

BIOSYNTHESIS OF VOLATILES BY *Pleurotus ostreatus* (Jacq.:Fr.) Kumm. MUSHROOMS ON SUBSTRATES ENRICHED WITH VEGETABLE OILS

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The purpose of the study was to analyze the ways and intensity of synthesis of volatile flavor compounds by *Pleurotus ostreatus* (Jacq.:Fr.) Kumm. mushrooms in the process of intensive cultivation on sunflower husk and barley straw with the addition of vegetable oils (sunflower and corn) as a potential source of unsaturated fatty acids. Methods of sensory profile analysis and ultraviolet spectroscopy were used. Sensory profile analysis of dried samples of fruit bodies showed an increase in the intensity of mushroom, meat and grassy notes of flavor on substrates with the addition of vegetable oils in a concentration of 1% and 5% of the weight of the substrate. For the strain IBK-551 marked increase in the intensity of sweet and floral attributes of the aroma on both substrates with the addition of corn oil. UV spectroscopy of hexane extracts of dried samples of fruit bodies revealed maxima of light absorption in the range of 200–210 nm and 260–300 nm. There was a difference in intensity of light absorption of samples of different strains cultivated on substrates with the addition of vegetable oils.

Key words: *Pleurotus ostreatus*, volatile aroma compounds, sunflower oil, corn oil, sensory profile analysis, UV spectroscopy.

In many countries, mushrooms and dishes from them are considered to be delicacies due to their specific aroma, which is provided by hundreds of volatiles such as octane derivatives and octenes, lower terpenes, benzaldehyde, sulfur compounds, and others. Fatty C8-compounds such as 1-octanol, 3-octanol, 3-octanone, 1-octen-3-ol, 2-octen-1-ol, 1-octen-3-one are formed in lipoxygenase pathway from polyunsaturated fatty acids.

Mushrooms have long been used as food or food flavoring materials because of their unique and flavor. Fresh and preserved mushrooms are consumed in a lot of countries as a delicacy, particularly for their specific aroma and texture [1].

Consumers highly appreciate the characteristic flavor of mushrooms, which consists of hundreds of odorous compounds such as derivatives of octane and octenes, lower terpenes, derivatives of benzaldehyde, sulphur compounds and others [1]. Volatile aroma substances of edible mushrooms may be divided into three groups: carbon-eight (C8)

derivatives impart the “characteristic mushroom flavor”, terpenoid volatiles and sulfur-containing odour compounds [2].

Eight-carbon volatile compounds are a key contributor to mushroom flavor and have been described by many authors in fungi [3]. 1-Octanol, 3-octanol, 3-octanone, 1-octen-3-ol, 2-octen-1-ol, and 1-octen-3-one are the main of them. They account for 44,3–97,6% of the total volatile fraction. [4].

The aliphatic alcohol 1-octen-3-ol (first called “matsutake alcohol”) is the principal compound contributing to the unique mushroom aroma and flavor [3].

Isoprenoids (also known as terpenoids) belong to a group of secondary metabolites that are synthesized in mushrooms. Twenty-four isoprenoids have recently been reported in fruiting bodies of the white *Tuber magnatum*. Limonene and cedrol were the most abundant of them [5]. Aromadendrene, alpha-farnesene and other terpenoid compounds were detected in *T. borchii* fruit body [6].

Sulfur volatile organic compounds (S-VOCs) are key contributors to truffle aroma. The diversity of sulfur volatiles in truffles is large, ranging from relatively small compounds, such as dimethyl mono- (DMS), di- (DMDS) and tri- (DMTS) sulfides, which are produced by most truffle species, to complex S-volatiles such as 2-methyl-4,5-dihydrothiophene, characteristic of the white truffle *T. borchii*, and bis(methylthio) methane, characteristic of the white truffle *T. magnatum*. The latter species contain further 27 sulfur volatiles [5].

The characteristic “sulfurous” note of Shiitake mushroom is composed from S-compounds. They include the straight chain compounds dimethyl disulfide, dimethyl trisulfide, 1-(methylthio)dimethyl disulfide and cyclic compounds lenthionine (1,2,3,5,6-pentathiepane, $C_2H_4S_5$), 1,2,4-trithiolane ($C_2H_4S_3$), 1,2,4,5-tetrathiane ($C_3H_6S_4$) and 1,2,3,4,5,6-hexathiepane (CH_2S_6) [7].

Eight-carbon volatiles are enzymatically formed by oxygenation of polyunsaturated fatty acids (PUFAs). This reaction is catalyzed by lipoxygenases (LOXs), thus starting the so-called lipoxygenase (LOX) pathway [8]. Metabolites originating from these pathways are collectively named oxylipins [9].

The purpose of the study was to analyze possible ways of synthesis of volatile flavor compounds in mushrooms and to determine the intensity of this synthesis by *Pl. ostreatus* (the oyster mushroom) in the process of intensive cultivation on substrates with the addition of vegetable oils as a potential source of unsaturated fatty acids, which are the main substrates for the synthesis of aliphatic aroma compounds.

Sunflower (*Helianthus annuus* L.) and corn (*Zea mays* L.) oils as vegetable oils were used in this work. Sunflower oil contains up to 90% unsaturated fatty acids (linoleic and oleic) and up to 10% saturated fatty acids (palmitic and stearic) [10]. Quantitatively the predominant components of the fatty acid composition of corn oil are palmitic, oleic and linoleic acids [11].

The composition of fatty acids of sunflower [12] and corn [13] oils is given in Table 1.

Materials and Methods

Mushroom strains. Three strains of the edible mushroom *Pleurotus ostreatus* (Jacq.:Fr.) Kumm.: IBK-549, IBK-551 and IBK-1535 from the mushroom collection of the Kholodny Institute of Botany of the National Academy of Sciences of Ukraine were objects of the study [14]. This mushroom belongs to the *Pleurotaceae* family of *Agaricales* of the *Agaricomycetes* class of the *Basidiomycota*, regnum *Fungi*.

Solid-phase cultivation. The substrate for the production of fruiting bodies was the agricultural waste: sunflower husk and barley straw. As additives to substrates, sunflower and corn oils were used at a concentration of 1% and 5% of the weight of the wet substrate. The sunflower oil for unrefined cold pressing of the first spin of the first grade [12] and the corn oil refined deodorized grade P [13] were used in the research.

Preparation and sterilization of substrates were carried out according to commonly accepted methods [15]. The substrate was evaporated for 2 hours, $CaCO_3$ was added in an amount of 1% to the mass of the substrate and sterilized twice autoclaving at 121 °C for 30 minutes with an interval of 24 hours. Straw was pre-minced to a size of 2–3 cm. The cooled substrate was inoculated with *Pl. ostreatus* mycelium in an amount of 5% by weight of the substrate. Seeding mycelium was obtained on barley grain. Cultivation was carried out in glass jars at 26–28 °C and 70–80% humidity to the full mycelial overgrowth of the substrate. The weight of the wet substrate on the basis of sunflower husk in one jar was 150 g, and on the basis of barley straw — 110 g. Containers with the substrate were transferred to a growth room with a temperature of 15–16 °C, humidity of 80–90% and 8-hour photoperiod after full growth of the substrate by mycelium. The 1st and 2nd flushes were harvested. Mushrooms were dried at 40–45 °C in a dry oven for 24–48 hours.

Growing strains of *Pl. ostreatus* on the substrates without the addition of oils was used as a control experiment.

Table 1. Fatty acid composition of vegetable oils

Kind of oil	Mass fraction of fatty acid,% to the sum of fatty acids				
	Palmitic $C_{16:0}$	Stearic $C_{18:0}$	Oleic $C_{18:1}$	Linoleic $C_{18:2}$	Linolenic $C_{18:3}$
Sunflower oil	3.0–10.0	1.0–10.0	14.0–35.0	50.0–75.0	<1.0
Corn oil	9.0–14.0	0.5–4.0	24.0–42.0	34.0–62.0	<2.0

During the cultivation process, the following growth parameters of the *Pl. ostreatus* mycelium were determined: the time of the mycelial development on the substrate, the time of primordia formation, the number of formed bunches per 100 g of wet substrate, and the yield of the first and second flushes of fruitage per 100 g of wet substrate. Cultural and morphological mushroom features were studied in order to establish a relationship between them and the synthesis of aroma-forming substances.

Sensory profile analysis. The sensory profile of the aroma of dried mushroom samples was studied according to [16].

The panel consisted of 5 experts trained for organoleptic analysis. First, the characteristic attributes of the aroma were determined, and then the intensity of each of them on a 5-point scale: 0 — not present; 1 — just recognizable or threshold; 2 — weak; 3 — moderate; 4 — strong; 5 — very strong. The studied samples were evaluated three times.

The organoleptic evaluation of different strains of dried mushrooms, collected at the same stages of maturation, was carried out in specially prepared, well-ventilated rooms at the Department of Biotechnology of the Ukrainian State University of Chemical Technology.

Microsoft Office Excel 2007 software was used to construct the aroma profiles of dried mushroom samples.

Spectrophotometric analysis. For a spectrophotometric study, the dried fruiting bodies of the first flush were crushed on an

electric mill to a powder. 1 g of the obtained material was placed in the extractor, then 100 cm³ of solvent were added (the hydromodule was 1:100). Hexane was used as a solvent. Extraction was carried out at boiling point (69 °C) of the solvent for 30 minutes. The extracts were cooled in a fume hood, filtered through a paper filter on a Buchner funnel and transferred quantitatively into a volumetric flask of 250 cm³. Then the solvent volume was adjusted to the mark. Absorption spectra were recorded using a spectrophotometer SF-2000 in the 200–350 nm wavelength range. Pure hexane was used as a comparative solution.

Statistical analysis. The obtained data were processed statistically using one-way analysis of variance [17]. All samples were carried out in triplicate. Values are presented as means ± standard error of the mean. Differences at $P \leq 0,05$ were considered to be significant.

Results and Discussion

Possible ways of synthesis of volatile flavor compounds in mushrooms.

Taking into account the data of other authors and our analysis, the most probable way of synthesis of 1-octen-3-ol is presented in Figure 1.

Fatty acids are precursors in the reaction of eight-carbon volatile synthesis and are key components in a variety of lipids [3].

Lipoxygenases (linoleate: oxygen oxidoreductase, EC 1.13.11.12; LOXs) are

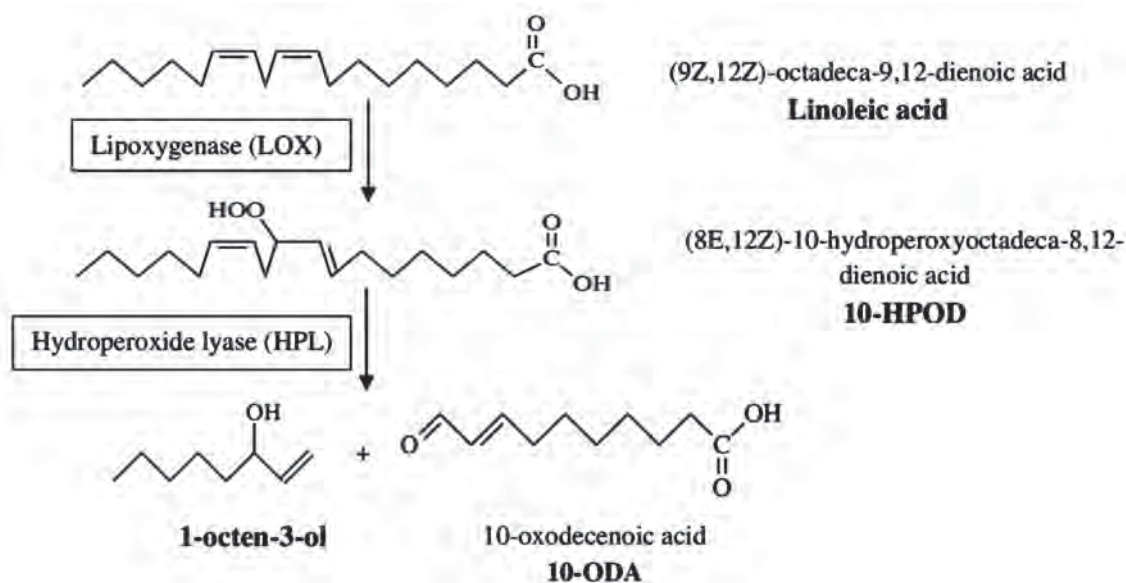


Fig. 1. Lipoxygenase pathway of biosynthesis of 1-octen-3-ol: (based on [3, 19, 21–23] with our modifications)

a family of enzymes found ubiquitously in plants and mammals, but have also been detected in coral, algae, fungi, yeast and a number of bacteria. In general LOXs are non-heme iron-containing dioxygenases [9]. This group of enzymes catalyzes the insertion of molecular oxygen into polyunsaturated fatty acids containing a (1Z,4Z)-pentadiene system, such as linoleic acid, linolenic acid, and arachidonic acid, yielding the corresponding hydroperoxides [18].

Although free fatty acids is the preferred substrate of LOX, it was recently reported that fatty acids acylated to phospholipids are slowly oxygenated by lipoxygenases [19].

A lot of researches conducted to study the structure and properties of plant and animal lipoxygenases.

Oxygen insertion into PUFAs by LOX is regio- and stereo-specific, and this specificity is used as the decisive criterion for LOX classification. In plants, linoleic acid and linolenic acid are the primary substrates of LOX, because they are the most abundant fatty acids. Plant LOXs are classified as 9- or 13-LOXs with respect to their positional specificity of linoleic acid oxygenation. Animal LOXs are classified as 5-, 8-, 9-, 11-, 12-, or 15-LOXs with respect to their positional specificity of the oxygenation of arachidonic acid, the predominant substrate of animal LOXs. Furthermore, LOXs are classified as S- or R-LOXs on the basis of the chirality of their hydroperoxide products [18].

Fungal LOXs, similar to plant LOXs, act mainly on C₁₈ fatty acids. Linoleic acid (18:2, $\Delta^{9,12}$) is the most abundant in widely appreciated cultivated mushrooms, followed by palmitic and stearic acids. Most fungi convert C₁₈ fatty acids into 9-hydroperoxy or 13-hydroperoxy fatty acids (i.e. they contain 9-LOX and 13-LOX, respectively). Interestingly, 10-hydroperoxy and 12-hydroperoxy fatty acids can also be formed as possible products of fungal polyunsaturated fatty acid metabolism [20].

Products formed by LOXs can be converted by a hydroperoxide lyase (HPL), an allene oxide synthase (AOS), a peroxygenase or a reductase.

Hydroperoxide lyases are said to perform either homolytic or heterolytic cleavage of hydroperoxides, yielding different short-chain volatiles, depending on their cleavage mode. The first cleavage mechanism, homolytic, involves the cleavage of the hydroperoxide between the carbon bearing the hydroperoxide group and the saturated carbon. This mechanism has been observed in algae and

mushrooms. The second mechanism is called heterolytic. It is found in most plants: the enzyme cleaves the hydroperoxide between the carbon bearing the hydroperoxide group and the unsaturated carbon [3].

Wurzenberger and Grosch showed that after incubation of the 9-, 10-, 12- and 13-hydroperoxide isomers of linoleic with a protein fraction of mushroom *Psalliota bispora* only 10-hydroperoxide isomer was cleaved to 1-octen-3-ol and 10-oxo-*trans*-8-decenoic acid [21].

Results of Akakabe et al. indicated a stereochemical correlation between (R)-1-octen-3-ol and (S)-10-hydroperoxy-(8E,12Z)-8,12-octadecadienoic acid [(S)-10-HPODE] with homogenates of *Lentinula edodes* and *Tricholoma matsutake* [22].

Assaf et al. showed that 13-hydroperoxy-*cis*-9,*trans*-11-octadecadienoic acid (13-HPOD) and 10-oxo-*trans*-8-decenoic acid (10-oxo-acid) were found to be the major nonvolatile metabolites of *Pl. pulmonarius* submerged culture, associated with the enzymatic cleavage of linoleic acid to 1-octen-3-ol. But despite its accumulation, 13-HPOD was found not to be the precursor of 1-octen-3-ol. These results suggest the involvement of two different lipoxygenases in 1-octen-3-ol and 13-HPOD formation [23].

But besides 1-octen-3-ol, other aliphatic saturated and unsaturated C₆-C₁₀ compounds, which are likely to be formed with the participation of other lipoxygenases and hydroperoxidilases, are responsible for the formation of the characteristic aroma of mushrooms. Probably there are several metabolic pathways of the synthesis of volatile aroma compounds in fungal cells.

Terpenoids constitute the most abundant and structurally diverse group of plant secondary metabolites [24] that is why the metabolic pathways for the formation of volatile aroma compounds of terpenoid nature are sufficiently studied and highlighted in the literature on the example of plant organisms. The scheme of biosynthesis of terpenoid compounds is shown in Fig. 2.

Terpenoids are derived from the universal C₅ precursor isopentyl diphosphate (IPP) and its allylic isomer dimethylallyldiphosphate (DMAPP), which in higher plants are generated from two independent pathways located in separate intracellular compartments. In cytosol, IPP is derived from the mevalonic acid (MVA) pathway that starts with the condensation of acetyl-CoA. In plastids, IPP is formed from pyruvate and glyceraldehydes-3-

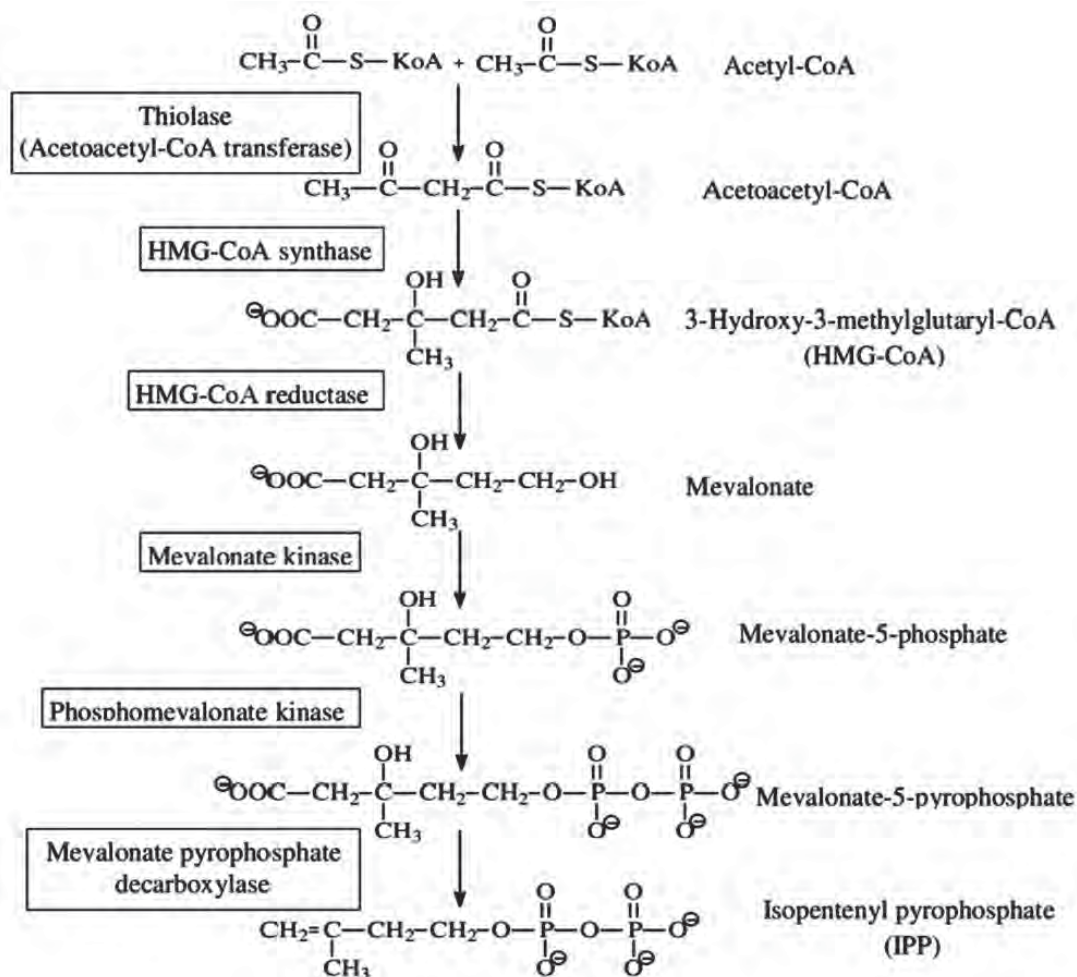


Fig. 2. Mevalonate pathway of biosynthesis of terpenoids: based on [24–26] with our modifications

phosphate. This MVA-independent pathway, also called MEP pathway after the key intermediate methylerythritol phosphate (MEP), was discovered [24–26].

Two main S-VOC biosynthetic pathways (Fig. 3), both relying on L-methionine (Met) catabolism, have been investigated on bacteria and ascomycetes: the one-step conversion of L-methionine to metanethiol (MTL) by methionine lyase (a typical bacterial rather than yeast enzyme) or by cystathionine lyase; and a two-step pathway, initiated by L-methionine transamination to 4-methylthio-2-oxobutyric acid, which is then converted to 3-(methylthio)propanal (also known as methional) via decarboxylation, with the ultimate formation of MTL [5].

Lenthionine (1,2,3,5,6-pentathiepane), a cyclic sulfur compound found in *L. edodes*, is derived from a γ -L-glutamyl-cysteine sulfoxide precursor (lenticinic acid) in a two-step enzymatic reaction. Lenticinic

acid is first activated by the removal of its γ -glutamyl moiety catalyzed by γ -glutamyl transpeptidase (GGT) producing a L-cysteine sulfoxide derivative, which then undergoes α , β -elimination catalyzed by cysteine sulfoxide lyase, resulting in a highly reactive sulfenic acid intermediate. The sulfenic acid is then rapidly condensed to form thiosulfinate, and the thiosulfinate is often further transformed into other sulfur compounds including lenthionine (Fig. 4) [28].

The analysis of possible ways of synthesis of volatile flavor compounds in mushrooms showed that the precursors of this synthesis are organic acids, including those that are part of vegetable oils.

Culture and morphological characteristics of fungal growth depending on the type of investigated substrate

The term of overgrowing of the substrate by mycelium was on the studied substrates

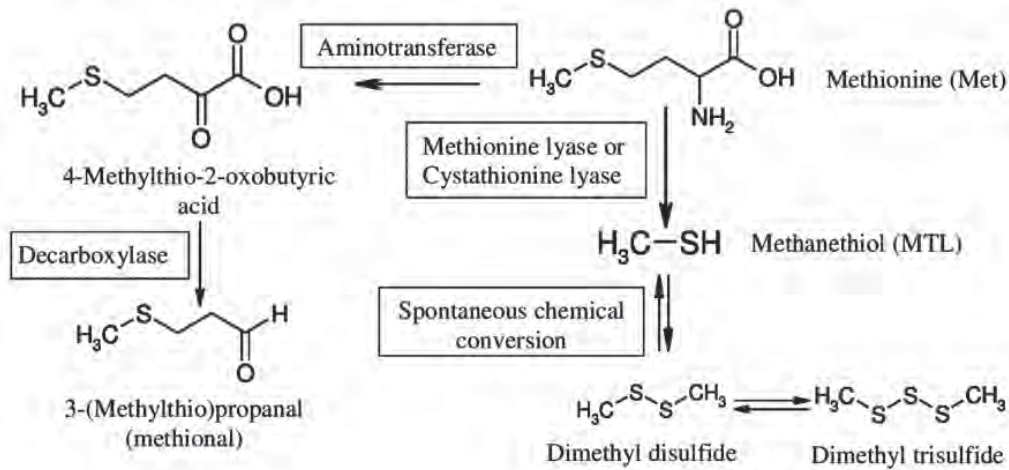


Fig. 3. Biosynthetic pathways for the synthesis of sulfur volatiles: based on [5, 27] with our modifications

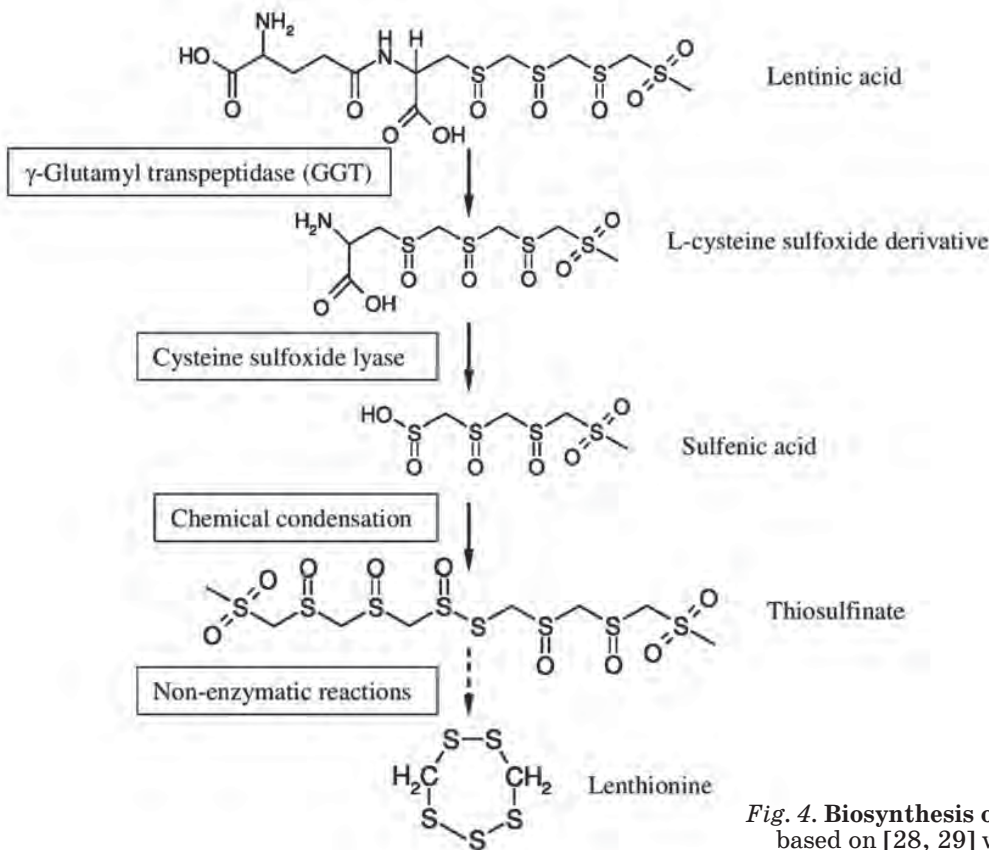


Fig. 4. Biosynthesis of cyclic sulfur volatiles: based on [28, 29] with our modifications

from 6 to 7 days, that is, there was no significant difference between this growth rate in different experimental variants compared with the control. According to the morphological features, *Pl. ostreatus* mycelia of all examined strains were white, fluffy, and denser in sunflower husk.

Pl. ostreatus fruit bodies cultivated on substrates with the addition of sunflower oil (A) and corn oil (B) are shown in Fig. 5.

Fruit bodies, obtained on different substrate variants within the mushroom strain, did not differ morphologically.

The growth parameters of *Pl. ostreatus* IBK-549, IBK-551 and IBK-1535 on different substrates are given in Table 2.

The period of primordial emergence varied depending on the mushroom strain from 18 to 30 days and did not differ significantly in various variants of substrates within the strain

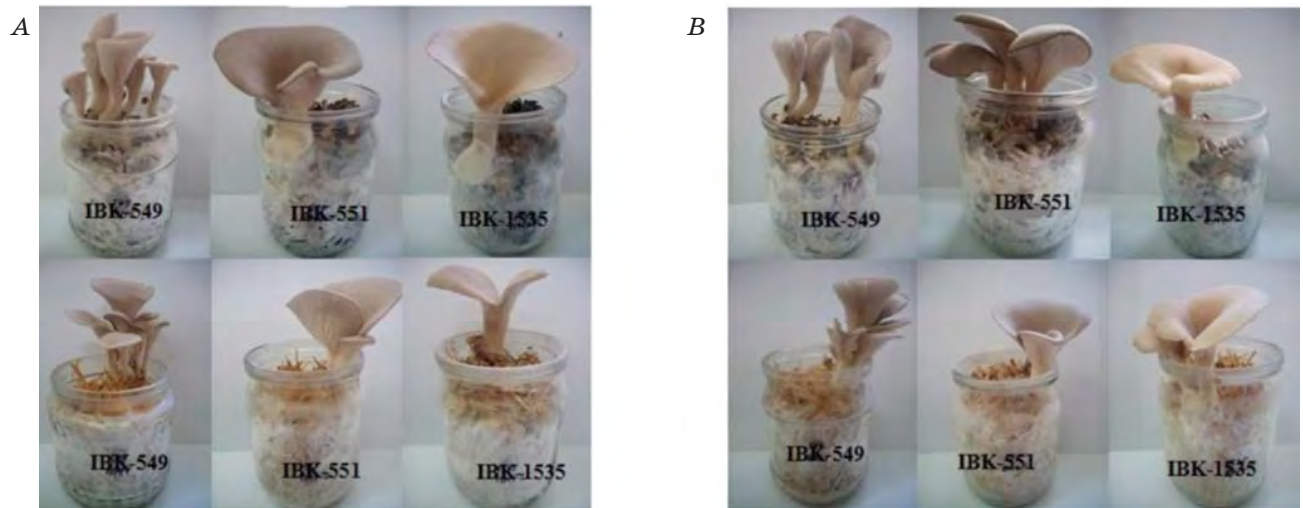


Fig. 5. Fruiting bodies of various strains of *Pleurotus ostreatus* (Jacq.:Fr.) Kumm. cultivated on substrates with: the addition of sunflower oil (A) and corn oil (B) (the upper line — the substrate is sunflower husk; the lower line — the substrate is barley straw)

Table 2. The growth parameters of *Pleurotus ostreatus* strains on various substrates

Substrate version	Time of primordial emergence, day	First flush of fruitage, day	Number of mushroom bunches per 100 g of substrate, pcs	Mushroom yield of the 1 st flush, g/100 g	Mushroom yield of the 2 nd flush, g/100 g	Time of primordial emergence, day	First flush of fruitage, day	Number of mushroom bunches per 100 g of substrate, pcs	Mushroom yield of the 1 st flush, g/100 g	Mushroom yield of the 2 nd flush, g/100 g
	Sunflower husk					Barley straw				
<i>Pl. ostreatus</i> , strain IBK-549										
SO 1%	18	25	11.6±1.4	10.8±0.4*	3.8±0.3	17-18	25	12.7±1.1	13.0±0.4	5.6±1.0
SO 5%	18-20	25	10.4±0.7	10.9±0.4*	4.2±0.5	18	25-27	15.5±2.3	14.9±0.9	5.5±0.5
CO 1%	19-20	25	17.1±1.0	12.7±0.5*	3.4±0.4	16-17	24-25	18.5±1.0	13.9±0.7	5.6±0.8
CO 5%	20	25	15.1±2.0	10.5±0.3*	3.9±0.4	18-19	24-28	15.2±0.4	12.4±0.5	9.1±1.1*
Control	18-20	25-27	12.2±0.7	9.2±0.4	3.4±0.2	17	25-27	14.8±1.6	13.0±0.6	4.5±0.2
<i>Pl. ostreatus</i> , strain IBK-551										
SO 1%	18-20	28	13.8±1.8	10.2±0.2*	3.7±0.3	18-20	28-29	13.0±2.1	13.1±0.4*	4.7±0.2
SO 5%	20-22	28-32	11.6±2.0	11.9±0.6*	3.4±0.1	20-22	27-29	15.8±2.6	13.2±0.4*	5.9±1.2
CO 1%	19-21	25	14.2±1.0*	10.8±0.4*	3.5±0.1	19-20	28	14.8±0.4	15.4±1.2*	4.8±0.4
CO 5%	21-23	25-29	8.9±0.7	11.4±0.5*	4.0±0.4	23	30	19.7±1.0	16.8±1.8*	6.1±0.4*
Control	20-22	27-28	10.4±0.5	9.0±0.4	3.1±0.3	22-23	29	19.7±2.6	11.6±0.3	4.4±0.3
<i>Pl. ostreatus</i> , strain IBK-1535										
SO 1%	22	29	11.1±0.3*	9.7±0.3*	3.3±0.1	22	32-36	16.7±1.0*	14.9±1.6	4.9±0.4
SO 5%	25-26	32-36	14.9±1.5*	10.6±0.5*	3.5±0.4	22	29-36	14.5±1.3*	14.2±0.5	5.4±1.1
CO 1%	30	39	11.8±0.3*	11.3±0.4*	4.1±0.4	25	30-34	18.5±0.4*	12.8±0.5	5.3±0.3
CO 5%	25-29	30-36	8.4±0.7	10.8±0.1*	4.0±0.4	25-26	33-36	20.6±2.7*	17.1±0.8*	4.9±0.3
Control	24-28	30-36	8.4±0.3	7.8±0.5	3.5±0.4	22-24	30-34	8.8±0.7	11.4±1.5	5.4±0.4

Note: * — $P < 0.05$ with compared to control ; SO — sunflower oil; CO — corn oil.

of control. The primordia were formed first by the strain IBK-549, 2–3 days later by IBK-551, and 4–5 days later by IBK-1535.

The examined strains had significant differences by fruitage time. Fruit bodies were most rapidly formed by strain IBK-549, 2–3 days later by the strain IBK-551 and 4–8 days later by IBK-1535. Also, there was no significant effect of additives to the substrate on the terms of fruiting.

There was an increase in the formation of bunches on sunflower husk with the addition of corn oil at a concentration of 1% for all strains. The strain IBK-1535 produced 1.3–2.3 times more bunches on both substrates with additives of vegetable oils in both concentrations as compared to control.

Yield of the first flush was higher for all strains grown on sunflower husk with both vegetable oils. On barley straw the increase of yield in the first flush was observed only for the strain IBK-551.

Analysis of literary data on the application of oils in the cultivation of mushrooms has shown that the addition of soybean oil at a concentration of 3 g/l in submerged cultivation of *Pl. mutilis* contributed to an increase of mushroom biomass yield and an increase in the synthesis of fatty acids [27–30].

The stimulating effect on the growth of mycelium *A. bisporus* was also revealed in agar medium with sesame oil additives at 1% concentration [31].

Profile analysis of the fungal aroma

During the sensory analysis by the panel, the following attributes of the aroma of dried mushroom samples were determined: mushroom, sweet, woody, herbaceous, sour, fish, meat, earthy, floral, and putrescent.

The results of sensory analysis of dried samples of different strains of *Pl. ostreatus* are presented in circle plots at Fig. 6.

From the provided data it is evident that the aroma profile of mushroom samples varied depending on the substrate, the strain of mushroom and additives. For all strains with both additives there was an increase in the intensity of mushroom notes in 1.2–1.5 times.

Samples of all strains grown on sunflower husk had a 1.4–2.4 times higher intensity of herbaceous notes and in samples obtained on barley straw, the intensity of the herbaceous attribute was higher in 1.5–2.4 times when only corn oil was added to the substrate.

As for meat notes, their higher intensity (in 1.4–1.8 times) was noted in comparison with the control in samples of dried mushrooms of all strains cultivated on barley straw with

additives of both oils. The higher intensity of meat notes was recorded for the strain IBK-551 (in 1.3–1.5 times) on sunflower husk with the addition of both oils, and for strain IBK-549 (in 1.5 times) with the additive of sunflower oil.

There was an increase in the intensity of sweet (in 1.4 times) and floral (in 1.8–2.6 times) notes for the strain IBK-551 and earthy (in 1.3–1.8 times) for strains IBK-549 and IBK-1535 on both substrates with the addition of corn oil.

For some samples there was a slight increase in the sour and putrescent characteristics of the aroma on the substrates with additives of vegetable oils. And the nature and strength of woody and fish notes were almost unchanged compared to control for any of the samples.

Statistical processing of the sensory analysis data showed that the standard error does not exceed ± 1 point, indicating the statistical homogeneity of the set of expert assessments [32].

Ultraviolet spectroscopy

The registered UV absorption spectra of hexane mushroom extracts are presented in Figures 7 and 8.

Hexane extracts of dried samples of *Pl. ostreatus* fruit bodies had light absorption maxima in ranges of 204–210 nm and 250–290 nm. Such spectral properties are characteristic of solutions of unsaturated compounds with unbound double bonds, saturated and unsaturated aldehydes and ketones, as well as derivatives of benzene [33]. As it was found in previous studies, the solution of 1-octen-3-ol in hexane has a maximum absorption at $\lambda = 207$ nm [34].

A 1.2–1.4 times higher light absorption intensity was observed throughout the studied range of wavelengths for samples of strain IBK-549 obtained on sunflower husk, with the addition of both oils at a concentration of 1%, and in the range of 250–290 nm only for the strain IBK-551.

Mushroom extracts of the strain IBK-1535 cultivated on husk with sunflower and corn oil additives in both concentrations also showed an increase in light absorption compared to control. In addition, it is higher in 1.4–1.6 times as at 207 nm (the maximum is typical for 1-octen-3-ol), and 1.5–2.5 times higher in the range of 250–300 nm (the maxima inherent in aldehydes and ketones).

A similar dependence was observed for the strain IBK-1535, cultivated on barley straw with additives of oils. And also for the strain IBK-549, whose extracts revealed more intense (in 1.2 times) light-absorption maxima

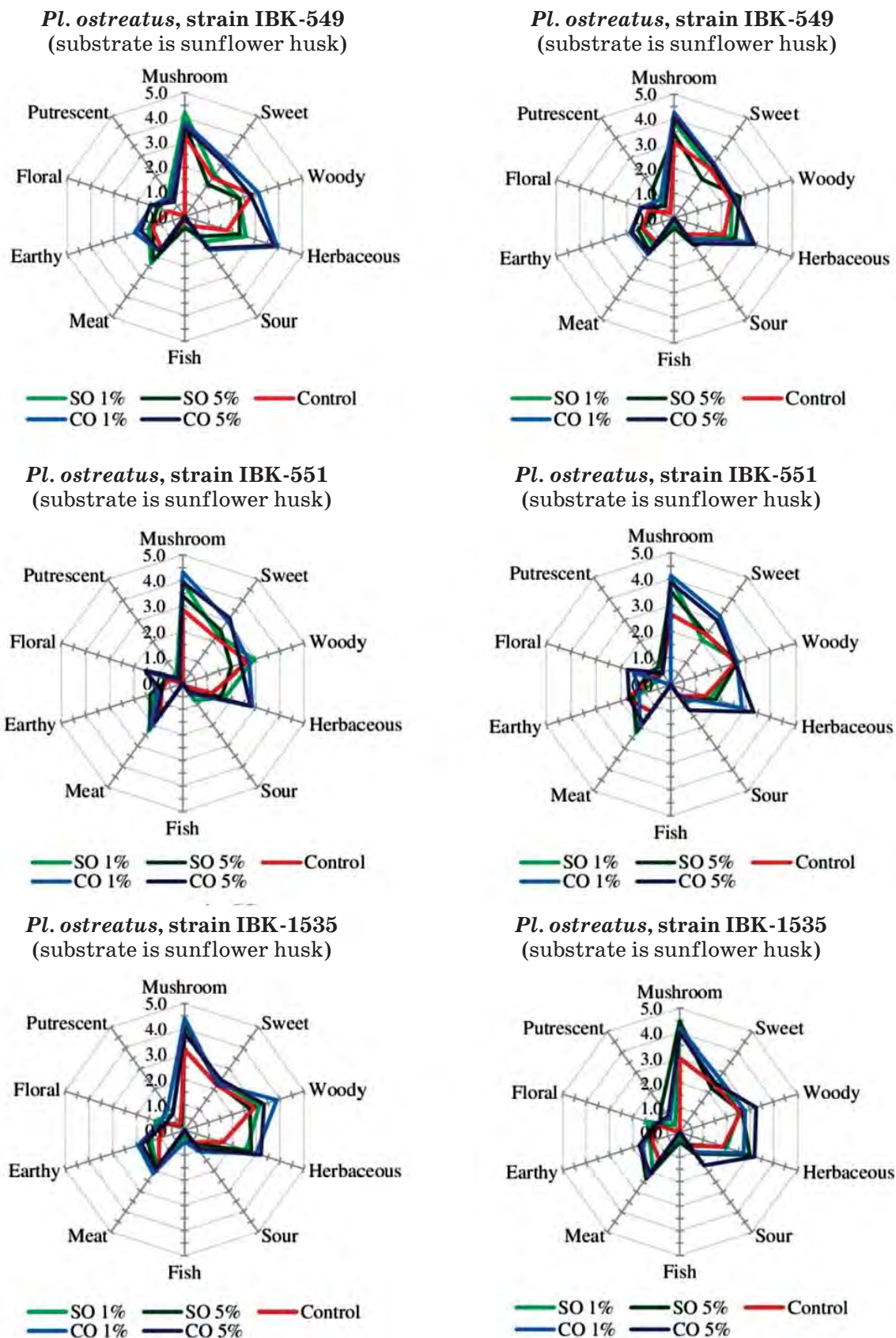


Fig. 6. Sensory profile of aroma of dried samples of *Pleurotus ostreatus* strains

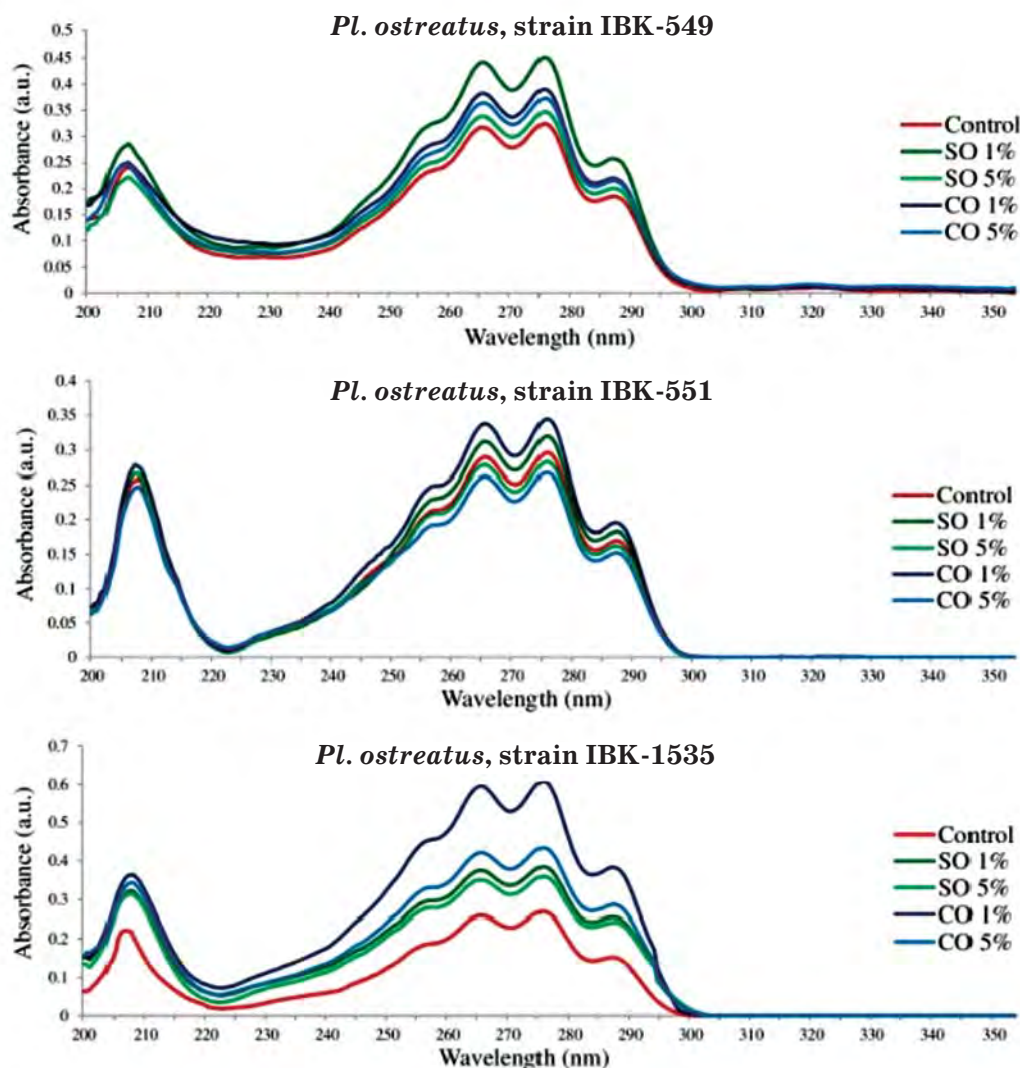


Fig. 7. UV spectra of hexane extracts of *Pleurotus ostreatus* strains: the substrate is sunflower husk

for strains grown on straw with both oils at a concentration of 1%.

The intensity of light absorption was 1.1–1.2 times higher only in the range of 260–290 nm for samples of the strain IBK-551, collected from substrates with additives of both oils at a concentration of 1%.

Comparison of the intensity of light absorption of various strains of *Pl. ostreatus* showed the highest level for the strain IBK-549 on both substrates compared to control.

It should also be noted that the ratio of the intensity of the light-absorption maxima at $\lambda = 207$ nm (typical for 1-octen-3-ol) and at $\lambda = 260$ –280 nm (typical for aldehydes, ketones and benzene derivatives) for different substrates and strains was dissimilar. Investigated extracts of the strain IBK-549 cultivated on sunflower husk had in 2,3 times

higher optical density at 260–280 nm than at near ultraviolet light. And for samples of this strain grown on barley straw, the intensity of the maxima in these ranges is almost the same. Both other strains showed almost the same ratio of intensity of light absorption on both ranges.

Thus, as a result of the study, it was found that the addition of vegetable oils to the substrate, as precursors of the synthesis of flavor compounds by the *Pl. ostreatus* strains, promotes the formation of aroma compounds by mushrooms during solid phase cultivation.

The sensory profile analysis of dried samples of the fruit bodies obtained on substrates with the addition of sunflower and corn oils at concentrations of 1% and 5% showed an increase in the intensity of mushroom, meat and herbaceous notes of

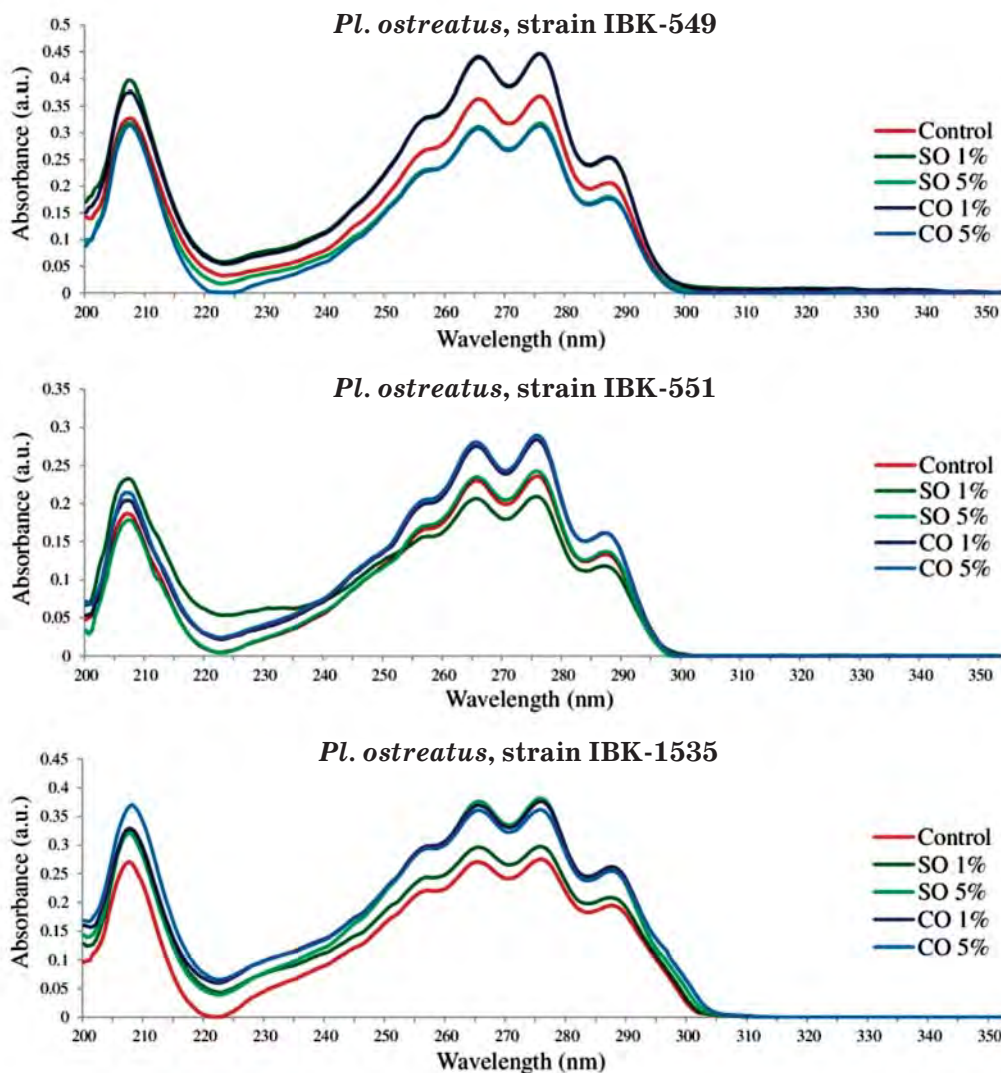


Fig. 8. UV spectra of hexane extracts of *Pleurotus ostreatus* strains: the substrate is barley straw

aroma. Spectrophotometric study of hexane extracts of dried fruit bodies of studied strains showed an increase in the intensity of light absorption of samples cultivated on substrates with the addition of vegetable oils compared to control.

The results of the study can be used in mushroom cultivation to increase the organoleptic quality of *Pl. ostreatus* fruit bodies through enriching the composition of lignocellulose substrates with vegetable oils.

REFERENCES

1. Kalac P. A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms. *J. Sci. Food Agric.* 2013, 93, 209–218. doi: 10.1002/jsfa.5960.
2. Fraatz M.A., Zorn H. Fungal flavours. Industrial applications. The mycota (A comprehensive treatise on fungi as experimental systems for basic and applied research). Hofrichter M. (eds). Springer, Berlin, Heidelberg. 2011, 10, 249–268. doi: 10.1007/978-3-642-11458-8_12.
3. Combet E., Henderson J., Eastwood D. C., Burton K. S. Eight-carbon volatiles in mushrooms and fungi: properties, analysis, and biosynthesis. *Mycoscience.* 2006, 47, 317–326. doi: 10.1007/S10267-006-0318-4.
4. Cho I. H., Namgung H.-J., Choi H.-K., Kim Y.-S. Volatiles and key odorants in the pileus and stipe of pine-mushroom (*Tricholoma matsutake* Sing.). *Food Chem.* 2008, 106, 71–76. doi: 10.1016/j.foodchem.2007.05.047.

5. Splivallo R., Ottonello S., Mello A., Karlovsky P. Truffle volatiles: from chemical ecology to aroma biosynthesis. *New Phytologist*. 2011, 189 (3), 688–699. doi: 10.1111/j.1469-8137.2010.03523.x.
6. Zeppa S., Gioacchini A. M., Guidi C., Guescini M., Pierleoni R., Zambonelli A., Stocchi V. Determination of specific volatile organic compounds synthesised during *Tuber borchii* fruit body development by solid-phase microextraction and gas chromatography-mass spectrometry. *Rapid Commun. Mass Spectrom.* 2004, 18, 199–205. doi: 10.1002/rcm.1313.
7. Wu C.-M., Wan Z. Volatile compounds in fresh and processed shiitake mushrooms (*Lentinus edodes* Sing.). *Food Sci. Technol. Res.* 2000, 6 (3), 166–170. doi: 10.3136/fstr.6.166.
8. Feussner I. Oxylipins in fungi. *FEBS J.* 2011, 278, 1047–1063. doi: 10.1111/j.1742-4658.2011.08027.x.
9. Senger T., Wichard T., Kunze S., Gobel C., Lerchl J., Pohnert G., Feussner I. A multifunctional lipoxygenase with fatty acid hydroperoxide cleaving activity from the moss *Phycomitrella patens*. *J. Biol. Chem.* 2005, 280 (9), 7588–7596. doi: 10.1074/jbc.M411738200.
10. Zheljaskov V. D., Vick B. A., Ebelhar M. W., Buehring N., Baldwin B. S., Astatkie T., Miller J. F. Yield, oil content, and composition of sunflower grown at multiple locations in Mississippi. *Agr. J.* 2008, 100 (3), 635–642. doi: 10.2134/agronj2007.0253.
11. Tymchuk D. S., Muzhylko V. V., Demchenko D. A. Content and fatty acid composition of oil in grain of corn endosperm mutants. *Visnyk Harkivskoho natsionalnoho ahrarnoho universytetu, serii biologii*. 2017, 2 (41), 85–91. (In Ukrainian).
12. DSTU 4492:2005 Sunflower oil. Specifications. Existing from 01. 01. 2007. *Kyiv: Derzhspozhyvstandart Ukrainy*. 2006, 22 p. (In Ukrainian).
13. DSTU GOST 8808:2003 Corn oil. Specifications. Existing from 01. 01. 2004. *Kyiv: Derzhspozhyvstandart Ukrainy*. 2003, 12 p. (In Ukrainian).
14. Bisko N. A., Lomberg M. L., Mytropolska N. Yu., Mykchaylova O. B. The IBK Mushroom Culture Collection. *Kyiv: Kholodny Institute of Botany of the National Academy of Sciences of Ukraine: Alterpres*. 2016, 120 p.
15. Buhalo A. S., Bis'ko N. A., Solomko E. F., Poedinok N. L., Mihajlova O. B. The cultivation of edible and medicinal mushrooms. *Kyiv: Chornobylinterinform*. 2004, 127 p. (In Russian).
16. Vlasenko K. M., Kuznetcova O. V. The use of sensory analysis in biotechnology of the cultivation of macromycetes. *Visn. Dnipropetr. Univ. Ser. Biol. Ekol.* 2016, 24 (2), 347–352. doi: 10.15421/011645. (In Ukrainian).
17. Atramentova L. O., Utevs'ka O. M. Statistics for biologists. *Harkiv: Vydavnytstvo "NTMT"*. 2014, 331 p. (In Ukrainian).
18. Tasaki Y., Toyama S., Kuribayashi T., Joh T. Molecular characterization of a lipoxygenase from the basidiomycetes mushroom *Pleurotus ostreatus*. *Biosci. Biotechnol. Biochem.* 2013, 77 (1), 38–45. doi: 10.1271/bbb.120484.
19. Gardner H. W. Recent investigations into the lipoxygenase pathway of plants. *Biochim. Biophys. Acta.* 1991, 1084, 221–239. doi: 10.1016/0005-2760(91)90063-N.
20. Reis F. S., Barros L., Martins A., Ferreira I. Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: an inter-species comparative study. *Food Chem. Toxicol.* 2012, 50 (2), 191–197. doi: 10.1016/j.fct.2011.10.056.
21. Wurzenberger M., Grosch W. The formation of 1-octen-3-ol from the 10-hydroperoxide isomer of linoleic acid by a hydroperoxide lyase in mushrooms (*Psalliota bispora*). *Biochim. Biophys. Acta.* 1984, 794 (1), 25–30. doi: 10.1016/0005-2760(84)90293-5.
22. Akakabe Y., Matsui K., Kajiwara T. Stereochemical correlation between 10-hydroperoxyoctadecadienoic acid and 1-octen-3-ol in *Lentinula edodes* and *Tricholoma matsutake* mushrooms. *Biosci. Biotechnol. Biochem.* 2005, 69 (8), 1539–1544. doi: 10.1271/bbb.69.1539.
23. Assaf S., Hadar Y., Dosoretz C. G. 1-Octen-3-ol and 13-hydroperoxylinoleate are products of distinct pathways in the oxidative breakdown of linoleic acid by *Pleurotus pulmonarius*. *Enz. Microb. Technol.* 1997, 21 (7), 484–490. doi: 10.1016/S0141-0229(97)00019-7.
24. Cheng A.-X., Lou Y.-G., Mao Y.-B., Lu S., Wang L.-J., Chen X.-Y. Plant terpenoids: biosynthesis and ecological functions. *J. Integr. Plant Biol.* 2007, 49 (2), 179–186. doi: 10.1111/j.1672-9072.2006.00395.x.
25. Heldt G.-V. Plant biochemistry. *Moskva: Binom. Laboratoriya znaniy*. 2011, 471 p. (In Russian).
26. Dudareva N., Klempien A., Muhlemann J. K., Kaplan I. Biosynthesis, function and metabolic engineering of plant volatile organic compounds. *New Phytologist*. 2013, 198, 16–32. doi: 10.1111/nph.12145.
27. Lee W.-J., Banavara D. S., Hughes J. E., Christiansen J. K., Steele J. L., Broadbent J. R., Rankin S. A. Role of cystathionine β -lyase in catabolism of amino acids to sulfur volatiles by genetic variants of *Lactobacillus helveticus* CNRZ 32. *Appl. Environm. Microbiol.* 2007, 73 (9), 3034–3039. doi: 10.1128/AEM.02290-06.
28. Liu Y., Lei X.-Y., Chen L.-F., Bian Y.-B., Yang H., Ibrahim S. A., Huang W. A novel cysteine desulfurase influencing organosulfur compounds in *Lentinula edodes*. *Sci. Rep.* 2015, 5, 10047. doi: 10.1038/srep10047.

29. Yasumoto K., Iwami K., Mitsuda H. Enzyme-catalyzed evolution of lenthionine from lenthionine acid. *Agricult. Biol. Chem.* 1971, 35 (13), 2070–2080. doi: 10.1080/00021369.1971.10860188.
30. Hu C., Zou Y., Zhao W. Effect of soybean oil on the production of mycelial biomass and pleurotulin in the shake-flask culture of *Pleurotus mutilis*. *World J. Microbiol. Biotechnol.* 2009, 25, 1705–1711. doi: 10.1007/s11274-009-0064-9.
31. Kalyoncu I. H., KaSik G., Ozcan M., Ozturk C. Effects of sesame and bitter almond seed oils on mycelium growth of *Agaricus bisporus* (Lange) Sing. *Grasas y Aceites.* 1999, 50 (5), 392–394.
32. Rodina T. G. Sensory analysis of food products. *Moskva: Akademiya.* 2004, 208 p. (In Russian).
33. Sil'vertejn R., Bassler G., Morril T. Spectrometric identification of organic compounds. *Moskva: Mir.* 1977, 590 p. (In Russian).
34. Vlasenko K. M., Kuznetcova O. V., Stepnevs'ka Ja. V. Influence of mineral substances on the synthesis of volatile organic compounds by *Pleurotus ostreatus* in the process of solid phase cultivation. *Regul. Mech. Biosyst.* 2017, 8 (4), 489–496. doi: 10.15421/021775. (In Ukrainian).

БІОСИНТЕЗ ЛЕТКИХ СПОЛУК ГРИБАМИ *Pleurotus ostreatus* (Jacq.:Fr.) Kumm. НА СУБСТРАТАХ, ЗБАГАЧЕНИХ РОСЛИННИМИ ОЛІЯМИ

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Метою дослідження було проведення аналізу можливих шляхів синтезу запашних сполук грибами *Pleurotus ostreatus* (Jacq.: Fr.) Kumm. за культивування на соняшниковому лушпинні та солomé ячменю з додаванням рослинних олій (соняшникової та кукурудзяної) як джерела ненасичених жирних кислот. Сенсорний профільний аналіз висушених зразків плодів тіл показав підвищення інтенсивності грибних, м'ясних та трав'янистих нот запаху на субстратах з додаванням рослинних олій у концентрації 1% і 5%. Для штаму ІВК-551 відзначено зростання інтенсивності солодких і квіткових складових запаху на обох субстратах із додаванням кукурудзяної олії. УФ-спектроскопія гексанових екстрактів висушених зразків плодів тіл виявила максимуми світлопоглинання у діапазоні 200–210 нм та 260–300 нм. Спостерігали неоднакове збільшення інтенсивності світлопоглинання зразків різних штамів, культивованих на субстратах із додаванням рослинних олій.

Ключові слова: *Pleurotus ostreatus*, леткі запашні сполуки, соняшникова олія, кукурудзяна олія, сенсорний профільний аналіз, УФ-спектроскопія.

БІОСИНТЕЗ ЛЕТУЧИХ СОЕДИНЕНІЙ ГРИБАМИ *Pleurotus ostreatus* (Jacq.:Fr.) Kumm. НА СУБСТРАТАХ, ОБОГАЩЕНИХ РАСТИТЕЛЬНИМИ МАСЛАМИ

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Целью исследования было проанализировать возможные пути синтеза летучих душистых соединений *Pleurotus ostreatus* (Jacq.: Fr.) Kumm. при культивировании на подсолнечной лузге и соломе ячменя с добавлением растительных масел (подсолнечного и кукурузного) в качестве источника ненасыщенных жирных кислот. Сенсорный профильный анализ высушенных образцов плодовых тел показал повышение интенсивности грибных, мясных и травянистых нот запаха на субстратах с добавлением растительных масел в концентрации 1% и 5%. Для штамма ІВК-551 отмечен рост интенсивности сладких и цветочных составляющих запаха на обоих субстратах с добавлением кукурузного масла. УФ-спектроскопия гексановых экстрактов высушенных образцов плодовых тел обнаружила максимумы светопоглощения в диапазоне 200–210 нм и 260–300 нм. Наблюдалось неодинаковое увеличение интенсивности светопоглощения образцов различных штаммов, культивируемых на субстратах с добавлением растительных масел.

Ключевые слова: *Pleurotus ostreatus*, летучие душистые соединения, подсолнечное масло, кукурузное масло, сенсорный профильный анализ, УФ-спектроскопия.