

STUDIES ON THE AROMA OF DIFFERENT SPECIES AND STRAINS OF *PLEUROTUS* MEASURED BY GC/MS, SENSORY ANALYSIS AND ELECTRONIC NOSE

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Abstract. The aroma of several strains of *Pleurotus ostreatus*, *Pleurotus citrinopileatus* and *Pleurotus djamor* was studied by GC/MS. Three main mushrooms aroma constituents: 3-octanol, 3-octanone and 1-octen-3-ol were taken into account for quantitative measurements. The highest amount of 1-octen-3-ol was recorded in *P. ostreatus*, while considerably lower amounts in *P. citrinopileatus*. Sensory profile analysis as well as the electronic nose also varied between the three species of *Pleurotus*. Chiral gas chromatography showed the high optical purity of (R)-(-)-1-octen-3-ol in *P. ostreatus* and *P. djamor* (the highest one) in contrast to *P. citrinopileatus*. Carpophores of *P. djamor* was characterized relatively high dry matter and protein contents.

Key words: *Pleurotus* spp. aroma, 1-octen-3-ol, chirality, sensory testing, electronic nose

INTRODUCTION

World-wide, commercial mushroom production comprises about 5×10^6 tonnes fresh weight per year, although at present only several basidiomycetes (*Agaricus*, *Leontinus*, *Pleurotus*, *Auricularia*, *Volvariella*, *Flammulina*, *Tremella* and a few others) are being grown [Kües and Liu 2000]. The cultivation of *Pleurotus* as well as other mushrooms has a long tradition in East Asia, particularly in China. *Pleurotus* species (oyster mushroom) stands next to *Agaricus bisporus* in the production statistics of widely cultivated and consumed mushrooms. Information on flavour profiles of the cultivated edible mushrooms is essential for the improvement of their sensory quality and assessment of their potential in bioproduction of natural flavour [Venkateshwarlu et al. 1999]. Mushrooms flavours have been studied by many authors [Venkateshwarlu et al. 1999, Wąsowicz 1974, Maga 1981, Chen and Wu 1984, Chen et al. 1984, Fischer and Grosch 1987, Mau et al. 1992, 1997, Lizárraga-Guerra et al. 1997, Assaf et al. 1977, Zawirska-

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-Wojtasiak 2004]. The main odorants of the mushroom aroma are eight carbon atoms (C8) compounds, of which the most important is 1-octen-3-ol. It is found in two optically active forms [Mosandl et al. 1986, Bauer et al. 1990, Chambers et al. 1998]. Studies of chemicals indicated that (R)-(-)-1-octen-3-ol has a fruity mushroom-like characteristic, whereas (S)-(+)-1-octen-3-ol has a mouldy, grassy note [Mosandl et al. 1986]. The first one has been described as the character impact flavour compound of mushrooms. *Pleurotus flavor* has been attributed also to the C8 compounds, mainly 1-octen-3-ol [Drawert et al. 1983]. In *P. florida* [Venkateshwarlu et al. 1999] this compound is estimated at the range of 68% of total volatiles. According to Zawirska-Wojtasiak [2004] the optical purity of (R)-(-)-1-octen-3-ol in *P. ostreatus* is very high and amounts to 97.3%. However, in *P. eryngii* [Mau et al. 1998] the major volatile compound is benzaldehyde.

A number of research workers reported significant differences in the chemical composition between various species and strains of cultivated mushrooms [Weaver et al. 1977, Crisan and Sands 1978, Chang et al. 1981, Bano and Rajarathnam 1982, Raguanthan and Swaminathan 2003, Shashirekha et al. 2002, 2005]. At present, a growing interest has been observed in Europe in new mushroom species [Oei 2003]. There is an increasing demand on the market for packaged mixtures of fresh mushrooms consisting of several species, characterized by different colours of their carpophores.

Pleurotus is cultivated now under various growing conditions and in various species and strains. One of these species is *P. ostreatus*; also two other, more popular in Asia, i.e. *P. citrinopileatus* (yellow caps) and *P. djamor* (pink caps) are offered in retail now. There is limited data in literature on the flavour of these mushrooms.

P. spp., as primary wood rot fungi, are able to colonise different types of agricultural wastes as substrates [Raguanthan and Swaminathan 2003]. Some studies have been conducted concerning the effect of various substrates on the characteristics of mushroom *P. spp.*, such as the yield, biological efficiency or chemical composition including proteins, carbohydrates, dietary fibre [Raguanthan and Swaminathan 2003, Shashirekha et al. 2002, 2005], but not flavour.

The aim of the study was to test the aroma of various strains of *P. ostreatus* as well as *P. citrinopileatus* and *P. djamor* grown on wheat straw, under particular conditions, using GC/MS, chiral GC, sensory analysis and the electronic nose.

MATERIALS AND METHODS

Materials

Mushrooms, reference compounds. Mushroom samples were collected from an experimental cultivation plot at the Department of Vegetable Crops, Poznań University of Life Sciences, in two experiments (E1, E2). The following strains of *P. ostreatus* were analysed: K2, K22, HK35, HK35/K22, B22, B101, while of *P. citrinopileatus* there were B74, B83 and CRN131, as well as *P. djamor* B62. Experiments were carried out on an agricultural farm in Łobez near Jarocin. In the experiment the substrate was wheat straw cut into chaff 2.5-5 cm long. The straw was prepared using the xerothermic method (95°C for 2 hours) and then it was moistened to a moisture content of about 70%. The prepared substrate was mixed with grain mycelium which constituted 3%

in relation to the wet weight of the substrate and placed in bags of perforated foil. Each bag contained 16 kg of the substrate. Mycelium incubation was conducted at the temperature of 22-24°C. Once the substrate was totally overgrown with the mycelium of the mushroom, it was transferred to cultivation room, in which different thermal conditions were maintained depending on the species requirements. In the case of the *P. ostreatus* strains, the temperature was 17-18°C, whereas for the *P. djamor* and *P. citrinopileatus* strains it ranged from 20 to 21°C. Humidity was maintained in all rooms at 80-85% and the culture was lighted with fluorescent light (Day-Light) of 500 lx intensity for 10 hours per day. Two of the tasted strains, i.e. K22 and HK35, were harvested at two flushes of crop (I, II) in one of the experiments.

The experiments were carried out in a random design in four repetitions in the form of a single pressed block of the experimental substrate. Before the analyses mushrooms were stored no more than 3 days at 1-2°C. Standards of volatiles were purchased from Sigma-Aldrich and were of 99% degree of purity. SPME fiber used for samples evaluation was obtained from Supelco (Bellefonte, PA).

Methods

Estimation of dry matter content. The content of dry matter in the carpophores of chosen mushroom samples from I plot of cultivation (strains K22 and HK35 of *P. ostreatus*, strain B83 of *P. citrinopileatus* and strain B62 of *P. djamor* – all from both experiments E1 and E2) was determined in four repetitions by the gravimetric method [Rutkowska 1979]. Carpophores with stems were pre-dried at 40°C for 4 h and then forced-dried to constant weight at 105°C.

Statistical analysis of data was performed using the analysis of variance for factorial experiments. Means were compared using the Newman-Keuls test at $\alpha = 0.05$.

Estimation of total nitrogen concentration. The concentration of total nitrogen in chosen mushroom samples from I plot of cultivation (strains K22, HK35 and HK35/K22 of *P. ostreatus*, strains B74 and B83 of *P. citrinopileatus* and strain B62 of *P. djamor* – all from both experiments E1 and E2) was determined in four repetitions by the Kjeldahl method [Rutkowska 1979] and then it was converted into protein nitrogen using the coefficient of 4.38, which is commonly applied for mushrooms [Shashirekha et al. 2002].

Statistical analysis of data was performed using the analysis of variance for factorial experiments. Means were compared using the Newman-Keuls test at $\alpha = 0.05$.

Isolation of volatiles by the microdistillation-extraction procedure. The volatiles from mushrooms and mushroom-like products were isolated by the microdistillation extraction procedure in a Likens-Nickerson apparatus [Bouseta and Collin 1995], with ether-pentane (1:1 v/v) as the extraction solvent; 75 g of fresh mushrooms were used for isolation. Pentadecane as the internal standard was added (at 0.6 mg) before distillation. Fresh mushrooms were cut into pieces and homogenized with 150 ml of distilled water for 5 min. The homogenate was left for 15 min to maximize the enzymatic production of flavor [Venkateshwarlu et al. 1999] and subjected to simultaneous distillation and solvent extraction in a Likens-Nickerson apparatus. The flavour extract was dried over anhydrous sodium sulphate and concentrated to 0.5 ml at room temperature. A 76% recovery of added 1-octen-3-ol was recorded in case of this method.

Gas chromatography analysis. A Hewlett-Packard HP 6890 gas chromatograph with a split/splitless injector and an FID detector was used for the analyses. Compounds

were separated using capillary column a ZB-wax (60 m × 0.53 mm × 1 μm) and RESTEK chiral capillary column an RtβDex sa (30 m × 0.32 mm × 0.25 μm). The identity of separated compounds was confirmed on a Hewlett-Packard HP 5890 II gas chromatograph coupled to an HP 5971MSD quadrupole mass spectrometer. Injection of volatiles was performed in the split mode. Analysis parameters on a ZB-wax column were the following: initial temp. 60°C, then 8°C per min to 200°C, while on the chiral column they were initial temp. 60°C, 3°C per min to 150°C and 10°C per min to 200°C. The flow rate of hydrogen, used as a carrier gas, was 1.6 ml per min. The Kovats' indices (retention indices RI) were calculated using n-alkanes (C8-C18) as standards (Sigma-Aldrich). The calculated retention indexes for the separated compounds were compared with those for the standards. The concentrations of volatiles were calculated on the basis of known amounts of the internal standard added to the sample prior to the distillation. Volatiles were measured in all the mushroom samples mentioned in Materials.

One way analysis of variance by F-function was performed for the data of measured volatiles concentration in different varieties of *Pleurotus ostreatus*.

Electronic nose. Selected samples of *P. ostreatus*, *P. citrinopileatus* and *P. djamora* from experiment E1 were analysed with electronic nose device. A Fox 4000 electronic nose with 18 metal oxide sensors in three chambers was used for analysis (Alpha M.O.S., Toulouse, France). Average samples prepared from homogenized mushrooms' carpophores were placed in 10 ml vials (1 g to every vial), capped, and placed in a Combipal type autosampler (HS-100). Samples were incubated for 5 min at 35°C and than volatiles (500 μl) were transferred automatically to the electronic nose with the gastight syringe. A pure synthetic air flow of 150 ml per min was used to sweep samples through the electronic nose chambers. Each sample was analysed in triplicate – three vials were prepared from the same sample lot. Sensor optimization and data treatment were performed using the Alpha Soft 0.8 software package (Alpha M.O.S.). Operation on signals included signal pre-processing to build libraries with the default values for statistical data processing, the selection of sensors providing the highest degree of sample differentiation and principal component analysis (PCA) of obtained data. For the sensor array data, the library was built with the values in $\Delta R/R_0$ and maximum of the sensor intensity. $\Delta R/R_0$ displayed the value of the sensors in relative resistance change. In that mode the displayed value was $(R_0 - R)/R_0$, where R_0 was resistance at $t = 0$ (baseline resistance) and R was resistance at a selected time. When the maximum value option was selected, the library was built with the value taken from the top of the response peak for each sensor (even if the maximum did not occur at the same time for all the sensors).

Sensory analysis. Selected samples of *P. ostreatus*, *P. citrinopileatus* and *P. djamora* from experiment E1 were analysed by sensory panel. Sensory analysis was performing according to Zawirska-Wojtasiak et al. [1992]. A panel of 10 people experienced in profile sensory analysis analysed of *Pleurotus* samples. A vocabulary of descriptors was developed for the evaluation of mushroom odors. The following odour descriptors were offered for examined samples: (1) mushroom-like (typical for fresh edible mushroom e.g. *Boletus edulis*), (2) woody/fungal, (3) earthy, (4) musty, (5) putrid, (6) fishy and (7) meaty. Mushroom samples (30 g) were presented to panel members in closed 100 ml vials. The vials with samples were preheated at 40°C to liberate volatile compounds. Mushroom samples were evaluated during three sessions. Panel members marked the intensity of each odour descriptor on a 0-10 graphic line scale. Thirty measurements for each descriptor were processed using principal component analysis (PCA).

RESULTS AND DISCUSSION

Dry matter and protein content

Dry matter of carpophores was measured, since it is a major marker of mushrooms processability. Significant differences were observed in the carpophore dry matter content between the examined oyster mushroom species. On the other hand dry matter content of fruiting bodies of the examined strains within the same species was similar. Carpophores of *P. ostreatus* were found to possess the lowest dry matter content in comparison with the two other studied oyster mushroom species. Strains of this species developed carpophores with uniform morphological traits very similar to one another and were also characterized by a firm, although not very hard, parenchyma of the carpophores. The highest dry matter content was found in carpophores of *P. djamor*, which were characterized by a considerable hardness of the parenchyma of the pileus and the stem, while a lower dry matter content was recorded in carpophores of *P. citrinopileatus*, whose strains developed carpophores characterized by a thin and delicate parenchyma of the cap but a hard parenchyma of the stem.

Dry matter content of the analysed samples of *P.* strains recorded in experiments E1 and E2 is compared in the Table 1. The content of the carpophore dry matter of the *P.* genus can vary from 7.4 to 26.2 g·100 g⁻¹, depending on the species and strain [Chang and Miles 2004, Siwulski et al. 2007]. The carpophore dry matter content of all the examined species and strains of oyster mushroom ranged from 9.2 to 9.5 g·100 g⁻¹ for *P. ostreatus*. The carpophore dry matter content recorded in *P. citrinopileatus* ranged from 10.9 to 11.6 g·100 g⁻¹ and in *P. djamor* from 13.9 to 14.1 g·100 g⁻¹.

Table 1. Dry matter content of carpophores of some *Pleurotus* species and strains, g·100 g⁻¹

Experiment	<i>P. ostreatus</i> strain		<i>P. citrinopileatus</i> strain	<i>P. djamor</i> strain	Mean
	K22	HK35	B83	B62	
E1	9.2	9.5	11.6	13.9	11.0 a
E2	9.4	9.3	10.9	14.1	10.9 a
Mean	9.3 a	9.4 a	11.2 b	14.0 c	

Data marked by the same letter do not differ significantly at $\alpha = 0.05$.

Crude protein content was determined in selected samples of the examined *P.* species (Table 2). According to different researchers, the content of protein in the carpophores of different species of *Pleurotus* can fluctuate within very wide intervals, namely from 7.1 to 44.3 g·100 g⁻¹ DM [Raguanthan and Swaminathan 2003, Shashirekha et al. 2005, Oei 2003, Chang and Miles 2004]. In our own investigations, this content was contained within a narrower interval, i.e. 14.9 to 24.0 g·100 g⁻¹. The highest protein content was determined in the carpophores of *P. djamor*. The lower protein content was found in the carpophores of *P. citrinopileatus* followed by *P. ostreatus*. According to Oei [2003], the protein content in the carpophores of *P. djamor* ranges

Table 2. Crude protein (N · 4.38) content of carpophores of some *Pleurotus* species and strains, g·100 g⁻¹ d.m.

Experiment	<i>P. ostreatus</i> strain			<i>P. citrinopileatus</i> strain		<i>P. djamor</i> strain	Mean
	K22	HK35	HK35/K22	B74	B83	B62	
E1	14.9	15.4	15.8	19.3	18.9	23.2	17.9 ^a
E2	15.2	15.6	15.2	19.7	19.0	24.0	18.1 ^a
Mean	15.0 a	15.5 a	15.5 a	19.5 b	18.9 b	23.6 c	

Data marked by the same letter do not differ significantly at $\alpha = 0.05$.

from 13 to 17 g·100 g⁻¹ and is lower in comparison with that determined in our experiments (23.2-24.0 g·100 g⁻¹). The protein content reported by Chang and Miles [2004], i.e. 25 g·100 g⁻¹, is much closer to that determined in our investigations. On the other hand, the protein content in the carpophores of *P. citrinopileatus* was considerably lower (18.9-19.7 g·100 g⁻¹) in comparison with the results of experiments reported by Raguanthan and Swaminthan [2003], namely 30.1-40.6 g·100 g⁻¹. It is rather difficult to account for such differences because the same conversion factor (4.38) of the total determined nitrogen into protein was employed in all experiments. It is possible that the above differences could be attributed to differences in the composition of the cultivation substrate used in the experiments, as well as the applied cultivation conditions. Shashirekha et al. [2005] reported an approximately 90 g·100 g⁻¹ increase in protein content in the carpophores of *P. florida* in their experiments in which they supplemented the substrate of rice straw with cotton seed powder.

No significant differences in the protein content were observed between the strains of the examined species.

Mushroom volatiles – *Pleurotus ostreatus*

The resolution of volatiles in mushroom was obtained using a ZB-wax column. Figure 1 presents the chromatogram for *P. ostreatus*. Identified compounds included hexanal (RI 1119), 3-methyl butanol (RI 1225), 3-octanone (RI 1291), 2-octanone (RI 1323), hexanol (RI 1375), 3-octanol (RI 1408), 1-octen-3-ol (RI 1473), 1-octanol (1580) and 2-octen-1-ol (RI 1703), also mentioned by other authors in mushrooms generally including *Pleurotus* [Venkateshwarlu et al. 1999]. The identity of separated compounds was confirmed by mass spectrometry. According to the data in Table 3 in the majority of samples 1-octen-3-ol was found in relatively very high or the highest amounts (from 1.14 to 2.83 mg·100 g⁻¹ of fresh weight). This compound is well known as the most odorous mushroom component and was reported by other authors [Maga 1981, Fischer and Grosch 1987, Mau et al. 1992] as the main odorant of various mushroom species. In the studies conducted by Venkateshwarlu et al. [1999] the concentration of 1-octen-3-ol in *P. florida* was recorded at the level of 1.9 mg·100 g⁻¹ of fresh mushrooms.

Three main eight-carbon atom volatiles (3-octanone, 3-octanol and 1-octen-3-ol) were taken into consideration for quantitative comparisons of several strains *P. ostreatus*.

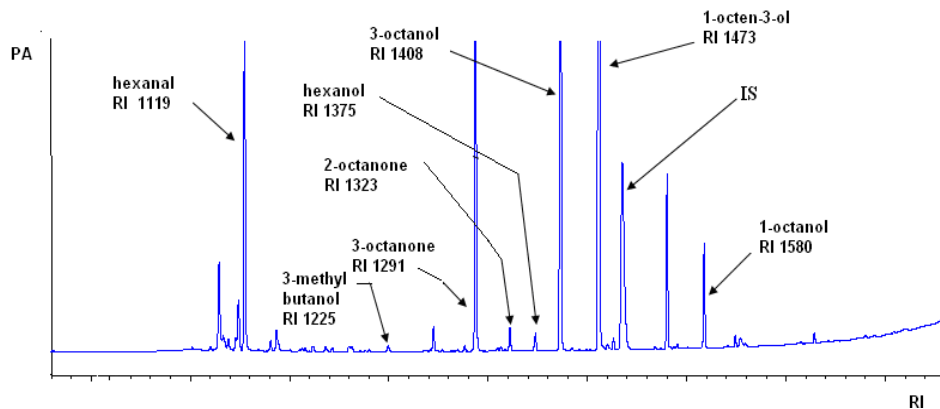


Fig. 1. Gas chromatographic resolution of volatiles from *Pleurotus ostreatus* (strain B22 from experiment E1) on ZBwax column: RI – Kovats' retention index, IS – internal standard

The data obtained for the three selected compounds in various experiments, strains, and cultivation plots of *P. ostreatus* grown under conditions described above are presented in Table 3. The highest observed concentration of 1-octen-3-ol in the samples reached $2.83 \text{ mg} \cdot 100 \text{ g}^{-1}$ of mushrooms (HK35 in experiment E1). 1-octen-3-ol were recorded at a lower concentration in flush II than in flush I. The observation is worth conducting further investigations, not performed in this study, but similar findings were well documented in studies concerning experimental cultivation of *Agaricus bisporus* [Zawirska-Wojtasiak et al. 2007].

Optical purity of 1-octen-3-ol was established for all the mushroom samples. The data for *P. ostreatus* are presented in Table 3. In most edible mushrooms the predominant form of 1-octen-3-ol is (R)-(-)octen-3-ol. Despite significant differences in the concentration of this compound between species, the optical purity of the minus form was very high – the highest one was measured in *Agaricus bisporus* (over 98.5%), while the lowest in *Xerocomus badius* (82%) [Zawirska-Wojtasiak 2004].

Table 3. Concentration of the main volatile compounds in different strains of *Pleurotus ostreatus*, $\text{mg} \cdot 100 \text{ g}^{-1}$ f.w.

Experiment	Cultivation plot	3-octanone* RI 1291	3-octanol* RI 1408	1-octen-3-ol* RI 1473	Enantiomeric ratio	
					R(-)1-octen-3-ol RI 1224	S(+)-1-octen-3-ol RI 1230
1	2	3	4	5	6	
K22						
E1	I	1.68	3.81	2.36	95.2/4.8	
	II	1.44	3.10	1.14		
E2	I	1.63	2.66	2.16	95.7/4.3	

Table 3 – cont.

1	2	3	4	5	6
HK35					
E1	I	2.57	4.34	2.83	94.7/5.3
	II	2.45	2.83	1.57	
E2	I	2.38	4.16	2.58	94.8/5.2
K22/HK35					
E1	I	1.93	3.32	2.29	92.7/7.3
K2					
E1	I	1.12	2.61	1.76	95.3/4.7
B22					
E1	I	0.56	1.45	2.56	94.3/5.7
B101					
E1	I	0.49	1.23	1.72	91.1/8.9

*The values represent means of four repetitions, coefficient of variation 2-5%.

Pleurotus showed a rather high purity of (R)-(-)-octen-3-ol; in a previous study it was over 97% in *P. ostreatus* [Zawirska-Wojtasiak 2004], now not lower than 91% (B101), while the highest value amounted to 95.7% (K22).

Concerning the three estimated 8-carbon atoms components it can be stated, that the highest concentration were recorded more frequently for 3-octanol, but the concentrations varied in the wide range (from 1.23 to 4.34 mg · 100 g⁻¹ in *P. ostreatus* – Table 3). On the basis of statistic F-function calculations done in all experiments for *P. ostreatus*, it could be concluded the concentration of the three measured compounds depended on the variety.

Mushroom volatiles – *Pleurotus citrinopileatus* and *Pleurotus djamor*

The gas chromatogram on Figure 2 pertains to the separation of volatiles in *P. djamor*, while the data of measured compounds in the species of *P. citrinopileatus* and in *P. djamor* in Tables 4 and 5 respectively. In these species apart from all the mentioned compounds, 2-pentylfuran (IR 1259) was identified in relatively high amounts in *P. citrinopileatus*. The concentration of this compound was recorded at 0.06 mg · 100 g⁻¹ in *P. djamor* (Table 5), while in *P. citrinopileatus* it was from 0.80 in sample B83 to 2.60 mg · 100 g⁻¹ in B74 (Table 4). Two other compounds were identified in relatively high amounts, but only in *P. citrinopileatus*. They were 3-nonen-1-ol (IR 1711) and 4-methyl-4-nonanol (IR 1920). Their concentration amounted to 1.13/100 g in B74 (3-nonen-1-ol) and 1.17 mg · 100 g⁻¹ in B83 (4-methyl-4-nonanol).

It needs to be stressed that the concentration of the main odorant 1-octen-3-ol (Tables 4-5) was very low in *P. citrinopileatus* (0.03-0.08 mg · 100 g⁻¹), while in *P. djamor* it was similar to that in *P. ostreatus* (1.76 mg · 100 g⁻¹). Another interesting finding was the very high optical purity of this compound in *P. djamor*, even higher than

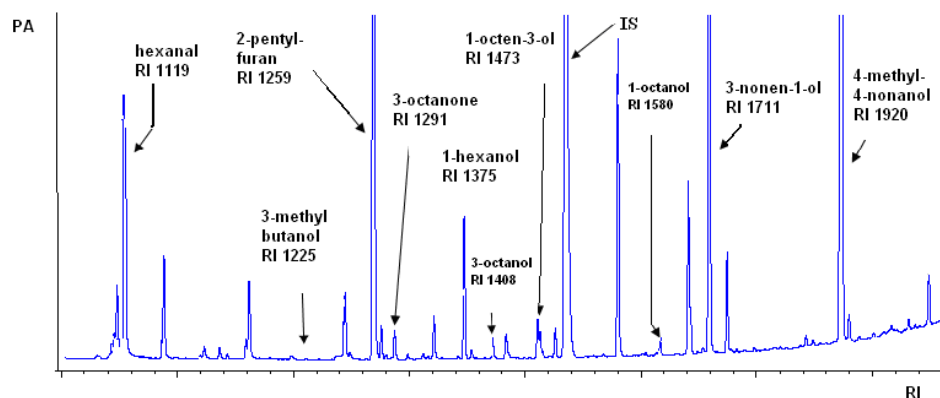


Fig. 2. Gas chromatographic resolution of volatiles from *Pleurotus djamor* (strain B62 from experiment E1) on ZBwax column: RI – Kovats' retention index, IS – internal standard

in *P. ostreatus* (over 98%), in the contrast to *P. citrinopileatus*, which was characterized by an almost racemic ratio. According to Zawirska-Wojtasiak [2004], all tested species of edible mushrooms, cultivated and growing wild showed a high enantiomeric excess of the 1-octen-3-ol minus form. Concentration of 3-octanone and 3-octanol were much lower in both species than in *P. ostreatus*.

Table 4. Concentration of the main volatile compounds in different strains of *Pleurotus citrinopileatus*, mg·100 g⁻¹ f.w.

Experiment	3-octanone* RI 1291	3-octanol* RI 1408	1-octen-3-ol* RI 1473	Enantiomeric ratio R(-)1-octen-3-ol RI 1224 S(+)1-octen-3-ol RI 1230
B83				
E1	1.11	0.08	0.04	
B83				
E2	0.02	0.02	0.03	50.1/49.9
B74				
E1	0.18	0.13	0.08	51.7/48/3
CRN131				
E1	0.25	0.07	0.07	50.4/49.6

*The values represent means of four repetitions, coefficient of variation 2-5%.

Table 5. Concentration of the main volatile compounds in one strain of *Pleurotus djamor*, mg·100 g⁻¹ f.w.

Experiment	3-octanone* RI 1291	3-octanol* RI 1408	1-octen-3-ol* RI 1473	Enantiomeric ratio R(-)1-octen-3-ol RI 1224 S(+)-1-octen-3-ol RI 1230
		B62		
E1	0.32	0.19	1.76	98.4/1.6
		B62		
E2	0.19	0.14	1.76	98.1/1.9

*The values represent means of four repetitions, coefficient of variation 2-5%.

Sensory profile analysis of *Pleurotus* species

Samples of *P. ostreatus* (p1-p4), *P. citrinopileatus* (p5-p7) and *P. djamor* (p8-p9) were selected for the sensory profile analysis performed according to the method described above. Figure 3 presents the graphic illustration of the PCA interpretation of the data reported by panelists. PCA was able to discriminate mostly between samples of

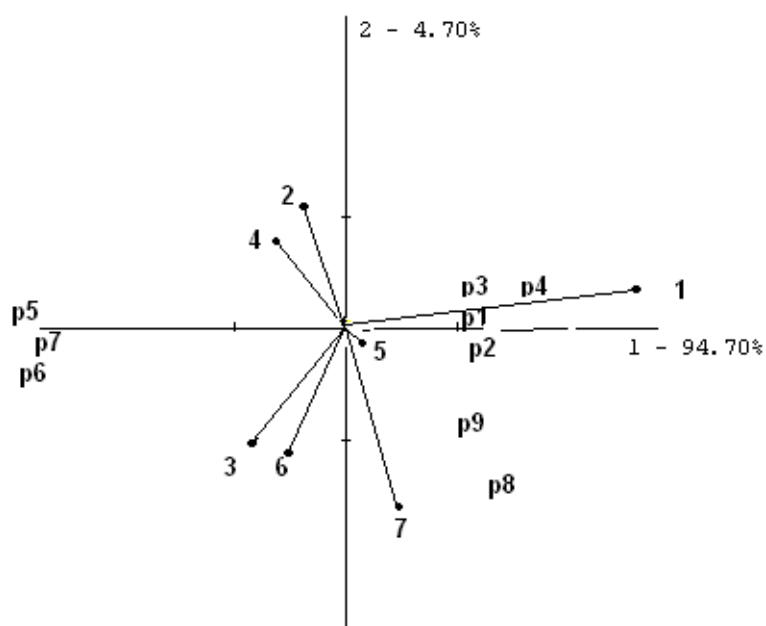


Fig. 3. PCA plots of sensory data. Sample codes: p1-p4 – *Pleurotus ostreatus*, p5-p7 – *Pleurotus citrinopileatus*, p8-p9 – *Pleurotus djamor*. Descriptors: 1 – mushroom like, 2 – woody, 3 – earthy, 4 – musty, 5 – putrid, 6 – fishy, 7 – meaty

P. citrinopileatus and the two other strains: *P. ostreatus* and *P. djamor* (PCA component 1 – 94.7%). The considerable grouping of samples mainly depended on attribute (1) – mushroom-like. *P. citrinopileatus* was much less intensive in terms of mushroom-like character, also possessing a little bit of the fishy/earthy notes (6, 3). A slight distinction between *P. djamor* and *P. ostreatus* was based on meaty note (7), perceived in *P. djamor*. However, in the olfactory measurements performed for distillates no fraction responsible for fishy or meaty odour was found.

Discrimination of *Pleurotus* species by the electronic nose

Samples of *P. ostreatus* (A), *P. citrinopileatus* (B) and *P. djamor* (C) were selected for the analysis with the electronic nose device. For electronic nose measurement optimization of sensors was done as the first step in order to select sensors providing the highest responses and differentiation between samples. After sensor optimization, 5 sensors out of 18 were used to discriminate between mushroom samples (LY/AA, LY/gCTI, P30/2, T40/2, TA2). The RSD value for the sensor P30/2 was estimated as 0.95% for the samples of *P. ostreatus* while it was 8.74% for the sensor LY/cCT1 in the case of *P. citrinopileatus*. Figure 4 shows response curves of gas sensors (Graph I) and radar/plot graphs as a sample fingerprint (Graph II) corresponding to A, B and C.

Figure 5 (Graph I) shows the PCA projection of electronic nose data in the samples of mushrooms using a combination of sensors. PCA provided good separation of samples with 99.64% of the variation accounted for by PC1 and 0.24% accounted for by PC2. A discrimination index of 89% was recorded for tested samples. Discriminant factorial analysis (DFA) was used to separate groups mentioned in PCA discrimination (Fig. 5, Graph II). After cross-validation of the model, a percentage of recognition of 100% was achieved. PCA was able to discriminate in similar way, although not the same as PCA based on sensory data. On the basis of the euclidean distances between groups of *P. spp.* measured it can be stated that the highest differentiation was found between *P. ostreatus* and *P. citrinopileatus* (0.371183).

Partial least squares analysis (PLS) was used to correlate sensory attributes with the electronic nose responses for the same samples of mushrooms. The correlations between the electronic and human data were from 0.816 to 0.968 ($p < 0.05$). Angerosa et al. [1996] using the dynamic head space sampling and artificial neural network to examine oil quality in order to predict panel test scores, obtained 96% correct answers and suggested that sensory evaluation could be replaced by those techniques. The electronic nose was also applied in the preliminary research on the differentiation between nine wild species of freeze-dried mushrooms in comparison with other instrumental data such as GC/sniffing and AromaScan A20S [Schaller et al. 1998]. The MOS electronic nose sensors differentiated between four groups of samples, similarly as GC equipped with a sniffing-port. However, sensory analysis provides the closest approximation to the consumers' approach, the electronic nose which is a faster method – is also though to be a more objective one.

Taking into account the concentration of the main mushroom volatiles determined in this study, a difference in the aroma of analyzed *P.* strains may be concluded. The highest 1-octen-3-ol content was recorded in *P. ostreatus*, followed by *P. djamor*. However, a very high optical purity of this compound in *P. djamor* (over 98% of (R)-(-)-1-octen-3-ol) needs to be emphasized. The most typical mushroom-like odour of edible mushrooms

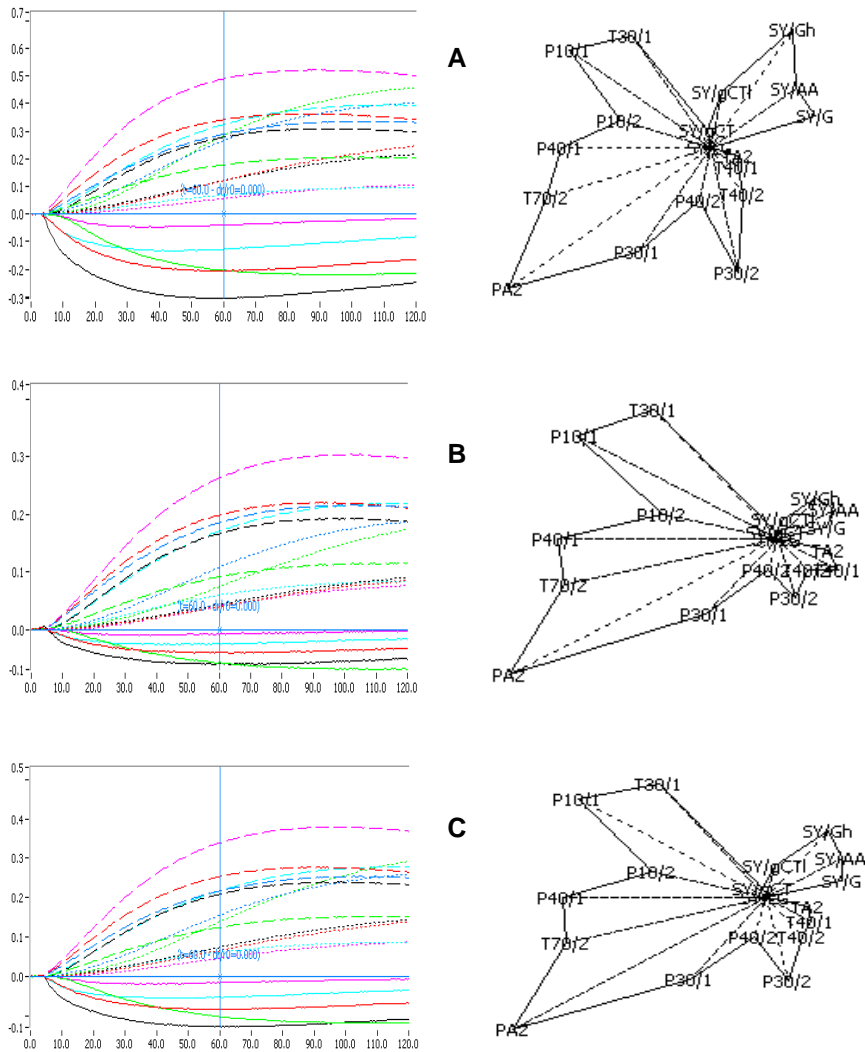


Fig. 4. Typical gas sensors responses curves (Graphs I – left side) and radar/plot graphs (Graphs II – right side) obtained for different samples: A – *Pleurotus ostreatus*, B – *Pleurotus citrinopileatus*, C – *Pleurotus djamor*

depends on the odour of this levorotatory antipode. The aroma of *P. djamor* was distinguished from the other species by sensory and electronic nose analyses. This species seems to be attractive for consumers also for the nice pink colour of carpophores. The relatively high protein content and particularly high dry matter content also needs to be stressed. This means that these mushrooms may both be sold directly to consumers and be processed.

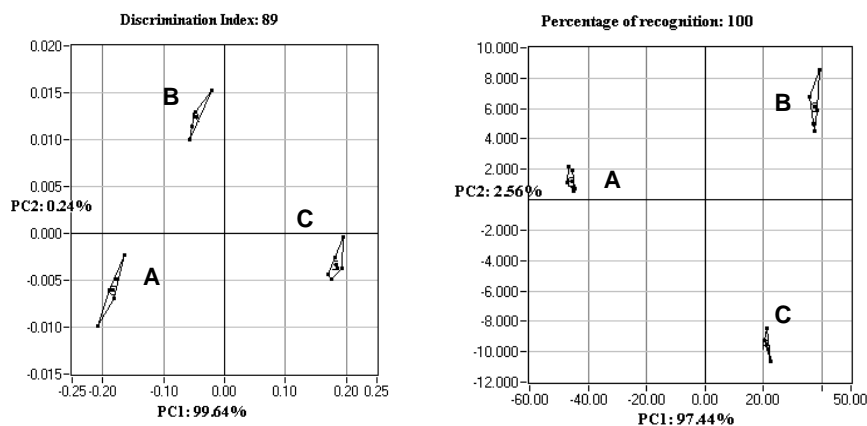


Fig. 5. The PCA interpretation (Graph I – left side) and Discrimination Factorial Analysis (Graph II – right side) of electronic nose data of mushroom samples using combination of sensors: A – *Pleurotus ostreatus*, B – *Pleurotus citrinopileatus*, C – *Pleurotus djamor*

REFERENCES

- Angerosa F., Di Giacinto L., Vito R., Cumitini S., 1996. Sensory evaluation of virgin olive oils by artificial neural network processing of dynamic head-space gas chromatographic data. *J. Sci. Food Agric.* 72, 323-328.
- Assaf S., Hadar Y., Dosoretz C.G., 1997. 1-octen-3-ol and 13-hydroperoxylinolate are products of distinct pathways in the oxidative breakdown of linoleic acid by *Pleurotus pulmonarius*. *Enzym. Microb. Technol.* 21, 484-490.
- Bano Z., Rajarathnam S., 1982. *Pleurotus* mushroom as a nutritious food. *Tropical mushrooms-biological nature and cultivation methods*. Eds S.T. Chang, T.H. Quimio. Chinese Univ. Press Hong Kong, 363-380.
- Bauer K., Garbe D., Surburg H., 1990. *Common fragrance and flavor material*. VCH Verlagsgesellschaft Weinheim.
- Bouseta A., Collin S., 1995. Optimized Likens-Nickerson methodology for quantifying honey flavors. *J. Agric. Food Chem.* 43, 1890-1897.
- Chambers IV E., Smithz E.C., Seitz L.M., Sauer D.B., 1998. Sensory properties of musty compounds in food. In: *Food flavors: formation, analysis and packaging influences*. Eds E.T. Contis, C.T. Ho, C.J. Mussinan, T.H. Parliment, F. Shahidi, A.M. Spanier. Elsevier Amaster, 173-180.
- Chang S.T., Miles P.G., 2004. *Mushrooms. Cultivation, nutritional value, medicinal effect and environmental impact*. CRC Press Boca Raton.
- Chang S.T., Lau O.W., Cho K.Y., 1981. The cultivation and nutritional value of *Pleurotus sajor-caju*. *Eur. J. App. Microbiol. Biotechnol.* 12, 58-62.
- Chen C.-C., Wu C.-M., 1984. Studies on the enzyme reduction of 1-octen-3-ol in mushroom (*Agaricus bisporus*). *J. Agric. Food Chem.* 32, 1342-1344.
- Chen C.-C., Chen S.-D., Chen J.-J., Wu C.-M., 1984. Effects of pH value on the formation of volatiles of shiitake (*Lentinus edodes*) and edible mushroom. *J. Agric. Food Chem.* 32, 999-1001.
- Crisan E.V., Sands A., 1978. Nutritional value. In: *The biology and cultivation of edible mushrooms*. Eds S.T. Chang, W.A. Hayes. Academic Press New York, 137-168.

- Drawert F., Berger R.G., Neuhausre K., 1983. Biosynthesis of flavor compounds by microorganisms. 4. Characterization of the major principles of the odor of *Pleurotus euosmus* [Food industry]. Eur. J. A. Microbiol. Biotechnol. 18, 124.
- Fischer K.H., Grosch W., 1987. Volatile compounds of importance in the aroma of mushrooms (*Psalliota bispora*). Lebensm.-Wiss. Technol. 20, 233-236.
- Kües U., Liu Y., 2000. Fruiting body production in basidiomycetes. Appl. Microbiol. Biotechnol. 54, 141-152.
- Lizárraga-Guerra R., Guth H., Lopez M.G., 1997. Identification of the most potent odorants in huitlacoche (*Ustilago maydis*) and austern pilzen (*Pleurotus* sp.) by aroma extract dilution analysis and static head-space samples. J. Agric. Food Chem. 45, 1329-132.
- Maga J.A., 1981. Mushroom flavor. J. Agric. Food Chem. 29, 1-4.
- Mau J.-L., Beelman B.B., Ziegler G.R., 1992. 1-octen-3-ol in the cultivated mushroom *Agaricus bisporus*. J. Food Sci. 57, 704-706.
- Mau J.-L., Chyau C.-C., Li J.-Y., Tseng Y.H., 1997. Flavor compounds in straw mushrooms *Volvariella volvacea* harvested at different stages of maturity. J. Agric. Food Chem. 45, 4726-4729.
- Mau J.-L., Lin Y.-P., Wu Y.-H., Peng J.-T., 1998. Flavor compounds in king oyster mushrooms *Pleurotus eryngii*. J. Agric. Food Chem. 46 (11), 4587-4591.
- Mosandl A., Heusinger G., Gessner M., 1986. Analytical and sensory differentiation of 1-octen-3-ol enantiomers. J. Agric. Food Chem. 34, 119-122.
- Oei P., 2003. Mushroom cultivation. Backhuys Publ. Leiden, Netherlands.
- Raguanthan R., Swaminthan K., 2003. Nutritional status of *Pleurotus* spp. grown on various agro-wastes. Food Chem. 80, 371-375.
- Rutkowska U., 1979. Wybrane metody badania składu i wartości odżywczej żywności [Chosen methods for estimation of composition and nutritive value of food]. PZWL Warszawa [in Polish].
- Schaller E., Bosset J.O., Escher F., 1998. 'Electronic Noses' and their application to food. Lebensm.-Wiss. Technol. 31, 305-316.
- Shashirekha M.N., Rajarathnam S., Bano Z., 2002. Enhancement of bioconversion efficiency and chemistry of mushroom, *Pleurotus sajor-caju* (Berk and Br.) Sacc. produced on spent rice straw substrate, supplemented with oil seed cakes. Food Chem. 76, 27-31.
- Shashirekha M.N., Rajarathnam S., Bano Z., 2005. Effects of supplementing rice straw growth substrate with cotton seeds on the analytical characteristics of the mushroom, *Pleurotus florida* (Block & Tsao). Food Chem. 92, 255-259.
- Siwulski M., Czerwińska A., Sobieralski K., 2007. Comparison of mycelium growth and yielding of some *Pleurotus* sp. strains. In: Spontaneous and induced variation for the genetic improvement of horticultural crops. Ed. P. Nowaczyk. Univ. Press. Univ. Techn. Life Sci. Bydgoszcz, 335-339.
- Venkateshwarlu G., Chandravadana M.V., Tewari R.P., 1999. Volatile flavour components of some edible mushrooms (*Basidiomycetes*). Flav. Fragr. J. 14, 191-194.
- Wąsowicz E., 1974. Identification of the volatile flavor compounds in mushrooms *Agaricus bisporus*. Bull. l'Acad. Pol. Sci. 22, 143-151.
- Weaver J.C., Kröger M., Kneebone L.R., 1977. Comparative protein studies (Kjeldahl, dye binding, amino acid analysis) of nine strains of *Agaricus bisporus* (Lange) Imbach mushrooms. J. Food Sci. 42, 364-366.
- Zawirska-Wojtasiak R., 2004. Optical purity of (R)-(-)-1-octen-3-ol in the aroma of various species of edible mushrooms. Food Chem. 86, 113-118.
- Zawirska-Wojtasiak R., Kamiński E., Rogalska M., 1992. Application of sensory profile analysis for quality evaluation of cereal grain during storage. In: Aroma production and application. Eds M. Rothe, H.P. Kruse. Deuts. Inst. Ernaerungsf. Potsdam-Rehbrücke, 351-366.
- Zawirska-Wojtasiak R., Siwulski M., Wąsowicz E., 2007. Volatile compounds of importance in the aroma of cultivated mushrooms *Agaricus bisporus*. Pol. J. Food Nutr. Sci. 3, 329-334.

**BADANIE AROMATU RÓŻNYCH GATUNKÓW I ODMIAN *PLEUROTUS*
Z ZASTOSOWANIEM GC/MS, ANALIZY SENSORYCZNEJ
I NOSA ELEKTRONICZNEGO**

Streszczenie. Badano aromat kilku odmian *Pleurotus ostreatus*, *Pleurotus citrinopileatus* i *Pleurotus djamor* metodą GC/MS. W oznaczeniach ilościowych brano pod uwagę trzy główne związki zapachowe grzybów: 3-oktanon, 3-oktanol i 1-okten-3-ol. Największą zawartość 1-okten-3-olu odnotowano w *P. ostreatus*, natomiast znacząco mniejszą w *P. citrinopileatus*. Aromat badanych trzech gatunków *Pleurotus* był także różnicowany w profilowej analizie sensorycznej oraz na nosie elektronicznym. Chiralna chromatografia gazowa wykazała wysoką czystość optyczną (R)-(-)-1-okten-3-olu w *P. ostreatus* i *P. djamor* (najwyższa) w przeciwieństwie do *P. citrinopileatus*. Owocniki *P. djamor* charakteryzowały się poza tym relatywnie dużą zawartością suchej substancji i białka.

Słowa kluczowe: *Pleurotus* spp. aromat, 1-okten-3-ol, chiralność, ocena sensoryczna, nos elektroniczny

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