Chapter 33

PLEUROTUS OSTREATUS VOLATILE AROMA COMPOUNDS IDENTIFIED FROM FRUIT-BODY AND FROM MYCELIUM GROWN IN SUBMERGED AND SOLID-STATE CULTURES

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Abstract

Comparative analyses of volatile aroma compounds of the *Pleurotus ostreatus* JMO.95 fruit-body and its corresponding mycelium grown in liquid, on agar surface, or on solid support cultures have been carried out by dynamic headspace concentration using GC/MS and GC/sniffing. The aroma of the fruit-body was due essentially to the presence of octan-3-one and, in a lesser extent, to the presence of octan-3-ol. Other compounds, such as oct-1-en-3-ol, oct-1-en, 2-methylbutanol and α-pinene were also present in low concentrations. The comparison of the aromatic spectra of the fruit-body with the aromatic spectra of mycelia obtained under different culture conditions indicated that the main aromatic compounds present in the *P. ostreatus* fruit-body were also produced by the mycelium and generally in the same proportions, except for culture under submerged conditions.

Key words: *Pleurotus ostreatus*, mushroom fruit-body, mycelium, oct-1-en-3-ol, aroma, headspace concentration, GC/MS, GC/sniffing.

Introduction

Edible mushroom cultivation has greatly increased worldwide in the last decade (**1**). This increase in production has resulted from the interesting flavour characteristics of mushrooms and improvements in their growth processes. Mushrooms such as *Agaricus bisporus, Pleurotus ostreatus* and *Lentinus edodes* are currently cultivated on industrial scale according to well-defined processes. *P. ostreatus* is the second most important mushroom produced in Europe, after *A. bisporus*. The fructification of *P. ostreatus* is simpler than that for *A. bisporus* utilising less elaborated compost over a shorter incubation period (**2**).

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Several papers describing and comparing mushroom flavour compounds produced by the fruit-bodies andby the mycelial biomass exhibiting aromatic properties have been published. Most of them were in relation to the genera *Agaricus*, *Morchella* and *Pleurotus* (**3-9**). Other studies which have focused mainly on widespread species such as *Agaricus bisporus* (**10-14**) revealed the importance of the eight carbon atom compounds (C8) series and notably one molecule: oct-1-en-3-ol. These compounds play a major role in the development of the mushroom's aromatic properties and could represent up to 90% (w:w) of the volatile fraction issued from different fresh fruit-bodies (**11,15,16**). Pyysalo & Suihko (**11**) have shown that the aromatic specificity of seven edible fruit-bodies can be mainly explained by variations in the relative concentrations of these C8 molecules.

Little information is presently available on the aroma composition of both the fruit-body of *P. ostreatus* and its mycelium. The *P. ostreatus* strain JMO 95, which has the ability to fructify in controlled conditions, was chosen for its mycelial vigour and its strong aromatic note.

The aim of the present work was to compare, by means of headspace concentration and GC/SM identification, the volatile compounds exhaled from the *Pleurotus* sporocarpe and from its corresponding mycelium grown in agar surface, in liquid medium and on solid support impregnated with a nutritive solution.

Materials and Methods

Material and maintenance.

P. ostreatus (Jacq.:Fr) Kummer, strain INRA-JMO.95 was isolated in 1995 from dead elm trees in Brittany (France). It is characterised by small caps, a still grey colour, a very short stipe and an abundant fructification with well-isolated fruit-bodies. It was selected among other *Pleurotus* for its fragrant potential. The mycelium was maintained on Potato Dextrose Agar (PDA, Difco) before inoculation. Fruit-bodies and mycelial fractions were treated in duplicate. All mycelial fractions recovered from liquid, surface or solid-state cultures were frozen until chromatographic analysis was performed.

Submerged culture

Mycelial colonies incubated for 5 to 7 days on Raper solid medium (**17**) and having a diameter up to 3 or 4 cm in Petri dishes were carved in aseptic conditions, pounded in a Polytron homogenizer at high speed, in fresh sterile Raper solid medium and cooled on ice. 5 ml of this mycelial suspension were transferred to a 250 ml Erlenmeyer containing 100 ml of liquid Raper medium and incubated at 24-25°C on a shaker with intermittent orbital agitation (100 rpm) with 48 hours periods.

Surface culture.

Cultures of *P. ostreatus* JMO.95 mycelium were grown on agar Raper medium (1.5%).

Solid support culture

The culture system was composed of sugarcane bagasse as an inert support impregnated with the nutritive solution with up to 78% of initial humidity (**18**). The composition of the nutritive solution was (in $g.L^{-1}$): glucose, 30.0; urea, 2.68; yeast extract, 6.0; KH_2PO_4, 2.0; $MgSO_4.7H_2O$, 2.0; $NaH_2PO_4.H_2O$, 1.8. Impregnated sugarcane bagasse fractions (35 g) were introduced in 250 ml flasks and sterilised at 120°C for 20 minutes. Inoculation was carried out with 3 pieces of 1 cm^2 of mycelial colonies of *P. ostreatus* JMO.95 cultivated for 10 days on PDA at 25°C. These inoculum pieces were equally distributed into the flask. Cultures were incubated at 25°C for 14 days without forced aeration.

Culture and fruiting on lignocellulosic substrates

The substrate used for *P. ostreatus* JMO.95 cultivation consisted of pasteurised wheat straw, according to Laborde (1987). Spawn was prepared on rye grains cooked and autoclaved. The spawning rate was 3% (w:w). Each culture unit consisted of a plastic bag filled with 5 Kg of spawned substrate. Spawn running conditions for 16 days were, 25°C, no light, no fresh air and 80% for the relative air humidity. Fruiting was induced by decreasing the temperature to 14-15°C, supplying fresh air at 150 m^3 h^{-1}, and 12 hours of cycled illumination a day (by day light lamps) and increasing air humidity up to 92%. First fruit-bodies were picked 16 days after incubation and the fructification continued over a 4-month period with a weak flush effect. Fruit-bodies used for aroma analysis were collected twice during the first flush, early stage (with a rolled margin) and 2 days later (with a flat margin).

Dynamic headspace concentration

Fruit-body or mycelial samples (on an average of 20 g) were placed in a glass cell (0.25 L capacity) directly connected to a dynamic headspace concentrator (CHISA device, SGE). Volatile compounds were concentrated on a TENAX trap with a stripping gas (helium) flow rate of 30 $mL.min^{-1}$ for 20 min at room temperature. Samples were desorbed with a headspace injector (CHISA device, SGE) directly connected to the analytical column. The temperature for desorption was 210°C and volatile compounds were cryofocused at -20°C in the column's head before being injected directly into the column (**19**).

Gas chromatography / Mass spectrometry

Analyses were carried out using a gas chromatograph (5890, Hewlett-Packard) and a mass selective detector (5971, Hewlett-Packard) with a potential of 70 eV for ionisation by electron impact. Headspace analyses were performed by a 50 m x 0.22 mm x 1μm dimethylpolysiloxane BP1 (SGE) fused silica capillary column. The

pressure of the carrier gas (helium) was fixed at 22 psi. The injector and detector temperatures were 210°C and 250°C, respectively. The temperature of the oven was programmed as follows: 50°C to 220°C (3° C min^{-1}).

Gas-chromatography / sniffing

Headspace analysis was carried out by a purge and trap injector (DCI device, DELSI Instruments) connected to a gas chromatograph (DELSI 30) and performed with a 50 m x 0.32 mm x 1µm dimethylpolysiloxane SBP1 fused silica capillary column. Pressure of carrier gas (helium) was 14.5 psi. The detector temperature was 230°C and the temperature of the oven was programmed from 50°C to 220°C (3°C min^{-1}). Temperatures of the trap system for concentration and desorption were -20°C and 250°C respectively (**19**). Odour profile description was obtained using sniffing-port (olfactory detector, SGE) with a ratio FID 30% / Sniffing, 70%. GC/sniffing was performed by three persons using the olfactory referential "Le champ des odeurs" (**20,21**).

All compounds were identified by comparison with the mass spectral library NBS (**22**), literature spectra (**23-25**), Kovats indices data from the literature (**24,25**) and from our own data bank. The Kovats indices were calculated using n-alcanes (C_5-C_{18}); for the headspace technique, 1 µL of the mix was deposited in the glass cell and analysis was carried out as previously described.

Results and Discussion

Direct headspace analysis allowed trapping of the most volatile compounds present in the gaseous state in the closed atmosphere of the mushroom. This analysis reflected an image of the aroma perceived by the human olfactory system (**19**).

The identification of aroma compounds of *P. ostreatus* JMO.95 fruit-bodies collected twice during the first flush (Table 1), demonstrated the presence of octan-3-one as the major volatile molecules, representing 80% of the total GC/MS integrated area. This compound, whose odour was described as sweet (**10**), fruity or mildewy at high concentration (**11**), is responsible for the fruity lemon-like odour of *P. ostreatus*. Octan-3-ol, whose concentration corresponded to an average of 14.3% of the integrated area, participated in the hazelnut and herbaceous sweet note. Nevertheless, the oct-1-en-3-ol, generally described as the key compound of the fresh mushroom aroma, was found to constitute only a very low proportion in the fruit-body profile. The absence of other molecules having eight carbon atoms such as oct-1-en-3-one, oct-2-en-1-ol and octan-1-ol was noted. Furthermore, the composition of the aroma of the *P. ostreatus* fruit-body during the mature stage changed slightly with respect to the proportions of oct-1-en-3-ol and benzilic acid (increase) and of 2-methylbutanol and 2-methylbutanal (decrease).

The *P. ostreatus* mycelium grown in liquid medium, on agar surface, or on solid support cultures, were shown to be able to produce most of the aroma compounds

involved in fruit-body aroma. However, the proportions between these different molecules changed significantly according to the conditions used (Table 1).

Mycelium from solid-state culture showed the highest diversity of aroma compounds, even more than the fruit-body. Nevertheless, the olfactory impact corresponded to the characteristic *Pleurotus* aroma. Thus, oct-1-en-3-one appeared to be responsible for the cooked mushroom note, octanal for a honey-orange like odour and octanol for an orange-rose like odour. Traces of 1,3-octadiene were also present. This compound, having a fruity-green odour, was rarely found among C8 molecules that constituted the typical mushroom aroma. A great similarity in the proportions of octan-3-one and octan-3-ol was observed between aromatic profiles issued from the fruit-body and the mycelium cultivated on sugarcane bagasse impregnated with a nutritive solution.

Table 1. Headspace analysis of aroma compounds from the fruit-body and the mycelium of *P. ostreatus* grown in liquid and solid-state conditions.

Identified compounds [a]	Kovats indices	Fruit-Body (3 days storage)		Mycelium culture			Odour notes on the sniffing port
		Young	Mature	Solid support (16 days)	Agar surface (21 days)	Liquid medium (21 days)	
2-methylbutanal	639	[b] 0,2	-	-	-	-	-
1-heptene	683	-	-	0,06	-	-	-
3-methylbutanol	716	-	-	-	-	5,4	-
2-methylbutanol	721	4,1	1,6	9	-	16,2	-
pentan-1-ol	747	-	-	-	0,5	-	-
1-hexanal	777	-	-	2		-	green
oct-1-ene	787	0,9	0,8	3,9	0,1	-	fruity - etherous
*(E)*1,3-octadiene	817	-	-	0,7	-	-	-
*(Z)*1,3-octadiene	819	-	-	0,7	-	-	-
benzaldehyde	939	-	-		1,9	-	mild spicey
a-pinenc	940	-	traces	0,01	-	-	piney
oct-2-en-3-one	957	-	-	3,9	traces	-	fungal
oct-1-en-3-ol	964	0,6	1,6	0,3	1	38,5	fungal
octan-3-one	968	79,9	80,2	72,5	67,4	36,2	fungal - citrus
octan-3-ol	980	14,8	13,8	11,3	26,5	1,7	fungal
octan-1-al	983	-	-	0,4	-	-	orange, honeyed
benzilic acid	1047	traces	0,6	-	-	-	amine like
oct-2-en-1-ol	1050	-	-	1,3	traces	-	orange - rose like
octan-1-ol	1052	-	-	0,04	0,7	-	amine like
anisaldehyde	1234	-	-	-	0,9	-	anised

[a] The identifications of fragmentation spectra from GC-MS analysis were based on Hewlett Packard NBS data.

[b] Relative percentage of the volatile compounds based on the total integrated chromatographic area

On solid agar surface, the aromatic profile of the mycelium resembled that of the fruit-body showing presence of several C8 molecules (Table 1). In this case, the relative proportion of octan-3-ol was the highest comparatively to the solid support and the fruit-body profiles. In liquid culture, the volatile fraction of *P. ostreatus*

mycelium was characterised by the predominance of oct-1-en-3-ol and of octan-3-one (Table 1). Meanwhile, the proportion of octan-3-ol was very low. 2-methylbutanol, found at a relatively high concentration (16.2%), brought a characteristic spicy and repellent odour. These variations in the composition of the aromatic fraction of the mycelium, as compared to these of *P. ostreatus* JMO.95 fruit-body, explain the differences observed in the final aroma. Furthermore, the aromatic intensity perceived for liquid cultures was very low compared to that of fruit-body.

It can be concluded that the composition of the aroma produced by *P. ostreatus* JMO.95 mycelium is directly dependent on the type of culture. Various aromatic profiles were produced under liquid, surface and solid-state culture conditions, but the best aromatic similarities with the fruit-bodies of *P. ostreatus* JMO.95 were obtained with the mycelium grown on sugarcane bagasse impregnated with nutritive solution. Furthermore, the difference noticed between the aromatic spectra of the fruit-bodies and those of the mycelia, developed on agar surface or issued from liquid culture, both obtained with the same culture medium composition (except agar) and environmental conditions, could be explained by the mode of growth.

In liquid culture, mycelial growth involved pellet formation. The microorganism was probably under stress culture conditions, particularly with the low availability of dissolved oxygen. In solid-state conditions, agar surface and solid support, the growth was apical and mycelium development was related with the natural hyphal growth.

The relatively long cycle of fructification, as well as the output of fruit-body production, constituted major disadvantages for *P. ostreatus* industrial aroma production. The production of *Pleurotus* aroma by the mycelium of the selected *P. ostreatus* JMO.95 growing on solid support, which occurred in a shorter period of time and at lesser costs, would be considered as an excellent alternative.

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References

1. Chang S.T., Miles P.G. (1991) *Mushrooms J.* **504**, 15-15.
2. Laborde J. (1987) P.H.M.- *Revue Horticole* **278**, 13-19.
3. Humfeld H., Sugihara F. (1952) *Mycologia* **44**, 605-620.
4. Litchfield J.H. (1967) *Biothechnol. Bioeng.* **9**, 289-304.
5. Chahal D.S., Khan S.M. (1991) *In* Science and cultivation of edible fungi, (ed. Maher), Balkema, Rotterdam, pp. 709-716.

6. Hadar Y., Cohen-Arazi E. (1986) *Appl. Environ. Microbiol.* **51**, 1352-1354.
7. Hadar Y., Dosoretz C.G. (1991) *Trends Food Sci. and Technol.* **2**, 214-218.
8. Bensoussan M., Tisserand E., Kabbaj W., Roussos S. (1995) *Crypt. Mycol.*, **16**:65-75.
9. Kabbaj W., Bensoussan M., Roussos S. (1997). *In* Advances in Solid State Fermentation (eds. S. Roussos, B.K. Lonsane, M. Raimbault and G. Viniegra-Gonzalez), pp 437-448. Kluwer, Dordrecht.
10. Cronin D.A., Ward M.K. (1971) *J. Sci. Food Agric.* **22**, 477-479.
11. Pyysalo M., Suihko M. (1976) *Lebensm.-Wiss. u.-Technol.* **9**, 371-373.
12. Buchbauer G., Jirovetz L., Wasicky M., Nikiforov A. (1993) *Z. Lebensm. Unters. Forsch.* **197**, 429-433.
13. Grove J.H. (1981) *Phytochem.* **20**, 2021-2022.
14. Fisher K.H., Grosch W. (1987) *Lebensm.-Wiss. u.-Technol.* **20**, 233-236.
15. Latrasse A., Degorce-Dumas J.R., Leveau J.Y (1985) *Sciences des Aliments* **5**, 1-26.
16. Vidal J.P., Toulemonde B., Richard H. (1986) *Lebensm.-Wiss. u.-Technol.* **19**, 353-359.
17. Raper C.A., Raper J.R. (1972) *Mycologia* **64**, 1088-1093.
18. Roussos S., Bresson E., Saucedo-Castañeda G., Martinez P., Olivier J.M., Guimberteau J. (1997) *In* Advances in Solid State Fermentation (eds. S. Roussos, B.K. Lonsane, M. Raimbault and G. Viniegra-Gonzalez), pp 483-500. Kluwer: Dordrecht.
19. Talou T., Roule K., Gaset A. (1996) *Arômes Ingrédients Additifs* **3**, 34-36.
20. Jaubert J.N., Gordon G., Dore J.C. (1987) *Parfums, Cosmet., Aromes*: **78**, 71-82.
21. Jaubert J.N., Tapiero G., Dore J.C. (1995) *Perfum. Flavor.* **20**, 1-16.
22. McLafferty F.W., Stauffer D.B. (1989) The Wiley NBS Registry of Mass Spectral Data. Wiley J. & Sons : New York, USA.
23. Stenhagen E., Abrahamson S., McLafferty F.W. (1976) Registry of Mass Spectral Data. Wiley J. & Sons: New York, USA.
24. Jennings W., Shibamoto T. (1980) Qualitative analysis of flavor and fragance volatiles by glass capillar gas chromatography. Academic Press : New-York, USA.
25. Adams R.P. (1989) Identification of essential oils by ion trap Mass Spectroscopy; Academic Press, Inc.: San Diego, California, USA.

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