VOLATILE COMPOUNDS IN STINKHORN (*PHALLUS IMPUDICUS* L. EX PERS.) AT DIFFERENT STAGES OF GROWTH

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Abstract

Volatile compounds released from stinkhorn (*Phallus impudicus* L. ex Pers.) at its different stages of maturity (immature "egg" form, mature fruit body, over-ripe fruit body) were examined. A total number of 59 components were identified with the use of headspace solid-phase microextraction (HS-SPME) method followed by gas chromatography-mass spectrometry (GC-MS). The research showed a considerable qualitative difference of major components, according to their stages of maturity. Dimethyl trisulfide, *cis*-β-ocimene, *trans*-β-ocimene, 2-phenylacetladehyde and 2-phenylethanol were the most abundant volatiles in mature fruit bodies. Dimethyl oligosulfides, the most significant contributors to the strong and foul odor of stinkhorn fruit bodies, were absent at egg-shaped and over-ripe stages.

Keywords: Phallus impudicus, stinkhorn, mushroom, volatiles, GC-MS, SPME

Introduction

The stinkhorn (*Phallus impudicus* L. ex Pers.) of the family *Phallaceae* is a common and widely distributed fungus in Europe and North America. Its fetid smell and the unmistakable appearance make it one of the most easily recognizable species of fungi. The mature fruit body (an inedible white hollow stalk-like stipe, 10-30 cm high, with a cap 2-4 cm wide, covered with olive brown to dark brown slime containing the spores) evolves from the egg-shaped form (a whitish to yellowish round structure, up to 6 cm across, usually partly submerged in the ground). The "eggs", which lack the unpleasant scent, are edible and regarded as delicacies in some countries.

Several stinkhorn volatiles were reported by List and Freund (1966, 1967a, 1967b) and particularly dimethyl oligosulfides (dimethyl disulfide, dimethyl trisulfide) are considered to make a major contribution to the strong and foul odor of mature fruit bodies 0 et al., 1994). To our knowledge, no information about volatile constituents of immature and over-ripe fruit bodies has been published.

This research was designed to examine major volatile compounds of stinkhorn at different stages of maturity using headspace solid-phase microextraction (HS-SPME). HS-SPME is a simple, rapid, solvent-free sample preparation technique, which combines extraction, concentration and injection in one step 0 and Pawliszyn, 1990). HS-SPME in the combination with gas chromatography-mass spectrometry (GC-MS) has gained a wide acceptance as an effective way for analyzing volatile compounds in plant materials (Cong et al., 2007, Wu et al., 2004) foods (Kataoka et al., 2000, Wilkes et al., 2000) and other biological samples (Ulrich, 2000).

Experimental:

Material:

Stinkhorn fruit bodies at different stages of growth were collected in autumn 2005 in Brdské lesy Woods, the Czech Republic. The specimens were brushed clean of forest debris and sorted into three maturity categories as follows: egg-shaped form; mature fruit body; over-ripe fruit body. After being sorted out, the samples from each category were used for the analysis of volatile compounds.

SPME fiber and extraction conditions:

The sampling of volatiles was performed by means of headspace solid-phase microextraction (SPME). A manual SPME holder with a 65 μm PDMS-DVB fiber from Supelco (Bellefonte, PA, USA) was used for the SPME procedure. The SPME fiber was conditioned following the supplier's instructions prior to use.

Samples from each category were cut into small cubes, placed into 10 ml glass vials and sealed with septum-type caps from Supelco (Bellefonte, PA, USA). For each extraction, after the SPME needle pierced the septum, the fiber was extended through the needle and exposed to the headspace above each sample at 25 °C for 30 min. After the extraction time, the fiber was pulled into the needle, and then the needle was removed from the septum and was directly inserted onto the injection port of the GC. The desorption of analytes from the fiber coating was performed by heating the fiber in the injection port at 230 °C for 2 min.

GC-MS conditions

Chromatographic separation was performed with the usage of a Fisons GC8000 apparatus (Fison Instruments, Italy) equipped with a HP-5 capillary column (25 m × 0.2 mm i.d. × 0.33 μm film thickness, Hewlett-Packard, USA) and connected to a Fisons MD800 mass selective detector (Fison Instruments, Italy). The GC oven temperature was programmed as follows: from 50 °C (2 min isothermal delay) to 220 °C at a rate of 5 °C/min and kept for 10 min. The injection temperature was 230 °C. Helium of high-purity was used as the carrier gas at a flow rate of 1 ml/min. The mass spectrometer was operated at electron impact mode at 70 eV and mass spectra were recorded in the full acquisition mode.

The identification of the volatile compounds was based on the comparison of the obtained mass spectra with those of authentic standards from the NIST and Wiley mass spectral libraries with the percentage resemblance over 85 %.

Results and discussion

The fast development of stinkhorn (*Phallus impudicus* L. ex Pers.) fruit bodies, even after the harvesting, is represented by significant metabolism changes in the fungus during a small period of time (hours). Although the abundance of volatiles varies considerably during ripening and ageing, this research was focused only on qualitative changes.

It was possible to identify 59 components from various classes of compounds such as hydrocarbons, alcohols, aldehydes, ketones, acids, esters, terpenoids, sulfuric compounds and others. The total ion chromatograms of the volatile compounds at different stages of growth (egg-shaped, mature, over-ripe) obtained by the HS-SPME-GC-MS method are shown in Fig.1 and the corresponding volatile compounds identified by this method are listed in Tab. 1.

The total amount of volatile compounds at the egg-shaped form stage of the stinkhorn was very low (Fig.1a). The prevailing components were 1,4-dimethoxybenzene, which has sweet floral scent (Dötterl et al., 2005), followed by 3-octanone, 2-hexanone and a series of alkanes (undecane, dodecane, tridecane, tetradecane). 3-Octanone has been reported as one of the major contributors to the characteristic mushroom flavor (Fischer and Grosch, 1987, Pyysalo and Suihko, 1976, Venkateshwarlu et al., 1999) due to its mushroom-like, sweet buttery odor (Cho et al., 2006).

Figure 1: Volatile compounds of stinkhorn (*Phallus impudicus* L. ex Pers.) at different stages of growth obtained by HS-SPME-GC-MS method

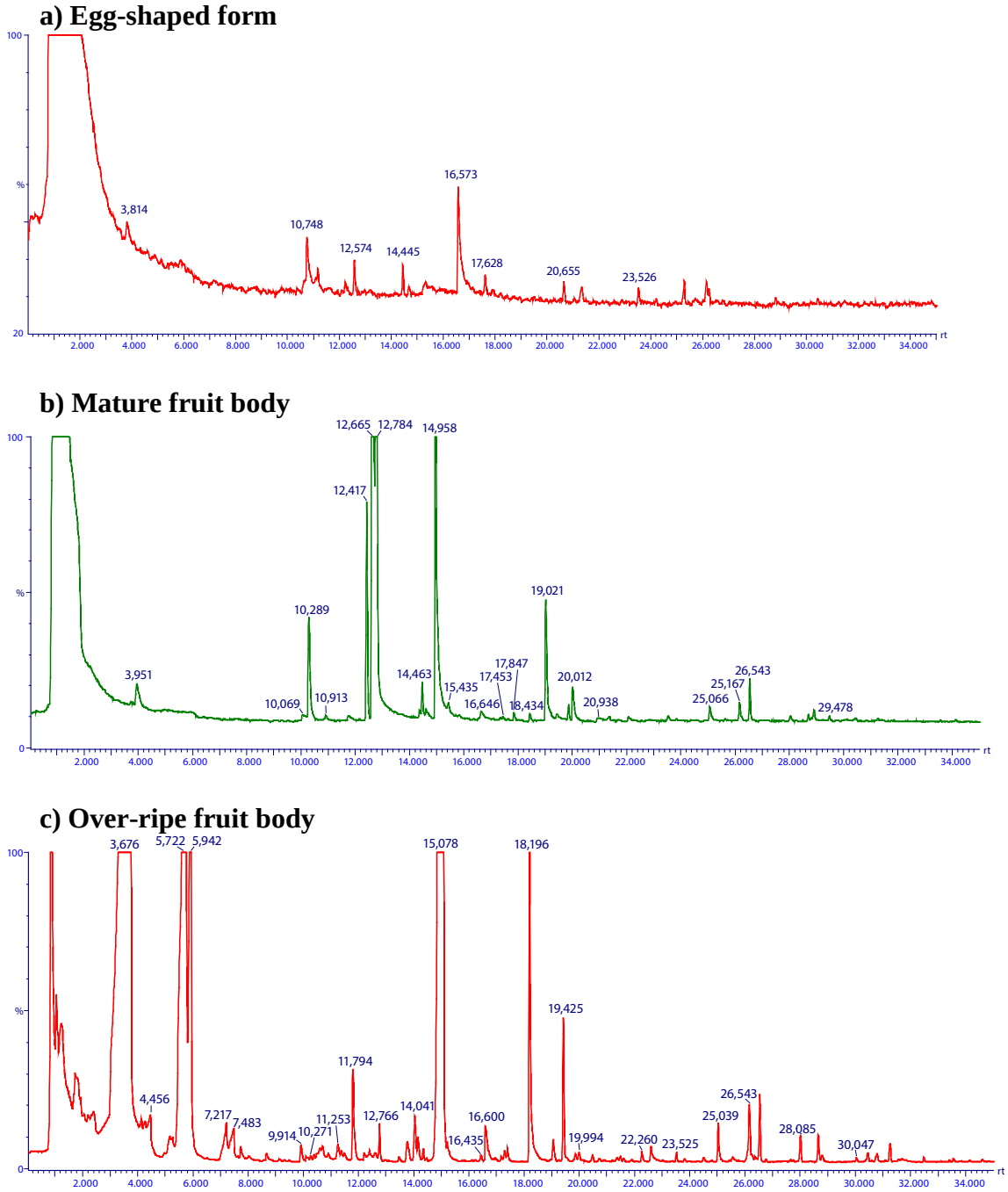

Table 1: Volatile compounds of stinkhorn (*Phallus impudicus* L. ex Pers.) at different stages of growth obtained by HS-SPME-GC-MS method

No.	Compound	Retention time (min)			Note
		Egg-shaped form	Mature fruit body	Over-ripe fruit body	
1	Acetic acid	-	-	3,676	
2	2-Hexanone	3,814	-	-	
3	Dimethyl disulfide	-	3,951	-	
4	Propanoic acid	-	-	4,456	
5	2-Methylpropanoic acid	-	-	4,969	
6	2,3-Butanediol	-	-	5,722	Isomeric form*
7	2,3-Butanediol	-	-	5,942	Isomeric form*
8	Hexanoic acid	-	-	7,217	
9	Heptanoic acid	-	-	7,483	
10	2-Heptanone	-	-	7,639	
11	2,5-Dimethylpyrazine	-	-	8,373	
12	2,6-Dimethylpyrazine	-	-	8,583	
13	Benzaldehyde	-	10,069	9,914	
14	Dimethyl trisulfide	-	10,289	10,271	
15	Phenol	-	-	10,611	
16	3-Octanone	10,748	-	10,693	
17	2-Pentylfuran	-	-	10,904	
18	β-myrcene	-	10,913	-	
19	2,3,5-Trimethylpyrazine	-	-	11,253	
20	Dipropylene Glycol Methyl Ether	-	-	11,794	
21	*cis*-β-Ocimene	-	12,417	-	Isomeric form*
22	Methyl 2-ethylhexanoate	12,574	-	-	
23	2-Phenylacetaldehyde	-	12,665	-	
24	*trans*-β-Ocimene	-	12,784	12,766	Isomeric form*
25	2,3,5,6-Tetramethylpyrazine	-	-	14,041	
26	2-Nonanone	-	-	14,160	
27	Undecane	14,445	-	-	
28	Linalool	-	14,463	-	
29	Nonanal	-	14,600	-	
30	2-phenylethanol	-	14,958	15,078	
31	allo-Ocimene	-	15,435	-	
32	2-Ethyl-2,3,5-trimethylpyrazine	-	-	16,435	
33	1,4-Dimethoxybenzene	16,573	-	-	
34	4-Ethylphenol	-	-	16,600	
35	3-Phenylpropanal	-	16,646	-	
36	2-Decanone	-	17,453	17,398	
37	Decanal	-	17,847	-	
38	Dodecane	17,628	-	-	
39	4-Methylbenzaldehyde	-	-	18,196	
40	Dimethyl tetrasulfide	-	18,434	-	
41	Photocitral B	-	19,021	-	

42	Phenethyl acetate	-	-	19,425	
43	2-Phenyl-2-butenal	-	20,012	19,994	
44	2-Undecanone	-	-	20,480	
45	Tridecane	20,655	-	-	
46	Indole	-	20,938	-	
47	Phenethyl propanoate	-	-	22,260	
48	Phenethyl 2-methylpropanoate	-	-	23,525	
49	Tetradecane	23,526	-	-	
50	Geranyl acetone	-	25,066	25,039	
51	β-Farnesene	-	25,167	25,168	Isomeric form*
52	α-Farnesene	-	26,543	26,543	Isomeric form*
53	2,6-bis(1,1-dimethylethyl)-4-methylphenol	-	-	26,773	
54	Phenethyl 2-methylbutanoate	-	-	28,085	
55	Isopropyl dodecanoate	-	29,478	-	
56	Phenethyl pentanoate	-	-	30,047	
57	2-Pentadecanone	-	-	31,258	
58	Phenethyl hexanoate	-	-	34,835	
59	Phenethyl 2-phenylacetate	-	-	36,642	

* Isomeric forms not fully characterized

Sulfuric odorous compounds (dimethyl oligosulfides), which are insects attractants, were absent since the slime covered cap containing the spores is enclosed in the mushroom's veil. However the unpleasant scent might evolve during culinary preparation of stinkhorn "eggs".

22 volatile compounds (including dimethyl oligosulfides, 2-phenylacetaldehyde, 2-phenylethanol, Photocitral B, possibly formed during degradation of citral (Szente and Szejtli, 1987), 2-phenyl-2-butenal, a potential indicator of roasting intensity (Fadel et al., 2006), and a number of terpenoids) were identified in mature fruit bodies (Fig. 1b). 2-phenylacetaldehyde and 2-phenylethanol, both important aroma-active compounds derived from phenylalanine (Tieman et al., 2007), were also reported as insects attractants (Ragusoa et al., 2003, Zhu et al., 2005). Major terpenoids identified were *cis*-β-ocimene and *trans*-β-ocimene, commonly described as responsible for fresh floral odors (Füssel et al., 2007).

A series of dimethyl oligosulfides (dimethyl disulfide, dimethyl trisulfide, dimethyl tetrasulfide) were identified dimethyl trisulfide being the most potent. Sulfuric odorous compounds are known to be formed during degradation of sulfur-containing amino acids cysteine, cystine and methionine (Herbert et al., 1971, Kiene and Hines, 1995, Mackie et al., 1998). Although present at lower abundances, these sulfuric compounds are the most significant contributors to the foul odor of mature fruit bodies since their sensory threshold values are very low (Golovjna and Rothe, 1980, O'Neill and Phillips, 1992).

Volatile compound composition of over-ripe fruit bodies (Fig. 1c) differed significantly from those of mature fruit bodies. 41 components were identified, the main volatiles being acetic acid, 2,3-butanediol, 2-phenylethanol and 4-methylbenzaldehyde, also reported as an insect attractant (Miles et al., 1975). Furthermore a number of other important flavor contributors, e. g. phenethyl esters and alkylpyrazines, were identified. Dimethyl oligosulfides were found only in traces since majority of the smelling slime was carried away by insects. Therefore over-ripe fruit bodies can have slightly honey-like roasty aroma over their unpleasant odor.

Conclusion

The composition of stinkhorn (*Phallus impudicus* L. ex Pers.) volatiles changes significantly during the fast growth of the fungus. Volatile analyses showed obvious differences of major components, both in qualitative and relative abundance, all this according to the stage of maturity. A total number of 59 volatiles from various classes of compounds (including hydrocarbons, alcohols, aldehydes, ketones, acids, esters, terpenoids, sulfuric compounds and others) were identified. Dimethyl oligosulfides, carriers of the foul smell, were absent both in egg-shaped and over-ripe forms of the fungus.

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