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# Study of the chemical composition of the lipophilic extract of fruit bodies of *Pleurotus ostreatus* (Jacq. Ex Fr.) P. Kumm.

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**Abstract**

The aim of the work was to study the changes in the chemical composition of the fruiting bodies of *Pleurotus ostreatus* depending on the growth stage of the fungi. The fruiting bodies of wild and cultivated *Pleurotus ostreatus* of the Euromycel PL132 tile variety were used as the object of the study. Changes in the qualitative and quantitative composition of the lipophilic fraction were investigated by gas chromato-mass spectrometry. There was a gradual decrease in the amount of squalene and bis (2-ethylhexyl) phthalate from young to overripe fungi. Young fungi have a high content of the phytohormone gibberellin A3, which is not found in mature and overripe fungi. At the same time, overripe fungi have 18% of ergosta-5,7,22-trien-3-ol, whose precursor is squalene. No fatty acids were detected in young fungi. They occur in mature fungi, and their content, in particular palmitic, stearic and linolenic acids, increases from mature to overripe fruiting bodies of fungi. At the same time, the yield of lipophilic extract from wild mushrooms is five times higher than that of the cultivated ones (0.98% against 0.14–0.35%). This extract is characterized by the presence of vitamin E and is characterized by a high content of squalene, linolenic acid and ergosterols. It was determined that the range of chemicals increases with the growth of mushrooms and decreases with their aging.

**Keywords** – bis (2-ethylhexyl) phthalate – ergosterols – fatty acids – gas chromatography-mass spectrometry – gibberellin A3 **–** squalene

## Introduction

*Pleurotus ostreatus* (type: *Basidiomycota*, class: *Agaricomycetes*, order: *Agaricales*) is a fungus that grows wild on dead wood and weakened trees, mostly deciduous trees. This mushroom is edible, eaten boiled, fried and dried. It is grown on an industrial scale. It is undemanding to climatic conditions and has a viable mycelium that can withstand prolonged storage (Muswati et al. 2021). The fungus is able to grow on a variety of agricultural substrates, including corn cobs, millet straw and bamboo waste (Fufa et al. 2021), tea waste, linden sawdust, alder sawdust, sawdust/hornbeam chips, wheat stalk straw (Karataş 2022), and on a variety of pulp and paper waste (Grimm et al. 2021**)**. Today, a large number of mushroom varieties have been bred that differ in morphological characteristics and chemical composition of their fruiting bodies (Lin et al. 2022)

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According to the literature, the fruiting bodies of the fungus contain up to 8% of mineral elements, the main share of which is potassium, magnesium, sodium and phosphorus, although their level depends on their content in the substrate on which these mushrooms are grown (Karataş 2022, Mleczek et al. 2021), and include the whole complex of B vitamins and fat-soluble vitamins $D_2$ and E (Torres-Martinez 2022). It is believed that polysaccharides, in particular β-glucans of the mycelium and fruiting bodies of the fungus, possess medicinal properties. The polysaccharide fraction of the fruiting bodies of *P. ostreatus* showed antiviral activity against the influenza A virus (Ilyicheva et al. 2020). Pleuran (insoluble β-1,3/1,6-D-glucan *P. ostreatus*) significantly reduced the duration of herpes simplex symptoms in patients (Urbancikova et al. 2020). Pleuran-based cream reduced UV-induced erythema on human skin, and long-term use of this cream for 30 days improved all controlled facial and body skin parameters (Schiano et al. 2021). The polysaccharide complex of the fruiting bodies of the fungus also contains chitin, from which chitosan can be obtained with a lower molecular weight than chitosan from shrimp and crabs (Antonyuk et al. 2020).

Biologically active substances obtained from *P. ostreatus* have radioprotective, immunostimulatory, antioxidant, antitumor, and antisclerotic action (Dargude Namrata & Patil Rupali 2021). It was found that the *P. ostreatus* is a good sorbent of heavy metals, radionuclides and toxins (Dicks & Ellinger 2020).

The mycelium and fruiting bodies of *P. ostreatus* contain enzymes of industrial interest. Xylanase, laccases and cellulases were found in aqueous extracts (Trejo-López et al. 2021, Zamora Zamora et al. 2021). An enzyme complex derived from spent *P. ostreatus* substrate was added to goats' feed to study its effects on goat's milk composition and yield. It was concluded that the addition of the enzymatic extract of the spent *P. ostreatus* substrate into the diet of goats increased the yield of fresh cheese and also influenced its color and texture (Trejo-López et al. 2021). Untreated laccases from *P. ostreatus* can be used for the purification of wastewater from organic micro-pollutants, such as drugs (Hultberg et al. 2020).

Lipophilic substances, which include ergocalciferols, tocopherols, squalene and higher fatty acids, are of interest to medicine and the economy. However, their content, according to various researchers, is very different. One of the possible reasons for this is the collection of raw materials in different phases of fungal growth. Therefore, we aimed to investigate changes in the chemical composition of the fruiting bodies depending on the stage of growth of fungi.

## Materials and methods

### Sample collection and preparation

The fruiting bodies of Euromycel PL132 pleurot fungi were taken from Dobry Hryb LLC (Zhydachiv district, Lviv region, Lavrykiv village). Mushrooms were grown in a dimly lit, cool room (t = +10–15ºC). Three portions of samples were prepared, which differed in appearance and growth time on straw substrate: 1) young mushrooms, fruiting bodies of which were formed in no more than 7 days; 2) mature mushrooms that enter the trade network (7–12 days) and 3) overripe mushrooms, the nutritional value of which is considered to be reduced (more than 14 days of growth). The fruiting bodies of wild *P. ostreatus* were collected in November 2020 in the forest on a maple stump in the Skole district of the Lviv region.

### Sample extraction

The exact masses of separate portions of fruiting bodies of mushrooms were spread in a thin layer in an oven with a fan and were dried overnight at a temperature of + 52ºC. After drying, the fruit bodies were weighed, ground and the resulting powder was passed through a sieve (D = 1.0 mm). The powder was then placed in paper capsules in a Soxhlet extractor and extracted for one hour with petroleum ether (boiling point - 40–60 °C). After extraction, the petroleum ether was distilled off, the extract was filtered through anhydrous sodium sulfate, and the solvent residue was removed by evaporation in an oven at + 52°C. The resulting dry residue was weighed, and the

residue was dissolved in hexane. The resulting solution was filtered, and the clear filtered solution was analyzed by gas chromatography-mass spectrometry (GC-MS).

**GC-MS analysis**

Identification of substances in the obtained extracts was carried out using a mass spectrometer 6C / MS Agilent Technologies 6890 N / 5975 B (USA) attached to the chromatographic column HP-5MS model, 30 m length, 0.25 mm diameter, filler: 95% dimethylpolysiloxane + 5% diphenylpolysiloxane; carrier gas - helium with a constant flow of 1.5 ml/min. The column was washed with methanol. Gas chromatography was programmed for a temperature rise of 15 °C / min from 75 to 300 °C. The initial temperature was maintained for 1 min, and the final temperature for 8 min. A mass-selective detector with interface temperature T = 250 °C was used. The ionization was performed by electron impact, the ionization energy was 70 eV, the ion source temperature was 230°C, quadrupole temperature was 150 °C.

The NIST05 and WILEY 2007 mass spectrum libraries, with a total number of spectra of more than 470,000, were used to identify the components in combination with computer programs for AMDIS identification. The relative error in determining the above components does not exceed 10% with a confidence level of 0.95.

**Results**

**Yield of dry and lipophilic residue**

Depending on the stage of growth of fungi, the yield of dry matter from fruiting bodies differed slightly (Table 1).

**Table 1** Yield of dry residue from young, mature and overripe fruiting bodies of *P. ostreatus.*

| Experiment number | **Wild mushrooms** | | | **Cultivated mushrooms** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Mature* | | | *Young* | | | *Mature* | | | *Overripe* | | |
| | Mass (g) | | | Mass (g) | | | Mass (g) | | | Mass (g) | | |
| | before drying | after drying | dry residue (%) | before drying | after drying | dry residue (%) | before drying | after drying | dry residue (%) | before drying | after drying | dry residue (%) |
| 1 | 220.0 | 21.01 | 9.55 | 700.0 | 52.96 | 7.56 | 700.0 | 48.74 | 6.96 | 700.0 | 64.12 | 9.16 |
| 2 | 340.0 | 33.56 | 9.87 | 700.0 | 54.66 | 7.81 | 700.0 | 46.78 | 6.68 | 700.0 | 63.49 | 9.07 |
| 3 | 170.0 | 17,05 | 10.03 | 700.0 | 55.86 | 7.98 | 700.0 | 51.88 | 7.41 | 700.0 | 63.77 | 9.11 |
| 4 | 170.0 | 17,10 | 10.06 | 700.0 | 56.14 | 8.02 | 700.0 | 53.26 | 7.60 | 700.0 | 62.72 | 8.96 |
| 5 | 150.0 | 15.18 | 10.12 | 700.0 | 55.16 | 7.88 | 700.0 | 51.81 | 7.40 | 700.0 | 63.7 | 9.06 |
| | | | **9.93± 0.38** | | | **7.85± 0.30** | | | **7.21± 0.53** | | | **9.07± 0.11** |

As can be seen from Table 1, the dry matter content of mature mushrooms is slightly lower than that of young mushrooms, and in overripe mushrooms, it increases again, most likely due to loss of moisture. In wild mushrooms, the yield of dry residue is approximately 37% higher compared to cultivated mushrooms.

The mass of lipophilic substances in wild mushrooms was five times greater than the mass of similar substances in cultivated mushrooms. The yield of lipophilic substances from the fruiting bodies of *P. ostreatus* cultivar Euromycel PL132 is insignificant and tends to decrease during the growth and maturation of fungi (Table 2).

**Qualitative and quantitative composition of the lipophilic fraction**

The chemical composition of the lipophilic residue of fungi at different stages of growth differed significantly both qualitatively and quantitatively. In young fungi (Table 3), only six substances were detected in the lipophilic residue by GC-MS (Table 3). The third lipophilic residue (34.19%) was bis (2-ethylhexyl) phthalate, and the growth stimulant Gibberellin A3 was 28.11%. The squalene content was 8.42%. No higher fatty acids or ergocalciferol were found in this residue.

**Table 2** Percentage of lipophilic residue at different stages of growth of fruiting bodies of *P. ostreatus* (Euromycel PL132 variety).

| Stages of fungi growth | Experiment number | Mass of samples | Weight of lipophilic residue (g) | Content (%) |
|---|---|---|---|---|
| **Cultivated mushrooms** | | | | |
| Young mushrooms | 1 | 31.0 | 0.117 | 0.38 |
| | 2 | 27.2 | 0.087 | 0.32 |
| | 3 | 26.1 | 0.094 | 0.36 |
| | Average value 0.35 | | | |
| Mature mushrooms | 1 | 20.5 | 0.034 | 0.17 |
| | 2 | 22.1 | 0.042 | 0.19 |
| | 3 | 21.8 | 0.046 | 0.21 |
| | Average value 0.19 | | | |
| Overripe mushrooms | 1 | 29.9 | 0.041 | 0.14 |
| | 2 | 30.0 | 0.048 | 0.16 |
| | 3 | 27.8 | 0.036 | 0.13 |
| | Average value 0.14 | | | |
| **Wild mushrooms** | | | | |
| Mature mushrooms | 1 | 12.0 | 0.074 | 0.62 |
| | 2 | 10.0 | 0.128 | 1.28 |
| | 3 | 14.3 | 0.147 | 1.03 |
| | Average value 0.98 | | | |

**Table 3** Qualitative and quantitative composition of the lipophilic fraction of young fruiting bodies of *P. ostreatus* cultivar Euromycel PL132.

| No | Retention time (min) | Name of the substance | Content (%) |
|---|---|---|---|
| 1 | 12.600 | Butyraldehyde | 7.53 |
| 2 | 13.718 | 9-Decen-1-ol | 18.47 |
| 3 | 16.103 | Bis(2-ethylhexyl) phthalate | 34.19 |
| 4 | 16.798 | But-2-enyl Peop-2-ynyl Ether | 3.28 |
| 5 | 17.494 | Squalene | 8.42 |
| 6 | 20.423 | Gibberellin $A_3$ | 28.11 |

Notes: No. – is the order of the substance eluting from the column.

Lipophilic residue obtained from mature marketable fungi contained a much wider range of substances. In total, according to GC-MS, 45 of them were detected, although the content of 30 of them was less than 1%. Significantly less gibberellin A3 (1.11%) was found in this residue, while the content of bis (2-ethylhexyl) phthalate was almost 2.5 times lower, and squalene was approximately four times lower (Table 4). In the lipophilic residue obtained from mature fungi, several higher fatty acids (palmitic, stearic, and linolenic) were identified, with a total content reaching 12.54%, along with dibasic sebacic acid. Hydrocarbons (heptadecane, tetratriacontane) and their derivatives were also found in the residue.

**Table 4** Quantitative and qualitative composition of the lipophilic fraction of mature fruiting bodies of *P. ostreatus* of Euromycel PL132 variety.

| No | Retention time (min) | Name of the substance | Content (%) |
|---|---|---|---|
| 7 | 9.211 | Heptadecane | 1.07 |
| 8 | 9.366 | Phenol, 2,4-bis(1,1-dimethylethyl) | 1.44 |
| 16 | 12.606 | Palmitinic acid | 4.98 |
| 17 | 12.707 | Dibutyl phthalate | 1.73 |
| 18 | 12.850 | Cyclohexanol | 2.56 |
| 20 | 13.724 | Linoleic acid | 5.44 |
| 21 | 13.748 | 2-Methylcyclohexanone oxime | 4.87 |
| 22 | 3.873 | Stearic acid | 2.12 |
| 26 | 15.246 | Tetratriacontane | 1.54 |
| 31 | 16.102 | Bis(2-ethylhexyl) phthalate | 13.69 |
| 34 | 16.911 | Sebacic acids | 1.42 |
| 36 | 17.500 | Squalene | 2.09 |
| 37 | 17.809 | Gibberellin $A_3$ | 1.11 |

Notes: No. – is the order of the substance eluting from the column.
* The table does not include substances with a content of less than 1%.

With the aging of the fruiting bodies of oyster mushrooms, the range of lipophilic fraction was reduced to 21 substances, apparently due to reduced metabolic processes. The squalene content was reduced by about 15%, and the bis (2-ethylhexyl) phthalate content was reduced by more than two times compared to mature mushrooms. At the same time, the amount of higher fatty acids increased, and their total amount in the lipophilic residue exceeded 60% (Table 5). Ergosta-5,7,22-trien-3-ol appeared in the lipophilic residue of overripe fungi, the content of which reaches 18% by weight of the lipophilic residue.

**Table 5** Basic substances and their quantity in the lipophilic fraction of overripe fruiting bodies of *P. ostreatus* of Euromycel PL132 variety.

| No | Retention time (min) | Name of the substance | Content (%) |
|---|---|---|---|
| 9 | 12.618 | Palmitinic acid | 15.37 |
| 10 | 12.708 | Butyl-2-ethylhexyl phthalate | 1.71 |
| 11 | 13.742 | Linoleic acid | 41.00 |
| 12 | 13.879 | Octadecanoic acid | 4.51 |
| 14 | 16.103 | Bis(2-ethylhexyl) phthalate | 5.84 |
| 17 | 17.500 | Squalene | 1.78 |
| 19 | 20.414 | Ergosta-5,7,22-trien-3-ol | 18.00 |

Notes: No. – is the order of the substance eluting from the column.
* The table does not include substances with a content of less than 1%.

The analysis of the lipophilic fraction of wild fruiting bodies of *P. ostreatus* identified 18 substances, the main ones of which are presented in Table 6. It is noteworthy that wild mushrooms exhibit a very high content of linolenic acid, accounting for 57.14% of the total lipophilic residue, along with a significantly higher content of squalene, the presence of tocopherol acetate (vitamin E), and a broader range of ergosterols. The total ergosterol content (13.83%) is comparable to that found in overripe cultivated mushrooms.

**Table 6** Basic substances and their quantity in the lipophilic fraction of wild mature fruiting bodies of *P. ostreatus.*

| No | Retention time (min) | Name of the substance | Content (%) |
|---|---|---|---|
| 2 | 12.054 | Pentadecanoic acid | 1.35 |
| 3 | 12.707 | Dibuthyl phthalate | 0.89 |
| 4 | 12.606 | Palmitinic acid | 5.69 |
| 5 | 13.724 | Linoleic acid | 57.14 |
| 7 | 16.102 | Bis(2-ethylhexyl) phthalate | 3.42 |
| 8 | 17.500 | Squalene | 7.73 |
| 9 | 19.206 | α-tocopherol acetate | 1.53 |
| 10 | 19.241 | 3,5-Cholesta-6,8(14 )-dien | 0.90 |
| 13 | 20.188 | 22E-Ergosta-5,7,9,22-tetraen-3β-ol | 0,73 |
| 15 | 20.414 | Ergosta-5,7,22-trien-3-ol | 9.19 |
| 16 | 20.485 | Ergosta-5,22-dien-3-ol | 1.03 |
| 18 | 20.830 | γ-Ergostenol | 1.13 |

Notes: No. – is the order of the substance eluting from the column.
* The table does not include substances with a content of less than 1%.

**Discussion**

The most controversial issue is the presence of phthalic acid derivatives in lipophilic extracts of the studied samples. Recently, a number of review papers have been published on the distribution of phthalates in lower and higher plants, bacteria and fungi. Phthalates have been detected in a very large number of higher plants, algae and fungi (Ortiz & Sansinenea 2018, Huang et al. 2021, Thiemann 2021). These authors show that in most cases, phthalates are of anthropogenic origin (from fertilizers, agrochemicals, and polluted water and air). On the other hand, phthalates that are not produced industrially have been found in a number of natural objects. In addition, experiments on the red alga *Bangia atropurpurea* showed that $C^{14}$ from $NaH^{14}CO_3$ is converted to dibutyl phthalate and diethyl phthalate (Chen 2004).

Phthalates began to be found in small quantities in real mushrooms with the development of modern sensitive methods of analysis, in particular, GC-MS. Thus, in 1976, a work was published in which phthalates were described as components of the specific odor of wild edible mushrooms of Finland. In particular, diethyl phthalate and dibutyl phthalate were detected in *Cantharellus cibarius* and *Gyromitra esculenta*, and dibutyl phthalate was detected in *Agaricus bisporus* (Pyysalo 1976). At that time, phthalate production in northern Europe was not yet developed, and it is unlikely that phthalates in these mushrooms could be the result of pollution. In our opinion, phthalates, as secondary metabolites, can play an important protective role in certain groups of mushrooms. When analyzing the milky juice of mushrooms of the genus *Lactarius*, phthalates were detected in all analyzed mushrooms (Tsivinska et al. 2015; Antonyuk et al. 2023). The study of the chemical composition of the milky juice of mushrooms of the genus *Lactarius* Pers. allowed us to make the statement that it can be considered as a stable balanced emulsion containing a large number of substances, one part of which is responsible for the toxic effect on other living organisms, and the other determines the stability of this emulsion. This creates an effective system of protection of mushrooms of the genus *Lactarius* from microorganisms, insects, mollusks and animals. Phthalates, in particular, may be responsible for the insecticidal effect (Antonyuk et al. 2023).

The results refer to fungi grown in a cool (t = +10 −15ºC), dimly lit room. At the same time, there are data from the literature that show that the amount of certain substances in the fruiting bodies of *P. ostreatus* is influenced by various factors: substrate composition (Garuba et al. 2017, Mleczek et al. 2021), substrate humidity (Deacon 2006), air temperature, light level (Krings & Berger 2014, Schmidt 2006), salt concentration in the substrate (Mousa 2021), substrate pH (Ahmad & Hussien 2021), amount and composition of mineral (Sianturi & Sabrina 2021) and

organic (Orngu et al. 2021) fertilizers. In particular, it was noted that the best pH value for fungal growth is 6.0 and a temperature of + 30 °C (Ahmad & Hussien 2021).

Light is a signal that indicates that the mycelium has reached the surface, when spores can be produced in an environment suitable for their release into the outside world (Deacon 2006). Short wavelengths (blue light) stimulate the growth of fruiting bodies, and longer wavelengths are ineffective. The amount of light required for the growth of fruiting bodies is insignificant; it is lower than the amount of full moonlight in the clear sky (Schmidt 2006). For example, even short-term (within an hour) ultraviolet light significantly changes the amount of ergosterols (Keflie et al. 2019) and fatty acids in the fruiting bodies of the *P. ostreatus* (Krings & Berger 2014). At the same time, a study of the effect of green light (515−530 nm) on the growth of mycelium of five species of *Pleurotus* (*P. citrinopileatus, P. djamor, P. eryngii, P. ostreatus* and *P. pulmonarius*) found that it reduced the growth of biomass of mycelium of these fungi, but increased their cellulolytic and xylanolytic activity. The cellulolytic activity of most of the studied strains increased in the presence of green light, with an increase of 1.5 times (endoglucanase *P. ostreatus*) to 8 times (total cellulase and endoglucanase *P. citrinopileatus*). The green light reduced laccase activity for most strains studied. The specific enzymatic activity of cellulase and endoglucanase from *P. citrinopileatus* increased 31 times and 30 times, respectively, compared to the dark. In addition, under the green light, the specific laccase and xylanase activity of *P. pulmonarius* increased 4.4 times and 6.8 times, respectively (Araújo et al. 2021).

The moisture content in the substrate is an important factor in the growth and metabolic activity of fungi (Schmidt 2006, Stienen et al. 2014). The optimal moisture content in the substrate for the growth of the fungus *Pleurotus* is from 50% to 80% (Mohammadi Goltapeh & Pourjam 2010). Substrate moisture is important for the activity of fungal enzymes and the rate of degradation of bonds in lignin in the cell walls of wood. This lignin is broken down by enzymes produced by *P. ostreatus* (Aghajani et al. 2018, Bari et al. 2018).

Although we did not study all these effects on the chemical composition of fungi, all studies were performed on cultivated fungi grown under the same conditions of temperature, humidity and light. Therefore, we can be sure that the dynamics of these changes will be the same for other experimental conditions. Wild mushrooms grew in different temperature conditions, on different substrates and in the presence of sunlight. This can be the cause of the greater quantity of lipophilic substances and differences in chemical composition.

## Conclusions

The qualitative and quantitative chemical composition of lipophilic extracts of young, mature and overripe fruiting bodies of *P. ostreatus* differs significantly. As fungi grow, the range of chemicals increases, and then with aging, decreases. With age, the amount of fatty acids and steroids increases, and the amount of squalene and phytohormone decreases. It is noteworthy that the yield of lipophilic extract from wild mushrooms is five times higher than that of cultivated ones. Vitamin E, more ergosterols, squalene and linolenic acid were found in this extract. A possible reason for this is the growth of wild mushrooms in sunlight, other temperatures and humidity.

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