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AMINO ACIDS AND CAROTENOIDS PROFILE OF EDIBLE MUSHROOMS (*Marasmius oreades* AND *Cantharellus aurora*)

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ABSTRACT

Mushrooms are important food resources valued for its high quality protein content, health promoting properties and potential to enhance food palatability. There is not enough or lack of information in the literature particularly on the amino acid composition of mushrooms in the study area. The aim of this study was to investigate the carotenoid and amino acid content of two mushroom species (*Marasmius oreades* and *Cantharellus aurora*). The study revealed found out seventeen amino acids, lycopene and β-carotene by high performance liquid cromatography with reversed-phase and DAD (Diode Array Detector). The highest quantities of essential amino acids in *Marasmius oreades* specie were leucine (6.76 ± 0.12 g 100 g^{-1} protein) and isoleucine (4.47 ± 0.25 g 100 g^{-1} protein) followed by lysine, phenylalanine, valine, threonine, methionine, tyrosine and cysteine. The most abundant essential amino acid in *Cantharellus aurora* was leucine (4.12 ± 0.34 g 100 g^{-1}) followed by phenylalanine + tyrosine (5.23 ± 0.24 g 100 g^{-1}) and isoleucine (3.36 ± 0.23 g 100 g^{-1}).

Keywords: amino acid composition, carotenoids content, wild edible mushroom, HPLC analysis.

AIMS AND BACKGROUND

Mushrooms have been used for thousands of years, dating back to ancient Egyptian and Chinese cultures, to promote longevity and general health and treat various dis-

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eases. Mushrooms can be found in many forests of the tropical countries. Wild edible mushrooms are widely consumed and cultivated in many countries particularly in central and east Europe. The mushroom is a fungus typically produced on the soil or a suitable food substrate. It consists of a fleshy and spore bearing fruiting body. Nowadays, edible mushrooms are eaten by people for their flavour, texture as well as for the health benefits. Mushrooms are with of increasing importance in modern nutrition and medicine, due to the presence of metabolites with pharmacological potential. Wild mushrooms have been considered as highly nutritious tasty food items from ancient time[1–6] besides nutritional importance wild edible mushrooms are now well known for their pharmaceutical constituents[7–11]. Major bioactive compounds extracted from mushrooms are well known for their antioxidant, antitumor, and antimicrobial properties[12–17]. The nutritive nutraceuticals present in mushrooms are dietary fibres, polyunsaturated fatty acids (PUFA), proteins, amino acids, antioxidative vitamins and minerals[18–26].

Amino acid is the basic unit of composition of proteins and peptides in the body of living organisms, eight of them essential and that are not created by the human body, so they can be obtained from animals and plants source. The rest of amino acids are non-essential that (can be manufactured within the human body, provided to healthy nutrition). Despite of the ability to manufacture unessential amino acids, in some cases the body have to take supplements of the essential amino acids to ensure their optimum quantity, more resintly a third section is a semi-essential amino acids, which the body manufactures these acids, but in limited quantities.

The nutritional value of mushroom was primarily in the content of the proteins that make up 5% of the weight of fresh mushroom, which is equivalent to 40–35% of the weight of dry matter and characterised by a high quality of these proteins. That why, the amino acids making the proteins found in mushrooms were very similar to animal proteins in meat, milk, eggs, and can compensate the animal ones at 100% while other vegetable proteins of cereals, pulses and vegetables compensate them, but only to 40–50%. This makes mushroom real and strong competitor of the meat and the other products[27–31].

The aim of study was to determine protein, amino acids and carotenoids of the both species mushroom – *Marasmius oreades* and *Cantharellus aurora*. No literature data for these wild edible mushrooms in Bulgaria have been reported, as well as data on essential amino acid and carotenoid compositions.

In the present study, high performance liquid chromatography (HPLC) with diode area detection (DAD) method is applied for the determination of carotenoids and amino acids in mushrooms.

EXPERIMENTAL

MUSHROOM SAMPLES

Mushroom samples were collected in 2018 and 2019 from the Batak Mountain, Bulgaria personally by the authors. Fresh stipes of mushroom were removed, samples were stored at 4°C within 12 h before drying. Prior to dehydration, mushrooms were thoroughly washed to remove the dirt and graded by size (2 mm in diameter) to eliminate the variations in respect to the exposed surface area.

REAGENTS

All chemicals and solvents used in the study were analytical grade. Methanol, ethanol, *n*-hexane and ethyl acetate were HPLC grade and were purchased from Merck (Darmstadt, Germany). The standards of lycopene and β-carotene were obtained from Sigma Aldrich (Germany). The mix of amino acids standard was from Waters (USA).

DETERMINATION OF PROTEIN CONTENT

The Kjeldahl method was performed according to method 976.06 of the AOAC International. Approximately 1 g of ground dried sample powder was hydrolysed with 15 ml concentrated sulphuric acid (H_2SO_4) and added 7 g anhydrous potassium sulphate (K_2SO_4) and heat in a block (Kjeltec system digestor, Tecator Inc., Herndon, VA, USA) at 420°C for 60 min. After cooling, H_2O was added to the hydrolysates before neutralisation and titration. Distillation and titration used the unit UDK 152. Titrant 0.2 N HCl. The amounts of total nitrogen in the samples were multiplied with both the traditional conversion factor of 6.25.

DETERMINATION OF AMINO ACID COMPOSITION

Amino acid analysis was performed with a high performance liquid chromatography (HPLC). Sample equivalent 30 mg was weighed into the conical flask and mixed with 2 ml 6M HCl. The flask was placed in oven at 105°C for 24 h. The flask was then opened and added 3 ml deionised water and the hydrolysed amino acids were evaporated to dryness at 60°C and the residue was dissolved in 2 ml 20 M HCl. After passing through a 0.45 μm filter, the samples were collected and processed to derivatisation.

DERIVATISATION OF AMINO ACIDS

The sample (20 μl) was derivatiaed with AccQ-Fluor reagent kit WAT052880 (Waters, USA). AccQ-Fluor borate buffer (60 μl) was added by micropipette to the sample and vortexed. Then, 20 μl of AccQ-Fluor reagent were added and the sample was additionally vortexed for 30 s. The sample was heated in a waterbath MLW W3 (Labexchange, Burladin-gen, Germany) at 55°C for 10 s before separation of amino acids using HPLC system.

The AccQ-Fluor amino acid derivates were separated on ELITE LaChrom HPLC system (VWR™ Hitachi, Tokyo, Japan). Sample of 20 µl was injected into an HPLC reversed phase AccQ-Tag™ silica-bonded amino acid column C18, 3.9 mm × 150 mm (Waters). The elution of the amino acids was performed by gradient system with mobile phase, eluent A, buffer WAT052890 (Waters) and mobile phase, eluent B, 60% acetonitrile (Sigma-Aldrich, Merck), in a separation gradient with a flow rate of 1.0 ml min^{-1} The amino acids were detected using a diode array detector (DAD) at 254 nm with the column condition set at 37°C for 40 min. For qualitative and quantitative determination of amino acids were used the retention time and calibration curves for each amino acids with linearity range shown in Table 1.

Table 1. Retention time and linearity range for each amino acids

No	Amino acid	Retention time (min)	Linearity (µg ml^{-1})
1	Aspartic acid (Asp)	$Y = 5407.5x - 217871$	50–500
2	Serine (Ser)	$Y = 5467.6x - 223160$	50–750
3	Glutamic acid (Glu)	$Y = 7850.2x - 259738$	50–500
4	Glycine (Gly)	$Y = 11613.5x - 47469$	50–750
5	Histidine (His)	$Y = 10251x - 99045$	50–500
6	Arginine (Arg)	$Y = 10108x - 38768$	50–500
7	Threonine (Thr)	$Y = 9270.5x + 12004$	50–500
8	Alanine (Ala)	$Y = 5407.5x - 217871$	50–500
9	Proline (Pro)	$Y = 9300x - 29864$	50–500
10	Cysteine (Cys)	$Y = 7690.2x - 55813$	50–500
11	Tyrosine (Tyr)	$Y = 7761.4x + 174003$	50–500
12	Valine (Val)	$Y = 9752.6x - 109900$	50–750
13	Methionine(Met)	$Y = 8162.9x - 43359$	50–500
14	Lysine (Lys)	$Y = 10052x + 373765$	50–750
15	Isoleucine (Ile)	$Y = 7391.9x - 56099$	50–500
16	Leucine (Leu)	$Y = 71775x - 60398$	50–500
17	Phenylalanine (Phe)	$Y = 8276.6x - 105385$	50–500

Amino acid score (AAS) was calculated as a ratio of the amount of each essential amino acid in a sample (g 100 g^{-1} protein) and the amount of the respective amino acid in an 'ideal' protein (g 100 g^{-1} protein) as defined by the World Health Organisation[35]. The results were multiplied by 100 to express in percentage.

EXTRACTION OF LYCOPENE AND B-CAROTENE

To the 0.1 g lyophilised sample was added 2 ml of methanol (1:20), 5 ml of a mixture of carbon tetrachloride and methanol in ratio 3:1. The solution must contain also 0.5% BHT. The sample was placed for 15 min in ultrasonic bath (35 kHz) and after extraction 1 ml of 10% NaCl solution was added. The carbon tetrachloride fraction

was separated and passed through a column of anhydrous Na_2SO_4. The samples were collected in a 5-ml volumetric flask.

DETERMINATION OF LYCOPENE AND B-CAROTENE

Qualitative and quantitative determination of carotenoids was performed by using Elite LaChrome (Hitachi) HPLC system equipped with DAD and ELITE LaCHrome (Hitachi) software. Separation of the carotenoids was performed by Discovery® HS C18 (5 μm, 25 cm × 4.6 mm). Split temperature 30°C, wavelengths λ = 270 nm and 290 nm. Elution was performed by gradient mobile phase system A – methanol: acetonitrile in a ratio of 8:2 and mobile phase B MTBE (methyl *tert*-butyl ether) and two wavelengths (λ = 270 nm, 290 nm).

RESULTS AND DISCUSSION

Crude protein is particularly found in high levels in edible mushrooms and range can vary between 15.2 g 100 g^{-1} dried weight in *Lentinus edodes* to 80.93 g 100 g^{-1} dried weight in *Agaricus bisporus*[2,32].

The protein percent of tested mushrooms (*Marasmius oreades* and *Cantharellus aurora*) is shown in Table 2.

Table 2. Protein content (%) in mushrooms (mean ± SD)

Mushroom	Protein (%)
Marasmius oreades	36.49 ± 0.45
Cantharellus aurora	25.37 ± 0.45

The protein content in our study is lower than the value found in *Marasmius oreades* (40.19%) and higher than the value found in *Cantharellus cibarius* (16.19%) (Ref. 33).

The results showed that the obtained percentages of protein in the mushrooms are higher than all kinds of meat, eggs and milk, reported in different sources[34]. We can conclude that it is approaching the protein content of the sources of meat (cows, sheep, chicken, shrimp, fish, eggs and other animal products), so it is one of the healthy foods that have to be recommended to maintain the overall health of the body.

Mushrooms are important and necessary components of human nutrition. They are energy source and also contain amino acids – compounds essential for human health. Although the levels of essential amino acids in mushrooms widely vary among species, generally the biological quality of mushroom proteins (considered to be in similar quality as animal proteins but, accompanied by less fat content) is also high[2,35]. According to FAO and WHO (Ref. 36), they are considered rich in glutamic acid, aspartic acid and arginine, however, their proteins are deficient in methionine and cysteine.

The chromatograms of HPLC analysis of 17 amino are shown in Fig. 1.

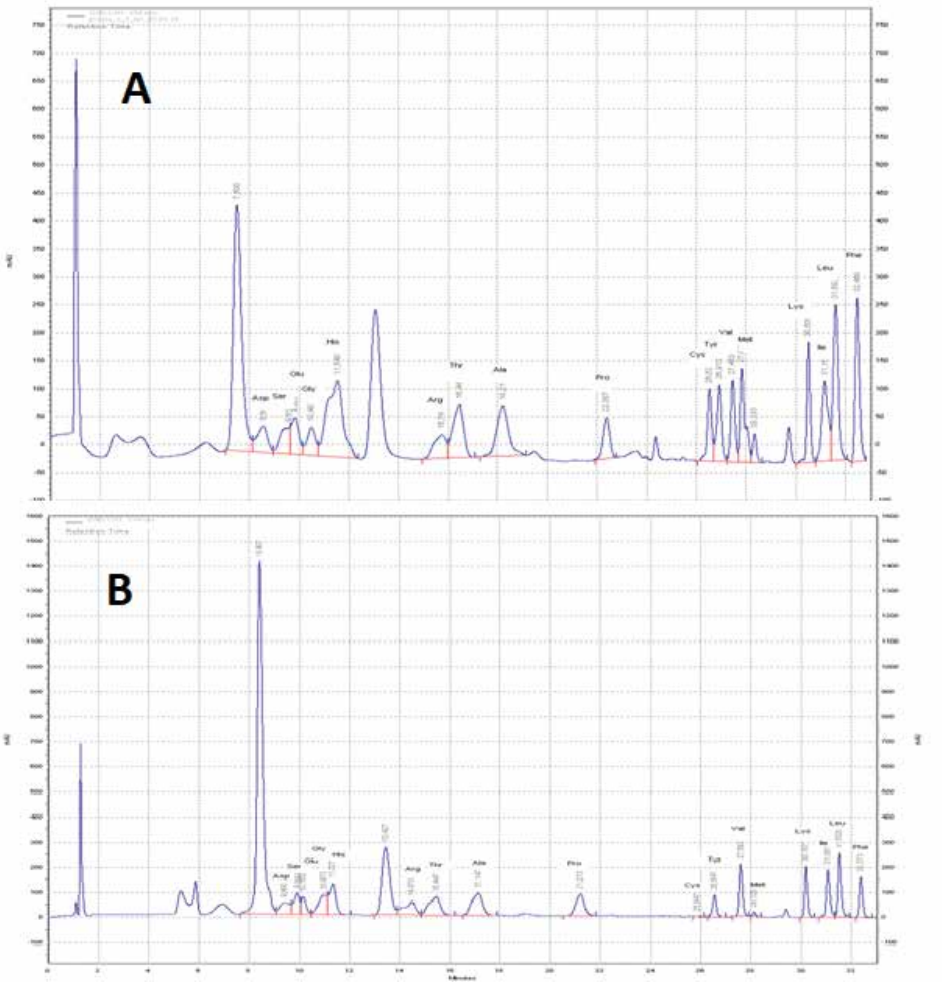

Fig. 1. Chromatograms of amino acid analysis by RP-HPLC: A – *Marasmius oreades*; B – *Cantharellus aurora*

Essential and non-essential amino acid compositions of *Marasmius oreades* and *Cantharellus aurora* are presented in Tables 3 and 4, respectively.

Table 3. Essential amino acid composition and amino acid score of *Marasmius oreades* and Cantharellus aurora

Essential amino acids	*Marasmius oreades*		*Cantharellus aurora*	
	content (g 100 g^{-1} protein)	amino acid score (%)	content (g 100 g^{-1} protein)	amino acid score (%)
Val	3.77 ± 0.23	96.67	2.4 ± 0.12	61.54
Leu	6.76 ± 0.12	114.57	4.12 ± 0.34	69.83
Ile	4.47 ± 0.25	149.00	3.36 ± 0.23	112.00
Thr	3.40 ± 0.34	147.82	2.90 ± 0.14	126.08
Met + Cys	2.29 ± 0.52	114.25	2.02 ± 0.43	91.82
Phe + Tyr	6.37 ± 0.34	167.63	5.23 ± 0.24	137.63
Lys	4.10 ± 0.45	91.11	2.65 ± 0.12	58.89

The protein present in mushrooms contains all nine essential amino acids (EAAs), in contrast to most other plant-based protein options which are typically missing one or more EAAs.

The results showed that three most abundant EAAs are determined in our mushrooms. Leucine was found to be most abundant in both mushroom species such as contained in *Marasmius oreades* was higher (6.76 ± 0.12 g 100 g^{-1} protein) compared to *Cantharellus aurora* (4.12 ± 0.34 g 100 g^{-1} protein). Leucine was reported as the most abundant amino acid in others wild mushrooms[37]. Leucine has been reported to be particularly important for immunity, reproduction, extra-endocrine signalling, neurological function, bloodflow, osmoregulation, growth and development[38]. Isoleucine were found to be the second essential amino acids with 4.47 ± 0.25 g 100 g^{-1} in *Marasmius oreades* and 3.36 ± 0.23 g 100 g^{-1} in *Cantharellus aurora*. The third predominant acid is lysine (4.10 ± 0.45 g 100 g^{-1}) for *Marasmius oreades* and threonine (2.90 ± 0.14 g 100 g^{-1}) for *Cantharellus aurora*. The same results for essential amino acids content is in *Cantharellus cibarius* reported by Deepak K. Rahi and Deepika Malik[39]. On the other hand, Akindahunsi and Oyetayo[40] reported valine was the most abundant amino acid in *Pleurotus* species while threonine and leucine were found to be the second and third most abundant, respectively. It is possible that the differences of amino acids found in the same mushroom species could be a consequence of the genetic variation and cultivation process applied in commercial practices which was also revealed by Manzi et al.[2] and Colak et al.[27]

Table 3 shows the amino acids score of wild edible mushrooms (*Marasmius oreades* and *Cantharellus aurora*) as calculated based on FAO/WHO. Chemical score provides an estimate of the nutritive value of a protein by comparing the levels of essential amino acids between samples and standard proteins Phenylalanine (or + tyrosine) score was highest in both fungal species, followed by isoleucine, tryptophan and methionine (or + cysteine). *Cantharellus aurora* mushroom exhibited lowest scores (up 90%) in valine and lysine and isoleucine. The amino acids score of the essential amino acids of the samples have over 100% values so they can be a good food source.

Table 4. Non-essential amino acids composition of *Marasmius oreades* and *Cantharellus aurora* (mean ± SD)

Non-essential amino acids	*Marasmius oreades* (g 100 g^{-1} protein)	*Cantharellus aurora* (g 100 g^{-1} protein)
Aspartic acid (Asp)	11.18 ± 0.12	15.00 ± 0.45
Glutamic acid (Glu)	6.07 ± 0.32	7.18 ± 0.20
Glycine (Gly)	2.32 ± 0.45	3.98 ± 0.16
Arginine (Arg)	6.07 ± 0.13	9.47 ± 0.34
Alanine (Ala)	11.91 ± 0.23	9.23 ± 0.15
Proline (Pro)	6.21 ± 0.30	7.00 ± 0.17
Histidine (His)	13.04	5.30
Serine (Ser)	5.35	16.69

Eight non-essential amino acids were detected (Table 4). The study revealed alanine and aspartic acid as the most abundant non-essential amino acid in the both mushroom species, but significant variation was detected for histidine and serine. The value of serine varied from 5.53 g 100 g^{-1} in *Marasmius oreades* to16.69 g 100 g^{-1} in *Cantharellus aurora*, while histidine ranged in value from 5.30 g 100 g^{-1} in *Cantharellus aurora* to 13.04 g 100 g^{-1} in *Marasmius oreades.*

Our result for aspartic acid in *Cantharellus aurora* is the same as that reported by Guo et al.[41] in *Pleurotus djamor* mushrooms.They reported that the most abundant non- essential amino acid is aspartic acid comprising 19% of total non-essential amino acids.

The content of arginine and aspartic acid in *Marasmius oreades* mushroom species observed in this study is in accordance with previous reports[42], but the values of other non-essential amino acids obtained in the present study are generally higher compared to the values reported by these authors.

CAROTENOIDS

In this study lycopene was found in 0.024 mg g^{-1} and 0.026 mg g^{-1} for *Cantharellus aurora* and *Marasmius oreades* respectively and β-carotene not detection (Table 5).

Table 5. Content of lycopene and β-carotene of edible mushrooms *Cantharellus aurora* and *Marasmius oreades* (mg g^{-1} of dried mushrooms)

Mushroom	Lycopene (mg g^{-1})	β-carotene
Cantharellus aurora	0.024	LOQ
Marasmius oreades	0.026	LOQ

Other authors have also presented lycopene and β-carotene contents of the mushroom samples within the ranges: *Marasmius oreades* – 0.012–0.086 – lycopene (mg g^{-1}) and 0.08–0.32 – β-carotene (mg g^{-1}); *Cantharellus aurora* – 0.016 – 0.108 lycopene (mg g^{-1}) and 0.06–0.57 β-carotene (mg g^{-1}) (Refs. 2,40,43). The results

obtained in the current study, indicated that lycopene and β-carotene contents of the investigated mushroom samples were comparable with those reported in the literature.

CONCLUSIONS

In conclusion, the both mushroom species posses high protein quality, since they contain most of the essential and non-essential amino acids required for human health. Furthermore, most of these amino acids are presented in quantities sufficient to satisfy metabolic needs for maintenance, normal growth and other functions of adequate protein uptake. Mushroom species could be recommended as a dietary supplement for essential amino acids, especially *Marasmius oreades*.

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