Volume 6 Number 3
December, 2003

ISSN 0972-060X

Journal of Essential Oil Bearing Plants

JOURNAL OF ESSENTIAL OIL-BEARING PLANTS

The Journal devoted to the promotion of research on Essential Oil

Jeobp 6 (3) 2003 pp 153-160

ISSN 0972-060X

Volatile components of fresh *Pleurotus ostreatus* and *Termitomyces shimperi* from Cameroon

Maximilienne Nyegue[1], Paul-Henri Amvam Zollo[1], Jean-Marie Bessière[2] and sylvie Rapior[3*]

[1] Laboratoire de phytobiochimie, Université de Yaoundé I, B.P. 812, Yaoundé, Cameroon.

[2] École nationale supérieure de chimie, 8 rue de l'École normale, F-34296 Montpellier cedex 5, France

[3] Laboratoire de botanique, phytochimie et mycologie, mycologie/UMR-CNRS (CEFE) Faculté de Pharmacie, B.P. 14491, 15 avenue Charles Flahault, F-34093 Montpellier cedex 5, France

Abstract: Odorous wild *Pleurotus ostreatus* and *Termitomyces shimperi* from Cameroon are highly appreciated for both their culinary value and their good taste. The edible mushrooms were investigated for volatile constituents by GC-MS using organic solvent extraction. Twenty-eight and 24 volatile components were identified by solvent method for *P. ostreatus* and *T. shimperi*, respectively, and biosynthesized from the lipidic, shikimic and terpenic pathways. The major odorous compounds identified in fresh *P. ostreatus* organic extract were C_8 components (mushroom odors) as octen-3-ol (59.3%), octen-3-one, octan-3-one, 3-octanol, n-octanal, (E)-2-octenal and n-octanol. Benzaldehyde (almond odor), benzyl alcohol (sweet-spicy odor) and phenylethanol (rose odor) as well as monoterpenes, i.e., linalool and linalool oxide detected from the Common Oyster Mushroom may also contribute to its pleasant flavour.

Many aliphatic compounds dominate the volatile content of fresh *T. shimperi*, i.e., C_6 derivatives (hexan-2-one, hexan-3-ol, hexanal, hexan-2-ol), C_7 derivative (hept-2-enol) and C_8 derivatives (octen-3-ol, 1-octen-3-one, octan-3-one, (E)-2-octenal). Within the aromatic components, phenylethanol was the main component identified from *T. shimperi*. Most of the volatiles identified from *P. ostreatus* and *T. shimperi* are widely used as aroma components in flavor industry due to their strong pleasant flavours.

Key Words: Basidiomycota, *Pleurotaceae*, *Tricholomataceae*, aroma, mushroom

Introduction: The increasing request for flavours since the 1980's has been supported by studies aiming to their extraction from natural sources of both plant and fungus

---

*Corresponding author (Sylvie Rapior)
e-mail address : srapior@ww3.pharma.univ-montp1.fr

kingdoms. These materials are often seasonal and of inconstant quality because of variations of climatic conditions, but they lead to define natural products highly appreciated by consumers. Producing pleasant and various odours, higher fungi constitute an attractive source of valorisation for food processing, cosmetic industries and medicinal value[3,16,17]. Several laboratories are interested in volatile metabolites produced by *Basidiomycota* in order to select odorous components and undergo bioconversion process[14,15]. Within the Basidiomycetes, mushrooms as *Pleurotus* and *Termitomyces* species represent a worldwide potential for the production of natural flavours[1,4].

For the present work, chemical studies on liposoluble components were carried out on two edible African Basidiomycetes, i.e., *Pleurotus ostreatus* and *Termitomyces shimperi*, highly appreciated by local populations in Cameroon. Both higher mushrooms were investigated for volatile constituents by solvent extraction and analysed using gas chromatography/mass spectrometry.

Experimental

Fungal material: *Pleurotus ostreatus* (Jacq. : Fr.) Kummer (*Basidiomycota, Agaricomycetideae, Pleurotaceae*[9]) grows in tropical areas in compact clusters on stumps and trunks of leaved trees during the raining season[18]. This species is highly appreciated for both its culinary value and good taste. The sporophores were collected and identified in the Laboratory of Mycology at the University of Yaounde[1].

Widespread in Cameroon and largely appreciated for its culinary value, *Termitomyces shimperi* (Pat.) Heim (*Basidiomycota, Agaricomycetideae, Tricholomataceae*[24]) grows in rural areas on termitaries built by *Macrotermes natalensis*[7,10]. The specimens were collected in Foulassi and identified in the Laboratory of Mycology at the University of Yaounde[1]. These samples were separated into three sets as the whole fungi, only the caps and only the stems.

Sample preparation: Fresh mushroom species were sliced and completely immerged in previously distilled hexane (Table 1, w/3v). Maceration was carried out once for 48 hours. Then the organic phases were evaporated under normal pressure. Hexane extracts of *P. ostreatus* and *T. shimperi* were stored at low temperature (4°C) and used directly for Gas Chromatography/Mass Spectrometry (GC-MS) analyses.

GC-MS analyses: GC/MS analyses were carried out using a gas chromatograph (5892-Hewlett-Packard) and a mass selective detector (5971-Hewlett-Packard) with a potential of 70 eV for ionization by electron impact. Analyses of volatile constituents from *P. ostreatus* and *T. shimperi* were performed using a 25 m x 0.25 mm x 0.13 µm polydimethylsiloxane DB-1 column fused silica capillary column. The carrier gas (helium) rate was 0.9 ml/min. Injector and detector temperatures were 200°C and 270°C, respectively. Temperature was programmed from 60°C (2 min) to 200°C at a rate of 4°C/min[22,23]. Chemical components were identified by comparison with retention indexes reported in literature [2,12].

Results and Discussion : Hexane extracts of *P. ostreatus* and *T. shimperi* are lightly yellow. Yields of the volatile components from fresh *P. ostreatus* and *T. shimperi* are reported in Table 1. Fresh whole *P. ostreatus* had the lowest yield (0.16%). For *T. shimperi*, yields were higher in the cap extract (0.41%) than in the stem (0.19%) or the whole sporophore (0.25%). Twenty-eight and 24 volatile components were identified by solvent method for *P. ostreatus* (Table 2) and *T. shimperi* (Table 3), respectively; they were biosynthesized from the lipidic, shikimic and terpenic pathways.

The volatile components of *P. ostreatus* extract are listed in Table 2. Our data show that the main volatile components from the Common Oyster Mushroom have three different pathways. The major C_8 components are octen-3-ol (59.3%) as well as octen-3-one, octan-3-one, 3-octanol, n-octanal, (E)-2-octenal and n-octanol. Combined benzaldehyde (almond odour), benzyl alcohol (sweet-spicy odour) and phenylethanol (rose odour) detected from *P. ostreatus* may contribute to the complex pleasant flavour of the mushroom as described for *Agaricus augustus*[27], *A. subrufecens*[8] and *Gyrophragmium dunalii*[22]. Moreover, monoterpenes, i.e., α -pinene, *p*-cymene as well as linalool and linalool oxide were also identified and possessed pleasant odours [5].

A comparative study was done with Korean *P. ostreatus*; the specimens were also characterised by a high rate of C_8 components, i.e., octen-3-ol (67%), octen-3-one (5.5%) and octan-3-ol (4.9%) as reported by authors[11,13]. All of these components were also identified from fresh Cameroon mushroom. Otherwise, there is a close volatile composition between the flavour profile of *P. ostreatus* and those of *P. florida* and *Calocybe indica* from India[25,26].

The composition of volatiles of *T. shimperi* was reported for the first time in Table 3. Numerous aliphatic compounds dominate the volatile content of the mushroom, i.e., C_6 derivatives (hexan-2-one, hexan-3-ol, hexanal, hexan-2-ol) and C_7 derivative (hept-2-enol) as well as C_8 derivatives (octen-3-ol, 1-octen-3-one, octan-3-one, (E)-2-octenal). Aromatic components also occurred from *T. shimperi* mainly phenylethanol that was present in both cap and stem aromas. No terpene derivatives were detected from *T. shimperi*.

Finally, our studies showed that many components such as hexanal, octen-3-one, octen-3-ol, octan-3-one, octan-3-ol, and phenylethanol, identified in the Cameroon mushroom were also reported for aromatic extracts of American, Asian and European Basidiomycetes[5,6,20-23,25-27].

Conclusion : *P. ostreatus* and *T. shimperi* from Cameroon are highly appreciated for both culinary value and good taste as another African related species *T. striatus* from Ivory Coast[19]. Aromatic extracts of fresh *P. ostreatus* and *T. shimperi* (whole plant, cap and stem) were obtained by organic solvent maceration. Yields of volatiles from whole fungi clearly depended on the investigated species, i.e., 0.16% for *P. ostreatus* and 0.25% for *T. shimperi* (Table 1). Within the *T. shimperi* specimens, yields varied according to the organ extracted with the organic solvent as 0.41% for the cap extract and 0.19% for the stem extract.

In terms of volatile composition, the GC/MS analyses revealed the presence of C_8 derivatives as well as arenic compounds and terpenes (Tables 2, 3). Many of them were identified in other Basidiomycetes and well known for their specific pleasant flavour. C_8-Derivatives (fungal odours) as well as 2-phenylethanol (rose odour) and benzaldehyde (almond odour) are widely used as aroma components in flavour industry due to their strong odours[1,15,21-23]. Higher mushrooms as *P. ostreatus* and *T. shimperi* are available and valuable sources of natural volatile components in Cameroon. Production of flavours could be locally carried out in order to exploit the broad odorous spectrum of both African mushrooms.

References

1. **Abraham, B., Krings, U. and Berger, R.G.(1993).** Dynamic extraction, an efficient screening procedure for aroma producing Basidiomycetes. Chem. Mikrobiol. Technol. Lebensm. 15: 178-181.
2. **Adams, R.P.(2000).** Identification of essential oil components by Gas Chromatography/Quadripole Mass Spectroscopy. Allured Publishing Corporation, Illinois. 442 pp.
3. **Bano, Z. and Rajarathnan, S.(1988).** *Pleurotus* mushrooms. Part II. Chemical composition, nutritional value, post-harvest physiology, preservation, and role as human food. Critical Reviews in Food Science and Nutrition 27: 87-158.
4. **Beltrangarcia, M.J., Estarronespinosa, M. and Ogura, T.(1997).** Volatile compounds secreted by the oyster mushroom (*Pleurotus ostreatus*) and their antibacterial activities. J. Agric. Food Chem. 45: 4049-4052.
5. **Breheret, S., Talou, T., Rapior, S. and Bessière, J.M.(1997a).** Monoterpenes in the aromas of fresh wild mushrooms (Basidiomycetes). J. Agric. Food Chem. 45: 831-836.
6. **Buchbauer, G., Jirovetz, L., Wasicky, M. and Nikiforov, A.(1993).** Zum Aroma von Speisepilzen. Zeitschr. Lebensmit-Untersuch. und -Forsch. 197: 429-433.
7. **Ceballos, L.(1992).** Le genre *Termitomyces* Heim en Afrique. Thèse Pharmacie, Université Montpellier 1. 128 p.
8. **Chen, C.C. and Wu, C.M.(1984).** Volatile components of mushroom (*Agaricus subrufecens*). J. Food Sc. 49: 1208-1209.
9. **Courtecuisse, R._and Duhem, B.(2000).** Guide des champignons de France et d'Europe. Delachaux et Niestlé (eds), Lausanne. 476 pp.
10. **Heim, R.(1977).** Termites et champignons. Boubée (ed.), Paris. 205 pp.
11. **Hong, J.S., Lee, J.Y., Kim, Y.H., Kim, M.K., Jung G.T. and Lee, K.R.(1986).** Studies on the volatile aroma components of *Pleurotus ostreatus*. Kor. J. Mycol. 14: 31-36.
12. **Jennings, W. and Shibamoto, T.(1980).** Qualitative analysis of flavour and fragrance volatiles by capillary gas chromatography. Academic Press, New York. 472 pp.
13. **Jung, S.T. and Hong, J.S.(1991).** Volatile components of Oyster Mushrooms (*Pleurotus* sp.) cultivated in Korea. Kor. J. Mycol. 19: 299-305.
14. **Krings, U. and Berger, R.G.(1988).** Biotechnological production of flavours and fragrances. Appl. Microbiol. Biotechnol. 49: 1-8.
15. **Lesage-Meesen, L., Delattre, M., Haon, M., Thibault, J.F., Colonna Ceccaldi, B., Brunerie, P. and Asther, M.(1996).** A two-step bioconversion process for

vanillin production from ferulic acid combining *Aspergillus niger* and *Pycnoporus cinnabarinus*. J. Biotechnol. 50: 107-113.

16. **Ogundana, S.K. and Fagade, O.E.(1982).** Nutritive value of some Nigerian edible mushrooms. Food Chemistry 8: 263-268.
17. **Pegler, D.N. and Piearce, G.D.(1980).** The edible mushrooms of Zambia. Kew Bull. 35: 475-492.
18. **Phillips, R.(1981).** Les champignons. Solar (ed.), Hong Kong. 182-183.
19. **Rapior, S., Pélissier, Y., Vassas, A., Greuet, J. and Kone-Bamba, D.(1993).** *Termitomyces striatus* (Beeli) Heim *forma annulatus*. Identification et étude préliminaire de la composition chimique : Recherche des polyols, des sucres, des alcaloïdes, des acides phénols et des toxines. Le Pharmacien d'Afrique 77: 19-22.
20. **Rapior, S., Cavalié, S., Andary, C., Pélissier, Y., Marion, C. and Bessière, J.M.(1996).** Investigation of some volatile components of fresh wild mushrooms (Basidiomycetes). J. Essent. Oil. Res. 8: 199-201.
21. **Rapior, S., Breheret, S., Talou, T. and Bessière, J.M.(1997).** Volatile flavor constituents of fresh *Marasmius alliaceus* (Garlic *Marasmius*). J. Agric. Food. Chem. 45: 820-825.
22. **Rapior, S., Mauruc, M.J., Guinberteau, J., Masson, C.L. and Bessière, J.M.(2000).** The volatile composition of *Gyrophragmium dunalii*. Mycologia 92: 1043-1046.
23. **Rapior, S., Breheret, S., Talou, T., Pélissier, Y. and Bessière, J.M.(2002).** The anise-like odour of *Clitocybe odora*, *Lentinellus cochleatus* and *Agaricus essettei*, Mycologia 94: 373-376.
24. **Rouland-Lefèvre, C.(2003).** Du champignon pour les termites. Spécial Champignons Magazine 35: 18-21.
25. **Venkateshwarlu, G., Chandravadana, M.V. and Tewari, R.P.(1999).** Volatile flavour components of some edible mushrooms (Basidiomycetes). Flavour Fragr. J. 14: 191-194.
26. **Venkateshwarlu, G., Chandravadana, M.V., Pandey, M. and Tewari, R.P.(2000).** Volatile flavour compounds from oyster mushroom (*Pleurotus florida*) in submerged culture. Flavour Fragr. J. 15: 320-322.
27. **Wood, W.F., Watson, R.L. and Largent, D.L.(1990).** The odor of *Agaricus augustus*. Mycologia 82: 276-278.

Table 1: Yield of volatile components of wild *P. ostreatus* and *T. shimperi* from Cameroon

Mushroom specie	Place of harvesting	Date of harvesting	Date of maceration	Mushroom material's weight (g)	Extract's weight (g)	Yield (%)
P. ostreatus	Obala	05/04/1997	05/06/1997	110	0.18	0.16
T. shimperi (whole fungi)	Foulassi	07/24/1997	07/26/1997	1000	2.49	0.25
T. shimperi (cap)	Foulassi	07/24/1997	07/26/1997	375	1.55	0.41
T. shimperi (stem)	Foulassi	07/24/1997	07/26/1997	450	0.86	0.19

Table 2: Volatile composition of *P. ostreatus*

Volatile components	Retention Index	Percentage[a]
3-methylbutanol	782	0.2
hexana l	792	2.6
butyl acetate	812	0.3
3-methylbutyric acid	871	0.9
2-methylbutyric acid	901	0.3
2-acetyl-2-pyrroline	902	0.5
α-pinene	934	0.1
benzaldehyde	958	0.3
octen-3-one	965	1.2
octen-3-ol	973	59.3
octan-3-one	980	5.3
3-octanol	987	5.8
n-octanal	998	1.3
hexanoic acid	1000	0.3
p-cymene	1003	0.1
hexyl acetate	1009	0.4
benzyl alcohol	1020	0.2
(E)-2-octenal	1050	1.2
n-octanol	1053	1.1
linalool oxide	1063	0.2
linalool	1092	0.2
nonanal	1097	0.2
phenylethanol	1105	0.7
benzyl acetate	1152	0.3
octanoic acid	1180	0.3
nonanoic acid	1282	0.2
(Z,E)-deca-2,4-dienal	1286	0.2
(E,E)-deca-2,4-dienal	1302	0.5

[a] Relative percentage of the identified volatile component based on the GC-MS chromatographic area

Table 3 : Volatile composition of *T. shimperi*

Volatile components	Percentage[a]			
	RI	Whole plant	Cap	Stem
2-methylpentan-ol	755	0.8	0.2	tr.[b]
3-methylpentan-ol	767	0.4	0.3	0.1
hexan-2-one	779	0.4	0.2	tr.
hexan-3-ol	787	3.4	4.5	3.1
hexanal	792	11.8	16.5	11.3
hexan-2-ol	800	7.4	12.4	5.3
butyl acetate	812	0.5	0.9	0.4
(E)-hept-2-enol	942	1.3	2.9	1.0
octen-3-one	965	1.0	0.9	1.5
octen-3-ol	973	34.3	33.7	36.7
2-pentylfuranne	975	0.2	0.4	0.3
ethyl hexanoate	979	0.1	0.1	0.5
octan-3-one	980	2.1	2.7	1.4
(E)-2-octenal	1050	0.8	0.6	1.1
methyl benzoate	1069	0.9	0.4	1.2
nonanal	1097	1.6	0.8	0.8
phenylethanol	1105	19.5	18.5	22.6
octanoic acid	1180	-	0.1	0.1
(E,E)-nona-2.4 dienal	1187	1.3	2.4	0.9
decanal	1195	-	tr.	0.1
phenylethyl acetate	1223	0.3	0.2	0.8
(Z,E)-deca-2,4-dienal	1286	0.2	0.3	0.2
(E,E)-deca-2,4-dienal	1302	0.6	1.6	0.5
ethyl-(E)-cinnamate	1313	-	-	0.3

[a] Relative percentage of the identified volatile component based on the GC-MS chromatographic area, [b] traces