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PROXIMATE COMPOSITION OF WILD EDIBLE MUSHROOMS FROM THE BATAK MOUNTAIN, BULGARIA

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ABSTRACT

Aim: The aim of the study was to be thoroughly examined the chemical and lipid composition of three species of wild edible mushrooms (*Boletus pinophilus*, *Cantharellus cibarius* and *Craterellus cornucopioides*) grown in Bulgaria as well as performing a principal component analysis in order to clarify specific relationships between the species and their nutritional compositions. Methods: Standard methods following the ISO procedures were used for determination of the components. Results: Moisture, crude fat, proteins, total carbohydrates, crude fibre and ash were determined in the examined mushroom species. Potassium and phosphorus were the essential elements in the mushrooms. Unsaturated fatty acids predominated in the lipid fraction of triacylglycerols, and oleic acid was the main one. Ergosterol was the major sterol in the lipid fraction of all mushrooms (42.4 – 72.8%). Phospholipid composition of the mushrooms differed, and phosphatidic acids were the main class in *B. pinophilus* and *C. cibarius*, while in *C. cornucopioides* all phospholipid classes were present in similar quantities. Fatty acid composition of the main phospholipid classes of the mushrooms was determined for the first time and was observed that saturated fatty acids (51.2 – 73.3%) predominated in all main phospholipids except in phosphatidic acids from *C. cornucopioides*, where unsaturated fatty acids prevailed (54.9%). Conclusion: Some key differences were observed in the chemical and lipid composition of the examined mushroom species but all of them were abundant in valuable fat-soluble biologically active components which made them suitable for possible application as food additives in many products and functional foods.

Keywords: Biologically active components, Chemical composition, Lipids, Wild edible mushrooms

INTRODUCTION

Mushrooms are widespread in nature and their number is over 10 000 species worldwide. About 200 types of edible mushrooms have been established in Bulgaria, but the population of the country uses as food no more than 20-30 types of wild mushrooms, of which only a few types are widely consumed. The most common edible mushrooms in the country are: porcini mushroom (*Boletus edulis*), field mushroom (*Agaricus campestris*), slippery jack mushroom (*Suillus luteus*) and parasol mushroom (*Macrolepiota procera*) (**Brzezicha-Cirocka *et al.*, 2019**). Mushrooms are unique food worldwide with specific taste and flavor. Many species can produce myriads of novel constituents that possess biological properties as well. Recently, mushrooms are viewed as a source of nutraceuticals and antioxidants that lead to their anti-microbial, prebiotic, immune modulating, anti-inflammatory, cardiovascular, anti-carcinogenic and anti-diabetic activity (**Ebrahimzadeh *et al.*, 2015; Figueiredo and Régis, 2017; Gąsecka *et al.*, 2017; Jabłornska-Ryrs *et al.*, 2016; Kanagasabapathy *et al.*, 2011; Kathiravan and Krishnakumari, 2017; Kosanić *et al.*, 2016; Wasser, 2017**).
Functional foods are enriched or modified foods which are consumed as normal diet to provide healthful benefit (**Demková *et al.*, 2017; Fiket *et al.*, 2017; Shirur *et al.*, 2017; Türkmen and Budur, 2018**). Wild mushrooms are considered to be functional foods because they contain substances that could be used in the human diet in order to promote healthiness such as phenolic compounds, minerals, vitamins, tocopherols, ascorbic acid and carotenoids, but also are rich in some macro- and micronutrients (**Adebayo *et al.*, 2018; Barros *et al.*, 2008a; Dospatliev and Ivanova, 2017a; Dulay *et al.*, 2015; Jiang *et al.*, 2012; Mattila *et al.*, 2001; Nasiry *et al.*, 2017; Türkekul *et al.*, 2017**).
The chemical composition of mushrooms has been the subject of several studies, and it has been established that the content of the main components varies within different limits depending on the species, the soil and the climatic conditions in which they have grown. According to different authors, the oil content of mushrooms varies widely - from 0.4 to 27.5% (**Beluhan and Ranogajec, 2011**; **Kalac, 2009**). The amount of oil in *Cantharellus cibarius* has been found to be between 1.9 and 4.5% (**Barros *et al.*, 2008a**; **Beluhan and Ranogajec, 2011**; **Kalac, 2009**), while in *Craterellus cornucopioides* it is about 4.9 % (**Barros *et al.*, 2008a**; **Beluhan and Ranogajec, 2011**).
Total protein content is relatively high – from 16.5 to 69.5% and their amount is considerable in *C. cibarius* and *C. cornucopioides* (30.9 - 69.0% and 47.2 - 69.5%, respectively) (**Barros *et al.*, 2008a; Beluhan and Ranogajec, 2011**, **Kalac, 2009**). The carbohydrate content of different types of mushrooms also varies widely – from 16.4 to 74.3%, with an average amount of 59.9% (**Kalac, 2009**). On the other hand, their content in *C. cibarius* is between 14.3 and 32.0% (**Barros *et al.*, 2008a; Kalac, 2009**), while in *C. cornucopioides* it is lower - about 13.4% (**Barros *et al.*, 2008a**). The content of the reducing sugars in the last two species of mushrooms is found to be 2.5 - 2.7 % (**Barros *et al.*, 2008a**).
Copper, iron, zinc, cobalt, selenium, and manganese are some of the essential elements that play an important role in the catalytic processes of the living organisms (**Dospatliev and Ivanova, 2017c; Falandysz and Drewnowska, 2015; Keen *et al.*, 2004; Khalili *et al.*, 2015; Kuka *et al.*, 2014; Massadeh and Al-Massaedh, 2018; Sumaira *et al.*, 2016; Wang *et al.*, 2017**). Despite that, high levels of these metals can be very dangerous to human health. (**Dospatliev and Ivanova, 2017b; Koyyalamudi *et al.*, 2013; Ouzouni *et al.*, 2009; Širić *et al.*, 2014; Soylak *et al.*, 2005; Stefanović *et al.*, 2016; Tuzen *et al.*, 2007; Valverde *et al.*, 2015**). Total ash content of *C. cibarius* was reported to vary between 11.5 and 12.1%, while in *C. cornucopioides* it was about 12.2% (**Barros *et al.*, 2008a; Kalac, 2009**).
Lipid composition of the mushrooms is also abundant in some fat-soluble biologically active components. Unsaturated fatty acids (61.0 - 77.4%) prevailed in the oil from *C. cibarius*, and some authors reported that, the main representative was oleic acid (about 35.4%), followed by linoleic acid (17.3%) (**Kavishree *et al.*, 2008**). According to other studies, polyunsaturated linoleic acid predominated (53.4%), and the content of monounsaturated oleic acid was significantly low (10.9%) (**Barros *et al.*, 2008a**). Saturated fatty acids were from 22.6 to 39.0%, the main ones among them were palmitic (13.1 – 18.3%) and stearic (6.0 – 6.5%) acids (**Barros *et al.*, 2008a; Kavishree *et al.*, 2008**).
In the oil from *C. cornucopioides*, unsaturated fatty acids (83.6%) also predominated, of which the main representative was oleic acid (51.8%), followed by linoleic acid (23.7%) (**Barros *et al.*, 2008a**). The content of saturated fatty acids (about 16.4%) was lower than that in the lipids from *C. cibarius*, with stearic (7.8%) and palmitic acid (6.7%) being the main ones (**Barros *et al.*, 2008a**).
Previous studies on the sterol composition of mushrooms were scarce, but according to most authors ergosterol predominated (304 μg/100 g and 24.7 mg/100 g in *C. cibarius*, respectively) (**Mattila *et al.*, 2002**; **Teichmann *et al.*, 2007**).
It is well-known that the chemical and lipid composition of the same species of mushrooms may differ depending on the region they grow. On the other hand, the

information about the chemical and lipid composition of wild edible mushrooms grown in Bulgaria is rather scarce. Therefore, the aim of this study was to determine proximate composition of three species wild edible mushrooms (*Boletus pinophilus, Cantharellus cibarius* and *Craterellus cornucopioides*) grown in the Batak Mountain, Bulgaria, as well as to characterize the isolated lipids, i.e., the content of biologically active substances (essential fatty acids, sterols and phospholipids). The present work provides data about the nutritional value of the investigated species of mushrooms and reveals them as a potential source of beneficial bioactive components.

MATERIAL AND METHODS

Mushroom samples

Mushrooms were collected in 2017 from the Batak Mountain area. Fifteen samples of entirely matured fruiting bodies from the tree species were collected, cleaned, and washed. They were air-dried in an oven at 60 °C, homogenized, then ground in order to pass through a 40-mesh sieve and stored at room temperature before analysis. The Batak Mountain is located in western Rhodopes, which is defined by the Chepinska river to the west, by Dospatska river and Dospat dam to the south, by Vacha river to the east and by the Thracian Plane to the north (GPS41°46'02.6"N 24°08'48.4"E) (Figure 1). The region is industry-free and is characterized with forests, lands, and low buildings.

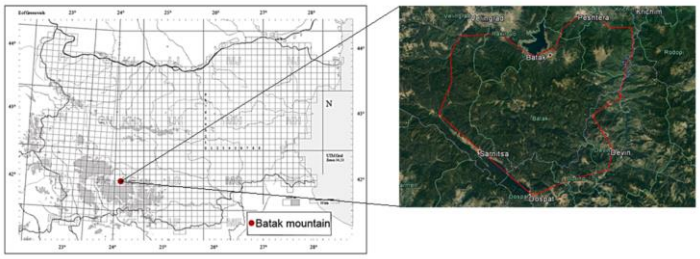

Figure 1 Location of the sampling sites

Standards and reagents

Reagents were purchased from Merck and Fluka. In order to validate the method for accuracy and precision the certified reference material (CRM) - Virginia Tobacco Leaves (CTA-VTL-2) was analysed for the corresponding elements. Water was deionized in a Milli Q system (Millipore, Bedford, MA, USA) to a resistivity of 18.2 MΩ cm.

Chemical composition

Protein, crude fiber, moisture, and ash were determined according to **AOAC (2016)**. The carbohydrate content was calculated by the following formula: Total carbohydrates (g/100g) = 100 - (g crude protein + g crude fat + g ash) (**FAO, 2003**). The soluble carbohydrates and the starch content were identified by the methods described in **BS 7169:89** and **BS 13488:76**, respectively. Total energy was calculated: Total energy (kJ /100g) = 17 × (g protein +g carbohydrate) + 37 × (g lipid) (**Dir. 90/496/EEC**).

Mineral and trace elements

Mineral and trace elements were determined by Perkin Elmer AAnalyst 800 atomic absorption spectrometer with deuterium background corrector and ICP Optima model 7000 DV. For the sample digestion was used Multiwave 3000 closed vessel microwave system. Mushroom samples (0.25 g) were digested with HNO_3 (65%) and H_2O_2 (30%) in the system and then diluted with deionized water. A blank digest was carried out as well.

Determination of glyceride oil content

Dry mushrooms (100 g sample) were subjected to extraction with hexane in a Soxhlet apparatus for 8 h in order to isolate the glyceride oil. The solvent was removed with rotary vacuum evaporator and the oil content was determined gravimetrically (**ISO 659, 2014**).

Analysis of fatty acids

Glyceride oil was subjected to transmethylation with 2% H_2SO_4 in CH_3OH at 50 °C (**ISO 12966-2, 2011**). Fatty acid methyl esters (FAME) were determined on a HP 5890 series II (Hewlett Packard GesmbH, Vienna, Austria) gas chromatograph with a flame ionization detector and a capillary column Supelco (75 m × 0.18 mm (I.D.) × 25 μm (film thickness)). The column temperature was: from 140 °C (5 min), at 4 °C/min to 240 °C (3 min); injector and detector temperatures were 250 °C and hydrogen was the carrier gas (**ISO 12966-1, 2014**).

Analysis of sterols

Unsaponifiable matter was isolated after saponification of the glyceride oil and extraction with hexane (**ISO 18609, 2000**). Total sterols were determined spectrophotometrically (**Ivanov *et al*., 1972**). Individual sterol composition was determined on HP 5890 gas chromatograph (Hewlett Packard GmbH) with flame ionization detector and DB - 5 capillary column 25 m × 0.25 mm. Temperature was: from 90 °C (hold 2 min) up to 290 °C at a rate of change 15 °C/min and then up to 310 °C at a rate of 4 °C/min (hold 10 min); detector temperature was 320 °C; injector temperature was 300 °C and carrier gas was hydrogen. For the identification was used a standard mixture of sterols (cholesterol – stabilized, purity 95%, New Jersey, USA; stigmasterol – Sigma-Aldrich, purity 95%, St. Louis, MO, USA and β-sitosterol with ca 10% campesterol, ca 75% β-sitosterol, New Jersey, USA) (**ISO 12228-1, 2014**).

Analysis of phospholipids

Air-dried mushrooms (50 g) were extracted with chloroform: methanol (2:1, v/v) (**Folch *et al*., 1957**). The phospholipid classes were isolated by a two-dimensional TLC (**Schneiter and Daum, 2006**) and their quantity was determined spectrophotometrically at 700 nm (**ISO 10540-1, 2014**).
The isolation of phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylethanolamine (PE) from the phospholipid fraction was performed by TLC. The main phospholipids were subjected to saponification and acidifycation (**Arutyunyan and Kornena, 1986**) in order to obtain free fatty acids which were esterified with 2% H_2SO_4 in absolute CH_3OH at 50 °C to methyl esters of the fatty acids (FAME) (**ISO 12966-2, 2011**). FAMEs of the main phospholipid classes were run in GC under the same conditions described above.

Statistical analyses

Statistical analysis and generation of all graphs were performed using the R program version 3.4.4 (2018-03-15). The results are shown as mean value and standard deviation (SD) and analyzed through one-way analysis of variance (ANOVA) followed by Duncan's test with $p < 0.05$. The relationships between mushroom species and their nutritional composition were examined using a principal component analysis (**Sharma, 1996**).

RESULTS

Chemical composition of mushrooms

The contents of the main components in the mushrooms, glyceride oil, proteins, carbohydrates (soluble sugars, starch and fibers), minerals and moisture, are presented in Table 1.

Table 1 Moisture (% of fresh weight), macronutrients (% of DW*), and total energy (kJ/100g of DW) in the wild edible mushrooms

Components	Species		
	Boletus pinophilus	*Cantharellus cibarius*	*Craterellus cornucopioides*
Moisture	90.40±1.22^b	89.59±0.06^b	87.95±0.55^a
Crude fat	4.42±0.07^a	3.48±0.10^b	3.26±0.12^c
Crude protein	22.82±0.59^a	22.95±0.27^a	23.70±0.44^a
Total carbohydrates	66.81±0.63^b	65.73±0.29^a	65.89±0.38^a
» Soluble sugars	6.98±0.16^a	2.50±0.51^b	1.46±0.49^c
» Starch	18.25±0.11^a	17.71±0.62^a	20.78±0.48^b
Crude fiber	7.49±0.04^a	9.05±0.49^b	7.98±0.54^a
Ash	5.95±0.09^a	7.84±0.10^b	7.15±0.08^c
Total energy	1687.23±0.53^a	1636.27±2.79^b	1643.68±2.83^c

Each value represented mean ± standard deviation (SD) of three samples (n = 3). Different letters in the same row indicate significant difference at $p < 0.05$ levels by Duncan's Multiple Range Test.
* DW - dry weight

The moisture content was the lowest in *C. cornucopioides* (87.95%) and the highest in *B. pinophilus* (90.40%). Crude fat was relatively low varying between 3.26 and 4.42%. Crude protein was similar in all examined species and varied from 22.82 to 23.70%. Total carbohydrate content was higher in *B. pinophilus* (66.81%) than in *C. cornucopioides* (65.89%) and *C. cibarius* (65.73%). A high content of soluble sugars was found in *B. pinophilus* (6.98%), but in the other two species were 2.50 and 1.46%, respectively. On the other hand, *C. cornucopioides* distinguished as a high content of starch (20.78%) while in the other species its amount was lower (18.25 and 17.71%). Crude fiber and ash contents ranged from 7.49 to 9.05% and from 5.95 to 7.84%, respectively, in all samples. The values of total energy were 1687.23, 1636.27 and 1643.68 kJ /100g of dry weight (DW).
The content of essential elements of the examined varieties of mushrooms is shown in Table 2.

Table 2 Essential element content in mushrooms (mg/kg DW basis)

Elements, mg/kg	Species		
	Boletus pinophilus	*Cantharellus cibarius*	*Craterellus cornucopioides*
N	36517.33±945.43^{a}	36725.33±429.14^{a}	37923.20±703.85^{a}
P	7347.93±807.51^{a}	2713.62±168.84^{b}	2762.51±113.69^{b}
K	30555.6±2735.73^{a}	31308.13±1185.24^{a}	31454.93±1219.66^{a}
Na	94.75±23.57^{a}	139.43±5.02^{b}	140.36±4.32^{b}
Ca	256.96±50.86^{a}	459.59±17.35^{b}	460.52±18.86^{b}
Mg	647.26±98.79^{a}	626.06±65.29^{a}	635.01±68.22^{a}
Fe	98.42±32.56^{a}	82.29±6.75^{a}	82.87±6.9^{a}
Pb	0.58±0.29^{a}	0.27±0.11^{a}	0.41±0.06^{a}
Cd	0.61±0.21^{a}	0.33±0.16^{ab}	0.09±0.01^{b}
Ni	0.59±0.11^{a}	0.54±0.16^{a}	0.61±0.15^{a}
Mn	10.42±1.57^{a}	18.32±4.24^{b}	19.31±4.32^{b}
Co	0.26±0.08^{a}	1.48±0.14^{b}	1.62±0.11^{b}
Cu	23.67±6.15^{a}	27.57±4.96^{a}	4.58±1.02^{b}
Zn	69.01±6.29^{a}	78.83±3.86^{b}	11.43±1.62^{c}

Each value represents mean ± SD of fifteen samples (n = 15). Different letters in the same column indicate significant difference at $p < 0.05$ levels by Duncan's Multiple Range Test.

According to the results can be considered that nitrogen was the main essential element in all samples (36517.33 – 37923.20 mg/kg DW), followed by potassium (30555.6 – 31454.93 mg/kg). Phosphorus was found to be from 2713.62 to 7347.93 mg/kg. The quantity of magnesium varied from 626.06 to 647.26 mg/kg, while those of calcium and sodium were from 256.96 to 460.52 mg/kg and from 94.75 to 140.36 mg/kg, respectively. Iron was found to be from 82.29 to 98.42 mg/kg and the amount of zinc was higher in *C. cibarius* (78.83 mg/kg) and *B. pinophilus* (69.01 mg/kg), but much lower in *C. cornucopioides* (11.43 mg/kg). The same tendency was observed in the content of copper where this element was higher in the first two species (23.67 and 27.57 mg/kg) and again lower in *C. cornucopioides* (4.58 mg/kg). On the other hand, the quantity of manganese was 18.32 and 19.31 mg/kg in *C. cibarius* and *C. cornucopioides*, while it was two times lower in *B. pinophilus* (10.42 mg/kg). The amount of the other essential elements was relatively minor from 0.26 to 1.62 mg/kg.

Studies on lipid composition of the glyceride oil from mushrooms

The data about the biologically active substances (sterols, phospholipids) in the glyceride oils as well as in the dry extracts are presented in Table 3.

Table 3 Content of biologically active substances in the oil and dry extract of the studied species of mushrooms

Compounds	Species		
	Boletus pinophilus	*Cantharellus cibarius*	*Craterellus cornucopioides*
Unsaponifiable substances			
- in oil, %	10.1±0.2^{a}	9.1±0.3^{a}	3.7±0.1^{b}
- in dry extract, %	0.5±0.01^{a}	0.2±0.01^{b}	0.2±0.01^{b}
Sterols			
- in oil, %	7.3±0.3^{a}	0.5±0.1^{b}	0.6±0.2^{b}
- in dry extract, %	0.3±0.01^{a}	0.01±0.002^{b}	0.03±0.01^{b}
Phospholipids			
- in oil, %	9.3±0.4^{a}	7.2±0.2^{b}	1.5±0.1^{c}
- in dry extract, %	0.4±0.02^{a}	0.2±0.01^{b}	0.1±0.01^{c}

Each value represented mean ± SD of three samples (n = 3). Different letters in the same row indicate significant difference at $p < 0.05$ levels by Duncan's Multiple Range Test.

Total unsaponifiable substances in the oil from *B. pinophilus* and *C. cibarius* were found to be 10.1 and 9.1%, respectively, which was almost three times more than in the oil from *C. cornucopioides* (3.7%). The highest sterol content was observed in *B. pinophilus* oil, while their amount in the other two species were significantly lower (0.5 – 0.6%).
Phospholipids were also established in a high quantity in the oils from *B. pinophilus* and *C. cibarius* (9.3 and 7.2%, respectively), while their amount was 1.5% in the oil from *C. cornucopioides*.

Fatty acid composition

The data about the fatty acid composition of the triacylglycerols in the oils from the studied species of mushrooms are presented in Table 4.

Table 4 Fatty acid composition of oils from the studied species of mushrooms

Fatty acids, %		Species		
		Boletus pinophilus	*Cantharellus cibarius*	*Craterellus cornucopioides*
C 10:0	Decanoic	0.1±0.05^{a}	-*	-
C 12:0	Lauric	0.1±0.02^{a}	0.1±0.03^{a}	-
C 13:0	Tridecanoic	-	0.1±0.02^{a}	0.1±0.02^{a}
C 14:0	Myristic	0.2±0.05^{a}	-	0.1±0.01^{a}
C 15:0	Pentadecanoic	0.2±0.03^{b}	0.5±0.1^{a}	0.4±0.1^{a}
C 16:0	Palmitic	14.6±0.2^{c}	27.3±0.3^{a}	11.7±0.2^{b}
C 16:1	Palmitoleic	0.5±0.03^{a}	1.2±0.2^{b}	0.7±0.1^{a}
C 17:0	Heptadecanoic	0.2±0.02^{a}	0.4±0.1^{b}	0.3±0.05^{ab}
C 17:1	Heptadecenoic	0.1±0.01^{a}	-	-
C 18:0	Stearic	4.2±0.2^{c}	10.5±0.5^{b}	13.2±0.2^{a}
C 18:1	Oleic	52.6±0.5^{b}	31.7±0.6^{a}	63.4±0.4^{c}
C 18:2	Linoleic	25.8±0.6^{a}	25.2±0.2^{a}	8.9±0.2^{b}
C 18:3	Linolenic	0.8±0.1^{a}	0.6±0.1^{a}	0.4±0.1^{b}
C 20:0	Eicosanoic	0.4±0.1^{b}	0.4±0.1^{b}	0.5±0.1^{a}
C 20:2	Eicosadienoic	0.2±0.05^{a}	0.2±0.05^{a}	0.3±0.03^{a}
C 20:4	Arachidonic	-	1.6±0.2^{a}	-
C 22:0	Behenic	-	0.2±0.05^{a}	-

Each value represented mean ± SD of three samples (n = 3). Different letters in the same row indicate significant difference at $p < 0.05$ levels by Duncan's Multiple Range Test; * - Not identified.

The main fatty acid in all of the examined varieties of mushrooms was oleic acid, which was higher in the oil from *C. cornucopioides* (63.4%) than in the oil from *B. pinophilus* (52.6%) and *C. cibarius* (31.7%). The following fatty acids in the triacylglycerols of the latter variety were palmitic (27.3%), linoleic (25.2%) and stearic acid (10.5%); in *B. pinophilus* were linoleic (25.8%), palmitic (14.6%) and stearic acid (4.2%); in *C. cornucopioides* were stearic (13.2%), palmitic (11.7%) and linoleic acid (8.9%). The differences in amount of the main fatty acids between the three species might be due to the origin of the mushrooms and the environmental conditions under which they had grown. The other fatty acids were presented in the oil in small quantities (0.1 – 1.6%).

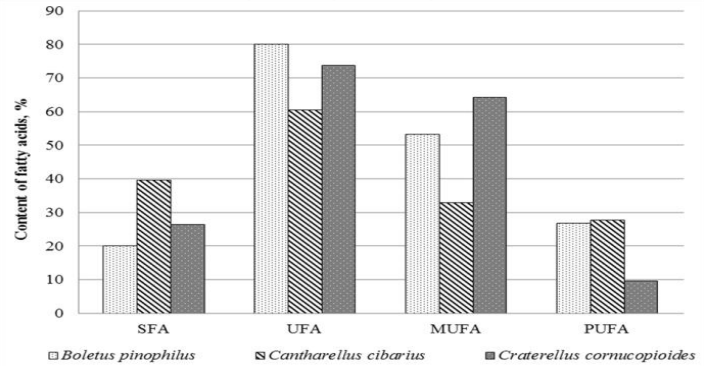

Figure 2 Content of saturated (SFA), unsaturated (UFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in the oils from the studied species of mushrooms

Content of saturated (SFA), unsaturated (UFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids is shown in Figure 2.
Unsaturated fatty acids predominated in all examined species of mushrooms (60.5 – 80.0%), while saturated fatty acids were varying from 20.0 to 39.5%. The amount of MUFA in *C. cornucopioides* (64.1%) was higher than the same fatty acids in the other species. On the other hand, the oil from this mushroom distinguished with its lowest quantity of PUFA (9.6%). The amount of MUFA (53.2%) in *B. pinophilus* was two times higher than those of PUFA (26.8%), while in *C. cibarius* they were 32.9 and 27.6%, respectively.

Sterol composition

Individual sterol compositions in the lipids are presented in Table 5.

Table 5 Individual composition of sterols in lipids from the studied species of mushrooms

Sterols, (% of total)	Species		
	Boletus pinophilus	*Cantharellus cibarius*	*Craterellus cornucopioides*
Cholesterol	4.0±0.2[a]	6.2±0.1[b]	3.6±0.1[a]
Brassicasterol	0.9±0.1[c]	5.6±0.1[a]	2.8±0.1[b]
Campesterol	5.7±0.1[a]	7.7±0.2[c]	6.2±0.2[b]
Stigmasterol	15.2±0.3[b]	3.3±0.05[a]	3.7±0.1[a]
Ergosterol	66.8±0.5[b]	42.4±0.4[a]	72.8±0.4[c]
β-Sitosterol	6.1±0.1[b]	31.1±0.3[c]	3.8±0.1[a]
Δ^5 – Avenasterol	0.5±0.1[a]	-[*]	4.6±0.2[b]
Δ^7 – Stigmasterol	0.8±0.1[a]	3.7±0.2[c]	2.5±0.1[b]

Each value represented mean ± SD of three samples (n = 3). Different letters in the same row indicate significant difference at $p < 0.05$ levels by Duncan's Multiple Range Test; [*] - Not identified

Ergosterol was the main sterol comprising from 42.4 to 72.8% of the total sterol content of all examined mushrooms. β-Sitosterol was in a relatively higher quantity in the lipids from *C. cibarius* (31.1%), while its amount in the lipids from the other mushrooms was lower (3.8 – 6.1%). The content of stigmasterol in the oil from *B. pinophilus* was found to be 15.2%, but in the other samples was about five times lower (3.3 – 3.7%). Surprisingly, the amount of cholesterol was relatively higher in the lipids from all mushrooms ranging from 3.6 to 6.2%. The quantity of the other sterols was identified to be from 0.5 to 7.7%.

Phospholipid composition

The composition of the phospholipid fraction of the mushrooms is presented in Table 6.

Table 6 Individual phospholipid composition of the studied species of mushrooms

Phospholipids, (% of total)	Species		
	Boletus pinophilus	*Cantharellus cibarius*	*Craterellus cornucopioides*
Lysophosphatidylcholine	8.9±0.2[a]	-[*]	11.9±0.2[b]
Lysophosphatidylethanolamine	8.1±0.2[a]	9.2±0.2[b]	11.2±0.1[c]
Sphingomyelin	9.9±0.1[b]	10.3±0.2[b]	17.1±0.2[a]
Phosphatidylinositol	8.9±0.1[c]	10.3±0.1[b]	13.1±0.1[a]
Phosphatidylcholine	8.8±0.2[a]	12.4±0.2[b]	15.9±0.3[c]
Phosphatidylethanolamine	14.0±0.3[b]	8.1±0.1[a]	15.7±0.1[c]
Monophosphatidylglycerol	-	6.6±0.1[a]	-
Diphosphatidylglycerol	8.3±0.1[a]	-	-
Phosphatidic acids	33.1±0.5[a]	32.4±0.4[a]	15.1±0.3[b]

Each value represented mean ± SD of three samples (n = 3). Different letters in the same row indicate significant difference at $p < 0.05$ levels by Duncan's Multiple Range Test.
[*] - Not identified

Phosphatidic acids were the main components in lipids from *B. pinophilus* (33.1%) and *C. cibarius* (32.4%), while all phospholipid classes in *C. cornucopioides* were present in similar quantities (11.2 - 17.1%). The content of the other phospholipids in *B. pinophilus* and *C. cibarius* was from 6.6 to 14.0%. Monophosphatidylglycerol was identified only in *C. cibarius* (6.6%), while diphosphatidylglycerol was present in *B. pinophilus* (8.3%).

Fatty acid composition of main classes of phospholipids

Fatty acid composition of the main classes of phospholipids was investigated for the first time. The results about the fatty acid composition of the main phospholipid classes of the studied mushrooms are presented in Figure 3.

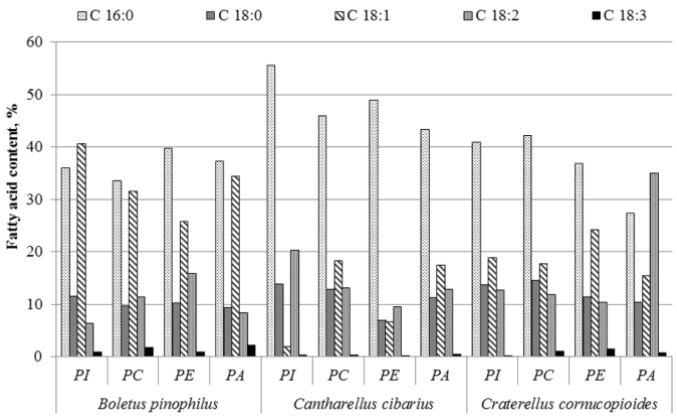

Figure 3 Content of the main fatty acids in the phospholipids from the studied species of mushrooms
[*] - PI – phosphatidylinositol; PC – phosphatidylcholine; PE – phosphatidylethanolamine; PA – phosphatidic acids; C16:0 - Palmitic acid; C18:0 - Stearic acid; C18:1 - Oleic acid; C18:2 - Linoleic acid; C18:3 - Linolenic acid.

The content of SFA, MUFA and PUFA of the main phospholipids is shown in Figure 4. These results were compared to the fatty acid composition of the triacylglycerols of the examined mushrooms.

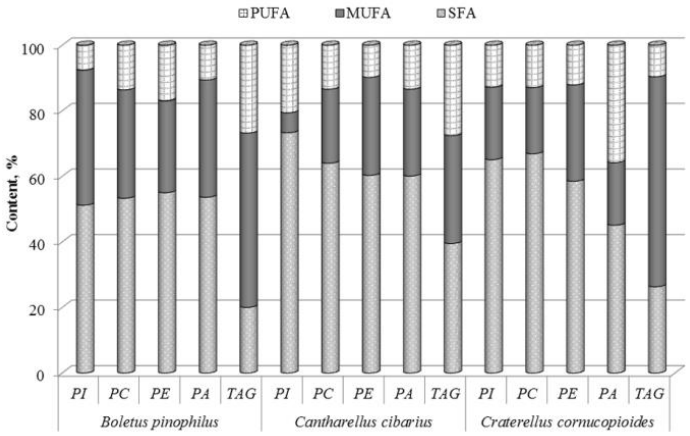

Figure 4 Content of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in the phospholipids and triacylglycerols (TAG) from the studied species of mushrooms
[*] - PI – phosphatidylinositol; PC – phosphatidylcholine; PE – phosphatidylethanolamine; PA – phosphatidic acids; TAG – triacylglycerols; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.

Effects between mushroom species and their nutritional compositions

To clarify specific relationships between nutritional compositions and wild edible mushroom species (*B. pinophilus*, *C. cibarius* and *C. cornucopioides*) a principal component analysis was used regarding only the effects of the first two principal compounds.

Figure 5 gives a global view of the effect of all nutritional variables based on the results of the principal component analysis. K, Mn, crude fat, Co, Na, Ca, P, sterols, Fe, total energy, total carbohydrates, Cd, Ash, Mg, moisture, Pb, phospholipids, unsaponifiable substances, N and crude protein are the most important variables for the formation of the first principal compound (Dim1) judging from the values of the correlation coefficients with that Dim1, which are greater than 0.7 (-0.99, -0.99, 0.99, -0.99, -0.99, -0.99, 0.98, 0.98, 0.98, 0.96, 0.96, 0.94, -0.87, 0.84, 0.83, 0.82, 0.79, 0.72, -0.71 and -0.71, respectively). For the same reason, MUFA, Ni, Cu, Zn, UFA and SFA are the most important variables of the second principal compound (Dim 2) (0.99, 0.99, -0.87, -0.86, 0.75 and -0.75, respectively).
Mushroom species positioned close to an arrow of a variable show strong relationship. Thus, high SFA content is indicative of *C. cibarius* presence, whereas *C. cornucopioides* is the richest Crude protein and N, and *B. pinophilus* - Sterols, P, T. energy, T. carbohydrates and Fe.

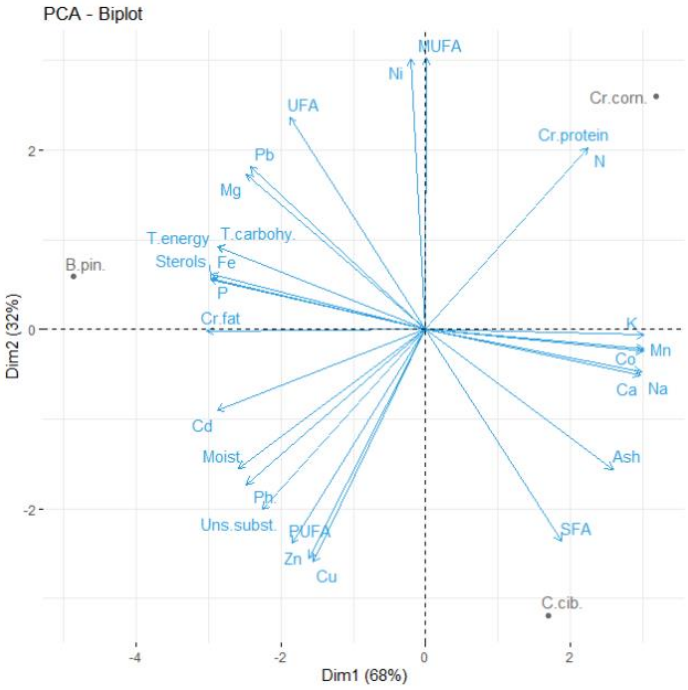

Figure 5 Biplot based on principal component analysis of mushroom nutritional compositions and species arrangement.
Legend: B. pin. - Boletus pinophilus, C. cib. - Cantharellus cibarius, Cr. corn. - Craterellus cornucopioides, Cr. protein - crude protein, T. carbohy - total carbohydrates, T. energy - total energy, Cr. fat - crude fat, Moist. – moisture, Ph. – phospholipids, Uns. subst. - unsaponifiable substances.

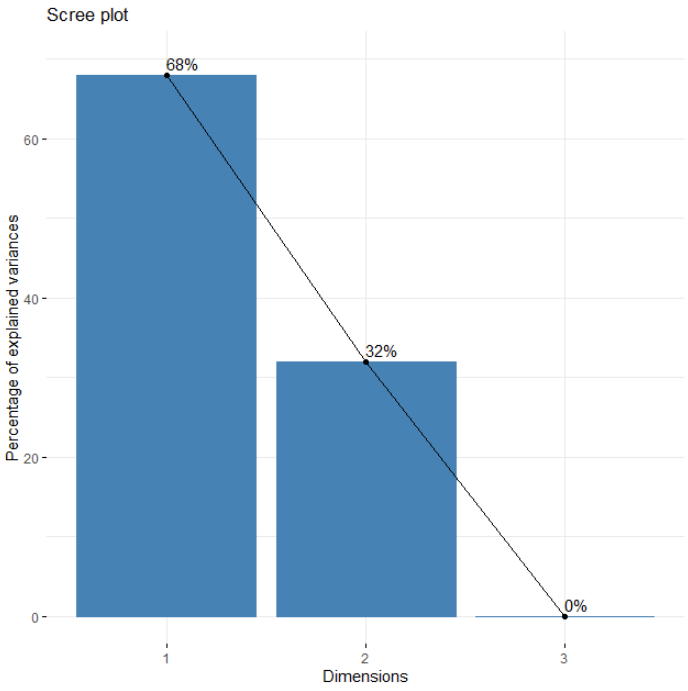

Figure 6 Scree plot of the eigenvalues/variances of the dimensions

From the scree plot graph of eigenvalues/variances of the dimensions (Figure 6) we may notice that the first principal compound explained 68% of the variance, while the second principal component contributed 32% of the total variance.

DISCUSSION

The fat contents were in agreement with the results obtained by **Wang *et al.* (2014)** and **Beluhan and Ranogajec (2011)** (from 1.0 to 6.7%). On the other hand, the fat amount of the examined mushrooms was much higher than those of some widely appreciated cultivated mushrooms from Portugal (**Reis *et al.*, 2012**) with crude fat from 0.14 to 0.35%. The crude protein of the examined mushrooms was lower than those reported by **Beluhan and Ranogajec (2011)** (30.91 and 47.21%). **Barros *et al.* (2008b)** also established high protein content in *C. cibarius* (53.7% of DW). The results for the total sugars were also in agreement with previous studies on the same species (**Beluhan and Ranogajec, 2011**). The ash content of *C. cibarius* in the present study was similar to the reported by **Beluhan and Ranogajec (2011)** (8.8 g/100g) while the same authors established that the ash content in *C. cornucopioides* (10.08 g/100g) was higher than our results.

It is known that ash content does not give precise information about the mineral content of mushrooms (**Wang *et al.*, 2014**). For this reason, the essential elements were determined by atomic absorption spectrometry. **Wang *et al*. (2014)** revealed that the wild mushroom had the ability to accumulate different macro- and microelements, including some toxic elements such as As, Hg, Cd and Pb. The main element that was identified in the present study was N, a finding that differed from those reported by some previous studies (**Wang *et al.*, 2014; Okoro and Achuba, 2012**) in which K and P prevailed in the fruiting body. According to **Brzezicha-Cirocka *et al.* (2019)** the content of K was found to be the highest in the species *B. pinophilus* and *C. cibarius* (9300 – 45000 mg/kg and 6200 – 59000 mg/kg, respectively), followed by Mg (220 – 2100 mg/kg and 410 – 1400 mg/kg, respectively). These results were similar to the amount of K and Mg found in the examined species of mushroom in the present study (30555.6 – 31454.93 mg.kg for K and 626.06 – 646.26 mg/kg for Mg). The same authors also established that in *B. pinophilus* the content of Na (635 mg/kg), Zn (125 mg/kg) and Mn (24.2 mg/kg) were higher than in the current study, but the quantity of Ca (175 mg/kg) and Fe (25.0 mg/kg) was lower than ours. For *C. cibarius* the content of Na, Ca, Fe, Mn, Cu and Zn was lower than the amount of the same elements in a previous study by **Brzezicha-Cirocka *et al.* (2019)** – 158 mg/kg, 747 mg/kg, 218 mg/kg, 35.3 mg/kg, 49.1 mg/kg and 100 mg/kg, respectively. Similar to our results for the content of Cu were reported by **Yildiz *et al.* (2019)** (31.2 mg/kg) and **Sarikurkcu *et al.* (2020)** (32.90 mg/kg). According to **Yildiz *et al.* (2019)** the amounts of Ca (673.2 mg/kg), Fe (588.5 mg/kg) and Ni (35.1 mg/kg) were higher, but these of Mg (106.3 mg/kg), Mn (4.6 mg/kg), Co (0.37 mg/kg) and Zn (49.4 mg/kg) were lower than the present results for *C. cibarius.* The same authors established also that Ca predominated in mushroom *C. cornucopioides* and its amount (935.5 mg/kg) was twice higher than our findings (460.52 mg/kg). In higher amounts they also established Fe (255.3 mg/kg), Ni (58.4 mg/kg), Cu (40.5 mg/kg) and Zn (91.5 mg/kg), while the quantity of Mg (151.6 mg/kg) and Mn (13.7 mg/kg) were lower than ours for *C. cornucopioides*. On the other hand, **Sarikurkcu *et al.* (2020)** reported that the content of all identified elements (Cd, Pb, Cu, Mn, Zn, and especially Fe) was higher than in the present study, apart from Co which had similar content – 1.62 mg/kg vs. 1.12 mg/kg (**Sarikurkcu *et al.*, 2020**).

As can be seen in Table 3 the oil from *B. pinophilus* is rich in unsaponifiable matters, sterols and phospholipids, while *C. cibarius* is abundant in unsaponifiable matters and phospholipids. The share of the sterols in the fat from mushroom *B. pinophilus* was approximately 72% of the total unsaponifiables, while those of the other two species was 5.5 and 16.2%, respectively for *C. cibarius* and *C. cornucopioides*. The obtained results differed from these of previous studies on total sterol and phospholipid content of mushrooms from genus Boletus (**Hanuš *et al.*, 2008**), which may be due to the differences of the species as well as the climatic conditions and geographic origins of the mushrooms.

All investigated species were abundant in unsaturated fatty acids among which oleic acid predominated. The results about the fatty acid composition of *C. cibarius* were in agreement with those reported by **Kavishree *et al.* (2008)**. The fatty acid composition of the species of mushroom *C. cornucopioides* differed from the results reported in a previous study (**Barros *et al.*, 2008a**) in which the amount of oleic acid in the triacylglycerols was slightly lower (51.85%) than in our samples (63.4%). In all examined mushrooms linoleic acid varied from 8.9 to 25.8%, and linolenic ranged from 0.4 to 0.8%. It is reported that these fatty acids contribute to reducing the incidence of heart disease and cancer (**Parker *et al.*, 2003**).

The obtained results on the sterol composition differed from the previous studies where only ergosterol, ergosta-7,22-dienol, ergosta-5,7-dienol, ergosta-7-enol, fungisterol, brassicasterol and campesterol were identified (**Mattila *et al.*, 2002; Phillips *et al.*, 2011; Teichmann *et al.*, 2007**). Ergosterol variation could be explained by the differences of the species as well as the impact of some external factors such as light, substrate, climate conditions, etc. (**Huang *et al.*, 1985; Mattila *et al.*, 2002**).

Individual phospholipid composition of the examined mushrooms differed from those reported by **Hanuš *et al.* (2008)**, where was established that the main phospholipid in the oils from genus Boletus was phosphatidylcholine (33.7 – 83.5%).

Differences between the main phospholipid classes were observed within one given variety. The main fatty acid in all phospholipid classes of the examined mushrooms was palmitic (33.6 – 55.6%) with the exception of phosphatidylinositol of *B. pinophilus* where the main fatty acid was found to be oleic acid (40.6%), and phosphatidic acids of *C. cornucopioides* where linoleic acid (35.1%) was the major one. The content of palmitic acid in variety *B. pinophilus* increased in direction phosphatidylcholine (PC) < phosphatidylinositol (PI) < phosphatidic acids (PA) < phosphatidylethanolamine (PE) ($p < 0.05$), which was at the expense of lessening of the quantity of the other major component – the oleic acid (from 40.6 to 25.8%), trend not observed for the other two species. The amount of palmitic acid in *C. cibarius* increased in the following direction: PA < PC < PE < PI ($p < 0.05$), but in *C. cornucopioides* followed the pattern PA < PE < PI < PC ($p < 0.05$). Fatty acid composition of PI in both *B. pinophilus* and *C. cibarius* as well as those of PA in *C. cornucopioides* differed from the fatty acid composition of the other phospholipid classes of the examined mushrooms. The first one was abundant in

oleic acid, followed by palmitic (36.1%), stearic (11.5%) and linoleic (6.4%). Phosphatidylinositol in *C. cibarius* depicted to have the higher content of palmitic acid (55.6%), followed by linoleic (20.3%) and stearic (13.9%), while the amount of oleic acid was considerably lower (2.0%). Phosphatidic acids in *C. cornucopioides* were characterized by higher content of linoleic acid, followed by palmitic (27.4%), oleic (15.4%) and stearic (10.4%). A relatively high content of heptadecanoic acid was observed in phosphatidylethanolamine of *C. cibarius* (13.6%), while in the other phospholipids its amount was from 0.1 to 4.5%.

Saturated fatty acids (SFA) predominated in phospholipid fraction in all of the examined mushrooms. Their content in *B. pinophilus* ranged from 51.2 (in PI) to 55.0% (in PE); in *C. cibarius* was from 60.1 (in PA) to 73.3% (in PI); in *C. cornucopioides* was from 45.1 (PA) to 66.9% (in PC). Monounsaturated fatty acids (MUFA) (20.2 – 41.2%) were in higher amount than the respective quantity of PUFA (7.6 – 13.6%), with the exception of PI in *C. cibarius* and PA in *C. cornucopioides*, where the content of PUFA (20.6 and 35.8%, respectively) was higher than MUFA (6.1 and 19.1%). Fatty acid composition of the phospholipids differed from those of triacylglycerols. While SFA predominated in the phospholipids, unsaturated fatty acids prevailed in triacylglycerols. On the other hand, the amount of SFA in triacylglycerols (20.0 – 39.5%) was about two times lower than that in the main phospholipid classes.

CONCLUSION

A thorough analysis of the chemical and lipid composition of three species of wild edible mushrooms (*Boletus pinophilus*, *Cantharellus cibarius* and *Craterellus cornucopioides*) grown in Bulgaria (the Batak Mountain) was conducted for the first time. The examined mushrooms are abundant in proteins and carbohydrates but possess lower oil content. They were rich in phytosterols and phospholipids as well as in unsaturated fatty acids, with oleic acid as a major fatty acid. Based on the present results, we can conclude that the examined species of mushrooms are potential sources of valuable biologically active components and may take part as a functional food ingredient or food additives in different products.

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