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# The anise-like odor of *Clitocybe odora*, *Lentinellus cochleatus* and *Agaricus essettei*

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***Abstract:*** The fruiting bodies of fresh and wild *Clitocybe odora*, *Lentinellus cochleatus* and *Agaricus essettei* were investigated for volatile compounds by gas chromatography-mass spectrometry analysis using hydro-distillation and solvent extraction techniques. The three mushroom species are well known to possess anise odors. The main volatile compounds of the three species were aromatic derivatives. Anise fragrance was due either to a single impact aroma compound, or to mixtures of volatile constituents. *p*-Anisaldehyde was identified as the key odorous component responsible for the pure anise fragrance of *C. odora*. *p*-Anisaldehyde, methyl *p*-anisate, methyl (*Z*)-*p*-methoxycinnamate and methyl (*E*)-*p*-methoxycinnamate were responsible for the aniseed smell of *L. cochleatus*. Benzaldehyde and benzyl alcohol may contribute to the anise-like odor of *A. essettei*.

***Key Words:*** Anisaldehyde, Basidiomycota, fruiting bodies, natural fragrance

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## INTRODUCTION

Distinctive odors have long been used as taxonomic markers for mushroom species identification (Bessette et al 1997, Claus 1978, Courtecuisse 1999, Læssøe et al 1996, Lincoff 1998, Moreau and Roux 2001, Pacioni 1982). Key compounds directly responsible for fruiting bodies aromas have been studied in some mushroom species. These compounds have odors described as musty-earthy (Breheret et al 1999), alliaceous-sulfureous (Rapior et al 1997a), fenugreek (Rapior et al 2000a), cucumber (Wood et al 1994), sweet (Wood et al 1992), candy-like (Largent et al 1990), anise and almond (Chen and Wu 1984, Rapior et al 2000b, Wood et al 1988, 1990). The potential of higher fungi for the industrial production of natural anise aroma compounds has been largely overlooked. Anise-like odors are reported from fruiting-bodies of *Agaricus arvensis*, *A. essettei*, *Clitocybe odora*, *C. suaveolens*, *Hydnellum suaveolens* and *Lentinellus cochleatus*. Coumarin and anisaldehyde appeared to be responsible for the fragrant anise aroma of *H. suaveolens* (Wood et al 1988). Fruiting bodies of *Agaricus augustus* and *Gyrophragmium dunalii* possessing an anise-like odor, occasionally mixed with a bitter-almond smell, were investigated for volatile compounds by Wood et al (1990) and Rapior et al (2000b), respectively. These authors showed that a mixture of benzaldehyde and benzyl alcohol contributed to the complex aroma of both species.

In the present study, fresh and wild sporophores of *Clitocybe odora* (Bull. : Fr.) Kummer (anise-scented *Clitocybe*), *Lentinellus cochleatus* (Pers. : Fr.) P. Karsten (cockle-shell *Lentinus*) and *Agaricus essettei* M. Bon (woodland *Agaricus*) were investigated for volatile constituents by hydro-distillation and solvent extraction using gas chromatography (GC) and mass spectrometry (MS) to identify the compounds responsible for their anise-like odor.

## MATERIALS AND METHODS

Fresh and wild fruiting bodies with an anise smell were collected in the fall of 1999 in Languedoc-Roussillon (France). *Clitocybe odora*, *Lentinellus cochleatus* and *Agaricus essettei* were wrapped in waxed paper bags after identification. The specimens were brushed clean of forest debris and treated immediately after collection. Fruiting bodies were investigated for volatile compounds by hydro-distillation and organic solvent extraction, and analyzed by gas chromatography/mass spectrometry (GC/MS) as detailed by authors (Breheret et al 1999, Rapior et al 2000a, b).

Samples of *C. odora* (12 g), *L. cochleatus* (115 g) and *A. essettei* (90 g) fruiting bodies were subjected to a three-hour hydro-distillation with a Lickens-Nickerson apparatus using dichloromethane as solvent. Solvent extraction was performed on 30, 50, and 150 g of fresh fruiting bodies (cut into cubes) for *C. odora*, *L. cochleatus* and *A. essettei*, respectively. Volatile compounds were extracted with 100, 150, and 350 mL dichloromethane for the *Clitocybe*, cockle-shell *Lentinus* and woodland *Agaricus*, respectively. Both organic extracts were then concentrated to a small volume (0.5 mL) under nitrogen stream and directly analyzed (1.0 μL) in duplicate by GC/MS (Rapior et al 2000b).

GC/MS analyses were carried out using analytical 30 m × 0.20 mm × 1 μm polydimethylsiloxane DB-5 column fused silica capillary column. Volatile compounds were identified by their mass spectra and retention indices (Adams 1989, Jennings and Shibamoto 1980, National Institute of Standard and Technology 1994, The Mass Spectrometry Data Centre 1986), and with reference to our own data bank.

## RESULTS AND DISCUSSION

TABLES I, II, and III list the volatile composition of fresh fruiting bodies of *C. odora*, *L. cochleatus* and *A. essettei*, respectively. A strong anise aroma was detected in the distillates and solvent extracts from the fresh *C. odora*, *L. cochleatus* and *A. essettei*. The major volatile components of the three mushroom species were aromatic derivatives, i.e., *p*-anisaldehyde (= 4-methoxybenzaldehyde), methyl *p*-anisate, benzaldehyde, and benzyl alcohol.

The main volatile component in fruiting bodies of *C. odora* was identified as *p*-anisaldehyde by GC/MS (81.4% in the distillate and 66.8% in the organic extract; TABLE I). This volatile compound is well known for possessing a characteristic anise odor (Arctander 1994, Jaubert et al 1995), and we conclude that it is responsible for the pure anise fragrance of *C. odora* which has been reported by numerous mycologists (Claus 1978, Courtecuisse 1999, Læssøe et al 1996, Lincoff 1998, Moreau and Roux 2001). The intense anise aroma of *p*-anisaldehyde hides other flavoring compounds such as benzaldehyde and 1-octen-3-ol. Significant amounts of *p*-anisaldehyde were previously detected in frozen fruit bodies of *C. odora* (20.0%) (Rapior et al 1996) and *H. suaveolens* (30.0%) (Wood et al 1988) using solvent extraction. In the present work, the high anisaldehyde content detected in fresh specimens of *C. odora* offers promise for *p*-anisaldehyde production using Basidiomycota.

*L. cochleatus* is another widely-distributed mushroom species having a pleasant aniseed-like smell (Læssøe et al 1996, McIlvaine and Macadam 1973, Moreau et al 1999, Moser 1983). Whether or not the aniseed note is the predominant odor from *L. cochleatus* extracts, *p*-anisaldehyde is not the major volatile component: only 23.0 and 11.8% of *p*-anisaldehyde were detected in the distillate and solvent extract, respectively (TABLE II). However, the aniseed aldehyde derivative aroma of the cockle-scented *Lentinus* is strengthened by the aniseed odor-activity of methyl *p*-anisate as previously reported in other mushroom species (Jong and Birmingham 1993, Lomascolo et al 1999). Other volatile components also contributed to the fragrance of *L. cochleatus*, which is described as anise-like with a cinnamic note by Hanssen and Abraham (1987). This is consistent with our GC/MS analysis in which two methyl *p*-methoxycinnamate stereoisomers were identified in *L. cochleatus* extracts. These derivatives were previously detected in *Neolentinus lepideus* (Buxbaum ex Fr. : Fr.) Redhead & Ginns (= *Lentinus lepideus*) (Latrasse et al 1986, Sprecher and Hanssen 1985), whose fruiting bodies have an anise-like odor (Moser 1983, Pacioni 1982). The significant amounts of *p*-anisaldehyde, methyl *p*-anisate, methyl (*Z*)-*p*-methoxycinnamate and methyl (*E*)-*p*-methoxycinnamate suggest that all of the compounds may contribute to the pleasant anise-like odor of *L. cochleatus*.

TABLE I. Volatile composition of fresh *Clitocybe odora* fruiting bodies

| Volatile compounds | RI[a] | Hydro-distillation %[b] | Solvent extraction %[b] |
|---|---|---|---|
| Hexanal | 790 | — | 0.2 |
| 2-(5H)-Furanone | 815 | — | 2.0 |
| 3-Methylbutanoic acid | 876 | — | 3.0 |
| 2-Methylbutanoic acid | 891 | — | 0.2 |
| Benzaldedyde | 953 | 8.0 | 5.2 |
| 1-Octen-3-one | 967 | 0.1 | 0.3 |
| 1-Octen-3-ol | 972 | 2.5 | 3.0 |
| 3-Octanone | 980 | 5.4 | 11.0 |
| 3-Octanol | 988 | — | 0.1 |
| n-Pentylfurane | 997 | — | 0.2 |
| Limonene | 1028 | 0.5 | 0.4 |
| 2-Phenylethanal | 1033 | 0.3 | — |
| (*E*)-2-Octenal | 1056 | — | 0.1 |
| Linalool | 1095 | 0.4 | 0.2 |
| *p*-Anisaldehyde | 1240 | 81.4 | 66.8 |
| *p*-Anisyl alcohol | 1275 | — | 0.1 |
| 5-Pentyl-γ-lactone | 1335 | — | 0.1 |
| 2-Phenyl-5-methylhex-2-enal | 1445 | — | 0.1 |
| (*E*)-Nerolidol | 1565 | — | 0.2 |

[a] Retention indices on polydimethylsiloxane DB-5 column.

[b] Percentage of total ion current (TIC).

TABLE II. Volatile composition of fresh *Lentinellus cochleatus* fruiting bodies

| Volatile compounds | RI[a] | Hydro-distillation %[b] | Solvent extraction %[b] |
|---|---|---|---|
| Hexanal | 790 | — | 0.3 |
| 3-Methylbutanoic acid | 876 | — | 3.5 |
| 2-Methylbutanoic acid | 891 | — | 0.7 |
| Benzaldehyde | 953 | 18.0 | 27.0 |
| 1-Octen-3-one | 967 | 1.0 | 2.5 |
| 1-Octen-3-ol | 972 | 7.5 | 9.2 |
| 3-Octanol | 988 | 0.5 | 0.1 |
| Octanal | 995 | — | 0.2 |
| 2-Phenylethanal | 1033 | 2.0 | 5.8 |
| 1-Octanol | 1065 | 0.7 | 1.0 |
| Nonanal | 1095 | 0.9 | 2.4 |
| Decanal | 1195 | 0.8 | 1.1 |
| Benzoic acid | 1196 | — | 0.2 |
| *p*-Anisaldehyde | 1240 | 23.0 | 11.8 |
| (*E,Z*)-2,4-Decadienal | 1288 | <0.1 | 0.3 |
| (*E,E*)-2,4-Decadienal | 1310 | 0.3 | 1.1 |
| Methyl *p*-anisate | 1359 | 13.8 | 7.6 |
| *p*-Hydroxybenzaldehyde | 1414 | — | 2.7 |
| Methyl (*Z*)-*p*-methoxycinnamate | 1631 | 8.8 | 6.3 |
| Methyl (*E*)-*p*-methoxycinnamate | 1750 | 18.2 | 14.5 |

[a] Retention indices on polydimethylsiloxane DB-5 column.
[b] Percentage of total ion current (TIC).

TABLE III. Volatile composition of fresh *Agaricus essettei* fruiting bodies

| Volatile compounds | RI[a] | Hydro-distillation %[b] | Solvent extraction %[b] |
|---|---|---|---|
| Hexanal | 790 | — | 0.1 |
| Benzaldehyde | 953 | 35.4 | 29.3 |
| 1-Octen-3-ol | 972 | 0.5 | 0.1 |
| 3-Octanone | 980 | 2.1 | 0.1 |
| Benzyl alcohol | 1027 | 57.3 | 66.2 |
| Methyl benzoate | 1086 | 0.2 | 0.1 |
| 2-Phenylethanol | 1106 | 0.3 | 0.1 |
| Benzoic acid | 1210 | — | 0.2 |
| *p*-Anisyl formate | 1327 | — | 0.2 |
| *p*-Hydroxybenzaldehyde | 1414 | 3.1 | 1.4 |
| (*E*)-Stilbene | 1602 | — | 0.1 |

[a] Retention indices on polydimethylsiloxane DB-5 column.
[b] Percentage of total ion current (TIC).

Unlike *C. odora* and *L. cochleatus*, *p*-anisaldehyde is absent from *A. essettei*. The volatile fraction from fresh fruiting bodies of *A. essettei* contained 96.3 and 97.5% of benzylic structures after hydro-distillation and solvent extraction, respectively (TABLE III). GC/MS analyses showed that the extracts contained two major volatile compounds identified as benzaldehyde and benzyl alcohol. Amounts of both volatiles detected in the distillate and solvent extract were 35.4 and 29.3% for benzaldehyde, and 57.3 and 66.2% for benzyl alcohol, respectively. The volatile composition of fresh fruiting bodies of woodland *Agaricus* also included minor aromatic derivatives: *p*-hydroxybenzaldehyde, 2-phenylethanol, methyl benzoate, benzoic acid, and *p*-anisyl formate.

GC/MS carried out on *A. essettei* extracts did not indicate that a single compound was directly responsible for the anise-like odor of this mushroom species. Two main odor-active compounds were identified as benzaldehyde (bitter almond odor) and benzyl alcohol (sweet-spicy odor). Benzaldehyde and benzyl alcohol mixtures based on the natural ratio released by sporophores of *A. augustus* were evaluated by an odor panel (Wood et al 1990). At concentrations which were high enough that the odor of the mixture was easily perceived, a number of judges in this panel reported that a benzaldehyde/benzyl alcohol mixture produced an anise-like odor. The authors of this study also showed that different benzaldehyde and benzyl alcohol mixtures were perceived by judges as anise-like or almond-like odors depending on the ratio of volatile constituents. Likewise, Rapior et al (2000b) showed that a benzaldehyde and benzyl alcohol mixture may contribute to the complex almond odor with an anise note of *G. dunalii*. In the present study, high concentrations of benzaldehyde and benzyl alcohol could have been responsible for the anise smell of *A. essettei*, variously reported initially as "*odore complexo anisato-benzoylato*" (Bon 1983), as an almond-anise odor (Lincoff 1998), and as a bitter almond smell (Courtecuisse 1999).

*C. odora*, *L. cochleatus* and *A. essettei* produce aromatic volatile metabolites, i.e., *p*-anisaldehyde, methyl *p*-anisate, benzaldehyde, benzyl alcohol, 2-phenylethanol as reported for other mushroom species (Chen and Wu 1984, Rapior et al 1997b, 2000b, Wood et al 1990, 1992). All of these aromatic derivatives are synthetized via the shikimate pathway from cinnamic acids to phenylpropanes as clearly reviewed by Manitto (1981) and recently detailed for *Polyporus tuberaster* (Kawabe and Morita 1994). Thus it seems that the similarity between the fragrances of *C. odora*, *L. cochleatus* and *A. essettei* tallies with a common biochemical pathway of anise-active aromatic components.

While plant materials have been used as sources of essential oils for centuries, the potential of higher fungi for the industrial production of natural aroma components has been overlooked. Clearly, *C. odora*, *L. cochleatus* and *A. essettei* possess the enzymatic capacity for synthesis of aromatic compounds. Mushroom species could represent a valuable source of aromatic molecules for the flavor industry.

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