Dynamic Headspace−Gas Chromatography−Olfactometry Analysis of Different Anatomical Parts of Lovage (*Levisticum officinale* Koch.) at Eight Growing Stages

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Volatiles of five different parts of lovage (leaves, stems, flowers, seeds, and roots) were isolated by dynamic headspace (DHS) method and analyzed by GC−FID and GC−olfactometry (GC−O) techniques. In total, 98 compounds were identified in the samples, of which 41 are reported as lovage volatiles for the first time. Qualitative differences in the composition of DHS constituents of various anatomical parts of the plants were not significant, whereas the amounts of a number of identified volatile compounds were different in leaves, stems, flowers, seeds, and roots. Seasonal variations in the composition of headspace volatiles were also determined. Except for roots, β-phellandrene was found to be the most abundant headspace component in all anatomical parts of lovage constituting from 36.50% to 79.28% of the total GC peak area. The sniffing panel characterized effluents from the GC column, and odor descriptors were attributed to the recognized constituents. α-Pinene and α-phellandrene/myrcene were the most frequently recognized constituents among 11 GC effluents constituting 12 identified compounds and 1 unknown compound, which were detected by the members of the sniffing panel. None of the detected constituents was recognized as a lovage character impact aroma compound.

Keywords: *Levisticum officinale Koch.; Apiaceae; lovage; volatiles; aroma; dynamic headspace; gas chromatography; olfactometry; growth phase*

INTRODUCTION

Studies on the lovage (*Levisticum officinale* Koch.) volatiles obtained by different isolation procedures, such as distillation, solvent extraction, and dynamic headspace (DHS) techniques, have resulted in the identification of approximately 200 compounds. Most detailed investigations (Toulemonde and Noleu, 1988; Bylaitė et al., 1998; Venskutonis, 1995) were performed on the volatiles collected by solvent extraction and distillation. However, the most important goal in aroma analysis is the identification of volatile compounds released from the product and perceived by the human olfactory system (Guth and Grosch, 1993). This can be achieved by using different kinds of headspace-gas chromatography techniques. Static headspace (SHS) reflects accurately the equilibrium composition of volatile compounds, which are perceived as a wholesome characteristic odor of the product, but its use is limited by the detection levels of GS detectors, in particular for less volatile substances. DHS techniques permit collection of larger amounts of volatile compounds for their qualitative and quantitative analysis by instrumental methods. Nevertheless, many widely used detectors, such as flame ionization (FID), flame photometry (FPD), electron capture (ECD), and mass spectrometry (MS), are not as sensitive for the detection of odorants as the human olfactory system (Acree and Barnard, 1994). For this reason, gas chromatography− olfactometry (GC−O) analysis using sniffing ports for column effluents seems to be an interesting approach to obtain important information about the contribution of individual compounds to the overall flavor of the product. In many cases this technique could be successfully used for the determination of character impact compounds. In general, sniffing of GC effluents, which is the final step in the determination of odor-active compounds, is almost similar in all published reports; however, various modifications in sample preparation and data treatment have been used (Acree et al., 1984, 1994; Blank and Grosch, 1991; Ulrich and Grosch, 1987; Abbott et al., 1993; Guth and Grosch, 1993; Moio et al., 1993, 1994; Etiévant et al., 1983, 1994, 1999; Hinterholzer and Shieberle, 1998; Masanetz and Grosch, 1998a, b). The extract dilution method was very popular in establishing odor-activity values expressed as flavor dilution (FD)-factor and Charm value. In most cases flavor extracts isolated by various methods were analyzed. In agriculture the GC−O method may also contribute to important decisions concerning selection of cultivars, cultivation and

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harvesting techniques, and storage conditions (Dirinck and De Winne, 1994).

DHS composition of lovage volatiles was reported earlier. Nine components were identified in DHS of fresh lovage (De Pooter et al., 1985) constituting mainly mono- and sesquiterpenes, whereas root composition was dominated by ligustilide and β-phellandrene (Cu et al., 1990). Blank and Schieberle (1993) reported odorants of the acidic fraction of a commercial lovage extract. Sotolon was a main aroma compound of the acidic fraction because of its characteristic seasoning-like flavor. Some results on lovage aroma were presented in our previous paper (Bylaite et al., 1996) reporting 25 volatiles in different anatomical parts (leaves, stems, blossoms, and seeds) of the plant. Some aldehydes and terpenes were considered to contribute to lovage flavor.

The present study on DHS−GC−O analysis of volatiles isolated from 5 anatomical parts of a plant at different growth stages is aimed to expand reported data, to provide a better understanding of the formation of lovage aroma during the vegetation period, and to look for the volatile constituents which could contribute to the overall odor of lovage.

MATERIALS AND METHODS

Plant material was harvested in the experimental garden of the Lithuanian Institute of Horticulture from the middle of May until the end of September 1995 at 8 different growth phases: 1, May 15; 2, May 25; 3, June 9; 4, June 16; 5, June 28; 6, July 7; 7, July 19; and 8, September 21. Raw material was air-dried at 30 °C, packed in glass containers, and stored at room temperature in the absence of light for 3−6 months until further analysis. It should be noted that plant material used in this study was similar to the samples used in previously reported data on the analysis of the essential oil (Bylaitė et al., 1998) and on the preliminary investigations of sensory characteristics (Bylaitė et al., 1996). The present study is aimed at expanding published data with new information; on the other hand, the results obtained can be easily compared with those previously reported.

Essential oils were hydrodistilled in a Clevenger apparatus for 2 h. Isolation of volatile compounds by DHS technique was performed by purging with purified nitrogen gas in order to trap the released compounds on a Tenax TA, as described elsewhere (Bylaitė et al., 1996). Volatile compounds were released from Tenax by a thermal desorption (210 °C, 5 min) and cryofocused on a cold trap (−120 °C/240 °C) Carlo Erba TDAS 5000 device. The compounds were analyzed on a Carlo Erba MEGA 5300 gas chromatograph equipped with a FID heated at 275 °C by using a Supelcowax 10 column (Supelco Inc., Bellefonte, PA), 60 m length, 0.25 mm i.d., and 0.25 μm film thickness. The oven temperature was programmed from 40 °C (4 min hold) to 92 °C at a rate of 2 °C min^{-1}, and then to 272 °C at a rate of 6 °C min^{-1} with a final hold of 5 min. At the end of the column the effluents were split 1:2:2 for FID and 2 sniffing ports, respectively, and assessed by a sniffing panel consisting of 10 assessors (Ruth and Roozen, 1994; Linssen et al., 1993). GC−MS analyses were performed on a Varian 3400-Finnigan MAT 95 instrument equipped with a thermal desorption/cold trap device (TCT injector 16200, Chrompack) at 70 eV electron impact ionization mode and scanned from $m/z = 24$ to 300 at 0.5 s $decade^{-1}$. GC conditions for MS were the same as in the GC−FID−O analysis. Selected samples for the identification purposes were also analyzed on a nonpolar column under the conditions described earlier (Bylaitė et al., 1998).

Identification was based mainly on the comparison of retention indices (RI) (Adams, 1995; Davies, 1990) and mass spectra (NIST and the Wageningen Collection of Mass Spectra of Natural Products). Positive identification was considered in case of a match of MS and RI on at least of one of the columns, tentative in case of a very good match of MS or RI.

RESULTS AND DISCUSSION

In total, 98 compounds were identified in different anatomical parts of lovage, and they are listed in Tables 1 and 2 in order of their elution from the Supelcowax 10 column; 41 of them are reported as constituents of lovage aroma for the first time (all compounds in Table 2 were identified positively). These data substantially expand the information on lovage headspace composition presented in our previous work (Bylaitė et al., 1996).

DHS−GC−FID analysis has revealed considerable differences in the compositions of the analyzed anatomical parts of the plant. The quantitatively dominant compound in DHS samples from lovage leaves, stems, flowers, and seeds was the hydrocarbon monoterpene β-phellandrene. The relative content of β-phellandrene was 47−66% in the leaves, 36−47% in the stems, 61% in the flowers, 79% in the seeds, and 15% in the roots. The content of the second major constituent, α-terpinyl acetate was, respectively, in the leaves, stems, and flowers 9.2−16.1%, 13.7−22.4%, and 5.3%. However, this compound was among the minor constituents in the seeds and in the roots constituting 0.6% and 2.0%, respectively. It is interesting to note that the quantitative composition of DHS volatiles from lovage roots was rather different as compared with that of the other parts. Pentylbenzene was the major constituent (23.6%) in the roots, followed by β-pinene (20.21%), β-phellandrene (15.11%), and α-pinene (12.75%).

Changes in the total amounts of DHS volatile compounds and α-phellandrene, and changes in the total amount of hydrodistilled essential oil during the vegetation period from May 15 till September 21 are presented in Figure 1. In general, the changes in the amount of total DHS volatiles and of β-phellandrene in particular are in good agreement with the changes in the essential oil content (Bylaitė et al., 1998). The concentration of DHS volatiles from the seeds, which contain the highest amount of the essential oil, was approximately 3−10 times higher than those of the leaves, stems, and flowers. The amounts of DHS constituents released from lovage roots were very small because of a very low content of essential oil in the roots. At this point it is interesting to compare the composition of DHS with the composition of essential oils obtained by hydrodistillation from the same lovage samples and reported in our previous work (Bylaitė et al. 1998). Thus, α-terpinyl acetate was a major constituent in the essential oils from all anatomical parts of lovage (48−70%) except for flowers and seeds, whereas in DHS samples the amounts of β-phellandrene were several times higher than the amounts of α-terpinyl acetate. The molecular weight of β-phellandrene (FW = 136, $bp_{101.3kPa}$ = 172 °C for its close isomer α-phellandrene) is lower than that of α-terpinyl acetate (FW = 196, $bp_{5.3kPa}$ = 140 °C) and consequently, the volatility of the monoterpene hydrocarbon is considerably higher than the volatility of the acetic acid ester of α-terpineol (Bauer, 1990).

It should be emphasized that neither phthalides, constituting a significant part of the lovage essential oil (Bylaitė et al., 1998), nor sotolon, which was identified as the most important odorant in the acidic fraction of lovage (Blank and Shieberle, 1993), were detected in DHS samples of different anatomical parts of the plant

Table 1. Volatile Compounds Identified[a] in DHS−GC of Different Anatomical Parts of Lovage

no.	compound[b]	identification[d]	leaves	stems	seeds	flowers	roots	earlier reported as lovage volatile[c]
1	2-methyl prop-2-enal	c	+			+		−
2	2-methyl butanal	c	+	+		+	+	+
3	salvene (Z)	ac		+				−
4	tricyclene	abc	+	+		+		+
5	hexanal	ac	+	+	+	+	+	+
6	*iso*-limonene	ac		+				−
7	undec-1-ene	c		+			+	+
8	Δ-3-carene	abc			+	+		+
9	butan-1-ol	c	+				+	+
10	heptanal	ac					+	+
11	pentan-1-ol	ac					+	+
12	3-methylbutyl 2-methylbutanoate	c		+				−
13	2-methylbutyl 2-methylbutanoate	c	+	+				+
14	octan-2-one	ac		+			+	−
15	octanal	ac	+	+	+	+	+	+
16	3-methylbutyl 3-methylbutanoate	c	+	+		+		−
17	2-ethyl hex-2-enal	c	+			+		−
18	nonan-4-one	c		+				−
19	6-methyl hept-5-en-2-one	ac		+	+	+		−
20	hexan-1-ol	ac			+	+	+	+
21	nonan-2-one	c					+	−
22	nonanal	abc		+	+	+	+	+
23	α-thujone	abc		+				−
24	*p*-mentha-1,3,8-triene	ac			+	+		−
25	*β*-thujone	abc		+				−
26	α-cubebene	abc			+	+	+	+
27	*trans*-sabinene hydrate	ac		+		+		−
28	fenchyl acetate	ac	+	+				+
29	α-copaene	abc	+		+	+	+	+
30	decanal	ac	+	+	+	+	+	+
31	hexylbenzene	c					+	−
32	phellandral	ac	+	+		+	+	+
33	undecan-6-one	c		+				−
34	propanoic acid	ac	+					−
35	non-2-enal (E)	ac					+	−
36	*β*-cubebene	abc			+	+		+
37	2-methyl 6-methyleneocta-1,7-dien-3-one	c	+	+	+	+		−
38	pinocarvone	c		+				−
39	thymol methyl ether	ac		+				−
40	*β*-elemene	abc					+	+
41	terpinen-4-ol	abc		+				+
42	*β*-caryophyllene	abc			+	+		+
43	butanoic acid	ac	+	+		+	+	−
44	α-terpineol	abc				+		+
45	*p*-2-butyl anisole	c					+	−
46	dec-2-enal (E)	c					+	+
47	borneol propanoate	ac		+				+
48	*β*-farnesene	abc			+			+
49	estragole	ac		+		+		−
50	humulene	abc		+				−
51	germacrene D	abc			+	+		+
52	pentanoic acid	ac	+	+				−
53	cuminaldehyde	ac	+	+	+	+		+
54	*β*-selinene	abc					+	+
55	decan-1-ol	c	+					−
56	α-phellandrene epoxide	c			+			−
57	*p*-mentha-1,3-dien-7-al	c			+			−
58	*δ*-cadinene	abc					+	+
59	7-*epi*-α-selinene	ac					+	−
60	methyl salicylate	ac				+		+
61	hexanoic acid	ac	+	+	+	+	+	−
62	dibutyl phthalate	ac	+				+	−
63	octanoic acid	ac	+			+		−
64	nonanoic acid	ac				+		−
65	carvacrol	abc	+					−
66	4-pentylphenol	c	+		+			−
67	5-hydroxy-*p*-menth-6-en-2-one	c	+					−
68	benzoic acid	ac				+		−

[a] Other identified compounds are listed in Table 2. [b] Compounds are listed in order of their elution from Supelcowax 10 column. [c] All compounds noted as identified earlier in lovage (in Tables 1 and 2) were reported by Toulemonde and Noleau (1988), except for acetic acid (Blank and Shieberle, 1993), bornyl propaonate, and methyl salycilate (Bylaitė et al., 1998). [d] a, RI match with RI provided in the literature on nonpolar column. b, RI match with RI provided in the literature on polar column. c, MS match with reference spectra.

Table 2. Concentration (GC peak area units, $n = 6$) of Compounds[a] in DHS of Lovage at Various Growth Phases (1−8)

	leaves								stems								flowers	seeds	roots
compound	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	6	7	8
butanal	0.34	0.47	0.58	0.31	0.24	0.44	0.33	0.38	0.60	0.62	0.75	0.63	1.10	0.49	1.53	1.14	1.15	1.20	1.77
3-methyl butanal	0.71	0.96	0.99	0.70	1.06	0.75	0.85	3.96	0.33	0.62	0.33	0.35	0.44	0.52	0.37	1.28	0.21	1.24	0.68
pentanal	0.31	0.31	0.33	0.32	0.35	0.37	0.34	0.33	0.28	0.30	0.34	0.40	0.26	0.28	0.18	0.11	0.45	nd	tr
α-pinene	10.93	12.45	13.25	11.69	4.02	5.46	4.82	7.50	3.52	7.23	9.02	13.03	1.10	1.49	1.53	1.14	4.68	46.80	5.50
α-thujene	1.11	1.39	1.69	1.26	0.84	1.29	1.23	1.30	0.48	0.64	0.87	1.43	0.80	0.76	0.84	0.48	0.78	6.35	nd
toluene	0.65	0.79	0.86	0.81	0.48	0.60	0.57	0.68	0.47	0.55	0.74	0.94	0.80	0.64	0.73	0.50	1.55	2.26	tr
camphene	3.13	3.40	3.24	3.22	1.01	1.32	0.97	1.84	0.70	2.74	2.62	4.10	1.27	2.24	1.87	1.41	1.40	9.12	1.20
β-pinene	2.74	2.92	2.75	2.58	1.26	1.52	1.38	2.26	3.04	4.16	3.60	2.43	2.21	2.05	2.19	2.44	1.81	9.61	8.72
sabinene	16.11	17.19	18	15.78	8.14	11.46	11.90	11.76	4.78	7.00	6.68	7.93	2.23	4.76	4.98	3.64	4.70	15.77	0.12
α-phellandrene	12.10	14.74	15.31	15.43	6.33	10.10	10.65	6.73	1.31	1.55	1.88	2.08	2.22	2.8	2.4	2.50	9.45	38.63	nd
myrcene	26.24	32.26	33.1	36.16	33.66	36.58	32.45	31.17	9.85	10.12	12.61	16.25	13.40	12.25	14.44	19.75	36.17	32.46	0.36
α-terpinene	2.91	3.07	3.25	3.12	1.71	2.45	2.03	1.99	0.37	1.24	1.37	1.55	0.71	1.35	1.15	0.96	1.70	21.24	nd
limonene	5.11	4.18	7.82	9.83	9.81	12.09	13.31	10.56	20.45	30.16	37.02	25.10	26.17	27.44	24.3	26.11	18.94	22.94	1.12
β-phellandrene	319.35	415.55	394.27	333.69	188.25	205.96	205.45	229.27	94.40	106	108	167	88.78	95.79	85.83	78.89	272.71	1775.24	6.52
β-ocimene (Z)	3.53	4.02	5.19	5.95	7.91	8.19	7.71	8.68	0.81	0.93	2.23	1.82	1.46	1.31	1.55	1.31	6.77	32.90	nd
γ-terpinene	2.67	5.64	7.22	7.69	6.18	7.86	12.59	10.37	0.65	0.78	0.93	0.91	1.00	0.99	0.96	0.75	7.04	15.15	tr
β-ocimene (E)	0.51	0.60	1.11	0.54	0.69	0.58	0.63	0.81	0.49	0.36	0.40	0.36	0.49	0.39	0.34	0.30	1.24	12.05	nd
p-cymene	10.72	12.63	14.92	12.79	10.89	18.71	18.1	18.24	5.30	5.41	5.33	6.34	4.62	5.89	5.36	5.52	16.55	51.34	0.49
terpinolene	2.47	3.51	2.18	5.95	3.57	2.68	3.19	3.40	2.99	3.18	4.32	2.66	2.59	2.58	2.52	2.60	0.95	3.65	tr
pentylcyclohexadiene	3.14	4.52	4.75	6.24	6.37	4.67	5.71	2.49	1.25	1.40	1.22	2.06	1.53	1.67	0.68	1.00	2.06	10.15	1.94
allo-ocimene	2.64	2.75	3.4	4.45	5.08	4.82	5.34	5.17	1.31	1.40	1.82	1.23	1.55	0.69	0.84	0.81	3.96	9.51	nd
undeca-1,3,5-triene	0.45	0.43	0.83	0.41	0.63	0.59	0.59	0.58	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
pentylbenzene	2.11	3.10	2.45	3.52	2.45	2.16	1.59	1.26	1.44	1.72	1.53	2.04	1.75	2.36	1.17	1.29	1.30	7.06	10.20
p-cymenene	1.09	0.84	0.87	1.03	0.54	0.65	0.54	0.72	1.11	1.12	1.23	1.25	1.15	1.16	0.94	0.83	nd	2.47	tr
acetic acid	1.61	2.33	3.35	3.13	2.74	1.84	2.53	2.32	2.35	2.07	2.65	1.65	1.52	1.74	1.79	1.73	0.42	1.84	tr
linalool acetate	0.42	0.32	0.33	0.65	0.27	0.48	0.34	0.45	0.33	0.36	0.42	0.47	0.33	0.30	0.56	0.41	0.74	3.30	nd
bornyl acetate	1.08	1.46	1.10	1.30	0.80	1.23	1.11	0.80	2.21	2.51	2.06	2.35	1.64	2.02	1.87	2.08	0.70	2.98	nd
acetophenone	0.50	0.51	0.82	0.74	0.41	0.51	0.46	0.36	0.59	0.58	0.72	1.07	0.48	0.61	0.64	0.59	0.61	0.64	0.42
α-terpinyl acetate	47.15	58.07	74.83	70.91	63.97	65.33	61.07	63.86	40.51	59.68	61.52	48.13	42.6	34.89	42.19	48.45	23.7	12.41	0.86
2,6-dimethyl octa-1,5,7-trien-3-ol	0.37	0.36	0.39	0.40	0.41	0.43	0.32	0.57	0.57	0.58	0.65	0.46	0.47	0.52	0.56	0.63	0.53	nd	nd
total	507.84	630.96	648.35	606.05	397.92	432.75	425.1	449.12	209.75	273.72	288.5	351.68	208.75	224	217.55	216.09	446.91	2239.08	43.14
average coefficient of variance (%)	26	25	23	34	35	35	27	33	23	26	23	22	28	30	26	25	29	30	34
dry matter in used plant material, %	10.5	11.4	12.1	10.6	12.3	8.9	8.6	8.7	9.6	12.8	10.5	9.7	10.5	9.6	10.2	7.8	3.9	6.7	8.0

[a] All compounds were reported by Toulemonde and Noleau (1988) except undeca-1,3,5-triene, acetophenone, and 2,6-dimethyl octa-1,5,7-trien-3-ol; tr, traces; nd, not detected.

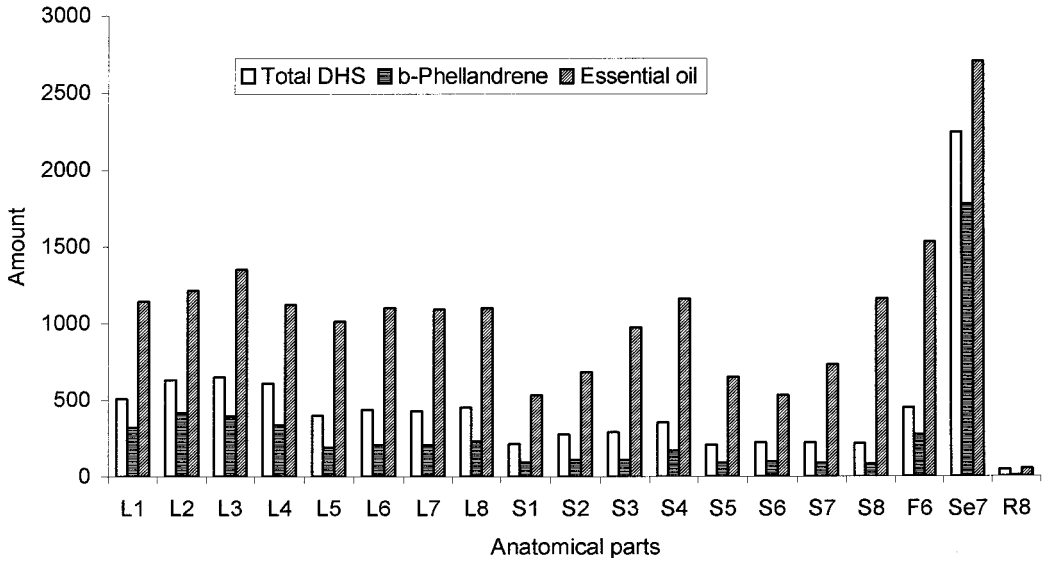

Figure 1. Amount of total DHS volatile compounds, β-phellandrene, and total essential oil in lovage leaves (L), stems (S), flowers (F), seeds (Se) and roots (R) at different growing stages (1−8). Amounts of total DHS and β-phellandrene are reported in arbitrary units (GC peak area; a.u.); amount of essential oil is reported in mg 100 g^{-1} w/w of dry weight.

Table 3. Retention Indices for the Main Lovage Phthalides

compound	DB-5 (Adams, 1995)	Supelcowax 10
(*Z*)-3-butylidene phthalide	1668	2558
(*E*)-3-butylidene phthalide	1711	2672
(*Z*)-ligustilide	1730	2621
(*E*)-ligustilide	1790	not detected

in our study. First of all it was assumed that the headspace concentration of the main phthalide ((*Z*)-ligustilide) was below FID detection level. Even selected ion traces for characteristic masses (*m/e* 148, 161, and 190) did not reveal any trace of (*Z*)-ligustilide. Also, a sample of the essential oil injected on Tenax and analyzed in the same way as the DHS samples failed to show ligustilide under analytical TDAS−GC−FID−O conditions. This prompted us to determine the retention index for phthalides on the Supelcowax column, because the retention indexes for phthalides in the literature were available only for a nonpolar DB5 column (Adams, 1995). The results obtained (Table 3) showed that the RI's for phthalides on a polar column are extremely high and strongly temperature-program-dependent as compared with the RI's obtained on a nonpolar column. It is obvious from these results that phthalides will not elute from the Supelcowax column under conditions applied to TDAS−GC−FID−O.

Eleven peaks and/or their combinations were detected among the effluents during GC−FID−O analysis of lovage DHS (Table 4). Retention times of the compounds β-pinene/sabinene and α-phellandrene/myrcene were very close and the panelists perceived these compounds as a one-odor effluent. In general, it could be expected that with the increase of a compound concentration (i.e., its peak area on the chromatogram) the possibility of its detection by the sniffing panel also increases. For instance, β-pinene/sabinene was detected by 3 and 4 panelists in the leaves 1 and 2, respectively, at the concentration of 18.85 GC peak area units (a.u.) and 20.75 a.u. respectively. This fraction was not detected by the panel in the leaves 5 (9.40 a.u.) and 7 (13.28 a.u.), in the stems 1 (7.84 a.u.), 4 (10.36 a.u.) and 8 (7.44 a.u.), and in the flowers (6.51 a u.). This peak was detected again by 4 panelists in the DHS samples from the seeds where the concentration was highest (25.38 a.u.). Four members of the sniffing panel also detected this fraction in the roots, where the sum of β-pinene and sabinene was only 8.84 a.u. However, in the latter case β-pinene was dominating (8.72 a.u.) compared with sabinene (0.12 a.u.), but in all other lovage anatomical parts the amount of sabinene (4.70−18.00 a.u.) was higher than that of β-pinene (1.26−9.61 a.u.). Unfortunately, very little data exist on the odor threshold values of terpenes determined in air (Table 5).

It was already said that phthalides were not detected in DHS by GC−FID−O because of a very high retention time of these compounds on the Supelcowax column and also because of the very low concentrations in the DHS samples. Therefore, sensory evaluation of separately eluting phthalides and consequently their effect on the lovage aroma remains an open question. The existing presumption that phthalides, particularly ligustilide, are the important aroma constituents of lovage roots (α-terpinyl acetate is considered as one of the most important aroma constituents in the leaves) (Bauer, 1990; Gijbels et al., 1982; Segebrecht and Schilcher, 1989; Toulemonde et al., 1987), should be tested by using a nonpolar column. However, it should be noted that the success of such tests is also doubtful because phthalides were not detected in DHS on a Supelcowax column even by using favorable conditions for their elution on GC/MS. Extract dilution sniffing analysis can be suggested for further investigations to determine odor threshold values for the main phthalides, however, the composition of plant extracts and essential oils is usually very different from the composition of their HS volatiles (Venskutonis, 1997). The reliability of GC/O results depends on various factors, such as the composition of aroma, abilities of panelists, and odor assessment procedure (temperature, elution frequency, etc.). For instance, some points of uncertainty were observed in the treatment of data from extract dilution sniffing analysis (Abbott et al., 1993).

Odor active constituents were characterized by using odor descriptors, which are partly provided in Table 5 with the available odor threshold values. The specific flavor of lovage roots is described in different sources by very general and abstract characteristics, such as

Table 4. DHS Constituents of Lovage Harvested at Different Growth Phases (1−8) Which Were Detected by Sniffing Panel[a]

		leaves				stems			flowers	seeds	roots
RI	constituent	1	3	5	7	1	4	8	6	7	8
827	unknown	**4** (bdt)	**5** (bdt)	**3** (bdt)	**4** (bdt)	**0** (bdt)	**4** (bdt)	**3** (bdt)	**6** (bdt)	**3** (bdt)	**0** (bdt)
878	butanal	**3** (0.34)	**3** (0.58)	**0** (0.24)	**4** (0.33)	**4** (0.60)	**3** (0.63)	**3** (1.14)	**6** (1.15)	**5** (1.20)	**0** (1.77)
880	2-methyl prop-2-enal	**0** (bdt)	**3** (bdt)	**0** (bdt)	**4** (bdt)	**0** (bdt)	**0** (bdt)	**0** (bdt)	**6** (bdt)	**0** (bdt)	**0** (bdt)
923	2/3-methyl butanal	**0** (0.71)	**3** (0.99)	**0** (1.06)	**3** (0.85)	**3** (0.33)	**5** (0.35)	**0** (1.28)	**5** (0.21)	**0** (1.24)	**3** (0.68)
985	pentanal	**0** (0.31)	**4** (0.33)	**0** (0.35)	**3** (0.34)	**3** (0.28)	**3** (0.40)	**5** (0.11)	**6** (0.45)	**0** (bdt)	**3** (tr)
1002	α-pinene	**3** (10.93)	**5** (13.25)	**3** (4.02)	**4** (4.82)	**3** (3.52)	**5** (13.03)	**5** (1.14)	**4** (4.68)	**6** (46.80)	**3** (5.50)
1102	*β*-pinene	**3** (2.74)	**4** (2.75)	**0** (1.26)	**0** (1.38)	**0** (3.04)	**0** (2.43)	**0** (2.44)	**0** (1.81)	**4** (9.61)	**4** (8.72)
1117	sabinene	(16.11)	(18.00)	(8.14)	(11.90)	(4.78)	(7.93)	(4.98)	(4.70)	(15.77)	(0.12)
1166	α-phellandrene	**7** (12.10)	**8** (15.31)	**4** (6.33)	**6** (10.65)	**5** (1.31)	**5** (2.08)	**4** (2.50)	**7** (9.45)	**6** (38.63)	**0** (bdt)
1172	myrcene	(26.24)	(33.10)	(33.66)	(36.58)	(9.85)	(16.25)	(19.75)	(36.17)	(32.46)	(0.36)
1228	*β*-phellandrene	**0** (319.3)	**3** (394.3)	**0** (188.3)	**4** (205.5)	**0** (94.40)	**4** (167.0)	**0** (78.89)	**4** (272.7)	**5** (1775)	**0** (6.52)
1247	(Z)-*β*-ocimene	**0** (3.53)	**4** (5.19)	**0** (7.91)	**3** (7.71)	**0** (0.81)	**0** (1.82)	**0** (1.31)	**3** (6.77)	**4** (32.9)	**0** (0.86)
1690	α-terpinyl acetate	**0** (47.15)	**3** (74.83)	**0** (63.97)	**0** (61.07)	**0** (40.51)	**0** (48.13)	**0** (48.45)	**3** (23.7)	**0** (12.4)	**0** (0.86)

[a] Number of panelists simultaneously recognizing GC effluent is shown in bold; amount of constituent in DHS, in GC peak area units, shown in brackets; bdt, below GC−FID detection threshold; tr, traces.

Table 5. Odor Threshold Values and Odor Descriptors of the Detected Lovage DHS Compounds by GC−O

constituent	odor threshold values	descriptors attributed by panelists	reference descriptors
butanal	0.01−0.03 mg m^{-3} (van Gemert and Nettenbreijer, 1977); 0.0022 mg dm^{-3}, 0.046 ppm, 0.0092 ppm, 9 ppb: air; 9 ppb, 0.07 ppm: water (Fazzalari, 1974)	chocolate, chemical	fruity, meaty, ethereal (Aldrich, 1993)
2-methyl prop-2-enal	not found	grassy, spicy	
2-methylbutanal	0.004 mg kg^{-1}: water (Guth and Grosch, 1994)	chocolate, spicy, chemical	malty (Masanetz and Grosch, 1998)
3-methylbutanal	0.0019 mg kg^{-1}: water (Rychlik and Grosch, 1996)		malty (Masanetz and Grosch, 1998)
pentanal	0.07 mg m^{-3} (van Gemert and Nettenbreijer, 1977); 12 ppb (Fazzalari, 1974)	caramel, butter, sour	woody, vanilla, fruity, nutty on dilution (Aldrich, 1993)
α-pinene	0.02 mg m^{-3} (van Gemert and Nettenbreijer, 1977); 6 ppb: water; 6 ppb, 140 ppb: air (Fazzalari, 1974)	pine, grassy, floral	sharp, pine (Aldrich, 1993)
β-pinene	140 ppb: water (Fazzalari, 1974)	pine, chemical, spicy	woody, pine (Aldrich, 1993)
sabinene	75 ppb (Fazzalari, 1974)		woody, terpy, citrus, pine-like with a spice nuance (natural) (Mosciano et al., 1993)
α-phellandrene	13 ppb: water, air (Fazzalari, 1974)	pine, grassy, chemical	minty, herbaceous, (Aldrich, 1993); citrus, terpenic, slightly green, black-pepper-like (from Givaudan) (Mosciano et al., 1991)
myrcene	0.01 mg kg^{-1}: water (Masanetz and Grosch, 1998)		sweet, balsamic, plastic (Aldrich, 1993); metallic, herbaceous (Masanetz and Grosch, 1998)
β-phellandrene	0.036 mg kg^{-1}: water (Masanetz and Grosch, 1998)	grassy, chemical	Terpene-like (Masanetz and Grosch, 1998)
(Z)-*β*-ocimene	not found	mushrooms, musty, chemical	tropical, green, terpy and woody with vegetable nuances (natural) (Mosciano et al., 1990)
α-terpinyl acetate	not found	floral sweet	herbal, citrus, spicy, woody, floral, waxy and clean (natural) (Mosciano, 1997)

medium aromatic (Heath and Reineccius, 1986) or by attributing several descriptors, e. g., strong impact warmly aromatic with sweet, yeasty, musky, lemon-like, celery-like notes (Heath, 1981). The list of 17 different descriptors was prepared for the effluents of lovage DHS during four preliminary panel sessions. Data provided in Table 5 also demonstrate that there is a significant diversity in the reference descriptions of the odor of the same compound. Sometimes odor descriptors in various sources are very controversial. For instance, the odor of butanal was characterized with such strikingly different descriptors as fruity, meaty, and ethereal (Aldrich, 1993), and the odor of α-phellandrene was described with descriptors such as minty, herbaceous, (Aldrich, 1993), citrus, terpenic, slightly green, and black-pepper-like (Mosciano et al., 1991). An interesting observation could be made concerning (*Z*)-*β*-ocimene, which among others was attributed the descriptor "mushroom-like". Mushroom-like odor has also been attributed to the different anatomical parts of lovage (Baranauskienė, 1995). Odor threshold values also can vary in a wide range depending on testing media, conditions, and other factors. All these aspects make accurate assessment of such complex flavors as lovage rather difficult.

CONCLUSIONS

GC/MS analysis of a great number of DHS samples from different anatomical parts of lovage has led to the

identification of 41 constituents not previously reported in this plant. The dominant constituent was β-phellandrene in most of the DHS samples, however, its impact on the lovage aroma does not seem to be the most significant. In general, DHS−GC−O analysis of all anatomical parts of lovage did not reveal character impact aroma compounds which could be unambiguously used for the characterization of lovage aroma.

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LITERATURE CITED

Abbott, N.; Etiévant, P. X.; Issanchou, S.; Langlois, D. Critical evaluation of two commonly used techniques for the treatment of data from extract dilution sniffing analysis. *J. Agric. Food Chem*. **1993**, *41*, 1698−1703.

Acree, T. E.; Barnard, J. Gas chromatography-olfactometry and Charm Analysis. In *Trends in Flavour Research*; Maarse, H., Van der Heij, D. G., Eds.; Elsevier Science Publishers B. V.: Amsterdam, The Netherlands, 1994; pp 211−220.

Acree, T. E.; Barnard, J.; Cunningham, D. G. A procedure for the sensory analysis of gas chromatographic effluents. *Food Chem*. **1984**, *14*, 273−286.

Adams, R. P. *Identification of Essential Oil Components by GC/MS*. Allured Publishing Corp.: Carol Stream, IL, 1995.

Aldrich. *Flavors & Fragrances, International Edition*; Aldrich Chemical Co.: Milwaukee, WI, 1993.

Baranauskienė, M. *Spicy Plants (Lithuan.)* Ûkininko patarėjas, Kaunas, Lithuania, 1995.

Bauer, K.; Garbe, D.; Surburg, H. *Common Fragrance and Flavour Materials. Preparation, Properties and Uses*. VCH Verlagsgesellschaft GmbH: Weinheim, Germany, 1990.

Belitz, H.-D.; Grosch, W. *Food Chemistry*. Springer-Verlag: Berlin, Heidelberg, Germany, 1987.

Blank, I.; Grosch, W. Evaluation of potent odorants in dill seed and dill herb (*Anethum graveolens* L.) by aroma extract dilution analysis. *J. Food Sci*. **1991**, *56*, 63−67.

Blank, I.; Schieberle, P. Analysis of the seasoning-like flavour substances of a commercial lovage extract (*Levisticum officinale* Koch.). *Flavour Fragrance J*. **1993**, *8*, 191−195.

Bylaitė, E.; Legger, A.; Roozen, J. P.; Venskutonis, P. R. Dynamic headspace gas chromatography of different botanical parts of lovage (*Levisticum officinale* Koch.) In *Developments in Flavour Science;* Taylor, A. J., Mottram, D. S., Eds.; The Royal Society of Chemistry: UK, 1996; pp 66−69.

Bylaitė, E.; Venskutonis, P. R.; Roozen, J. P. Influence of harvesting time on the composition of volatile components in different anatomical parts of lovage (*Levisticum officinale* Koch.*). J. Agric. Food Chem*. **1998**, *46*, 3735−3740.

Compilation of Odor and Taste Threshold Values Data. Fazzalari, F. A., Ed.; ASTM Data Series DS 48A; American Society for Testing and Materials: Philadelphia, PA.

Cu, J.-Q.; Pu, F.; Shi, Y.; Perineu, F.; Delmas, M.; Gaset, A. The chemical composition of lovage headspace and essential oil produced by solvent extraction with various solvents. *J. Essent. Oil Res*. **1990**, *2*, 53−59.

Davies, N. W. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. *J. Chromatogr*. **1990**, *503*, 1−24.

De Pooter, H. L.; Coolsaet, B. A.; Dirinck, P. J.; Schamp, N. M. GLC of the headspace after concentration on Tenax GC and of the essential oils of apples, fresh celery, fresh lovage, honeysuckle and ginger powder. In *Essential Oils and Aromatic Plants;* Baerheim-Svendsen, A., Scheffer, J. J. C., Eds.; Nijhoff/Junk: Dordrecht, The Netherlands, 1985; pp 67−77.

Dirinck, P.; De Winne, A. Advantages of instrumental procedures for measurement of flavours characters. In *Trends in Flavour Research*; Maarse, H., Van der Heij, D. G., Eds.; Elsevier Science Publishers B. V.: Amsterdam, The Netherlands, 1994; pp 249−258.

Etiévant, P. X.; Issanchou, S. N.; Bayonove, C. L. The flavour of Muscat wine: the sensory contribution of some volatile compounds. *J. Sci. Food Agric*. **1983**, *34*, 497−504.

Etiévant, P. X.; Callement, G.; Langlois D.; Issanchou, S.; Coquibus, N. Odor intensity evaluation in gas chromatography-olfactometry by finger span method, *J. Agric. Food Chem*. **1999**, *47*, 1673−1680.

van Gemert, L. J.; Nettenbreijer, A. H. *Compilation of Odour Threshold Values in Air and Water*. CIVO-TNO: Zeist, The Netherlands, 1977.

Gijbels, M. J. M.; Scheffer, J. J. C.; Baerheim-Svendsen, B. Phthalides in the essential oil from roots of *Levisticum officinale. Planta Medica* **1982**, *44*, 207−211.

Guth, H.; Grosch W. Identification of potent odourants in static headspace samples of green and black tea powders on the basis of aroma extract dilution analysis (AEDA). *Flavour Fragrance J*., **1993**, *8*, 173−178.

Heath, H. B. *Source Book of Flavors*. AVI Publishing Co., Inc.: New York, 1981.

Heath, H. B.; Reineccius, G. *Flavor Chemistry and Technology*. Macmillan Publishers: Riverside, NJ, 1986.

Hinterholzer, A.; Schieberle, P. Identification of the most odour-active volatiles in fresh, hand-extracted juice of Valencia late oranges by odour dilution techniques. *Flavour Fragrance J*. **1998**, *13*, 49−55.

Linssen, J. P. H.; Janssens, J. L. G. M.; Roozen, J. P.; Posthumus, M. A. Combined gas chromatography and sniffing port analysis of volatile compounds of mineral water packed in laminated packages. *Food Chem*. **1993**, *46*, 367−371.

Masanetz, C.; Grosch, W. Key odorants of parsley leaves (*Petroselinum crispum* [Mill.] Nym. spp. *crispum*) by odour-activity values. *Flavour Fragrance J*. **1998a**, *13*, 115−124.

Masanetz, C.; Grosch, W. Hay-like off-flavour of dry parsley. *Z. Lebensm.-Unters.-Forsch. A* **1998b**, *206*, 114−120.

Moio, L.; Langlois, D.; Etiévant, P. X.; Addeo, F. Powerful odorants in water buffalo and bovine Mozzarella cheese by use of extract dilution sniffing analysis. *Italian J. Food Sci*. **1993**, *5*, 227−237.

Moio, L.; Etiévant, P. X.; Langlois D.; Dekimpe, J.; Addeo, F. Detection of powerful odorants in heated milk by use of extract dilution sniffing analysis. *J. Dairy Res*. **1994**, *61*, 385−394.

Mosciano, G. Organoleptic characteristics of flavor materials, *Perfum. Flavor*. **1997**, *22*, 75−78.

Mosciano, G.; Fasano, M.; Michalski, J.; Sadural S. Organoleptic characteristics of flavor materials. *Perfum. Flavor*. **1990**, *15*, 69−73.

Mosciano, G.; Fasano, M.; Michalski, J.; Sadural S. Organoleptic characteristics of flavor materials. *Perfum. Flavor*. **1991**, *16*, 49−55.

Mosciano G.; Fasano M.; Cassidy J.; Connelly C.; Mazeiko P.; Montenegro A.; Michalski, J.; Sadural S. Organoleptic characteristics of flavor materials. *Perfum. Flavor*. **1993**, *18*, 43−45.

Ruth van, S. M.; Roozen, J. P. Gas-chromatography/sniffing port analysis and sensory evaluation of commercially dried bell peppers (*Capsicum annuum*) after rehydration. *Food Chem*. **1994**, *51*, 165−170.

Segebrecht, S.; Schilcher, H. Ligustilide: guiding component for preparation of *Levisticum officinale* roots. *Planta Medica* **1989**, *55*, 572−573.

Toulemonde, B.; Noleau, I. Volatile constituents of lovage (*Levisticum officinale* Koch.) In *Flavors and Fragrances: a World Perspective;* Lawrence, B. M., Mookherjee, B. D., Willis, B. J., Eds; Elsevier Science Publishers B. V.: Amsterdam, The Netherlands, 1988; pp 641−657.

Toulemonde, B.; Paul, F.; Noleau, I. Phthalides from lovage (*Levisticum officinale* Koch.) In *Flavour Science and Technology;* Martens, M., Dalett, A., Russwurm, H., Eds.; John Wiley & Sons: New York, 1987; pp 89−94.

Ulrich, F.; Grosch, W. Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. *Z. Lebensm.-Unters.-Forsch.* **1987**, *184*, 277−282.

Venskutonis, P. R. Essential oil composition of some herbs cultivated in Lithuania. In *Flavours, Fragrances and Essential Oils;* vol. 2, Baser, K. H. C., Ed.; AREP: Istanbul, 1995; pp 108−123.

Venskutonis, P. R. Effect of drying on the volatile constituents of thyme (*Thymus vulgaris* L.) and sage (*Salvia officinalis* L.). *Food Chem.* **1997**, *59*, 219−227.

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