# Volatile Constituents of the Giant Puffball Mushroom (Calvatia gigantea)

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#### **KEYWORDS**:

#### Calvatia gigantea, GC-MS, Headspace Volatiles, Anthranilates, VOCs

#### **ABSTRACT:**

The volatile compounds of fresh and air dried fruiting bodies of the wild edible giant puffball mushroom (*Calvatia gigantea*) were investigated by high resolution GC-MS using a sequential purge & trap technique. A total of 235 compounds were identified in this study with significant changes occurring during the ageing & sequential purging of the samples. Major volatiles in the "fresh" samples were anisole (methoxybenzene), 3-octanone, 3-methylbutan-1-ol, 3-octanol, methyl N,N-dimethylanthranilate and methyl N-methylanthranilate. Major volatiles in the "air dried" samples initially were 3-methylbutan-1-ol, 3-methylbutanal and 2-Methylbutanal; however, after the final 48 hour purge of the air dried sample, the 3-methylbutan-1-ol, 3-methylbutanal and 2-Methylbutanal had decreased dramatically with substantial increases of isovaleric acid, 2-methylbutyric acid, 2-Phenylethyl alcohol, phenol, methyl N,N-dimethylanthranilate, several lactones, methionol and 4-Hydroxy-2-pentanone. The isolation of the two aforementioned anthranilate esters along with a lesser amount of methyl anthranilate appears to be the first report of anthranilate esters in a mushroom species. Potential environmental contaminants, including VOC air pollutants in such analyses are discussed.

#### INTRODUCTION

The giant puffball (*Calvatia gigantea*) is one of the largest of the edible mushroom species. A member of the fungi family of the class Homobasidiomycetes and family Lycoperdaceae, it is usually found in late summer and autumn in meadows, fields, and forests worldwide. It is notable, and noticeable, because of its size and shape. Resembling a white soccer ball, this puffball has been known to reach a size as big as 150 cm in diameter, although more commonly they are 20-60 cm in diameter.

The white fruiting body develops rapidly over a period of several weeks after which it quickly turns brown and releases millions of spores.

This species is only edible when fresh and is usually consumed within 24 hours of harvest as the flavor becomes disagreeable on aging. Normally, it is sliced and pan-fried in butter, sometimes in batter, and optionally with garlic, herbs and spices. The consistency of the cooked slices resembles tofu and its inherent flavor is relatively mild.

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Only a few references to the volatile constituents of *Calvatia gigantea* have been reported. Dijkstra reported that 1-octen-3-ol was the major volatile both in an extract of the fresh puffball and in a fermented submerged culture of the mycelium [1,2]. Subsequently, Overton reported that the giant puffball *Calvatia* contained a high concentration of methoxybenzene (Anisole) but did not find 1-octen-3-ol [3]. In addition, a few lipid components have been identified by Buckley [4]. A review of the ethnomycology and biotechnological potential of the genus *Calvatia* ('Gasteromycetes', Lycoperdaceae), including *C. gigantea* has recently been published [5].

Herein, we report the GC-MS study of the volatiles of both fresh and air-dried samples of this puffball mushroom by a sequential purge & trap technique.

Our interest in the giant puffball was generated by the observation of the powerful force generated by the growth of one of these puffballs under a slab of asphalt driveway. As the puffball grew, it lifted a circular area of the driveway to form a large hemispherical asphalt mound about 15 cm in height, beneath which was a giant puffball. While this obviously was not a candidate for analysis because of potential hydrocarbon contamination, several pristine puffballs averaging between 28-42 cm in diameter were harvested more than a hundred feet from the asphalt driveway in a meadow near a tree line. Nevertheless, as these were harvested in an urban area, part of this study included, not only identification of the total volatiles, but assessment of potential environmental contaminants – including VOC air pollutants.



Photos of the *Calvatia gigantea* Samples

# MATERIALS & EXPERIMENTAL METHODS

*Calvatia* Samples: The *Calvatia gigantea* puffball mushrooms were harvested in a suburban area of Louisville Kentucky during late September 2005.

**Sample Preparation:** Four analyses of "fresh" slices of *Calvatia gigantea* were conducted by sequential dynamic purge and trap as described below. Similarly, three analyses of the "air dried" slices of *Calvatia gigantea* were conducted by sequential dynamic purge and trap. All samples were purged at air-conditioned room temperature of about 25-26° C.

A) A 4-5 gram sample of sliced "fresh" *Calvatia gigantea* was placed in 16 fl. oz. glass jar fitted with a metal lid having both inlet and outlet gas tubes. The inlet tube was connected to a helium tank and the outlet tube connected to a stainless tube with Swagelok nuts & ferrules attached. The top of the tube was fitted with a 1/4" Swagelok union with the top nut hole diameter increased to allow the easy introduction of a Hewlett-Packard injection port liner (HP part

#5181-3316) and sealed with an injection port O-Ring. The injection port liner had been packed with 100 mg of Tenax TA (20-35 mesh) (glass-wool plugs on top and bottom) and previously baked out at 260C for 2.5 hours. Helium (50 cc/min) was introduced into the bottom of the glass jar containing the *Calvatia* slices. The sample was purged trapping the volatiles on the Tenax injection port liner. This was successively done four times at room temperature for periods of (FR-1) 9 hrs, (FR-2) 3 hrs, (FR-3) 9 hrs and (FR-4) 24 hrs, changing the Tenax trap between each purge. The Tenax liner was then placed directly into the GC injection port for thermal desorption of the volatiles onto the GC column, for each analysis.

B) In a similar manner, a sample of 4-5 grams of sliced *Calvatia gigantea* that had been air-dried for 48 hours was sequentially purged for (AD-1) 6 hrs, (AD-2) 24 hrs and (AD-3) 48 hrs for each analysis.

**GC and GC-MS:** The GC-MS was a Hewlett-Packard 6890/5973 High Performance combination. A Varian 60m X 0.32mm I. D. fused silica column coated with a 0.25 micron film thickness of VF-5MS (Varian part No. CP8961), a DB-5 equivalent column, was used in all analyses. The column was held isothermally at 30°C for 2 minutes, and then programmed from 30°C to 260°C at 2°C/min, with a final hold time of 28 minutes to give a total analysis time of 145 minutes. The Injection port was held at 260°C. Helium Carrier Gas was used with a column flow rate of 2.9 ml/min. The Mass spectrometer was scanned in the EI mode from 26m/z to 350m/z using 70eV ionizing voltage. Additionally, FID spectra and sulfur detection using a PFPD (Pulsed Flame Photometric Detector) were employed. Percentages, corrected for known system artifacts, are FID percentages without correction for response factors.

**Component Identifications:** Identifications were based on mass spectra from the Wiley and NIST MS libraries as well as from the authors MS library. Where available, retention time comparisons were used employing primarily the Retention Indices compilations of the NIST Mass Spec Data Center [6], as well as those of Boelens [7] and Adams [8]. Initial analysis was done on the Agilent MS Enhanced Chemstation program employing both normal and selective ion modes. The NIST AMDIS deconvolution program (Ver. 2.65) was also employed in both normal and high resolution modes using the MSP file format from data imported from the Wiley and NIST MS libraries, as well as the authors library. For purposes of comparison, RI values for DB-5, HP-5, HP5-MS, etc. were considered valid (and were comparable to our prior experience using values obtained using an HP-5 column).

Retention Indices: Kovats RI Indices were derived using the classic (isothermal) formula of Kovats [9]:

$$RIx = 100n + 100[log(tx) - log(tn)] / [log(tn+1) - log(tn)]$$

LRI Indices (often referred to as Linear Temperature Programmed Retention indices [LTPRI] or LRI) were derived by the formula of van den Dool and Kratz [10]:

$$LRIx = 100n + 100(tx-tn) / (tn+1 - tn)$$

where  $t_n$  and  $t_{n+1}$  are retention times of the reference n-alkane hydrocarbons eluting immediately before and after chemical compound "X";  $t_x$  is the retention time of compound "X".

Both the calculated Kovats and the LRI retention indices are provided in our tables because much of the reported literature does not specify which RI calculations were employed. The NIST values used [6] for comparison were primarily the van den Dool and Kratz temperature programmed LRI values while the Adams values [8] used the Kovats isothermal equation even though temperature programming was utilized in their analyses. The Boelens values in the ESO 2000 (Update 2006) [7] that were derived from the literature do not specify the method of RI calculation. In most cases the RI values by both methods are quite similar, but some varied by as much as 6-7 RI units. As with all such reported RI values, those between 400-700 must be considered as somewhat approximate as such values are subject to some instrumental experimental fluctuations between laboratories.

# **RESULTS & DISCUSSION**

The volatile compounds identified by sequential dynamic headspace analyses of both fresh and air dried *Calvatia gigantea* consisted of 34 aromatic hydrocarbons, 33 alkanes, 29 alcohols, 28 monoterpenoids, 23 ketones, 16 acids, 15 aldehydes, 11 esters, 9 lactones, 6 sulphur compounds, 5 furans, 6 sesquiterpenes, 4 alkenes, 4 phenolics, 4 aromatic ethers, 3 anthranilate esters, 2 aniline formamides (tentative identifications), 1 pyrrole and 1 cycloalkane.



# Fig. 1. Headspace Chromatogram of Fresh (FR-1) Calvatia gigantea

(1) 3-Methylbutanal (2) 2-Pentanone (3) Isoamyl alcohol (4) 2-Methyl-1-butanol (5) Toluene (6) 1-Octene (7) Anisole (8) 1-Octen-3-one (9) 1-Octen-3-ol (10) 3-Octanone (11) 3-Octanol (12) Limonene (13) N-(2-methylphenyl)formamide<sup>tent</sup> (14) Methyl anthranilate (15) Methyl N,N-dimethylanthranilate (16) Methyl N-methylanthranilate



#### Fig. 2. Headspace Chromatogram of Air-dried (AD-1) Calvatia gigantea

(1) 3-Methylbutanal (2) 2-Methylbutanal (3) 2-Pentanone (4) Isoamyl alcohol (5) 2-Methyl-1-butanol (6) 4-Hydroxy-2pentanone (7) Isovaleric acid (8) 1-Hexanol (9) Anisole (10) Phenol (11) 3-Octanone (12) 3-Octanol (13) Limonene (14) Methyl anthranilate (15) Methyl N,N-dimethylanthranilate (16) Methyl N-methylanthranilate

Table 1 summarizes these 235 volatile compounds and their retention indices.

						Fr	esh		Air-Dr	ied (48 ]	Hours)	
					FR-1	FR-2	FR-3	FR-4	AD-1	AD-2	AD-3	
Actual	VF-5MS	VF-5MS		Purge Time>	9 Hr	3 Hr	9 Hr	24 Hr	6 Hr	24 Hr	48 Hr	
R.T.	Kovats RI	LRI	Compounds		%	%	%	%	%	%	%	Ident.
2.550	412	412	Acetaldehyde <sup>e</sup>		0.05	0.10	0.01	0.01	0.01	0.01	0.01	MS,RI
2.793	429	426	Ethanol <sup>e</sup>		0.04	0.11	0.16	0.21	0.01	0.09	ND	MS,RI
3.000	496	496	2-Propanone <sup>e</sup>		0.05	0.16	0.15	0.21	0.02	0.03	0.04	MS,RI
3.572	553	549	1-Propanol <sup>e</sup>		0.05	0.14	0.11	0.10	trace	ND	ND	MS,RI
3.623	558	554	Isobutanal <sup>e</sup>		ND	ND	ND	ND	0.23	0.16	trace	MS,RI
3.634	559	555	2-Methylpentan	e <sup>d</sup>	0.10	0.14	ND	ND	ND	ND	ND	MS,RI
3.983	588	587	Acetic acid <sup>e</sup>		0.01	0.01	0.02	0.01	1.10	0.82	1.40	MS,RI
4.077	592	592	2-Butanone <sup>e</sup>		0.39	0.58	0.23	0.06	0.34	trace	ND	MS,RI
4.189	598	596	2-Butanol <sup>e</sup>		0.08	0.31	0.12	0.10	ND	ND	ND	MS,RI
4.390	607	604	Ethyl acetate <sup>e</sup>		ND	ND	trace	0.20	ND	ND	ND	MS,RI
4.634	618	614	Tetrahydrofuran	d?	0.19	0.34	0.17	0.04	ND	ND	ND	MS,RI
4.705	621	616	Isobutanol <sup>e</sup>		0.21	0.40	0.19	0.16	0.47	0.15	ND	MS,RI
5.436	651	645	3-Methylbutana	l <sup>e</sup>	0.82	0.49	0.17	0.02	30.71	7.29	1.24	MS,RI
5.654	659	653	2-Methylbutana	le	0.44	0.45	0.09	0.07	12.13	5.17	0.30	MS,RI
6.279	680	678	2-Pentanone <sup>e</sup>		0.72	0.69	0.82	0.30	0.33	0.19	0.02	MS,RI

Table 1. Volatiles of Calvatia gigantea by Sequential Purge & Trap Dynamic Headspace Analysis

	6.643	700	700	Heptane <sup>e</sup>	0.18	0.24	ND	ND	ND	ND	ND	MS,RI
	6.739	703	702	Pentanal <sup>e</sup>	ND	ND	ND	ND	0.10	0.14	0.02	MS,RI
	6.798	704	703	2-Pentanol <sup>e</sup>	0.14	0.22	0.23	0.38	0.04	trace	ND	MS,RI
	7.114	712	710	1,2-Ethanediol	ND	ND	ND	ND	0.03	trace	ND	MS
	7.190	714	711	Acetoin <sup>e</sup>	ND	ND	ND	ND	ND	ND	0.03	MS,RI
	7.470	721	717	Propanoic acid	ND	ND	ND	ND	ND	ND	0.04	MS
	7.692	726	721	Methylcyclohexane <sup>d</sup>	0.15	0.27	ND	ND	ND	ND	ND	MS,RI
	8.369	742	735	Isoamyl alcohol <sup>e</sup>	11.78	29.76	40.48	32.14	35.71	52.03	3.72	MS,RI
	8.502	744	738	2-Methyl-1-butanol <sup>e</sup>	0.95	3.02	3.77	3.21	3.32	3.04	0.78	MS,RI
	8.572	746	739	Dimethyl disulfide <sup>e</sup>	trace	MS,RI						
	9.098	757	750	Pyrrole <sup>e</sup>	trace	0.23	0.61	0.25	trace	ND	ND	MS,RI
	9.452	764	757	Ethyl isobutyrate	ND	ND	trace	0.30	ND	ND	ND	MS,RI
	9.482	764	758	Propylene glycol <sup>e</sup>	ND	ND	ND	ND	0.09	trace	trace	MS,RI
	9.735	769	763	Toluene <sup>a,d,e</sup>	1.00	0.11	ND	ND	0.23	trace	trace	MS,RI
	9.990	773	768	1-Pentanol <sup>e</sup>	trace	trace	0.11	0.11	0.08	0.30	0.02	MS,RI
	10.290	779	774	Isobutyric acid <sup>e</sup>	ND	ND	ND	ND	0.18	0.17	0.25	MS,RI
	10.471	782	778	2,4-Pentanedione <sup>e</sup>	0.11	0.10	0.23	0.23	0.09	0.43	0.18	MS,RI
	11.030	791	789	2-Hexanone <sup>e</sup>	ND	ND	trace	trace	trace	ND	0.04	MS,RI
	11.050	792	789	1-Octene <sup>d,e</sup>	1.14	0.09	ND	ND	ND	ND	ND	MS,RI
	11.215	794	793	Cyclopentanone	trace	trace	0.07	0.05	ND	ND	ND	MS,RI
	11.574	800	800	Octane <sup>e</sup>	0.19	0.09	0.07	0.08	0.03	0.03	0.05	MS,RI
	11.757	803	803	Butyric acid <sup>e</sup>	ND	ND	ND	ND	0.02	0.06	0.02	MS,RI
	11.822	804	803	Ethyl butanoate <sup>e</sup>	ND	ND	0.06	0.07	ND	ND	ND	MS,RI
	11.849	805	804	Hexanal <sup>e</sup>	0.20	0.28	ND	0.01	0.17	0.09	0.24	MS,RI
	12.836	822	818	4-Hydroxy-2-pentanone	ND	trace	0.06	0.16	0.26	1.22	8.10	MS
	13.773	836	831	Furfural <sup>e</sup>	trace	trace	trace	trace	0.04	trace	0.01	MS,RI
	14.680	850	844	2-Methylthioethanol	ND	ND	ND	ND	ND	0.01	0.02	MS
	14.710	850	844	4-Hydroxy-4-methyl-2- pentanone <sup>e</sup>	ND	ND	ND	ND	0.02	0.06	0.21	MS,RI
	15.374	859	854	Ethyl isovalerate	ND	ND	trace	0.04	ND	ND	ND	MS,RI
	15.490	861	855	Furfuryl alcohol <sup>e</sup>	ND	ND	ND	ND	0.02	trace	0.41	MS,RI
	15.686	864	858	Ethylbenzene <sup>d</sup>	0.17	ND	trace	trace	0.07	trace	trace	MS,RI
	16.330	872	867	Isovaleric acid <sup>e</sup>	ND	ND	ND	0.01	0.51	1.71	19.15	MS,RI
	16.354	872	867	p-Xylene <sup>d,e</sup>	0.32	0.09	0.06	0.02	0.10	trace	trace	MS,RI
	16.455	874	869	m-Xylene <sup>d,e</sup>	0.16	0.06	0.01	ND	0.05	ND	ND	MS,RI
	16.623	876	871	1-Hexanol <sup>e</sup>	trace	trace	0.07	0.12	0.20	1.26	0.89	MS,RI
	16.869	879	875	2-Methylbutanoic acid <sup>e</sup>	ND	ND	ND	ND	0.07	0.64	5.58	MS,RI
	17.196	883	879	Isoamyl acetate <sup>e</sup>	ND	ND	ND	0.03	0.01	0.30	0.29	MS,RI
_	17.353	885	882	Allyl isothiocyanate	ND	ND	ND	ND	trace	0.02	0.03	MS,RI
_	17.683	889	886	3-Heptanone	0.06	ND	0.02	0.03	trace	ND	ND	MS,RI
	17.919	891	889	Cyclohexanol	ND	ND	ND	trace	0.02	ND	ND	MS,RI
	17.961	892	890	2-Heptanone <sup>e</sup>	ND	ND	ND	trace	0.02	trace	ND	MS,RI

17.983	892	890	Styrene <sup>d</sup>	0.10	0.05	0.03	0.05	0.04	ND	trace	MS,RI
18.023	893	891	o-Xylene <sup>d,e</sup>	0.21	0.09	0.05	0.10	0.06	ND	ND	MS,RI
18.365	897	896	Cyclohexanone	ND	ND	ND	ND	0.04	ND	ND	MS,RI
18.484	898	897	Pentanoic acid <sup>e</sup>	ND	ND	ND	ND	0.03	0.03	0.21	MS,RI
18.664	900	900	Nonane <sup>e</sup>	0.41	0.12	0.06	0.04	0.04	ND	ND	MS,RI
18.980	905	904	Heptanal <sup>e</sup>	ND	ND	ND	trace	0.02	trace	ND	MS,RI
19.092	906	905	2-Butoxyethanol <sup>d,e</sup>	ND	ND	ND	0.01	0.06	0.19	0.09	MS,RI
19.460	912	910	Methional <sup>e</sup>	ND	ND	ND	ND	trace	trace	trace	MS,RI
19.514	912	911	2-Acetylfuran <sup>e</sup>	ND	ND	ND	ND	trace	trace	ND	MS,RI
19.684	915	913	gamma-Butyrolactone <sup>e</sup>	ND	ND	ND	ND	0.02	0.04	0.16	MS,RI
20.094	921	918	Anisole <sup>a,e</sup>	23.65	18.92	15.08	11.72	1.64	0.43	0.37	MS,RI
20.481	926	923	Isopropylbenzene <sup>d,e</sup>	0.13	0.07	0.07	0.05	0.03	ND	ND	MS,RI
20.678	929	925	alpha-Thujene	trace	trace	ND	ND	trace	trace	ND	MS,RI
21.216	936	932	alpha-Pinene <sup>e</sup>	0.21	0.20	0.02	trace	0.03	trace	ND	MS,RI
21.284	937	933	2-Methyl-5-isopropenylfuran	0.07	0.03	0.08	0.10	0.02	0.03	0.06	MS,RI
21.286	937	933	2,6-Dimethyloctane <sup>d</sup>	0.14	0.01	ND	ND	0.02	ND	ND	MS,RI
21.719	942	938	3-Ethyl-2-methylheptane <sup>d</sup>	0.11	ND	ND	ND	0.02	ND	ND	MS,RI
21.933	945	941	1-Butoxy-2-propanol <sup>d</sup>	ND	ND	ND	trace	0.02	0.01	ND	MS,RI
22.618	954	949	2-Methylcyclohexanone <sup>e</sup>	ND	ND	ND	ND	ND	0.01	0.02	MS,RI
22.904	957	953	Propylbenzene <sup>d</sup>	0.11	trace	0.02	0.01	0.26	ND	ND	MS,RI
22.940	958	953	gamma-Valerolactone <sup>e</sup>	ND	ND	trace	0.01	0.26	0.29	1.80	MS,RI
23.318	962	958	4-Methylnonane <sup>d</sup>	0.12	ND	trace	trace	0.02	ND	ND	MS,RI
23.494	964	960	1-Ethyl-3-methylbenzene <sup>d</sup>	0.28	0.11	0.09	0.09	0.17	ND	trace	MS,RI
23.548	965	961	delta-Valerolactone	trace	0.01	0.02	0.03	0.08	0.47	4.60	MS,RI
23.726	967	963	Benzaldehyde <sup>e</sup>	0.04	0.08	0.03	0.03	0.20	0.59	0.30	MS,RI
23.748	967	963	1-Ethyl-4-methylbenzene <sup>d</sup>	0.08	0.06	0.04	0.04	0.04	ND	ND	MS,RI
23.801	968	964	2-Methylnonane <sup>d</sup>	0.14	ND	ND	ND	ND	ND	ND	MS,RI
24.139	972	968	Dimethyl trisulfide <sup>e</sup>	trace	trace	0.01	0.02	trace	0.02	0.02	MS,RI
24.176	972	969	1,3,5-Trimethylbenzene <sup>d</sup>	0.08	0.04	0.03	0.02	0.04	0.01	ND	MS,RI
24.572	977	973	1-heptanol <sup>e</sup>	ND	ND	ND	0.03	0.05	0.08	ND	MS,RI
24.326	974	970	3-Methylnonane <sup>d</sup>	0.13	ND	ND	0.03	0.08	ND	ND	MS,RI
24.464	976	972	Sabinene <sup>e</sup>	trace	trace	0.02	0.03	0.06	0.06	ND	MS,RI
24.830	980	977	beta-Pinene <sup>e</sup>	0.09	0.06	0.02	0.01	0.01	trace	ND	MS,RI
24.900	980	978	1-Ethyl-2-methylbenzene <sup>d</sup>	0.15	0.07	0.03	0.02	0.07	0.08	ND	MS,RI
24.979	981	979	1-Octen-3-one <sup>e</sup>	0.11	ND	ND	ND	0.04	ND	0.01	MS,RI
25.173	984	981	Methionol	ND	ND	ND	trace	0.01	0.88	4.96	MS,RI
25.396	986	984	1-Octen-3-ol <sup>b.e</sup>	0.45	trace	trace	trace	0.15	trace	ND	MS,RI
25.650	989	987	Phenol <sup>e</sup>	trace	0.70	2.24	4.61	0.33	2.81	6.69	MS,RI
25.799	990	989	3-Octanone <sup>e</sup>	25.75	15.45	12.68	9.20	0.70	0.12	0.16	MS,RI
25.932	992	990	beta-Myrcene <sup>e</sup>	0.19	trace	trace	trace	0.01	0.08	ND	MS,RI

26.010	993	991	2-Pentylfuran <sup>e</sup>	0.19	trace	0.02	trace	0.11	trace	trace	MS,RI
26.046	993	992	2-Octanone <sup>e</sup>	ND	ND	trace	trace	0.01	0.01	ND	MS,RI
26.227	995	994	1,2,4-Trimethylbenzene <sup>d</sup>	0.38	0.25	0.19	0.20	0.22	0.22	ND	MS,RI
26.296	996	995	Hexanoic acid <sup>e</sup>	ND	ND	ND	0.02	0.07	0.06	0.02	MS,RI
26.698	1000	1000	3-Octanol <sup>e</sup>	4.97	5.06	4.60	8.04	0.21	0.09	0.03	MS,RI
26.706	1000	1000	Decane <sup>e</sup>	0.06	trace	trace	ND	0.11	0.05	trace	MS,RI
27.153	1006	1006	Octanal <sup>e</sup>	0.13	0.14	0.07	0.10	0.05	0.06	0.01	MS,RI
27.196	1007	1006	alpha-Phellandrene <sup>e</sup>	trace	ND	0.07	trace	trace	trace	ND	MS,RI
27.372	1009	1008	delta-3-Carene	0.22	0.09	0.10	0.07	0.08	0.03	trace	MS,RI
27.821	1015	1014	Isoamyl isobutyrate	ND	ND	ND	ND	0.02	0.02	ND	MS,RI
28.311	1022	1020	meta-Cymene	0.03	0.01	0.01	0.02	0.01	0.01	trace	MS,RI
28.355	1023	1020	1,2,3-Trimethylbenzene <sup>d</sup>	0.12	0.03	0.09	0.08	0.06	0.01	trace	MS,RI
28.472	1024	1022	2,6-Dimethylnonane <sup>d</sup>	0.17	ND	0.11	trace	0.04	ND	ND	MS,RI
28.717	1027	1025	para-Cymene <sup>e</sup>	0.14	0.09	0.05	0.05	0.06	0.01	0.02	MS,RI
29.085	1032	1029	Limonene <sup>a,e</sup>	2.31	1.24	0.85	0.72	0.68	0.28	0.16	MS,RI
29.199	1034	1031	beta-Phellandrene <sup>e</sup>	0.01	0.08	0.09	0.06	0.07	0.03	trace	MS,RI
29.209	1034	1031	2-Ethyl-1-hexanol <sup>e</sup>	0.43	0.34	0.67	1.53	0.23	1.31	3.23	MS,RI
29.333	1035	1032	1,8-Cineole <sup>e</sup>	0.13	trace	0.03	0.02	0.16	0.17	ND	MS,RI
29.554	1038	1035	ortho-Cymene	trace	ND	trace	trace	ND	trace	ND	MS,RI
29.681	1040	1037	Benzyl alcohol <sup>e</sup>	ND	trace	0.03	0.05	0.05	0.30	1.41	MS,RI
29.880	1042	1039	5-Methyl-5-vinyldihydrofuran- 2(3H)-one	ND	ND	ND	ND	trace	0.06	0.01	MS,RI
30.428	1049	1046	Phenylacetaldehyde <sup>a,e</sup>	ND	ND	ND	ND	0.06	0.08	ND	MS,RI
30.440	1049	1046	2-Hydroxybenzaldehyde <sup>e</sup> + unknowns	trace	trace	trace	0.01	ND	trace	trace	MS,RI
30.528	1050	1047	1,3-Diethylbenzene <sup>d</sup> + unknown	0.11	0.10	0.06	ND	0.04	0.04	trace	MS,RI
30.811	1054	1051	1-Methyl-3-propylbenzene <sup>d</sup>	0.14	0.06	0.03	0.03	0.04	0.05	trace	MS,RI
31.100	1057	1054	gamma-hexalactone <sup>e</sup>	ND	ND	ND	trace	0.06	0.07	2.41	MS,RI
31.267	1059	1056	1-Methyl-4-propylbenzene <sup>d</sup> + unknown	0.12	0.05	0.04	0.06	0.04	0.05	trace	MS,RI
31.356	1061	1057	1-Ethyl-3,5-dimethylbenzene <sup>d</sup>	0.08	0.06	0.06	0.07	0.06	0.05	trace	MS,RI
31.477	1062	1059	gamma-Terpinene <sup>e</sup>	0.03	trace	0.02	trace	trace	0.02	trace	MS,RI
31.581	1063	1060	4-Methyldecane <sup>d</sup>	0.08	0.05	0.02	trace	0.02	0.03	ND	MS,RI
31.800	1066	1063	1-Phenylethanol <sup>e</sup>	trace	ND	trace	0.02	0.01	0.02	0.08	MS,RI
31.940	1067	1065	1-Methyl-2-propylbenzene <sup>d</sup>	0.13	0.08	0.04	0.03	0.05	0.06	trace	MS,RI
32.156	1070	1067	Acetophenone <sup>e</sup>	0.07	0.07	0.04	0.05	0.02	0.04	0.11	MS,RI
32.421	1073	1070	3-Methyldecane <sup>d</sup>	0.08	trace	trace	ND	0.04	0.05	ND	MS,RI
32.500	1074	1071	cis-Linalool oxide (furanoid) <sup>e</sup>	ND	ND	ND	trace	ND	0.03	ND	MS,RI
32.648	1076	1073	1-Octanol <sup>e</sup>	0.06	0.05	0.04	0.02	0.03	0.06	0.18	MS,RI
32.660	1076	1073	Heptanoic acid <sup>e</sup>	ND	ND	0.01	0.01	0.01	0.02	ND	MS,RI
32.815	1078	1075	1-Ethyl-2,4-dimethylbenzene <sup>d</sup>	0.06	ND	0.04	0.03	0.04	0.05	trace	MS,RI
33.539	1086	1084	1-Ethyl-2,5-dimethylbenzene <sup>d</sup>	0.09	0.08	0.04	0.02	0.04	0.05	trace	MS,RI

33.653	1087	1086	Terpinolene <sup>e</sup>	0.04	trace	0.02	trace	trace	0.04	trace	MS,RI
33.760	1088	1087	Guaiacol <sup>e</sup>	ND	ND	trace	trace	trace	trace	trace	MS,RI
33.793	1089	1087	trans-Linalool oxide (furanoid) <sup>e</sup>	ND	ND	ND	trace	trace	0.04	trace	MS,RI
33.842	1089	1088	1-Ethyl-3,4-dimethylbenzene <sup>d</sup> (or isomer)	0.05	0.03	trace	trace	0.02	trace	ND	MS,RI
33.890	1090	1089	α,α-Dimethylbenzyl alcohol	ND	ND	trace	trace	0.02	0.06	0.17	MS,RI
33.981	1091	1090	Fenchone <sup>e</sup>	trace	ND	trace	0.01	0.02	trace	ND	MS,RI
34.086	1092	1091	1-isopropenyl-4-methylbenzene	ND	ND	ND	0.01	0.02	0.01	trace	MS,RI
34.160	1093	1092	2-Nonanone <sup>e</sup>	ND	ND	ND	0.01	0.02	0.01	0.02	MS,RI
34.364	1095	1094	5-Ethyl-5-methyldihydrofuran- 2(3H)-one <sup>tent</sup>	ND	ND	ND	ND	trace	0.01	0.03	MS
34.459	1096	1096	Methyl benzoate <sup>e</sup>	ND	ND	ND	0.02	0.01	0.01	0.05	MS,RI
34.820	1100	1100	Undecane <sup>e</sup>	0.43	0.37	0.14	0.10	0.17	0.12	0.02	MS,RI
34.869	1101	1101	Linalool <sup>e</sup>	0.14	trace	0.08	0.13	0.11	0.18	0.13	MS,RI
35.314	1107	1106	Nonanal <sup>a,e</sup>	0.22	0.28	0.07	0.10	0.09	0.11	ND	MS,RI
35.942	1116	1114	2-Phenylethyl alcohol <sup>e</sup>	trace	0.05	0.05	0.13	0.06	1.99	7.42	MS,RI
36.079	1118	1116	1,2,4,5-Tetramethylbenzene <sup>d</sup>	0.06	0.04	0.03	0.04	0.04	0.06	trace	MS,RI
36.338	1121	1119	1,2,3,5-Tetramethylbenzene <sup>d</sup>	0.04	0.09	0.03	trace	0.04	0.06	trace	MS,RI
36.477	1123	1121	exo-Fenchyl alcohol	trace	trace	trace	0.02	0.02	0.03	trace	MS,RI
36.625	1125	1123	Isophorone <sup>e</sup>	ND	ND	ND	trace	trace	0.03	0.07	MS,RI
36.680	1126	1124	2-Ethylhexanoic acid <sup>e</sup>	ND	ND	0.01	0.02	0.05	0.03	0.24	MS,RI
38.091	1144	1142	Benzeneacetonitrile	ND	ND	ND	ND	ND	0.04	0.06	MS,RI
38.473	1149	1147	4-Ketoisophorone	ND	ND	ND	trace	ND	0.01	0.04	MS,RI
38.669	1152	1149	Camphor <sup>e</sup>	0.04	trace	0.06	0.07	0.11	0.04	0.02	MS,RI
38.777	1153	1151	1,2,3,4-Tetramethylbenzene <sup>d</sup>	0.04	0.02	0.03	0.03	0.03	0.02	0.04	MS,RI
39.094	1157	1155	gamma-heptalactone	ND	ND	ND	ND	ND	trace	0.03	MS,RI
39.336	1160	1158	Menthone <sup>e</sup>	0.03	trace	0.06	0.02	0.02	trace	0.01	MS,RI
39.638	1164	1162	1,2,3,4-Tetrahydronaphthalene	ND	ND	trace	0.03	0.02	0.02	trace	MS,RI
39.853	1167	1165	Benzyl acetate <sup>e</sup>	0.08	0.02	0.03	0.03	trace	0.01	0.01	MS,RI
40.529	1175	1173	1-Nonanol <sup>e</sup>	ND	0.02	0.02	0.03	ND	0.05	0.17	MS,RI
40.614	1176	1174	Borneol	0.03	0.04	0.04	0.04	0.02	0.04	0.08	MS,RI
40.738	1178	1176	Octanoic acid <sup>e</sup>	0.06	0.18	0.06	0.05	0.05	0.01	ND	MS,RI
41.069	1182	1180	Menthol <sup>e</sup>	trace	0.05	0.05	0.08	0.07	0.10	0.24	MS,RI
41.254	1184	1182	4-Terpineol	ND	ND	0.01	0.02	0.02	0.03	0.04	MS,RI
41.562	1188	1186	Naphthalene <sup>e</sup>	trace	0.02	0.06	0.05	0.07	0.04	0.06	MS,RI
42.078	1194	1193	2-Decanone <sup>e</sup>	ND	ND	ND	0.03	trace	trace	ND	MS,RI
42.157	1195	1194	Methyl salicylate	trace	trace	trace	0.01	trace	0.01	0.01	MS,RI
42.406	1198	1197	alpha-Terpineol <sup>e</sup>	0.03	0.05	0.04	0.05	0.03	0.08	0.19	MS,RI
42.620	1200	1200	Dodecane <sup>e</sup>	0.25	0.24	0.14	0.11	0.15	0.04	0.02	MS,RI
42.634	1200	1200	Methyl chavicol	0.02	0.01	0.02	0.03	0.02	0.02	0.04	MS,RI
43.202	1208	1208	Decanal <sup>e</sup>	0.08	0.12	0.03	0.03	0.03	0.02	trace	MS,RI
43.353	1211	1210	Verbenone	ND	ND	ND	0.02	0.02	0.02	0.07	MS,RI
44.185	1223	1221	N-Phenylformamide <sup>tent</sup>	0.11	0.06	0.04	0.06	0.01	0.03	0.01	MS

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44.207	1223	1221	2-Phenoxyethanol <sup>d</sup>	0.04	0.04	0.04	0.08	0.04	0.05	0.15	MS
46.109	1249	1247	Carvone	trace	trace	trace	0.02	0.01	0.02	0.03	MS,RI
46.349	1252	1250	Linalyl acetate <sup>e</sup>	trace	0.09	0.03	0.06	0.04	0.08	0.05	MS,RI
47.000	1261	1259	gamma-Octalactone <sup>e</sup>	trace	ND	trace	0.03	0.01	0.02	0.04	MS,RI
47.392	1266	1265	(E)-2-decenal <sup>a,e</sup>	trace	0.06	0.02	ND	ND	0.01	ND	MS,RI
47.907	1273	1272	Nonanoic acid <sup>e</sup>	trace	0.17	0.08	0.03	0.05	ND	ND	MS,RI
48.079	1275	1274	1-Decanol <sup>e</sup>	trace	ND	trace	0.03	0.01	0.02	0.01	MS,RI
48.671	1283	1282	N-(2-methylphenyl)formamide <sup>tent</sup>	1.24	0.75	0.56	0.60	0.10	0.40	0.64	MS
48.997	1287	1286	Isobornyl acetate	trace	trace	trace	trace	trace	trace	0.01	MS,RI
49.188	1290	1289	Bornyl acetate	0.03	trace	0.02	0.02	0.01	0.02	0.03	MS,RI
49.232	1290	1289	(E)-Anethole	0.01	trace	0.01	0.02	0.01	0.02	0.05	MS,RI
49.557	1294	1294	2-undecanone <sup>e</sup>	0.03	trace	0.02	0.03	0.02	0.04	0.05	MS,RI
49.903	1299	1299	2-methylnaphthalene <sup>e</sup>	0.03	0.04	0.04	0.10	0.04	0.13	0.20	MS,RI
50.012	1300	1300	Tridecane <sup>e</sup>	0.08	0.07	0.06	0.08	0.05	0.04	trace	MS,RI
50.978	1315	1314	1-methylnaphthalene	0.03	0.03	0.03	0.05	0.02	0.04	0.06	MS,RI
51.301	1319	1318	2,2,4,4,6,8,8- Heptamethylnonane <sup>d?</sup>	0.28	0.23	0.19	0.27	0.22	0.22	0.14	MS,RI
53.155	1346	1345	Methyl anthranilate	0.11	0.13	0.12	0.28	0.03	0.11	0.21	MS,RI
53.870	1357	1355	Eugenol <sup>e</sup>	ND	ND	ND	trace	trace	trace	trace	MS,RI
54.460	1365	1363	gamma-Nonalactone <sup>e</sup>	trace	trace	trace	0.03	0.01	0.04	0.08	MS,RI
54.710	1368	1367	Decanoic acid <sup>e</sup> + unknown	0.06	0.08	0.06	0.07	0.02	ND	ND	MS,RI
55.429	1378	1377	alpha-Copaene	ND	ND	trace	0.05	trace	0.02	trace	MS,RI
56.290	1390	1390	beta-Cubebene	0.82	0.62	0.50	0.77	0.04	0.09	0.11	MS,RI
56.487	1393	1392	1-Tetradecene	0.01	ND	trace	0.02	0.01	0.02	0.02	MS,RI
57.020	1400	1400	Tetradecane <sup>e</sup>	0.18	0.20	0.15	0.20	0.10	0.16	0.20	MS,RI
57.266	1404	1404	Methyl N,N-dimethylanthranilate	4.84	4.28	4.08	7.11	1.33	4.51	9.93	MS
57.307	1405	1404	Diphenyl ether <sup>d?</sup>	0.04	0.08	0.06	0.15	0.03	0.12	0.21	MS,RI
57.330	1405	1405	Vanillin	ND	ND	trace	trace	trace	trace	0.01	MS,RI
57.792	1412	1412	Methyl N-methylanthranilate	4.19	3.89	3.79	7.01	0.64	2.15	3.67	MS,RI
62.041	1477	1476	1-Dodecanol <sup>e</sup>	0.11	0.18	0.10	0.17	0.04	0.05	0.09	MS,RI
62.580	1485	1485	Germacrene D	0.06	trace	0.06	0.11	trace	trace	trace	MS,RI
63.595	1500	1500	Pentadecane <sup>a,e</sup>	0.17	0.22	0.14	0.19	0.09	0.19	0.30	MS,RI
63.697	1502	1502	alpha-Muurolene	0.01	ND	trace	0.01	trace	trace	trace	MS,RI
63.867	1505	1504	2,6-di-tert-butyl-4-methylphenol (BHT) <sup>d,e</sup>	ND	trace	0.01	0.03	0.05	0.05	0.06	MS,RI
64.941	1522	1522	delta-Cadinene <sup>e</sup>	0.06	trace	0.04	0.06	ND	0.01	0.02	MS,RI
67.644	1566	1565	Dodecanoic acid <sup>e</sup>	0.27	0.38	0.13	ND	ND	ND	ND	MS,RI
69.839	1600	1600	Hexadecane <sup>e</sup>	0.15	0.18	0.13	0.17	0.07	0.12	0.25	MS,RI
70.797	1617	1616	Cedrol	0.03	0.07	trace	0.04	0.01	0.01	0.07	MS,RI
71.949	1637	1636	Benzophenone	0.03	trace	0.03	0.04	trace	0.01	0.06	MS,RI
75.756	1700	1700	Heptadecane <sup>e</sup>	0.17	0.14	0.05	0.13	0.03	0.07	0.11	MS,RI
76.524	1714	1714	2,3-dihydro-1,1,3-trimethyl-3- phenyl-1H-Indene <sup>d</sup>	0.03	0.05	trace	0.04	0.01	0.03	0.05	MS

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79.259	1763	1762	Tetradecanoic acid <sup>e</sup>	0.18	0.36	0.14	0.13	0.12	0.05	ND	MS,RI
81.380	1800	1800	Octadecane <sup>e</sup>	0.07	0.10	0.05	0.07	0.02	0.03	0.04	MS,RI
85.821	1883	1883	1-Nonadecene	0.16	0.30	0.04	0.03	0.02	ND	0.02	MS,RI
86.740	1900	1900	Nonadecane <sup>e</sup>	trace	0.06	0.04	0.04	0.01	0.02	0.04	MS,RI
88.095	1927	1927	Methyl palmitate <sup>e</sup>	trace	trace	trace	0.01	ND	trace	trace	MS,RI
89.902	1963	1962	Palmitic acid <sup>c,e</sup>	trace	0.19	0.21	0.35	0.03	ND	ND	MS,RI
91.840	2000	2000	Eicosane <sup>e</sup>	trace	0.09	0.03	0.04	0.01	0.02	0.03	MS,RI
92.984	2024	2023	Isopropyl palmitate	0.11	0.27	0.04	0.06	0.03	0.03	0.06	MS,RI
96.090	2087	2087	1-octadecanol	trace	0.04	0.01	ND	ND	ND	ND	MS,RI
96.719	2100	2100	Heneicosane <sup>e</sup>	0.06	0.12	0.03	0.06	0.01	0.02	0.04	MS,RI
101.392	2200	2200	Docosane <sup>e</sup>	0.10	0.16	0.03	0.05	0.01	0.02	0.03	MS,RI
105.867	2300	2300	Tricosane <sup>e</sup>	0.11	0.19	trace	0.02	trace	0.01	trace	MS,RI
110.161	2400	2400	Tetracosane <sup>e</sup>	0.10	0.17	trace	trace	trace	ND	ND	MS,RI
114.290	2500	2500	Pentacosane <sup>e</sup>	0.10	0.16	trace	trace	trace	ND	ND	MS,RI
118.266	2600	2600	Hexacosane <sup>e</sup>	0.11	0.17	trace	trace	trace	ND	ND	MS,RI
122.094	2700	2700	Heptacosane <sup>a,e</sup>	0.11	0.13	0.01	0.02	trace	ND	ND	MS,RI
125.824	2800	2800	Octacosane <sup>e</sup>	0.09	0.11	trace	0.04	0.01	ND	ND	MS,RI
126.362	2813	2813	10-Demethylsqualene	0.24	0.25	0.11	0.11	0.57	0.01	0.01	MS
129.925	2900	2900	Nonacosane <sup>e</sup>	0.08	0.06	0.05	0.05	0.02	ND	<u>ND</u>	MS,RI
			Total % Identified	99.27	99.08	98.59	96.85	99.66	97.64	96.68	

<sup>a</sup> Previously identified in *Calvatia gigantean* by Overton [3]

<sup>b</sup> Previously identified in *Calvatia gigantea* by Dijkstra [1,2]

<sup>c</sup> Previously identified in *Calvatia gigantea* by Bentley [4]

<sup>d</sup> Probable or potential environmental contaminant, including VOC air pollutants [11,12, 13,14,15]

<sup>e</sup> Previously identified in other mushroom varieties [16,17]

ND = not detected; trace = <0.01%; tent = tentative identification

#### Major Headspace Constituents and Changes with Sequential Purges & Sample Age

Perhaps the most surprising finding was the presence of Methyl anthranilate, Methyl N,N-dimethylanthranilate and Methyl N-methylanthranilate, with the latter two comprising 4.8-7.1% and 4.2-7.0% respectively in the fresh samples, and 1.3-9.9% & 0.6-3.7% respectively in the air-dried samples. The variations by analysis are shown in Fig. 3.





Fig. 3 Anthranilate Esters - % by Analysis

While methyl anthranilate has been produced in the laboratory by the basidiomycete *Pycnoporus cinnabarinus* in liquid cultures using maltose and diammonium tartrate as the carbon and nitrogen source [18], we believe that none of the three anthranilate esters identified in our analyses have ever previously been reported as naturally occurring in mushroom species.

Methyl N-methylanthranilate has been shown to undergo N-demethylation to methyl anthranilate using both fungi cultures of *Polyporus zonalis* ATCC 10089 [19] as well as by the topsoil bacterium *Bacillus megaterium* [20]. As no significant increase of methyl anthranilate was observed either between the airdried vs. fresh sample analyses, there is nothing to suggest that such N-demethylation was occurring in our samples.

As to occurrence in natural products, methyl N-methylanthranilate primarily is found in citrus petitgrain oils (e.g. mandarin petitgrain) where it occurs up to a 47-65% level [21,22] while methyl anthranilate is known to be important to concord grape [23], genét [24] and bitter gourd [25]. We believe the only prior reported isolation of methyl N,N-dimethylanthranilate from a natural product was from an Italian mandarin petitgrain oil at a 0.02-0.4% level [22].

Considerable changes occur, both in composition and amounts, between analyses, presumably due to <u>both</u> catabolic reactions during the rapid senescence of the mushroom as it ages as well as by the expected depletion of certain constituents during the sequential purge analysis process. For example, 1-octen-3-ol which is considered to be the typical odor constituent of many mushroom species, was "only" found in measurable quantities in the initial fresh sample (FR-1) and the initial air-died sample (AD-1) at levels of 0.45% and 0.15%, respectively. Anisole, which is one of the major components in the fresh *Calvatia* declines with each purge as the samples age while phenol (which was less than 0.01% in the initial fresh *Calvatia*) increases over time in the subsequent purges as shown in Fig. 4. Whether anisole is converted to phenol by microbial O-demethylation in our studies is unclear [26]. Similarly, Fig. 5 shows that isoamyl alcohol tends to initially increase with sample age in the fresh *Calvatia*, while in the air-dried samples a large amount 3-methylbutanal has appeared that then declines rapidly in subsequent purges. The isoamyl alcohol also tends to initially increase followed by a precipitous decrease in the air-dried samples.



# Fig. 4. Changes in Anisole & Phenol by sequential purge analysis

Fig. 5. Changes in Isoamyl alcohol & 3methylbutanal by sequential purge analysis

Similarly, 2-Methyl-1-butanol increases with each successive purge in the fresh *Calvatia* while in the air-dried samples a large amount 2-methylbutanal had appeared that then declines rapidly.



Fig. 6. Changes in 3-Octanone & 3-Octanol

Figure 6 shows that 3-Octanone, which was initially the largest component in the fresh sample, decreases with each purge while 3-octanol increases in the FR-4 analysis. Both were significantly decreased in the air-died samples.



Fig. 7. Changes in Major acids

Fig. 8. Changes in Methionol & 2-Phenethanol



Fig. 9. Changes in Lactones

Figures 7, 8 & 9 show other major changes in the air-dried samples for compounds that were either absent or very minor constituents in the fresh *Calvatia* samples.

The finding that Anisole was the major constituent in the fresh *Calvatia* sample is consistent with that reported by Overton [3], but we also found many other major constituents not found in his analysis. While Dijkstra reported that 1-octen-3-ol was the major volatile [1,2], we only found modest amounts in both the "fresh' and "air-dried" samples.

We previously noted that this mushroom is only edible when fresh and becomes disagreeable on aging. In view of the major compositional changes that occur in the sequential purges and aging, especially of the air-dried samples, such an observation is not unexpected. The significant increase in the amounts of isovaleric & 2-methylbutanoic acids, as well of methionol, phenol, and lactones (Figures 7-9) would be expected to add a disagreeable (spoiled) flavor. See Table 2 for the odor descriptions of some important volatiles.

## **Potential Environmental Contaminants**

As mentioned, the concern about potential environmental contaminants in an urban area, including VOC air pollutants was a potential issue, especially because of the high surface area of fungi species and the potential affinity of such fungi to absorb volatile organic compounds (VOCs). Accordingly, a number of potential or probable environmental contaminants are noted in our tables. Potential environmental biogenic VOCs such as terpenoids and sesquiterpenoids from trees and other vegetation are more difficult to assess and are not addressed in our analyses.

There has been debate [27,28] as to whether or not items such as 2,6-di-tert-butyl-4-methylphenol (BHT), a prior mushroom isolate and a reported isolate of at least 38 other natural products [7,16], is "natural" and the concerns about how to handle such items which are known environmental contaminants in publications. We are aware that at least one paper has addressed this problem by noting potential environmental contaminants in their tabulations [11] and we have adopted a similar protocol.

In this study, we identified 44 compounds that are probable or potential environmental contaminants [11,12,13,14,15], including 25 substituted (C1-C4) benzenes, 9 branched alkanes and 10 miscellaneous.

#### Odor Descriptions of Important Calvatia gigantea Constituents

Finally, in Table 2 we provide the organoleptic odor descriptions for the important constituents found in these analyses using the descriptors of Boelens "Perfumery Materials and Performance 2001" database [29], which has been ranked as the most reliable of the available large olfactory databases [30].

Compound	Odor Descriptors
3-Methylbutanal	fruity, peach- & cocoa-like in dilution
2-Methylbutanal	fresh, fruity, apple-like; somewhat herbal, fermented
Isoamyl alcohol	fresh, ethereal, fusel-like, fermented & yeasty
2-Methyl-1-butanol	soft ethereal, somewhat fermented, apple-like
Isovaleric acid	acidic, caprylic, sweat-like, somewhat cheesy
2-Methylbutanoic acid	acidic, caprylic, sweat-like, somewhat cheesy
Anisole	sweet, balsamic, gasoline, anise, somewhat phenolic
gamma-Valerolactone	fruity, lactonic, buttery; slightly fatty
delta-Valerolactone	lactonic, fruity
gamma-Hexalactone	herbaceous, tobacco-like, creamy
Methionol	sulfurous, meaty, soup-like aroma
Phenol	strongly phenolic, medicinal, antiseptic
1-octen-3-ol	earthy, mushroom-like
1-Octen-3-one	aromatic, mushroom-like aroma
3-Octanone	herbaceous-fruity; slightly spicy, buttery; top note of lavender
3-Octanol	oily, herbaceous, somewhat nut-like
Limonene	harsh, terpene-like; citrusy, fruity, orange, berry-like, tarty
2-Ethyl-1-hexanol	mild ethereal, somewhat yeasty & fermented

Table 2.	<b>Odor Descrit</b>	otors (Boelens	s 2001) of Im	portant <i>Calvatia</i>	gigantea	Constituents
I ubic 2.	Out Deserry			por tant Carrain	Sisanca	constituents

2-Phenylethyl alcohol	fresh, sweet aromatic floral, strongly reminiscent of rose with a hyacinth nuance
Methyl anthranilate	orangeflower-like, sweet fruity, tangerine & grape-note
Methyl N,N- dimethylanthranilate	sweet aromatic, citrusy, mandarin & tangerine note
Methyl N-methylanthranilate	sweet aromatic, citrus-fruity, musty; reminiscent of mandarin & tangerine

# CONCLUSIONS

A sequential purge and trap technique was applied to the GC-MS analyses of both fresh and air-dried samples of the giant puffball mushroom (*Calvatia gigantea*) to examine the potential constituents contributing to its flavor. The presence of Methyl anthranilate, Methyl N,N-dimethylanthranilate and Methyl N-methylanthranilate are reported for the first time in a mushroom species. Consideration of probable, or potential, environmental contaminants, including VOC air pollutants, allowed us to identify 44 compounds that met that criteria.

## REFERENCES

1. F.Y. Dijkstra, Z. Lebensm. Unters. Forsch. A. 160(2), 401-405 (1976)

2. F.Y. Dijkstra, Submerged cultures of mushroom mycelium as sources of protein and flavour compounds. [Ph.D. Thesis]. Delft Technical University, Delft NL (1976). Retrieved on Sept. 18, 2010 from http://www.fransdijkstra.nl/diss/diss.doc

3. S. V. Overton, Determination of volatile organic compounds in mushrooms. Mass Spec Source 18(2), 4-7 (1994); Scientific Instrument Services, Inc. Retrieved on Sept. 20, 2010 from http://www.sisweb.com/news/mar1994.pdf

4. R. Bentley, W.V. Lavate, C.C. Sweeley. Comp. Biochem. Phys. 11(2), 263-264 (1964)

5. J. C. Coetzee, A. E. van Wyk. African J. Biotechn. 8(22), 6007-6015 (2009)

6. S.E. Stein, director, NIST Mass Spec Data Center, "Retention Indices", in NIST Chemistry WebBook, NIST Standard Reference Database Number 69 (P.J. Linstrom and W.G. Mallard, eds.), National Institute of Standards and Technology, Gaithersburg MD, 20899, http://webbook.nist.gov, (retrieved September 20, 2010).

7. ESO 2000 (Update 2006) - The complete database of essential oils, Boelens Aroma Chemical Information Service. 2006, Leffingwell & Associates, Pub., Canton GA, USA

8. R.P. Adams. Identification of essential oil components by gas chromatography/quadrapole mass Spectrometry, 3d. Ed. Allured Pub. Corp., Carol Stream IL (2004)

9. E. Kovats. Helv. Chim. Acta 41, 1915-32 (1958)

10. H. van den Dool, P. D. Kratz. J. Chromatogr. 11, 463–471 (1963)

11. A. Vallat, S. Dorn. J. Agric. Food Chem. 53(10), 4083–4090 (2005)

12. R. G. Derwent. In Volatile Organic Compounds in the Atmosphere. (R.E. Hester, R.M. Harrison, eds.), pp. 1-15. The Royal Society of Chemistry, Cambridge (1995), RSC eBook Collection. Retrieved on Sept. 18, 2010 from http://www.rsc.org/ebooks/archive/free/BK9780854042159/BK9780854042159-00001.pdf.

13. R. Friedrich, A. Obermeier. Anthropogenic emissions of volatile organic compounds. In Reactive Hydrocarbons in the Atmosphere, (C. N. Hewitt, ed.), pp. 1-38. Academic Press, London. (1998)

14. X. Xu, L. L. P. van Stee, J. Williams, J. Beens, M. Adahchour, R. J. JVreuls, U. A. Th. Brinkman, J. Lelieveld. Atmos. Chem. Phys. Discuss. 3, 1139–1181 (2003). Retrieved on July 19, 2008 from http://www.atmos-chem-phys-discuss.net/3/1139/2003/acpd-3-1139-2003.pdf.

15. M. E. Fleming-Jones, R. E. Smith. J. Agric. Food Chem. 51(27), 8120-8127 (2003)

16. VCF 2000 - Volatile compounds in food database, Boelens Aroma Chemical Information Service. 2000, Leffingwell & Associates, Pub., Canton GA, USA

17. S. Breheret, T. Talou, S. Rapior, J-M. Bessiere. J. Agric. Food Chem. 45(3), 831-836 (1997)

- 18. B. Gross, G. Yonnet, D. Picque, P. Brunerie, G. Corrieu, M. Asther. Appl. Microbiol. Biotechnol. 34, 387-391 (1990)
- 19. G.V. Page, B. Scire, M.I. Farbood. US Patent 5200330, Filed 14 Sept. 1990, Issued 06 April 1993.
- 20. M. Taupp, D. Harmsen, F. Heckel, P. Schreier. J. Agric. Food Chem. 53, 9586-9589 (2005)
- 21. Z. Fleisher, A. Fleisher. J. Essent. Oil Res. 2, 331-334 (1990)
- 22. L. Mondello, A. Basile, P. Previti, G. Dugo. J. Essent. Oil Res. 9, 255-266 (1997)
- 23. J-W. R. Liu, J. F. Gallander. J. Food Sci. 50(2), 280-282 (1985)

24. D. Joulain. In Progress in essential oil research (Proc. Intern. Symp. on Ess. Oils). (E. J. Brunke, ed.), pp. 57-67. Walter de Gruyter, Berlin (1986)

- 25. L. N. Fernando, I. U. Gruen. Flav. Fragr. J. 16, 289-293 (2001)
- 26. G. F. White, N. J. Russell, and E. C. Tidswell, Microbiol. Rev., 60(1), 216-232 (1996)
- 27. P. Davoli, F. Bellesia, A. Pinetti. J. Agric. Food Chem. 51(15), 4483-4483 (2003)
- 28. E. Ibanez, F. J. Senorans, P. Diaz, G. Reglero. J. Agric. Food Chem. 51(15), 4484-4484 (2003)

29. PMP 2001 - Database of perfumery materials & performance, Boelens Aroma Chemical Information Service. 2001, Leffingwell & Associates, Pub., Canton GA, USA

30. M. Pintore, C. Wechman, G. Sicard, M. Chastrette M, N. Amaury, J. R. Chretien. J. Chem. Inf. Model. 46(1), 32-38 (2006)