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Volatile composition of *Gyrophragmium dunalii*

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Abstract: *Gyrophragmium dunalii* was investigated for volatile compounds by GC/MS. Thirty volatile components were identified. The major volatile constituents of mushrooms from the Mediterranean basin and Atlantic Ocean sand dunes were benzaldehyde (44.2 and 28.0%, respectively) and benzyl alcohol (11.0 and 38.6%, respectively). The volatile component proportions correspond to 410 and 960 $\mu g\ g^{-1}$ of fresh weight from Mediterranean and Atlantic specimens, respectively. Combined benzaldehyde and benzyl alcohol content may contribute to the complex almond odor with an anise note of *G. dunalii*.

Key Words: Agaricaceae, almond-like odor, anise-like odor, Basidiomycota, benzaldehyde, benzyl alcohol, Podaxaceae

Higher fungi can generate a broad spectrum of odors (Maga 1981, Mazza 1998). Typical pleasant and unpleasant aromas of mushrooms are well known including bitter almond (Chen and Wu 1984), anise-like (Wood et al 1990), fungal (Fischer and Grosch 1987, Rapior et al 1997b, Tressl et al 1982), garlic (Rapior et al 1997a), fenugreek (Rapior et al 2000), and musty-earthy (Breheret et al 1999) odors.

Gyrophragmium dunalii is a worldwide species growing on sand dunes (Guinberteau 1997, Kreisel 1973, Pilát 1958). An epigeous ochraceous fungus with a large annulus and typical funnel-shaped volva at the base of a very long hypogeous stipe, *G. dunalii* has dingy dark-brown anastomosing lamellae when old (Honrubia et al 1982, Moser 1978, Zeller 1943). According to authors, *G. dunalii* develops either *Agaricus bisporus* odor (Kreisel 1973) or cyanic (Pacioni 1982) and bitter almond smell with an anise-like note (Guinberteau 1999).

As far as we know, this species was first considered as a hymenomycetous species and named *Agaricus ocreatus* (Raffeneau-Delile 1820–1840). According to Montagne (1843), Fries named the mushroom as *Montagnites delilei* but published the species as *M. dunalii* (Fries 1836–1838). Then Montagne (1843) classified the fungus as *Gyrophragmium delilei* (type genus) into the tribe Podaxinées (Gasteromycetes) using macroscopic characteristics of young basidiomes from Algeria. Later Zeller (1943) called it *G. dunalii* (Fr.) Zeller according to International Rules of Botanical Nomenclature. Recently, according to Hawksworth et al (1995) and Honrubia et al (1982), *G. dunalii* belongs either to Agaricales Clements (*Podaxaceae* Corda, secotioid species) or Podaxales Zeller (*Secotiaceae* Tul., agaricoid species), respectively.

The present work is the first chemical study on the complex almond odor with an anise note of *G. dunalii*. The fresh mushroom was investigated for volatile components by solvent extraction using gas chromatography/mass spectrometry (GC/MS). Thirty-one and 56 g of fresh *G. dunalii*, representing a combination of young and mature sporophores, were collected on sand dunes from the Mediterranean basin in Dec 1998 in the National Reserve of Camargue (France) and Atlantic Coast in Nov 1999 in the Oléron Island (France), respectively. The specimens were brushed clean, cubed and treated immediately after collection with diethyl ether (120 mL and 220 mL, respectively) to stop enzymatic activity. The organic extracts were gently concentrated to a small volume (0.5 mL) under nitrogen stream and analyzed (1.0 μL) in duplicate by GC/MS.

Solvent extract analyses from fresh *G. dunalii* were carried out with a gas chromatograph (5890-Hewlett-Packard) and a mass selective detector (5971-Hewlett-Packard) with

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TABLE I. Volatile composition of fresh *Gyrophragmium dunalii*

Volatile compounds	RI[a]	Location: Mediterranean %[b]	Mediterranean µg g^{-1}[c]	Atlantic %[b]	Atlantic µg g^{-1}[c]
3-Methylbutanol	716	0.4	1.6	0.1	1.0
2-Methylbutanol	720	0.3	1.2	—	—
Hexanal	797	0.6	2.4	0.2	2.0
3-Methylbutanoic acid	846	0.6	2.4	0.2	2.0
2-Methylbutanoic acid	854	0.2	0.8	—	—
Styrene	870	0.4	1.6	—	—
1-Hexanol	904	0.1	0.4	—	—
Benzaldehyde	953	44.2	181.0	28.0	273
Methylstyrene[d]	977	0.2	0.8	0.1	1.0
2-*n*-Pentylfurane	985	0.3	1.2	—	—
Phenol	999	0.3	1.2	0.3	2.9
Benzyl alcohol	1036	11.0	45.0	38.6	376
2-Phenylethanal	1037	2.0	8.0	3.1	30.7
Acetophenone	1060	0.4	1.6	—	—
n-Octanol	1087	0.1	0.4	0.1	1.0
Nonanal	1098	0.5	2.0	—	—
Undecane	1100	0.1	0.4	—	—
2-Phenylethanol	1112	2.1	8.6	6.2	61.4
Benzoic acid	1197	10.5	43.0	7.0	69.3
Phenylethanoic acid	1254	0.6	2.4	0.3	3.0
2-Undecanone	1294	0.1	0.4	—	—
Tridecane	1300	0.6	2.4	—	—
Indole	1303	1.5	6.1	0.4	4.0
Benzamide	1342	1.8	7.4	—	—
p-Hydroxybenzaldehyde	1398	0.6	2.4	—	—
p-Aminobenzaldehyde[e]	1420	8.0	33.0	4.8	47.5
2-(2′-thienyl)-thiophene	1432	0.5	2.0	—	—
Cinnamic acid	1453	0.7	2.9	6.0	59.4
Unidentified	1481	0.5	2.0	0.3	3.0
Pentadecane	1500	0.7	2.9	—	—
Unidentified	1567	4.9	20.0	1.2	11.9
3-Carboxyindole	1758	4.0	17.0	1.2	11.9

[a] Retention indices on polydimethylsiloxane Optima 5 column.
[b] Percentage of total ion current (TIC).
[c] Amount of volatile compounds (µg g^{-1} of fresh weight).
[d] Unidentified isomer.
[e] Tentatively identified.

a potential of 70 eV for ionization by electron impact. GC/MS analyses were performed by a polydimethylsiloxane Optima 5 (Macherey-Nagel, 25 m length, 0.20 µm i.d., 0.13 µm phase thickness), fused silica capillary column (J & W). The carrier gas was helium with a linear gas velocity of 28 cm s^{-1}. The injector and detector temperatures were 200 C and 270 C, respectively. The column was temperature programmed as follows: 50 C (3 min) to 200 C (3 C/min).

Thirty volatile components were identified by GC/MS (TABLE I). The volatile compound proportion corresponds to 410 and 960 µg g^{-1} of fresh weight for Mediterranean and Atlantic locations, respectively. Retention indices and experimental mass spectra of volatiles were compared to those available from literature spectra (Adams 1989, Jennings and Shibamoto 1980), mass spectral library NBS (MacLafferty and Stauffer 1989), and our own data bank. There is no significant qualitative difference from the major volatile compounds of specimens collected from the Mediterranean Sea and Atlantic Ocean dunes. But quantitative differences in volatile components are reported in TABLE I: the main difference is relative to benzyl alcohol content (11.0% and 45.0 µg g^{-1}, and 38.6% and 376 µg g^{-1}, respectively).

The main volatile compounds of *G. dunalii* were aromatic derivatives classified in two groups. Benzylic derivatives (benzaldehyde, benzyl alcohol, benzoic acid, benzamide, *p*-hydroxybenzaldehyde, *p*-aminobenzaldehyde) represented 76.1 and 84.9% of the volatile substances listed in TABLE I, while phenylethyl derivatives (2-phenylethanal, 2-phenylethanol, phenylethanoic acid) constituted only 4.7 and 10.2% of the volatile fraction from Mediterranean and Atlantic specimens, respectively.

The major volatile components of *G. dunalii* were quantitatively benzaldehyde (44.2 and 28.0%, respectively) and benzyl alcohol (11.0 and 38.6%, respectively) as well as the volatile compound (RI: 1420, 8.0 and 4.8%, respectively) tentatively identified as *p*-aminobenzaldehyde. The experimental mass spectrum (ms) of the latter matches the ms expected according to its chemical structure and is identical to the ms published for *m*-aminobenzaldehyde (MacLafferty and Stauffer 1989). The calculated retention indices of the three aminoacetophenone isomers confirm the para structure of the aminobenzaldehyde component. No odor description was reported for the latter volatile. It is not commercially available due to its instability.

Among the flavor volatile components, many aliphatic alcohols (3-methylbutanol, 2-methylbutanol, 1-hexanol, *n*-octanol) and aldehydes (hexanal, nonanal) were identified from *G. dunalii* (TABLE I). Other minor fragrant components, i.e., indole and phenol, with low odor threshold may significantly contribute to fungus aroma despite low contents. However, the similarity between aroma specimens from Mediterranean and Atlantic sites leads us to think that the main volatile constituents (benzaldehyde and benzyl alcohol) also characterized by low odor thresholds have a major contribution to the complex aroma of *G. dunalii*.

Investigations show that a close and demonstrable affinity exists between certain *Secotiaceae* and certain Agaricales (Singer 1958). An evaluation of *Gyrophrag-*

mium microscopic characters supports its inclusion in the *Agaricaceae* as a secotioid genus (Kreisel 1973). According to Guinberteau (1999), the positive Schaeffer reaction on the epicutis of *G. dunalii* as well as the almond/anise-like odor exhibit demonstrable affinities with agarics especially from the section *Arvenses* Konrad & Maublanc. Wood et al (1990) identified benzaldehyde (almond odor) and benzyl alcohol (sweet-spicy odor) as well as hydroxybenzaldehyde isomers (sweet-woody balsamic odor) in *Agaricus augustus*. The authors demonstrated the odor of benzaldehyde and benzyl alcohol mixtures as to be either almond-like or anise-like depending on the relative concentrations of these two chemicals. High contents of benzaldehyde and benzyl alcohol were also reported in *A. bisporus* (Fischer and Grosch 1987), *A. campestris* (Buchbauer et al 1993), and *A. subrufecens* (Chen and Wu 1984).

Benzaldehyde and benzyl alcohol may contribute to the specific almond odor with an anise note of fresh *G. dunalii*. Benzaldehyde with its almond odor is widely used as an aroma component in food, cosmetics and flavor industries. Considering consumer preference for natural aroma components, the potential for biotechnological production of volatiles from the Basidiomycota is currently being developed as reported in the literature (Lomascolo et al 1999, Schindler and Schmid 1982).

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LITERATURE CITED

Adams RP. 1989. Identification of essential oils by ion trap mass spectroscopy. San Diego: Academic Press. 469 p.

Breheret S, Talou T, Rapior S, Bessière JM. 1999. Geosmin, a sesquiterpenoid compound responsible for the musty-earthy odor of *Cortinarius herculeus, Cystoderma amianthinum,* and *Cy. carcharias.* Mycologia 91:117–120.

Buchbauer G, Jirovetz L, Wasicky M, Nikiforov A. 1993. Zum Aroma von Speisepilzen. Kopfraum-Analyse mittels GC/FID und GC/FTIR/MS. Z Lebensm Unters Forsch 197:429–433.

Chen CC, Wu CM. 1984. Volatile components of mushroom (*Agaricus subrufecens*). J Food Sc 49:1208–1209.

Fischer KH, Grosch W. 1987. Volatile compounds of importance in the aroma of mushrooms (*Psalliota bispora*). Lebensm Wiss Technol 20:233–236.

Fries EM. 1836–1838. *Epicrisis Systematis Mycologici seu Synopsis Hymenomycetum.* Uppsala, Sweden: Typographia Academica. p 240–241.

Guinberteau J. 1997. La mycoflore des écosystèmes dunaires du Bas-Médoc (Gironde, France). Approche écodynamique des mycocoenoses dunaires atlantiques. In: Favennec J, Barrère P, eds. Biodiversité et protection dunaire. Bordeaux, France, 17–19 avril 1996. Paris: Lavoisier. p 242–257.

———. 1999. *Gyrophragmium dunallii.* Un cousin mutant des agarics. Spécial Champignons Mag 16:19.

Hawksworth DL, Kirk PM, Sutton BC, Pegler DN. 1995. Ainsworth & Bisby's dictionary of the fungi. Oxon, UK: CAB International. 616 p.

Honrubia M, Calonge FD, Demoulin V, Moreno G, Llimona X. 1982. Aportacíon al conocimiento de los hongos del SE. de España. VI- Esclerodermatales, Licoperdales, Nidulariales, Falales, Himenogasterales, Podaxales (Gasteromicetes, Basidiomicetes). Anal Univ Murcia 38(1–4):101–132.

Jennings W, Shibamoto T. 1980. Qualitative analysis of flavor and fragrance volatiles by glass capillar gas chromatography. New York: Academic Press. 472 p.

Kreisel H. 1973. Die Gattung *Gyrophragmium* Mont. und ihre Stellung im System der Basidiomycetes. Feddes Repert 83:577–583.

Lomascolo A, Stentelaire C, Asther M, Lesage-Meessen L. 1999. Basidiomycetes as new biotechnological tools to generate natural aromatic flavours for the food industry. Trends Biotechnol 17:282–289.

MacLafferty FW, Stauffer DB. 1989. The Wiley NBS Registry of mass spectral data. New York: Wiley & Sons. 256 p.

Maga JA. 1981. Mushroom flavor. J Agric Food Chem 29: 1–4.

Mazza R. 1998. Introduzione alla «micosmologia». Pagine Micol 10:29–96.

Montagne C. 1843. Considérations générales sur la tribu des Podaxinées, et fondation du nouveau genre *Gyrophragmium,* appartenant à cette tribu. Ann Sc Nat 2nd Ser 20:69–82.

Moser M. 1978. Keys to Agarics and Boleti (Polyporales, Boletales, Agaricales, Russulales). London: Kibby. 535 p.

Pacioni G. 1982. Guia de Hongos. Barcelona: Grijalbo. 523 p.

Pilát A. 1958. Flora CSR. B1—Gasteromycetes. Prague: Nakladatelstí Ceskoslovesnké Akademie Ved. p 551–552.

Raffeneau-Delile A. 1820–1840. *Icones ineditae.* Iconographies inédites des champignons de Montpellier peints par Node-Veran TF et Fils. Service des Herbiers, Institut de Botanique de Montpellier, France: Original publication by authors. 6:pl 268.

Rapior S, Breheret S, Talou T, Bessière JM. 1997a. Volatile flavor constituents of fresh *Marasmius alliaceus* (Garlic *Marasmius*). J Agric Food Chem 45:820–825.

———, Fons F, Bessière JM. 2000. The fenugreek odor of *Lactarius helvus.* Mycologia 92:305–309.

———, Fruchier A, Bessière JM. 1997b. Volatile aroma con-

stituents of Agarics and Boletes (a review). Recent Res Devel Phytochem 1:567–584.

Schindler J, Schmid RD. 1982. Fragrance or aroma chemicals—microbial synthesis and enzymatic transformation—a review. Process Biochem 35:2–8.

Singer R. 1958. The meaning of the affinity of the *Secotiaceae* with the Agaricales. Sydowia (Annales Mycologici) 12:1–43.

Tressl R, Bahri D, Engel KH. 1982. Formation of eight-carbon and ten-carbon components in mushrooms (*Agaricus campestris*). J Agric Food Chem 30:89–93.

Wood WF, Watson RL, Largent DL. 1990. The odor of *Agaricus augustus*. Mycologia 82:276–278.

Zeller SM. 1943. North american species of *Galeropsis, Gyrophragmium, Longia,* and *Montagnea*. Mycologia 35: 409–421.