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Comparison of the fragrance constituents of *Tuber aestivum* and *Tuber brumale* gathered in Hungary

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Summary

Scent components from two species of *Tuber* truffles gleaned in Hungary were examined with gas chromatography-mass spectrometry subsequent to Likens-Nickerson simultaneous distillation extraction. The analysis revealed several differences between them. In *Tuber aestivum* the abundant presence of aliphatic alcohols was the most characteristic feature, while in *Tuber brumale* methoxy methyl benzenes proved to be the most dominant aroma constituents. The latter components were totally absent from *Tuber aestivum*, so they could be the main distinctive key-substances between the Hungarian summer and winter truffle species. Of the characteristic C_8 compounds present in most mushrooms only 3-octanone, 1-octen-3-one and 3-octanol were identified in both species, in *T. aestivum* they were found in a larger proportion.

Introduction

Owing to their favourable organoleptic properties, truffles are highly beloved and appreciated in gastronomy and possess huge commercial value. Their sensory qualities and man's inability to control the development of the mycelium make these mushrooms attract both economic and scientific interest intensely (HARKI et al., 2006).

The most important distinctive and quality criterion of truffles – aside from morphological structure – is the aroma. By attracting different animals (*e.g.* flies, pigs, dogs) their fragrance is the characteristic feature that reveals their presence in their habitat. The thorough assessment of truffle volatiles can support to avoid fraud. Comprehensive knowledge about truffles is necessary to distinguish the *Tuber* species, because at times slightly odorous and less valuable fungi are sold and deceive the customers. For instance, *Tuber borchii* is used as an imitation of the precious *Tuber magnatum* Pico, since they fairly resemble each other on the surface. A similar situation there exists in the case of *Tuber indicum* and *Tuber melanosporum*, the first truffle possesses much weaker quality (MASHAYEKHI, 2005).

Several authors have tried to reveal and identify the volatile compounds of the various truffle species. MARCH et al. (2006) examined six kinds of French truffles with headspace analysis. All the examined six species exhibited 19-25 compounds, with *T. aestivum* producing the lowest (19) and *T. rufum* displaying the highest number (*i.e.* 25). These authors observed that alcohols and esters present formed homologous series each, and a number of esters was detected with some of them, unique for the truffle species in question. DÍAZ et al. (2003) tried to describe the aroma of the different type *Tubers* and to characterize their origin by their scent. The comparison of *T. melanosporum* and *T. aestivum* clarified that the first species had a stronger smell in terms of total amount and number of volatile compounds detected. The investigation of summer truffles of different geographical origin (two distinct geographical zones of Spain, Valladolid and Soria) led to compounds that can be associated with the origin (factors such as growing conditions, ecology, etc.). In the above study among the components -found only in one of the examined samples and not reported in other species before-, the most important were: 2-furancarboxaldehyde, 2-propenoic acid and decanal. The presence of 2-propenoic acid was attributed to the use of pesticides thus merely the two compounds left could be used to discriminate between the origins. For a summer truffle of an another provenance, the presence of 3-methyl-3-penten-2-one was considered a distinctive component since it had never been detected in any of other *Tuber* species previously. Although in the latter case the examined truffles belonged to the same species significant differences were found in their aroma features attributable to the different place of origin.

Another important field in truffle investigation is the study of ageing during storage. Freshness is extremely important for both the consumers' safety and commercial points of view. It determines the quality and price of the product. Truffle's ageing implies several biochemical reactions, which cause the change of the flavour with time. In these „*post harvest*" processes an increase of alcoholic and sulphur compounds attributed to spontaneous fermentation has been observed (FALASCONI et al., 2005). These authors examined the change of the fresh white truffle's aroma (*Tuber magnatum*, Pico) in the days following the harvest, in order to determine the maximum preservation time for this truffle. Two sulphur containing substances, namely 2,4-dithiapentane and thiobismethane were the most representative compounds of the fresh product. In the work of MASHAYEKHI (2005) dithiapentane is mentioned as a typical volatile constituent identified in white truffle. A relevant change of truffle's aroma has been observed after 5 days from harvesting. The concentration of 2,4-dithiapentane remained quite constant with ageing while other compounds changed their abundance in the head-space substantially. In particular, the variation during storage has been attributed to an increase of four compounds in the head-space: acetic acid, ethanol, 2-methyl-1-butanol, 2-methyl-1-propanol.

Numerous articles have already been published on the aroma compounds of different truffle species grown in France, Italy or Spain. Our present study written on the summer and winter truffles would like to complete the knowledge about *Tuber* volatiles of different geographic derivations in Hungary.

Materials and methods

Samples and chemicals

The summer truffles (*Tuber aestivum* Vitt.) studied were collected in August 2007, while winter truffles (*Tuber brumale* Vitt.) were picked in February 2008. The provenance of the samples were in the Somogy district *i.e.* in the south-west part of Hungary. The gleaned truffle samples were placed in aluminium-foil covered (inner surface) aroma tight plastic bags and kept at 4 °C until analysis performed within 24 hours. The applied chemicals and solvents were of analytical and HPLC grade and were purchased from Merck (Darmstadt, Germany). Teflon™-valve equipped distillation-extraction apparatus and glassware of thermoresistant Pyrex quality were used during sample preparation.

Sample preparation

Volatile compounds of the truffle samples were extracted in a modified Likens-Nickerson SDE apparatus. Cleaned, sliced 200 g of the samples were placed into a 4000 ml round-bottom flask with 180 g of salt, 900 ml of distilled water and 150 µl of 0,8 mg/ml undecanol-1 (ethanolic) solution as an internal standard (ISTD). The NaCl was added to the mixture to expel the steam volatile aroma constituents from the water phase more efficiently (salting out effect). The distillation was conducted for 1 hour after boiling and was repeated in triplicate. Pentane was used as the organic solvent in the second flask and was heated at 60 °C. Three consecutive measurements were perfomed and the volatiles were collected to the same pentane solvent. Subsequent to distillation-extraction, the extract was frozen to remove water (in the form of ice) and the remainder (dry pentane extract) was evaporated to 1 ml. Eventually, 3 x 1 µl of the essence was injected into the gas chromatograph – mass spectrometer consecutively.

GC-MS analysis

An HP 5890/II gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with a 60 m x 0,25 mm x 0,25 µm AT-WAX fused silica capillary column and a 5971/A mass selective detector was used to analyse the volatile compounds of truffle extracts. The initial oven temperature was 60 °C and immediately increased to 280 °C at a rate of 4 °C/min. The injector was operated in splitless mode at 270 °C, with a 100:1 split ratio at 0.1 min. delay time. The detector was run in electron impact mode (70 eV) at 280 °C. Helium (4.6) was used as a carrier gas with the flow rate of 30 cm/s. The detection was performed in the 35-350 mass range at 390 mass/s scan speed. The identification of the fragrance constituents were accomplished using the NBS49k.L, Wiley138.L, Wiley275.L and NIST05.L spectrum libraries.

The aromaspectra method

For the interpretation of the composition data the *„aromaspectrum"* method developed at the Department of Food Chemistry and Nutrition of Corvinus University of Budapest (KORÁNY, 2005) was adopted. The method eliminates the distorting effects of the measuring process by normalizing both axes of the total ion chromatograms (TICs). As a consequence the comparison of the fragrance features becomes unambiguously executable. In the process the retention times are replaced with programmed temperature retention indices -PTRIs, calculated on the base of n-alkanes' elution of a model solution-, while on the vertical axis area-ratios related to the total relative intensity sum (referring to the undecanol-1 ISTD) are depicted instead of absolute abundances. This mode of expression represents the participation of the individual volatile constituents from the total aroma (area). With a special unit enlarging technique (not discussed in details) small peaks „invisible" on the TICs were converted into „visible" ones and shown on the diagrams. These normalized graphs – called aromaspectra – were compared instead of the total ion chromatograms recorded by the GC-MS equipment.

Results and discussion

By GC-MS analysis subsequent to the simultaneous distillation-extraction method the detection of 102 compounds in *T. aestivum* and 104 components in *T. brumale* was possible. Of them 26 common compounds were found in both species. The number of the identified compounds extracted with distillation-extraction method exceeded those performed with headspace methods found in literature (MARCH et al., 2006; FALASCONI et al., 2005; DÍAZ et al. 2009). The recognized components were sorted into chemical classes in accordance with their structure and are listed in Tab. 1. Comparing the total ion chromatograms of the examined truffle species (Fig. 1.) at first glance *T. aestivum* seems to be more fragrant, because its record contains peaks in greater number and higher abundance.

Both samples exhibit a lot of substances belonging to the class of „Open chain alcohols, aldehydes, ketones" among the identified constituents. These components generally possess relatively high volatility and they appear early in the chromatogram. Aldehydes play an important role in the aroma formation of many edible raw materials, so their presence is not surprising in the diverse *Tuber* species. MASHAYEKHI (2005) identified several kind of aldehydes in the different truffles. Of the most important aldehydes contributing to the overall aroma of *Tubers* hexanal, heptanal and 2-butenal were identified in our samples as well. Though alcohols generally do not belong to the characteristic compounds of the organoleptic profiles, they contribute – like the aldehydes – to the total aroma of the truffles (MASHAYEKHI, 2005). Our investigations show, that in the case of *T. aestivum*, alcohols receive a share of nearly the half of the total aroma (measured in peak area). A number of C_8 compounds – including alcohols, aldehydes and ketones – were found in both varieties. The presence of these substances in the examined samples is almost expected, because these constituents – especially 1-octen-3-ol and 1-octen-3-one – are of great significance in developing the specific mushroom odour (BAUER et al., 2001; MARSILI, 2002). In our scent measurements we also succeeded in identifying numerous C_8 compounds in other mushrooms *e.g. Agaricus bisporus*, *Agaricus blasei*, *Boletus edulis*, *Cantharellus cibarius* and *Hericium erinaceus* as well. Data are intended to be published in the near future. For example, 1-octen-3-ol – known as „mushroom alcohol" –, also contributes to the familiar earthy note of mushrooms. The unsaturated ketone 1-octen-3-one found along with the *„alcohol of mushrooms"* has got a much more intense odour with a reported sensory threshold of 0.005 ppb compared to the 1 ppb level of the 1-octen-3-ol alcohol (ROWE, 2005). In fungi, 1-octen-3-ol is a product of the enzymatic breakdown of linoleic acid by lipoxygenase and a hydroperoxide lyase (BELITZ et al., 2009). This compound is detected in raw mushrooms especially when they are damaged (CHITARRA et al., 2004). In the truffle species examined by us the C_8 compounds achieved a share of 6,91 % in the total aroma of the summer *T. aestivum*. Contrary to the winter one where the quota of these substances amounted to 0,33 % expressed in the total fragrance area, merely.

The aroma of many vegetables is due to volatile sulphur compounds obtained by a sort of enzymatic reactions (BELITZ et al., 2009). In previous truffle investigations (DÍAZ et al., 2003; FALASCONI et al., 2005; TIRILLINI et al.,2000; SPLIVALLO et al., 2007), a number of sulphur compounds were reported as the most characteristics of *Tubers*. In our present examination, several kind of sulphur constituents were identified in both species. The components belong to various chemical classes: thiazoles, thiophenes, dithiazines and their derivatives. The skeletons of the identified most important heterocyclic components of high odour activity are depicted in Tab. 2. Although these volatiles are normally present in trace quantities relative to other components, their low odour thresholds and high impact nature make these substances of crucial importance to the overall aroma and flavour of truffles (ROWE, 2005). On the basis of our examinations, *T. aestivum* comprises more sulphur containing constituents (4,90 %), than *T. brumale* (3,64 %). Similar ratio was observed in case of N-containing compounds (cyclic and non-cyclic together), these kind of components occurred in summer truffle in more significant amount than in the winter truffle (5,46 % vs. 1,29 %).

Both species contain compounds bearing benzene ring. These constituents usually possess high odour activity, a property that can

Tab. 1: Identified volatile constituents in *T. aestivum* and *T. brumale* species. The area ratio (share of the areas in the sum of relative intensities related to the ISTD) is determined for each component

N°.	PTRI	Compounds	*T. aestivum* Area %	*T. brumale* Area %
		Open chain alcohols, aldehydes, ketones		
1	1019	2-butanol	5,48	0,19
2	1033	3-ethylpentan-2-one, (3-ethyl-2-pentanone)		0,14
3	1037	(E)-2-butenal	0,02	
4	1043	2,3-pentanedione	0,75	0,45
5	1059	2-methyl-1-propanol, (isobutyl alcohol)	4,29	0,43
6	1066	hexanal	0,07	0,13
7	1076	2-methyl-(E)-2-butenal, (tiglaldehyde)	0,03	
8	1081	2-pentanol, (pentan-2-ol)	0,06	0,04
9	1095	(E)-3-penten-2-one, (trans-ethylidene-acetone)	0,24	0,06
10	1104	1-butanol	0,18	
11	1155	n-heptanal	0,11	1,38
12	1158	2-methyl-1-butanol	32,88	4,38
13	1167	2,4-pentanedione (acetoacetone)		0,17
14	1206	1-pentanol	0,07	
15	1224	3-octanone	0,49	0,11
16	1255	octyl aldehyde		0,06
17	1274	1-octen-3-one (vinyl amyl ketone)	0,17	0,04
18	1283	2-methyl-2-buten-1-ol, (2-methyl-but-2-ene-1-ol)	0,03	
19	1368	3-octanol	0,56	0,02
20	1398	propionoin (4-hydroxy-3-hexanone)	0,79	0,22
21	1413	2-octenal (trans-2-octenal)		0,10
22	1434	1-octen-3-ol	5,16	
23	1458	2,6-dimethyl-4-heptanol (2,6-dimethyl-4-heptanol)		0,16
24	1534	(E)-2-nonenal (trans-2-nonenal)		0,03
25	1556	1-octanol	0,04	
26	1605	2-undecanone		0,03
27	1622	2-octen-1-ol (oct-2-en-1-ol)	0,49	
28	2180	1-tridecanol	0,11	
29	2355	1-tetradecanol	0,15	
30	2758	2-ethyl-2-methyl-tridecanol		0,32
			52,17	**8,46**
		Furans and derivatives		
31	1198	2-pentyl-furan, (2-amylfuran)	**0,04**	
		S-containing compounds		
32	1449	3-(methylthio)-propanal, (methional)	0,20	0,18
33	1532	4-methylthio-2-butanone	0,07	
34	1632	3,5-dimethyl-1,2,4-trithiolane		0,04
35	1713	2-methylmercaptomethylbut-2-enal	0,09	
36	1733	methionol (3-methyl thiol propanol)	0,10	
37	1968	2-methyl-2-(phenylthio)propanal		0,08
38	2334	1,4-dimethoxy-2-(methylthio)-benzene		0,32
			0,46	**0,62**
		Thiazoles and derivatives		
39	1196	2-methyl-thiazole		0,05
40	1265	2-ethyl-thiazole		0,08
41	1350	4-ethyl-2-methyl-thiazole, (2-methyl-4-ethylthiazole)		0,16
42	1355	2-isopropyl-4,5-dihydro-thiazole		0,11
43	1358	2,4,5-trimethyl-thiazole	0,07	
44	1373	2-sec-butyl-thiazole		0,75
45	1433	2-isopropyl-4,5-dimethyl-thiazole		0,04
46	1651	2-acetylthiazole, (1-(2-thiazolyl)-ethanone)	0,35	0,53
			0,42	**1,72**

		Thiophenes and derivatives		
47	1535	dihydro-2-methyl-3(2H)-thiophenone	0,03	
48	1557	dihydro-3-(2H)-thiophenone		0,08
49	1573	4,5-dihydro-3(2H)-thiophenone	0,05	
50	1685	3-thiophenecarboxaldehyde	0,22	0,08
51	1701	2-thiophenecarboxaldehyde (2-formylthiophene)	0,23	0,16
52	1738	2-formyl-3-methyl-thiophene	0,07	
53	1755	5-methyl-thiophene-2-carboxaldehyde	0,04	
54	1833	2,5-diformyl-thiophene	1,29	0,45
55	1916	2-acetyl-5-formyl-thiophene	0,11	
56	1963	2-thiophenemethylamine (2-thiophenemethanamine)		0,07
57	2538	2-[2-(4-methylphenyl)cyclopropanyl]-thiophene		0,46
58	2826	1,4-dihydrodibenzo-thiophene	0,26	
			2,30	**1,30**
		Dithiazines and derivatives		
59	1786	5,6-dihydro-2,4,6-trimethyl-4H-1,3,5-dithiazine	**0,63**	
		N, and S containing in variable form		
60	1805	2-(methylthio)-3-pyridinol, (3-hydroxy-2-methiopyridine)	**1,09**	
		N-containing open chain compounds		
61	1101	2-methyl-N-(2-methylbutylidene)-1-butanamine		0,19
62	1122	allyldiethylamine (N,N-diethyl-2-propen-1-amine)		0,04
63	1130	3-methyl-N-(3-methylbutylidene)-1-butanamine	0,06	
			0,06	**0,23**
		Terpenes and derivatives		
64	1130	β-myrcene		0,06
65	1611	tricyclo[5.3.0.0.(2,8)]decan-4-one		0,93
66	1692	alpha-humulene		0,10
67	2335	trans-farnesol	0,18	
			0,18	**1,09**
		N-containing heterocyclic compounds		
68	1123	2,3-dimethyl-piperidine (6 atom ring with NH group)	0,04	
69	1576	3-methoxy-pyridine (3-methoxypyridine)		0,08
70	1884	(S)-3-(1-methyl-2-pyrrolidinyl)-pyridine, (nicotine)		0,07
71	2120	4-methoxy-2-(1-methylethyl)-pyrimidine	0,10	
72	2190	2-ethyl-3,5-dimethyl-pyrazine	0,38	
73	2194	3,4,5-trimethyl-1H-pyrazole, (3,4,5-trimethylpyrazole)	3,80	
74	2197	1,3,5-trimethyl-1H-pyrazole, (1,3,5-trimethylpyrazole)	0,74	
75	2247	3-phenyl-pyridine, (3-phenylpyridine)	0,17	
76	2274	2-methyl-5-phenyl-pyridine, (5-phenyl-2-picoline)	0,12	
77	2302	5-methoxyimidazo[1,5-a]quinoline		0,76
78	2311	2-phenyl-4-tertiobutyl-pyridine	0,05	
79	2503	5-methyl-4-(1-methylethylidene)-2-phenyl-2,4-dihydro-3H-pyrazol-3-one		0,15
			5,40	**1,06**
		Compounds bearing benzene ring		
80	1516	benzaldehyde	0,28	0,14
81	1520	3-ethyl-5-methyl-phenol, (3-ethyl-5-methylphenol)		0,24
82	1648	benzeneacetaldehyde, (hyacinthin)	0,11	0,31
83	1671	1-phenyl-ethanone, (acetophenone)	0,09	
84	1893	1-(2,4-dihydroxy-3-methylphenyl)-ethanone		0,05
85	1899	benzenemethanol, (benzyl alcohol)	0,04	
86	1921	benzeneethanol, (phenethyl alcohol)	6,35	0,18
87	1961	α-ethylidene-benzeneacetaldehyde, (2-phenyl-2-butenal)	0,16	
88	2134	1-fluoro-2,4,6-trimethyl-benzene	0,25	
89	2211	4-fluoro-1,2-Xylene, (4-fluoro-1,2-dimethyl-benzene)	0,57	
90	2312	N-ethyl-2-methyl-N-phenyl-benzenamine	0,10	
91	2432	(R)-3,4-dihydro-8-hydroxy-3-methyl-1H-2-benzopyran-1-one		0,14
			7,95	**1,06**

		Compounds of naphthalene skeleton		
92	1927	4a.beta.-methyl-3,4,4a,5,6,7,8a.alpha.-hexahydronaphthalene-1-(2H),7(8H)-dione		**0,11**
		Polycyclic condensed aromatic heterocycles		
93	2432	1H-9-methoxy-benz[f]indole, (9-methoxy-1H-benz[f]indole)	**0,10**	
		Polycyclic condensed hydrocarbons		
94	2106	1-methyl-9H-fluorene		0,34
95	2616	phenanthrene		0,38
				0,72
		Phenol and derivatives		
96	1520	3-ethyl-5-methyl-phenol, (5-ethyl-m-cresol)		0,24
97	1967	2-methoxy-4-methylphenol, (p-methylguaiacol)	0,07	
98	1980	2-methoxy-4-methylphenol, (2-methoxy-4-methyl-phenol)	0,07	
99	2007	phenol		0,04
100	2021	2-methyl-phenol (o-cresol)	0,08	
101	2103	3-methyl-phenol, (m-cresol)	4,09	
102	2120	4-ethyl-2-methoxy-phenol (p-ethylguaiacol)	0,10	
103	2127	4,5-dimethoxy-2-methylphenol		0,16
104	2171	3-ethyl-phenol	0,14	0,17
105	2185	4-vinyl-2-methoxy-phenol		0,05
106	2246	3-propyl-phenol		0,39
107	2250	3,4-dimethoxy-phenol, (3,4-dimethoxyphenol)		0,46
108	2349	4-methoxy-phenol		0,23
			4,55	**1,74**
		Methoxy-methyl-benzenes		
109	1310	methoxy-benzene		0,56
110	1426	1-methoxy-3-methyl-benzene		3,93
111	1731	1,2-dimethoxy-benzene		0,18
112	1752	1,4-dimethoxy-benzene		8,53
113	1761	1,3-dimethoxy-benzene		0,34
114	1811	2,5-dimethoxytoluene, (1,4-dimethoxy-2-methyl-benzene)		0,18
115	1818	2,3-dimethoxytoluene, (1,2-dimethoxy-3-methyl-benzene)		0,18
116	1861	2,5-dimethoxyethylbenzene		0,09
117	1864	3,5-dimethoxytoluene, (1,3-dimethoxy-5-methyl-benzene)		0,44
118	1971	1,2,3-trimethoxybenzene		0,17
119	2094	1,2,4-trimethoxybenzene		51,19
120	2101	1,2,3-trimethoxy-5-methyl-benzene		0,38
121	2133	1,2,4-trimethoxybenzene		0,14
122	2140	1,2,3,4-tetramethoxybenzene		0,26
123	2278	2,5-dimethoxy-benzaldehyde		0,06
				66,63
		Cyclic carbonyl compounds		
124	1193	2-pentyl-furan		0,04
125	1399	5-ethylcyclopent-1-ene-carboxaldehyde		0,10
126	1441	2-furancarboxaldehyde, (furfural)	0,07	0,09
127	1728	5-ethyldihydro-2(3H)-furanone (γ-hexalactone)	0,06	
128	2057	dihydro-5-pentyl-2(3H)-furanone, (γ-nonalactone)	0,06	
129	2071	7,7-dimethoxy-2,3-dimethylidenebicyclo[2.2.1]heptane	0,09	
130	2096	2-allyl-5,5-dimethyl-1,3-cyclohexanedione	0,79	
131	2114	2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	0,05	
132	2369	(Z)-dihydro-5-(2-octenyl)-2(3H)-furanone	0,70	0,43
133	2477	4,5,6,7,8,9-hexahydrocycloocta[c]furan-1(3H)-one		0,13
			1,82	**0,79**
		Esters of open chain acids		
134	1906	2-methyl-propanoicacid1-(1,1-dimethylethyl)-2-methyl-1,3-propanediylester	0,09	0,33
135	2063	isopropyl myristate, (tetradecanoic acid 1-methylethyl ester)	0,09	
136	2472	(Z,Z)-9,12-octadecadienoic acid methyl ester, (methyl linoleate)	0,26	
			0,44	**0,33**

		Open chain acids		
137	2451	dodecanoic acid, (lauric acid)	0,15	
138	2609	tetradecanoic acid, (myristic acid)	0,80	
139	2686	pentadecanoic acid	0,30	
140	2749	hexadecanoic acid, (palmitic acid)	8,19	0,59
			9,44	**0,59**
		Open chain and cyclic hydrocarbons		
141	1548	cyclooctane (octamethylene)		0,04
142	2462	pentacosane		0,09
143	2467	7-tetradecyne		0,07
144	2865	2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene	0,65	0,43
			0,65	**0,63**

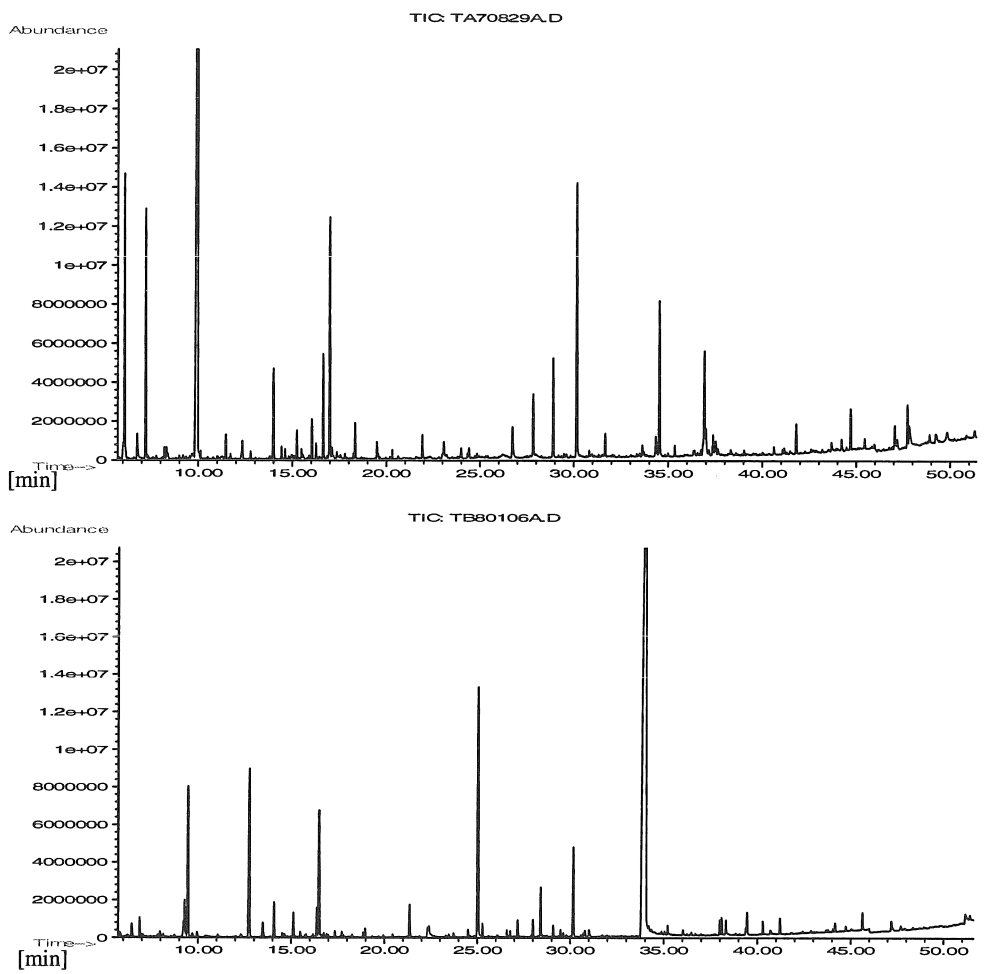


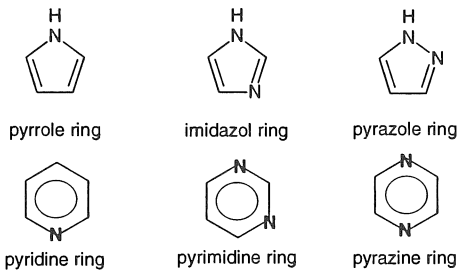
Fig. 1: Total ion chromatograms of the volatiles extracted from the examined truffle species: *Tuber aestivum* (upper) and *Tuber brumale* (lower).

presumably be assigned to the aromatic ring, since substances of this chemical structure have low odour thresholds in general (RYCHLIK et al., 1998; BURDOCK, 2005; BELITZ et al., 2009). The ratio of these substances to the total aroma was almost eightfold in summer truffle than was in the winter one, with the 6,35 % share of the benzeneethanol. The methoxy-(alkyl)-benzenes – as one of the most original categories reported by FLAMENT et al. (1992) also present in the *Tuber melanosporum* abundantly – were sorted into an independent, individual chemical group in Tab. 1. They have been separated from the other, called „simple" benzene consituents. An amazing experience of our work was, whilst these methoxy-methyl-benzenes were fully absent from *T. aestivum* their contribution to the total aroma was more than 60 % in the Hungarian *T. brumale*. In this truffle the ratio of 1,2,4-trimethoxybenzene alone exceeded 50 %.

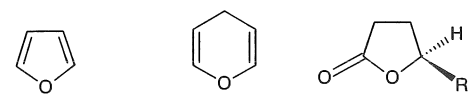
The amount of terpenes and their derivatives was insignificant in the samples investigated. This fact is not unexpected being these components the primary marker ones of plant origin in many cases and truffles do not belong to plants. Otherwise they predominantly bear pleasant fragrance, spicy taste and may exhibit specific pharmacological activities (BREITMAIER, 2006). In the winter species their ratio in the total aroma was sixfold higher than in the summer one.

Tab. 2: The skeletons of the identified main hetrocyclic molecules in the order of atomic number of the hetero-atom.

N-containing heterocycles

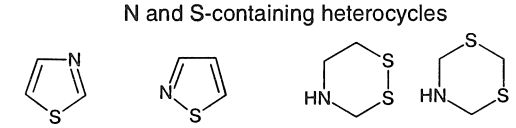


O-containing heterocycles

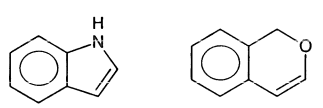


S-containing heterocycles

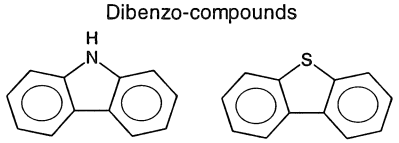
N and S-containing heterocycles



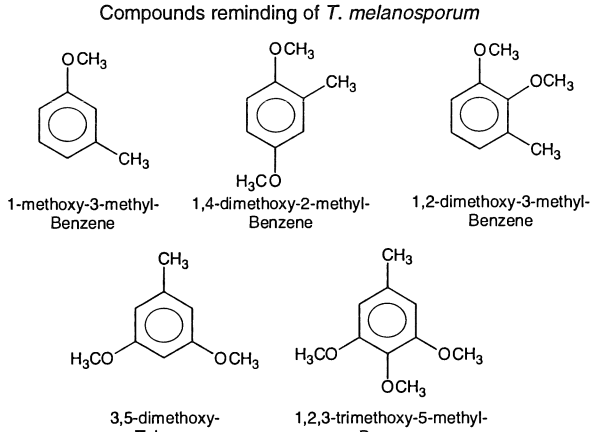
Heterocycles combined with benzene ring



Dibenzo-compounds



Compounds reminding of *T. melanosporum*



Contrary to the results of MARCH et al. (2006) we did not succeed in identifying a great number of esters. This group of compounds is widespread and abundant in plant kingdom (*e.g.* fruits), but in truffles – according to our measurements – their presence was not significant.

Furthermore, the examined *Tuber* species contained several open chain acids, the odour of what is not remarkable owing to their elevated chain length (C_{12}-C_{16}). Considering these substances, expressive difference exists between their ratios in the two truffle species analysed: in *T. aestivum*, their share from the total aroma is more than 9 % – with the 8,19 % portion of hexadecanoic acid alone –, while this value is about 0,6 % in *T. brumale*, merely.

To facilitate the review of the results and the recognition of the differences, modified aromaspectra were created, by the help of the method developed at the Department of Food Chemistry of BCE (KORÁNY et al., 2005). Thus the results can be liberated from the proportional distortion effects (efficiency of sample peparation, accuracy of injection, changing sensitivity of the instrument etc.). The resulting graphs (Fig. 2.) are very similar to mass spectra, and the main differences between the species can be illustrated more plausibly. The dominant fragrance constituents are annotated with numbers. Studying the aromaspectra and the data of Tab. 1, significant differences can be found among the odour properties of the two truffle species examined.

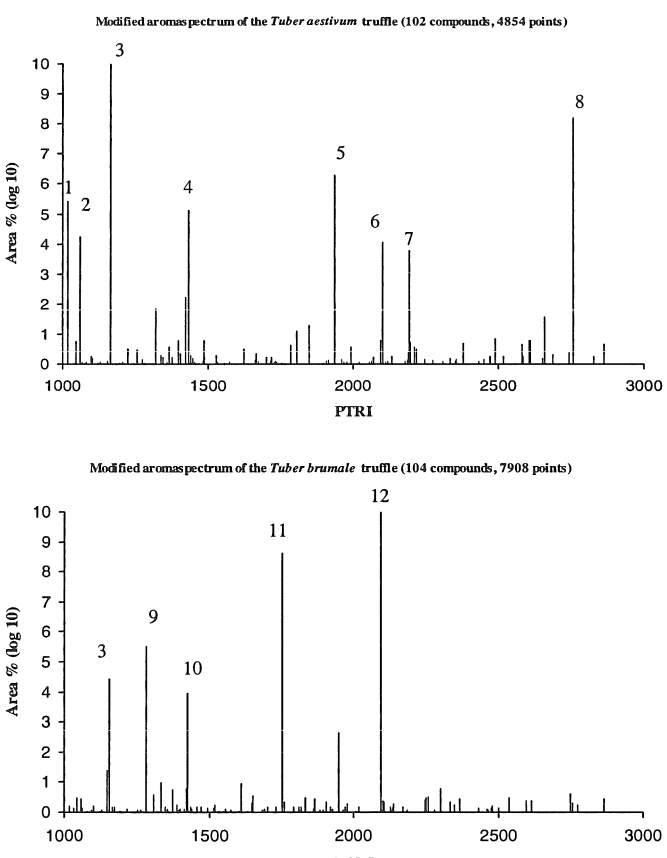


Fig. 2: The modified aromaspectra of the examined varieties: *T. aestivum* (above) and *T. brumale* (below).
The most intense peaks are as follows: 1: 2-butanol, 2: 2-methyl-1-propanol, 3: 2-methyl-1-butanol, 4: 1-octen-3-ol, 5: benzeneethanol, 6: 3-methyl-phenol, 7: 3,4,5-trimethylpyrazole, 8: hexadecanoic acid, 9: Tub.brumale-A (unknown), 10: 1-methoxy-3-methyl-benzene, 11: 1,4-dimethoxy-benzene, 12: 1,2,4-trimethoxybenzene.

In the summer truffle, the most abundant constituent in the chromatogram is an alcohol, 2-methyl-1-butanol, which exhibits approximately one third of the total aroma value alone. The component group containing this constituent – 'Open chain alcohols, aldehydes, ketones' – amounts to more than the half (52,17 %) of the truffle's entire fragrance. Furthermore this tuber contains notable amount of hexadecanoic acid (8,19 %), benzeneethanol (6,35 %), 2-butanol (5,48 %), 1-octen-3-ol (5,16 %), 2-methyl-1-propanol (4,29 %), m-cresol (4,09 %) and 3,4,5-trimethylpyrazole (3,80 %). Fig. 3. shows the share of the individual component groups from the total odour of the truffle samples. In case of *T. aestivum*, the ratio of 'Open chain alcohols, aldehydes, ketones', 'N-containing heterocyclic compounds', 'Compounds bearing benzene ring', 'Phenol and derivatives' and 'Open chain acids' were significantly greater than in *T. brumale*. Summer truffle contains numerous fragrance compounds of great intensities and several minor (often possessing large odour activities, *e.g.* sulphur components) scent constituents.

In the winter truffle the most significant compound is the 1,2,4-trimethoxybenzene. This substance dominates the chromatogram, followed by two additional benzene constituents: 1,4-dimethoxybenzene and 1-methoxy-3-methyl-benzene. Furthermore it contains notable amount of 2-methyl-1-butanol, the most intense component of *T. aestivum*. Unexpectedly, 1-octen-3-ol, that is considered the character-impact flavor compound in mushrooms (BAUER et al., 2001; MARSILI, 2002), could not be detected in *T. brumale* at all, though its abundance in summer truffle was fairly high (5,16 %). The ratio of 1-octen-3-one – the other key scent-compound in mushrooms – was relatively small (0,04 %) as well. The number of intense peaks was smaller in winter truffle, and a surplus of minor volatile compounds was present in this sample.

Summarizing our efforts to clear up the differences between the fragrance structures of summer and winter truffles of Hungarian south-west region origin led to the next results of interest. Among the various kinds of chemical classes that take part in forming the aroma features of the *Tubers* investigated, the sulphur compounds with great odour activities, the C_8 compounds considered the creators of the characteristic „mushroom-note" and the methoxy-alkil benzenes are of the greatest significance. The measurements show that the definite lack of C_8 open chain constituents and the wealth of methoxy-methyl benzenes in *T. brumale* compared to *T. aestivum* are the main distinctive features between summer and winter truffle varieties of Hungary. The revealed distribution of constituents makes the Hungarian winter truffle *i.e. Tuber brumale* similar to the French *Tuber melanosporum* Vitt. from a compositional point of view.

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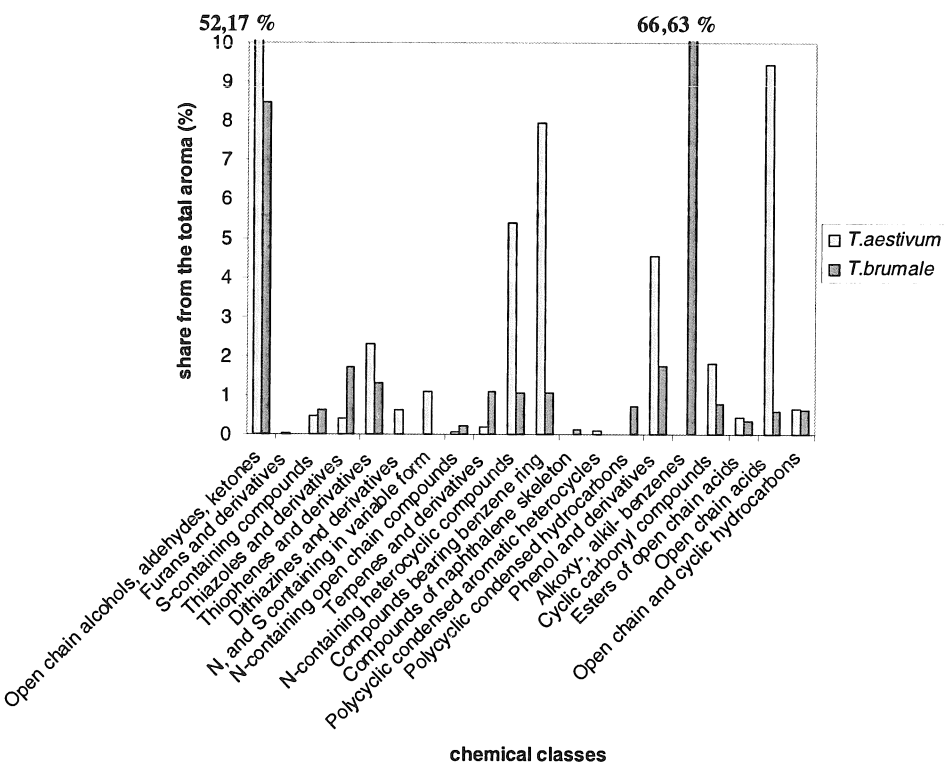

Fig. 3: The participation of the single chemical classes from the total aroma in the two examined *Tuber* species.

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