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SYNTHESIS OF AROMA COMPOUNDS BY *Pleurotus ostreatus* (Jacq.:Fr.) Kumm. CULTURED ON VARIOUS SUBSTRATES

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The aim of the study was to determine the intensity of synthesis of volatile aroma compounds by *Pleurotus ostreatus* (oyster mushroom) on sunflower husks and barley straw using sensory profile analysis and UV spectroscopy. The main cultural and morphological characteristics of the mycelial growth and development of fruiting bodies are determined: the period of mycelial development on the substrate, the time of primordial formation, the number of mushroom bunches per unit volume of substrate, the morphology of carpophores. Characteristic attributes of the aroma of dried fruiting bodies (mushroom, woody, sweet, herbaceous, fish, meat, floral, earthy, acidic, putrescent) are established and their aroma profiles are built. Sensory profile analysis of flavor of dried samples showed that the mushroom flavor of fungi cultivated on the sunflower husk is more pronounced than of those grown on barley straw. The light absorption maxima are recorded in the ranges 204–210 and 250–290 nm according to UV absorption spectra. Optimal conditions for extracting aromatics from dried fungi samples are the extraction time of 20–35 min at the boiling point of the solvent. Analysis of the UV spectra of fungal alcohol and hexane extracts showed that the intensity of the synthesis of volatile compounds is higher for strains cultivated on sunflower husks than for samples obtained on barley straw.

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

The quality of edible mushrooms depends on factors such as their flavor, taste, texture, and color. The flavor is indeed of the quality's most defining features [1].

Due to the large number of volatile components, the aroma profile is the so-called "business card" of a product and can be used to determine its organoleptic quality and authenticity [2]. Edible mushrooms are no exception. They have been used by humans since ancient times as food and fragrances due to their characteristic taste and aroma [3].

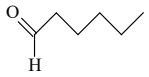
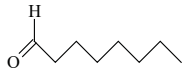
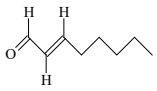
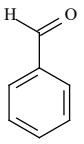
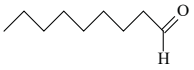
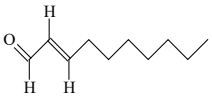
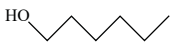
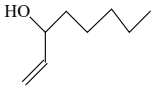
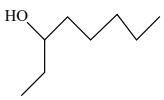
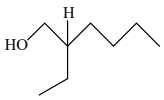
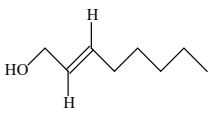
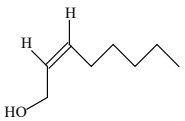
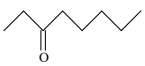
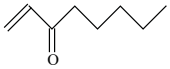
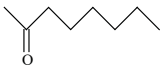
The key compounds determining the mushroom flavor are aliphatic organic substances (alcohols, aldehydes, ketones) characterized by 6 to 10 carbon atom numbers. Their concentration in the total fraction of aroma-causing substances can vary from 44.3 to 97.6% [4]. The profile of mushroom aroma varies by species and strain, and may also change under the influence of cultivation conditions. The content of volatile compounds also depends on the sampled part of the fruiting body. It is significantly different in the cap and stipe [1] and varies in different stages of maturity of the fruiting bodies [5].

To date, more than 200 different volatile organic compounds which determine the flavor of edible mushrooms have been identified [6]. Extracts of *Pleurotus ostreatus* contain 59.3% 1-octene-3-ol, 5.8% 3-octanol, 5.3% 3-octanone, 2.6% hexanal, 1.3% *n*-octanal and 1.2% (E)-2-octenal. The content of other components in extracts is negligible [7]. Other studies showed, per 100 g of sample, up to 4.34 mg of 3-octanol, 2.83 mg of 1-octene-3-ol, 2.57 mg of 3-octanone [8].

Table 1 presents the main characteristics of the most common aroma compounds of mushrooms [9].

There are many methods for extraction, concentration, and separation of volatile compounds. The main methods of extraction are hydrodistillation, organic solvent extraction,

Table 1. **The main aroma compounds of mushrooms**

Aroma compound	Structural formula	Physical and chemical properties	Characteristic aroma
	Aldehydes		
Hexanal ($C_6H_{12}O$)		Colorless liquid, well soluble in ethanol, ether, soluble in acetone, benzene, slightly soluble in water	Fresh herbal scent, apple, oily
n-Octanal ($C_8H_{16}O$)		Colorless or yellowish liquid, soluble in ethanol and other organic solvents, insoluble in water	Citrus, herbaceous, oily
Trans-2-octenal ($C_8H_{14}O$)		Yellowish liquid, soluble in ethanol and other organic solvents, insoluble in water	Fruit, herbaceous, spicy
Benzaldehyde (C_7H_6O)		Colorless or yellowish liquid soluble in ethanol, ether and other organic solvents, solubility in water 0.3%	Almond, cherry, malt, roasted peppers
Nonanal ($C_9H_{18}O$)		Colorless or yellowish liquid, soluble in ethanol and other organic solvents, insoluble in water	Fatty, floral, grassy, lemon
2-decanal ($C_{10}H_{18}O$)		Colorless, slightly oily liquid, soluble in ethanol, poorly soluble in water	Orange, fatty, fishy
	Alcohols		
1-hexanol ($C_6H_{14}O$)		Colorless liquid, soluble in ethanol and other organic solvents, solubility in water 5.9 g/l (20°C)	Herbaceous, floral
1-octene-3-ol ($C_8H_{16}O$)		Colorless liquid, soluble in ethanol, poorly soluble in water	Mushroom, earthy
3-octanol ($C_8H_{18}O$)		Colorless oily liquid, soluble in ethanol and ether, poorly soluble in water	Mushroom, oily, walnut, citrus
2-ethyl-1-hexanol ($C_8H_{18}O$)		Colorless liquid soluble in most organic solvents, solubility in water 0.07%	Rose, herbaceous
Trans-2-octene-1-ol (C_8H_{16O})		Colorless liquid insoluble in water	Mushroom
Cis-2-octene-1-ol ($C_8H_{16}O$)		Colorless liquid poorly soluble in water, soluble in most organic solvents and ethanol	Floral, herbaceous
	Ketones		
3-octanone ($C_8H_{16}O$)		Colorless transparent liquid, solubility in water 0.7 g/l (20°C)	Oily, herbaceous, moldy
1-octene-3-on ($C_8H_{14}O$)		Colorless transparent liquid, insoluble in water	Mushroom, earthy
2-octanone ($C_8H_{16}O$)		Colorless transparent liquid, solubility in water 0.9 g/l (20°C)	Oily, fatty

solid-phase microextraction, and headspace analysis. For separation, gas chromatography is most often used [10].

Qualitative and quantitative analyses of volatile organic compounds are carried out using gas chromatography with mass spectrometric identification, as well as spectroscopic methods such as UV, IF and NMR spectroscopy, and X-ray diffraction analysis [11].

UV spectroscopy is one of the instrumental methods of analysis that allows qualitative and quantitative identification of organic compounds containing chromophore groups (double bonds, carbonyl groups, etc.) [12]. These substances include mushroom volatile aroma compounds.

Organoleptic methods of analysis are used to assess the consumer characteristics of mushroom products. Sensory analysis is the most widespread, simple, accessible and sufficiently informative method of research [13].

The purpose of the study was to determine the intensity of the synthesis of volatile flavor compounds by *Pleurotus ostreatus* (the oyster mushroom) in the process of intensive cultivation using sensory profile analysis and UV spectroscopy.

Materials and Methods

Mushroom strains

The objects of the study were 3 strains of the edible *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. The 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. Preparation and sterilization of substrates were carried out according to commonly accepted methods [14]. The substrate was evaporated for 2 hours, $CaCO_3$ was added in an amount of 1% to the mass of the substrate and sterilized by twice autoclaving at 121 °C for 30 min at an interval of 24 hours. Straw was pre-minced to a size of 2–3 cm. The cooled substrate was inoculated with *P. ostreatus* mycelium. Seeding mycelium was obtained on barley grain. Cultivations were carried out at 26 ± 1 °C and 70–80% humidity to the full mycelial overgrowth of the substrate. Then the containers with the substrate were for 24 hours transferred to a growth room with a temperature of 15–16 °C, humidity of 80–90% and 8 hours of illumination. The 1st and 2nd flushes were harvested. Mushrooms were dried at 40–45 °C in a dry oven for 24–48 hours.

During the cultivation process, the following growth parameters of the *P. ostreatus* mycelium were determined: the time of the mycelia development on the substrate, the time of primordial formation, the number of formed bunches, and the yield of the first and second flushes. 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 by [15].

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.

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. A 1 g weight of raw material was placed in the extractor, then 100 cm^3 of solvent were added (the hydromodule was 1:100). Ethyl alcohol and hexane were used as polar and nonpolar solvents respectively. Extraction was carried out at boiling point of the solvent for 15, 30 and 45 min. 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 cm^3. 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. As a comparative solution, pure solvent was used.

The presence of α-amino acids in alcohol and hexane extracts of dried mushrooms was determined using a qualitative ninhydrin reaction [16].

The obtained data were processed statistically using dispersion analysis [17].

Results and Discussion

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

The growth parameters of *Pleurotus ostreatus* IBK-549, IBK-551 and IBK-1535 on different substrates are given in Table 2 (M ± m, $n = 5$).

By the time of overgrowing of the substrate by mycelium, the studied substrates did not differ much. The period of primordial emergence varied depending on the fungus strain from 17 to 26 days and did not differ by substrate variants. 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. According to the morphological features, *P. ostreatus* mycelia of all examined strains were white, fluffy and denser in sunflower husk. There were no differences in the morphology of the obtained fruiting bodies of different fungal strains. The *P. ostreatus* IBK-549 carpophores had long stipes, small caps of light gray-brown color and very soft flesh. *P. ostreatus* IBK-551 caps were bigger, gray-brown, stipes were shorter, the flesh was very dense and elastic. The caps of *P. ostreatus* IBK-1535 were yellow-brown, dense, fleshy, elastic and their stipes were long.

P. ostreatus fruiting bodies of different strains are presented in Fig. 1.

The examined strains had a significant difference by fruitage times. The fastest fruiting bodies appeared in the strain IBK-549, 4–6 days later in the strain IBK-551 and 6–10 days later in IBK-1535. However, the substrate type did not significantly affect the fruitage periods of these strains.

In the strain IBK-549, bunches formed on both substrates almost in the same numbers, while in the strains IBK-551 and IBK-1535, more bunches were observed on sunflower husk than on barley straw, in 1.5 and 2.1 times respectively.

Yield of the first flush was higher for all strains grown on sunflower husk: 1.6 times for IBK-549, 1.4 times for IBK-551, and 2 times for IBK-1535. Yields apparently decrease in the IBK-549 > IBK-551 > IBK-1535 row.

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 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.

The results of sensory analysis of dried samples of different strains of *P. ostreatus* are presented in circle plots at Fig. 2.

From the provided data it is evident that the aroma profile of the mushroom samples varied depending on the culture substrate. Mushroom and fish notes of flavor were most pronounced

Table 2. **The growth parameters of *Pleurotus ostreatus* IBK-549, IBK-551 and IBK-1535 at various substrates**

Substrate version/ fungal strain		Time of mycelial development on substrate, day	Time of primordial emergence, day	First flush, day	Number of mushroom bunches per substrate volume unit	Yield of the first flush, g	Yield of the second flush, g
Sunflower husk	549	6–7	18–20	26–32	12.00 ± 0.71	20.30 ± 2.41	3.03 ± 0.10
	551	6–7	20–24	30–36	13.40 ± 1.57*	15.09 ± 1.57	3.57 ± 0.20
	1535	6–7	22–26	32–36	20.40 ± 2.39*	14.70 ± 1.51*	4.69 ± 0.98
Barley straw	549	6	17–20	26–32	12.60 ± 1.96	12.40 ± 2.66	5.36 ± 1.57
	551	6–7	20–24	30–35	9.00 ± 1.00*	10.69 ± 5.98	5.98 ± 2.23
	1535	6	22–24	34–40	9.75 ± 1.52*	7.53 ± 0.43*	2.41 ± 0.62

Note: * — $P < 0.05$.

IBK-549 IBK-551 IBK-1535

Fig. 1. **Fruiting bodies of various strains of *Pleurotus ostreatus* (Jacq.:Fr.) Kumm.**

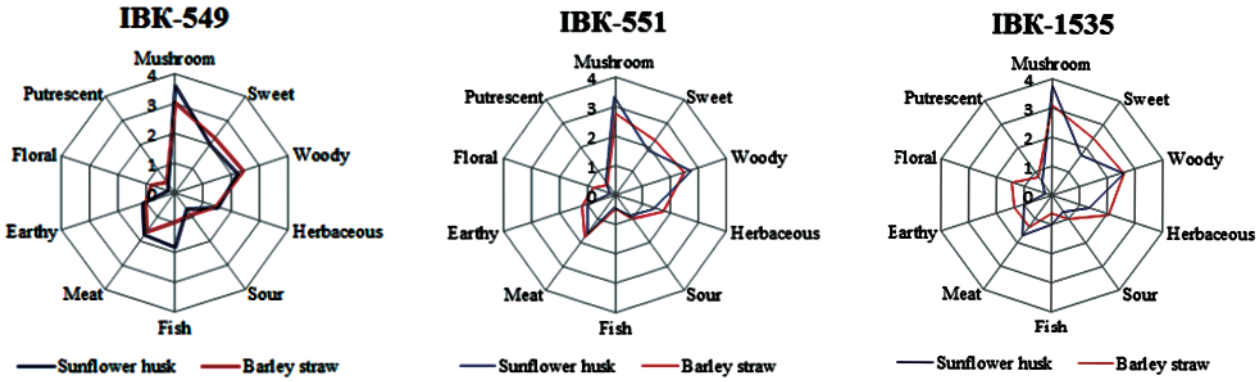


Fig. 2. **Sensory profile of aroma of dried samples of *Pleurotus ostreatus* strains**

in the fruiting bodies obtained on sunflower husk. This dependence has been noted for all strains. The aroma profiles of the strain IBK-551 on the two substrates were similar, but the intensity of the characteristic aroma profile was higher on husk. The intensity of floral, sweet and herbaceous notes was higher in samples obtained on straw. The aroma profiles of the IBK-549 and IBK-1535 strains cultivated on sunflower husk were inclined towards mushroom, fish and meat notes. The fruiting bodies of these strains cultured on barley straw showed more pronounced sweet, grassy, woody, earthy and floral notes. Putrescent and acidic odor components were poorly expressed in all mushroom samples.

Table 3 provides the statistically processed results of assessing the intensity of each characteristic for each dried mushroom sample ($M \pm \delta$, $n = 15$).

According to the sensory analysis method, the standard deviation characterizes the consistency of experts' assessments [13]. As can be seen from Table 3, the deviation does not exceed ± 1 point, indicating the statistical homogeneity of the set of expert assessments.

It was also found during the sensory analysis that fungi with a brown colored cap had a more pronounced mushroom aroma. But a detailed study of this fact will be carried out in further research.

Ultraviolet spectroscopy

As extractants we used different solvents, polar (ethyl alcohol) and nonpolar (hexane) to evaluate all groups of odorants.

The registered UV absorption spectra of hexane and alcohol mushroom extracts are presented in Figs. 3 and 4.

Alcohol extracts had light absorption maxima in the ranges of 204–210 nm and 267–269 nm. Hexane extracts showed the presence of the main maxima of light absorption at 202-205, 252, 262, 272, 282, and 292 nm. Such spectral properties are characteristic of solutions of unsaturated compounds with unbound double bonds, and saturated and unsaturated aldehydes and ketones [18].

The intensity of formation of volatile odorants by *P. ostreatus* strains decreased in the following sequence: IBK–549 > IBK–1535 > IBK–551.

Absorption maxima in the range of 200-220 nm for alcohol extracts are more intense than for hexane. However, the maxima of 260-295 nm for hexane extracts are more pronounced indicating a greater variety of aroma substances.

Table 3. **The intensity of flavor attributes of *Pleurotus ostreatus* IBK-549, IBK-551 and IBK-1535 depending on substrate type**

Substrate type/ fungal strain		Aroma attribute intensity, points									
		Mushroom	Sweet	Woody	Herbaceous	Acidic	Fish	Meat	Earthy	Floral	Putrescent
Sunflower husk	549	3.6±0.51*	2.0±0.65	2.2±0.41	1.5±0.52	0.7±0.49	1.8±0.77*	1.7±0.46	1.1±0.52	0.3±0.46*	0.4±0.51
	551	3.3±0.62	1.9±0.59	2.7±0.46	1.4±0.74	0.9±0.74	0.4±0.51	1.6±0.51	1.0±0.65	0.2±0.41*	0.5±0.52
	1535	3.8±0.68*	1.7±0.80*	2.6±0.51	1.3±0.49*	0.7±0.49	1.0±0.65	1.7±0.49	1.0±0.65	0.3±0.46*	0.6±0.63
Barley straw	549	3.1±0.59*	2.3±0.59	2.4±0.51	1.5±0.52	0.9±0.52	0.9±0.70*	1.6±0.51	1.1±0.59	0.9±0.35*	0.5±0.52
	551	2.8±0.77	2.3±0.62	2.5±0.52	1.7±0.46	1.0±0.53	0.5±0.52	1.7±0.70	1.2±0.77	0.8±0.41*	0.5±0.52
	1535	3.1±0.52*	2.5±0.52*	2.6±0.51	2.1±0.59*	1.0±0.53	0.6±0.51	1.3±0.49	1.3±0.49	1.5±0.52*	0.8±0.41

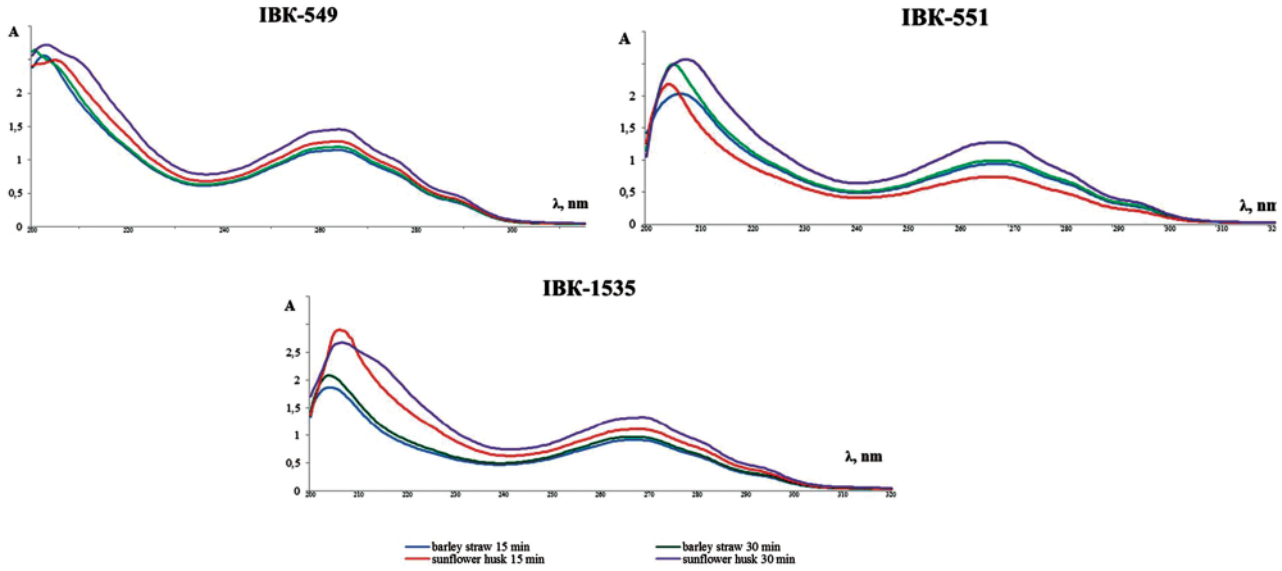


Fig. 3. **UV spectra of alcohol extracts of *Pleurotus ostreatus* strains**

The qualitative ninhydrin reaction showed the presence of α-amino acids in alcohol extracts, which testifies to the ability of ethyl alcohol to extract not only volatile organic substances from the fungal fruiting bodies, but also a complex of other biologically active substances such as α-amino acids. In hexane extracts of fungal fruiting bodies, α–amino acids were not detected.

It is known that α–amino acids have light absorption maxima in the same range as the flavor compounds of *Pleurotus ostreatus*: 205–220 nm and 240–280 nm [17]. Thus, the presence of amino acids in alcohol mushroom extracts does not allow quantifying the content of all groups of aroma substances due to the overlap of their light absorption maxima. Though, the conducted studies allow us to conclude that it is expedient to use ethyl alcohol as a universal extractant for the removal of all groups of biologically active substances from the raw mushroom material.

Hexane is a more selective solvent of volatile organic compounds, as evidenced by the maxima of light absorption at λ_{max} = 262, 270, 280, 295 nm, and the absence of amino acids in the extract confirmed by qualitative reactions.

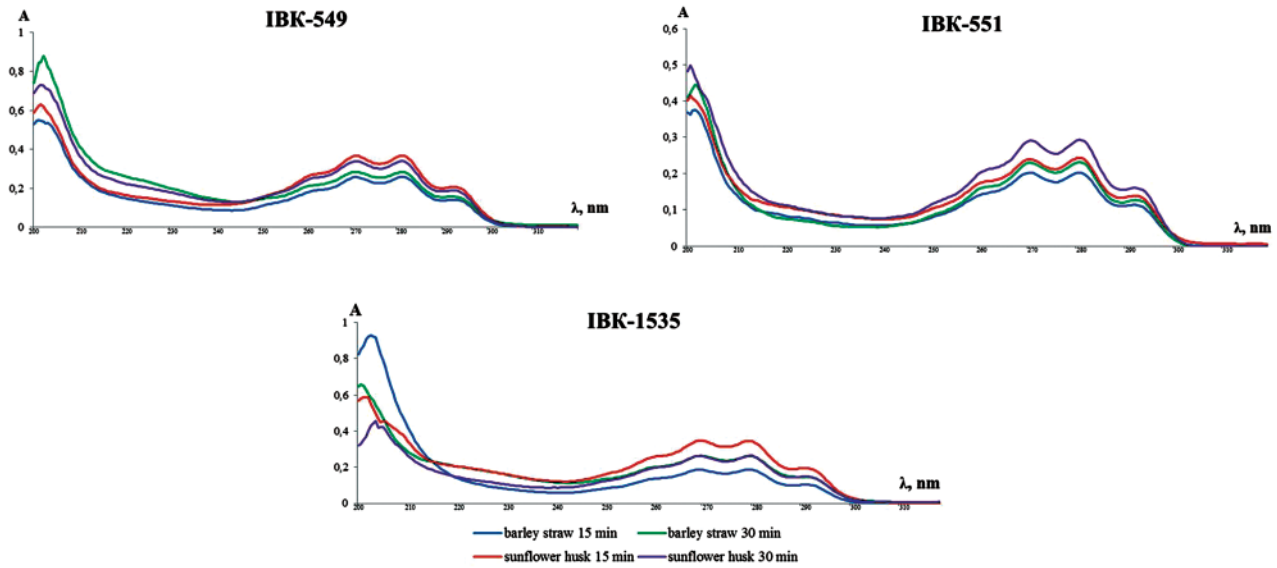


Fig. 4. **UV spectra of hexane extracts of *Pleurotus ostreatus* strains**

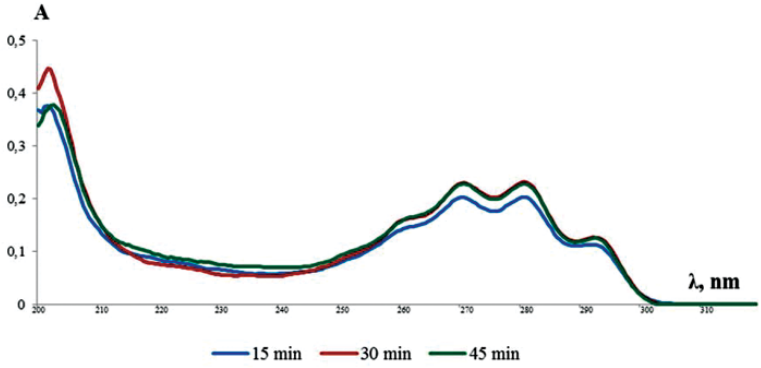


Fig. 5. **UV spectra of hexane extractions of *Pleurotus ostreatus* IBK-551 strains with different extraction times** (substrate is barley straw)

Thus, for the maximum extraction of all groups of biologically active compounds from the fungal raw material, ethyl alcohol may be used as an extractant, and for selective extraction of only odorous substances, non-polar solvents such as hexane are recommended.

The extraction time for the release of volatile compounds that are also susceptible of decomposition and temperature isomerization, is also an important factor to be studied. Therefore, we researched the conditions for the extraction of aromatic substances from raw *Pleurotus ostreatus* mushrooms. Fig. 5 shows the UV spectra of hexane extracts of *P. ostreatus* strain IBK-551 cultured on barley straw. The extraction time was 15, 30 and 45 min.

It was established that with an increase in the extraction time from 15 to 30 min, the intensity of the maxima of light absorption did not significantly increase. This indicates the inexpediency of increasing the extraction time from 30 to 45 min. A similar dependence was observed for both solvents and all investigated *P. ostreatus* strains.

The study of culture and morphological parameters of the growth of mycelium and fruiting bodies of *Pleurotus ostreatus* (strains IBK-549, IBK-551 and IBK-1535) on two substrate types revealed slight differences in growth and yield characteristics depending on the substrate, and a certain variation of the morphological characteristics of growth and development depending on the fungal strain.

The sensory profile analysis of the flavor of dried samples showed that mushrooms cultivated on sunflower husk have a more pronounced mushroom aroma than those grown on barley straw. The obtained results correlate with the spectrophotometric analysis of alcohol and hexane mushroom extracts and confirm the fact that the intensity of the synthesis of volatile compounds is higher for all strains grown on sunflower husk than for the strains obtained on barley straw.

It is also established that for selective extraction of volatile organic compounds, the best solvent is hexane. Selected optimal extraction conditions are extraction time 20–35 min at the boiling point of the solvent.

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СИНТЕЗ ЗАПАШНИХ СПОЛУК *Pleurotus ostreatus* (Jacq.:Fr.) Kumm. ЗА КУЛЬТИВУВАННЯ НА РІЗНИХ СУБСТРАТАХ

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Метою дослідження було визначення за допомогою сенсорного профільного аналізу та УФ-спектроскопії інтенсивності синтезу летких запашних сполук грибами *Pleurotus ostreatus* (глива звичайна) у процесі культивування на соняшниковому лушпинні та соломі ячменю.

Визначено основні культурально-морфологічні характеристики росту міцелію та розвитку плодових тіл: термін освоєння субстрату міцелієм, час появи примордіїв, кількість зростків на одиницю об'єму субстрату, морфологія карпофорів. Встановлено характерні атрибути аромату висушених плодових тіл (грибний, деревний, солодкий, трав'янистий, рибний, м'ясний, квітковий, земляний, кислий, гнильний) та побудовано їхні профілі аромату. Сенсорний профільний аналіз запаху висушених зразків показав, що гриби, культивовані на соняшниковому лушпинні, мали більш виражений грибний аромат, ніж ті, що їх вирощено на соломі ячменю. За допомогою УФ-спектроскопії зареєстровано максимуми світлопоглинання у діапазонах 204–210 та 250–290 нм. Підібрано оптимальні умови екстрагування ароматоутворювальних сполук із висушених зразків грибів — час екстракції 20–35 хв за температури кипіння розчинника. Аналіз УФ-спектрів спиртових та гексанових екстрактів грибів показав, що інтенсивність синтезу летких ароматних сполук вище для штамів, які культивували на соняшниковому лушпинні, ніж для зразків, отриманих на соломі ячменю.

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

СИНТЕЗ ДУШИСТЫХ СОЕДИНЕНИЙ *Pleurotus ostreatus* (Jacq.:Fr.) Kumm. ПРИ КУЛЬТИВИРОВАНИИ НА РАЗЛИЧНЫХ СУБСТРАТАХ

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Целью исследования было определение с помощью сенсорного профильного анализа и УФ-спектроскопии интенсивности синтеза летучих душистых соединений грибами *Pleurotus ostreatus* (вешенка обыкновенная) в процессе культивирования на подсолнечной лузге и соломе ячменя.

Определены основные культурально-морфологические характеристики роста мицелия и развития плодовых тел: срок освоения субстрата мицелием, время появления примордиев, количество сростков на единицу объёма субстрата, морфология карпофоров. Установлены характерные атрибуты аромата высушенных плодовых тел (грибной, древесный, сладкий, травянистый, рыбный, мясной, цветочный, земляной, кислый, гнилостный) и построены их профили аромата. Сенсорный профильный анализ запаха высушенных образцов показал, что грибы, культивируемые на подсолнечной лузге, имели более выраженный грибной аромат, чем те, которые выращены на соломе ячменя. С помощью УФ-спектроскопии зарегистрированы максимумы светопоглощения в диапазонах 204–210 и 250–290 нм. Подобраны оптимальные условия экстрагирования ароматобразующих соединений из высушенных образцов грибов — время экстракции 20–35 мин при температуре кипения растворителя. Анализ УФ-спектров спиртовых и гексановых экстрактов грибов показал, что интенсивность синтеза летучих душистых веществ выше для штаммов, которые культивировали на подсолнечной лузге, чем для образцов, полученных на соломе ячменя.

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