DETERMINATION PHOSPHOLIPIDS OF WILD EDIBLE MUSHROOMS (*SUILLUS LUTEUS*)

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Abstract: Mushroom samples were collected in 2017 and 2018 from the Batak mountain by the authors themselves. The aim of this study was to determine phospholipids composition of species wild edible mushrooms (*Suillus luteus*) grown in the Batak mountain, Bulgaria. Phosphatidic acids were the main components in lipids from *Suillus luteus* (34.2 %). The content of the other phospholipids in the oils from *Suillus luteus* was from 8.4 to 19.6%. Phosphatidylcholine was identified in *Suillus luteus* (19.6 %). Based on the results, can be concluded that the examined species of mushrooms is a potential source of phospholipids and can be used as a functional food or as food additives in many products.

Keywords: Wild mushrooms, *Suillus luteus*, Phospholipids, Bulgaria.

1. INTRODUCTION

Wild mushrooms are considered as functional foods because they contain substances that might be used directly in diet and promote healthiness. They are rich in protein, carbohydrates, minerals, vitamins, unsaturated fatty acids, phenolic compounds, tocopherols, ascorbic acid and carotenoids that fit the definition of food supplement [1-10]. 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 system [11-20]. Living organisms require traces of some heavy metals, including iron, cobalt, copper, manganese, chromium and zinc. Excessive levels of these metals, however, can be detrimental to human health. Lead and cadmium are non-essential metals as they are toxic, although their trace amounts are not known for any beneficial effects on organisms. Furthermore, deficiency or imbalance of essential or non-essential minerals above threshold concentration levels can put the individual at risk of disease development [21-32].

Suillus luteus, so-called *Slippery Jack*, belongs to the *Boletales*, a well studied order of *Basidiomycota*, with recently sequenced genomes within the genera *Serpula*, *Paxillus* and *Pisolithus*. The large genus *Suillus* is a sister group of the genus *Rhizopogon*. Both genera are differentiated by a distinct ontogeny of the reproductive organs. Comparative analysis of these *Boletales* genomes will contribute to the exploration of the intra-clade variability in symbiosis-related gene repertoire. *Suillus luteus* is a cosmopolitan ectomycorrhizal (ECM) fungus whose natural range of distribution matches the range of distribution of its host plants, the *Pinus* species. It is particularly abundant in young pine forest or planted stands. The species is a pioneer, which quickly starts sexual reproduction from large edible sporocarps that produce massive quantities of basidiospores, spread by wind and mammals. It forms conspicuous, though relatively few mycorrhizas from which an extensive external mycelium

(long distance exploration type) develops into the mineral soil. It appears frequently on man-disturbed sites wherever pines are planted or start primary succession. It grows easily in culture, rapidly colonizes roots and can be used for inoculation of containerized pine seedlings (<https://genome.jgi.doe.gov/Suilu1/Suilu1.home.html>). [33]

Suillus luteus - Cap up until 120 (150) mm, initially hemispheric then convex, pulvinate, rarely; margin curving at the extremes towards the base, regular, acute, a little excessive, often decorated with whitish remnants of the partial veil; smooth cuticle, very viscous, separable, brown, yellow brown, brown violet, chocolate brown, often with darker radial fibrils when dry.

Tubes up to 12 mm, adnate or decurrent, yellow, then chrome yellow, finally yellow brownish, small, round pores, only angled in advanced maturation, concolour with tubes, uniform when pressed.

Stipe 40-70 × 12-30 mm, longer than the diameter of the cap when young, then the same length as the diameter of the cap or shorter, cylindrical, often a little widened towards base, full; with a broad membranous annulus, whitish in colour, then violet brown, it is possible to find adherent volviform residues at the base, from whitish to white-grey-violet. A fine yellow lattice can be found above the annulus, below the annulus there are yellow points which then become concolour with cap.

Flesh initially firm soon becoming soft and watery in cap, more fibrous in stipe; white, then yellowing; uniform to gill edge. Pleasant, fruity odour, sweet flavour.

Microscopy: ellipsoidal spores, 7.0-9.2 × 3.0-4.0 µm, pale yellow under microscope. Spores brown olive rusty in colour.

Habitat: only in groups in *Pinus woods*, recurrent from late summer to late autumn.

Edibility: edible note - this is a very common *Suillus*, the only one which has an annulus adorning its stipe and which grows with two-fascicle (*P. nigra*, *P. sylvestris*, more rarely anche *P. pinaster*) and three-fascicle (*P. radiata*) *Pinus* trees. Grows tendentially in hills and mountains and is not usually found under coastal pines. As it matures and ages, the cuticle has a tendency to become dehydrated and at that stage it often assumes an appearance very similar to that of *S. collinitus*. Several forms and varieties have been described: fo. albus, completely white, and fo. volvaceus, with a short stipe whose annulus has the appearance of a volva.

The information on the chemical, the aim of this study was to determine phospholipids composition of species wild edible and lipid composition of wild edible mushrooms grown in Bulgaria is rather scarce. Therefore mushrooms (*Suillus luteus*) grown in the Batak mountain, Bulgaria.

2. EXPERIMENTAL

2.1. Sampling

Mushroom samples were collected in 2017 and 2018 from the Batak mountain by the authors themselves.

Mushroom samples were washed with distilled water and dried at 105 °C for 24 h. The dried samples were ground, then homogenized using an agate pestle and stored in polyethylene bottles until analysis [34].

2.2. Reagents

All reagents were of analytical reagent grade unless otherwise stated. Double deionized water (Milli-Q Millipore 18.2 MΩ cm resistivity) was used for all dilutions. All the plastic and glassware was cleaned by soaking in dilute HNO₃ (1+9) and rinsed with distilled water prior to use.

2.3. Analysis of phospholipids

Air-dried mushrooms (10 g) were subjected to Folch extraction [35]. The phospholipid classes were isolated by a variety of two-dimensional TLC on 20 cm × 20 cm glass plates with 0.2 mm Silica gel 60 G layer impregnated with aqueous (NH₄)₂SO₄ (1 g in 100 mL water). In the first direction the plate was developed with chloroform:methanol:ammonia, 65:25:5 (v/v/v) and in the second – with chloroform: acetone:methanol:acetic acid:water, 50:20:10:10:5 (v/v/v/v/v) [36]. The identification of the individual phospholipids 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 (v/v) [37].

2.4. Statistical processing

Statistical analysis and all chartings were performed within the SPSS for Windows software. The data were presented as mean value and standard deviation (SD).

3. RESULTS AND DISCUSSION

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 in blue in Figure 1), and a long, hydrophobic tail (containing fatty acids or fatty acid-derived hydrocarbons, shown in orange in Figure 1). Phospholipids are the predominant lipids of cell membranes. In 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 (Figure 1). Membrane phospholipids also function as a reservoir for intracellular messengers, and, for some proteins, phospholipids serve as anchors to cell membranes. Nonmembrane-bound phospholipids serve additional functions in the body, for example, as components of lung surfactant 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 membranes of organelles or the plasma membrane, or are secreted from the cell by exocytosis.

Figure 1. Structures of some glycerophospholipids.

B. Phosphatidic acid. P = phosphate, PO_4^{-1}

3.1. Synthesis of phosphatidic acid (PA)

PA is the precursor of many other phosphoglycerides. The steps in its synthesis from glycerol phosphate and two fatty acyl coenzyme A (CoA) molecules were illustrated in Figure 2, in which PA is shown as a precursor of triacylglycerol. Essentially all cells except mature erythrocytes can synthesize phospholipids, whereas triacylglycerol synthesis occurs essentially only in liver, adipose tissue, lactating mammary glands, and intestinal mucosal cells.

Figure 2. Synthesis of triacylglycerol

3.2. Synthesis of phosphatidylethanolamine (PE) and phosphatidylcholine (PC)

PC and PE are the most abundant phospholipids in most eukaryotic cells. The primary route of their synthesis uses choline and ethanolamine obtained either from the diet or from the turnover of the body's phospholipids.

3.2.1. Synthesis of PE and PC from preexisting choline and ethanol -amine:

These synthetic pathways involve the phosphorylation of choline or ethanolamine by kinases, followed by conversion to the activated form, CDP-choline or CDP-ethanolamine. Finally, choline-phosphate or ethanolamine-phosphate is transferred from the nucleotide (leaving CMP) to a molecule of diacylglycerol (Figure 3a and 3b).

Figure 3. a) Activation of either diacylglycerol or an alcohol by linkage to a nucleoside diphosphate (CDP) promotes phospholipid synthesis; b) Synthesis of phosphatidylcholine from phosphatidylserine in the liver.

3.2.2. Phospholipid composition

The composition of the phospholipid fraction of the mushroom oils is presented in Table 1.

Table 1. Individual phospholipid composition of oils from the studied species of mushrooms (n = 3)

Phospholipids, (% of total)	<i>Suillus luteus</i>
Lysophosphatidylcholine	9.1 ± 0.2
Lysophosphatidylethanolamine	8.4 ± 0.2
Sphingomyelin	10.5 ± 0.2
Phosphatidylinositol	8.4 ± 0.1
Phosphatidylcholine	19.6 ± 0.3
Phosphatidylethanolamine	14.8 ± 0.4
Monophosphatidylglycerol	-
Diphosphatidylglycerol	8.5 ± 0.1
Phosphatidic acids	34.2 ± 0.6

* - Not identified

Phosphatidic acids were the main components in lipids from *Suillus luteus* (34.2 %). The content of the other phospholipids in the oils from *Suillus luteus* was from 8.4 to 19.6%. Phosphatidylcholine was identified in *Suillus luteus* (19.6 %). The results about phospholipids of the examined mushrooms differ from those of previous study made by Hanus et al. [38], who observed that phosphatidylcholine (33.7 – 83.5 %) was the main phospholipid in the oils from genus *Boletus*. The causes of lower phosphatidylcholine content in mushrooms seems to be affected by environmental and fungal related factors. Environmental factors such as organic matter content, pH and metal concentration in soil and fungal factors such as the species, morphological part of the fruit body, developmental stages, age of mycelium, intervals between fructifications and biochemical composition [39-46].

4. CONCLUSIONS

Based on the results, can be concluded that the examined species of mushrooms is a potential source of phospholipids and can be used as a functional food or as food additives in many products.

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