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Chemical composition and stability of the hydrosols obtained during essential oil production. I. The case of *Melissa officinalis* L. and *Asarum canadense* L.

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Abstract
The chemical composition of the oils and hydrosols obtained from either wild or cultivated plantations was determined by GC-FID and GC/MS analyses. The components obtained from the hydrosols are mainly monoterpenic alcohols, aldehydes and ketones and as such the composition of the hydrosols are very different of the corresponding oils: the oil with the most important fraction of hydrocarbon compounds, the composition of the two phases is the most different. It appears that the composition of hydrosols undergoes important change over a two-year period of storage at normal temperature. In both cases, a complex equilibrium involving nerol, geraniol, linalool, linalool oxides and several diols is not achieved. The same kind of behaviour involving several menthadienols is working with citral. Also, it is observed that phenylpropanoid molecules partly disappear during the same time.

Keywords: *Melissa officinalis*, *Asarum canadense*, hydrosol, shelf-life, essential oil, nerol, geraniol, linalool, citral, methyleugenol, degradation.

1. Introduction
Although hydrosols are produced in the same extraction process than essential oils, their analyses were the subject of a limited number of publications [1, 2]. In a previous paper, we compared the composition of the essential oils and of the fresh hydrosols obtained from three different species of the *Pinaceae* family of conifers [3]. The aim of this work is to explore the possibility of commercialization of a quantitatively important sub-product of local oil production: the hydrosols of *Melissa officinalis* L. and *Asarum canadense* L. At least two questions must be addressed. First, what is their composition and second is their stability reliable over a long period of shelf-life?

2. Materials and methods
2.1 Plant material
Roots of *A. canadense* (Canadian snakeroot), Aristolochiaceae, were collected from spontaneous plants growing wild in the Grondines region and *M. officinalis* (Lemon balm), Lamiaceae, was cultivated.

2.2 Oil and hydrosol isolation
All the samples were produced in a small plant located in Grondines, on the North side of the St-Lawrence River, between Quebec City and Three-Rivers. Typical batch involves 300 kg of fresh material and produces 50 kg of hydrosol. A 4-liter bottle of each hydrosol was kept at room temperature during the time of experimentation. 100 mL hydrosols are submitted three times to extraction using 24 mL of chloroform HPLC grade solvent. These three fractions are gathered and concentrated by partial evaporation until 2 mL and kept over dry $MgSO_4$. Liquid samples are stored in a room at -5 °C before the first GC or GC/MS analysis and then kept at room temperature for shelf stability studies.

2.3 Oil analysis
Fresh essential oils are analyzed by gas chromatography on a HP 5890, equipped with a

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flame ionisation detector (GC-FID) and two capillary columns: a Supelcowax 10 and a DB-5 column (30 m × 0.25 mm × 0.25 µm). Samples are also analyzed by gas chromatography, HP 5890, coupled with an HP 5972 mass spectrometer at 70 eV (GC/MS) and equipped either with a DB-5 or a Supelcowax column (same as above). Injection port and detector temperature are 220 and 260 °C, respectively. The temperature program for both GC-FID and GC/MS is 40 °C for 2 min, then 2 °C/min until 210 °C and held under constant temperature for 33 min. Identification of the components is done by comparison of their retention indices (RI) with standards, by comparison of their mass spectra with literature data [4-6] and with our own data bases. An internal standard, 400 µL (tetradecane: chloroform solution – 0.4:100), was added to the hydrosol before each GC analysis. Quantitative data are obtained electronically from GC-FID area percentages. The FID response factors for compounds relative to tetradecane are taken as one.

2.4 Hydrosol Analysis

They are submitted to GC and GC/MS analyses using the same procedure as that used for essential oils. All the samples were tested for aerobic and facultative anaerobic heterotrophic bacteria to estimate the density of the bacterial population. The measured values are essentially 0, far below the admitted value for drinking water: <5 CFU/100 mL. Detection of coliforms or atypical bacteria is essentially negative in each case.

3. Results and discussion
3.1 *Melissa officinalis*

Oil of balm is well known and has been described in literature for a long time. For example, in the Guenther's book, the main compounds of this oil are: geraniol (12.38%), linalool (14.21%), citronellol (8.23%), citronellal (3.90%) and citral (0.96%) [7]. There is variation in the composition according to the geographical origin of the plant [8, 9]. The composition of the sample analyzed in this work appears in Tables 1 and 2. Almost half of this oil is characterized by aldehydes: citral is the main compound (43%) followed by citronellal (4.5%). Geraniol (8.5%), nerol (4.2%), and citronellol (3.5%) are the main alcoholic compounds. Several minor compounds must be added to the compounds contained in Tables 1 and 2. They are: *cis-* and *trans-*β-ocimene (0.1 and 1.1%, respectively), 3-(methylthio)propanal (<0.1%), camphor (0.1%) and borneol (0.6%).

The fresh hydrosol contains 240 mg/L (± 6%). This figure is in line with reported values: quantities of VOCs included in hydrosols lie between 0.3 and 1.2 g/L [2, 3]. pH values of each sample are 4.3 ± 0.1. About 90% of these VOCs appear in Table 2. The analyzed VOC quantities show some variations over the observation period. Several other compounds at concentration lower than 0.5 mg/L were also observed. They are (RI on Swax-10 column): 1-penten-3-ol (1167), 2,3-dehydro-1,8-cineole (1189), 1,8-cineole (1193), isopentanol (1211), 3-hydroxy-2-butanone (1293), *trans*-3-hexen-1-ol (1371), 3-methylthiopropanal (1458), isopulegol (1564), α-cyclogeraniol (1676), (*E*)-3-hexenoic acid (1962), (*E*)-2-hexenoic acid(1973), octanoic acid (2072), and citronellic acid (2255). As it can be seen none of the hydrocarbon compounds set out in Table 1 is observed in the hydrosol. Several new oxygenated compounds are found as well as several unidentified compounds. There is a short description in literature of the lemon balm hydrosol. It corresponds roughly to the present description: citral (43%) is the main component along with *cis-* and *trans-p-*menth-1-en-3-acetoxy-8-ol (15.1 and 12.9%, respectively) [2]. After a period of two years, one can observe the decrease of the concentration of nerol and geraniol, about 85%. During the same period of storage, the percentage of linalool increases from *ca.* 3% to 12%. This is illustrated in Fig. 3A. Isomerization of alcohol in aqueous medium from primary to tertiary position in the case of allylic alcohol is a well-known process especially in the presence of catalytic additives [10]. α-Terpineol is also a product of the isomerization of nerol and geraniol [11, 12]. There is a small increase in the *p*-cymen-8-ol concentration and a strong increase in the concentration of *cis-* and *trans-p-*menth-2-ene-1,8-diol (Fig. 3B). For example, in aqueous oxalic acid solutions, geraniol rearranges and forms a variety of compounds such as α-terpineol (26.2%), citronellol (16.3%) [11], etc.

Moreover, linalool can be transformed in other compounds. Thus, it was established that during the hop steam distillation, (during a very short time and at a temperature close to that of boiling water) linalool is transformed into isomeric furan and pyran linalool oxides, 2,6-dimethyl-7-octene-2,6-diol, and hodiendiol (2,6-dimethyl-3,7-octadiene-2,6-diol) [12]. The linalool oxides (pyr.) are measured in low quantities. 2,6-Dimethyl-7-octene-2,6-diol is positively identified. However, hodiendiol was not identified in the present study. Finally, it is impossible to say if the reverse isomerization process from linalool to nerol and geraniol occurs as it was observed in the presence of various metal catalysts [13].

The total of linalool, linalool oxides, α-terpineol, 3,6-dimethyl-7-octene-2,6-diol, nerol and geraniol shows a decrease of 30% during the two years of shelf conservation (Fig. 3A). Although some unknown processes can be active, this observation is consistent with the expected behaviour of nerol, geraniol and linalool as discussed above.

Citral (neral and geranial) is an important compound in the fresh *M. officinalis* hydrosol: 12 and 13.5%, respectively. In the hydrosol, these concentrations practically vanish over a 24 month period of storage (Fig. 3B). As far as the fate of citral is concerned, several studies reported in literature were made in various conditions. For example, in aqueous citric acid solution *p*-mentha-1,5-dien-8-ol and *p*-mentha-l (7),2-dien-8-ol as well as small amounts of 2,3-dehydro-1,8-cineole were isolated from citral as major components [14]. It is known that these products degrade over time in aqueous solutions due to an acid-catalyzed reaction which leads to loss of desirable flavor notes and formation of off-flavor notes [15]. At 40°C and at pH 3.0, citral disappears in less than 7 days. During that time concentrations of *p*-mentha-1,5-dien-8-ol, *p*-mentha-1(7),2-dien-8-ol and *p*-menth-2-ene-1,8-diol pass through a maximum around the third day. 8-Hydroxy-*p*-menthene does the same around the 7th day. The concentrations of *p*-cymen-8-ol, *p*-cymene, *p*-methylacetophenone and *p*-cresol as well as minor quantities of α,*p*-dimethylstyrene and 4-(2-hydroxy-2-propyl)benzaldehyde are still increasing after 14 days [15]. In the essential oil itself, over a 4-month period at normal temperature, the concentration of citral decreases from 45.9 to 8.6%. Even at temperature as low as -20 °C, a significant reduction, from *ca.* 46 to 41%, in citral concentration was

observed over the same period of time showing the need of very cold conditions to keep this oil with its genuine quality[16].

As mentioned above, it was shown that *p*-mentha-1, 5-dien-8-ol and *p*-mentha-l(7),2-dien-8-ol are also unstable. On incubation at 38 °C, they disappear almost after 68 hours. In agreement with that, the various menthadienols mentioned in these papers are barely observed in this study. Another well-documented study shed some light on the overall degradation process of citral [17]. In a sample containing 4 ppm of citral, maintained at 25 °C and at a pH value of 3.15, the first step is described as a cyclisation one followed by hydration leading to the formation of *cis*- and *trans*-*p*-menth-1-en-3,8-diol. Then, these last compounds form several equilibria leading to the formation of *p*-mentha-1,5-dien-8-ol, *p*-mentha-1(7),2-dien-8-ol, *cis*- and *trans*-isopiperitenol, *cis*- and *trans*-*p*-menth-2-ene-1,8-diol, etc. These two last compounds are by far the main

compounds observed after 28 days: *ca.* 2.5 ppm. These processes are still active after 69 days: the concentrations of 2, 3-dehydro-1,8-cineole (71 ppb), α,*p*-dimethylstyrene (16 ppb), and *p*-cymen-8-ol (277 ppb) are still increasing. Due to the low quantities formed in the over-all process and its low solubility in hydrosol α,*p*-dimethylstyrene is not observed in the present study. The presence of relatively rare compounds, isopiperitenols [18] mentioned above and *p*-mentha-1, 8-dien-5-ols [19] is noteworthy.

Autoxidation of pure geranial at room temperature was published [20]. Geranic acid, 6,7-epoxygeranial and a dioxolane hydroperoxide were observed. This latter compound, not analyzed in this study, was identified as mainly responsible for the skin sensitization potency of air-exposed geranial. A small increase of geranic acid is observed (Table 2).
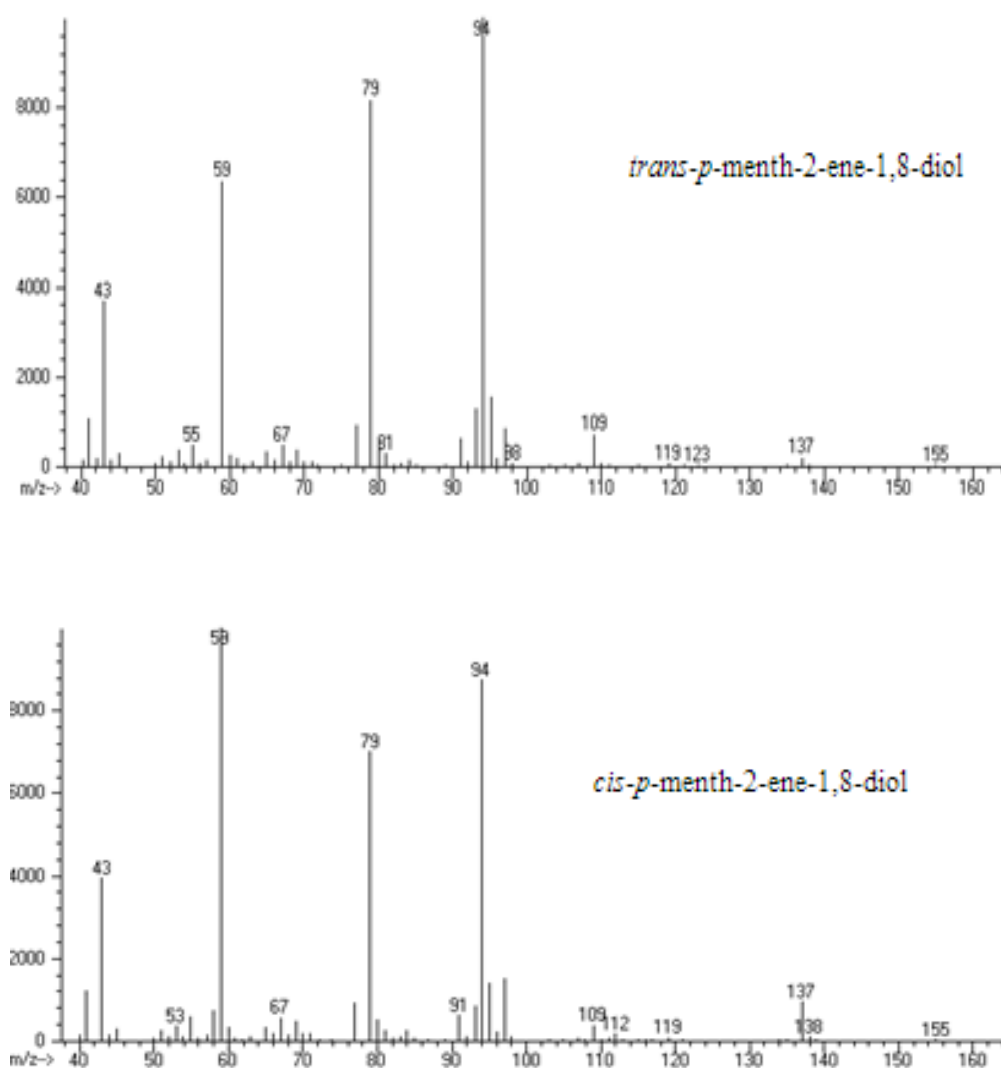
Fig 1: Identification of *cis*- and *trans*-*p*-menth-2-ene-1,8-diol in the hydrosol of *M. officinalis* (see text).
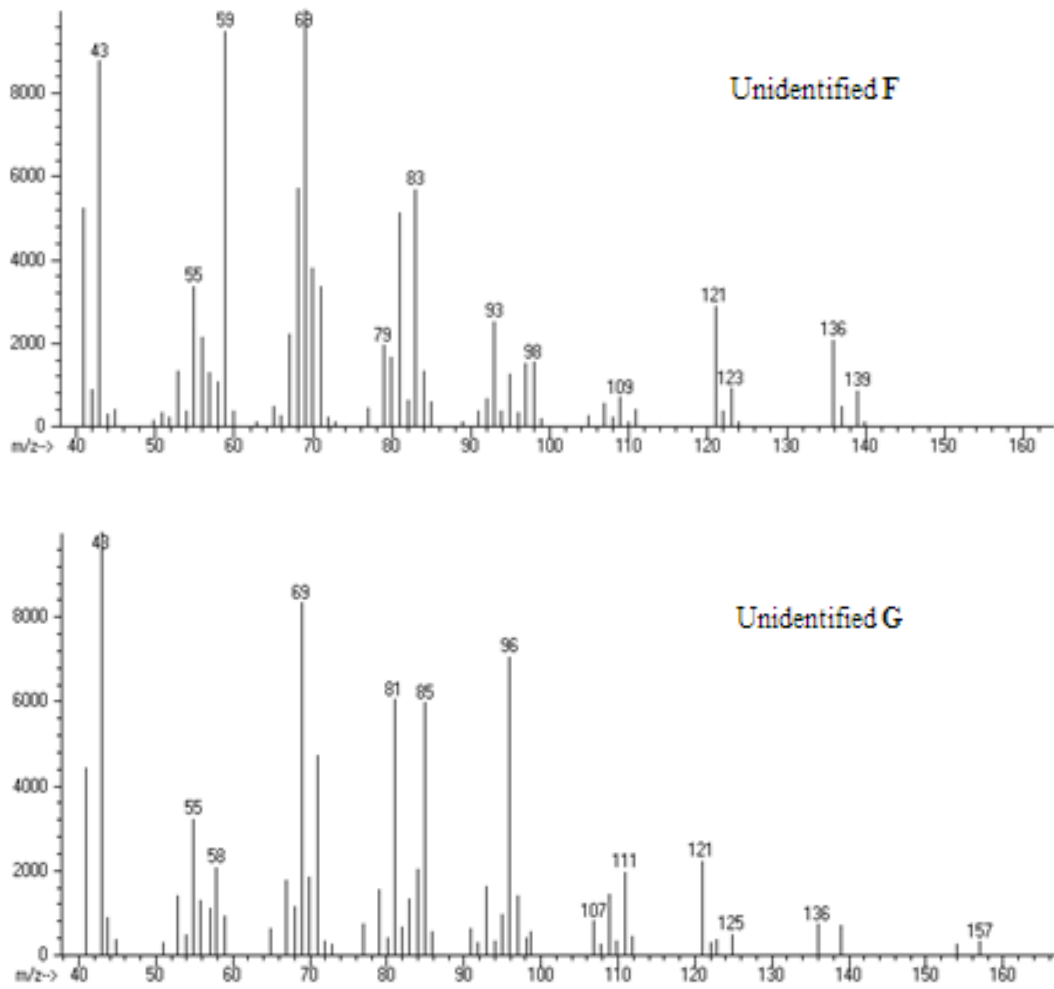
Fig 2: Identification of unidentified compounds F and G in the hydrosol of *M. officinalis*.
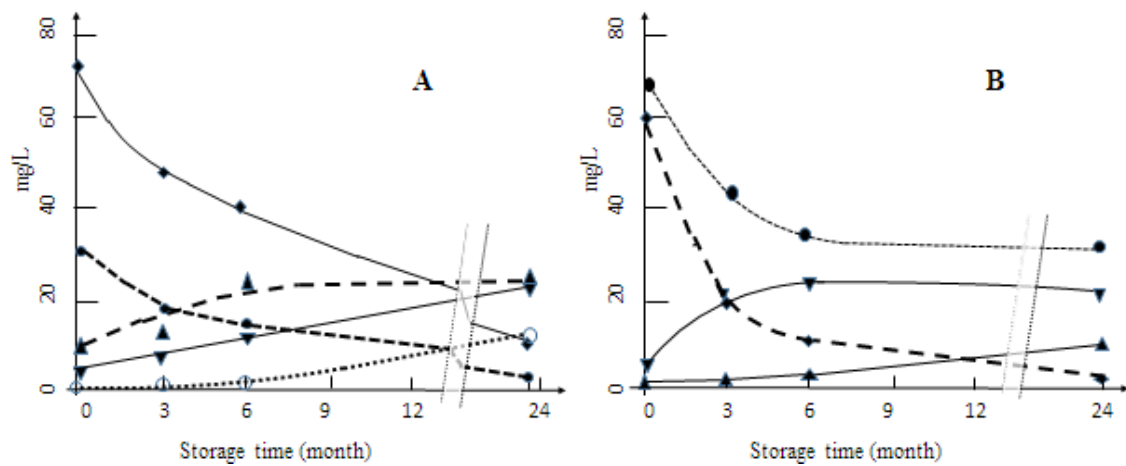
Fig 3: Effect of shelf-life on the composition of the *M. officinalis* hydrosol.

A: ♦: geraniol; ●: nerol; ▲: α-terpineol; ▼: linalool; ○:2,6-dimethyl-7-octene-2,6-diol;

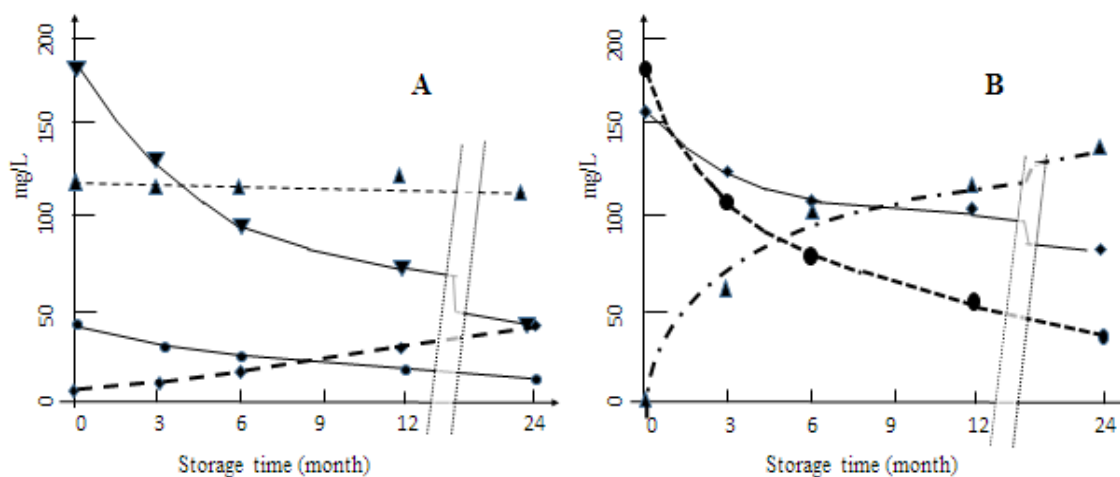B: ▲:*p*-cymen-8-ol; ▼:*cis-* + *trans-p*-menth-2-ene-1,8-diol; ♦: citral; ●: total of three preceeding compounds + geranic acid.

Fig 4: Effect of the shelf-life on the composition of the *A. canadense* hydrosol.

A: ▼: linalool; ●: geraniol + nerol; ◆: 2,6-dimethyl-7-octene-2,6-diol; ▲: α-terpineol;
B: ●: methyleugenol; ◆: [elemicin + (E)-isoelemicin] × 10; ▲: the decrease of the measured VOCs× ½

Table 1: Composition (%) of the sesquiterpene fraction of the *M. officinalis* essential oil

Identification	RI[1]	RI[2]	%
α-copaene	1376	1488	0.6
β-bourbonene	1383	1514	0.1
β-caryophyllene	1416	1583	12.3
α-humulene	1454	1656	0.9
trans-β-farnesene	1463	1671	0.3
germacrene D	1483	1698	3.8
bicyclogermacrene	1498	1723	0.2
germacrene A + ?	1503	1752	0.3
α-muurolene + ?	1505	1718	0.2
unidentified	1516		0.3
γ-cadinene	1517	1749	0.2
δ-cadinene	1527	1749	1.0
caryophyllene oxide	1579	1958	1.3
unidentified	1628		0.4
τ-muurolol	1640	2171	0.2
τ-cadinol	1640	2157	0.2
α-cadinol	1654	2213	0.4
Other compounds, see text			2.0
Total from Table 2 (%)			67.7
Total (%)			92.2

[1]: retention index on a DB-5 column; [2]: retention index on a Swax-10 column.

Table 2: Composition (mg/L) of the hydrosol and partial composition (%) of the essential oil of *M. officinalis*

Identification	RI[1]	RI[2]	Shelf-life (month)				% in oil
			0	3	6	24	
trans-hex-2-en-1-al	858	1221	n.m.[3]	1.6	1.6	1.5	-
cis-2-penten-1-ol	773	1333	n.m.	-	0.7	0.8	-
6-methyl-5-hepten-2-one	989	1347	1.5	9.0	10.4	8.6	0.8
hexanol	878	1362	1.0	1.8	2.1	2.0	-
trans-3-hexen-1-ol	859	1371	-	0.4	0.5	0.5	-
cis-3-hexen-1-ol	863	1388	6.4	33	36	38	0.2
trans-2-hexen-1-ol	875	1408	1.6	3.8	4.3	4.6	-
trans-linalool oxide (fur.)	1083	1445	1.5	2.3	2.7	3.6	-
1-octen-3-ol	983	1462	1.5	2.3	2.7	2.8	0.4

furfural	834	1471	n.m.	1.2	1.3	1.4	-
cis-linalool oxide (fur.)	1100	1476	0.9	1.3	2.2	2.3	-
citronellal	1161	1486	-	-	-	-	4.5
linalool	1113	1558	7.2	15.5	24	26	0.8
terpinen-4-ol	1177	1599	n.m.	0.3	0.4	0.4	-
trans-p-mentha-2,8-dien-1-ol	1130	1625	0.8	1.0	1.0	0.4	-
benzene acetaldehyde	1047	1636	2.0	1.2	1.7	1.1	-
neral	1246	1662	28	-	-	-	17.5
cis-p-mentha-2,8-dien-1-ol	1144	1671	0.9	0.9	1.0	0.3	-
trans-p-mentha-1,8-dien-5-ol[5]	1177	1687	1.8	1.6	2.2	2.0	-
α-terpineol	1190	1699	3.3	6.4	11.8	23	-
unidentified **A**	1106	1706	1.0	1.0	1.1	1.2	-
geranial	1280	1714	32.5	12.4	6.4	-	24.3
p-mentha-1,5-dien-8-ol	1171	1729	1.7	2.4	5.1	3.8	-
cis-p-mentha-1,8-dien-5-ol [5]	1202	1733	1.5	1.7	2.1	1.2	-
isopiperitenol B (trans ?) [6, 7]	1217	1728	0.4	0.4	0.2	0.2	-
isopiperitenol A (*cis* ?) [6, 7]	1197	1735	0.6	0.6	0.8	0.1	-
trans-linalool oxide (pyr.)	1177	1740	1.8	1.6	0.4	0.5	-
geranyl acetate	1386	1746	-	-	-	-	2.0
p-methylacetophenone	1182	1764		-	0.2	0.5	-
cis-linalool oxide (pyr.)	1175	1765		-	0.2	0.4	-
citronellol	1234	1770	4.6	3.4	3.9	1.8	3.5
β-phellandren-8-ol		1778		3.3	6.6	4.0	-
trans-p-mentha-1(7),8-dien-2-ol	1188	1791		0.3	0.3	0.2	-
nerol	1234	1798	33	19	17	2.4	4.2
(*Z*)-iso-geraniol	1247	1808	0.6	0.4	0.5	0.3	-
(*E*)-iso-geraniol	1236	1813	0.6	0.4	0.5	0.4	-
p-cymen-8-ol	1185	1849	1.0	0.8	1.1	8.1	0.9
geraniol	1266	1851	73	44	41.0	9.4	8.5
benzyl alcohol	1038	1873	1.1	1.0	1.1	1.1	-
cis-p-mentha-1(7),8-dien-2-ol	1234	1906		0.5	0.7	0.7	-
phenylethyl alcohol	1122	1921	0.7	0.6	0.7	0.8	0.1
unidentified **B** (double peak)		1938	t[4]	0.9	1.1	1.3	-
2,6-dimethyl-7-octene-2,6-diol	1237	1983	t	0.5	1.6	12.4	-
(*E*)-cinnamaldehyde	1272	2025	n.m.	0.4	0.6	0.6	-
8-hydroxyneomenthol	1338	2093	0.7	0.3	1.7	2.4	-
p-menthane-1,8-diol	1190	2093	-	-	0.1	1.6	-
trans-p-menth-2-ene-1,8-diol [6, 8]	1290	2118	6.3	12.0	13.8	11.1	-
8-hydroxyneomenthol (isomer)		2169		0.2	0.5	1.0	-
unidentified **C**	1359	2156	2.6	1.7	3.7	2.1	-
unidentified **D**	1362	2176	1.7	1.2	1.3	1.0	-
cis-p-menth-2-ene-1,8-diol [6, 8]	1310	2186	5.6	9.0	9.1	7.8	-
hydroxycitronellol	1366	2203	-	0.2	1.4	1.8	-
unidentified **E**	1380	2266	0.7	0.6	1.1	2.2	-
neric acid	1332	2300	-	0.5	0.6	0.6	-
unidentified **F**	1390	2310	0.0	0.6	2.2	4.2	-
unidentified **G**	1417	2310	0.0	0.2	0.8	1.4	-
geranic acid	1366	2345	-	1.5	3.0	2.8	-
Other compounds: see text			0.2	2.8	6.0	3.2	-
Total of above compounds (mg/L)			**230**	**211**	**248**	**217**	**67.7%**
Total of GC compounds (mg/L)			**240**	**223**	**266**	**230**	**-**

[1]: retention index on the DB-5 column; [2]: retention index on a Swax-10 column; [3]: not measured; [4]: traces, < 0.05%; [5]: partial MS [19]; [6]: RI values are in agreement with those measured on OV-101 column;[7]: MS are available [18]; [8]: Although the MS of *cis-p*-menth-2-ene-1,8-diol is quite similar to the one given in the SciFinder database [22], there are differences between our spectrum and that appearing in [22]: see Fig. 1; Mass spectrum of unidentified compounds, *m/z*(intensity):

A. 43(100), 55(98), 81(80), 41(58), 95(50), 74(48) … 152(2);

B. 59(100), 43(64), 84(50), 81(42), 41(24), 127(24) … 171? Two peaks with similar MS;

C. 94(100), 79(95), 43(68), 59(48), 69(38), 41(36) … 152(4).

D. 109(100), 79(64), 59(50), 94(32), 41(20) …

E. 9(100), 69(96), 109(90), 43(66), 94(60), 41(44) …

F. and **G.:** see Fig. 2.

Table 3: Composition of the hydrocarbon monoterpene fraction of the essential oil of *A. canadense*

Identification	RI[1]	RI[2]	%
α-pinene	940	1021	1.3
camphene	953	1066	0.4
sabinene	976	1124	0.6
β-pinene	977	1108	1.3
myrcene	992	1169	1.3
α-phellandrene	1001	1165	0.2
p-cymene	1026	1277	0.3
limonene	1032	1195	0.5
β-phellandrene	1032	1202	0.1
cis-β-ocimene	1046	1243	0.5
trans-β-ocimene	1058	1261	0.9
γ-terpinene	1067	1249	0.2
terpinolene	1098	1288	0.4
Total			8.0

[1]: retention index on DB-5; [2]: retention index on Swax-10.

Table 4: Composition (mg/L) of the hydrosol and partial composition (%) of the essential oil of *Asarum canadense*

Storage time (month)			0	3	6	12	24	%
Total analyzed amount of VOCs (mg/L)			590	463	389	372	306	in
Identification	RI[1]	RI[2]						oil
1,8-cineole	1035	1199	2.1	1.8	0.5	1.4	1.1	0.3
trans-linalool oxide (fur.)	1084	1442	3.7	3.8	3.0	4.0	4.0	-
cis-linalool oxide (fur.)	1100	1473	2.8	3.0	3.6	3.1	3.0	--
linalool	1117	1558	184	133	95	77	41	19.4
3,7-dimethyl-1,5,7-octatrien-3-ol	1119	1581	1.3	1.1	0.8	0.5	0.4	-
sabina ketone	1162	1611	0.8	0.8	0.8	0.7	0.8	--
trans-linalol oxide (pyr,)	1172	1763	0.5	0.5	0.6	0.6	0.6	--
terpin-1-en-4-ol	1179	1594	8.4	7.2	6.1	6.2	5.1	0.5
p-cymen-8-ol	1186	1848	0.6	0.4	0.5	0.6	1.6	-
α-terpineol	1193	1699	117	114	111	123	108	5.9
hodiendiol *	1193	1951		2.0	2.8	2.8	2.5	-
nerol	1233	1796	10.8	6.8	5.8	3.5	1.7	1.2
2,6-dimethyl-7-octene-2,6-diol	1235	1980	1.8	8.6	16	31	39	-
geraniol	1265	1850	34	23	19	14	7.0	3.6
cuminol	1298	1688	0.6	0.6	0.6	0.7	0.1	-
terpin hydrate/*p*-menthane-1,8-diol	1311	2090	0.7	3.1	6.1	14.4	21.6	-
p-mentha-1,4-dien-7-ol	1326	2044	0.8	0.4	0.4	0.5	0.5	-
terpin hydrate isomer	1329	2167		0.4	0.6	1.5	2.2	--
unidentified acetate	1366	1738	0.8	0.6	0.6	0.6	0.3	
neryl acetate	1370	1730	0.4	0.4	0.8	2.0	2.8	1.4
geranyl acetate	1386	1759	0.6		0.1			-
unidentified **A**	1394	2309	0.2	1.9	3.2	6.4	7.5	-
methyl eugenol	1409	2011	180	104	78	53	30	38.5
3,4-dimethoxybenzaldehyde	1484	2377	1.2	1.2	1.3	1.3	1.4	-
1-hydroxylinalol?	1503	2093	0.7	0.4	0.1	-	-	-
elemicin	1562	2222	9.7	8.1	7.4	7.2	6.1	1.1
methoxyelemicin	1600	2437	0.9	0.4	0.4	0.2	0.2	0.6
5-epi-7-α-eudesmol + ?	1612	2033	0.6	0.6	0.5	0.2		2.4
hydroxyelemicin	1614		0.8	0.2	0.2	0.2		-
γ-eudesmol ?	1648	2437	1.1	0.3	0.3	0.2	-	0.9
(*E*)-isoelemicine	1655	2390	6.0	4.2	3.7	3.3	2.4	1.3
(*E*)-methoxyisoelemicin	1696	2359	0.7	0.4	0.4	0.3	0.3	-
unidentified **B**	1773	2552	1.0	1.3	1.5	1.7	1.7	-
unidentified **C**	1804	2687	1.6	0.8	0.8	1.1	1.1	-
Total			575	435	373	360	295	76.7

[1]: retention index on the DB-5 column; [2]: retention index on the Swax-10 column; *: = 2,6-dimethyl-3,7-octadiene-2,6-diol; Mass spectrum of unidentified compounds, *m/z*(intensity):

A- 59(100), 69(94), 43(94), 81(68), 41(63), 83(52), 68(50) …;
B- 126(100), 112(68), 43(60), 59(58), 55(45), 109(43) …207(24) … 240(4);
C- 149(100), 59(90), 43(80), 109(44), 71(40), 81(36), 164(32)… 225(2).

Citronellal, an important compound in the *M. officinalis* oil, is not observed in the hydrosol as it has already been reported [2]. Either this compound is completely insoluble or it disappears quickly from the acidic hydrosol. To our knowledge, there is no known experimental value for the citronellal solubility in water. Calculated values indicate that this solubility is about half of that of geraniol [21, 22]. It can be assumed that the fraction of citronellal included in hydrosol disappears very quickly. The mechanism for this degradation in acidic water seems unknown. In the gas phase, citronellal reaction with OH radical led to the formation of 3-methylhexanedial and 2-oxopropanal (methylglyoxal) [23]. These compounds are not identified in this study.

Finally, during the shelf storage, there is a small increase in the percentage of 6-methyl-5-hepten-2-one. It was suggested that, under specific conditions, the presence of oxygen and moisture at higher temperature, methylheptenone could be a product of citral in coriander oil [24]. Thus, the increase in the concentration of this molecule could partly offset the decrease of citral.

The fate of these VOCs in the gas phase was studied. As a general rule, they react quickly with the OH atmospheric radical, with similar rate constants: $2.0 \pm 0.5 \times 10^{-12}$ cm^3 $mol.^{-1}$ s^{-1} [23, 25-27]. Obviously, in this hydrosol, the disappearance of citronellal is much faster than that for other VOCs and, as such, includes other processes. With regard to citral, its stability decreases with the decrease of pH [28]. This is an indication of the participation of H^+ ions in the degradation/oxidation processes for citronellal and several VOCs. The reference to the behaviour of VOCs in atmospheric gas-phase seems irrelevant to what is happening in hydrosol.

3.2 *Asarum canadense*

The composition of the essential oil obtained from the rhizomes and roots of *A. canadense* was published [29-31]. It contains several of the compounds listed in Tables 3 and 4 and about two dozens of sesquiterpenes in low concentration (0.1 to 3.0%): they are not observed in this study. Methyl eugenol was the main products in the rhizomes essential oil (*ca.* 50%). (*E*)-isoelemicin was the main product extracted from the roots (*ca.* 20%). The compositions of essential oil and hydrosol of the roots of *A. canadense* appear in Tables 3 and 4. The oil contains 8% of hydrocarbon monoterpenes and several oxygenated products also observed in the hydrosol. Several derivatives of elemicin are also positively identified.

The fresh hydrosol contains 590 mg/L of VOCs of which 97.5% appear in Table 4. pH values are fairly constant: 3.9 ± 0.1 lower than 5.4, a value reported in literature [32]. Minor compounds (<0.5 mg/L) are not included in Table 4. They are (RI on DB-5): (*Z*)-3-hexenol (859), bois de rose oxide (975), *trans*-pinocarveol (1145), (*Z*)-isoelemicin (1576), and oplopanone (1736). Contrary to what is observed in the case of *M. officinalis*, there is an important decrease in the quantities of VOCs with the shelf storage time. Formation of dimeric or polymeric compounds is not excluded, products here not analyzed.

Again, due to their low solubility in water [21], the hydrocarbon monoterpene molecules are absent in the hydrosol. As in *M. officinalis* hydrosol, the same behaviour of nerol, geraniol, and linalool is observed while the total of involved compounds is not as stable as it is in the case of *M. officinalis*: it decreases by

approximately 40% over a two-year period (Table 4).

In the fresh hydrosol of *M. officinalis*, geraniol and nerol were the main alcoholic compounds. As a result of what was reported above, the concentration of linalool increases with storage time. On the contrary, in the *A. canadense* hydrosol linalool is the major compound: its concentration decreases strongly with shelves conservation time. The low concentration of nerol + geraniol is not able to maintain the linalool concentration (Fig. 4A and 4B). However, it can be deduced that the isomerization of nerol and geraniol to linalool is a reversible process. Now, 2,6-dimethyl-7-octene-2,6-diol, a product expected from the degradation of linalool is now well established. Finally, the α-terpineol concentration seems less sensitive to the nerol + geraniol degradation than that of linalool.

Among the other compounds, one can observe a decrease of methyl eugenol (*ca.* 85%), elemicin (35%) and of its derivatives: (*E*)-isoelemicin (60%), hydroxyelemicin (75%) and (*E*)-methoxyisoelemicin (50%). At least two studies mentioned the formation of dimers of phenylpropanoids from eugenol and methyl eugenol in the presence of H_3PO_4 [33] or palladium chloride in acetic acid [34]. Thus, and although this may be fortuitous, the disappearance of these compounds could explain about 57% of the observed decay of the total analyzed VOCs (Fig. 4B). To our knowledge, the reaction between phenylpropanoid molecules and other VOCs is not documented; it could be part of the explanation for the decrease of the total VOCs.

4. Conclusion

The composition of hydrosols obtained in the vapor distillation of *Melissa officinalis* and *Asarum canadense* are time dependent. Their composition changes significantly over a two year period of shelf-life at room temperature. Nerol, geraniol, linalool, α-terpineol and other by-products form a complex mixture whose composition is far from that observed in fresh hydrosols. The same observation can be done for citral. As it is known since a long time, the stability of citral is also temperature dependant. Several menthadienols are involved in its degradation. Finally, as it is observed in the hydrosol of *A. canadense*, the phenylpropanoid compounds tend to disappear during the same period of conservation.

5. Acknowledgment

6. References

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