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AMINO ACID COMPOSITION AND ANTIOXIDANT PROPERTIES OF FIVE EDIBLE MUSHROOM SPECIES FROM KASTAMONU, TURKEY

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Abstract

Background: *Hydnum repandum*, *Cantharellus cibarius*, *Ramaria fennica*, *Boletus edulis*, and *Craterellus cornucopioides* are all wild edible mushroom species from the Kastamonu province. The aim of this study was to investigate antioxidant properties and amino acid contents of these mushrooms.
Materials and Methods: The mushrooms were analyzed for their free amino acid compositions using a high performance Amino Acid analyzer (Biochrom 30). Also, antioxidant activity and total phenolic concentrations of five different mushroom species were studied by using spectrophotometric methods.
Results: The mushrooms contained 17 amino acids (g/100 g): Glutamic acid (2.56–1.11), Alanine (1.49–0.54), Arginine (1.62–6.77), Aspartic acid (1.45–0.81), Leucine (1.08–0.64), Methionine (1.05–0.06), Valine (1.05–0.66), Lysine (1.01–0.57), Serine (0.68–0.38), Cysteine (0.88–0.11), Isoleucine (0.61–0.39), Glycine (0.82–0.35), Threonine (0.82–0.44), Phenylalanine (0.66–0.42), Proline (0.60–0.47), Tyrosine (0.58–0.38), and Histidine (0.48–0.22). The total free amino acid (TAA) contents ranged from 169.2 mg/g in *Boletus edulis* to 89.1 mg/g in *Hydnum repandum*. These five different mushroom species contain eight essential amino acid species except tryptophan. The antioxidant activity of mushroom extracts was expressed as the percentage of DPPH radical inhibition and IC_{50} values (mg/mL). The percentage of inhibition ranged from 2.38% to 88.05% and IC_{50} values ranged from 0.03 to 13.98 mg/mL. The total phenolic content ranged from 0.66 to 7.74 mg/g of dry mushroom extract, expressed as gallic acid equivalents.
Conclusion: Methanolic extract of *Boletus edulis* showed the highest phenolic content and strong antioxidant activity. As a result, the significant linear correlation between the values for the total phenolic content and antioxidant activity of mushroom extracts was corroborated in all of the investigated mushroom species, with the exception of *Craterellus cornucopioides*.

Key words: Edible mushrooms, Amino acids, Total phenolics, Antioxidant properties, Kastamonu

Introduction

Edible mushrooms are grown and consumed because of their delicious taste and a wide variety of aromatic flavors (Bailey and Day 1989, Breene 1990). They are used in traditional medicines because of their chemical properties. Mushrooms are rich in protein and carbohydrates and low in fat and calories. In addition, they contain high dietary fibers; edible vitamins (B1, B2, B12, C, D2 and E); and minerals (Kalač 2009). Phenolic acids are the major phenolic compounds found in mushrooms (Alispahić *et al.* 2015, Sun *et al.* 2017). Mushrooms are a good source of essential and non-essential amino acids (Chang and Miles 1989). The amino acid contents vary according to mushroom species (Ferreira *et al.* 2016) and are an indicator of the quality of the mushrooms and their nutritional value (Sudheep and Sridhar 2014, Teklit 2015). The taste of a mushroom is determined by the concentrations of aspartic and glutamic acids; sweet amino acids (alanine, glycine, and serine-threonine); bitter amino acids (arginine, histidine, isoleucine, leucine, methionine, phenylalanine, and valine); and tasteless amino acids (lysine and tyrosine) (Pomeranz 2012, Kalač 2016).

Mushrooms are useful candidates in the search for an effective antioxidant because of their naturally occurring composition (Jean-Philippe 2005). Certain mushrooms have been used as supplements to improve human health. Foods rich in antioxidants are now consumed more regularly because antioxidants or molecules with radical scavenging capacity are thought to exert a potential protective effect against free radical damage. These biomolecules prevent coronary and vascular diseases and tumor formation by inhibiting oxidative reactions.

Although edible wild mushrooms are more expensive than cultivated mushrooms, their taste, nutrition, and pharmacological properties are becoming increasingly important in our diet. Cultivated and wild edible mushroom species have been widely investigated in the northern hemisphere; however, little is known about the antioxidant properties of wild edible mushrooms from Turkey (Gursoy *et al.* 2009), in particular, the amino acid and phenolic contents of the species *Hydnum repandum* (*Hre*), *Cantharellus cibarius* (*Cci*), *Ramaria fennica* (*Rfe*), *Boletus edulis* (*Bed*), and *Craterellus cornucopioides* (*Cco*) from the Kastamonu region. The aim of this study was to determine the antioxidant properties, phenolic compounds, and amino acid contents of these five mushroom species.

Materials and Methods

Hre, *Cci*, *Rfe*, *Bed*, and *Cco* were the five wild edible mushroom species studied herein. They are consumed regularly and are available at local markets in the Kastamonu province.

All analytical grade chemicals were purchased from Sigma–Aldrich Co. LLC. Deionized purity water was used at each stage. Absorbents were measured using a Shimadzu UVM-1240 UV-Visible spectrophotometer (Shimazu Corp., Kyoto, Japan), and Biochrom 30 was used for amino acid analysis.

Sample collection and preparation

Fresh mushrooms (*Hre*, *Cci*, *Rfe*, *Bed*, and *Cco*) were collected from the towns of Inebolu, Taşköprü, and Devrekani of the Kastamonu province, Turkey (Table 1). The collected mushrooms were divided into 100 g samples. The samples were then dried at 30°C for 48 h. The dried mushrooms were homogenously powdered for use in the analyses.

Table 1: Collection points and voucher numbers for each mushroom species

Muhroom species	Voucher Numbers*	Collection point
Boletus edulis	370012	Taşköprü – Kastamonu, Turkey
Cantharellus cibarius	370013	Inebolu– Kastamonu, Turkey
Craterellus cornucopioides	370015	Devrekani– Kastamonu, Turkey
Hydnum repandum	370018	Inebolu– Kastamonu, Turkey
Ramaria fennica	370019	Taşköprü– Kastamonu, Turkey

*****Registration number for each mushroom species at Mushroom Research and Application Center of Kastamonu University.

Mushroom identification

The mushroom species were identified by Prof. Dr. Sabri ÜNAL at the Kastamonu University Mushroom Research and Application Center.

Boletus edulis

The average cap width of *Bed* is 24 cm. The cap is reddish brown, flat, and slightly sticky to touch. The stipes is 17 cm long, club-shaped and yellowish white. The underside of the cap comprises thin tubes.

Cantharellus cibarius

Cci is orange or yellow, meaty, and is funnel-shaped mushroom. Beneath the smooth cap, gill-like ridges run almost all the way down the stipe. The average cap width and stipe length are 6.8 and 5 cm, respectively.

Craterellus cornucopioides

Cco is funnel shaped and gray, brown, or black. They often grow in small bunches, although sometimes they grow singly. It comprises a flower-like cap with an average width of 3.4 cm. The stipe is hollow and very thin with an average height of 6.2 cm.

Hydnum repandum

The tan-colored cap of *Hre* is generally irregular in shape with an average width of 11.6 cm. The underside of cap is covered with small white spines that resemble those of a hedgehog. The stipe is 7.3 cm long and white.

Ramaria fennica

The average height and width of the fruiting body of *Rfe* are 10.8 cm and 7.2 cm, respectively. The base is 4.8 cm wide, white, and well developed. The many branches are vertically oriented and elongated. The cap is smooth and olive-yellow.

Analysis of free amino acid concentration

Amino acid analysis was performed with a high performance amino acid analyzer (Biochrom 30). Hydrolysis was performed in a closed conical flask to determine all amino acids except tryptophan. 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 was 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. The pH was adjusted to 2.20 using sodium hydroxide solution. Sodium citrate buffer (pH 2.20) was added to the hydrolyzed sample. Once all the soluble material was completely dissolved, the sample was ready for analysis (AOAC 2012).

Analysis of antioxidant components

Preparation of mushroom extracts for antioxidant components

For methanolic extractions, 5 g samples of each mushroom species were extracted by mixing with 30 mL of 80% methanol at 25°C for 3 h and filtration through Whatman filter paper (No.1). The residue was then extracted with two 20 mL portions of methanol. The combined ethanolic extracts were filtered through Whatman filter paper (No.1) after storing the extracts for 2 h at room temperature. The resulting homogenate was centrifuged at 5000 rpm for 10 min (4°C). The derived supernatant was centrifuged at 7500 rpm for 10 min. The final supernatant (100 mg/mL) was used for 1,1-diphenyl-2-picrylhydrazyl (DPPH) measurements and stored at 4°C (Pedraza-Chaverri *et al.* 2004, Lee *et al.* 2004).

Evaluation of antioxidant properties using the DPPH method

The method proposed by Shimada *et al.* (1992) and Brand-Williams *et al.* (1995) was slightly modified, and free radical scavenging ability on DPPH was performed using the modified method. Different volumes (X: 0.05–0.20 mL) of the extracts in methanol (3-X mL) were mixed with 3 mL of the methanolic solution containing DPPH (Sigma) radicals; the total volume of the reaction mixture was 6 mL. This resulted in a final concentration of (stock 4.0×10^{-4} M) DPPH. The mixture was shaken vigorously and left to stand for 30 min in the dark; the absorbance was then measured at 517 nm against a blank. The percentage radical scavenging activity was calculated from the following formula:

$$\% \text{ scavenging [DPPH]} = [(A_0 - A_1) / A_0] \times 100,$$

Where A_0 is the absorbance of the control solution (no antioxidant added) and A_1 is the absorbance of the sample solution (when antioxidant was present) (Huang *et al.* 2005). The amount of antioxidant required to reduce the DPPH concentration by 50% is a commonly used parameter to measure the antioxidant activity and is called IC_{50} (mg/mL) (Frankel and Meyer 2000). The IC_{50} value was determined from the graph slope "y = mx + c" formula obtained from the inhibition (%) concentration graph for mushroom extracts (Mukherjee *et al.* 2011).

Determination of total phenolic content

The concentration of phenolics in the mushroom extracts was determined using a spectrophotometric method (Singleton *et al*. 1999). A methanolic solution was used at a concentration of 1 mg/mL. The reaction solution was prepared by mixing 0.5 mL of methanolic solution of mushroom extracts, 2.5 mL of 10% Folin Ciocalteu's reactant, which was dissolved in water, and 2.5 mL 7.5% $NaHCO_3$. The blank was prepared by adding 0.5 mL methanol to 2.5 mL 10% Folin Ciocalteu's reactant dissolved in water, and 2.5 mL of 7.5% $NaHCO_3$. The samples were then incubated at 45°C for 45 min. The same procedure was repeated for the gallic acid solution. The absorbance was determined using a spectrophotometer at $\lambda max = 765$ nm. The phenolic content of the extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract) after the concentration of phenolics (ppm) was read from the calibration line.

Statistical analysis

For each mushroom species, three samples were used; the antioxidant content of each was calculated using descriptive statistical analysis with Microcal Origin Pro 8.5.1 (Origin Lab. Corp., Northampton, MA, USA). Statistically significant effects were investigated using SPSS software (SPSS Inc., Chicago, IL, USA) for Windows version 13.

Results and Discussion

Free amino acid concentrations in the five mushroom species

In this study, eight essential and nine non-essential amino acid species were identified in the five wild mushroom species. In all five of the mushroom species, glutamic acid was the predominant non-essential amino acid. It was the main compound in *Bed* followed by *Ref*, *Cci*, *Cco*, and *Hre*. These results clearly indicate that glutamic acid, which is the major compound in all species, contributes to the primary taste of the mushrooms (Yamaguchi *et al.* 1971, Dutta *et al.* 2013). Several reports have shown that glutamic acid, which is an anti-cancer agent, is the highest total free amino acid (TAA) in mushrooms (Sato *et al.* 1985).

The free amino acid content ranged from 5.8 ± 0.01 to 25.6 ± 0.05 mg/g in *Bed*, from 0.6 ± 0.00 to 11.1 ± 0.03 mg/g in *Hre*, from 1.1 ± 0.00 to 17.5 ± 0.02 mg/g in *Cci*, from 1.8 ± 0.00 to 19.4 ± 0.04 mg/g in *Ref*, and from 1.1 ± 0.00 to 13.7 ± 0.04 mg/g in *Cco* (Table 2 and Figure 1).

Table 2. Free amino acid content in *Bed*, *Hre*, *Cci*, *Ref*, and *Cco*

Amino acid	Content (mg/g dry weight)				
	Hydnum repandum	*Cantharellus cibarius*	*Ramaria fennica*	*Boletus edulis*	*Craterellus cornucopioides*
Aspartic acid (Asp)	8.1 ± 0.03	10.9 ± 0.02	11.5 ± 0.04	14.5 ± 0.04	9.4 ± 0.03
Threonine (Thr)	4.4 ± 0.01	5.9 ± 0.01	7.0 ± 0.03	8.2 ± 0.03	4.6 ± 0.01
Serine (Ser)	4.5 ± 0.01	5.9 ± 0.01	6.4 ± 0.03	6.8 ± 0.03	3.6 ± 0.01
Glutamic acid (Glu)	11.1 ± 0.03	17.5 ± 0.02	19.4 ± 0.04	25.6 ± 0.05	13.7 ± 0.04
Glycine (Gly)	3.5 ± 0.01	4.5 ± 0.01	5.8 ± 0.01	8.2 ± 0.03	4.5 ± 0.01
Alanine (Ala)	7.2 ± 0.03	7.4 ± 0.03	9.4 ± 0.03	15.1 ± 0.03	6.3 ± 0.02
Valine (Val)	7.7 ± 0.03	7.9 ± 0.03	8.8 ± 0.03	10.5 ± 0.04	6.6 ± 0.03
Isoleucine (Iso)	3.9 ± 0.01	4.8 ± 0.01	5.2 ± 0.01	6.1 ± 0.03	4.1 ± 0.01
Leucine (Leu)	7.1 ± 0.03	8.1 ± 0.03	8.8 ± 0.03	10.8 ± 0.04	6.4 ± 0.03
Tyrosine (Tyr)	4.7 ± 0.01	4.0 ± 0.01	4.3 ± 0.01	5.8 ± 0.01	3.8 ± 0.01
Phenylalanine (Phe)	5.0 ± 0.02	5.2 ± 0.02	6.1 ± 0.03	6.6 ± 0.02	4.6 ± 0.02
Histidine (His)	2.2 ± 0.01	3.0 ± 0.01	2.6 ± 0.01	4.8 ± 0.01	2.2 ± 0.00
Lysine (Lys)	6.0 ± 0.02	7.7 ± 0.03	6.5 ± 0.03	10.1 ± 0.03	5.7 ± 0.02
Arginine (Arg)	6.2 ± 0.02	14.9 ± 0.04	7.6 ± 0.03	10.9 ± 0.04	5.4 ± 0.02
Prolin (Pro)	5.3 ± 0.01	4.7 ± 0.01	6.0 ± 0.03	5.9 ± 0.01	5.1 ± 0.01
Cysteine (Cys)	1.6 ± 0.01	1.1 ± 0.00	2.7 ± 0.01	8.8 ± 0.03	4.5 ± 0.01
Methionine (Met)	0.6 ± 0.00	1.3 ± 0.00	1.8 ± 0.00	10.5 ± 0.04	1.1 ± 0.00

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

Several factors can affect the composition of mushrooms, including time of harvest, species, environmental factors, and method analysis (Manzi *et al.* 2001). Sun *et al.* (2017) reported that the total amino acid composition of 13 popular wild edible mushroom species from the Yunnan Province ranged from 14.62 to 131.06 mg/g. The synergistic effect of glutamic and aspartic acid are thought to contribute to the umami taste of mushrooms (Baars *et al.* 2014). We found the maximum concentration of aspartic acid in *Bed* (14.5 ± 0.04 mg/g) and the minimum aspartic acid concentration in *Hre* (8.1 ± 0.03 mg/g).

Aspartic acid had the second largest concentration in four of the studied species (except *Cci*), and the methionine concentration was low in *Hre*, *Cco*, and *Cci*, as reported by Ribeiro (2008).

Alanine was the most abundant compound in *Bed*. The concentration of serine was the highest in *Bed*, and lowest in *Cco* compared to all the other mushroom species. *Cci* had the highest concentration of arginine, and the maximum concentration of cysteine was recorded in *Bed*, proline in *Ref*, and tyrosine in *Bed*.

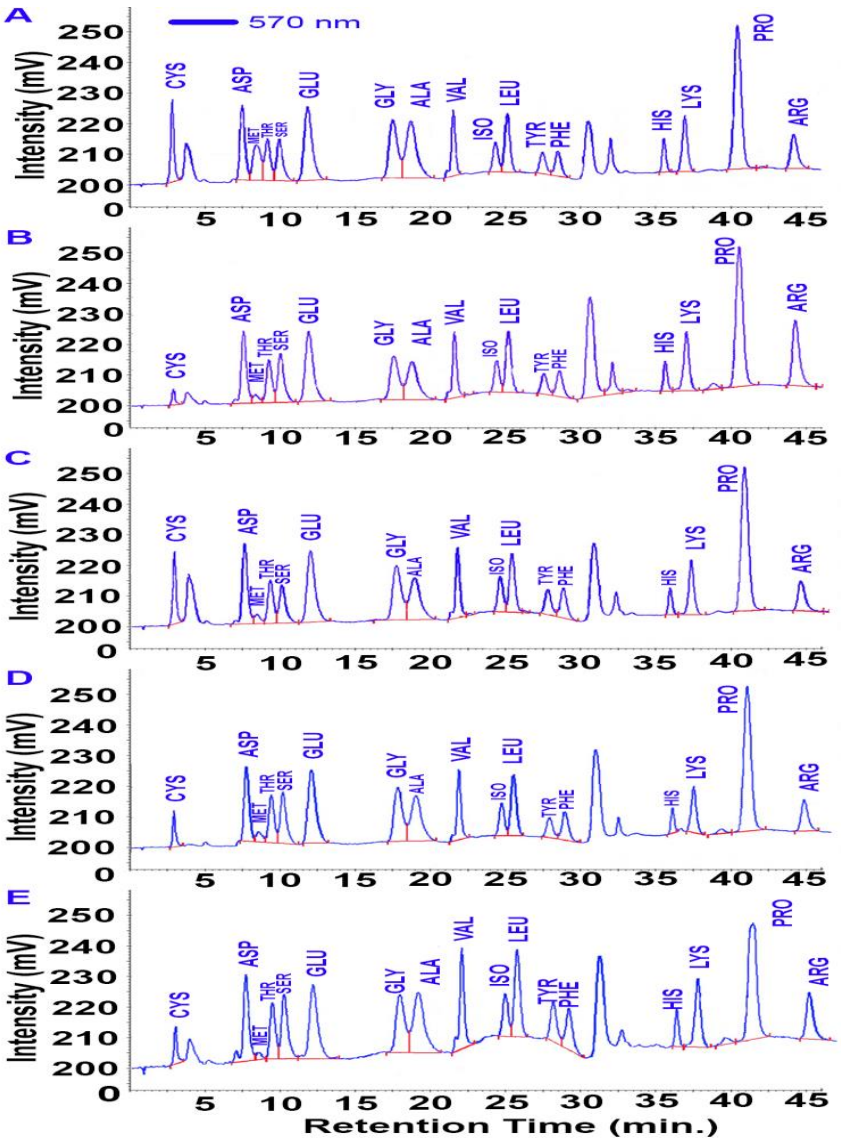

Figure 1. Typical chromatograms of free amino acids determined by high performance Amino Acid Analyzer Biochrom 30. **(A)** *Boletus edulis*, **(B)** *Cantharellus cibarius*, **(C)** *Craterellus cornucopioides*, **(D)** *Ramaria fennica*, and **(E)** *Hydnum repandum*. **Cys** (Cysteine), **Asp** (Aspartic acid), **Met** (Methionine), **Thr** (Threonine), **Ser** (Serine), **Glu** (Glutamic acid), **Gly** (Glycine), **Ala** (Alanine), **Val** (Valine), **Iso** (Isoleucine), **Leu** (Leucine), **Tyr** (Tyrosine), **Phe** (Phenylalanine), **His** (Histidine), **Lys** (Lysine), **Pro** (Proline), and **Arg** (Arginine).

Bed is the most abundant mushroom species in terms of essential amino acids. Histidine content was the lowest compared to the other amino acids. The highest concentrations of glutamic acid, methionine and valine were found in *Bed*. The lowest concentrations of valine and lysine were found in *Cco*; the lowest concentration of isoleucine (3.9 ± 0.01 mg/g), glycine (3.5 ± 0.01 mg/g), and threonine (4.4 ± 0.01 mg/g) were found in *Hre*. Phenylalanine concentration was the highest in *Bed* and lowest in *Cco*. These results agree with those reported by Petrovska (2002), and the *Boletus* species are similar to those reported by Sun *et al.* (2017). In contrast, Ribeiro *et al.* (2008) and Tsai *et al.* (2008) reported low amino acid levels in *Bed*. Our findings for the amino acid distribution in *Cci* are similar to those reported by Danell and Eaker (1992), whereas Mdachi *et al.* (2004), Agrahar-Murugkar and Subbulakshmi (2005) and Ribeiro *et al.* (2008) found different values. Our findings for *Cco* are in agreement with those reported by Liu *et al.* (2012); however, although Beluhan and Ranogajec (2011) reported similar results for threonine and lysine, their findings for the other amino acids were different.

The amino acid composition in *Rfe* was similar in comparison to other *Ramaria* species, e.g., León-Guzmán et al. (1997) reported similar results in *Ramaria flava*, and Agrahar-Murugkar and Subbulakshmi (2005) also found low levels of amino acids in *Ramaria brevispora*. The lowest amino acid content in the mushroom samples was 89.1 mg/g in *Hre*, which was in agreement with the results presented by Kalač (2016).

The correlation between each mushroom species was significant at $p<0.01$ and (r) values ranged from 0.728 to 0.962 for the 17 amino acids. When the correlation coefficients (r) were examined for the 17 amino acid species statistically, it was found that the lowest correlation existed between arginine (Arg) and proline (Pro). Arginine exhibited lower correlation values than the other amino acids. In contrast, the highest correlation coefficient was found to be 0.998 for ($p < 0.01$, $n = 5$) significance between aspartic acid (Asp) and glutamic acid (Glu).

Antioxidant properties and total phenolics

The antioxidant properties and total phenolics assayed here are summarized in Table 3, and the results were explained as IC_{50} values (mg dry weight of various extracts per mL) for comparison.

Table 3. Inhibition (%) and IC_{50} values calculated using the DPPH method and total phenolic values measured using Folin Ciocalteu method for mushroom species

	Concentration (mg/mL)*	Inhibition (%)	IC_{50} (mg/mL)	Total phenolics (mg/L)
**Boletus edulis*	0.83	50.40	0.03	7742.2
	1.67	86.53		
	2.50	87.91		
	3.33	88.05		
**Hydnum repandum*	0.83	12.80	13.98	1320.9
	1.67	13.95		
	2.50	14.71		
	3.33	20.61		
**Cantharellus cibarius*	0.83	10.09	12.98	2178.8
	1.67	12.38		
	2.50	15.85		
	3.33	18.09		
***Ramaria fennica*	0.83	17.42	2.87	6431.6
	1.67	39.17		
	2.50	40.36		
	3.33	57.45		
Craterellus cornucopioides	0.83	2.38	8.50	657.4
	1.67	8.09		
	2.50	12.52		
	3.33	18.09		

Mushroom extract concentrations (c*): 0.83, 1.67, 2.50, 3.33 mg/mL
The 95% confidence interval of the difference test for the concentration-inhibition pairs are significant at the *$p < 0.01$ and **$p < 0.05$ level.

Percent inhibitions for each mushroom species increased in direct proportion with increasing mushroom concentration (0.83–3.33 mg/mL). Bed showed the highest antioxidant activity and *Cco* showed the lowest antioxidant activity when the concentrations were considered separately. DPPH radical scavenging capacities for mushroom species were determined by IC_{50} value. In scavenging ability on DPPH radicals, the effectiveness was in descending order: (*Bed*) > (*Rfe*) > (*Cco*) > (*Cci*) > (*Hre*).

Total phenols were found in the range of 7.74–0.66 mg gallic acid equivalents/g for methanolic extracts as a major naturally occurring antioxidant component. The contents of total phenols were found in descending order of (*Bed*) > (*Rfe*) > (*Cci*) > (*Hre*) > (*Cco*). The order of total phenolic contents exhibited similar results with IC_{50} values, with the exception of *Cco*.

A relative correlation between concentration and the percent of inhibition for each mushroom species was obtained. *Cco* and *Cci*, which were measured by the DPPH method, showed a good significance ($p<0.01$, $n=4$). The values ofr were 0.999 and 0.996 between concentration and the percent of inhibition, respectively. Likewise, *Rfe* showed good significance ($p<0.05$, $r=0.955$). *Bed* and *Hre*, which were also measured by the DPPH method, the values of r were 0.797 and 0.895, respectively.

The 95% confidence interval of the difference test for the concentration-inhibition pairs was at a level of significance $p<0.01$ for *Bed*, *Hre*, *Cci*, and $p<0.05$ for *Rfe*. The standard deviation value (SD) for concentration-inhibition comparisons was 1.075.

Total phenolics are directly correlated with the change in antioxidant properties. Therefore, statistically correlation coefficients were found ($r= -0.887$) at the significant level ($p<0.05$) between total phenolic contents values and IC_{50} values for all mushrooms. Accordingly, the negative correlation found confirmed the inverse relation between IC_{50} and total phenolic concentration.

Ahmad and Mukhtar (1999) proposed good antioxidant properties of phenols because of their scavenging abilities on free radicals and chelating abilities on metal ions. Tsai *et al.* (2007) and Dubost *et al.* (2007) found a good correlation between total antioxidant components and polyphenols.

Conclusion

This study was conducted to assess the nutritional value of mushroom species that are widely consumed and form a commercial source of income in the Kastamonu region.

The results of the analysis show the essential and non-essential amino acid compositions of the mushrooms. In particular, non-essential amino acids such as glutamic acid, which also play a role in the formation of the essential

amino acids, were found extensively in the studied mushroom species. Among these five mushroom species, *Bed* and *Rfe* had the highest antioxidant activity and amino acid contents. In addition, antioxidant activity and total phenolic substance concentrations for these mushroom species followed a linear correlation, with the exception of *Cco*. As a result, it was found that the total phenolic contents had a significant effect on the antioxidant activity, but the antioxidant activity was also determined by the different compositions. Antioxidant activity may also be affected by the amino acid content, which is responsible for typical mushroom tastes and natural values of these five mushroom species.

Conflict of interest: The authors have no conflict of interest in terms of the publication of this work.

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