VOLATILE COMPONENTS OF FRESH *AGROCYBE AEGERITA* AND *TRICHOLOMA SULFUREUM*

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ABSTRACT: Two fresh wild mushrooms *Agrocybe aegerita* and *Tricholoma sulfureum* were investigated for volatile components by headspace concentration, organic solvent extraction and water-distillation using Gas Chromatography/Mass Spectrometry. Thirty-one and forty-two volatile constituents were identified from *A. aegerita* and *T. sulfureum*, respectively. C_8 derivative (3-octanone), phenylethyl derivatives (2-phenylethanal, 2-phenylacetamide, 2-phenylcrotonaldehyde) and monoterpenes (α-pinene, linalool were the main volatile components of fresh *A. aegerita*. 3-Hydroxybutan-2-one and 2-methylbutanol could have a role in the lees of wines flavour of this species. C_8 compound (1-octen-3-ol), benzene derivatives (ethylbenzene, styrene, xylene), monoterpenes (limonene, linalool) and indole derivatives were the major volatile compounds of *T. sulfureum*. Indole and 3-formylindole could be responsible for the gas-like odour of *T. sulfureum*. Comparison of extraction methods reveals large qualitative and quantitative differences in volatile metabolites for both fresh species.

KEY WORDS INDEX: Mushroom; *Basidiomycotina*; Volatile components; *Agrocybe aegerita*; *Tricholoma sulfureum*; Solvent extraction; Dynamic headspace concentration; Water distillation.

RÉSUMÉ: Les composés volatils de deux champignons sauvages frais, *Agrocybe aegerita* et *Tricholoma sulfureum* ont été étudiés par concentration des effluves, extraction par solvant et hydrodistillation, et identifiés par Chromatographie en phase Gazeuse couplée à la Spectrométrie de Masse. *A. aegerita* et *T. sulfureum* présentent respectivement 31 et 42 composés volatils. L'octan-3-one, le 2-phényléthanal, le 2-phénylacétamide, le 2-phénylcrotonaldéhyde ainsi que les monoterpènes (α-pinène, linalol) sont les principaux composés des sporophores d'*A. aegerita*. La 3-hydroxybutan-2-one et le 2-méthylbutanol pourraient jouer un rôle dans l'odeur de lie de vin de ce champignon frais. Par ailleurs, l'oct-1-èn-3-ol, les dérivés benzéniques (éthylbenzène, styrène, xylène), les monoterpènes (limonène, linalol) et les dérivés indoliques sont les composés volatils majoritaires de *T. sulfureum*. L'indole et le 3-formylindole pourraient être responsables de l'odeur de gaz d'éclairage de *T. sulfureum*.

INTRODUCTION

For ages, mycologists have been sniffing to their specimens for determining species. Considering the unique and subtle odours of mushrooms and because more and more industries are turning to a search for novel sources for natural aroma components, general reviews on mushroom flavour (Claus, 1978; Maga, 1981; Mau *et al.*, 1994; Rapior *et al.*, 1998) and studies relative to specific mushroom odours have been reported such as the anise-like aroma of *Hydnellum suaveolens* (Wood *et al.*, 1988; Solberg, 1989) and *Clitocybe odora* (Breheret *et al.*, 1996), the almond-like odour of *Agaricus augustus* (Wood *et al.*, 1990), the sweet odour of *Hebeloma sacchariolens* (Wood *et al.*, 1992), the cucumber odour of some *Tricholomataceae* and *Entolomataceae* species (Wood *et al.*, 1994), the garlic-like aroma of *Marasmius alliaceus* (Rapior *et al.*, 1997a) and the coal-tar odour of *Tricholoma inamoenum* (Watson *et al.*, 1986).

Although the fresh mushrooms have characteristic aroma properties, few published studies exist on comparative methods for the volatile composition of headspace concentrate, solvent extract and distillate from edible and non-edible species (Vidal *et al.* 1986; Buchbauer *et al.*, 1993). Comparison of volatile components from headspace concentrate and distillate was also reported by a few authors (Sulkowska & Kaminski, 1977; Yajima *et al.*, 1981; Charpentier *et al.*, 1986). Moreover, correlation between the odour and the profile of volatile components has been given little attention so far (Watson *et al.*, 1986; Rapior *et al.*, 1997a).

Only one study and none on volatile constituents was reported for odorous *Agrocybe aegerita* (Takama *et al.*, 1979) and *Tricholoma sulfureum* mushrooms, respectively. This paper focuses both on the extraction of volatile metabolites from fresh wild basidiocarps of *A. aegerita* and *T. sulfureum* by dynamic headspace concentration, organic solvent extraction and water-distillation, and identification of volatile substances using Gas Chromatography-Mass Spectrometry (GC/MS).

MATERIALS AND METHODS

Mushroom material

The fruit bodies representing a combination of young and old sporophores from two wild mushrooms, *Agrocybe aegerita* (Brig.) Fayod (= *Agrocybe cylindracea*, Poplar field cap) and *Tricholoma sulfureum* (Bull.:Fr.) Kummer (Gasworks Knight-cap) were collected in Fall 1996 in the South of France. *A. aegerita* is an edible mushroom with a fruity-wine flavour and *T. sulfureum* has a characteristic nauseating smell of gas (Courtecuisse & Duhem, 1994 ; Læssoe *et al.*, 1996). The fresh material was brushed clean of forest debris and treated immediately after collection.

Solvent extraction

The volatile components from 250 g of small mushroom cubes (approximately 100 mm^3) were extracted with 400 ml of dichloromethane in 500 ml sealed Erlenmeyer flask (Rapior *et al.*, 1996, 1997b). After maceration for seven days, the organic layer was separated and traces of water removed with anhydrous sodium sulfate as a drying agent. The dichloromethane extract was then filtered through a glass funnel with Whatman n°1 filter paper and concentrated to 1 ml into a bottle with teflon lined cap using a nitrogen stream.

Dynamic headspace concentration

Fresh chopped mushrooms, i.e., 45 g of *A. aegerita* and 13 g of *T. sulfureum* were placed in a glass cell (0.25 l capacity) directly connected to a dynamic headspace concentrator (CHISA device-SGE). Volatile components were concentrated on Tenax trap with a stripping gas (Helium) with a constant flow rate set close to 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 of desorption was 210°C; the volatile components were cryofocused at -20°C in the column head before to be injected directly in the column (Breheret *et al.*, 1997).

Water-distillation

The finely chopped fresh materials, i.e., 50 g of *A. aegerita* and 300 g of *T. sulfureum*, were water-distilled in an original glass apparatus for 4 h. The condensed water-distillates were extracted with dichloromethane and the solvent then removed *in vacuo* (Pélissier *et al.*, 1995).

Gas Chromatography-Mass Spectrometry

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.

Solvent extraction and water-distillation analyses were performed by a 25 m × 0.23 mm × 0.13 µm dimethylpolysiloxane OPTIMA 1 (M-N) fused silica capillary column. The carrier gas was helium with a constant flow rate set close to 0.6 ml/mm. The injector and detector temperatures were 200°C and 220°C, respectively. The column was temperature programmed as follows: 60°C (2 min) to 200°C (4°C/min).

Headspace analyses were performed by a 50 m × 0.22 mm × 1 µm dimethylpolysiloxane BP1 (SGE) fused silica capillary column. The carrier gas was helium and the pressure was fixed at 22 psi. The detector temperature was 250°C. The column was temperature programmed as follows: 50°C to 220°C (3°C/min).

Volatile component identification

The volatile constituents were identified by matching their mass spectra and retention indices with reference libraries (Stenhagen *et al*, 1976; Jennings & Shibamato, 1980; Adams, 1989; MacLafferty & Stauffer, 1989) and our own data bank.

RESULTS AND DISCUSSION

Volatile components of *A. aegerita*

The C_8 derivatives are the major volatile compound of fresh *A. aegerita* from headspace concentrate, organic extract and water-distillate. 3-Octanone (fungal-fruity odour) is the major component and 1-octen-3-ol (fungal odour) belongs to the minor fraction in the three extracts (Table 1) as described for *A. bisporus* headspace extract (Fischer & Grosch, 1987). These results are not in agreement with those reported by Takama *et al.* (1979) twho described the volatile 1-octen-3-ol as the main volatile component of *A. aegerita* organic extracts.

The phenylethyl derivatives, i.e., 2-phenylethanal (floral-green odour), 2-phenylacetamide and 2-phenylcrotonaldehyde represented more than 24 % of the volatile fraction from the extract. Monoterpenes such as α-pinene (resineous odour) in headspace concentrate and linalool (fresh odour) in both extract and distillate were detected at low levels (Breheret *et al.*, 1997).

3-Hydroxybutan-2-one and 2-methylbutanol with their respective etherous-fruity and dairy notes were detected at high levels in the dynamic headspace concentrate of *A. aegerita*. Thus, these volatile constituents seemed to have a role in the lees of wine aroma of fresh *A. aegerita*.

Volatile components of *T. sulfureum*

The C_8 compounds are also the main volatile metabolites of fresh *T. sulfureum* from headspace concentrate, solvent extract and water-distillate. The major volatile component is 1-octen-3-ol in all extracts (Table 2).

The benzene derivatives (ethylbenzene, styrene, xylene, propenylbenzene) represented *ca.* 5% of the volatile fraction from headspace extract.

Monoterpenes i.e., α-pinene, sabinene, β-pinene and *p*-cymene were detected in headspace concentrate. Limonene and linalool were identified in the three extracts (Breheret *et al.*, 1997).

Both indole and 3-formylindole were identified in the dichloromethane extract. Indole (animal odour) that was also detected in the water-distillate, is probably responsible for the town gas odour of *T. sulfureum*. Indeed, this volatile constituent was previously reported by Hilber (1968) for *T. inamoenum* (Fr.) Quél., a mushroom described as having a smell of illuminating gas (Moser, 1983). On the other hand, a mixture of benzaldehyde, 1-octen-3-ol and phenylacetaldehyde could also be the basic components of the repulsive "coal tar" aroma of *T. sulfureum* as described for *T. inamoenum* by Watson *et al.* (1986) using sensory tests.

Volatile Compounds	BP1 column RI[a]	BP1 column Headspace Sample[b]	Optima 1 column RI[a]	Optima 1 column Solvent Extract	Optima 1 column Steam Distillate
3-Hydroxybutan-2-one	707	16.1	715	0.2	
2-Methylbutanol	720	4.8	722	0.2	
Octene	785	7.8	789	0.3	
Ethylbenzene	850	2.1			
p- and/or *m*-Xylene	860	0.3			
Styrene	878	1.8	859	2.8	0.5
o-Xylene	883	0.1			
α-Pinene	938	0.3			
Benzaldehyde			938	0.8	0.2
1-Octen-3-one			958	0.9	2.2
1-Octen-3-ol	965	0.1	966	0.5	0.3
3-Octanone	966	64.5	962	47.9	37.6
3-Octanol	980	0.7	983	6.3	26.2
p-Cymene			1020		0.3
2-Phenylethanal			1024	1.1	0.8
Limonene			1028		0.1
(*E*)-2-Octenal			1038	0.1	0.2
(*E*)-2-Octenol			1056	1.9	11.1
Octanol			1060		4.6
Linalool			1095	2.0	0.9
2-Phenylethanol			1106	11.0	8.7
(*E*)-Non-2-enal			1136		0.1
2-Phenylcrotonaldehyde			1250	1.4	
Undecan-2-one			1293		0.1
(*2E,4Z*)-Decadienal			1304	0.1	0.2
2-Phenylacetamide			1330	11.5	
(*2E,4E*)-Decadienal			1345	0.3	0.7
4-Hydroxybenzaldehyde			1400	1.1	
3-Formylindole			1675	10.3	
Tetradecanol			1676	0.3	0.1
Pentadecan-2-one			1695	1.0	0.2

[a]Different retention indices due to the GC column used; [b]Relative percentage of the identified volatile based on the GC/MS chromatographic area

Table 1. Volatile composition (percentage) of three extracts from *A. aegerita*

Volatile Compounds	BP1 column RI[a]	BP1 column Headspace Sample[b]	Optima 1 column RI[a]	Optima 1 column Solvent Extract	Optima 1 column Steam Distillate
Hexan-2-ol			781	0.4	
Octene	785	3.9	789	0.1	
(*Z*)-1,3-Octadiene	817	3.0	823	0.1	
Ethylbenzene	850	1.7			
p — and/or *m*-Xylene	860	0.7			
Styrene	878	2.2	959	0.2	0.5
o-Xylene	883	0.2			
2-Propenylbenzene	934	0.2			
Benzaldehyde			938	3.7	6.2
α-Pinene	938	0.4			
Camphene	953	0.1			
1-Octen-3-one	956	0.8	958	3.1	5.3
1-Octen-3-ol	965	64.2	966	43.3	50.7
3-Octanone	966	11.5	962	0.2	0.8
Sabinene	972	0.1			
β-Pinene	979	0.1			
3-Octanol	980	2.6	980	0.3	0.5
2-Pentylfurane			986	0.1	
p-Cymene			1022		0.1
β-Phellandrene	1028	0.6	1025	0.5	
Limonene	1028	0.6	1026	0.6	1.4
2-Phenylethanal			1028	0.2	0.2
Benzyl alcohol			1035	2.0	0.5
(*E*)-2-Octenol	1049	0.5	1056		3.5
Octanol	1052	0.7	1060	0.4	1.9
(*E*)-Oct-3-en-1-ol			1062		3.7
Linalool	1084	0.8	1095	0.2	0.4
Undecene	1084	0.1			
Nonan-2-one			1090		0.1
2-Phenylethanol			1106	2.9	1.7
1,3-Undecadiene	1136	2.0			
Decan-2-one			1191		0.1
2-Phenylcrotonaldehyde			1250	0.7	
(*E*)-2-Decenal			1253	0.5	1.2
Undecan-2-one			1293		0.1
Indole			1320	3.4	15.8
2-Phenylacetamide			1330	24.1	
Gamma-nonalactone			1355		0.5
4-Hydroxybenzaldehyde			1416	5.1	
Benzopyrrolidone			1452	1.8	
Nerolidol			1565		0.2
3-Formylindole			1675	1.8	

[a]Different retention indices due to the GC column used; [b]Relative percentage of the identified volatile based on the GC/MS chromatographic area

Table 2. Volatile composition (percentage) of three extracts from *T. sulfureum*

Effect of extraction method on volatile composition

Three main isolation techniques were used, i.e., dynamic headspace concentration, liquid solid extraction into a organic solvent and water-distillation for the investigation of volatile substances from fresh *A. aegerita* and *T. sulfureum*. Only the volatile components unambigously identified by both GC and MS methods are reported in Tables 1 and 2.

The organic extracts from fresh mushrooms contained many primary and secondary metabolites such as lipids, sterols and fatty acids (Regerat *et al.*, 1976; De Simone *et al.*, 1979; Senatore *et al.*, 1988; Bonzom *et al.*, 1995). Tables 1 and 2 list the volatilizable metabolites detected in our GC/MS experiments at 200°C (injector temperature) from the organic solvent extracts. The water-distillate of fresh mushrooms contained both low and high volatile metabolites of fresh mushrooms. The headspace concentrates contained only volatile components with low volatility indice at room temperature. Thus, only the most volatile constituents of fresh mushrooms are detected at high levels in the headspace concentrates. It is why low molecular weight benzene derivatives, i.e., ethylbenzene, xylenes and styrene as well as monoterpenes are commonly identified in headspace extracts and more rarely reported in solvent extracts and distillates from fresh mushrooms.

Consequently, the volatile composition of mushroom extracts and water-distillates is close to the genuine composition of volatile constituents in fresh mushrooms. Indeed, the major volatile components of solvent extracts are similar to those of water-distillates as reported in Tables 1 and 2. Therefore, qualitative differences appeared for some aliphatic alcohols; 3-octanol, (*E*)-2-octenol and octanol levels are much lower in the extracts of both fresh mushrooms studied than in the water-distillates. These C_8 derivatives could be present in a linked form in mushrooms; water-distillation should be then responsible for the free aliphatic alcohols formation as already reported by Cravo *et al.* (1993).

In conclusion, many volatile secondary metabolites are produced by mushrooms. The volatile composition of *A. aegerita* and *T. sulfureum* is directly dependent of the extraction process as already described for other mushroom species (Charpentier *et al.*, 1986; Vidal *et al.*, 1986). Thus, the headspace concentrates, organic extracts and water-distillates of *A. aegerita* and *T. sulfureum* are attractive natural resources for aroma application in food, pharmacy and cosmetic industries.

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