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STUDY OF AROMATIC COMPONENTS IN THE COURSE OF INITIATING ENZYMATIC REACTIONS IN THE EDIBLE MUSHROOM *PLEUROTUS OSTREATUS*

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Abstract. Various branches of the food and medical industries widely use mushrooms, that is why their aromatic characteristics are quite important. This paper presents a comparative analysis of the aromatic and fatty acid composition of the mycelium of the edible oyster mushroom (*Pleurotus ostreatus* (Jacq.) P. Kumm., strain K-17) cultured on a glucose-peptone-yeast growth medium with wheat bran added. The quantitative and qualitative composition of the aromas has shown that wheat bran components affect the aroma-forming reactions during the growth of the mycelium of *P. ostreatus*. It has been established that the introduction of wheat bran increases the content of 1-octen-3-ol, the main fungal aromatic component. In the culture liquid, 1-octen-3-ol increases by 1.4 times, as compared with classic samples cultured on a growth medium without wheat bran. Also, in the mycelium cultured on a wheat bran-containing growth medium, the total quantity of identified aromatic components increases by 1.7 times. In this mycelium, the two main components of the mushroom's aroma are formed: 1-octen-3-ol and hexanal. In other samples, these important components are absent. The results of this study confirm that the formation of the two main fungal aromatic components, 1-octen-3-ol and hexanal, involves polyunsaturated fatty acids, namely linoleic acid. Its content decreased in parallel with the accumulation of aromatic components throughout culturing in the wheat bran-containing medium. The data obtained allow establishing how initiation of enzymatic oxidative reactions changes the aroma of *P. ostreatus* during surface culturing on a liquid medium. The research results reveal the regular patterns in the formation of aromatic components of macromycetes from lipid precursors. Addition of wheat bran to a growth medium unbalances the total content of aromatic components towards their accumulation in the mycelium. Since fruiting bodies and vegetative mycelium are similar in their biochemical composition, the results of studying the mycelium cultured on a liquid medium allow predicting how aromatic components will be formed in the fruiting bodies of oyster mushrooms.

Key words: mycelium, culture liquid, aromatic components, wheat bran, peroxidase, enzymatic reactions.

WB – wheat bran, CL – culture liquid, GPY – glucose-peptone-yeast medium, PUFA – polyunsaturated fatty acids

Introduction. Formulation of the problem

Synthesis and formation of aroma volatiles in mushrooms is a brunch of experimental science still involving a large body of research. Throughout decades, researchers have considered various additives to mushrooms under culturing, both to study the specific conditions of the aroma-forming process and to control it [1,2]. Studies of the aroma of mushrooms focused, generally, on their fruiting bodies [3-5]. Usually, researchers compared the chemical compositions of fruiting bodies and mycelium. However, the content of aromatic components in them has been the object of comparison far less frequently [6].

Aromatic components are secondary metabolites of plants and mushrooms [7]. Secondary metabolites

can accumulate not only in the mycelium, but also in the medium where they are cultured. Thus, studies of aroma formation under stationary conditions are more informative when mushrooms are cultured on liquid media, as compared with other methods. After the mycelium is separated, the culture liquid contains bioactive substances, in particular, aromatic components. So, recent research has made it possible to start using culture liquids as aromatising agents in beer and bread as well as nutritive supplements [8,9], and mycelium has found its application in sausage manufacture [10]. Thus, a topical, though understudied problem is establishing how aromatic components are finally distributed in the culture liquid and mycelium after the culturing process is completed. Formation of aromatic components should be studied in connection with the role of aroma precursors, the changes in them, and their reactions [11].

Analysis of recent research and publications

The paper [12] pays much attention to the lipoxygenase pathway of aroma formation resulting in genuine fungal aromatic components. The lipoxygenase pathway is based on the process of oxidation and depends on the activity of certain enzymes. These can be oxidative enzymes, usually lipoxygenases. However, oxidation processes involve peroxidases, too, though their role in the lipoxygenase pathway is still not wholly certain. Peroxidase is an inducible enzyme, which can be induced by physical, chemical, and biological factors [13, 14]. A. Kapich shows in his study that the manganese peroxidase (MnP) of mushrooms has not only an antioxidant effect, but pro-oxidant, too. This enzyme initiates peroxidation of polyunsaturated fatty acids (PUFA) [15]. The specific feature of oxidation of PUFA with an active MnP is more rapid oxidation of linoleic acid, as compared with less saturated fatty acids (linolenic, arachidonic) [16].

By now, quite a limited set of classes of substrates for peroxidases has been established [17]. It has been confirmed, though, that phenols can be a substrate for peroxidases [18]. Phenolic antioxidants (ArOH) are known to interact effectively with hydroperoxyl radicals of fatty acids and of unsaturated lipids in the reaction [19]:

$$ArOH + RO_2 \bullet \rightarrow ArO \bullet + ROOH$$

Interreaction of peroxidase with a phenolic substrate can cause indirect (chemical) oxidation of another substrate. While forming particles $A^{\bullet}$, phenolic substrates also produce $B^{\bullet}$ and H_2O_2, which can take part in the subsequent oxidation reactions. That is why the role of peroxidases in aroma formation through oxidising PUFA has not been duly considered [19]. Wheat bran is high in phenolic compounds, especially in cinnamic acid derivatives and alkylresorcinol [20,21].

Alkylresorcinols are a natural homologous series of phenolic lipids. The antioxidant activity of certain alkylresorcinol homologues in oils and oil-in-water emulsions was considered in the research [22]. Besides, it was established that specifically smelling acetic acid was a secondary metabolite of alkylresorcinol [23]. So, the contribution of wheat bran to aroma-forming processes is quite forecastable but needs further verification. K. Vlasenko considered wheat bran as an aroma-forming additive in solid-state cultivation [1]. The possible effect of this additive on aroma formation was studied in fruiting bodies. It should be noted that with solid-state cultivation, no significant effect of wheat bran on the aroma of *P. ostreatus* fruiting bodies was reported. Liquid-state culturing of the mycelia of basidiomycetes involves no expense on extraction of aromatic substances produced in liquid media, they can be concentrated or transferred onto a dry carrying agent, which is an advantage. That is why this research focuses on the formation of aromatic components and their distribution while culturing the mycelium of *P. ostreatus*. This will allow using the culture liquid as a mushroom aroma agent.

The **purpose of the research** was studying how the aroma of *P. ostreatus* cultured on growth media of various compositions changed in the resulting products of culturing (culture liquid and mycelium) after initiating lipid peroxidation reactions.

The research objectives were:

1. Studying the concentrations of aromatic components in the culture liquid and mycelium after culturing on liquid media of different compositions.
2. Comparing and analysing the fatty acid composition of mycelium grown on media with and without wheat bran added.
3. Describing the distribution of the aromatic components in the culture liquid and mycelium of *Pleurotus ostreatus* on completion of the culturing process.

Research materials and methods

The *raw material* was a pure culture of the edible mushroom *Pleurotus ostreatus*, strain K-17. It was cultured under stationary conditions on a liquid glucose-peptone-yeast (GPY) growth medium, g/l: glucose – 25.0; peptone – 3.0; yeast extract – 2.0; KH_2PO_4 – 1.0; K_2HPO_4 – 1.0; $MgSO_4 \cdot 7H_2O$ – 0.25, pH 6.5. The basic composition of the GPY medium was supplemented with 20 g/l of wheat bran (GPY+WB), then boiled, filtered, and bottled in culture flasks. All the media were prepared and sterilised by the conventional method.

The material under study was placed in one-litre culture flasks containing 150 ml of a growth medium. The inoculum was prepared using 7-day fungal cultures that had been grown on GPY media at 26±0.1 °C. Into each flask with the liquid medium GPY, five mycelial discs (d=6 mm) were placed. The cultures were incubated under stationary conditions at the incubation temperature 26±0.1 °C. The mycelium was cultured for 14 days. On completion of the culturing, the mycelium biomass was

filtered, and the final pH of the culture liquid was measured using indicator test kits.

The *wheat bran* was provided by the company *Farmakom* (Kharkiv, Ukraine).

Preparation of the samples:

– *aqueous extract of wheat bran* was prepared by the technology equivalent to that of wheat bran-containing GPY: 1 litre of distilled water was added to 20 g of wheat bran, followed by boiling and filtering;

– *culture liquid and mycelium grown on a GPY medium*: after culturing on a GPY medium, the mycelium biomass was separated from the culture liquid by filtering;

– *culture liquid and mycelium grown on a GPY medium, with wheat bran added (GPY+WB)*: after culturing on a wheat bran-containing GPY medium, the mycelium biomass was separated from the culture liquid by filtering.

To change the pH of the samples, hydrochloric acid 2 N (up to 2.5–3.5) and sodium bicarbonate 2 N (up to 7.3–7.8) were used.

The *transmission spectra and absorbance* of alcohol solutions of the samples were studied by means of, respectively, a spectrophotometer UV1280 (Shimadzy, Japan) at the wavelength 200–900 nm and the photometer KFK-3 at the wavelength 620 nm.

To determine the concentration and identify the aromatic components of the mycelium and culture liquid, we employed headspace gas chromatography using a chromatographer *Kristallyuks*-4000M (from the research and production company Meta-chrom). The chromatography column used was ZB-WAX (l=30 m, d=0.32 mm, film thickness 0.5 μm) with the sorbent 100% polyethylene glycol (Phenomenex, USA). The starting temperature of the column was 40°C for 5 min. Then it was increased to 140°C by 4°C/min. The temperature of the flame ionisation detector (FID) was 250°C, and that of the vaporiser was 200°C. The pressure on the capillary column was optimised within 0.5–0.8 atm, and the expenditure of the carrier gas (nitrogen), respectively, from 1.5 to 2.9 ml/min, for better separation of a sample's components. The chromatograms were recorded and processed using the software NetChrom v2.1. The volatile compounds were identified according to the retention time and indices, comparing the parameters of each peak of a sample with those of the peaks of analytical standards (98.0–99.8%, for chromatography): hexanal, heptane, 1-octen-3-ol, dimethyl trisulphide, 2-pentylfuran, acetic acid, etc.

Fatty acid composition. The fatty acid composition of the mycelium and culture liquid was analysed in compliance with State Standard of Ukraine (DSTU) ISO 5508-2001 "Fats and oils of animal and plant origin. Analysis of fatty acid methyl esters by the method of gas chromatography." Methyl esters of fatty acids were studied using a gas chromatographer 7890 B (Agilent Technologies, USA). The samples were prepared according to DSTU ISO 661:2004 "Fats of animal and plant origin and oils. Preparation of a test sample" and DSTU ISO 5509-2002 "Fats of animal and plant origin and oils. Preparation of fatty acid methyl esters."

Statistical treatment. In this study, the measurements were made in triplicate. All the data on the samples of the mycelium and culture liquid were presented as a mean value (n=3) and processed using analysis of variance. For the statistical analysis, the statistical software TableCurve 3D was used. The double graph was analysed to reveal and classify the relationship between the samples of mycelium or culture liquid (with or without wheat bran) on the basis of the profiles of their aromatic components.

Results and their discussion

Basidiomycetes are known to have a strong exoenzymatic system [24]. According to some studies, mycelium extract and culture liquid of submerged-cultivated *P. ostreatus* contain a complex of quite active enzymes that can coagulate milk and form cheese curd like rennins do [25]. Macromycetes are viewed as a new source of peroxidase to be used industrially, besides horseradish roots. When mycelium grows, the activity and quantity of enzymes increase.

Besides peroxidases, the complex of oxidases found in *P. ostreatus* includes laccases (EC 1.10.3.2, *p*-diphenol oxidase), which oxidise phenols [26]. In higher basidiomycetes (in particular, *P. ostreatus*), the activity of peroxidases and its changes caused by the action of coherent and incoherent light were investigated in N. Poyedinok's research work [27].

An increase in the activity of any enzyme is known to cause a decrease in the quantities of the respective components of the substrate in the test samples. Considering WB as a source of phenols and alkylresorcinols for the action of peroxidases of *P. ostreatus*, we should expect that after enzymolysis, phenolic substances will be present in lower concentrations or totally absent in the medium under study. The presence of phenolic substances is vividly demonstrated by a qualitative reaction to a change in the pH medium. In an acidic medium, a solution containing phenolic compounds becomes lighter in colour, and in alkaline, it darkens. This corresponds to different values of the light transmission coefficient or those of absorbance (optical density) during the changes in the pH medium. On the other hand, if the samples have the same light absorption coefficients before and after the pH changes, this can indicate the absence of phenolic components. The changing concentrations of phenolic substances in a liquid medium give a vivid picture of the process of enzymolysis with peroxidases while culturing *P. ostreatus* (Table 1).

Table 1 – Qualitative reactions to the content of phenolic components in the culture liquid

No.	Samples	Absorbance (λ = 620 nm)		
		pH 2.5–3.5	pH before the changes	pH 7.5–8.0
1	Aqueous extract of wheat bran before culturing (pH 6.5)	0.17	0.50	0.74
2	Culture liquid with wheat bran after culturing (pH 6.5)	0.15	0.26	0.26

As can be seen from Table 1, the qualitative reaction to the phenolic content in the wheat bran extract before culturing showed that the absorbance had changed, on average, by 2.2 times in the samples with the acidic and alkaline media. This means that there had been qualitative reactions to the presence of phenolic substances in the aqueous extract of wheat bran before culturing. When this extract was analysed after culturing in the culture liquid composition, it was found that in the alkaline medium, the absorbance had remained unchanged, and in the acidic one, it had decreased by 1.7 times. This means that on completion of the culturing, the qualitative reaction revealed no phenolic substances of wheat bran in the growth medium in the alkaline range. In addition, the light transmission coefficient of the alcoholic solution of the culture liquid (Fig. 1) was determined after culturing on the growth medium containing wheat bran (GPY+WB) and without it (GPY).

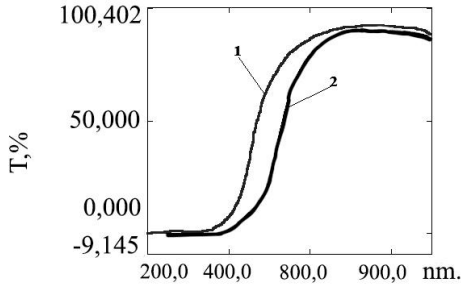

Fig. 1. UV transmission spectra (T, %) of the alcoholic solution of the culture liquid: GPY *(1)*; GPY+WB *(2)*

Analysis of the UV spectra has shown identical responses for alcoholic solutions of the culture liquid, both with and without wheat bran, at the wavelengths 200–900 nm. Wheat bran extract before culturing contains a mixture of various representatives of the classes of phenolic compounds. The sample of the culture liquid with wheat bran extract did not have distinctive shifts in the light transmission coefficient (T, %). This is another proof that the culture liquid samples contained no phenolic compounds after 14 days' culturing. It should be noted that after culturing with wheat bran added, the culture liquid is darker-coloured. In the interval 380–700 nm (visible spectrum), the difference in the response is 60–70 nm for the samples GPY and GPY+WB, which is characteristic of the presence of colouring pigments. Their accumulation can result from products that appear due to the Maillard reaction or the breakdown of carbon and nitrogen sources after autoclaving.

Thus, after culturing, phenolic compounds are identified neither by the qualitative reaction nor by the response of the UV spectra. The difference in the reactions of phenolic compounds in the culture liquid before and after culturing indicates that there are reactions where they take part. The reactions like the ones mentioned above are enzymatic reactions with the enzymes of *P. ostreatus* (peroxidases, laccases, lipoxygenases).

In the course of enzymatic aroma formation, the complex of oxidative enzymes takes part in the formation of carbonyl compounds from the PUFA of the medium under study, and this process follows a certain pattern (Fig. 2). Depending on what carbon atom of PUFA was involved in the oxidation reaction, formation of hydroperoxides takes place first, and then aromatic components of different chemical nature are formed from them. Aromatic components are final products of these oxidation reactions.

Wheat bran extract contains a mixture of various classes of phenolic compounds.

Lipid oxidation products are involved in the formation of the volatile composition of the aromas of roast fowl, potatoes, dry breakfast cereals, a great many cheeses. For example, of the 193 components that form the roast chicken aroma, 41 are aldehydes obtained by lipid oxidation, the predominant ones being hexanal and 2,4-decadienal [28]. The type of lipid decomposition products depends on the medium's fatty acid composition, and lipid oxidation has different effects on the sensory qualities [29].

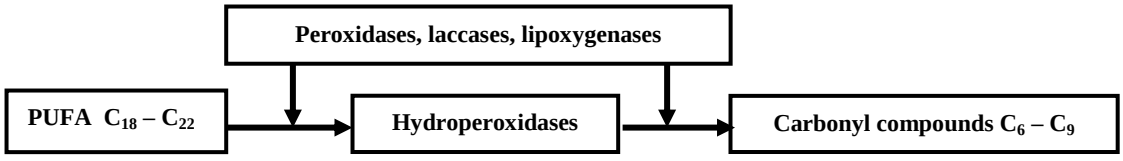

Fig. 2. Pattern of enzymatic transformation of PUFA by oxidative enzymes of *P. ostreatus* mushrooms

For example, oxidation of vegetable oils containing mainly ω-6 polyunsaturated fatty acids with a double bond between the sixth and seventh carbon atom (from the methyl end of the fatty acid chain), adds an off-odour of grass or beans to the product. And oxidation of high-molecular-weight ω-3 polyunsaturated fatty acids with a double bond at the third carbon atom makes the product smell of fish [19]. Interaction of lipids and oxidants is of a free-radical character, has three stages, and is described quite fully for many products containing lipids (vegetable oils and fish products) [29]. However, applying these data to mushroom mycelium is limited by the fact that lipids are dispersed as discontinuous phases throughout the heterogeneous matrix structure. That is why the nature of the formation of aromatic components in the fungal fruiting bodies or mycelia is studied in relation to the activity of enzymes that can oxidise PUFA.

Phenolic substances extracted from WB can activate the peroxidases of *P. ostreatus* and thus initiate lipid oxidation processes. Peroxidases are characterised by specific activity that can substantially increase as the concentration of substrates increases. The comparison of the aromatic profiles of *P. ostreatus* mycelium cultured on a liquid medium with (1) and without (2) wheat bran has shown that in the former case, there was an increase in the quantity of aromatic components (Fig. 3).

The changes in the profile of aromatic components of the GPY+WB-cultured mycelium (as compared with the GPY-grown one) result from different formation of the final products of PUFA oxidation reactions. This can be seen from the findings presented in Table 2. Enzymes break hydroperoxides with 12 and more carbons down to volatile compounds with 6–7 carbons according to the pattern shown in Fig. 2.

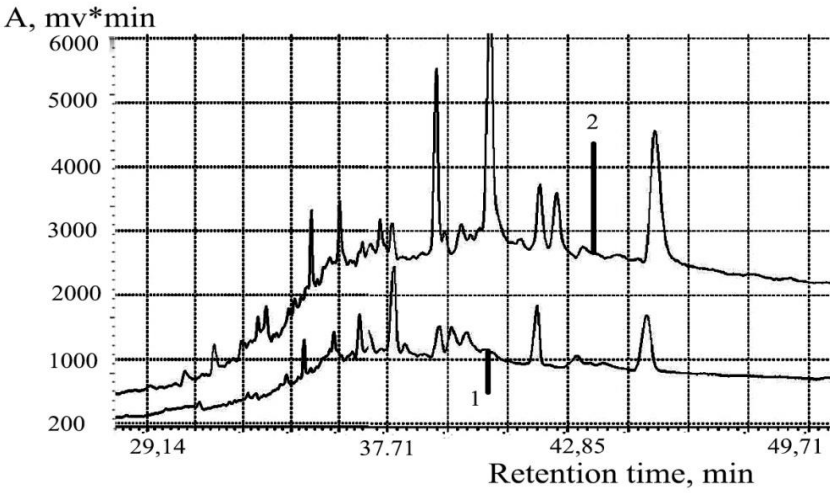

Fig. 3. Fragment of the chromatogram of aromatic components of *P. ostreatus* mycelium: GPY *(1)*; GPY+WB *(2)*

The culture liquid (CL) becomes of practical importance as the place where secondary metabolites accumulate. The CL is a transparent fluid with an intense aroma, the smell of mushrooms being its predominant component. The aroma of the grown mycelium is less intense than that of the culture liquid. The number of aromas identified in the CL and mycelium is different in the samples with and without wheat bran added (Table 2).

Analysis of the aromatic components identified has shown that there are two times as much of them in the GPY+WB mycelium as in the CL. However, as for 1-octen-3-ol (the main component responsible for the mushroom odour), its quantity in the CL is larger by 1.78 times than in the mycelium and by 1.4 times than in the CL without WB. In the mycelium grown on the WB-containing medium, the total quantity of the identified aromatic components is larger by 1.7 times. The concentration of aromatic components in the CL without WB is twice as high as in the CL with it. This means that with no enzymatic reactions initiated, the culture liquid accumulates a lot of aromatic components.

The identified secondary metabolites of the PUFA of *P. ostreatus* include C_7–C_9 aldehydes. The double bond of α and β-unsaturated aldehydes is always in a stable trans configuration, and for unbound aldehydes, cis configuration is natural. During oxidation, this cis-double bond easily isomerises to a trans-double bond in combination with a carbonyl group. These positional and geometric isomerisations of double bonds have a pronounced effect on both quantitative and qualitative aromatic reactions. That is why, despite the different quantitative and qualitative compositions of aromatic components, the sensory perception of the aroma intensity level was the same in both samples, GPY+WB *(1)* and GPY *(2)*. The comparative analysis of the peaks of the aromatic components of the CL with wheat bran and without it has shown that the main peaks are identical (Fig. 4).

Table 2 – Concentrations of aromatic components in the mycelium and culture liquid, μg/ml

No.	Component	SAMPLES			
		Culture liquid		Mycelium	
		GPY+WB	GPY	GPY+WB	GPY
1	volatile compounds C_6	1.890	1.652	22.47	20.4
2	volatile compounds C_7	1.435	0.6262	2.750	0.419
3	acetic acid	7.614	17.87	10.33	0.266
4	hexanal	0.00177	0.0101	0.00138	0
5	2-pentylfuran	0.00385	0.0491	0.001	0.0643
6	1-octen-3-ol	0.0141	0.0100	0.007967	0
7	octanol	0.01274	0.0245	–	–
8	styrene	0.009779	0	0	0.005526
	Total	10.97	20.25	35.56	21.15

The change of the aroma in the CL and mycelium of *P. ostreatus* indicates that the components of WB affect the distribution and accumulation of aromatic components in the GPY medium during stationary culturing on liquid growth media. When enzymatic processes are initiated by adding WB, the quantity of aromatic components accumulated in the mycelium is by 3.5 times larger (Table 2).

Fragments of chromatograms (Fig. 3, Fig. 4) show the degree of difference between the samples of mycelium and culture liquid with and without the addition of wheat bran. It can be stated that the degree of difference in the mycelium is much greater than in the culture liquid.

In the GPY growth medium (where initiation of enzymatic oxidation processes is impossible), the total contents of aromatic components in the mycelium and CL are, respectively, 21.15 μg/ml and 20.25 μg/ml, and they are distributed between these two locations in the ratio 50:50 (Table 2). Adding WB unbalances the total content of aromatic components towards their accumulation in the mycelium. Since fruiting bodies and vegetative mycelium are similar in their biochemical composition, the results of studying the mycelium cultured on a liquid medium allow predicting the aroma of the fruiting bodies of mushrooms. In general, mycelium and the CL contain a complex of identical aromatic substances and have similar chromatographic profiles, but the content of mushroom aroma components increases in the WB-containing samples.

The distribution of acetic acid (a possible secondary metabolite of transformations of wheat bran alkylresorcinols) in the samples with different growth media is not even either. In the WB-containing medium, 40% of acetic acid is accumulated in the mycelium and 60% in the CL. In the media without WB, almost all acetic acid is accumulated in the CL and is practically absent in the mycelium. This proves that WB can unbalance the total distribution of aromatic components towards their accumulation in mycelium.

E. Frankel's monograph presents data on the versatile character of the smell and taste of compounds obtained from lipids. The author believes that studies of the sources of volatile lipid oxidation products still remain contradictory and are often hard to interpret [30].

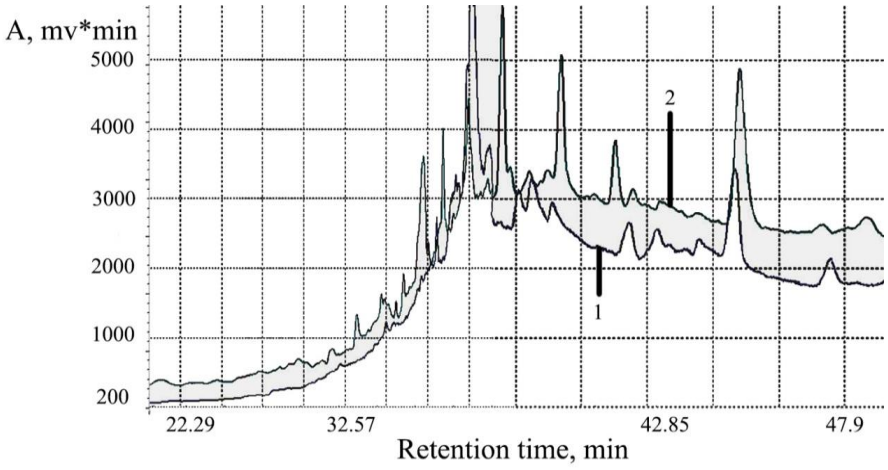

Fig. 4. Fragment of the chromatogram of aromatic components of the culture liquid: GPY+WB *(1)*; GPY *(2)*

Decomposition of hydroperoxides involves a very complicated set of reaction pathways for the formation of a lot of volatile and non-volatile products. Lipid metabolism is connected with a wide variety of aspects of the life activity of plants and fungi: photosynthesis, development, cell permeability, mineral metabolism, transition of a plant's organs to dormancy, resistance to adverse environments [24]. So, analysis of the fatty acid compositions of the culture liquid and mycelium is an important element of research.

The fatty acid composition of the CL after 14 days' culturing on GPY and GPY+WB reveals but a slight difference in the amount of fatty acids (1.7%) and in the ratio between the saturated and unsaturated ones (Table 3).

The fatty acid composition of the mycelium has shown that the sample GPY+WB is by 5.4% lower in PUFA than the GPY sample is (Table 4).

Analysis of the qualitative fatty acid composition of the mycelium proves that the decrease in the total PUFA content in the mycelia grown on GPY and GPY+WB is largely due to linoleic acid (Fig. 5, Fig. 6). As mentioned above, this is the acid that is more rapidly oxidised by peroxidase. Thus, the study of the mycelium cultured with WB added confirms the initiation of processes involving enzymes of the oyster mushroom.

Comparison of chromatograms in Fig. 5 and Fig. 6 shows that in the wheat bran-containing samples of mycelium, components C_6, C_8 are formed. Their formation results from enzymatic cleavage of long-chain polyunsaturated fatty acids (Fig. 2). In the mycelium, the content of linoleic acid is 73.18 μg/ml (no WB) and 64.79 μg/ml (with WB added). In the culture liquid, the content of linolenic acid is 23.06 μg/ml (no WB) and 18.04 μg/ml (with WB added). Addition of WB affects the distribution of linolenic acid between the mycelium and culture liquid. In the WB-containing samples, α- and γ-linolenic acids accumulate in the mycelium, and in those without WB, they accumulate in the culture liquid. The different character of PUFA distribution is responsible for the increase in the quantity of aromatic components in the mycelium, where they accumulate more intensively with WB added and are aroma precursors in subsequent enzymatic reactions.

Table 3 – **Fatty acid composition of the culture liquid of GPY and GPY+WB**

№	Group	Concentration, %	Area, mV*min	Concentration, %	Area, mV*min
		GPY medium with wheat bran		GPY	
1	Saturated FA	1.523	27.3721	1.836	25.0805
2	Unsaturated (MUFA, PUFA)	3.124	56.1312	4.475	61.1320
	Total	4.648	83.5033	6.312	86.2125

Table 4 – Fatty acid composition of the mycelium of GPY and GPY+WB

№	Group	Concentration, %	Area, mV *min	Concentration, %	Area, mV *min
		GPY medium with wheat bran		GPY	
1	Saturated fatty acids	55.4	396.6	47.7	184.7
2	Monounsaturated FA	32.1	239.0	34.17	136.8
3	Polyunsaturated FA	12.5	93.77	17.97	72.4
	Total	100		100	

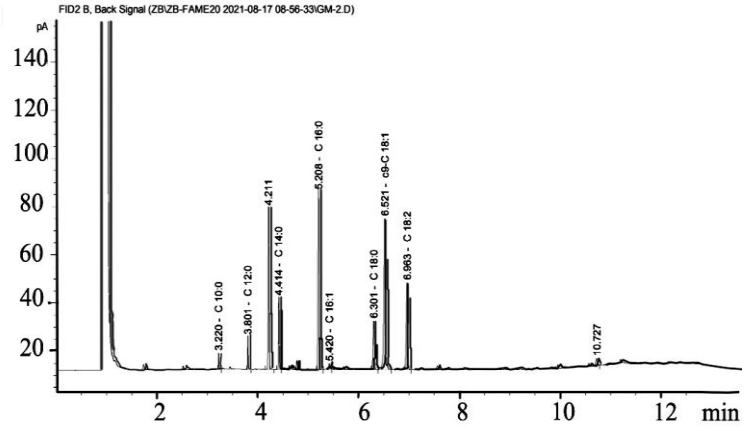

Fig. 5. Chromatograms of the fatty acid composition of the mycelium of GPY

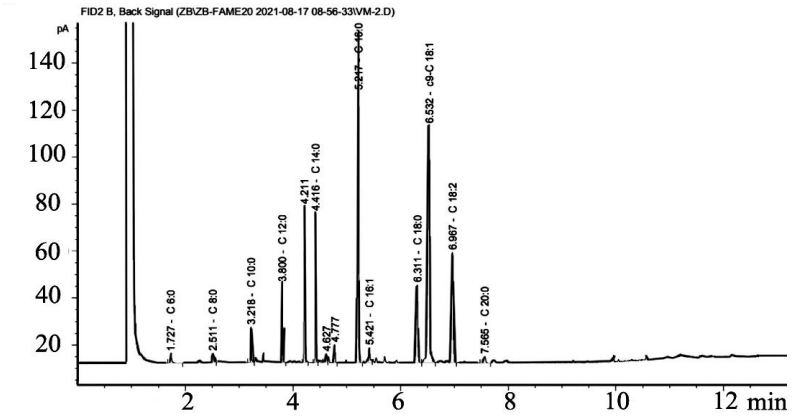

Fig. 6. Chromatograms of the fatty acid composition of the mycelium of GPY+WB

It is a well-known fact that reactive oxygen species can be an intermediate component of peroxidase oxidation. Their presence makes oxidation of PUFA 1,400 times more rapid and besides, causes changes in the composition of aromatic components [19]. When singlet oxygen attacks linoleic acid, hydroperoxides are formed at all carbons with double bonds. This process is different from β-cleavage, when an alkoxy radical is only formed at the tenth carbon atom. This means that when there are reactive oxygen species, hydroperoxides are also formed at the ninth and thirteenth carbon atoms, like they do in case of oxidation initiated by free radicals. Besides, hydroperoxides are formed at the tenth and twelfth carbons [19]. This significantly affects the qualitative composition of lipid peroxidation products and, consequently, the subsequent formation of aromatic components from hydroperoxides.

Formation of reactive oxygen species is a specific response to certain stressors. A shift in the tissular balance of antioxidants and pro-oxidants towards the latter is termed oxidative stress. The research conducted allows the conclusion that WB in the concentration 20 g/l, when used in a standard growth medium during *P. ostreatus* culturing, initiates oxidative stress and enzymatic reactions accompanied by aroma formation. The quantitative and qualitative study of the aromas of the mycelium and CL of the edible mushroom *P. ostreatus* has shown that wheat bran introduced as an additive affects the formation and distribution of aromatic components.

Conclusions

1. During culturing on a liquid growth medium under stationary conditions, aromatic components, as secondary metabolites, are present in the mycelium and CL. The quantitative and qualitative study of the aromas of the mycelium and CL of the edible mushroom *P. ostreatus* has shown that wheat bran introduced as an additive affects their aromatic components by initiating enzymatic oxidation reactions of lipids. With wheat bran added, the total amount of aromatic components is concentrated in the mycelium, and in a standard growth medium, it is evenly distributed between the mycelium and the culture liquid.

2. Wheat bran components initiate aroma-forming reactions in *P. ostreatus*. This is confirmed by the quantitative and qualitative composition of aromatic components in the mycelium and culture liquid. The culture liquid with wheat bran is more than 1.4 times higher in 1-octen-3-ol (which is the main component responsible for the mushroom aroma). The sample of mycelium with WB contains the two main mushroom aroma components, 1-octen-3-ol and hexanal, and in the sample without WB, these important aromatic components are absent. In the GPY+WB-cultured mycelium, the total quantity of identified aromatic components is larger by more than 1.7 times, as compared with the sample grown on GPY without wheat bran.

3. Analysis of the fatty acid composition of the culture liquid and mycelium after 14 days' culturing has shown that in the samples with WB, there is a decrease in linoleic acid, which is the first component to be oxidised when peroxidases initiate the process. The different character of distribution of PUFA (the aroma precursors) is responsible for the increase in the quantity of aromatic components in the mycelium, where they accumulate more intensively.

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ДОСЛІДЖЕННЯ АРОМАТИЧНИХ КОМПОНЕНТІВ ПРИ ІНІЦІЮВАННІ ФЕРМЕНТАТИВНИХ РЕАКЦІЙ ЇСТІВНОГО ГРИБА *PLEUROTUS OSTREATUS*

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Анотація. У зв'язку з широким використанням грибів у різних галузях харчової та медичної промисловості питання їх аромату є досить актуальними. У даній статті представлений порівняльний аналіз ароматичного та

жирнокислотного складу міцелію їстівного гриба Глива (*Pleurotus ostreatus* (Jacq.) P. Kumm., штам К 17), культивованого на глюкозо-пептонно-дріжджовому поживному середовищі з додаванням пшеничних висівок. Кількісний та якісний склад ароматів показав вплив компонентів пшеничних висівок у реакціях ароматоутворення під час росту міцелію гриба P. ostreatus. Встановлено, що внесення пшеничних висівок призводить до збільшення вмісту основного грибного ароматичного компонента 1-октен-3-олу. В культуральній рідині 1-октен-3-ол збільшується в 1,4 рази порівняно з класичними зразками, культивованих на поживному середовищі без пшеничних висівок. Також у міцелії, культивованому на поживному середовищі з пшеничними висівками, загальна кількість ідентифікованих ароматичних компонентів збільшується в 1,7 рази. У міцелії, культивованому на середовищі з пшеничними висівками, формуються два основних компоненти грибного аромату: 1-октен-3-ол і гексаналь, а в інших зразках ці важливі компоненти відсутні. Результати цього дослідження підтверджують, що в утворенні основних грибних ароматичних компонентів 1-октен-3-олу, гексаналю брали участь поліненасичені жирні кислоти, а саме лінолева кислота. Її вміст зменшувався протягом культивування в середовищі з пшеничними висівками одночасно з накопиченням ароматичних компонентів. Отримані дані дозволяють встановити роль ініціювання ферментативних окислювальних реакцій в зміни аромату P. ostreatus при поверхневому культивуванні на рідкому поживному середовищі. Результати дослідження показують процеси формування ароматичних компонентів макроміцетів із попередників ліпідної природи. Додавання пшеничних висівок до поживного середовища зсуває рівновагу між загальним вмістом ароматичних компонентів у бік їх накопичення в міцелії. Оскільки плодові тіла і вегетативний міцелій схожі за своїм біохімічним складом за результатами проведених досліджень міцелію культивованого на рідкому поживному середовищі можна прогнозувати утворення ароматичних компонентів плодових тіл грибів гливи.

Ключові слова: міцелій, культуральна рідина, ароматичні компоненти, пшеничні висівки, пероксидаза, ферментативні реакції.