0273-2289/02/102–103/0463/$11.75

Comparison of Volatile Compound Production in Fruit Body and in Mycelium of *Pleurotus ostreatus* Identified by Submerged and Solid-State Cultures

W. Kabbaj,[1] S. Breheret,[2] J. Guimberteau,[3] T. Talou,[2] J.-M. Olivier,[3] M. Bensoussan,[4] M. Sobal,[1] and S. Roussos*,[1]

[1]Equipe de Mycologie et de Fermentation en Milieu Solide IRD UR-119, IFR-BAIM, ESIL, Universités de Provence et de la Méditerranée, Case 925, 163 Avenue de Luminy, F-13288 Marseille Cedex 9, France, E-mail: roussos@esil.univ-mrs.fr; [2]Laboratoire de Chimie Agroindustrielle, I.N.P. de Toulouse, 31077 Toulouse, France; [3]Station de Recherche des Champignons INRA, BP. 81, 33883 Villenave d'Ornon Cedex, France; and [4]Laboratoire de Microbiologie-UA, ENSBANA, 21000 Dijon, France

Abstract

Comparative analyses of the production of volatile compounds by *Pleurotus ostreatus* JMO.95 fruit body and its corresponding mycelium grown in liquid, on agar surface, and on solid support cultures were carried out by dynamic headspace concentration using gas chromatography/mass spectrometry and gas chromatography sniffing. The aroma produced by fruit body was owing essentially to the presence of octan-3-one and, to a lesser extent, to 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. Comparison of aromatic spectra of the fruit body with that of mycelia obtained under different culture conditions indicated that the main aromatic compounds present in the *P. ostreatus* fruit body and mycelium were produced in the same proportions on agar surface and on solid support culture, but not under submerged conditions.

Index Entries: *Pleurotus ostreatus*; mushroom fruit body; mycelium; oct-1-en-3-ol; aroma; headspace concentration; gas chromatography/mass spectrometry; gas chromatography sniffing.

Introduction

Edible mushroom cultivation has greatly increased worldwide in the last decade *(1,2)*. This increase in production has been a result of interesting

*Author to whom all correspondence and reprint requests should be addressed.

flavor characteristics of mushrooms and improvements in their growth processes. Mushrooms such as *Agaricus bisporus*, *Pleurotus ostreatus*, and *Lentinus edodes* are currently cultivated on an 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 a simpler process than that for *A. bisporus*, utilizing less elaborated compost during a shorter incubation period *(2)*. Several articles describing and comparing mushroom flavor compounds produced by the fruit bodies with those resulting from mycelial biomass exhibiting aromatic properties have been published. Most are in relation to the genera *Agaricus*, *Morchella*, and *Pleurotus (3–9)*. Other studies that have focused mainly on widespread species such as *A. 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 aromatic note and could represent up to 90% (w/w) of the volatile fraction issued from different fresh fruit bodies *(14–16)*.

We 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 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 under controlled conditions, was chosen for its mycelial vigor and strong aromatic note. The aim of the present work was to compare, by means of headspace concentration and gas chromatography/mass spectrometry (GC/MS) identification, the volatile compounds released from the *Pleurotus* carpophore and from its corresponding mycelium grown in agar surface, in liquid medium, and on solid support impregnated with a nutritive solution.

Materials and Methods

Strain and Maintenance

P. ostreatus (Jacq.:Fr) Kummer, strain INRA-JMO.95 was isolated in 1995 from dead elm trees in Brittany, France. The fruit body is characterized by small caps, a steel gray color, a very short stipe, and an abundant fructification with well-isolated fruit bodies. This strain was selected among other *Pleurotus* isolates for its fragrant potential, growth velocity, and colonization of the medium. 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.

Submerged Culture

Mycelial colonies incubated for 5–7 d on Raper solid medium *(17)* and having a diameter up to 3 or 4 cm were carved in Petri dishes in aseptic

conditions; pounded to a homogenizer Polytron at high speed in fresh, sterile Raper solid medium; and cooled on ice. Five milliliters of this mycelial suspension was transferred to a 250-mL Erlenmeyer flask containing 100 mL of liquid Raper medium and incubated at 24–25°C in an orbital shaker with intermittent agitation (100 rpm) for 48-h periods.

Surface Culture

Cultures of *P. ostreatus* JMO.95 mycelium were grown on Raper medium containing 15 g/L of agar, incubated at 25°C for 7 d.

Solid-Support Culture

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

Culture and Fruiting on Lignocellulosic Substrates

The substrate used for *P. ostreatus* JMO.95 cultivation consisted of pasteurized wheat straw *(2)*. Spawn was prepared on rye grains cooked and autoclaved. The spawn 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 d, were 25°C, no light, no fresh air, and 80% relative air humidity. Fruiting was induced by decreasing the temperature to 14 to 15°C, supplying fresh air at 150 m^3/h, providing 12 h of cycled illumination per day (by daylight lamps), and increasing air humidity up to 92%. The first fruit bodies were harvested 16 d after incubation, and fructification continued for 4 mo 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 d later (with a flat margin).

Dynamic Headspace Concentration

Fruit-body or mycelial samples (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 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 headspace 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 ionization by electron impact. Headspace analyses were performed using a 50 m × 0.22 mm × 1 µm 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 and 250°C, respectively. The temperature of the oven was programmed at 50–220°C (3°C/min).

GC/Sniffing

Headspace analysis was carried out using a purge-and-trap injector (DCI device; Delsi) connected to a gas chromatograph (Delsi 30) and performed with a 50 m × 0.32 mm × 1 µm dimethylpolysiloxane SBP1 fused silica capillary column. Carrier gas (helium) pressure was 14.5 psi. The detector temperature was 230°C and the oven temperature was programmed at 50–220°C (3°C/min). Temperatures of the trap system for concentration and desorption were –20 and 250°C, respectively *(19)*. Odor profile description was obtained using a sniffing-port (olfactory detector; SGE) with a ratio of 30% FID/70% sniffing. GC/sniffing was performed by a panel of trained jury (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)*, and Kovats indices data from the literature *(24,25)* and from our own databank. 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 enabled trapping of the most volatile compounds present in the gaseous state in the closed atmosphere of the mushroom. This analysis could be considered a good representation of the aroma perceived by the human olfactory system *(19)*.

Identification of the aroma-related compounds of *P. ostreatus* JMO.95 fruit body collected twice during the first flush (Table 1) provided evidence of the presence of octan-3-one as the major volatile molecule, representing 80% of the total GC/MS integrated area. This compound, whose odor was described as sweet *(11)*, fruity, or mildewy at high concentration *(14)*, is responsible for the fruity lemon-like odor of *P. ostreatus*. Octan-3-ol, with an average concentration of 14.3% of the integrated area, resulted in a hazelnut and sweet herbaceous odor. Oct-1-en-3-ol, generally described as

Table 1
Headspace Analysis of Aroma Compounds from Fruit Body and Mycelium of *P. ostreatus* Grown in Liquid and Solid-State Conditions

Identified compound[a]	Kovats indices	Fruit body (3-d storage)[b]		Mycelium culture			Odor notes on sniffing port
		Young	Mature	Solid support (16 d)	Agar surface (21 d)	Liquid medium (21 d)	
2-Methylbutanal	639	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, spicy
α-Pinene	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 identification of fragmentation spectra from GC/MS analysis was based on Hewlett-Packard NBS data.
[b]Relative percentage of the volatile compounds based on the total integrated chromatographic area.

the key compound of 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 observed during the mature stage changed slightly with respect to the proportions of oct-1-en-3-ol and benzilic acid, which increased, and of 2-methylbutanol and 2-methylbutanal, which decreased.

P. ostreatus mycelium grown in liquid medium, on agar surface, or on solid support cultures produced most of the volatile compounds involved in fruit-body aroma. However, the proportions of these different molecules changed significantly according to the culture conditions (Table 1). Mycelium from solid-state culture showed the highest diversity of volatile compounds, even more than the fruit body. Nevertheless, the olfactory impact corresponded to the characteristic *Pleurotus* aroma, presumably owing to the constant concentration of oct-1-en-3-one in comparison with that in the fruit bodies. Thus, oct-1-en-3-one appeared to be responsible for the cooked mushroom note, octanal with a honey-orange-like odor, and octanol with an orange-rose-like odor. Traces of 1,3-octadiene were also present. This compound, having a fruity-green odor, was rarely found among C8 molecules that constituted the typical mushroom aroma.

On solid agar surface, the aromatic profile of the mycelium resembled that of the fruit body containing several C8 molecules (Table 1). However, the relative proportion of octan-3-ol was higher than that found in the solid support and the fruit-body profiles. In liquid culture, the volatile fraction of *P. ostreatus* mycelium was characterized 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 spicy characteristic and repellent odor. These variations in the composition of the aromatic fraction of the mycelium, as compared with those of *P. ostreatus* JMO.95 fruit body, explain the differences observed in the final aroma. Furthermore, the aromatic intensity perceived from liquid cultures was very low compared with 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 from the mycelium grown on sugarcane bagasse impregnated with nutritive solution. Furthermore, the differences noted between the aromatic spectra of the fruit bodies and those of the mycelia developed on agar surface or in liquid culture, both obtained with the same culture medium composition and environmental conditions, could be explained by the mode of growth. In liquid culture, mycelial growth involved pellet formation and growth under stress, particularly owing to the low concentration of dissolved oxygen. Under solid-state agar surface, and solid support conditions, the growth was apical and mycelium development was similar to normal hyphal growth in the fruit bodies.

The relatively long cycle of fructification and the low output of fruit-body production have constituted major disadvantages for industrial production of *P. ostreatus* aroma. 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 lower costs, should be considered as an excellent alternative method for industrial culture.

Acknowledgments

We thank Dr. T. C. Varzakas for helpful corrections. We are also grateful to IRD (ex-ORSTOM, France) for partial financial support and scholarship. This work was funded by the French Ministry of Research (no. 94 G 0086, aliment 2.002).

References

1. Chang, S. T. (1999), *Int. J. Medician Mushrooms* **1,** 1–7.
2. Martinez-Carrera, D., Aguilar, A., Martinez, W., Bonilla, M., Morales, P., and Sobal, M. (2000), in *Coffee Biotechnology and Quality*, Serra, T., Soccol, C. R., Pandey, A., and Roussos, S. (eds.), Kluwer, Dordrecht, The Netherlands, pp. 471–488.
3. Bensoussan, M., Tisserand, E., Kabbaj, W., and Roussos S. (1995), *Crypt. Mycol*. **16,** 65–75.
4. Chahal, D. S. and Khan, S. M. (1991), in *Science and Cultivation of Edible Fungi*, Maher, (ed.), Balkema, Rotterdam, pp. 709–716.
5. Hadar, Y. and Cohen-Arazi, E. (1986), *Appl. Environ. Microbiol*. **51,** 1352–1354.
6. Hadar, Y. and Dosoretz, C. G. (1991), *Trends Food Sci. Technol.* **2,** 214–218.
7. Humfeld, H. and Sugihara, F. (1952), *Mycologia* **44,** 605–620.
8. Kabbaj, W., Bensoussan, M., and Roussos, S. (1997), in *Advances in Solid State Fermentation*, Roussos, S., Lonsane, B. K., Raimbault, M., and Viniegra-Gonzalez, G. (eds.), Kluwer, Dordrecht, The Netherlands, pp. 437–448.
9. Litchfield, J. H. (1967), *Biotechnol. Bioeng.* **9,** 289–304.
10. Buchbauer, G., Jirovetz, L., Wasicky, M., and Nikiforov, A. (1993), *Z. Lebensm. Unters. Forsch*. **197,** 429–433.
11. Cronin, D. A. and Ward, M. K. (1971), *J. Sci. Food Agric.* **22,** 477–479.
12. Fisher, K. H. and Grosch, W. (1987), *Lebensm.-Wiss. u.-Technol*. **20,** 233–236.
13. Grove, J. H. (1981), *Phytochemistry* **20,** 2021, 2022.
14. Pyysalo, M. and Suihko, M. (1976), *Lebensm.-Wiss. u.-Technol.* **9,** 371–373.
15. Latrasse, A., Degorce-Dumas, J. R., and Leveau, J. Y (1985), *Sci. Aliments* **5,** 1–26.
16. Vidal, J. P., Toulemonde, B., and Richard, H. (1986), *Lebensm.-Wiss. u.-Technol.* **19,** 353–359.
17. Raper, C. A. and Raper, J. R. (1972), *Mycologia* **64,** 1088–1093.
18. Roussos, S., Bresson, E., Saucedo-Castañeda, G., Martinez, P., Olivier, J.-M., and Guimberteau, J. (1997), in *Advances in Solid State Fermentation*, Roussos, S., Lonsane, B. K., Raimbault, M., and Viniegra-Gonzalez, G. (eds.), Kluwer, Dordrecht, The Netherlands, pp. 483–500.
19. Talou, T., Roule, K., and Gaset, A. (1996), *Arômes Ingrédients Additifs* **3,** 34–36.
20. Jaubert, J. N., Gordon, G., and Doré, J. C. (1987), *Parfums, Cosmet. Aromes* **78,** 71–82.
21. Jaubert, J. N., Tapiero, G., and Doré, J. C. (1995), *Perfum. Flavor* **20,** 1–16.
22. McLafferty, F. W. and Stauffer, D. B. (1989), *The Wiley NBS Registry of Mass Spectral Data*, Wiley, New York.
23. Stenhagen, E., Abrahamson, S., and McLafferty F. W. (1976), *Registry of Mass Spectral Data*, Wiley, New York.
24. Jennings, W. and Shibamoto, T. (1980), *Qualitative Analysis of Flavor and Fragance Volatiles by Glass Capillary Gas Chromatography*, Academic, New York.
25. Adams, R. P. (1989), *Identification of Essential Oils by Ion Trap Mass Spectroscopy*, Academic, San Diego.