Volatile Components of Whole and Different Plant Parts of Bastard Balm (*Melittis melissophyllum* L., Lamiaceae) Collected in Central Italy and Slovakia

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The aim of this work was to trap the volatiles released from whole frozen and dry aerial parts, and, separately, from different organs (leaves, stems, corolla and calyx) of bastard balm (*Melittis melissophyllum* L., Lamiaceae) populations collected in Italy and Slovakia by HS-SPME, and to identify the headspace constituents responsible for the characteristic aroma impression by GC/FID and GC/MS techniques. Among more than 100 volatile components detected, the C_8 alcohol oct-1-en-3-ol, responsible for the typical mushroom-like odor, and the phenolic coumarin, with a characteristic sweet and creamy vanilla bean odor, played a major role in the aroma of whole aerial parts and different plant organ samples. In particular, dry calyx parts could be proposed as flavoring agent in food products as mushroom aroma enhancer. Multivariate chemometric techniques, such as cluster analysis and principal component analysis, were used to characterize the sample populations according to the geographical origin and processing of plant material.

Introduction. – Aroma and flavor are a combination of taste and olfaction, one of the most important factors in consumer acceptance of foods. They consist of a wide range of organic compounds possessing various polarities and reactivities, usually characterized by low odor threshold, and they influence the organoleptic quality of food and vegetable matrices at very low concentrations. Thus, the presence, contents, and composition of volatile substances in food and vegetable matrices have a substantial influence on their quality. Therefore, fragrance industry is interested in the characterization of plant volatiles and aroma compounds.

Although more than 400 volatile compounds have been identified, only a small number is essential and seems to contribute really to aroma profiles. Among the latter, the C_8 alcohol oct-1-en-3-ol and the phenolic coumarin 2*H*-1-benzopyran-2-one play a major role in the aromas of some food and vegetable samples [1][2]. On the other hand, different chemical families, including esters, ketones, aldehydes, and terpenes, have to be taken into account as main contributors to the aroma and flavor of food and vegetable samples.

The most frequently used analytical techniques to concentrate the volatile compounds of food and vegetable aroma are those based on headspace analysis, notably solid-phase microextraction (SPME) technique.

SPME is a nondestructive and noninvasive method to evaluate aroma compounds [3]. It has been largely applied to aroma analysis in combination with gas chromatography (GC) and GC/mass spectrometry (GC/MS), offering solvent-free and rapid sampling with low cost and ease of operation; moreover, it is sensitive, selective and also compatible with low detection limits [4].

The family of Lamiaceae comprises several species producing aroma and scented compounds. Among them, there is bastard balm (*Melittis melissophyllum* L., subfamily Lamioideae, subtribe Melittidinae), a perennial herb covered by abundant protecting trichomes mainly on the quadrangular stems. The plant has petiolate to subsessile, decussate, ovate to cordate, and dentate leaves that, when rubbed, emanate an unmistakable coumarin scent. Their similarity with those of lemon balm (*Melissa officinalis* L.) are at the basis of the specific name, while the name of the genus is based on the flowers which are visited by honey-producing pollinators. The flowers, of white, pink, or purple bilabiate corolla and with a persistent calyx, are the biggest in the Lamiaceae family. Bastard balm occurs in Middle, Southwestern, Southeastern, and Eastern Europe. In Italy, two subspecies occur: subsp. *albida* (Guss) P. W. Ball, larger than the nominate one (40–70 cm), with abundant glandular trichomes and only white corolla [5], distributed in Central-Southern Italy; subsp. *melissophyllum*, smaller than the latter (30–50 cm), with low-density glandular trichomes represented by peltate (type A) and capitate (types B and C) ones [6], and white to purple-stained corolla, distributed mainly in the North-Western and Central Italy [7]. Both subspecies grow in thermophilous copse broadleaf woodlands, mainly composed of oaks and hornbeams, belonging to the community of the class *Orno-Ostryetum*. In Slovakia, the subsp. *carpatica* (Klokov) P. W. Ball (*M. grandiflora* sensu Klokov) [8] occurs, even if the morphological border between the nominate subspecies and the subsp. *carpatica* is not sharp [9]. Compared to nominate subspecies, it has larger leaves (6)7–15 cm, with 20–32 teeth on each side. It grows in different substrates in lowland to hill, rarely to submontane level, in light deciduous forests, shrubs, and coupes, most often in communities of the class *Querco-Fagetea* and rarely in communities of the class *Rhamno-Prunetea*.

In the folk medicine of central Italy, *M. melissophyllum*, called '*Erba Lupa', 'Erba Limona'*, or '*Cedrina'*, was used as antispasmodic, and against insomnia and eye inflammations [10]. More, it is still used in Abruzzo by shepherds during transhumance, under a warm decoction used as digestive and to treat cough and sore throat [11]. In Serbia, it was used for its sedative properties, against nervous anxiety, insomnia, and hysteria [12]. In spite of these properties, people used to make an aromatic tea with the fresh or dry leaves to be drunk after meals as digestive and antispasmodic.

Our previous investigations revealed this plant unexpectedly as an important source of the mushroom-like aroma component oct-1-en-3-ol. This C_8 alcohol is produced by many species of edible fungi, and, owing to its low flavor threshold, is reported to be also an aroma component in several food products and beverages [13]. Along with oct-1-en-3-ol, another major volatile is coumarin, a phenolic compound occurring in many plants used as flavoring ingredients in foods as cinnamom, cassia, lavender, peppermint, and *Melilotus alba*, because of its sweet, aromatic, and creamy vanilla bean odor with nut-like tones [2]. Coumarin is used as a fixative and enhancing agent in perfumes, and is added to toilet soap and detergents, toothpaste, tobacco products, and some alcoholic beverages. Large quantities are used in rubber and plastic materials, and

in paints and sprays to neutralize unpleasant odors [14]. Due to its hepatoxicity, coumarin has been banned as a food additive in the US since 1956 [15]. Although the potentiality of this plant to produce economically interesting natural flavor has to be deeply investigated yet, we extended the analysis of the headspace volatiles to different European population of *M. melissophyllum* (Italy and Slovakia, see *Fig. 1* and *Table 1*), and, within one population, to different plant organs. The aim of this work was to trap the volatiles released by whole, frozen, and dried aerial parts, and different plant organs (leaves, stems, corolla, and calyx) of bastard balm by using SPME technique, and to identify the headspace constituents responsible for the characteristic aroma impression by GC/FID and GC/MS analyses. Moreover, we attempted to add useful data supporting the infraspecific taxonomy of the plant.

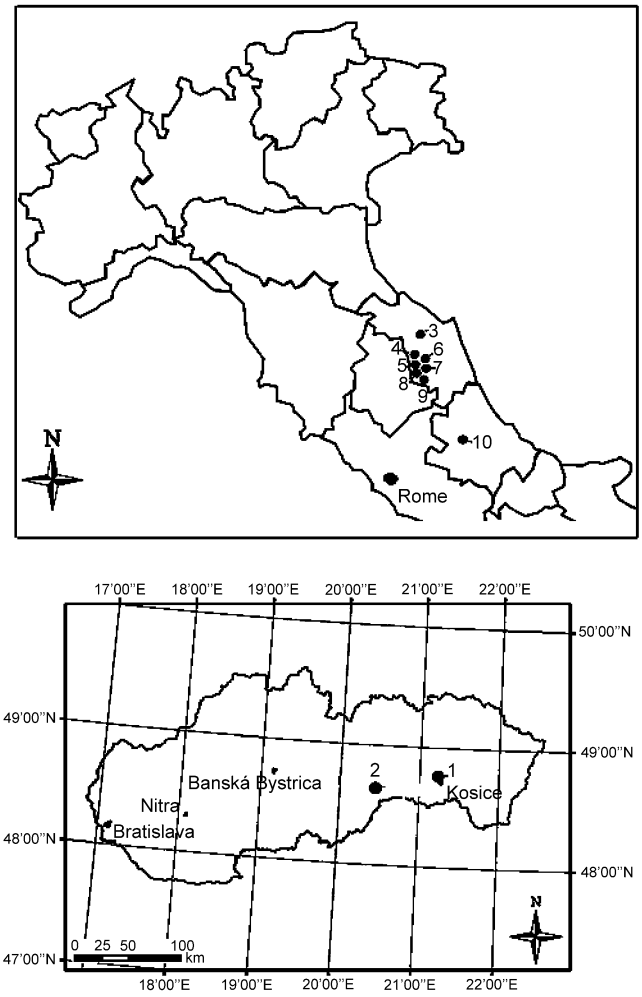

Fig. 1. *Collection sites of the investigated population of bastard balm* (*Melittis melissophyllum*) *in Italy and Slovakia*

Table 1. *Collection Localities of Bastard Balm* (*Melittis melissophyllum*) *Populations Investigated in This Study*

Sample	Collection site	Country	Date of collection	GPS coordinates	Altitude [m]	Voucher codes[a])
1	Hradova (Košice)	Slovakia	25/05/2009	N 48°45′42″; E 21°14′01″	431	KO 30343
2	Kruzna (Rožňava)	Slovakia	04/06/2009	N 48°38′31″; E 20°26′57″	537	KO 30344
3	Morello (Sassoferrato)	Italy	18/05/2009	N 43°29′34″; E 12°48′37″	440	CAME 16042
4	Palazzo (Esanatoglia)	Italy	14/05/2009	N 43°12′57″; E 12°56′08″	580	CAME 16044
5	Pioraco	Italy	14/05/2009	N 43°10′31″; E 13°00′08	420	CAME 16043
6	Crispiero (Castelraimondo)	Italy	13/05/2009	N 43°11′19″; E 13°06′27″	550	CAME 13429
7	Piedilapiaggia (Camerino)	Italy	13/05/2009	N 43°09′10″; E 13°07′18″	590	CAME 13430
8	Monte Igno (Camerino)	Italy	19/05/2009	N 43°06′33″; E 13°00′06″	700	CAME 16040
9	Gelagna bassa (Serravalle di Chienti)	Italy	19/05/2009	N 43°04′33″; E 13°01′35″	530	CAME 16041
10	Anversa degli Abruzzi (L'Aquila)	Italy	27/05/2009	N 41°58′59″; E 13°47.52″	1277	APP 39504

[a]) Accession No. in: KO, Herbarium of Department of Botany, P. J. Šafárik University, Košice, Slovakia; CAME, Herbarium Camerinensis, School of Environmental Sciences, University of Camerino, Camerino, Italy; APP, Herbarium of Centro Ricerche Floristiche dell'Appennino, Barisciano, Italy.

Results and Discussion. – 1. *Repeatability of the Method.* HS-SPME, as an equilibrium extraction technique, requires careful optimization to obtain high sensitivity and good repeatability of determination. The main factors influencing the whole analytical process, *i.e.*, optimal settings of extraction temperature and time, as well as the choice of suitable fiber stationary phase, were investigated in a previous study [16]. By using the previously optimized conditions, in this work all HS-SPME extractions were performed at 40° with an extraction time of 30 min, and an addition of 20 µl of H_2O in the case of dry samples.

The repeatability of the method was evaluated by performing three replicate analyses for fresh frozen and dry samples (whole aerial parts), as well as for different dry plant organ samples (leaves, stems, calyx, and corolla). RSD Values calculated for the major compounds (amounting to 56.0–88.9% of total volatiles released from whole aerial parts), *i.e.*, oct-1-en-3-ol and coumarin, in the headspace are compiled in *Table 2*. The RSD values for fresh frozen and dried samples were comparable, establishing that moisture content did not influence so much the repeatability of measurements. Repeatability of the method was demonstrated as the relative standard deviation (RSD) of three replicate analyses, for oct-1-en-3-ol peak area was lower than 13.0%. In the case of coumarin, RSD for peak area was always lower than 17.1%.

Table 2. *Relative Standard Deviation* (RSD) *Values* ($n=3$) *Obtained for Oct-1-en-3-ol and Coumarin Extracted from Whole Fresh and Dry Aerial Parts and Different Plant Parts of Bastard Balm*

Extracted Parts		RSD [%]	
		Oct-1-en-3-ol	Coumarin
Whole aerial parts (*Sample*)			
1	dry	12.4	12.1
2	dry	8.9	10.7
3	dry	7.3	5.7
	frozen	3.8	2.1
4	dry	6.8	6.1
	frozen	5.9	2.9
5	dry	3.0	4.7
	frozen	5.8	11.3
6	dry	5.0	10.3
	frozen	10.1	9.9
7	dry	4.2	9.0
	frozen	11.9	10.6
8	dry	8.8	4.4
	frozen	4.9	7.5
9	dry	6.6	4.0
	frozen	2.4	2.6
10	dry	4.7	4.8
Plant parts[a])			
Lv	dry	5.5	6.5
St	dry	5.5	11.1
Ca	dry	0.7	11.6
Co	dry	13.0	17.1

[a]) Lv, leaves; St, stems; Ca, calyx; Co, corolla.

Table 3. *Volatile Components of Whole Fresh and Dry Aerial Part Samples of Bastard Balm (Melittis melissophyllum) Populations Obtained by HS-SPME*

Component[a])	*RI*[b])	*RI* Lit.		Populations[c])																ID[d])
				1	*2*	*3*		*4*		*5*		*6*		*7*		*8*		*9*		*10*	
		ADAMS[e])	*NIST 08*[f])	dry	dry	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	
Hexanal	802	801	800	0.1	0.1	0.1	0.2	0.5	0.2	0.5	0.2		0.3	0.2	0.2	0.4	0.3	0.6	0.6	0.2	*RI*, MS
(*E*)-Hex-2-enal	859	855	857	tr.[g])	tr.	tr.	0.5	0.2	0.6	tr.	0.4	0.2	1.0	0.4	0.5	0.2	0.9	0.3	1.3	0.2	*RI*, MS
(Z)-Hex-3-en-1-ol	860	859	855	0.3	0.2															0.2	*RI*, MS
Hexan-1-ol	875	862	871	tr.	tr.		tr.						tr.				tr.		0.1		*RI*, MS
Heptanal	908	902	904		0.1	0.1		0.2		0.4				tr.		0.3		0.2		0.1	*RI*, MS
(2*E*,4*E*)-Hexa-2,4-dienal	909	909	909				tr.		tr.		tr.		tr.		tr.		tr.		tr.		*RI*, MS
α-Thujene	929	930	929	tr.		tr.	tr.	tr.	0.1	0.1	0.1	tr.	tr.		0.1		tr.		tr.	0.2	*RI*, MS
α-Pinene	935	939	934	2.8	1.7	0.3	1.1	0.3	7.4	3.3	4.3	4.2	10.2	0.8	4.7	5.1	2.8	0.6	3.8	0.4	Std
(*E*)-Hept-2-enal	947	947						tr.													*RI*, MS
Camphene	950	954	949				tr.		0.1		0.1		0.1		0.1		tr.		0.1		*RI*, MS
Thuja-2,4(10)-diene	956	953		0.1	0.1																*RI*, MS
Benzaldehyde	963	960	960	0.1	tr.	0.3	tr.	0.2	0.1	0.2	0.1	0.3	0.1	0.1	0.1		0.1	0.6	0.1	0.3	Std
β-Pinene	974	979	975	0.1	0.1	tr.	tr.		0.4	0.2	0.2	0.3	0.6	tr.	0.2	0.3	0.1		tr.		Std
Sabinene	975	975	975			tr.	0.3	0.2	0.2	0.1	0.4		0.1	0.2	0.5		0.4		0.7	4.3	*RI*, MS
Oct-1-en-3-ol	981	979	980	32.8	36.9	28.5	19.2	49.0	17.7	17.6	18.4	57.0	19.3	52.4	19.2	16.1	18.1	43.4	21.2	20.8	Std
Myrcene	991	990	992				0.5		0.9	0.8	0.7		1.1		0.7		0.6		0.8	0.8	Std
α-Phellandrene	995	1002	1002				tr.		0.3		0.3		tr.		0.3				tr.		*RI*, MS
Octan-3-ol	997	991	991	1.2	1.8	1.9	0.6	2.6	0.6	1.7	0.7	1.9	0.8	1.1	0.8	1.4	0.5	4.0	0.8	1.0	*RI*, MS
(2*E*,4*E*)-Hepta-2,4-dienal	1009	1007	1009	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	2.0	tr.	0.1	tr.	tr.	tr.	tr.	0.1	0.1	*RI*, MS
Δ-Car-3-ene	1009	1011	1009						0.2				0.2								*RI*, MS
Limonene	1027	1029	1026	tr.	tr.	tr.	0.1	tr.	0.4	0.8	0.3	0.2	3.7	tr.	0.3	0.3	0.2	tr.	0.3	0.2	Std
p-Cymene	1026	1024	1028	tr.	tr.	tr.	tr.	tr.	0.2	tr.	0.3		tr.	tr.	0.3	tr.	tr.	tr.	0.1	0.1	Std
Benzyl alcohol	1029	1026	1033	tr.	tr.																*RI*, MS
1,8-Cineole	1029	1031	1034				0.1		0.6	tr.	0.3		0.8		0.3	tr.	0.1		0.4		Std
Lavender lactone	1037	1034			tr.			tr.		tr.				tr.				tr.			*RI*, MS
(*Z*)-β-Ocimene	1040	1037	1040				tr.		0.1		tr.		1.0		tr.		tr.		tr.		*RI*, MS
Benzeneacetaldehyde	1041	1042	1042	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	0.3	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	Std

Table 3 (cont.)

Component[a])	*RI*[b])	*RI* Lit.		Populations[c])																	ID[d])
				1	*2*	*3*		*4*		*5*		*6*		*7*		*8*		*9*		*10*	
		ADAMS[e])	*NIST 08*[f])	dry	dry	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	
Salicylaldehyde	1044	1044	1041	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.		tr.	tr.	tr.	0.2	tr.	tr.	tr.	tr.	*RI*, MS
(*E*)-*β*-Ocimene	1053	1050	1050				tr.		tr.		tr.		0.3		tr.		tr.		tr.		*RI*, MS
(*E*)-Oct-2-en-1-ol	1062	1060	1069	tr.	tr.			tr.		tr.		0.2						tr.		tr.	*RI*, MS
γ-Terpinene	1062	1059	1061				tr.		tr.		tr.		0.1		tr.		tr.		0.1	0.3	Std
Octan-1-ol	1063	1063	1077	tr.																	*RI*, MS
cis-Sabinene hydrate	1069	1070					tr.	tr.	tr.		tr.			tr.	tr.		tr.		tr.	0.3	*RI*, MS
Octa-3,5-dien-2-one	1077		1076	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	0.8	tr.	1.3	0.1	tr.	tr.	0.3	0.1	tr.	*RI*, MS
Terpinolene	1088	1088	1088	tr.	tr.		tr.		0.5		0.5	0.1	0.7		0.6		0.2		0.2		Std
trans-Sabinene hydrate	1096	1098																		0.3	*RI*, MS
Linalool	1101	1096	1102	0.4	0.4	0.8	0.1	1.1	0.6	1.0	0.4	0.5	0.8	0.3	0.4	0.1	0.3	1.0	0.7	0.5	Std
Nonanal	1108	1100	1104	0.1	0.1	tr.	tr.	0.3	0.1	0.1	0.1	0.3	0.2	0.1	0.1	0.8	0.1	0.2	0.1	0.3	*RI*, MS
Oct-1-en-3-yl acetate	1114	1112	1112	0.1	tr.	tr.	tr.	0.1	tr.	tr.	tr.		tr.	0.1	tr.		tr.	0.1	tr.	tr.	*RI*, MS
2-Phenylethyl alcohol	1116	1107	1116	0.1	tr.	tr.	tr.	0.1	tr.	tr.	tr.		tr.	tr.	tr.	tr.	tr.	0.1	tr.	tr.	*RI*, MS
α-Campholenal	1127	1125		0.2	0.1		tr.	tr.	tr.	tr.	tr.		tr.	tr.	tr.	0.3	tr.	tr.	tr.	0.1	*RI*, MS
4-Acetyl-1-methyl-cyclohexene	1137		1137	tr.	tr.	tr.		tr.		tr.		tr.		tr.		tr.		tr.		tr.	*RI*, MS
trans-Pinocarveol	1139	1139	1141	0.2	0.1				tr.	tr.	tr.	0.5	0.1	0.1	tr.	tr.	tr.	tr.	tr.	tr.	*RI*, MS
cis-Verbenol	1142	1137	1140	tr.	tr.								tr.	0.1							Std
Camphor	1143	1146	1145	tr.	tr.	tr.	tr.	0.1	tr.	0.3	tr.		tr.	tr.	tr.	tr.	tr.	0.2	tr.	0.2	Std
trans-Berbenol	1149	1140	1145	0.2	0.2							0.3	tr.	0.4		0.9					*RI*, MS
(2*E*,6*Z*)-Nona-2,6-dienal	1154	1150	1152									0.4						0.1	0.1		*RI*, MS
Citronellal	1157	1153	1155	0.3	0.3		0.1		0.2		0.2		tr.		0.3		0.1				Std
Pinocarvone	1162	1164	1158	0.1	0.1	tr.	tr.	tr.	0.3	tr.	0.1	0.4	0.3	0.3	0.1	1.0	0.2	tr.	0.2	tr.	*RI*, MS
Terpinen-4-ol	1180	1177	1178																	0.1	Std
Dec-1-en-3-ol	1183	1180		tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.		tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	*RI*, MS
p-Cymen-8-ol	1186	1182	1182			tr.	tr.		tr.		tr.	0.1	tr.		tr.		tr.		tr.		*RI*, MS
Methyl salicylate	1188	1191	1185	tr.	tr.	0.1	tr.	0.1	tr.	0.1	tr.	0.1	tr.	0.1	tr.	tr.	tr.	0.2	tr.	tr.	*RI*, MS
α-Terpineol	1190	1188	1191	tr.	tr.	tr.	tr.	tr.	tr.	tr.	0.1		tr.	tr.	0.1	0.3	tr.	tr.	tr.	0.1	Std
Verbenone	1193	1204	1195	0.3	0.2	tr.	tr.	0.2	tr.	0.1		0.2		0.3		1.6	tr.	0.2	tr.	tr.	Std

Table 3 (cont.)

Component[a])	*RI*[b])	*RI* Lit.		Populations[c])																	ID[d])
				1	*2*	*3*		*4*		*5*		*6*		*7*		*8*		*9*		*10*	
		ADAMS[e])	*NIST 08*[f])	dry	dry	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	
Methyl chavicol	1195	1196	1198				tr.		tr.		tr.		tr.		tr.		tr.		tr.		*RI*, MS
Myrtenal	1197	1195	1193	0.1	0.1		tr.		tr.	tr.	tr.		tr.	tr.	tr.	0.3	tr.		tr.	tr.	Std
Myrtenol	1199	1195	1196									0.2									*RI*, MS
Dodecane	1200	1200	1209			tr.		tr.		0.1						0.3		0.1			*RI*, MS
Safranal	1200	1196	1197	0.1	tr.			0.2		tr.		0.1		tr.				tr.		0.1	*RI*, MS
Decanal	1206	1199	1206	0.2	0.1	0.1	tr.	0.4	tr.	0.2	0.1	0.5	0.1	0.2	0.1	0.9	0.1	0.2	0.1	0.3	*RI*, MS
(2*E*,4*E*)-Nona-2,4-dienal	1210	1210	1211		tr.	tr.	tr.		tr.		tr.			tr.	tr.	tr.	tr.	tr.	tr.		*RI*, MS
β-Cyclocitral	1223	1219	1220	tr.	0.1	0.1	tr.	0.3	tr.	0.2	tr.	0.2	0.1	0.2	tr.	0.4	0.1	0.2	0.1	0.1	*RI*, MS
Citronellol	1234	1223	1236																	0.1	*RI*, MS
Carvone	1239	1239	1241											tr.							Std
Neral	1240	1238	1240				tr.		tr.		tr.		tr.		tr.						*RI*, MS
Cuminaldehyde	1247	1238	1242																	tr.	*RI*, MS
Thymoquinone	1253		1258																0.1		*RI*, MS
Geraniol	1258	1252	1257	0.1	tr.		tr.		tr.		tr.		tr.		tr.				tr.		*RI*, MS
(*E*)-Dec-2-enal	1260	1263	1260					tr.										tr.	tr.		*RI*, MS
cis-Chrysanthenyl acetate	1261	1265	1280			2.0	0.3		0.5	3.0	2.3	1.9	0.3		2.5	0.8	0.4		0.9		*RI*, MS
(*E*)-Cinnamaldehyde	1274	1267	1272			tr.		0.3						tr.		0.6		1.1			*RI*, MS
Geranial	1276	1267	1271				tr.		tr.		tr.		tr.		tr.		tr.		tr.		*RI*, MS
Isobornyl acetate	1287	1283					tr.				tr.	tr.	tr.		tr.		tr.		tr.		Std
(*E*)-Anethole	1289	1282	1283			0.1								0.1		0.3					Std
Tridecane	1297	1300	1300	tr.	tr.	tr.	tr.	0.1	tr.	0.1	tr.	tr.	tr.	tr.	tr.	tr.	tr.	0.1	0.4	tr.	Std
(2*E*,4*E*)-Deca-2,4-dienal	1298		1297				tr.				tr.		tr.		tr.		tr.		tr.		*RI*, MS
Undecanal	1305	1306	1308	tr.	tr.	0.2	tr.	0.1	0.1	0.3	0.2	tr.	tr.	tr.	0.2	tr.	tr.	tr.	tr.	tr.	*RI*, MS
δ-Elemene	1334	1338	1338				tr.		0.2		tr.		tr.		tr.		tr.		tr.		*RI*, MS
α-Cubebene	1348	1348	1348	tr.			tr.		0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	tr.		tr.	0.2	Std
Eugenol	1357	1359	1361	tr.	tr.	tr.		tr.								0.1		0.2			Std
γ-Nonalactone	1361	1358	1359			tr.		tr.		tr.						tr.		tr.			*RI*, MS
4aα,7α,7aα-Nepetalactone	1362	1360					tr.		0.1		tr.		0.1		tr.		0.1		0.2		*RI*, MS
Undecanol	1364	1367	1365									0.1									*RI*, MS

Table 3 (cont.)

Component[a])	*RI*[b])	*RI* Lit.		Populations[c])																	ID[d])
				1	*2*	*3*		*4*		*5*		*6*		*7*		*8*		*9*		*10*	
		ADAMS[e])	*NIST 08*[f])	dry	dry	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	
2-(*p*-Methoxyphenyl)ethyl alcohol	1368		1374	tr.																	*RI*, MS
Piperitenone oxide	1369	1368					tr.		tr.		tr.		0.1		tr.		tr.		tr.		*RI*, MS
α-Copaene	1373	1376	1372	0.1	0.1	0.1	0.1	0.2	0.1	0.5	0.1	0.3	0.1	0.1	0.2	0.3	0.2	0.2	0.2	0.6	Std
3,4-Dihydrocoumarin	1380	1378		0.2	0.1	tr.							tr.			0.6	tr.	tr.	tr.	0.7	*RI*, MS
β-Bourbonene	1384	1388	1388	tr.	tr.	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.2	0.2	0.1		0.1	0.3	0.3	tr.	*RI*, MS
α-Isocumene	1387	1387					tr.	tr.	tr.	tr.	tr.			tr.	tr.						*RI*, MS
β-Cubebene	1387	1387	1388	tr.	tr.		tr.		tr.	tr.	0.1	tr.			0.1		tr.		tr.	0.6	*RI*, MS
β-Elemene	1388	1389	1388	0.2	tr.		0.1	0.1	0.1	0.2	tr.		0.1	tr.	tr.	0.8	0.1		0.1	tr.	*RI*, MS
Tetradecene	1390	1388	1393					tr.								tr.		tr.		0.1	*RI*, MS
(*Z*)-Jasmone	1395	1392	1393				tr.				tr.		tr.		tr.		tr.		tr.		*RI*, MS
6,10-Dimethylundecan-2-one	1399	1407	1407			tr.		tr.		tr.		tr.				tr.		tr.			*RI*, MS
n-Tetradecane	1400	1400	1400	tr.	tr.	tr.	tr.	tr.	0.1	tr.	tr.		tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	Std
Dodecanal	1403	1408	1405	0.1	0.1	0.3	tr.	0.7	0.2	0.1	tr.	0.1	0.1	0.2	tr.	1.1	0.1	0.5	0.1	0.2	Std
α-Gurjunene	1409	1409	1408										tr.				tr.				*RI*, MS
(*E*)-Caryophyllene	1417	1419	1412	0.7	0.7	1.8	1.8	0.4	3.3	2.1	1.1	0.2	5.7	1.2	1.2	1.1	1.9	0.4	2.6	6.5	Std
(*E*)-*α*-Ionone	1426	1428	1427	0.2	0.2	tr.		0.1	0.2	0.3	tr.	tr.	0.1	0.1	0.1	tr.	0.1	tr.	0.1	0.5	*RI*, MS
Epinepetalactone	1429										0.1				0.1		tr.		0.1		MS
β-Copaene	1429	1432	1430	tr.	tr.	0.1	0.1	tr.	tr.	tr.	tr.		tr.	tr.	tr.	0.2	tr.	tr.	tr.	tr.	*RI*, MS
α-*trans*-Bergamotene	1435	1434	1430									tr.									*RI*, MS
Coumarin	1442	1432	1432	51.8	51.9	56.5	69.7	31.8	58.2	55.4	64.7	17.9	44.2	35.8	62.4	43.8	66.5	37.7	57.5	35.2	Std
(*E*)-Cinnamyl acetate	1446	1443	1445													tr.					*RI*, MS
Geranyl acetone	1450	1453	1457		tr.			1.9		0.9			tr.	tr.		3.0		tr.		tr.	*RI*, MS
α-Humulene	1456	1454	1449	1.8	tr.	0.8	1.7	2.0	0.9	tr.	tr.	0.7	2.9	tr.	tr.	tr.	1.4	0.5	2.2	5.2	Std
cis-Cadina-1(6),4-diene	1462	1476																		1.6	*RI*, MS
9-Epicaryophyllene	1466	1464	1465										tr.								*RI*, MS
Dodecan-1-ol	1470	1469	1473		tr.	0.4		0.2	tr.	tr.				0.2		tr.		0.4		tr.	Std
γ-Gurjunene	1474	1475	1470				tr.		0.1	0.3	0.2	0.3	tr.	0.1	0.2	tr.	tr.		0.1	0.6	*RI*, MS
α-Amorphene	1475	1483	1475																	0.6	*RI*, MS

Table 3 (cont.)

Component[a])	*RI*[b])	*RI* Lit.		Populations[c])																	ID[d])
				1	*2*	*3*		*4*		*5*		*6*		*7*		*8*		*9*		*10*	
		ADAMS[e])	*NIST 08*[f])	dry	dry	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	
γ-Muurolene	1476	1479	1476			0.2	0.1		0.2	0.4	0.1	0.2	0.2	tr.	0.1	0.2	0.1		tr.		*RI*, MS
Germacrene D	1481	1485	1477	2.2	1.8	1.2	1.6	0.9	1.2	3.1	0.8	0.2	1.4	0.3	0.8	1.6	1.7	0.2	1.3	9.8	*RI*, MS
Viridiflorene	1483	1496	1489				0.1		0.3		tr.		tr.		tr.		tr.		tr.		*RI*, MS
(*E*)-β-Ionone	1485	1487		0.7	0.7	1.1	0.3	1.1	0.4	1.0	0.3	0.5	0.6	0.4	0.4	3.7	0.5	1.5	0.4	1.3	Std
Bicyclogermacrene	1491	1500	1499	0.3	0.3		tr.		tr.	0.3	0.4		0.2		0.4		0.4		0.2		*RI*, MS
cis-β-Guaiene	1492	1492	1492																	0.7	*RI*, MS
α-Muurolene	1495	1500	1500				0.2		0.1	0.6	tr.		0.3		tr.		tr.		0.1	0.6	*RI*, MS
(*E*,*E*)-α-Farnesene	1502	1505	1500	0.1	0.1	tr.	0.1		tr.	0.2	0.1		0.5	tr.	0.1	tr.	0.1		0.2		Std
β-Bisabolene	1504	1505	1506																	1.0	*RI*, MS
Tridecanal	1504	1509	1506	tr.	tr.	0.5	tr.	0.3	0.3	tr.	tr.		tr.	tr.	tr.	1.0	0.1	0.3	tr.		*RI*, MS
2,5-Bis(1,1-dimethyl-ethyl)phenol	1510		1514	0.1		tr.	0.1	tr.	0.1		0.1				0.1	0.7		tr.	0.1		*RI*, MS
γ-Cadinene	1512	1513	1509							0.3										0.3	*RI*, MS
7-*epi*-α-Selinene	1516	1520		0.1			tr.		0.1		tr.		0.2		tr.				tr.		*RI*, MS
δ-Eadinene	1521	1523	1521	0.2	0.1	0.1	0.1		0.1	0.7	0.1		0.1		0.1	0.3	0.2		0.1	0.7	*RI*, MS
cis-Ealamenene	1524	1528	1520									0.1									*RI*, MS
Ethyl 4-ethoxybenzoate	1525			0.1																	MS
Dihydroactinidiolide	1533		1532	0.2	0.2	0.6	tr.	0.5	tr.	0.8	tr.	0.5	tr.	0.1	tr.	2.1	tr.	0.9	tr.	0.3	*RI*, MS
(*Z*)-Nerolidol	1534	1531	1535												tr.						*RI*, MS
Elemicin	1554	1557	1554																tr.		*RI*, MS
(*E*)-Nerolidol	1563	1561	1564				tr.			0.1	tr.		tr.	0.2	tr.		tr.		tr.		Std
(*Z*)-Hex-3-enylbenzoate	1565	1565					tr.				tr.		tr.	tr.	tr.				tr.		*RI*, MS
Spathulenol	1578	1577	1578	0.1	0.1		tr.		0.1	tr.	tr.		tr.	tr.	tr.		tr.		tr.	tr.	*RI*, MS
Caryophyllene oxide	1582	1583	1578	tr.	0.1	tr.	0.1	0.5	0.1	0.3	tr.	0.1	0.2	0.7	tr.	0.4	0.1	0.4	tr.	0.1	Std
Hexadecene	1586	1588	1591					tr.		tr.						0.1				tr.	*RI*, MS
Viridiflorol	1591	1592	1589				tr.		tr.		tr.		tr.	0.6	tr.		tr.		tr.		*RI*, MS
Tetradecanal	1607	1612	1615	tr.	0.1	0.1	tr.	0.3	tr.	tr.	tr.		tr.	0.1	tr.	0.4	tr.	0.3	tr.	0.2	*RI*, MS
Caryophylla-4(12),8(13)-dien-5α-ol	1643	1640										0.4									*RI*, MS
cis-Methyl dihydro-jasmonate	1659			0.1	tr.	0.2	tr.	0.2	tr.	0.1	tr.		tr.	tr.	tr.	0.4	tr.	0.2	tr.	0.2	MS

Table 3 (cont.)

Component[a])	*RI*[b])	*RI* Lit.		Populations[c])																	ID[d])
				1	*2*	*3*		*4*		*5*		*6*		*7*		*8*		*9*		*10*	
		ADAMS[e])	*NIST 08*[f])	dry	dry	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	
(*Z*)-14-Hydroxycaryophyllene	1665	1667										0.2									*RI*, MS
α-Bisabolol	1683	1685	1687										tr.								Std
Heptadecane	1699	1700	1700	tr.	0.1	tr.	tr.	tr.	tr.	0.3	tr.		tr.	tr.	tr.	tr.	tr.	0.1	tr.	tr.	Std
Pentadecanal	1711		1714		tr.	tr.	tr.	tr.	tr.	tr.	tr.		tr.	tr.	tr.	tr.	tr.		tr.	tr.	*RI*, MS
Benzyl benzoate	1760	1760	1765						tr.		tr.				tr.	tr.		tr.	tr.		*RI*, MS
Octadecane	1793	1800	1800	0.1	tr.	tr.	tr.	0.3	tr.	tr.	tr.	tr.	tr.	tr.	tr.	0.2	tr.	0.1	tr.	tr.	Std
2,6,10,14-Tetramethylhexadecane	1810		1810		tr.					tr.					tr.						*RI*, MS
Hexadecanal	1820		1817	tr.	tr.	0.2	tr.	0.4	tr.	0.2	tr.		tr.	tr.	tr.	0.4	tr.	0.3	tr.	tr.	*RI*, MS
Isopropyl myristate	1827		1827	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.		tr.	tr.	tr.	tr.	tr.		tr.	tr.	*RI*, MS
6,10,14-Trimethylpentadecan-2-one	1844		1845	tr.	tr.	0.2	tr.	0.4	tr.	0.1	tr.	0.3	tr.	0.2	tr.	0.9	0.1	0.3	tr.	0.2	*RI*, MS
Nonadecane	1909	1900	1900	0.1	tr.	0.1	tr.	tr.	tr.	tr.	tr.		tr.	tr.	tr.	tr.	tr.		tr.	tr.	Std
Manool oxide	1987	1987					tr.		tr.		tr.		tr.		tr.		tr.		tr.		*RI*, MS
Icosane	1997	2000		tr.	tr.	tr.	tr.	0.1	tr.	tr.	tr.		tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	Std
13-Epimanool oxide	2013											tr.									MS
Heneicosane	2100	2100	2100	tr.	tr.	0.1	tr.	0.1	tr.	tr.	tr.	0.1	tr.	tr.	tr.	tr.	tr.		tr.	tr.	Std
Docosane	2195	2200		tr.	tr.	0.1	tr.	0.1	tr.	tr.	tr.	0.1	tr.	tr.	tr.	0.2	tr.	0.1	tr.		Std
Tricosane	2295	2300	2300				tr.		tr.		tr.	0.1	tr.		tr.		tr.				Std
Tetracosane	2359	2400	2400	tr.																	Std
Pentacosane	2485	2500	2500									tr.									Std
Aliphatics alcohols				34.3	39.0	30.8	19.8	52.5	18.3	19.4	19.1	58.8	20.1	53.7	20.0	17.4	18.6	47.8	22.2	22.0	
Alkanes				0.3	0.1	0.4	tr.	0.7	0.1	0.6	tr.	0.3	tr.	tr.	tr.	0.7	tr.	0.4	0.4	0.2	
Aldehydes and ketones				0.5	0.6	1.7	0.8	3.2	tr.	1.8	1.1	4.5	1.7	2.8	1.2	6.2	1.7	3.7	2.5	1.5	
Esters				0.1	tr.	tr.	tr.	0.1	tr.	tr.	tr.		tr.	0.1	tr.	tr.	tr.	1.1	tr.	0.0	

Table 3 (cont.)

Component[a])	Populations[c])																ID[d])
	1	*2*	*3*		*4*		*5*		*6*		*7*		*8*		*9*		*10*
	dry	dry	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry	fresh	dry
Aromatics	52.5	52.1	56.9	69.8	32.6	58.4	55.7	65.0	18.5	44.2	36.0	62.6	46.0	66.6	40.2	57.8	36.3
Monoterpene hydrocarbons	3.1	1.9	0.3	2.0	0.6	10.7	5.3	7.4	4.8	18.2	1.0	8.0	5.6	4.4	0.6	6.1	6.2
Oxygenated monoterpenes	1.9	1.3	2.9	0.7	3.5	2.7	5.2	3.5		2.7	1.7	3.8	8.0	1.3	1.4	8.8	1.8
Sesquiterpene hydrocarbons	5.6	3.3	4.4	6.2	3.8	7.3	9.2	3.2	2.2	12.0	2.1	3.5	4.7	6.5	1.7	7.5	29.0
Oxygenated sesquiterpenes	0.1	0.2	tr.	0.1	0.5	0.2	0.4	0.1	0.9	0.2	1.5	0.1	0.4	0.2	0.4	0.1	0.1
Diterpenes				tr.		tr.		tr.	tr.	tr.		tr.		tr.		tr.	
Norisoprenoids	1.1	1.2	1.7	0.4	2.0	0.6	2.2	0.5	1.2	0.8	0.8	0.5	6.2	0.7	2.7	0.5	2.3
Others	0.1	tr.	0.2	tr.	0.2	tr.	0.1	0.1		tr.		0.1	0.4	tr.	0.2	0.1	0.2
Total identified [%]	99.7	99.7	99.3	100.0	99.5	100.0	100.0	99.9	95.6	100.0	99.8	99.8	95.9	100.0	99.1	100.0	99.6
No. of identified compounds	83	82	72	100	75	99	86	102	59	104	80	103	79	98	69	105	84

[a]) Compounds are listed in order of their elution from a *HP-5* column; percentage values are means of three determinations with a SD range of 0.0–5.5; they were obtained at FID by peak-area normalization calculating the relative response factor. [b]) Retention index (*RI*) on *HP-5* column, experimentally determined using homologous series of C_8–C_{30} alkanes. [c]) Sample numbers correspond to bastard balm population indicated in *Table 1*. [d]) Identification (ID) methods: MS, by comparison of the mass spectrum with those of the computer mass libraries *WILEY*, *ADAMS* [17], and *NIST 08* [18]; *RI*, by comparison of *RI* values with those reported in [17][18]; Std, by comparison of the t_R and MS with those of available authentic standard. [e]) Relative *RI* taken from *ADAMS* [17]. [f]) Relative *RI* taken from *NIST 08* [18]. [g]) tr., traces (mean value below 0.1%).

2. *Analysis of Aroma Components in Whole Aerial Parts.* The aroma compounds of fresh frozen and dry whole aerial parts of *M. melissophyllum*, extracted by HS-SPME using PDMS fiber, and then analyzed by GC/FID and GC/MS, are listed in *Table 3*. In total, 159 volatile components were identified in the whole aerial parts of bastard balm; 118 compounds were identified in frozen samples, accounting to 99.8–100.0% of all the volatiles, whereas 136 compounds were identified in dry samples, accounting to 95.6–100.0% of total volatiles.

The aroma of fresh frozen samples consists largely of aromatic compounds (44.2–69.8%), represented mainly by coumarin (44.2–69.7%), and aliphatic alcohols (18.3–22.2%), mainly C_8 derivatives as the mushroom component oct-1-en-3-ol (17.7–21.2%). Monoterpene (2.0–18.2%) and sesquiterpene hydrocarbons (3.2–12.0%) gave a minor contribution to the total aroma, with α-pinene (1.1–10.2%) and (*E*)-caryophyllene (1.1–5.7%) being the most representative ones. Noteworthy is the release in the headspace of more than one hundred volatiles (112) occurring in scant amounts (traces or below 1%).

The volatile emission of dry samples was again characterized by coumarin (17.9–56.5%), but, in this case, aliphatic alcohols reached higher levels (17.4–58.8%). They were represented mainly by oct-1-en-3-ol (16.1–57.0%) and, to a lesser extent, by octan-3-ol (1.0–2.6%). This may be due to a simultaneous loss of H_2O-soluble compounds and an increase of oxidation products during drying. Other compounds responsible for typical mushroom aroma were represented by benzaldehyde (traces to 0.6%), benzyl alcohol (traces), (*E*)-oct-2-en-1-ol (traces to 0.2%), and octan-1-ol (traces). With the exception of sample *10*, also in this case monoterpene and sesquiterpene hydrocarbons (0.3–5.6% and 1.7–9.2%, resp.) provide lower contributions, with a little increase of oxygenated monoterpenes (1.3–8.0%) owing to oxidation processes occurring during drying. The only terpenes produced in appreciable amounts were α-pinene (0.3–5.1%), *cis*-chrysanthenyl acetate (0.8–3.0%), (*E*)-caryophyllene (0.2–2.1%), α-humulene (traces to 2.0%), and germacrene D (0.2–3.1%). In sample *10*, representing an Italian population occurring at a high altitude (1277 m), sesquiterpene hydrocarbons (29.0%) contribute more than aliphatic alcohols (22.0%), with (*E*)-caryophyllene (6.5%), α-humulene (5.2%), and germacrene D (9.8%) being the most frequent representative. As in frozen samples, also in dry bastard balm aerial parts, more than 100 volatiles (106) occurred in scant amounts (traces to below 1%).

3. *Effect of Drying Process.* Various physicochemical changes of aromatic volatiles (oxidation, evaporation) may take place during the drying process, influencing aroma intensity and the quality of the dried products. Compared with fresh frozen material (samples from populations *3–9*), a significant decrease in extracted volatiles ($p < 0.0005$), in terms of total peak areas and number of total components (from 101 to 71, mean values), was observed in dried whole parts of bastard balm, mainly as a result of the loss of aromatics (from 60.6 to 40.8%, mean values; $p < 0.01$), monoterpene hydrocarbons (from 8.1 to 2.6%, mean values; $p < 0.05$), and sesquiterpenes hydrocarbons (from 6.6 to 4.0%, mean values; $p < 0.05$; *Fig. 2*). This decrease that influenced mainly coumarin (from 60.5 to 39.8%, mean values; $p < 0.01$) and (*E*)-caryophyllene (from 2.5 to 1.0%, mean values; $p < 0.05$) was accompanied by an increase in oxygenated products, mainly aliphatic alcohols (from 19.7 to 40.1%, mean values;

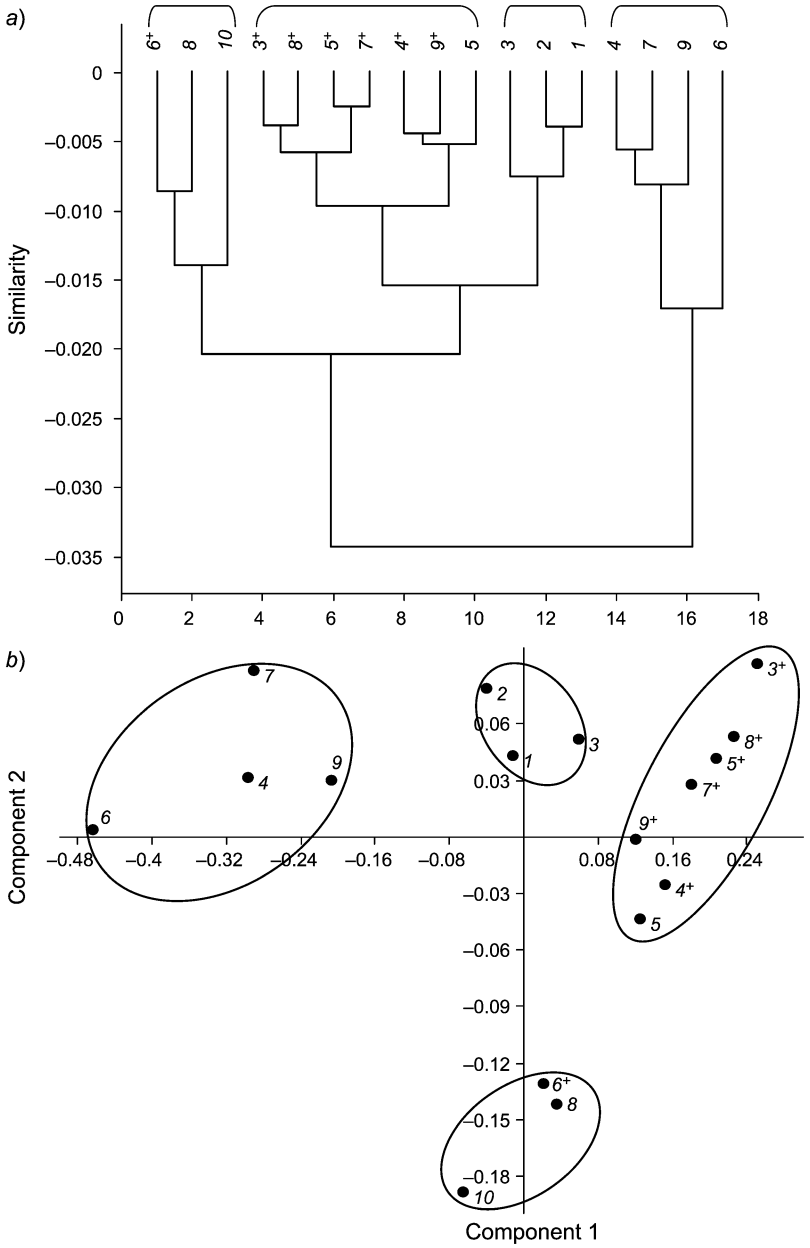

Fig. 2. *a*) *Dendrogram obtained by hierarchical cluster analysis of the percentage composition of volatiles from whole fresh frozen and dry aerial parts from ten provenances of* Melittis melissophyllum. Clustering method based on UPGMA with the Euclidean distance as dissimilarity coefficient. *b*) *Principal component analysis of the volatile compounds.* The first two principal components, PC1 and PC2, contain *ca.* 94.03% information. Samples are represented by numbers corresponding to population of origin (see *Table 1*); $^{+}$ indicate fresh frozen samples.

Table 4. *Percentages of the Main Compound Classes and Major Volatile Compounds Extracted by HS-SPME from Whole Fresh and Dry Aerial Parts, and Vegetative and Reproductive Organs of Bastard Balm*

Compound class	Whole aerial parts [%][a])		Plant parts (dry) [%][b])[c])			
	Fresh	Dry	Lv	St	Ca	Co
Oct-1-en-3-ol	19.0	37.7*	51.1	52.4	48.8	1.1
Coumarin	60.5	39.8**	40.8	16.6	1.9	tr.
(*E*)-Caryophyllene	2.5	1.0*	0.5	0.3	5.9	0.4
Aromatics	60.6	40.8**	41.2	19.9	3.4	1.2
Alcohols	19.7	40.1**				
Monoterpene hydrocarbons	6.6	2.6*	0.8	0.2	6.7	68.8
Sesquiterpene hydrocarbons	6.0	4.0*	1.7	0.4	8.7	5.6
Norisoprenoids	0.6	2.4*		3.0	2.5	0.2

[a]) Values are means of seven locality measurements (*Populations 3–9*) that are, in turn, means of three determinations each. *: $p < 0.05$ when compared with fresh material; **: $p < 0.01$ when compared with fresh material. [b]) Values are means of three determinations. [c]) Lv, leaves; St, stems; Ca, calyx; Co, corolla.

$p < 0.01$) as oct-1-en-3-ol (from 19.0 to 37.7%, mean values; $p < 0.05$; *Table 4*). Therefore, dry material may be preferable as mushroom-like aroma enhancer in food products.

Noteworthy is the increase in the dry material of norisoprenoids (from 0.8 to 6.2%, mean values; $p < 0.05$), notably (*E*)-*β*-ionone (0.4–3.7%). Norisoprenoids are C_{13} compounds derived from the degradation of carotenoids and also from the hydrolysis of glucosides occurring during drying processes [19]. They have an important sensorial impact as they have very low olfactory perception thresholds. In particular, (*E*)-*β*-ionone is one of the main contributors to the aroma of roses. A similar phenomenon was observed for geranyl acetone (traces to 3.0%), a terpene oxidation product and one possible precursor being squalene; it is found in fruits and essential oils, and is used in perfumery for its fresh-green, slightly penetrating, rose-like odor [20].

Moreover, dry samples of bastard balm were found to be a source of other important flavor compounds, which occur in scant amounts or traces, and completely lack in fresh frozen material, such as lavender lactone, safranal, (*E*)-cinnamaldehyde, (*E*)-anethole, and *γ*-nonalactone. Lavender lactone is presumably an oxidation metabolite of linalol oxide (furanoid), and it is one of the lactones that have been reported in lavender oil, wines, honey, and hop, and is well-known as an interesting olfactory natural product [21]. Safranal is a monoterpene aldehyde, constituting the main contributor to the aroma of saffron (*Crocus sativus* L.) stigma. (*E*)-Cinnamaldehyde is an aromatic compound occurring in the bark of cinnamon tree (*Cinnamomum zeylanicum* BLUME) and other species of the genus *Cinnamomum*, used widely in confectionery, baked foods, pickles, meat seasonings, soft drinks (cola-type), pharmaceuticals, oral-care products, *etc.* (*E*)-Anethole is an aromatic compound that contributes to the distinctive flavors of anise and fennel, myrtle, liquorice, and star anise. *γ*-Nonalactone is one of the important odor-active compounds in freshly cooked non-scented rice characterized by a strong, sweet, coconut-like aroma [22].

Other important odor-impact volatiles detected in scant amounts in the headspace of both frozen and dry bastard balm samples were benzaldehyde, benzyl alcohol, benzeneacetaldehyde, salicylaldehyde and 2-phenylethyl alcohol.

Of interest is the occurrence, only in fresh frozen samples, of 4aα,7α,7aα-nepetalactone (traces to 0.2%) and epinepetalactone (traces to 0.1%) that are bicyclic terpenoids, based on two fused rings, a cyclopentane and a lactone, first isolated from the catnip (*Nepeta cataria* L.), which exhibit feline attractant and insect repellent effects, in addition to some effects on humans as weak sedative, antispasmodic, febrifuge, and antibacterial activities [23]. Additionally, fresh frozen samples were found to emit some important aroma components, such as piperitenone oxide (traces to 0.2%), an important chemical constituent of the essential oil of many *Mentha* species, and (*Z*)-jasmone, a natural organic compound extracted from the volatile portion of the oil from jasmine flowers, acting either as an attractant or as a repellent for various insects, and commercially used in perfumes and cosmetics.

4. *Multivariate Analysis.* SPME Data from the analysis of whole fresh and dry aerial parts were subjected to hierarchical cluster analyses (CA). The UPGMA method (unweighted pair-group method using arithmetic averages), with the Euclidean distance as dissimilarity coefficient, and principal component analysis (PCA) were employed on percentages of volatiles. *Fig. 2*,*a*, shows a dendrogram formed by using the UPGMA method and the PCA projection of the same samples. PCA Visualization shows 83.28% of primary data variability on first axis and 10.75% on the second axis. The variability of these two axes is generated mostly by the content of oct-1-en-3-ol (values of eigenvectors: -0.66; -0.59) and coumarin (values of eigenvectors: 0.74; -0.56). Further important compounds, but with low total content in samples, are represented by α-pinene and germacrene D. Both methods indicated a similar grouping and clearly showed the differences between the populations.

From CA, four main groups could be delineated: group A, formed by Slovak populations (dry) and one dry Italian population (*3*), characterized by high content of coumarin (51.8–56.5%) and medium content of oct-1-en-3-ol (28.5–36.9%); group B, containing fresh frozen samples from populations 3^+, 4^+, 5^+, 7^+, 8^+, and 9^+, and population *5* (dry) characterized by high content of coumarin (55.4–69.7%) and low content of oct-1-en-3-ol (17.6–21.2%); group C composed of populations 6^+ (fresh), *8* and *10* (dry) with a medium content of coumarin (35.2–44.2%) and a low content of oct-1-en-3-ol (16.1–20.8%); group D include dry samples from populations *4*, *6*, *7*, and *9*, characterized by a low-to-medium content of coumarin (17.9–37.7%) and high content of oct-1-en-3-ol (43.4–57.0%). A similar arrangement was also observable in the plot from PCA (*Fig. 2*,*b*), where population *10* is linked to populations 6^+ and *8* because of the similar content in the two main volatiles. Interestingly, fresh frozen samples formed a distinct cluster, which was linked to the dry sample clusters at large distance value indicating a significant difference. This confirms that drying processes have a significant effect in changing qualitatively and quantitatively the volatile composition of the plant matrix. Finally, the volatile compositions of Slovak population, *1* and *2*, belonging to a different subspecies, were not sufficiently discriminable with respect to Italian populations, *i.e.*, sample *3*, meaning that aroma chromatograms from the HS-SPME sampling did not provide significant elements supporting the infraspecific variability of bastard balm. Therefore, other types of

phytochemicals have to be studied for their application in differentiating taxa at infraspecific level.

5. *Analysis of the Aroma Components in Different Plant Parts.* To investigate the volatile emissions by different parts of bastard balm, the leaves, stems, calyx, and corolla were collected from Piedilapiaggia (population *7*) and, once dried, analyzed by using the SPME-GC/FID and GC/MS procedure (three repeated analyses were performed).

In total, 120 volatile components were identified in the different aerial parts of bastard balm (64 in leaves, 63 in stems, 76 in calyx, and 84 in corolla), accounting to 97.5–99.8% of all the volatiles emitted (*Table 5*). While the volatile pattern emitted by calyx and stems was quite similar, with oct-1-en-3-ol as the major component (48.8–52.4%), in leaves coumarin reached maximum levels (40.8%). The overall emissions of volatiles from leaves were more than three times higher compared with other bastard balm parts (data not shown). Stems showed an overall volatile emission quite lower than that of leaves. Regarding blossom parts, corolla released a higher amount of volatiles than calyx, although oct-1-en-3-ol and coumarin were emitted in very scant amount (1.1% and traces, resp.). According to the ecological role of corolla in attracting pollinator insects, petals emitted more monoterpenes playing this role; notably, hydrocarbons (68.8%) represented mainly by α-pinene (52.0%). Calyx emitted a higher amount of oct-1-en-3-ol (48.8%), and octan-3-ol (5.0%), another contributor to the characteristic mushroom flavor, accompanied by a scant amount of coumarin (1.9%). This renders it the most suitable part of the plant giving a characteristic mushroom-like aroma.

Conclusions. – Application of HS-SPME procedure developed in this study allowed us to collect and analyze the volatiles emitted from the whole frozen and dried aerial parts, and different plant parts of bastard balm (*M. melissophyllum*). It permitted low extraction temperature and short sampling times, thus avoiding the formation of artifacts due to chemical changes in the plant matrix. The analytical method showed a high repeatability and good sensitivity, making it applicable to detect substantial changes in volatile emissions between different whole aerial parts and different organs of the plant. Oct-1-en-3-ol and coumarin were the major volatiles in all the samples of whole aerial parts, highlighting the potential of the plant as aroma enhancer to be used as flavoring in food processing. Various physicochemical changes of aromatic volatiles, such as oxidation and evaporation, took place during the drying process, influencing aroma intensity and the quality of the dried bastard balm samples. As a result, a lower amount of extracted volatiles, mainly aromatics, and monoterpene and sesquiterpene hydrocarbons, was found in dried whole aerial parts compared with fresh frozen material. However, this decrease was accompanied by an increase in oxygenated products, mainly oct-1-en-3-ol. Therefore, dry material is preferable in the case we would like to enhance the mushroom aroma of the plant matrix. Comparing ten populations of *M. melissophyllum* of different origin (Italy and Slovakia), and belonging to two different subspecies (subsp. *melissophyllum* and subsp. *carpatica*), no significant differences at infraspecific level were detected in volatile components sampled by SPME. Finally, SPME analysis permitted us to confirm the existence of spatial fragrance patterns within the vegetative and reproductive parts, with calyx

Table 5. *Volatile Aroma Components of Different Plant Organs of Bastard Balm* (*Melittis melissophyllum*) *Obtained by HS-SPME*

Component[a])	*RI* Lit.			Plant parts[b])				ID[c])	Odor description
	RI[d])	*ASAMS*[e])	*NIST 08*[f])	Lv	St	Ca	Co		
Hexanal	801	801	800	tr.[g])	1.2	1.0	1.0	*RI*, MS	Pungent
(*E*)-Hex-2-anal	857	855	857	0.3	tr.	2.9		*RI*, MS	Wet grass, rancid fat
Heptanal	907	902	904	tr.	0.2	0.4	0.2	*RI*, MS	Cheesy, caramel
α-Thujene	927	930	929				0.6	*RI*, MS	Cooked, nutty
α-Pinene	935	939	934	0.8	0.2	5.0	52.0	Std	Solvent
Camphene	949	954	949				0.8	Std	
Thuja-2,4(10)-diene	955	953					2.9	*RI*, MS	
Benzaldehyde	968	960	960	0.2	0.4	0.4	1.0	Std	Bitter almond
Sabinene	974	975	975	0.1		0.3	3.5	*RI*, MS	Oily, pepper, woody
β-Pinene	976	979	975	tr.		0.4	4.8	Std	Soapy, fragrant
Oct-1-en-3-ol	981	979	980	51.1	52.4	48.8	1.1	Std	Mushroom like
Octan-3-one	983	979	982		tr.			*RI*, MS	
Myrcene	991	990	992				0.7	Std	Slightly green
Octan-3-ol	997	991	991	2.6	3.2	5.0	0.5	*RI*, MS	Cod liver oil
(2*E*,4*E*)-Hepta-2,4-dienal	1006	1007	1009	tr.	tr.	tr.		*RI*, MS	Cooked meat
α-Terpinene	1017	1014	1015				0.2	Std	
Benzyl alcohol	1024	1026	1033			0.5	tr.	*RI*, MS	
p-Cymene	1026	1024	1028		tr.	tr.	0.4	Std	Week, citrus odor
1,8-Cineole	1029	1031	1034				tr.	Std	Fragrant, sweet
Limonene	1029	1029	1026	tr.	tr.	1.0	2.0	Std	Orange
Lavender lactone	1037	1034			tr.			*RI*, MS	Honey
Benzene acetaldehyde	1041	1042	1042	tr.	tr.	0.4	tr.	Std	Fruity, sweet
Salicylaldehyde	1044	1044	1041	tr.	tr.			*RI*, MS	Buckwheat groats
(*E*)-Oct-2-enal	1049	1060	1069		tr.	tr.		*RI*, MS	Medicinal
γ-Terpinene	1062	1059	1061				0.4	Std	Herbaceous, citrus
cis-Sabinene hydrate	1072	1070					0.7	*RI*, MS	
Octa-3,5-dien-2-one	1077		1076	tr.	tr.	tr.		*RI*, MS	
2-Ethyl-3,5-dimethyl-pyrazine	1080		1078		tr.			*RI*, MS	
Terpinolene	1088	1088	1088				0.5	Std	Plastic, petroleum
Camphen-6-one	1093	1095					0.2	*RI*, MS	
trans-Sabinene hydrate	1098	1098					tr.	*RI*, MS	
Linalool	1103	1096	1102	0.5	3.1	2.1	0.5	Std	Floral, fruity
Nonanal	1109	1100	1104	tr.	0.6	0.7	0.5	*RI*, MS	Plastic, soap
2-Phenylethyl alcohol	1113	1107	1116	tr.	2.2	tr.	0.1	*RI*, MS	Rose, floral
Oct-1-en-3-yl acetate	1114	1112	1112	tr.	tr.			*RI*, MS	
α-Campholenal	1128	1125				0.3		*RI*, MS	
trans-Pinocarveol	1141	1139	1141	tr.		tr.	2.1	*RI*, MS	
Camphor	1143	1146	1145	tr.	0.2	tr.		Std	Strong, aromatic
cis-Verbenol	1144	1137	1140				0.5	Std	
(2*E*,6*Z*)-Nona-2,6-dienal	1148	1150	1152		1.2	1.1		*RI*, MS	Cucumber
trans-Verbenol	1148	1140	1145			0.5	2.9	*RI*, MS	
Isopulegone	1150		1160				tr.	*RI*, MS	
Pinocarvone	1160	1164	1158	0.5	3.6	2.2	0.9	*RI*, MS	Minty
Menthol	1171	1167			tr.			Std	
Terpinen-4-ol	1174	1177	1178			tr.		Std	Flowers
p-Cymen-8-ol	1180	1182	1182			tr.	0.4	*RI*, MS	

Table 5 (cont.)

Component[a])	*RI* Lit.			Plant parts[b])				ID[c])	Odor description
	RI[d])	*ASAMS*[e])	*NIST 08*[f])	Lv	St	Ca	Co		
Dec-1-en-3-ol	1183	1180		tr.				*RI*, MS	
α-Terpineol	1186	1182	1182	tr.		tr.	0.2	Std	Cooling, fresh
Methyl salicylate	1188	1191	1185	tr.	tr.	0.2		*RI*, MS	Sweet, wintergreen
Myrtenal	1196	1195	1192	tr.	tr.	tr.	1.2	Std	Cooling, mint
Safranal	1200	1196	1197	tr.	tr.	0.2		*RI*, MS	Saffron
Verbenone	1201	1204	1195			0.9	0.9	Std	Spicy, mint
Decanal	1205	1199	1206	tr.	0.5	1.7	0.2	*RI*, MS	Green, nutty
(2*E*,4*E*)-Nona-2,4-dienal	1210	1210	1211		tr.			*RI*, MS	Sweet, unpleasant
β-Cyclocitral	1222	1219	1220	0.1	0.3	0.4		*RI*, MS	Tropical, saffron
cis-Carveol	1223	1226	1222				0.2	*RI*, MS	
Carvone	1245	1239	1241			tr.	0.3	*RI*, MS	Peppermint
Geranial	1264	1267	1271			tr.		Std	
Isobornyl acetate	1283	1283			tr.		tr.	Std	Camphoraceous
(2*E*,4*E*)-Deca-2,4-dienal	1298	1212	1297		tr.			*RI*, MS	Green, fat fried
Tridecane	1300	1300	1300	tr.	tr.	tr.	tr.	Std	
Undecanal	1307	1306	1308	tr.	tr.	tr.	tr.	*RI*, MS	Aldehydic
α-Cubebene	1346	1348	1348	0.1			0.2	Std	Herbal, waxy
γ-Nonalactone	1358	1358	1359		tr.		tr.	*RI*, MS	Coconut
Eugenol	1359	1359	1361				tr.	Std	Clove
4aα,7α,7aα-Nepetalactone	1360	1360					tr.	*RI*, MS	
Neryl acetate	1361	1359				tr.		*RI*, MS	
α-Copaene	1372	1376	1372	tr.		0.2	0.9	Std	Fragrant, fresh
3,4-Dihydrocoumarin	1381	1378		0.2	0.7			*RI*, MS	Sweet, creamy
β-Bourbonene	1385	1388	1388	tr.	0.1	0.4	0.1	*RI*, MS	Herbal, woody
β-Cubebene	1387	1387	1388				tr.	*RI*, MS	Citrus, fruity
β-Elemene	1391	1390	1389	tr.	tr.	tr.	0.2	*RI*, MS	Medium green
Tetradecane	1397	1400	1400	tr.	0.1	tr.	0.1	Std	
Dodecanal	1405	1408	1405	0.1	0.9	0.9	0.6	Std	Herbal, sweet
α-Gurjunene	1415	1409	1408				0.7	*RI*, MS	
(*E*)-Caryophyllene	1416	1419	1412	0.5	0.3	5.9	0.4	Std	Berry-fruit, orange
(*E*)-α-Ionone	1426	1428	1427	tr.	0.1	tr.		*RI*, MS	Rose
Epinepetalactone	1430					0.5	1.6	MS	
β-Copaene	1431	1432	1430	tr.		tr.		*RI*, MS	
Dihydronepetalactone	1439		1430				0.2	Std	
Coumarin	1442	1432	1432	40.8	16.6	1.9	tr.	Std	Creamy vanilla
Geranyl acetone	1450	1453	1457	tr.	6.6	tr.	tr.	*RI*, MS	Rose
α-Humulene	1453	1454	1449	tr.	tr.	0.6	0.3	Std	Woody
γ-Gurjunene	1470	1475	1470				0.4	*RI*, MS	
β-Chamigrene	1473	1476					1.1	*RI*, MS	
Dodecan-1-ol	1473	1469	1473	tr.	tr.	0.2		Std	Earthy, soapy
γ-Muurolene	1479	1479	1476	0.5		tr.	tr.	*RI*, MS	
(*E*)-β-Ionone	1484	1487		0.3	2.3	1.4		Std	Rose
Germacrene D	1484	1485	1477	0.6		1.5		*RI*, MS	Woody spice
β-Selinene	1488	1489					0.1	*RI*, MS	
n-Pentadecane	1490	1500	1500	tr.	tr.	1.7		Std	
α-Selinene	1494	1498					0.4	*RI*, MS	
α-Muurolene	1495	1500	1500				0.6	*RI*, MS	Cooling, fresh
(*E*,*E*)-α-Farnesene	1502	1505	1500			tr.		Std	Apple
Tridecanal	1502	1505	1506	tr.	tr.	0.8	0.2	*RI*, MS	Fresh, clean
2,5-Bis(1,1-dimethyl-ethyl)phenol	1503		1514				tr.	*RI*, MS	

Table 5 (cont.)

Component[a])	*RI* Lit.			Plant parts[b])				ID[c])	Odor description
	RI[d])	*ASAMS*[e])	*NIST 08*[f])	Lv	St	Ca	Co		
β-Bisabolene	1504	1505	1505			tr.		*RI*, MS	Balsamic
trans-Calamenene	1516	1521					0.1	*RI*, MS	
δ-Cadinene	1526	1523	1521	tr.				*RI*, MS	Warm, woody
Dihydroactinidiolide	1533		1532	0.2	0.3	0.7	0.2	*RI*, MS	Ripe apricot
(*E*)-Nerolidol	1563		1532			tr.		Std	Pungent, spicy
(*Z*)-Hex-3-enyl benzoate	1565	1561	1564			tr.		*RI*, MS	Green, sweet
Tridecan-1-ol	1570	1570				tr.		*RI*, MS	
Spathulenol	1578	1577	1578			0.7	0.2	*RI*, MS	Earthy, herbal
Caryophyllene oxide	1580	1583	1579	tr.	0.2	0.5	0.2	Std	Coriander
Hexadecene	1589	1588	1591			tr.	0.4	*RI*, MS	
Viridiflorol	1590	1592	1589	tr.		0.6		*RI*, MS	Fragrant
Tetradecanal	1607	1612	1615	tr.	tr.	0.3	0.3	*RI*, MS	Fatty, waxy
Heptadecane	1694	1700	1700	tr.	0.2	0.9	0.4	Std	
Pentadecanal	1711		1714	tr.	tr.	tr.	tr.	*RI*, MS	
Octadecane	1801	1800	1800	tr.	0.2	0.6	0.4	Std	
Hexadecanal	1822		1817	0.1	0.4	0.5	0.2	*RI*, MS	
Isopropyl myristate	1827		1827	tr.	tr.			*RI*, MS	
6,10,14-Trimethyl-pentadecan-2-one	1844		1845	0.1	0.1	0.3	0.5	*RI*, MS	
Nonadecane	1900	1900	1900	tr.	tr.			Std	
Manool oxide	1987	1987					tr.	*RI*, MS	
Icosane	2004	2000		tr.	tr.	tr.	0.1	Std	
Abietatriene	2047		2054				0.1	*RI*, MS	
Henicosane	2098	2100	2100	tr.	0.2	0.3	0.1	Std	
Docosane	2193	2200		tr.	0.4	0.3	0.2	Std	
Aliphatics alcohols				53.7	55.5	54.0	1.6		
Alkanes				0.1	1,1	3.8	1.5		
Aldehydes and ketones				0.5	5.1	10.6	3.7		
Esters				tr	tr				
Aromatics				41.2	19.9	3.4	1.2		
Monoterpene hydrocarbons				0.8	0.2	6.7	68.8		
Oxygenated monoterpenes				1.0	13.4	6.7	12.6		
Sesquiterpene hydrocarbons				1.7	0.4	8.7	5.6		
Oxygenated sesquiterpenes				0.1	0.2	1.8	0.4		
Diterpenes							0.1		
Norisoprenoids					3.0	2.5	0.2		
Others					tr	tr	1.6		
Total identified [%]				99.8	98.9	98.4	97.5		
No. of identified compounds				64	63	76	84		

[a]) Compounds are listed in order of their elution from a *HP-5* column; percentage values are means of three determinations with a SD range of 0.0–5.9; they were obtained at FID by peak area normalization calculating the relative response factor. [b]) Lv, Leaves; St, stems; Ca, calyx; Co, corolla. [c]) Identification (ID) methods: MS, by comparison of the mass spectrum with those of the computer mass libraries *WILEY*, *ADAMS* [17] and *NIST 08* [18]; *RI*, by comparison of *RI* value with those reported in [17][18]; std, by comparison of the t_R and MS with those of available authentic standard. [d]) Retention index (*RI*) on *HP-5* column, experimentally determined using homologous series of C_8–C_{30} alkanes. [e]) Relative *RI* taken from *ADAMS* [17]. [f]) Relative *RI* taken from *NIST 08* [18]. [g]) tr., Traces (mean value below 0.1%).

showing a volatile pattern profile with a more pronounced mushroom-like tone, thus, being culinary and economically the most appreciated. Therefore, SPME findings allowed us to state that collection of the plant as flavoring ingredient may be conducted also after the end of flowering, when corolla fall down and only sepals, responsible for the emission of the mushroom aroma, remain on the twigs. In this way, SPME resulted to be a very useful technique, which permits to choose the part of the plant which is the best source of a specific fragrance, and, therefore, to establish the best way of sampling in the industrial application of aromatic plants.

Experimental Part

Plant Material. Aerial parts of *M. melissophyllum* subsp. *melissophyllum* were collected at flowering in May/June 2009 in eight woody places in Italy (Appennino Umbro-Marchigiano, and Abruzzese, Central Italy); while those of *M. m.* subsp. *carpatica* were collected in the same period in two sites in Slovakia (*Fig. 1* and *Table 1*). Italian voucher specimens were identified by *F. M.* and *F. C.* using available literature and deposited with the *Herbarium Camerinensis* (CAME) and with the Herbarium of Centro Ricerche Floristiche dell'Appennino (APP) (both acknowledged in the *Index Herbariorum*) of School of Environmental Sciences, University of Camerino (Italy); while Slovak voucher specimens were authenticated by *P. M.* and deposited with the Herbarium of the Department of Botany (KO), Šafárik University in Košice (Slovakia).

Sample Preparation. After transportation to the laboratory, fresh leaves, stems, calyx, and corolla of the plant material collected in Piedilapiaggia (*Sample 7*) were manually separated and then stored in the dark and at r.t. (*ca.* 22°) until completely dry, while, for the other collection sites, aerial parts were collected and stored as a whole. For some localities (samples *3–9*), part of material was deep-frozen at −20° just after its collection and was kept at freezing temp. until extraction, in order to find out the potential changes of volatiles composition depending on the H_2O content. Whole and separated dry aerial parts were grounded with a blender *MFC* model *DCFH 48 IKA-WERK* (D-Staufen) using sieves of 1-mm diameter, whilst frozen material (whole parts) was homogenized with 10 ml of liquid N_2 for 5 min and reduced into a powder in a mortar. To estimate the H_2O content, fresh material from whole aerial parts was left in a stove at 110° for 24 h. Moisture was determined by the weighing of the plant material before and after drying. The experiment was performed three times. Moisture levels in percentage ranged from 76 to 81%.

Chemicals. Standards (Std.) used for identification (*Tables 3* and *5*) were purchased from *Sigma–Aldrich* (I-Milan). For retention index (*RI*) determination, a mixture of hydrocarbons ranging from octane (C_8) to triacontane (C_{30}) (*Supelco*, Bellefonte, PA, USA) was used and run under the experimental conditions reported below. All compounds were of anal. standard grade.

SPME Procedure. Thirty mg of dried and frozen samples were placed into a 4-ml headspace glass vial, and, after being capped with a polypropylene hole cap with PTFE/silicone septa (*Supelco*, Bellefonte, PA, USA), the vial was immersed in a thermostated water-bath. Once the desired temp. (40°) was reached, the polydimethylsiloxane (PDMS) fibre (*Supelco*), 100-μm-thick, 1-cm-long, was introduced into the vial by manually penetrating the septum and exposed to the headspace of the sample for 30 min, adding 20 μl of dist. H_2O in the case of dried samples. The SPME fibre was maintained 10 mm above the solid samples; after absorption, the fiber was retracted and inserted into the injection port with a SPME inlet liner (0.75 mm i.d., *Supelco*) of gas chromatograph in splitless mode. The extracted compounds were thermally desorbed at 250° for 3 min. These conditions were selected in a previous work where a complete optimization of the extraction conditions for the mushroom-like component oct-1-en-3-ol was carried out [16]. Prior to use, the PDMS fiber was conditioned in the GC injector at 250° in order to remove contaminants. All experiments and sample measurements were carried out in triplicate, and the average values were calculated. To obtain the highest reproducibility, all measurements were performed with the same fiber. No reconditioning was needed for the fiber before next sampling.

GC/FID and GC/MS Analysis. For GC separations, an *Agilent 4890D* instrument coupled to an ionization flame detector (FID) was used. Volatile components were separated on a *HP-5* cap. column (5% phenylmethylpolysiloxane, 25 m, 0.32 mm i.d.; 0.17 μm film thickness; *J & W Scientific*, Folsom, CA, USA). The oven temp. was initially maintained at 60° for 5 min and then programmed to 220° at a rate of 10°/min, then 20°/min up to 280°, held for 15 min, for a total run of 42 min. Temps. of the injector and detector were set to 250°. He was used as carrier gas at 1.4 ml/min under splitless mode. A mixture of aliphatic hydrocarbons (C_8–C_{30}; *Sigma*, I-Milan), diluted in hexane, was loaded onto the SPME fiber and injected under the above temp. program to calculate the *RI*s (as *Kovats* indices) of each extracted compound. The relative amounts of volatile components, expressed as percentages, were obtained by FID peak-area internal normalization, by calculating the response factor of the FID for different classes of volatiles occurring in the headspace of plant matrix [6]. Data were collected by using *HP3398A GC Chemstation* software (*Hewlett Packard*, Rev. A.01.01). GC/MS Analysis was performed with an *Agilent 6890N* gas chromatograph coupled to a *5973N* mass spectrometer equipped with a *HP-5MS* cap. column (5% phenylmethylpolysiloxane, 30 m, 0.25 mm i.d., 0.1 μm film thickness; *J & W Scientific*), using the same temp. program described above. Temps. of the injector, transfer line, and quadrupole were set to 250°. He was used as carrier gas at 1.0 ml/min under splitless mode. The mass spectrometer was run in the electron impact (EI) mode with electron energy at 70 eV, scanning the 29–400 amu. Whenever possible, aroma components were identified by comparing the t_R, *RI* and MS of the chromatographic peak with those of the standard analyzed under the same conditions. Otherwise, the peak assignment was based on computer matching with the *WILEY275*, *NIST 08*, *ADAMS* libraries, and a home-made (based on the analyses of reference oils and commercially available standards) library, taking into account the coherence of the *RI*s of the analyzed compounds with those reported by *ADAMS* [17] and *NIST 08* libraries [18]. Data were analyzed by using MSD ChemStation software (*Agilent*, Version G1701DA D.01.00).

Statistical Analysis. To statistically interpret the aroma-profile characteristics and discriminate amongst diverse volatile emission patterns of different populations, Cluster analysis (CA) and principal component analysis (PCA) methods were established to study the data of the corresponding aroma chromatograms from the HS-SPME sampling. CA is an unsupervised chemometric technique that reveals the natural groupings existing between sample populations characterized by the values of a set of measures. The computations were performed using PAST software package [24]. The handling data were the percent values of the identified compounds. Data with values under 0.1% or missing data were substituted for the purpose of statistical analyses by 0.01%. To ensure normality of data, primary matrix was transformed by arcsin transformation. For the data from the transformed matrix, hierarchical cluster analysis (CA)-UPGMA (unweighted pair-group method using arithmetic averages) with the Euclidean distance as dissimilarity coefficient was employed. A further multivariate method, principal component analysis (PCA) based on covariance matrix, was used for definition of principal components, which contribute most to the variability of the studied set. PCA enabled also two-dimensional visualization of the position of the samples relative to each other. Finally, analysis of variance (ANOVA) was performed by SPSS (v. 13.0) software package for *Windows* (*SPSS Inc.*, Chicago, IL, USA). Values of $p < 0.05$ were considered as statistically significant.

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