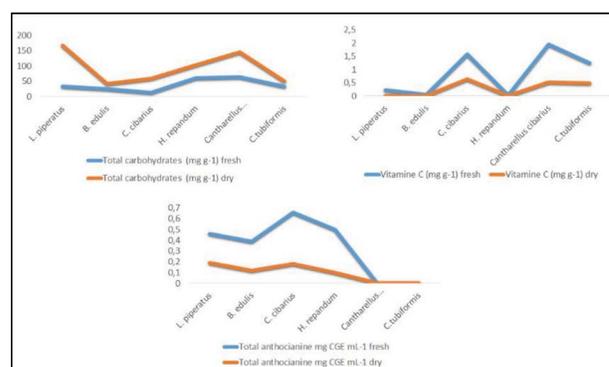
Edible mushrooms have a typical flavor, which comes from the presence of non-volatile constituents, including soluble carbohydrates and FAAs<sup>31,32</sup>. The total soluble carbohydrate content of fresh and dry mushroom extracts was determined using the Anthrone method. Total carbohydrate determination is based on the reaction of carbohydrates from samples with anthrone reagent by boiling in an acidic medium, which leads to the hydrolysis of complex carbohydrates into glucose, resulting in a green-blue color complex<sup>33,34</sup>. After cooling, the absorption is measured spectrophotometrically at 630 nm. The standard linear calibration curve was executed to obtain the linear range of analysis of dry and fresh mushroom extracts, the correlation factor for glucose was

accepted value ( $R^2=0.9988$ ) and the standard calibration curve was linear over the range of 0.02-0.10 mg/mL with the equation ( $y = 7.35x - 0.0154$ ).

The results of the total carbohydrates of extracts fresh and dry wild mushrooms are expressed in mg of total carbohydrates per gram of fresh and dry mass of the sample and are shown in Table 3 and Figure 1. Results show that the total carbohydrate content of mushrooms varied between 12.25-62.75 mg g<sup>-1</sup> for extracts of fresh mushrooms and 40.98-167.24 mg g<sup>-1</sup> for extracts of dry mushrooms. The highest total carbohydrates are found in *Cantharellus cibarius* and the lowest in *C. cibarius*. The total carbohydrate content of mushrooms can be affected by some factors such as genetic factors, the progress phase of the fruitful body, and the environment<sup>35,36</sup>. Our results for the total carbohydrate content were consistent with the results of Beluhan and Ranogajec (2011)<sup>37</sup>, but were lower than those of Turfan *et al.* (2018)<sup>36</sup> for some edible mushrooms. The content of total carbohydrates in mushrooms can deliver to their sweet flavor. Thus, the relatively high sugar content in edible mushrooms is a helpful feature. Our results showed that the total carbohydrate content in dry mushrooms is significantly higher than in extracts of fresh mushrooms, which corresponds to the available literature. Studies have shown that the carbohydrate content is between 35% and 70% of the dry matter, although there are differences between different types of mushrooms. Thermal treatment of mushrooms removes water and concentrates nutrients such as carbohydrates<sup>38,39</sup>.

## 4.3 Ascorbic Acid (vitamin C)

For the quantitative analysis of vitamin C in fresh and dry wild mushrooms, UV-Vis spectrophotometry was



**Figure 1.** Content of total carbohydrates, vitamin C, and total anthocyanins of fresh and dry mushrooms.

chosen. Vitamin C reacts in the sample with the acidic DNPH, whereby it is oxidized to dehydroascorbic acid in the presence of bromine water. Subsequently, L-dehydroascorbic acid reacts with DNPH to form osazone, which, treated with 85% H<sub>2</sub>SO<sub>4</sub>, gives the solution a red color. After cooling, the absorption is measured at 521.5 nm. The ascorbic acid content was calculated based on the calibration curve of the authentic L-ascorbic acid (0.05-0.10 mg/mL;  $y = 33.051x + 0.4343$ ;  $R^2 = 0.9851$ ).

The results for vitamin C of fresh and dry wild mushrooms were expressed in mg vitamin C per gram of fresh and dry wild mushrooms and are shown in Table 3 and Figure 1. Results show that the vitamin C content of mushrooms varied between 0.02-1.95 mg g<sup>-1</sup> for fresh mushrooms and 0.0-0.63 mg g<sup>-1</sup> for dry mushrooms. The highest vitamin C content was found in *Cantharellus cibarius* and the lowest in *H. repandum*. It is known that *Chanterelle* is a health-promoting nutrient with a high content of vitamins B and C, contains a large number of carbohydrates and proteins, and has a low content of fats, phenolic compounds, and organic acids<sup>40,41</sup> which corresponds to our results. The data obtained indicate that lower vitamin C content has been found in dry mushrooms, which is to be expected as the vitamin C content can also be influenced by the drying method<sup>42</sup>. Oxidation and degradation of vitamin C are stimulated by many factors such as the influence of light, elevated temperature, enzymes, heavy metals, and alkaline media<sup>43,44</sup>. Our results for vitamin C content were consistent with the results reported by Ferreire, *et al.* (2009)<sup>45</sup>, but were higher than those reported by Ozen *et al.* (2011)<sup>46</sup> for some edible mushrooms.

#### 4.4 Total Anthocyanins

The differential pH method was used to determine the total anthocyanin concentration, which is based on the change in anthocyanin color<sup>21</sup>. Therefore, the method is based on the knowledge that a change in the pH value causes a structural change in the anthocyanins. At pH = 1.0, anthocyanins are stained and at pH = 4.5, they are unstained. The proportion of total anthocyanins in the sample is calculated using the difference in absorptions according to the above formula. Results for total anthocyanins for fresh and dry wild mushroom extracts were expressed as equivalent to cyanidin-3-glucoside (mg CGE mL<sup>-1</sup>) of fresh and dry wild mushrooms and are presented in Table

3 and Figure 1. The results show that the total anthocyanins content in mushrooms varied between 0.39-0.66 mg CGE mL<sup>-1</sup> for fresh mushroom extracts and 0.10-0.19 mg CGE mL<sup>-1</sup> for dry mushroom extracts. In *Cantharellus cibarius* and *C. tubiformis* total anthocyanins were not found. The highest total anthocyanins content was found in *C. cibarius* and the lowest in *H. repandum*. Our results for the total anthocyanins content were consistent with those reported by Ozen *et al.* (2011) for some mushroom species<sup>46</sup>. Dry mushroom extracts have significantly lower total anthocyanins content, probably due to their drying destruction<sup>47</sup>. Studies have shown that a significant amount of anthocyanin is lost in the heat treatment of some fruits and foods<sup>48</sup>. In addition to heat and many other factors such as light, temperature, storage and tillage are also responsible for the degradation of anthocyanins<sup>49</sup>. Several factors can influence the composition of the mushrooms, including harvest time, mushroom species, environmental factors, and analysis methods<sup>50,51</sup>.

## 5. Conclusion

We have presented here amino acid compositions, the content of total carbohydrates, vitamin C, and total anthocyanins in the ethanol extracts of fresh and dry edible wild mushrooms, which are widely used in Bosnia and Herzegovina. Results show that fresh and dry analyzed mushrooms contain significant amounts of essential and non-essential amino acids. Our results show that the extracts of all six fresh and dry mushrooms have a significant amount of total carbohydrates and vitamin C, but anthocyanins, not only found in *Cantharellus cibarius* and *C. tubiformis*. Vitamin C content not found in extracts of dry *L. piperatus*, *B. edulis*, and *H. repandum*. Results of this study show that selected wild mushrooms, both fresh and dry, have considerable nutritional potential. By consuming these fresh or dry wild mushrooms, the recommended daily intake of most nutrients can be achieved. Knowledge of the nutritional and biologically active properties of wild edible mushrooms increases their consumption. However, knowledge of anti-nutrients and toxicity factors in wild edible mushrooms is also necessary to support their predominance in the diet.

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