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# FREE FATTY ACID COMPOSITION OF RAMARIA AUREA (SCHAEFF.) QUÉL.<sup>†</sup>

# *UDC 543.383*

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Abstract. Ramaria aurea (Schaeff) Quél., known as the golden coral, is an edible mushroom with a worldwide distribution, but there are scarce data on its chemical composition. Free fatty acids (FAs) from this fungal species have never been analyzed before, although it is known that FAs are linked with key physiological and biochemical processes, and thus, they essential for the proper functioning of various ecosystems. Moreover, there are no literature data on the chemical composition of R. aurea from Serbia. Herein, we report on the GC-MS characterization of a diethyl-ether extract of R. aurea fruit bodies collected in the region of Mt. Mali Jastrebac (central Serbia), with the focus on the free FAs profile, investigated using a method of FA derivatization by diazomethane. The GC-MS analysis enabled the identification of around 60 components, among which more than a half were methyl esters of saturated and unsaturated FAs, comprising 88% of the total extract. The rather diverse and complex free FA profile of R. aurea was dominated by monounsaturated FAs (ca. 35% of the total extract) with oleic acid as the most abundant constituent (ca. 34%). Polyunsaturated and saturated FAs were present in almost equal amounts, ca. 26% and 24%, respectively. The major polyunsaturated FA was linoleic (ca. 26%), while the principal saturated FA was palmitic acid (ca. 15%). The content of total saturated and mono- and polyunsaturated FAs, as well their ratio, could be used as an indicator of the potential nutritional and medicinal use of this mushroom.

Key words: mushrooms, Ramaria aurea (Schaeff) Quél., free fatty acids, methyl esters, diazomethane derivatization, GC-MS analysis.

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### **1. INTRODUCTION**

The history of mushroom consumption is probably as long as the history of food gathering (Boyden, 1973), but although mushrooms are considered to be one of the main pillars of primitive society's survival, they entered haute cuisine in the 19<sup>th</sup> century (Bertelsen, 2013). Some mushroom species represent highly valued foodstuff because of their nutritional properties; they are a good source of proteins (15-81% of dry weight) and carbohydrates (35-70% of dry weight) and make a useful contribution to the intake of vitamins and minerals (6-10% of dry weight) (Kalač, 2009). Even though they are not a choice source of lipids, due to their low content (2-6% of dry weight) (Kalač, 2009); the fat quality is good, as they mostly contain unsaturated fatty acids and they are considered to be an important source of essential fatty acids (Abugri et al., 2016).

Since ancient times, mushrooms have been used for their medicinal properties, as well, since they are rich in a variety of bioactive metabolites (e.g. phenolic compounds, polyketides, terpenes, steroids, and polysaccharides) with immunomodulatory, cardiovascular, liver protective, anti-fibrotic, anti-inflammatory, anti-diabetic, anti-viral, antimicrobial activities, and antitumor properties (Pereira et al., 2012, and references cited therein). Moreover, modern clinical practice continues to rely on mushroom-derived preparations. Purified bioactive compounds from mushrooms are a potential source of natural antioxidants that positively influence oxidative stress-related diseases such as cancer. In recent studies, it was demonstrated that unsaturated fatty acids, especially polyunsaturated ones, have an important contribution to the mushroom therapeutic properties (Abugri et al., 2016). During the last 20-30 years, gathering naturally growing edible and medicinal mushrooms has become a popular hobby worldwide, but also their cultivation has drawn much public interest due to their economic potential. In 2012, the production of edible mushrooms reached approximately 31 million tons, valued at 20 billion USD (Chang and Wasser, 2012).

The number of mushroom species is estimated to be around 140 000, but only about 10 to 15% have been well-characterized (Abugri et al, 2016). Supposing that among the unexplored mushrooms, there are only 5% of those that could have the potential nutritional and medicinal application, one can conclude that about 7 000 unrecognized species could be beneficial to humans (Abugri et al, 2016) representing a potential source of food and biologically active compounds. This points to the necessity of performing the chemical characterization of the uninvestigated species. Moreover, reevaluation of the chemical composition of known mushrooms with the development of new analytical techniques and instruments enables the discovery of new constituents that were either neglected during the previous characterizations or even misidentified.

The genus *Ramaria* (Gomphaceae) comprises approximately 200 species of coral fungi, some of which are cultivated for their edible properties (Petersen and Scates, 1988). Nonetheless, only few species have been thoroughly investigated. Besides edible ones, some *Ramaria* species are mildly poisonous causing nausea, vomiting, and diarrhea. Recently, a very unusual organoarsenic compound, homoarsenocholine, has been found in three *Ramaria* (*R. subbotrytis, R. largentii, R. pallida*) species (Braeuer et al., 2018).

*Ramaria aurea* (Schaeff) Quél., known as the golden coral, has a worldwide distribution. It is an edible mushroom when young, but there are scarce data on its chemical and nutritional composition. So far phenolic content, especially phenolic acids (Avramiuc M., 2018; Khatua et al, 2015; Olennikov and Penzina, 2014), triacylglycerol profile (Barreira et al., 2012), trace metals (Severoglu, 2013; Fujii et al., 2014), and nutritional composition (Kumar Sharma and

Gautam, 2017; Pereira et al., 2012), have been reported. Free fatty acids (FAs) from this fungal species have never been investigated before, although it is known that FAs are linked with key physiological and biochemical processes and thus, they are essential for the proper functioning of various ecosystems (Arts et al., 2001). Moreover, there are no literature data on the chemical composition of *R. aurea* from Serbia.

Herein, we report on the GC-MS characterization of a diethyl-ether extract of *R. aurea* fruit bodies collected in the region of Mt. Mali Jastrebac (central Serbia). The focus of the present study was on the free fatty acid profile of *R. aurea* investigated using a method of FA derivatization by diazomethane, followed by gas chromatography with mass spectrometry detection (GC-MS). Additionally, the ratio between saturated, monounsaturated, and polyunsaturated fatty acids was determined to estimate the quality of the lipids of this mushroom.

## 2. MATERIALS AND METHODS

## 2.1. General

All reagents and solvents were obtained from commercial sources (Sigma-Aldrich, St. Louis, Missouri, USA; Merck, Darmstadt, Germany; Carl Roth, Karlsruhe, Germany) and used as received, except that the solvents were purified by distillation.

## 2.2. Fungal material

*Ramaria aurea* (Schaeff) Quél. the fresh fruiting body was collected in the region of Mt. Mali Jastrebac (central Serbia), during summer 2019. Its identification was done according to the macroscopic features and the micromorphology of the reproductive structures (Courtecuisse, 1999). All samples were lyophilized in Alpha 2-4 LSCplus freeze-drying system (CHRIST, Osterode am Harz, Germany). The selected sample of the analyzed fungal species is maintained as exsiccate and deposited in the culture collection of the Innovation Centre (Faculty of Technology and Metallurgy, University of Belgrade).

### 2.3. Preparation of mushroom extract

Freeze-dried fungal material (*ca.* 20 g) was subjected to extraction with diethyl ether in an ultrasonic bath for 30 minutes and subsequently left to stand in the solvent, in the dark, overnight. The organic layer was washed with an aqueous solution of NaCl, the phases were separated and the diethyl-ether layer was dried with anhydrous MgSO<sub>4</sub>. The solvent was evaporated under a stream of dry nitrogen to yield 156 mg of the extract (0.78% of dry mass). The sample was transferred into a GC vial and subsequently analyzed by GC-MS. GC-MS analysis revealed that the extract was mainly composed of fatty acids. A portion of the extract (*ca.* 10 mg) was treated with diazomethane (*ca.* 3% Et<sub>2</sub>O solution, w/w) to convert free fatty acids to their methyl esters and further analyzed by GC-MS.

## 2.4. GC-MS Analyses

Gas chromatography-mass spectrometry (GC-MS) analyses were repeated three times for each sample using an HP 6890N gas chromatograph coupled with an HP 5975B massselective detector (Hewlett-Packard, Palo Alto, California, USA). The gas chromatograph was equipped with a DB-5MS fused silica capillary column (95% dimethyl-, 5% diphenyl-polysiloxane, 30 m × 0.25 mm, film thickness 0.25  $\mu$ m; Agilent Technologies, Palo Alto, California, USA). The oven temperature was raised linearly from 70 to 315 °C at a heating rate of 5 °C min<sup>-1</sup> and then held isothermally for 10 min. Helium at a flow rate of 1 ml min<sup>-1</sup> was used as the carrier gas. The injector and interface were maintained at 250 and 320 °C, respectively. The samples, 1  $\mu$ l of the solution, was injected in a split mode (the flow rate was 1 ml min<sup>-1</sup>; split ratio 10:1). The mass-selective detector was operated at the ionization energy of 70 eV in the *m/z* 35–750 range with a scanning speed of 0.34 s. The selected ion monitoring (SIM) mode was used for quantitation.

The percentage composition was computed from TIC-peak areas without the use of correction factors. The linear retention indices relative to the retention times of  $C_7-C_{33}$  *n*-alkanes on the DB-5MS column were calculated according to Van den Dool and Kratz (1963). Qualitative analyses of the mentioned samples were firstly based on the comparison of their retention indices with those from the literature (Ilić-Tomić, 2015; NIST Chemistry WebBook Database), the comparison of their mass spectra with those of authentic standards, as well as those from Wiley 11 and NIST14 databases, the analysis of the fragmentation patterns from their mass spectra. The double bond position was inferred from the MS fragmentation patterns of the corresponding DMDS adducts (Ilić-Tomić et al., 2014). Finally, wherever possible, identification was achieved by GC co-injection with an authentic sample.

#### 3. RESULTS AND DISCUSSION

GC-MS analysis of R. aurea diethyl-ether extract revealed that it was mainly composed of free fatty acids (FAs). To perform qualitative and quantitative analyses of these free FAs by GC-MS, we converted them to the corresponding fatty acid methyl esters (FAMEs), by treating the extract with an ethereal solution of CH<sub>2</sub>N<sub>2</sub>. The GC-MS analysis of this derivatized extract enabled the detection and identification of more than 60 components (the extent of identification of components being around 90% of the total detected total ion current (TIC) peak areas, Table 1). The inspection of the TIC chromatogram revealed, among other constituents (mostly alkanes, carbonyl compounds, and steroids), several series of FAMEs showing regularities in their GC retention behavior (constant retention index difference of ca. 100 units) and possessing analogous mass spectra. Among identified FAMEs the most abundant group of compounds were methyl esters of saturated and unsaturated FAs, comprising ca. 88% of the total extract. The identification of saturated normal chain FAMEs was based on a combination of data coming from their mass spectra and gas chromatographic retention behavior (Ilić-Tomić et al., 2012). In addition to this, the double bond position was inferred from the MS fragmentation patterns of the corresponding DMDS adducts (Ilić-Tomić et al., 2014). The identification was corroborated wherever possible by a subsequent GC-MS analysis of authentic standards.

Table 1 Chemical component	osition of the meth	ylated Ramaria a	aurea (Schaeff)	Quél. d	iethyl-
ether extract					

No	RI <sup>a</sup>	%	Component	Dessig-	Class	Method of
			L	nation		identification <sup>b</sup>
1.	789	0.18	2-Hexanone		0	RI, MS, CoI
2.	803	tr	<i>n</i> -Hexanal		0	RI, MS, CoI
3.	816	0.06	4-Hydroxy-2-pentanone		0	RI, MS, CoI
4.	824	tr	Methyl pentanoate (syn. methyl valerate)	5:0	SFAME	RI, MS, CoI
5.	854	0.11	1,3-Butanediol		0	RI, MS, CoI
6.	871	tr	4-Heptanone		0	RI, MS, CoI
7.	886	tr	3-Heptanone		0	RI, MS, CoI
8.	889	tr	2-Heptanone		0	RI, MS, CoI
9.	924	0.14	Methyl hexanoate (syn. methyl caproate)	6:0	SFAME	RI, MS, CoI
10.	971	tr	4-Octanone		0	RI, MS, CoI
11.	986	tr	3-Octanone		0	RI, MS, CoI
12.	1025	tr	Methyl heptanoate (syn. methyl enanthate)	7:0	SFAME	RI, MS
13.	1098	tr	Methyl benzoate		OAME	RI, MS, CoI
14.	1100	tr	Undecane		0	RI, MS, CoI
15.	1125	tr	Methyl octanoate (syn. methyl caprylate)	8:0	SFAME	RI, MS, CoI
16.	1179	tr	Methyl phenylacetate		OAME	RI, MS, CoI
17.	1200	tr	Dodecane		0	RI, MS, CoI
18.	1226	tr	Methyl nonanoate (syn. methyl pelargonate)	9:0	SFAME	RI, MS, CoI
19.	1300	tr	Tridecane		0	RI, MS, CoI
20.	1326	tr	Methyl decanoate (syn. methyl caprate)	10:0	SFAME	RI, MS, CoI
21.	1400	tr	Tetradecane		0	RI, MS, CoI
22.	1436	tr	Methyl 9-oxo-nonanoate		OFAME	RI, MS
23.	1519	tr	Methyl vanillate		OAME	RI, MS, CoI
24.	1527	tr	Methyl dodecanoate (syn. methyl laurate)	12:0	SFAME	RI, MS, CoI
25.	1551	tr	Dimethyl nonanedioate (syn. dimethyl azelate)		FADME	RI, MS, CoI
26.	1700	tr	Heptadecane		0	RI, MS, CoI
27.	1727	0.35	Methyl tetradecanoate (syn. methyl myristate)	14:0	SFAME	RI, MS, CoI
28.	1827	0.55	Methyl pentadecanoate (syn. methyl pentadecylate)	15:0	SFAME	RI, MS, CoI
29.	1854	tr	Dimethyl dodecanedioate		FADME	RI, MS
30.	1900	tr	Nonadecane		0	RI, MS, CoI
31.	1906	0.49	Methyl (Z)-9-hexadecenoate	16:1ω7c	MUFAME	RI, MS, CoI,
			(syn. methyl palmitooleate)			DMDS
32.	1927	15.13	Methyl hexadecanoate (syn. methyl palmitate)	16:0	SFAME	RI, MS, CoI
33.	1966	tr	Dimethyl tridecanedioate		FADME	RI, MS
34.	1998	tr	Ethyl hexadecanoate (syn. ethyl palmitate)		FAEE	RI, MS, CoI
35.	2000	tr	Eicosane		Ο	RI, MS, CoI
36.	2006	0.31	Methyl (Z)-9-heptadecenoate	17:1ω8c	MUFAME	RI, MS, DMDS
37.	2027	0.51	Methyl heptadecanoate (syn. methyl margarate)	17:0	SFAME	RI, MS, CoI
38.	2100	25.77	Methyl (Z,Z)-9,12-octadecadienoate	18:2 <i>w</i> 6c	PUFAME	RI, MS, CoI
			(syn. methyl linolenate)			
39.	2106	33.66	Methyl (Z)-9-octadecenoate (syn. methyl oleate)	18:1ω9c	MUFAME	RI, MS, CoI,
						DMDS
40.	2127	5.30	Methyl octadecanoate (syn. methyl stearate)	18:0	SFAME	RI, MS, CoI
41.	2165	0.25	Ethyl (Z,Z)-9,12-octadecadienoate		FAEE	RI, MS, Col
12	2170	0.24	(syn. curyf filloleate) Ethyl (Z)-9-octadecenoate (syn. ethyl oleate)		EVEE	RI MS Col
<i></i> +∠.	2170	0.24	$Lury (\Sigma)$ - $\beta$ -octaticentoate (Syll. Elliyi Oleate)		TALL	DMDS
43	2200	tr	Docosane		0	RI, MS, Col
44.	2227	tr	Methyl nonadecanoate (syn. methyl nonadecylate)	19:0	SFAME	RI, MS, CoI

A. MILTOJEVIĆ, I. MILOVANOVIĆ, N. RADULOVIĆ

45.	2299	0.26	Methyl (Z,Z)-11,14-eicosadienoate	20:2 <i>w</i> 6c	PUFAME	RI, MS, CoI	
46.	2306	0.79	Methyl (Z)-11-eicosenoate (syn. methyl gondoate)	20:1ω9c	MUFAME	RI, MS, CoI,	
						DMDS	
47.	2427	tr	Methyl heneicosanoate (syn. methyl heneicosylate)		SFAME	RI, MS, CoI	
48.	2506	0.25	Methyl (Z)-13-docosenoate (syn. methyl erucate)	22:1ω9c	MUFAME	RI, MS, CoI,	
						DMDS	
49.	2527	0.63	Methyl docosanoate (syn. methyl behenate)	22:0	SFAME	RI, MS, CoI	
50.	2627	0.19	Methyl tricosanoate (syn. methyl tricosylate)	23:0	SFAME	RI, MS	
51.	2706	0.56	Methyl (Z)-15-tetracosenoate (syn. methyl nervonate)	24:1ω9c	MUFAME	RI, MS, DMDS	
52.	2727	1.16	Methyl tetracosanoate (syn. methyl lingocerate)	24:0	SFAME	RI, MS	
53.	2827	tr	Methyl pentacosanoate (syn. methyl pentacosylate)	25:0	SFAME	RI, MS	
54.	2903	tr	Nonacosane		0	RI, MS	
55.	2927	tr	Methyl hexacosanoate (syn. methyl cerotate)	26:0	SFAME	RI, MS	
56.	3127	tr	Methyl octacosanoate (syn. methyl montanate)	28:0	SFAME	RI, MS	
57.	3166	0.82	Ergosta-5,7,9(11),22-tetraen-3β-ol		S	RI, MS	
58.	3205	3.84	Ergosta-5,7,22-trien- $3\beta$ -ol (syn. ergosterol)		S	RI, MS	
59.	3217	0.60	$5\alpha$ -Ergosta-7,22-dien-3 $\beta$ -ol (syn. 5-dihydroergosterol)		S	RI, MS	
		92.15	Total identified:				
		23.96	Saturated fatty acid methyl esters (SFAME)				
		36.06	Monounsaturated fatty acid methyl esters (MUFAME)				
		26.03	Polyunsaturated fatty acid methyl esters (PUFAME)				
		tr	Dioic fatty acid methyl esters (FADME)				
		tr	Other fatty acids methyl esters (OFAME)				
		tr	Other methyl esters (OAME)				
		0.49	Fatty acid ethyl esters (FAEE)				
		5.26	Steroids (S)				
		0.35	Others (O)				
tr - t	r – trace (<0.05%), syn. – synonym						

<sup>a</sup> RI – retention indices on a DB-5 column calculated against a series of co-injected *n*-alkanes (C<sub>7</sub>–C<sub>33</sub>) <sup>b</sup> RI – constituent identified by retention index matching, MS – constituent identified by mass spectra comparison, CoI – the identity of the constituent was additionally confirmed by co-injection of an authentic sample, DMDS – position of double bond was confirmed from the fragmentation patterns of the corresponding DMDS adducts

<sup>c</sup> Percentage composition

The rather diverse and complex free FA profile of R. aurea was dominated by monounsaturated FAs (MUFAMEs, ca. 35%) with oleic acid as the most abundant constituent, comprising 34% of the total extract. Polyunsaturated (PUFAMEs) and saturated fatty acids (SFAMEs) were present in almost equal amounts comprising approximately 26% and 24% of the total extract, respectively. The major polyunsaturated FA was linoleic (ca. 26%), while the principal saturated FA was palmitic acid (ca. 15% of the total extract). Oleic and linoleic acids are common in eukaryotic organisms such as fungi, while palmitic acid is common to different organisms. Oleic acid plays an important role in the biochemical and physiological pathways in cholesterol accumulation in the human body, as this monounsaturated FA can lower serum cholesterol concentrations (Abugri et al., 2016). Linoleic acid is an essential fatty acid to mammals. It is a precursor of arachidonic acid and of prostaglandins biosynthesis, which play important physiological activities (Nelson and Cox, 2008), as well as of 1-octen-3-ol, known as the alcohol of fungi, which contributes to mushroom flavor (Maga, 1981). Due to such a high linoleic acid content R. aurea, as other mushrooms, may be considered as a source of this essential fatty acid. Other unsaturated FAs were present in less than 1% of the total extract. Moreover, dimethyl esters of three dioic acids (nonadioic, dodecadioic, tridecadioic acids), as well as an oxoacid (9-oxo-nonanoic acid), were detected in traces. In addition to FAMEs, ethyl esters of the three mentioned most abundant FAs, oleic, linoleic, and palmitic acids, were identified.

The FA with the shortest chain detected in the analyzed sample was pentanoic acid. In previous researches on bound *R. aurea* FA composition, short-chain fatty acids were not detected. Kumar Sharma and Gautam (2017) detected nonanoic, while Pereira and coworkers (2012) detected palmitic acid as the one with the shortest chain. This might be due to the loss of short-chain fatty acids during the transesterification process. The methylation with  $CH_2N_2$ , performed herein, occurs at room temperature, not allowing short-chain fatty acids to evaporate from the sample.

Free fatty acids from R. aurea have never been investigated before. The fatty acid profile obtained after the transesterification of the fungal material was investigated on two previous occasions (Kumar Sharma and Gautam, 2017; Pereira et al., 2012). Pereira and coworkers identified only four FAMEs, while the number of FAMEs identified by Kumar Sharma and Gautam (2017) was nine; in this study, we identified 32 FAMEs in total. The relative abundance of FAME reported by a group led by Pereira (2012) is in good agreement with the results obtained herein. The most abundant are linoleic and oleic acids, followed by saturated palmitic and stearic acids. On the other hand, Kumar Sharma and Gautam (2017) identified two polyunsaturated FAs, linoleic and (Z,Z)-11.14-eicosadienic acids as the major ones. In our case, linoleic acid was the second most abundant, while (Z,Z)-11,14-eicosadienic acids was a minor constituent. This discrepancy may be due to the different sample preparation procedures (we analyzed free FAs and they analyzed transesterified fungal material). Moreover, variations in the FAs profiles may be attributed to differences in the abiotic and biotic factors, the geographical origin, pH of the soil, pressure, nutrients, moisture content, mushroom developmental stage, enzymatic activities in the mushroom itself and its symbiotic organisms (Abugri et al., 2016). The free fatty acid content of other Ramaria species was investigated only on one previous occasion (Leon-Guzman et al., 1997), where only four different free FAs (palmitic, stearic, oleic, and linoleic acid, all identified by us) of R. flava were detected, comprising 3.3% of the analyzed extract.

To conclude, in GC-MS analysis of methylated *R. aurea* diethyl-ether extract revealed *ca.* 60 constituents among which more than a half were fatty acids esters comprising more than 85% of the total extract. In *R. aurea*, free unsaturated fatty acids predominate over saturated ones and this is in agreement with the earlier findings concerning different mushroom species (Senatore et al., 1988). The linoleic to oleic, and linoleic to other unsaturated FAs ratio may be used as a biomarker in physiological, taxonomical, and cell differentiation studies of two similar or different species of mushrooms within the same family. Moreover, the content of total saturated and mono- and polyunsaturated fatty acids, as well their ratio, may be used as an indicator of the potential nutritional and medicinal use of a mushroom species in question (Abugri et al., 2016).

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# SASTAV SLOBODNIH MASNIH KISELINA VRSTE RAMARIA AUREA (SCHAEFF.) QUÉL.

Ramaria aurea (Schaeff) Quél. je jestiva gljiva rasprostranjena širom sveta, poznata pod nazivom zlatni koral. Međutim, hemijski sastav ove vrste je nedovoljno ispitan. Slobodne masne kiseline (MK) do sada nisu analizirane iako je poznato da MK imaju ključnu ulogu u mnogim fiziološkim i biohemijskim procesima i važne su za pravilno funkcionisanje različitih ekosistema. Pored toga, ne postoje literaturni podaci o hemijskom sastavu R. aurea iz Srbije. U ovom radu izvršena je GC-MS analiza dietil-etarskog ekstrakta plodonosnih tela vrste R. aurea sakupljene na planini Mali Jastrebac (centralna Srbija). Posebna pažnja posvećena je analizi profila slobodnih MK, nakon derivatizacije MK diazometanom. GC-MS analiza omogućila je identifikaciju oko 60 sastojaka, među kojima su dominirali metil-estri zasićenih i nezasićenih MK, čineći ca. 88% ukupnog ekstrakta. Profil MK vrste R. aurea bio je prilično raznolik i složen. Najzastupljeniji su bili metil-estri mononezasićenih MK (oko 35%) sa oleinskom kiselinom kao najzastupljenijim sastojkom (34%). Polinezasićene i zasićene MK

bile su prisutne u gotovo jednakim količinama, oko 26%, odnosno 24%. Glavna polinezasićeni MK bila je linoleinska (oko 26%), dok je glavna zasićena MK bila palmitinska kiselina (oko 15%). Dakle, kod gljive R. aurea slobodne nezasićene MK su zastupljenije od zasićenih. Sadržaj ukupnih zasićenih, mono- i polinezasićenih masnih kiselina, kao i njihov odnos, mogao bi se koristiti kao pokazatelj potencijalne upotrebe ove pečurke u nutritivne i lekovite svrhe.

Ključne reči: pečurke, Ramaria aurea (Schaeff) Quél., slobodne masne kiseline, metil-estri, derivatizacija diazometanom, GC-MS analiza