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Nutritional and Antioxidant Variability of Some Wild and Cultivated Edible Mushrooms from Kastamonu Rural Areas#

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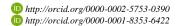
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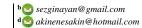
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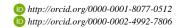
ABSTRACT

In this study, variation of some chemical components such as anthocyanin, β-carotene, lycopene, phenolic, nitrate, soluble protein, proline, glucose, sucrose and total carbohydrate level ad PAL activity in some wild and cultivated edible mushrooms was examined. For this, four different mushroom species (Agaricus campestris L., Cantharellus cibarius Fr., Hericium erinaceus (Bull.) Pers., Lactarius piperatus L. Pers) were supplied from local market, named Kuzeykent Semt Bazaar, in Kastamonu province of Turkey. Mushroom samples were collected from Araç, Daday, Devrekani and Tosya locations of Kastamonu. According to findings, the highest anthocyanin value and PAL activity were obtained from A. campestris collected from Arac location with 0.107 mg g⁻¹ and 6.99 EU, respectively. The amount of β -carotene (2.297 mg g⁻¹) and lycopene (0.644 mg g⁻¹) was the highest in C. cibarius collected from Tosya location, however; proline, soluble protein, nitrate and glucose level were the maximum in A. campestris collected from Devrekani location with 149.61 μmol g⁻¹, 55.49 mg, 159.963 mg g⁻¹ and 29.36 μg g⁻¹, respectively. While total carbohydrate was the highest in H. erinaceus collected from Araç location with 80.97 μg g⁻¹, sucrose concentration was the maximum with 39.22 mg g⁻¹ in *H. erinaceus* collected from Daday location. As a result, *A.* campestris collected from Devrekani location exhibited the highest nutrient in terms of chemicals analysed except anthocyanin and it was followed by *H. erinaceus* collected from Daday location. However, C. cibarius and H. erinaceus collected from Araç location had lower chemical components. It can be said that these mushroom species are valuable and important as major food sources and non-wood products for Kastamonu province.











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Introduction

Mushrooms have been consumed as food and sometimes used as medicine for centuries all over the world. They have cultivated for long years thanks to higher nutrients such as minerals, protein, amino acid, vitamins and fibres and also low in calories. Furthermore, mushrooms also contain antioxidant as phenolic, anthocyanin, enzymes, glucans which are extremely beneficial for human health (Kozarski et al., 2015; Taşkın et al., 2015; Bulam et al., 2019). They can consume as both fresh and dried. Recently, mushrooms have assessed as an attractive functional food mainly in daily nutrition because of their chemical and antioxidative properties (Heleno et al., 2015; Islam et al., 2017). It has been reported that antioxidant compounds as enzymatic and non-enzymatic precluded oxidative damage caused by free radicals regarding with aging and diseases such as atherosclerosis, diabetes, cancer and cirrhosis. Mushroom species can be used to decrease oxidative damage due to higher nutrient and chemical compositions (Patel and Goyal, 2013; Alispahić et al., 2015). There are over 100,000 mushroom species growing in nature. About 300 species of edible mushroom species growing in nature of Turkey (Anonymous, 2018). Turkey is a country extremely rich in terms of variety of wild mushrooms because of favourable climatic conditions. Turkey is also one of important wild mushroom exporters in the world (Peksen and Akdeniz,

2012; Bulam et al., 2018; Bengu et al., 2019). Among these, the most preferred in terms of taste are *Morchella* sp., *Boletus edulis* (Bull.), *Hydnum rufescens* Pers. Fr., *H. repandum* L. Fr., *Tuber melanosporum* Vittad, *T. magnatum* Picco, *T. aestivum* Vittad., *Terfezia claveryi* Chatin, *Lactarius deliciosus* (L.) Gray, *L. semisanguifluus* R. Heim & Leclair, *L. vellereus* (Fr.) Fr., *L. vinosus* (Quél.) Bataille, *Cantharellus cibarius* Fr., *Amanita caesaera* (Scop.) Pers. (Taşkın et al., 2012; Kucuker, 2019). In a study carried out by Inci and Kırbag (2018) on the antimicrobial effect of *T. claveryi*, it was found that this species is highly effective even at low concentrations.

Some of these mushroom species grow naturally in forest areas of Kastamonu-Turkey too. Especially, Pleurotus ostreatus (Jacq.) P. Kumm and Agaricus bisporus (J. E. Lange) Imbach having easy cultivation methods and also Lactarius deliciosus (L.) Gray collected from nature have been preferred by the local people (Ayaz et al., 2011; Bakır et al., 2017). There are many studies performed by the several authors for nutritional level, chemical composition and antimicrobial activity of edible, wild and cultivated mushroom species (Sevindik et al., 2017; Sevindik, 2018). However, there are limited researches related with nitrate, proline, total sugar, phenolic level and phenylalanine lyase (PAL) activity in some edible wild and cultivated mushroom species. In this study, mushroom species selected from wild and cultivated ones from different locations of Kastamonu were analysed for nitrate, total sugar, phenolic level and phenylalanine lyase activity. For this purpose, Agaricus campestris, Cantharellus cibarius, Hericium erinaceus, Lactarius piperatus were collected from Daday, Tosya, Devrekani and Arac districts of Kastamonu and were investigated for their chemical compounds.

Material and Method

Material

Mushroom samples of *Agaricus campestris* L., *Cantharellus cibarius* Fr., *Hericium erinaceus* (Bull.) Pers., *Lactarius piperatus* L. Pers. were provided from local markets of different districts of Kastamonu such as Daday, Arac, Tosya and Devrekani in the second week of July in 2019. Some information about mushroom samples used has been presented in Table1.

Method

In this study, variation of some chemical components such as β -carotene, lycopene, phenolic, proline, soluble protein, nitrate, glucose, sucrose and total carbohydrate level, which all of them may contribute in increasing taste, flavour, nutrients value and antioxidant capacity of mushrooms was determined in the mushroom species used.

The morphotaxonomic identification of the mushroom species were carried out according to Phillips (1994). Whole sporocarps (pileus+stipe) were used for chemical analysis. All of the measurements were carried out with three replications. Fresh mushroom samples (~500 g) were separated into small pieces and dried in an oven at 65°C to a constant weight. Then, the dried samples were ground into fine powder using a laboratory mill and were used for chemical analysis.

Anthocyanin level of mushroom samples was measured spectrophotometrically. β -carotene and lycopene content were measured according to Nagata and Yamashita (1992) method. Mushroom samples were extracted with acetone-hexane (4:6) at once, and then optical density of the supernatant at 663 nm, 645 nm, 505 nm and 453 nm was taken by spectrophotometer at the same time. The concentration of β -carotene and lycopene of extracts was determined spectrophotometrically using the following equations:

 $\beta\text{-Carotene} = 0.216 \times A663 - 1.22 \times A645 - 0.304 \times A505 + 0.452 \times A453$

 $Lycopene = -0.0458 \times A663 + 0.204 \times A645 + 0.372 \times A505 - 0.0806 \times A453$

The amount of proline was performed by the method of Bates et al. (1973). 500 mg mushroom samples were extracted in 3% aqueous sulfosalicylic acid and determined by using acidic ninhydrin reagent. Absorbance of homogenate was noted at 520 nm. Proline concentration was estimated by calibration curve and expressed as μ mol g⁻¹ fresh weight.

Nitrate content of mushrooms was estimated according to Cataldo et al. (1975) method using rapid colorimetric method. 500 mg dry samples were homogenized in 10 mL of de-ionized water and at 45°C for one hour. Then, homogenate was centrifuged at 5000 rpm for 20 min. The supernatant was used for nitrate estimation. 200 μL of the extract was mixed thoroughly with 800 μL of 5% (w/v) salicylic acid (prepared in concentrated H_2SO_4) in 50 mL test tubes. Samples were waited for 20 minutes at room temperature and 10 mL of 2N NaOH was put slowly. Then, all mixtures were cooled and absorbance was noted at 410 nm. The amount of nitrate (μg of NO3 g^{-1} dry weight) was estimated with a standard curve of KNO3.

The amount of total soluble protein content of dried mushroom samples was measured according to Bradford (1976).

Total phenols were measured spectrophotometrically with Folin-Ciocalteu reagent according to Waterhouse (2002). 500 mg of the powdered sample was dissolved in ethanol and mixed with 10 mL Folin-Ciocalteau reagent diluted 1/10 with distilled water. After waiting for few minutes, 8 mL sodium carbonate was added and all solution was waited in dark place for two hours. The absorbance was recorded at 765 nm and results are given in mg of gallic acid equivalents per gram (mg GAE g⁻¹) of mushrooms.

Determination of the total soluble carbohydrate was determined according to the Antron Method by spectrophotometry at 620 nm (McCready et al., 1950). Sucrose content was detected according to the Antron Method by spectrophotometry at 620 nm for sucrose (Handel, 1968). PAL activity was determined according to procedure given by Dickerson et al. (1984). 1 g sample was extracted with 3 mL of 0.1 M sodium borate buffer (pH 7.0) containing 1.4 mM of 2-mercaptoethanol in an ice bath. The extract was filtered and centrifuged at 10.000 g for 15 min. Then, the supernatant was used for PAL activity. Enzyme activity was assayed as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm. Enzyme activity was expressed as nmol transcinnamic acid min-1 mg-1 protein.

Statistical analysis

Analysis of variance (ANOVA) was applied for analysing the differences in the chemical composition of edible mushroom species using the SPSS program version 11.0 for Windows. Following the results of ANOVAs, Tukey's honestly significance difference (HSD) test ($\alpha = 0.05$) was used for testing differences between group means.

Result and Discussion

The analyses of variance showed that there were significant differences (P<0.05) in terms of the amount of anthocyanin, β -carotene, lycopene, phenolic and activity of phenylalanine ammonia-lyase in the examined mushrooms samples (Table 2). As shown in the Table 2, the amount of anthocyanin ranged from 0.006 mg g⁻¹ (*A. campestris*) to 0.107 mg g⁻¹ (*H. erinaceus*), which both was collected from Arac. β -caroten and lycopene concentrations were the highest in the *C. cibarius* with 2.297 mg g⁻¹ and 0.644 mg g⁻¹ collected from Tosya, however they were the lowest in the *A. campestris* with 1.640 mg and 0.232 mg g⁻¹ collected from Arac. Total phenolic concentration of the samples

examined varied between 0.325 mg g⁻¹ and 1.489 mg g⁻¹. The highest phenolic content was detected in C. cibarius samples collected from Tosya and Arac with 1.489 mg g⁻¹and 1.257 mg g⁻¹. It was followed by *H. erinaceus* collected from Daday with 1.123 mg g⁻¹ (Table 2). When considering nitrate, soluble protein and proline concentrations of mushrooms analysed, the highest nitrate and protein level were obtained from A. campestris collected from Devrekâni with 159.963 mg g⁻¹, 55.49 mg g⁻¹ and 55.49 μ mol g⁻¹, respectively (Table 3). It was followed by A. campestris collected from Araç with 50.66 mg g-1 and 147.10 μmol g⁻¹ proline (Table 3). PAL activity did not change significantly among the mushroom species. However, the highest PAL activity was obtained from A. campestris collected from Arac with 6.99 EU mg⁻¹ and the lowest value determined with L. piperatus with 5.79 EU mg⁻¹ collected from Daday (Table 2). In our study, the highest glucose concentration was determined in A. campestris with 29.96 μg g⁻¹ (Devrekani) and in *H. erinaceus* with 29.18 μg g⁻¹ (Daday), while the lowest value was obtained from *H. erinaceus* with 16.20 µg g⁻¹ and C. cibarius collected from Arac (Table 3).

Table 1. Scientific name, local name and location name of mushroom samples used in this study

Scientific name	Local name	Wild / Cultivated	Sample areas	
Agaricus campestris L.	Field mushroom	Cultivated	_	
Cantharellus cibarius Fr.	Girolle	Wild	Araç	
Hericium erinaceus (Bull.) Pers.	Lion's mane mushroom	Wild		
Lactarius piperatus L. Pers.	Blancaccio	Wild	Daday	
Hericium erinaceus (Bull.) Pers	Lion's mane mushroom	Wild	Daday	
Agaricus campestris L.	Field mushroom	Cultivated	Devrekani	
Cantharellus cibarius Fr.	Girolle	Wild	Tosya	

Table 2. Changing of anthocyanin, β -carotene, lycopene, phenolic level and PAL activity of selected wild growing and cultivated mushroom species.

Location	Species	Anthocyanin mg g ⁻¹	β-carotene μg g ⁻¹	Lycopene μg g ⁻¹	Phenolic mg g ⁻¹	PAL EU mg ⁻¹ protein
Araç	A. campestris	0.107±0.0001	1.640a±0.003	0.232 ± 0.001	0.827d±0.006	6.99±0.02
	C. cibarius	0.041 ± 0.0001	$1.837c\pm0.003$	0.282 ± 0.001	1.257f±0.006	6.36 ± 0.02
	H. erinaceus	0.006 ± 0.0001	$1.712b\pm0.001$	0.373 ± 0.001	$0.361b\pm0.016$	5.87 ± 0.05
Daday	L. piperatus	0.044 ± 0.0002	$1.770b\pm0.004$	0.388 ± 0.001	$0.325a\pm0.010$	5.79±0.08
	H. erinaceus	0.043 ± 0.0003	$1.969d\pm0.002$	0.474 ± 0.001	$1.123e\pm0.003$	6.49 ± 0.03
Devrekani	A. campestris	0.089 ± 0.0001	$1.650a\pm0.002$	0.374 ± 0.001	$0.614c\pm0.005$	6.70 ± 0.03
Tosya	C. cibarius	0.013 ± 0.0001	$2.297e\pm0.002$	0.644 ± 0.001	$1.489g\pm0.003$	6.24 ± 0.02
F value	·	65385.86	10230.64	35745.29	3137.945	109.95
Sig. level		0.000	0.000	0.000	0.000	0.000

Table 3. Changing of proline, soluble protein, nitrate, glucose, sucrose and total carbohydrate level of selected wild growing and cultivated mushroom species

Location	Species	Prolin	Protein	Nitrate	Glucose	Sucrose	Total
		μmol g ⁻¹	mg g ⁻¹	mg g ⁻¹	μg g ⁻¹	μg g ⁻¹	carbohydrate mg g -1
Araç	A. campestris	147.10d±0.55	50.66d±0.03	$75.952c\pm0.03$	20.25±0.02	24.55±0.33	50.62±0.05
	C. cibarius	122.49b±0.13	$47.49c\pm0.05$	$77.485c\pm0.65$	18.61 ± 0.04	34.93 ± 0.05	46.53 ± 0.10
	H. erinaceus	129.04c±0.09	$45.08b{\pm}0.02$	$88.947d\pm0.03$	16.20 ± 0.05	26.71 ± 0.77	80.97 ± 0.22
Daday	L. piperatus	146.32d±0.11	$46.56b \pm 0.07$	$66.166a\pm0.03$	24.06 ± 0.04	33.22 ± 0.19	60.15 ± 0.09
	H. erinaceus	131.94c±0.05	$42.53a\pm0.03$	$73.760b\pm0.06$	29.18 ± 0.13	39.22 ± 0.22	72.95 ± 0.33
Devrekani	A. campestris	149.61e±0.25	$55.49e\pm0.24$	$159.963e\pm0.14$	29.96 ± 0.09	34.56 ± 0.11	74.89 ± 0.22
Tosya	C. cibarius	114.00a±0.19	$47.97c\pm1.00$	73.225b±0.06	26.77 ± 0.04	38.41 ± 0.08	66.92 ± 0.11
F value		3069.67	81.305	16272.429	6493.15	271.25	5008.13
Sig level		0.000	0.000	0.000	0.000	0.000	0.000

The maximum sucrose level was found in *H. erinaceus* with 39.33 µg g⁻¹ collected from Daday. The lowest value was measured in *A. campestris* collected from Arac. Total carbohydrate concentration was ranged from 46.53 µg g⁻¹ to 80.97 µg g⁻¹. *H. erinaceus* had the highest level, while the lowest value was obtained from *C. cibarius*, which both sampled from Arac (Table 3).

It has been reported that a lot of mushrooms have high antioxidant capacity, which are synthesized naturally and it has been found in fruiting body, mycelium and culture as phenolic, carotenoids, ascorbic acid, polysaccharides such as glucose, sucrose and soluble carbohydrates (Kozarski et al., 2015; Bengü et al., 2019). All of them play an important role in the prevention of oxidative stress. On the other hand, intake of these compounds in daily nutrition may prevent oxidative stress induced diseases such as cancer, heart disease, macular degeneration and ageing (Johnson, 2002; Patel and Goyal, 2013). Our results related to anthocyanin, β-carotene, lycopene and phenolic are similar to data reported by Robaszkiewic et al. (2010), Johnsy and Kaviyarasan (2014), who stated that mushrooms are rich in terms of β-carotene, lycopene, phenolics and anthocyanin. Turfan et al. (2018) found that total phenolic concentration of mushrooms varied between 28.68 and 157.39 mg DW⁻¹. Results of Tajalli et al. (2015) showed that the amount of total phenolic and anthocyanin ranged from 3.61 to 9.61 mg g⁻¹ and from 0.087 mg 100 g⁻¹ to 7.70 mg 100 g⁻¹ respectively in six wild edible mushrooms. Hussein et al. (2015) determined that total phenolic level changed between 136.21 mg 100 g^{-1} and 431.03 mg 100 g^{-1} in seven wild edible mushrooms. On the other hand, β -carotene content was found between 5.35 and 48.15 mg 100⁻¹, and lycopene level was determined between 2.16 and 18.32 mg 100 g⁻¹. Robaszkiewic et al. (2010) showed that the amount of β -carotene ranged from 0.233 µg g⁻¹ to 15.256 µg g⁻¹, lycopene level varied between 0.001 μg g⁻¹ and 15.388 μg g⁻¹and also the content of total phenols differed between 0.02 µg mg⁻¹ to 4.85 µg mg⁻¹ of dried fruiting body in the edible mushroom species. Alispahić et al. (2015) showed that total phenolic content ranged from 4.94 mg g⁻¹ to 7.66 mg g⁻¹ and 35.56 mg g⁻¹. However, total anthocyanin level was very low with 0.134 mg g⁻¹ FW value.

Mushrooms are generally very rich in terms of nitrate and nitrogenous compounds, because they can absorb high amounts and accumulate (Nunes et al., 2012). These compounds are responsible for synthesis of amino acid, protein and enzymes, which provide a healthy life with their high protein supply for human (Bora and Asha, 2014; Sun et al., 2017). It has been reported that protein level of mushrooms is higher than some vegetables and fruits and also mushroom proteins have more essential amino acid (Adejumo and Awosanya, 2005). Nitrate, soluble protein and proline concentrations values of our study are in agreement with literature. Dunkwal and Jood (2009) examined Pleurotus sajor-caju grown on two substrates (wheat and brassica straw) and found that protein level was 26.99% in cultures including brassica straw. In terms of free amino acid, total lysine content changed between 6.00 and 6.25 g 100 g⁻¹ protein for two mushroom species, but methionine content varied between 1.80 and 1.75 mg 100 g⁻¹ protein in wheat straw and brassica straw, respectively. Turfan et al. (2018) investigated the amount of total soluble protein of selected wild growing and cultivated mushroom species. According to their result, protein level ranged from 33.57 mg to 126.57 mg g⁻¹. Beluhan and Ranogajec (2011) studied with Croatian wild edible mushroom species to determine chemical and non-volatile components and they found that all analysed mushrooms had high protein level varying in the ranges of 27.95-38.89 g 100 g⁻¹. Adedayo et al (2010) found that the amount of protein ranged from 3.25 to 10. 88 mg mL⁻¹ 10⁻² and free amino acid varied between 2.52 and 7.56 mg mL⁻¹ 10⁻² in some edible mushrooms. Ayaz et al. (2011) determined that nitrogen level ranged from 1.73 to 5.20 g 100 g⁻¹, while protein level changed between 10.80 and 32.50 g 100 g⁻¹ in some mushroom species collected from Black Sea region. The variation of nitrate content in our study may vary depend on growing conditions such as chemical properties of growing substrates, pileus size, cultivation time and strain (Membrillo et al., 2008; Jafarpour et al., 2010). Nunes et al. (2012) reported that nitrogen uptake and growing conditions affected yield and the chemical compounds of Oyster mushroom.

Phenylalanine lyase enzyme (PAL) catalyses the first step of the general phenylpropanoid pathway to product secondary metabolites such as coumarins, and phytoalexin having have diverse functions such as increasing stress tolerance (Jones, 1984; Hyun et al., 2011). The metabolism of phenylalanine lyase in plants has been well documented, however there is restricted information on the role of PAL in fungi. Recent studies show that PAL might also be involved in the production of phenolic compounds which perform some functions in the mushroom growth (Yun et al., 2015). In this study, PAL activity did not change significantly among the mushroom species. However, the highest PAL activity was obtained from A. campestris collected from Arac with 6.99 EU mg-1 and the lowest value determined with L. piperatus with 5.79 EU mg⁻¹ collected from Daday (Table 2).

Mushrooms have low sugar content and calories. Many authors expressed that mushrooms do not increase blood sugar level and help reduce more intake of food (Kim et al., 2009; Marsales et al., 2014). Because of this, mushroom consumption in daily dietary is important. Results of soluble sugars and total carbohydrate obtained from this study overlap with literature (Bora and Asha, 2014; Turfan et al., 2016). Butkhup et al. (2018) investigated some chemicals of twenty-five wild edible mushrooms and their results showed that sucrose concentration ranged from 0.15 g kg⁻¹ to 155.61 g kg⁻¹ and glucose level varied between 0.06 and 23.86 g kg⁻¹. Turfan et al (2016) compared chemical component of Ganoderma lucidum collected from nature with grown on orange stump. Result showed that glucose and sucrose level was higher in the samples cultured with 10.06 and 14.09 µg g¹. And also, they determined that sugar level was lower in the samples collected from nature with 708 and 2.54 µg g¹. Kumar et al. (2016) studied with Ganoderma lucidum strains to determine variation of sugar profile as reducing, non-reducing and simple sugar and they found that MS-1 strain has more carbohydrate and simple sugar with 40.04% and 1049%, respectively, while DARL-4 strain has more reducing sugar with 2.33%. When all chemical data evaluated, the amount of chemicals analysed showed significant variation between mushroom samples. Differences in the chemical components of mushroom species may result from location and ecological conditions. Many researchers stated that concentration of antioxidative chemicals varied depend on species, parts of the mushrooms and season (Ayaz et al., 2016; Turfan et al., 2018).

At the end of study, 3 edible mushroom species (*H. erinaceus*, *C. cibarius*, *A. campestris*) examined were found as a good source of nitrate, proline, soluble protein, phenolic, glucose, carotene and lycopene. According to result, *A. campestris* collected from Devrekani had higher nutrient in terms of analysed chemicals except β-caroten. It was followed by *H. erinaceus* collected from Daday. Among the mushrooms tested, *C. cibarius* and *H. erinaceus* collected from Arac had lower chemical components. It can be said that these mushroom species can be consumed as alternative food supplements. Also, results showed that the amount of chemicals examined varied depend on locations and mushroom species.

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