FATTY ACID COMPOSITION IN MEDICINAL MUSHROOMS (CRATERELLUS TUBAEFORMIS)

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Abstract: Bulgaria has a very rich fungal flora due to its phytogeographical position. The screening of chemical content and active substances of mushrooms becomes an important subject not only for Bulgaria but also for all over the world. In the last decade, Analyses on phytochemical and biological activity of fungi have gradually increased as a result of improvement in the number and quality of facilities. In the scope of the present research, medicinal mushrooms; *Craterellus tubaeformis* was analyzed for fatty acid compositions by GC-FID, tocopherols by HPLC-fluorescence, and Oil content by Soxhlet. The ongoing research will lead to a new generation of foods, and will certainly promote their nutritional and medicinal use.

Keywords: Oil content, Fatty acid, Tocopherols, *Craterellus tubaeformis*, Batak mountain, Bulgaria.

1. INTRODUCTION

Different mushrooms were studied by the scientific community, in searching for new therapeutic alternatives, and the results proved their bioactive properties. Mushrooms are rich sources of nutraceuticals responsible for their antioxidant, antitumor, and antimicrobial properties. Besides their pharmacological features, wild mushrooms are becoming more important in our diet due to their nutritional value, related to the high protein and low fat/energy contents.

Craterellus tubaeformis is a beneficial nutrient for human health with high levels of vitamins B and C [1]. Natural antioxidants can be used as an effective tool against oxidative damage which can result in ageing and degenerative diseases [2]. Some mushrooms are an important source of natural antioxidants and essential fatty acids. Many studies have been conducted individually on fatty acids, antioxidant activities, mineral and trace elements of mushrooms [3,4]. But fatty acid composition of mushrooms have not been evaluated altogether.

Our objective was to evaluate the fatty acid composition wild edible medicinal mushroom specie (*Craterellus tubaeformis*) collected from Bulgaria.

2. METHODS

2.1. Mushroom Samples

Fifteen mushroom samples were collected in 2014 and 2017 from the Batak mountain by the authors themselves.

The Batak mountain is located in western Rhodopes. Its western border is defined by the Chepinska river, the southern border – by Dospatska river and Dospat dam, the eastern border – by Vacha river and the northern border – by the Thracian Plane (GPS41°46'02.6"N 24°08'48.4"E). The regions is industry-free and is characterised with forests, land and low buildings.

2.2. Reagents

All chemicals were at least of analytical-reagent grade. Water was de-ionized in a Milli-Q system (Millipore, Bedford, MA, USA) to a resistivity of 18.2 MΩ cm.

All plastic and glassware were cleaned by soaking in diluted HNO₃ (1/9, v/v) and were rinsed with distilled water prior to use.

2.3. Determination of Oil content

The seeds (10 g sample) were air-dried and the oil was extracted with n-hexane in *Soxhlet* for 8 h [5]. The solvent was partly removed in rotary vacuum evaporator, the residue was transferred in pre-weight glass vessels and the rest of the solvent was removed under stream of nitrogen to a constant weight to determine the oil content.

2.4. Determination of Fatty acids

The fatty acid composition was determined by gas chromatography after transmethylation of the sample with 2% H₂SO₄ in absolute CH₃OH at 50°C [6]. Fatty acid methyl esters (FAME) were purified by thin-layer chromatography (TLC) on 20 x 20 cm plates covered with 0.2 mm silica gel 60 G (Merck) layer with mobile phase n-hexane:diethyl ether (97:3, v/v). GC was performed on a HP 5890 series II (Hewlett Packard GesmbH, Vienna, Austria) gas chromatograph equipped with a 75 m x 0.18 mm (I.D.) x 25 μm (film thickness) capillary column Supelco and a flame ionization detector. The column temperature was programmed from 140°C (5 min), at 4°C/min to 240°C (3 min); injector and detector temperatures were kept at 250°C. Hydrogen was the carrier gas at a flow rate 0.8 mL/min; split was 1:50. Identification of fatty acids was performed by comparison of retention times with those of a standard mixture of fatty acids subjected to GC under identical experimental conditions [7].

2.5. Determination of Tocopherols

Tocopherols were determined directly in the oil by high performance liquid chromatography (HPLC) on a Merck-Hitachi (Merck, Darmstadt, Germany) instrument equipped with 250 mm x 4 mm Nucleosil Si 50-5 column (Merck, Darmstadt, Germany) and fluorescent detector Merck-Hitachi F 1000. The operating conditions were as follows: mobile phase of n-hexane:dioxan, 96:4 (by volume), flow rate 1.0 ml/min, excitation 295 nm, emission 330 nm. 20 μl 2% solution of crude oil were injected. Tocopherols were identified by comparing the retention times with those of authentic individual tocopherols. The tocopherol content was calculated on the base of tocopherol peak areas in the sample vs. tocopherol peak area of standard tocopherol solution [8].

2.6. Statistical

SPSS (Statistical Package for Social Science) program for Windows was used for statistical data processing.

3. EXPERIMENTAL

The oil content, Tocopherol, Fatty acid compositions of mushrooms (*Craterellus tubaeformis*) were given in Table 1.

Chromatogram of FAMES of mushroom (*Craterellus tubaeformis*) is given in Figure 1.

Table 1. Oil content, Tocopherol, Fatty acid composition of mushrooms (*Craterellus tubaeformis*)

Mushrooms (<i>Craterellus tubaeformis</i>)		
Oil content, %		4.1
α -Tocopherol, %		51.7
γ -Tocopherol, %		-
δ -Tocopherol, %		48.3
Total tocopherols, mg/kg		17
Fatty acids, %		
C _{12:0}	Lauric	-
C _{14:0}	Myristic	0.3
C _{15:0}	Pentadecanoic	0.6
C _{16:0}	Palmitic	17.7
C _{16:1}	Palmitoleic	0.8
C _{17:0}	Margaric	0.3
C _{18:0}	Stearic	14.5
C _{18:1}	Oleic	54.9
C _{18:2}	Linoleic	7.5
C _{18:3}	Linolenic	0.2
C _{20:0}	Arachidic	0.6
C _{20:1}	Gadoleic	0.6
C _{20:2}	Eicosadienoic	0.9
C _{20:3}	Eicosatrienoic	0.1
C _{20:4}	Arachidonic	0.1
C _{20:5}	Eicosapentaenoic	0.4
C _{22:0}	Behenic	0.5
SFA		34.5
UFA		65.5
MUFA		56.3
PUFA		9.2

* - Not identified; SFA – saturated fatty acids;

UFA – unsaturated fatty acids;

MUFA – monounsaturated fatty acids;

PUFA – polyunsaturated fatty acids.

Figure 1.
Chromatogram of FAMES of mushroom (*Craterellus tubaeformis*)

1 – Lauric acid; 2 – Myristic acid; 4 – Pentadecanoic acid; 5 – Palmitic acid; 7 – Palmitoleic acid; 8 – Margaric acid; 10 – Stearic acid; 11 – Oleic acid; 14 – Linoleic acid; 15 – Arachidic acid; 16 – Linolenic acid; 17 – Gadoleic acid; 18 – Eicosadienoic acid; 19 – Behenic acid; 20 – Eicosatrienoic acid; 21 – Arachidonic acid; 25 – Eicosapentaenoic acid

4. RESULTS

Oil content of the sample was relatively low (4.1%) but higher than those of other species of mushrooms. The results obtained in this study are consistent with the previously reported results in the literature [4,9]. In addition to linoleic acid, the other fatty acids such as oleic acid, palmitic acid, pentadecenoic acid and linoelaidic acids were also found in the mushroom samples.

Total tocopherol content was found to be 17 mg/kg and the amount of α - and δ -tocopherol was about 50.0%.

Unsaturated fatty acids prevailed in the oil from mushroom (65.5%) and the content of MUFA consisted of 56.3%. On the other hand, the amount of PUFA was much lower (9.2%). Oleic acid predominated in the triacylglycerols (54.9%), followed by palmitic (17.7%) and stearic acid (14.5%). The quantity of linoleic acid was found to be 7.5% and the other fatty acids were from 0.1 to 0.9%.

5. CONCLUSIONS

The ongoing research will lead to a new generation of foods, and will certainly promote their nutritional and medicinal use.

6. REFERENCES

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