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Fatty acids and phospholipids of edible wild mushroom (*Amanita caesarea*) from the Batak Mountain, Bulgaria

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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. Samples were collected from the Batak Mountain, Bulgaria. The aim of this study was to investigate the chemical profile, and determine phospholipids and fatty acids in the wild edible mushroom *Amanita caesarea*. *Amanita caesarea* is rich in carbohydrates (57.86 g 100 g^{-1} dw), followed by proteins (24.81 g 100 g^{-1} dw), ash (8.92 g 100 g^{-1} dw) and fat 8.42 g 100 g^{-1} dw). Also moisture (88.12%), and total energy (1716.73 kJ 100 g^{-1} dw) were calculated. The content of saturated fatty acids consisted of 25.4%. Unsaturated fatty acids in the oil from the mushroom were 74.6% and the content of monounsaturated fatty acids consisted of 60.8%. On the other hand, the amount of polyunsaturated fatty acids was lower (13.8%). In the phospholipid fraction from mushrooms dominated phosphatidic fatty acids (16.3%) dominated phosphatidylcholine (10.5%) and phosphatidylethanolamine (10.5%) as major components, followed by phosphatidylinositol (9.7%). The quantities of lysophosphatidylcholine and lysophosphatidylethanolamine in the phospholipid fraction were from 8.2% to 8.5%.

Keywords: Fatty acids, Phospholipids, Mushroom (*Amanita caesarea*), Bulgaria

INTRODUCTION

Amanita caesarea, commonly known in English as Caesar's mushroom, is a highly regarded edible mushroom in the genus *Amanita*, native to southern Europe and North Africa. This mushroom can also be found in La Esperanza, Intibuca, Honduras, where a festival is annually celebrated in its honor. While it was first described by Giovanni Antonio Scopoli in 1772, this mushroom was a known favorite of early rulers of the Roman Empire. This mushroom has an orange-red cap, initially hemispherical before convex and finally flat. The surface is smooth, and margins striated, and it can reach 15 cm or rarely 20 cm in diameter. The free gills are pale to golden yellow, as is the cylinder-shaped stipe, which is 8 – 15 cm tall and 2 – 3 cm wide. The ring hangs loosely and is lined above and smooth below. The base of the stipe is thicker than the top and is seated in a greyish-white cup-like volva, which is a remnant of a universal veil. The spores are white [1].

Mushrooms have been viewed as gourmet food over the globe for their unique taste and inconspicuous flavor. As of late, it has been found that many mushroom species are miniature pharmaceutical factories producing thousands of novel constituents with exceptionally helpful biologic properties.

They have a long history of utilization in Oriental prescriptions, however their incredible impacts in advancement of good health and imperativeness are being upheld by contemporary reviews. Recently, mushrooms have developed as great wellsprings of nutraceuticals, anti-oxidants, anticancer, prebiotic, immune modulating, anti-inflammatory, cardiovascular, anti-microbial and anti-diabetic agents [2-10].

The known essential micronutrient minerals are iron, zinc, selenium, manganese, cobalt and copper. These microminerals play an important role in the catalytic processes within the enzyme system that includes a wide range of enzyme activities associated with metabolic, endocrine and immune systems [11-18].

The aims of this study were to investigate the chemical profile, and to determine phospholipids and fatty acid in wild edible mushroom *Amanita caesarea* growing in the Batak Mountain, Bulgaria.

EXPERIMENTAL

Samples

Mushroom samples were collected in 2018 from the Batak Mountain by the authors themselves.

The Batak Mountain is located in the western Rhodopes. Its western border is defined by the Chepinska river, the southern border – by Dospatska river and Dospat dam, the eastern border

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– by Vacha river and the northern border – by the Thracian Plane (GPS41°46'02.6"N 24°08'48.4"E). The region is industry-free and is characterised with forests, land and low buildings.

Fig. 1. *Amanita caesarea*

Reagents

Reagents are qualified "AR" (p.a. Merck & Fluka). Water was deionized in a Milli Q system (Millipore, Bedford, MA, USA) to a resistivity of 18.2 MΩ cm. All plastic and glassware was cleaned by soaking in dilute HNO_3 (1/9, v/v) and was rinsed with deionized water prior to use.

Sample preparation for nutritional analysis

The whole macrofungal samples were used in this study. Fresh samples, after the removal of extraneous material such as mud, bush, soil, plant, etc. by washing with deionized water, were air-dried between Whatman filter papers. Approximately 5 g of each sample was taken immediately for the determination of moisture. Remaining samples were stored in a deep-freezer until use [19]. While examining the nutritional composition of mushroom samples, their maturation stage was not considered.

Proximate composition analysis

For determination of proximate value, the following parameters were studied by using the mushroom material.

Crude protein content

Protein content was determined using folin phenol reagent. 0.5 g of the powdered mushroom sample was extracted with 50 mL of 2% NaCl in a water bath at 60°C for 1 h. The extract was filtered out and 50 mL of 3% copper acetate monohydrate was added to the filtrate to precipitate the protein. The precipitated protein was then centrifuged out and dissolved in 50 mL of 0.1M NaOH [20].

Carbohydrate content

Total carbohydrate was determined by adding 2 g of each sample in 50 mL distilled water, 0.2 mL of which was ten-fold diluted. To 1 mL of the resulting solution and serial dilutions of glucose stock (10 mg 100 mL^{-1}) solution, 4 mL of anthrone reagent was added and the absorbance of the solutions was measured by a spectrophotometer at 620 nm against a reagent blank [21].

Crude fat

Crude fat was determined by extracting 2 g of moisture-free samples with petroleum ether in a soxhlet extractor, heating the flask on a sand bath for about 6 h till a drop taken from the drippings left no greasy stain on the filter paper. After boiling with petroleum ether, the residual petroleum ether was filtered using Whatman No 40 filter paper and the filtrate was evaporated in a preweighed beaker. Increase in weight of beaker gave the crude fat [22].

Ash content

The powdered mushroom sample (3 g) was ashed in a previously ignited and cooled crucible of known weight in a Gallenkamp furnace at 55°C for 6 h. The fairly cooled crucibles were put in desiccators and weighed [23].

Energy

Total energy was calculated according to the following equations: Total energy (kJ 100 g^{-1} dw) = 17 (g protein + g carbohydrate) + 37 (g lipid). The weight of individual nutrients is g 100 g^{-1} dw sample [24].

Moisture content

The fresh weight of each mushroom sample was taken using a chemical balance. These samples were then oven-dried separately at 105°C for 24 h. The loss in weight obtained after drying was regarded as the moisture content .

Fatty acids

The fatty acid composition was determined by gas chromatography (GC) after transmethylation of the sample with 2% H_2SO_4 in CH_3OH at 50°C [25]. GC was performed on an HP 5890 series II gas chromatograph equipped with a 75 m × 0.18 mm × 25 μm capillary column Supelco and a flame ionization detector. The column temperature was programmed from 140°C (5 min), at 4°C min^{-1} to 240°C (3 min); injector and detector temperatures were kept at 250°C. Hydrogen was the carrier gas at a flow rate of 0.8 mL min^{-1}. 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 [26].

Phospholipids

Air-dried mushrooms (10 g) were subjected to Folch [27] extraction. Polar lipids were isolated from the total lipids by column chromatography according to Christie [28]. The phospholipid classes were isolated by a variety of two-dimensional thin-layer chromatography (TLC). In the first direction the plate was developed with chloroform: methanol:ammonia, 65:25:5 (by volume) and in the second – with chloroform:acetone:methanol:acetic acid:water, 50:20:10:10:5 (by volume). The identification was performed by comparing the respective R_f values with those of authentic commercial standards subjected to silica gel TLC under identical experimental conditions. The quantification was carried out spectrophotometrically at 700 nm after scrapping the respective phospholipid spot and mineralization of the substance with a mixture of perchloric acid and sulphuric acid, 1:1 (by volume) [29].

Statistics

All analyses were carried out in triplicate and the data were reported as means ± standard deviation (SD). All statistical computing, test and graphics were performed with the statistical software R version 3.5.1.

RESULTS AND DISCUSSION

Chemical composition of Amanita caesarea

Table 1. Moisture (g 100 g^{-1} of fresh weight), macronutrients (g 100 g^{-1} of dry weight) and total energy (kJ 100 g^{-1} of dry weight) in the wild edible mushrooms *Amanita caesarea* (n = 15).

Components	$\bar{X}$ (g 100 g^{-1})	SD (g 100 g^{-1})	-95% Confid.	+95% Confid.
Moisture	88.12	0.49	87.84	88.38
Ash	8.92	0.21	8.81	9.03
Crude protein	24.81	0.52	24.52	25.09
Crude fat	8.42	0.07	8.38	8.46
Total carbohydrates	57.86	0.61	57.52	58.20
Total energy	1716.73	2.16	1715.54	1717.93

The main components of the chemical composition of *Amanita caesarea* are presented in Table 1.

Amanita caesarea showed to be rich in carbohydrates (57.86 g 100 g^{-1} dw), which were the most abundant macronutrients. Proteins were present at 24.81 g 100 g^{-1} dw. Total energy value was established to be 1716.73 kJ 100 g^{-1} dw. The carbohydrates in mushrooms comprise various compounds: sugars (monosaccharides, their derivatives and oligosaccharides) and both reserve and structural polysaccharides [30].

Fatty acid composition

The content of saturated fatty acids (SFA) was 25.4 %. The content of unsaturated fatty acids (UFA) in the oil from mushroom was 74.6 % and that of monounsaturated fatty acids (MUFA) consisted of 60.8 %. On the other hand, the amount of monounsaturated fatty acids (PUFA) was lower (13.8 %).

The analysis of the obtained profiles showed that oleic (59.9%), palmitic (20.2%) and to a lesser extent linoleic (13.8%) acids were the main fatty acids in the studied species (Fig. 2). This is in agreement with the results reported for other edible mushrooms [31-33].

Oleic acid is a monounsaturated fatty acid included in the omega-9 family. Humans generally possess all the enzymes required for the synthesis of these metabolites, which means that oleic acid is not essential. Under severe conditions of essential fatty acids deprivation, mammals elongate and desaturate oleic acid to produce eicosatrienoic acid. Oleic acid is found in olive oil and is known for its effectiveness in reducing cholesterol levels, which promotes the decrease of cardiovascular diseases [34,35].

Linoleic acid is an essential fatty acid as it cannot be synthesised by the human organism, due to the lack of desaturase enzymes required for its production. It must be obtained from the diet and originates the omega-6 fatty acids series, which includes γ-linolenic, dihomo-γ-linolenic and arachidonic acids [39, 40]. It is known that linoleic acid is the precursor of eight-carbon volatiles in fungi, such as 1-octen-3-ol, 3-octanol, 1-octen-3-one and 3-octanone, which are the principal aromatic compounds in most species, contributing also to the flavour of most of the mushrooms. A deficient intake of essential fatty acids can be responsible for many problems, such as dermatitis, immunosuppression and cardiac disfunctions [36, 37].

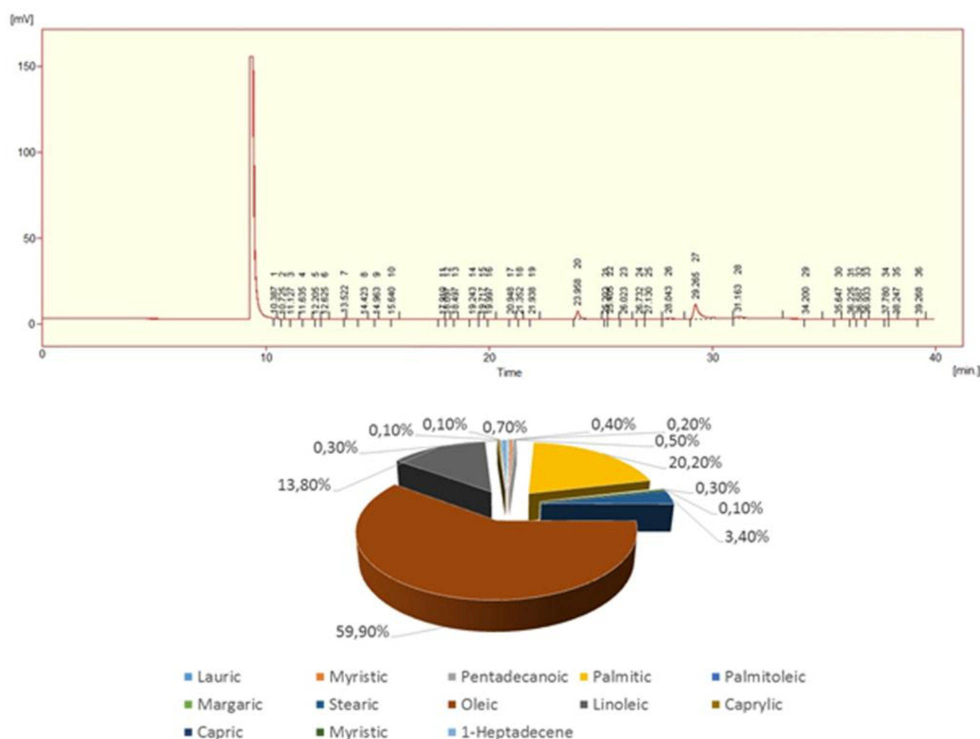

Fig. 2. The data of fatty acid composition in *Amanita caesarea* (n = 3)

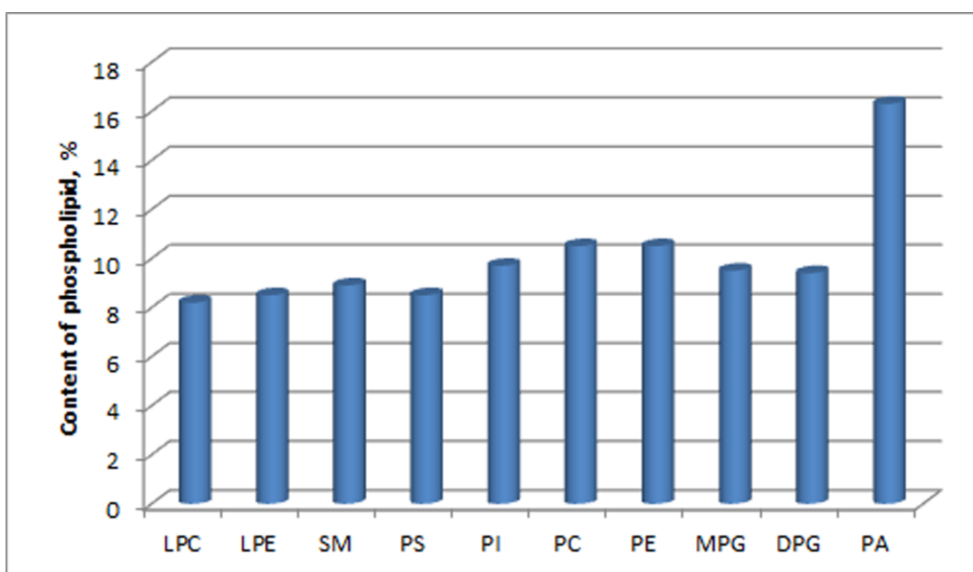

Fig. 3. Individual composition of phospholipid fraction of mushroom *Amanita caesarea* (n = 3)
Legend: LPC – Lysophosphatidylcholine; LPE – Lysophosphatidylethanolamine; SM – Sphingomyelin; PS – Phosphatidylserine; PI – Phosphatidylinositol; PC – Phosphatidylcholine; PE – Phosphatidylethanolamine; MPG – Monophosphatidylglycerol; DPG – Diphosphatidylglycerol; PA – Phosphatidic acids

Phospholipid composition

The composition of the phospholipid fraction of the mushrooms oils is presented in Fig. 3. In the phospholipid fraction of the mushrooms oils from different varieties, there were identified all major classes of phospholipids. On the grounds of the obtained data, it can be seen that in the phospholipid fraction from mushrooms predominated phosphatidic acids (16.3 %), phosphatidylcholine (10.5%) and phosphatidyl-ethanolamine (10.5%) as major components, followed by diphosphatidyllynositol (9.7%). The quantities of lysophosphatidylcholine and lysophosphatidylethanolamine in the phospholipid fraction were from 8.2% to 8.5%.The results obtained in this study are consistent with the previously reported results in the literature [38-41].

Phospholipids are polar ionic compounds composed of an alcohol that is attached by a phosphodiester bridge to either diacylglycerol or sphingosine. Like fatty acids, phospholipids are amphipathic in nature, that is, each has a hydrophilic head (the phosphate group plus whatever alcohol is attached to it, for example, serine, ethanolamine, and choline, highlighted), and a long, hydrophobic tail (containing fatty acids or fatty acid–derived hydrocarbons). Phospholipids are the predominant lipids of cell membranes. In the membranes, the hydrophobic portion of a phospholipid molecule is associated with the nonpolar portions of other membrane constituents, such as glycolipids, proteins, and cholesterol. The hydrophilic (polar) head of the phospholipid extends outward, interacting with the intracellular or extracellular aqueous environment. Membrane phospholipids also function as a reservoir for intracellular messengers, and, for some proteins, phospholipids serve as anchors to cell membranes. Non-membrane-bound phospholipids serve additional functions in the body, for example, as components of lung surfactants and essential components of bile, where their detergent properties aid in the solubilization of cholesterol. Most phospholipids are synthesized in the smooth endoplasmic reticulum. From there, they are transported to the Golgi apparatus and then to the membranes of organelles or the plasma membrane, or are secreted from the cell by exocytosis [42-44].

CONCLUSIONS

According to this study, the edible wild mushroom *Amanita caesarea* could be used in human nutrition due to its good parameters. The examined mushroom appeared to be abundant of proteins and carbohydrates. *Amanita caesarea* is rich in phospholipids, as well as in unsaturated fatty acids with oleic acid as the main fatty acid. The ongoing research will lead to a new generation of foods, and will certainly promote their nutritional and medicinal use.

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