

NUTRITIONAL AND PHENOLIC PROFILE OF SMALL EDIBLE FUNGAL SPECIES COPRINELLUS DISSEMINATUS (PERS.) J.E. LANGE 1938

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ABSTRACT: The aim of this work was to investigate nutritional profile in relation to proteins, amino acids, fatty acids and mineral composition, as well as phenolic profile of small edible fungal species Coprinellus disseminatus originated from Serbia. Total protein content in the analyzed fungal species was 9.72%. Fifteen protein fractions obtained by electrophoresis were identified in a range from 1.6 to 63.6 kDa. Chip-based separations showed the presence of protein fraction with molecular weight of 27.5 kDa that could possess antifungal activity. The total essential and non-essential amino acid contents were 29.57 and 96.69 mg/g DW, respectively. Among the essential amino acids, leucine was the most abundant. Fatty acid composition of C. disseminatus showed that polyunsaturated fatty acids (PUFA, 59.1% of total FA) predominated over saturated fatty acids (SFA, 23.1% of total FA) and monounsaturated fatty acids (MUFA, 17.9% of total FA). The dominant fatty acids were linoleic acid (56.6%), palmitic acid (13.9%), and oleic acid (12.0%). The most abundant macroelement in C. disseminatus was potassium, followed by calcium and magnesium, while iron dominated in microelements. Eight phenolic compounds were quantified in methanolic extract of C. disseminatus by LC-MS/MS with the highest amount of p-hydroxybenzoic acid and p-coumaric acid reaching 9.46 ± $0.2 \mu g/g$ DW and $7.8 \pm 0.1 \mu g/g$ DW, respectively.

Key words: mushroom, proteins, amino acids, fatty acid profile, phenolic profile, mineral composition

INTRODUCTION

Since earliest times mushrooms have been priced as a special kind of food, while during recent years they have become more attractive as functional food due to their proved medicinal values. They are valuable sources of physiologically beneficial compounds which provide their therapeutic effect and enable their usage as dietary supplements (Ferreira et al., 2009; Novaković et al., 2015). Mushrooms actually are mycofactories as they produce variety of secondary metabolites, including

phenolic compounds, polyketides, terpenes and steroids (Turkoglu et al., 2007). Numerous bioactive properties of mushrooms, such as antioxidant, antitumor (Ferreira et al., 2010; Puttaraju et al., 2006; Vaz et al., 2012; Wasser, 2002; Zaidman et al., 2005), and antimicrobial activities (Barros et al., 2007, Karaman et al., 2009; Karaman et al., 2012; Stojković et al., 2013) were discovered and addressed to abovementioned compounds. It is assumed that the presence of these com-

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pounds provides protection against certain chronic diseases such as cardiovascular diseases, cancer, diabetes, and neurological disorders (Zhou et al., 2009). It should be noted that the composition of mushrooms is affected by the diversity of their genetic makeup that is expressed by strains differences as well as by environmental conditions, including the nature of substratum they grow on (Miles and Chang, 2004).

Due to the possible application of edible mushrooms in the treatment and prevention of many diseases of modern life they have been thoroughly analyzed as therapeutic alternatives (Kalogeropoulos et al., 2013; Karaman et al., 2014; Zaidman et al., 2005). Their nutritional value is also of great interest as they can be used as ingredients in food formulations (Leal et al., 2013; Ribeiro et al., 2009).

The usage of mushrooms implies some advantages. Namely, the fruiting body can be produced in a short period of time, and the mycelium may also be rapidly produced in liquid culture and the culture medium can be manipulated to produce optimal quantities of active compounds (Elisashvili, 2012).

Coprinellus disseminatus (Pers.) J.E. Lange 1938 is a mushroom (phylum Basidiomycota, fam. Psathyrellaceae) whose different extracts (ethanolic, dichloromethane, ethanolic and aqueous extracts) were examined and evidenced to possess antioxidant (Han et al., 1999), antibacterial (Gu and Leonard, 2006) and antiproliferative activity (Novaković et al., 2016). Despite its small size, discovered bioactive properties of *C. disseminatus* may refer to its biotechnological exploitation, but there is an absence of its nutritional profile which can be useful in food creation. Therefore, the aim of this paper was to evaluate nutritional profile (proteins, amino acid composition, fatty acid profile, and mineral composition) of fungal species C. disseminatus originated from Serbia as well as its phenolic profile.

MATERIALS AND METHODS

Standards and reagents

Reference standards of the phenolic compounds were obtained from Sigma-Aldrich

Chem (Steinheim, Germany), Fluka Chemie GmbH (Buchs, Switzerland) and from Chromadex (Santa Ana, USA). HPLC gradient grade methanol was purchased from J. T. Baker (Deventer, The Netherlands) and p.a. grade formic acid was obtained from Merck (Darmstadt, Germany). The fatty acids methyl ester (FAME) reference standard mixture 37 (Supelco) was purchased from Sigma (Sigma-Aldrich, EU). Deionized water was produced using a Millipore water purification system (Darmstadt, Germany). All other chemicals and solvents were of analytical grade.

Mushroom samples

The species C. disseminatus (Pers.) 1938 was collected from an urban location of Novi Sad (Serbia) during 2012. Identification of the species was based on classical determination while voucher specimen (12-00662) was deposited at the mycological collection of the Herbarium BUNS (Faculty of Sciences, Department of Biology and Ecology, University of Novi Sad, Serbia). The samples were frozen at -20 °C and lyophilized during 48 h (BioAlpha, Martin Christ GmbH, Switzerland). Lyophilized samples were ground to a fine powder, packed in sealed plastic tubes and stored in a dark place at room temperature (23 ± 1 °C) prior to analysis.

Extraction with methanol

The entire lyophilized and powdered fruiting bodies of *C. disseminatus* (10 g) were extracted with 100 mL of methanol using a shaker (Thermofisher Scientific, USA) at 120 rpm for 24 h at room temperature (23 ± 1 °C). The extract was filtered through Whatman No. 4 filter paper and the solvent was removed by vacuum-evaporator at 40 °C (Büchi, Switzerland). The obtained extract was stored at -20 °C prior to phenolic profile analysis.

Protein content

The crude protein content N 4.38 of the samples was estimated by the macro-Kjeldahl method using the standard procedure (AOAC, 1995).

Determination of protein profile

Sample preparation was carried out according to Tidona et al. (2011) with some

modifications. The lyophilized and powdered fruiting bodies of C. disseminatus (15 mg) were dissolved in 100 µL buffer (0.125 M Tris HCl, 4% SDS, 2% glycerol, 2% βmercaptoethanol, pH 6.8) and heated at 100 °C for 5 min. The chip-based separations were performed on an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) in combination with the Protein 80 Plus LabChip kit and the dedicated Protein 80 software assav on 2100 expert software. Chips were prepared according to the protocol provided by the Protein 80 LabChip kit. Fractioning is sizebased, and the profiles show the smallest proteins emerging first in the profiles but at the bottom of the gel patterns, according to the convention for SDS-PAGE (Torbica et al., 2010). Bovine serum albumin was used as the standard for quantitation of the fungi proteins. All samples were analyzed in triplicate.

Determination of amino acid composition

Fungal samples were subjected to acid hydrolysis to determine the amino acid composition according to the modified method by Fountoulakis and Lahm (1998). The lyophilized samples (50 mg) were hydrolyzed by hydrochloric acid solution (6 M HCl containing 0.1% phenol and 3% thioglycolic acid, v/v) for 24 h, and the hydrolysis was done in sealed vessels under nitrogen atmosphere.

The amino acid composition of the samples was determined by high performance liquid chromatography (HPLC) on the HPLC Agilent 1200 Series instrument (Agilent Technologies, Palo Alto, USA) using Agilent Eclipse Plus C18 (5.0 µm, 3.0 x 250 mm) column. For peak detection, a fluorescence detector (FLD) was used, with excitation parameters at 340 nm and emission at 450 nm. Since previous derivatization is required for the analysis of amino acids under these conditions, this was achieved by pre-column derivatization of hydrolysates with ortho-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC). The pre-column derivatization and chromatographic analysis of amino acids was carried out according to Agilent Technologies application note No.

5990-4547EN (Henderson and Brooks, 2010), detailing the parameters and conditions of the chromatographic separation, the derivatization procedure with the ready reagent sets and the method of preparing the amino acid calibration standards.

A mixture of the amino acid standards used for calibration was prepared by diluting standard mixtures with 0.1 M HCl, and a dilution series in the range of 0.1–2 µmol/mL was obtained. The calibration curve was constructed for each individual amino acid, based on the obtained peak areas depending on the concentration of the standard (the linear coefficients for the individual calibration curves were in the range R = 0.9956 - 0.9993). The calculated values for each individual amino acid were corrected for the calculated value of the analytical yield (analytical yield values were from 67.2% for tryptophan, up to 106.7% for glycine). From the obtained linear dependence equation, the concentrations of individual amino acids in the samples were calculated and expressed as mg/g of the sample.

Fatty acid profile

To establish a fatty acid profile, samples were firstly subjected to extraction by the method of Folch (Folch et al., 1957). Extracted lipids were then submitted to trans-esterification using 14% boron(III)fluoride in methanol (Ackman, 1998) to obtain fatty acid methyl esters (FAMEs). Samples were then analyzed by a GC Agilent 7890A system. System was equipped with flame-ionization detector (FID) and with fused silica capillary column (Supelco SP-2560, 100 m, 0.25 mm, 0.20 μm). A carrier gas was helium (purity > 99.9997 vol %, flow rate was 1.00 mL/min). Two µL of sample were injected in split mode (25:1), with following oven temperature conditions: 140 °C for 5 minutes followed by temperature ramp of 3 °C/min to 240 °C and held for 10 minutes.

Identification of fatty acids in samples was done by comparison of their retention times with retention times of mixture with 37 standards (Supelco) and with data from internal data library. Final results were shown as gram of fatty acid or fatty acid group (g) per 100 g of total fatty acids.

Macro- and microelements analysis

Determination of selected macro- (K, Ca and Mg) and microelements (Cu, Mn, Fe and Zn) in lyophilized and powdered sample of C. disseminatus was done using flame atomic absorption spectroscopy (AAS). Approximately 0.3 g of oven-dried (70 °C for 24 h) material was ground and homogenized in a laboratory mill and then digested in 10 mL of nitric acid and 2 mL of 30% (w/v) hydrogen peroxide using a microwave-assisted digestion system (D series, Milestone, Bergamo, Italy) at 180 °C for 45 min with microwave power of 900 W. Digested samples were diluted to 25 mL with deionized water. Pre-treated samples were processed by atomic absorption spectrophotometer AAS240/GTA120, Varian) using the acetylene/air burner flame technique (with an atomization temperature of about 2300 °C) for Cu and Mg quantification, while a nitrous oxide (N2O)-acetylene flame (with a temperature of about 2700 °C) was used for Ca content determination. By using single element hollow-cathode lamps concentrations of Cu, Mg and Ca were determined at 324.8, 285.2 and 422.7 nm, respectively and expressed as mg/kg or μg/kg of dry weight of fungi material.

Phenolic compounds profile

Analysis of phenolic profile was done according to method described by Orčić et al. (2014). For separation of the phenolic compounds Agilent 1200 series HPLC-MS/MS chromatograph was employed equipped with reversed-phase column Zorbax Eclipse XDB-C18 4.6 mm x 50 mm x 1.8 mm (Agilent Technologies) that was held at 40 °C. Detection of analytes was done by Agilent series 6410A triple-quadrupole mass spectrometer using electrospray ionization (ESI). For instrument control and data analysis, MassHunter ver. B.03.01. software (Agilent Technologies) was used. Mobile phase used in analysis was made of 0.05% formic acid (A) and methanol (B) with a flow rate of 1 mL/min and solvent gradient: start with 70% A / 30% B, attaining 30% A / 70% B in 6.00 min, following 100 % B at 9.00 min, held

until 12.00 min, and then with 3 min to reequilibrate. Sample injection volume was 5 μ L. ESI has been done under following conditions: drying gas (N₂) temperature 350 °C; flow 9 L/min; nebulizer gas pressure 40 psi; capillary voltage 4 kV, negative polarity.

Dynamic MRM (multiple reaction monitoring) mode was used for quantification of all compounds. For analysis a mix of stock standard solutions was used. Starting concentration of each compound was 100 μ g/mL which was subsequently serially diluted (in methanol-water; 1:1). This resulted in working standard solutions with concentration range from 0.0015 μ g/mL to 25.0 μ g/mL. These standard solutions were used for construction of the calibration curves. Quantification of individual peaks (compounds) was done by using the equation for linear regression resulting from the calibration curves ($R^2 = 0.995$).

RESULTS AND DISCUSSION

Protein profile

The nutritional value of mushrooms is primarily related to their high quality protein content. It is not only dependent on environmental factors or stage of the fruiting body maturity, but also on the genotype specificity of the species (Wang et al., 2014). On a dry weight basis, mushrooms normally contain 19–35% of proteins (Miles and Chang, 2004).

Total protein content in analyzed fungal species was 9.72%, being in accordance with the previously detected content (11.00–11.24%) in *C. comatus* (Redhead et al., 2001).

Mushroom proteins are characterized by various biological activities such as antitumor, antiviral, antimicrobial, antioxidative, and immunomodulatory activity (Xu et al., 2011). Various pharmaceutical properties of mushroom proteins lead to the growing tendency in protein engineering focused on the protein specific activities (Ivanova et al., 2014).

C. disseminatus protein characterization obtained by electrophoresis identified 15 protein fractions and their molecular weights were in the range from 1.6 to 63.6 kDa. Results of the chip-based separation

showed the presence of protein fraction with molecular weight of 27.5 kDa (Fig. 1). According to Ngai and Ng (2003) the lentin, protein derived from shitake mushroom with a molecular mass of 27.5 kDa, inhibited mycelial growth in a variety of fungal species. The mentioned authors noted that it also exerted an inhibitory activity on HIV-1 reverse transcriptase and proliferation of leukemic cells.

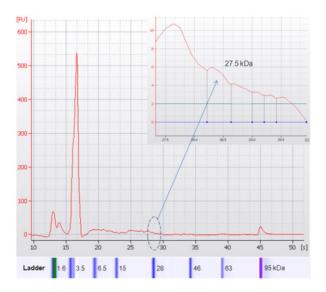


Figure 1. Electropherogram of *C. disseminatus* protein fraction with molecular weight of 27.5 kDa

The proteins of commonly cultivated mushrooms contain all nine essential amino acids (EAA) for humans - lysine, methionine, tryptophan, threonine, valine, leucine, isoleucine, histidine and phenylalanine, among which the most abundant is lysine and the lowest levels are those of tryptophan and methionine (Miles and Chang, 2004). Amino acid composition of C. disseminatus is presented in Table 1, showing that analyzed species was especially rich in arginine, alanine, cystein and glutamic acid. Among the essential amino acids, leucine was more abundant than valine and threonine. The total essential and non-essential amino acid contents were 29.57 and 96.69 mg/g DW, respectively. In a previous study done by Ayaz et al. (2011) mushrooms from the East Black Sea region contained a high amount of glutamic acid, while leucine was the most abundant among EAA, which is being in line with the obtained results.

Arginine is a precursor of nitric oxide, be-

ing important in people with angina and congestive heart failure (Appleton, 2002). It also acts by stimulating immune function and promoting the secretion of several hormones, such as glucagon, insulin and growth hormone (Meletis and Barker, 2005). Arginine is a semi-essential amino acid for humans, also is of great importance in babies, as they cannot produce it in their first months. In adults it becomes more important when body is submitted to a great physical stress, when additional arginine is required in a regular diet (Belitz and Grosch, 1999).

Fatty acid profile

Edible mushrooms are considered a good source of many different nutraceuticals such as unsaturated fatty acids (Yilmaz et. al., 2006) and *C. disseminatus* is not an exception. Fatty acid composition of *C. disseminatus* (Table 2) showed that polyunsaturated fatty acids (PUFA, 59.1% of total FA) predominated over saturated fatty acids (SFA, 23.1% of total FA) and monounsaturated fatty acids (MUFA, 17.9% of total FA). Among PUFA, the main was linoleic acid (56.6%), which was followed by SFA palmitic acid (13.9%), and MUFA oleic acid (12.0%).

The determined fatty acid profile of C. disseminatus was consistent with previously published data for *C. comatus* from Serbia (Stojković et al., 2013), Portugal (Vaz et al., 2011) and Turkey (Yilmaz et al., 2006). There is extensive scientific evidence showing that dietary PUFA may protect against several conditions such as cardiovascular, psychiatric, neurological, dermatological and rheumatologic disorders (Rubio-Rodríguez et al., 2010). Beside many phenolic compounds, eslow-molecular-weight phenolic pecially compounds that were identified to possesses antimicrobial properties (Karaman et al., 2009, Karaman et al., 2012; Karaman et al., 2014; Novaković et al., 2016), mushroom fatty acids, especially palmitic acid from G. applanatum fruiting bodies and *Dictyophora echino-volvatus*, showed strong antimicrobial activity against gramnegative bacteria (Moradali et al., 2008) as well as against many microorganisms -(S. aureus, E. coli, S. cerevisiae, C. albicans, Penicillium citrinum and A niger)

(Shen et al., 2017). These facts indicate the possible potential of *C. disseminates* interms of antimicrobial activity because it

possessed relatively high content of palmitic acid.

Table 1.

Amino acid composition of *C. disseminates* (mg/g DW)

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Val	Met	Phe	lle	Leu	Lys	Thr	Н	is
5.61±0.22	1.83±0.10	3.23±0.07	2.53±0.11	7.45±0.31	3.00±0.13	5.13±0.17	0.79	±0.05
Asp	Glu	Ser	Gly	Pro	Arg	Ala	Tyr	Cys
8.79±0.22	15.4±0.06	5.87±0.27	4.54±0.12	0.82±0.09	33.4±0.16	11.8±0.15	0.57±0.07	15.5±0.16

Values are means of three determinations ± standard deviation

Val – valine; Met –methionine; Phe – phenylalanine; Ile – isoleucine; Leu – leucine; Lys – lysine; Thr – threonine; His – histidine;

Asp – aspartic acid; Glu – glutamic acid; Ser – serine; Gly – glycine; Pro – proline; Arg – arginine; Ala – alanine; Tyr – tyrosine; Cys – cysteine;

DW - dry weight

Table 2.

Fatty acid profile of *C. disseminatus*

Fatty acid	(%)
C6:0	2.6 ± 0.01
C16:0	13.9 ± 0.19
C16:1	1.85 ± 0.04
C18:0	3.56 ± 0.02
C18:1n9c	12.0 ± 0.08
C18:2n6c	56.6 ± 0.26
C22:6n3	1.96 ± 0.01

Values are means of three determinations ± standard deviation

C6:00 – caproic acid; C16:00 – palmitic acid; C16:01 – palmitoleic acid;

C18:00 – stearic acid; C18:1n9c – oleic acid; C18:2n6c – linoleic acid;

C22:6n3 - docosahexaenoic acid

Table 3.

Mineral composition of C. disseminatus

Macroelement (mg/g DW)				
K	24.9 ± 0.30			
Mg	6.72 ± 0.02			
Ca	23.9 ± 0.26			
Microelement (μg/g DW)				
Cu	18.3 ± 0.20			
Zn	193 ± 0.74			
Mn	188 ± 0.58			
Fe	5.98 ± 0.01			

Values are means of three determinations ± standard deviation DW – dry weight

Table 4.

Phenolic compounds profile of *C. disseminatus*

Flavones (µg/g DW)				
chrysoeriol	0.3 ± 0.02			
luteolin7-O-glucoside	0.6 ± 0.001			
apigenin7-O-glucoside	0.2 ± 0.001			
Biflavonoid (μg/g DW)				
amentoflavone	0.4 ± 0.002			
Hydroxybenzoic and hydroxycinnamic acid (µg/g DW)				
<i>p</i> -hydroxybenzoic acid	9.46 ± 0.2			
<i>p</i> -coumaric acid	7.8 ± 0.10			
protocatechuic acid	0.7 ± 0.02			
chlorogenic acid	0.7 ± 0.02			

Values are means of three determinations ± standard deviation DW – dry weight

Macro- and microelements

Mushrooms are a good source of minerals which are taken up from the substrate by the growing mycelium and translocated to the fruiting bodies. As in higher plants, the dominant mineral is potassium (K), followed by phosphorus (P), sodium (Na), calcium (Ca), and magnesium (Mg) (Miles and Chang, 2004). Many mushroom species accumulate minor mineral elements e.g. trace elements to a considerably higher amounts than those in plants, such are copper (Cu), zinc (Zn), selenium (Se), iron (Fe), and molybdenum (Mo), which are involved in many physiological processes (Zaidman et al., 2005). The most abundant macroelement in C. disseminatus was K, followed by Ca and Mg (Table 3) and this finding is in accordance with the results of Heleno et al. (2015) who found K accounted for nearly 45% of the total mineral content in mushrooms.

Among microelements Fe was the most abundant element, followed by Zn, Mn and Cu (Table 3). The higher content of elements such as K and Fe in *C. disseminatus* is probably the consequence of accumulation of these elements caused by the adaptation to environmental conditions since fungi absorb nutritive elements from the growing substrates (Kalač, 2013; Karaman and Matavuli, 2005).

Phenolic profile

Forty-five phenolic compounds were identified in methanolic extracts of *C. disseminatus* using LC-MS/MS technique, among which eight were identified and quantified as flavones (apigenin 7-O-glucoside, chrysoeriol, luteolin 7-O-glucoside), biflavonoid (amentoflavone), and hydroxybenzoic and hydroxycinnamic acids (*p*-hydroxybenzoic acid, protocatechuic acid, *p*-coumaric acid, and chlorogenic acid) (Table 4).

p-Hydroxybenzoic acid was detected in higher amount in a wild growing *C. comatus* (61.53 μg/g) originated from Portugal (Vaz et al., 2011) and *C. micaceus* (25.06 μg/g) from Poland (Nowacka et al., 2014), while fungal species *C. comatus* from Serbia had lower *p*-hydroxybenzoic acids content (0.9 μg/g) (Stojković et al., 2013) compared to our sample (9.46 μg/g) (Table 4). Furthermore, *C. comatus* from

Portugal was characterized by higher content of p-coumaric acid (18.79 μ g/g) (Vaz et al., 2011), C. micaceus from Poland possessed lower content of the same compound (5.96 μ g/g) (Nowacka et al., 2014), and the wild sample of C. comatus from Serbia showed the lowest amount (1.5 μ g/g) of this phenolic compound (Stojković et al., 2013) in comparison to our result (7.8 μ g/g) (Table 4).

Phenolic compounds exhibit a wide range of physiological properties such as antiinflammatory, antimicrobial, cardioprotective, and vasodilatory effects, which have been related to their antioxidant activity (Alves et al., 2013; Ferreira et al., 2009). Antioxidant and antiproliferative activities of crude ethanolic and water extracts were previously determined for C. disseminatus by our research group and very strong and significant correlations ($r^2 = 0.99$, p < 0.01) between antiproliferative effect and total phenolic and total flavonoid content were found (Novaković et al., 2016).

CONCLUSIONS

According to the nutritional profile of fungal species *Coprinellum disseminatus* originnated from Serbia (proteins, amino acid composition, fatty acid profile, and mineral composition) and its phenolic profile this mushroom can be considered a functional food and can be used in a form of dietary supplement or spice in regular diet.

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НУТРИТИВНИ И ФЕНОЛНИ ПРОФИЛ МАЛЕ JECTUBE ГЉИВЕ COPRINELLUS DISSEMINATUS (PERS.) J.E. LANGE 1938

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Сажетак: Циљ овога рада био је да се испита нутритивни профил мале јестиве гљиве Coprinellus disseminatus пореклом из Србије са акцентом на протеине, аминокиселине, масне киселине и минерални састав, као и да се одреди њен фенолни профил. Садржај укупних протеина анализиране гљиве износио је 9.72%. Електрофорезом је идентификовано петнаест протеинских фракција молекулских маса у распону од 1.6 до 63.6 kDa, при чему присуство протеинске фракције молекулске масе 27.5 kDa указује на могућу антифунгалну активност. Садржај укупних есенцијалних и неесенцијалних аминокиселина износио је 29.57 и 96.69 mg/g СМ, респективно. Најзаступљенија есенцијална аминокиселина је леуцин. Маснокиселинкси састав C. disseminatus одликују полинезасићене масне киселине (PUFA, 59.1% од укупних масних киселина) које доминирају над засићеним (SFA, 23.1% од укупних масних киселина) и мононезасићеним масним киселинама (MUFA, 17.9% од укупних масних киселина). Доминантне масне киселине су линолна (56.6%), палмитинска (13.9%) и олеинска киселина (12.0%). C. Disseminatus је најбогатија калијумом, калцијумом и магнезијумом од макроелемената, док од микроелемента доминира гвожђе. У метанолном екстракту С. disseminatus квантификовано је осам фенолих једињења применом LC-MS/MS, при чему су највиши садржаји забележени за рхидроксибензоеву киселину (9.46 \pm 0.2 μ g/g CM) и *p*-кумаринску киселину (7.8 \pm 0.1 CM).

Кључне речи: *ељива, протеини, аминокиселине, профил масних киселина, фенолни профил, минерални састав*

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