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Essential Oil Composition of Sachalinmint from Norway Detected by Solid-Phase Microextraction and Gas Chromatography−Mass Spectrometry Analysis

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The essential oil of leaves and flowers of sachalinmint [*Mentha sachalinensis* (Briq.) Kudô] grown in Norway (Trondheim) has been studied by headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography−mass spectrometry analysis (GC-MS). The essential oil content increased linearly in acropetal direction from 1.08% (0−20 cm plant height) to 1.75% (60−80 cm; young leaves and flowers). The steam-distilled samples showed a minor complex matrix with a very high menthol and a much lower menthone content (87.89 and 4.05%, respectively). From testing of HS-SPME unequilibrated exposure times ranging from 10 s to 5 min, an extraction time of 30 s was found to be sufficient to detect both low- and high-eluting compounds. Comparison of HS-SPME and steam-distilled samples established that the same tendencies of increasing menthol/menthone content in the basipetal/acropetal direction could be detected by both analysis methods. With regard to the extraction efficiency, HS-SPME gave additional detailed information about less important terpenic compounds.

KEYWORDS: ***Mentha sachalinensis* (Briq.) Kudô; essential oil; leaves; flowers; headspace solid-phase microextraction; HS-SPME; GC-MS analysis**

INTRODUCTION

In the past 10 years there has been an increasing interest in growing medicinal plants and herbs in Norway. A research project, the Norwegian Herb Production (NUP), focusing on promoting, processing, marketing, and distribution, was carried out in the period 1995−1998. Of special interest were comparative analyses of essential oil content and quality related to developmental stages in aromatic plants such as chamomile, lemon balm, garden thyme, oregano, and yarrow (*1*). With regard to extensive demand and commercial interests, mint herbs such as peppermint and sachalinmint have been studied in detail in the framework of the project (*2*, *3*).

The present study has focused on the determination of the essential oil of leaves and flowers of sachalinmint [*Mentha sachalinensis* (Briq.) Kudô]. The plant originates from East Asia, and the essential oil of both sachalinmint and its hybrids has been exhaustively investigated by Eastern European research groups (*4*−*6*); other secondary metabolites such as flavonoids have also been studied (*7*). Recent reports from both Russia (*8*) and Finland (*9*) underscore the importance of this species in the *Mentha* genus with an exceptionally high menthol content (*3*−*5*). Simultaneously, the low content of the undesirable menthone makes this essential oil attractive as a potential source for menthol production. As already pointed out by different authors, the menthol/menthone ratio in *Mentha* × *piperita* is related to leaf maturation (*2*, *10*, *11*) with distinctly higher menthone concentrations in flowers of *M.* × *piperita* (*2*) and *M. arvensis* L. (*12*).

The headspace solid-phase microextraction technique (HS-SPME), as introduced by Zhang and Pawliszyn (*13*) and tested on mint herbs (*2*, *14*, *15*) and mint oil-based formulations (*16*−*19*), provides reliable results in the screening of the essential oil composition from other plant sources (*1*, *21*).

Comparative studies between HS-SPME and steam-distilled samples, coupled with gas chromatography−mass spectrometry analysis (GC-MS), have been carried out in order to determine if the increasing menthol/menthone ratio in the basipetal direction could likewise be detected by HS-SPME. The second goal of this study has been to determine the extraction and detection efficiency of the HS-SPME method by varying the SPME fiber exposure time with regard to the occurrence of characteristic terpenic compounds.

MATERIALS AND METHODS

Plant Material. The plant material (variety Mentolcsepp) was a gift from Bertalan Galambosi at the Agricultural Research Centre of Finland (MTT) and originated from the Research Institute for Medicinal Plants in Budakalasz (Hungary).

Plants were harvested from three trial plots at The Plant Biocentre in Trondheim (Norway) in late September 1998. Ten blooming plants (stems) with an overall plant height of ∼80 cm were taken from each

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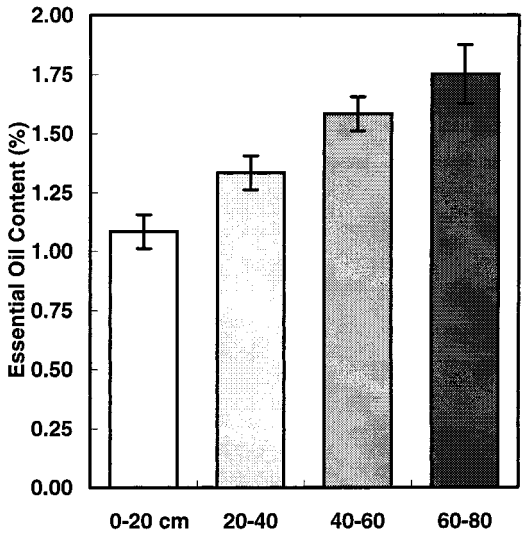

Figure 1. Distribution of the content of essential oil from steam distillation ($n = 3$) within sachalinmint plants with regard to stem positions of leaves (and flowers): 0–20, 20–40, 40–60, and 60–80 cm (vertical bars, ± SD), $p = 0.0000774$.

plot. The samples were dried at 35 °C in a drying cabinet with a fan (Termaks TS 5410) for 48 h and stored at room temperature prior to analysis in March 2000.

Steam Distillation. The samples were divided into four groups with regard to plant height (three repetitions each) in order to examine the essential oil content and composition at different locations from the plant roots: group A, 0−20 cm; group B, 20−40 cm; group C, 40−60 cm; group D, 60−80 cm. Leaves and flowers (only group D) were separated from the stems, pooled, and crushed before distillation. The distillation apparatus consisted of a heating cap, a 1 L distillation flask, a 3 mL graduated receiver (Dean & Stark), and a condenser (jacketed coil). Exactly 20 g of dried plant material and 600 mL of water were used, and each distillation was carried out for 1 h after the mixture had reached the boiling point.

GC Analysis. The gas chromatography samples were prepared by diluting 10 μL of essential oil in 1 mL of ethanol in brown autosampler flasks for analysis. The essential oil constituents were analyzed by using a Varian Star 3400 CX gas chromatograph coupled with a Saturn 3 mass spectrometer (Varian Inc.).

Steam-Distilled Sample GC-MS Conditions. A Chrompack CP Wax 52CB, 30 m × 0.32 mm i.d., 0.25 μm film thickness, capillary column was used: temperature, 60−210 °C at 2 °C/min with a 5 min hold at 210 °C; carrier gas, helium (5 psi); injector temperature, 220 °C; splitless; transfer line temperature, 210 °C; detector temperature, 175 °C.

SPME-GC-MS Samples. A PDMS coated fiber (100 μm) and a manual SPME holder (Supelco Inc.) were used for sample extraction. In a blank run, the fiber was inserted into the GC inlet for 3 min for thermal desorption at 250 °C before headspace sampling. One gram of each sample was sealed in a 10 mL screw-top vial with a phenolic cap and PTFE/silicone septa (Supelco Inc.) and stored in a drying cabinet at 45 °C for 10 min. Initializing sample tests were carried out to determine the extraction capacity of the SPME fiber by varying the extraction time: 10, 20, and 30 s and 1, 2, and 5 min ($n = 5$). Finally, an exposure time of 30 s was chosen for sampling by manually penetrating the septum to a depth of 0.25 cm. The SPME fiber was inserted into the injection port of the GC for 3 min for sample desorption. The GC-MS conditions were as follows: capillary column, as above; temperature, 35 °C for 2 min, then raised from 35 to 250 °C at 5 °C/min with a 5.0 min hold at 250 °C; carrier gas, helium (5 psi); injector temperature, 250 °C; split, 2 min (100 mL/min); transfer line temperature, 250 °C; detector temperature, 175 °C; mass range, *m*/*z* 40−300.

The active compounds from both steam-distilled samples and HS-SPME sampling were identified by mass spectrum database search

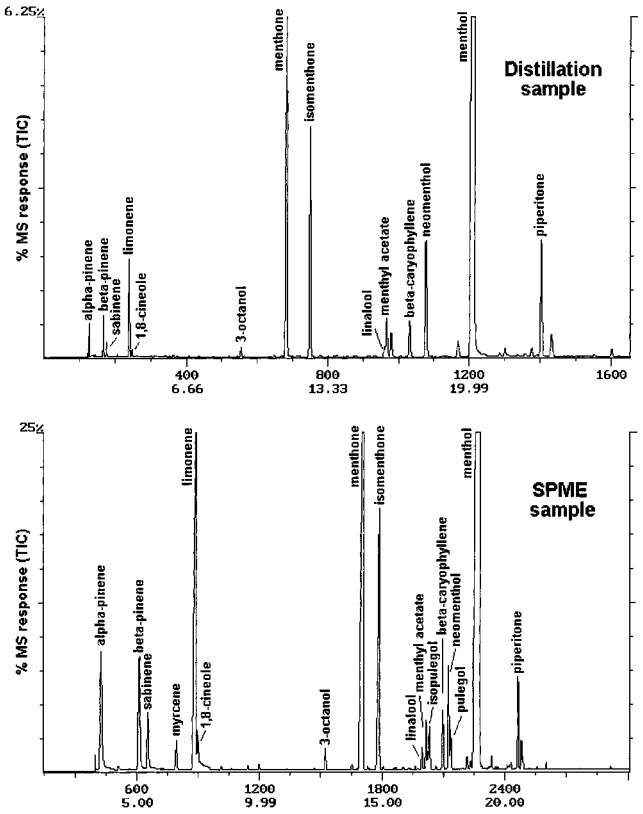

Figure 2. Characteristic GC-MS profiles of distilled and HS-SPME samples from leaves and flowers of sachalinmint (plant height = 60–80 cm).

(Varian NIST MS Database 1992 and IMS Terpene Library 1992) and on the basis of the relative retention index (ESO 97, Database of Essential Oils, Bacis). Quantitative analysis in percent was performed by peak area normalization measurements. Statistical analyses were carried out by using one-way ANOVA testing.

RESULTS AND DISCUSSION

HS-SPME has been used in routine and experimental research work in our laboratory and is being used as a screening tool for terpenic compositions in dried plant material from peppermint and sachalinmint (*2*, *3*), yarrow (*1*), and aroma volatiles from strawberries (*22*). In the present study, HS-SPME has been applied to sachalinmint plants by taking samples from various parts of the plant. In **Figure 1** the distribution of the essential oil content within sachalinmint plants with regard to plant leaves and flowers is illustrated. As shown, the content increases linearly in the acropetal direction, ranging from 1.08% in the lowest to 1.75% in the upper parts of the plant. These results were lower than detected in earlier trials within the NUP project (1995−1996) with oil concentrations on an average between 2 and 3.3% (*3*) and data reported by Eastern European research groups of 2.3−3.3% (*4*, *6*, *23*). The lower oil accumulation may be interpreted as an effect of unfavorable growing conditions in the cold and rainy summer season of 1998, but despite the minimal catabolic losses and volatilization (<1% monthly of the stored monoterpene pool), as reported for *M.* × *piperita* (*24*), sample storage time may play a role, too. With regard to the chosen sampling method, steam distillation seems to be a more effective extraction technique than supercritical fluid extraction and superheated water extraction reported by Ammann and co-workers (*25*).

Characteristic GC-MS profiles of HS-SPME and distilled samples from leaves and flowers of *M. sachalinensis* (plant height = 60−80 cm) are shown in **Figure 2**. The dominant

Table 1. GC-MS Detection of Terpenoids and Other Volatiles in Distilled Samples (DIST) and by Headspace Solid-Phase Microextraction Analysis (HS-SPME)

no.	compound	DIST[a]	SPME[a]	no.	compound	DIST[a]	SPME[a]
1	α-pinene	0.49	1.18	**30**	(*Z*)-sabinene hydrate	0.01	0.03
2	camphene	0.06	0.05	**31**	linalool	0.14	0.35
3	β-pinene	0.21	1.33	**32**	menthyl acetate	0.57	2.11
4	sabinene	0.09	0.59	**33**	isopulegol	0.31	0.82
5	3-carene	0.07	tr[b]	**34**	isopulegyl acetate	–[c]	0.01
6	β-myrcene	0.03	0.20	**35**	(*Z*)-dihydrocarvone	0.01	–
7	limonene	0.43	2.23	**36**	β-caryophyllene	0.37	0.77
8	1,8-cineole	0.05	0.34	**37**	neomenthol	1.60	2.08
9	β-phellandrene	tr	0.13	**38**	pulegol	0.01	0.63
10	(*Z*)-ocimene	0.02	0.05	**39**	isomenthol	0.17	0.41
11	γ-terpinene	0.01	tr	**40**	menthol	82.48	67.46
12	terpinolene	0.02	0.03	**41**	α-caryophyllene	tr	0.03
13	isopentyl isovalerate	0.02	0.10	**42**	neoisomenthol	0.05	0.07
14	6-methyl-5-hepten-2-one	tr	0.03	**43**	(*Z*,β)-farnesene	0.10	0.20
15	octyl acetate	–	0.03	**44**	(*E*)-verbenol	0.02	0.04
16	(*E*)-2-nonen-1-ol	tr	0.02	**45**	lavandulol	0.03	0.03
17	3-octanol	0.09	0.59	**46**	germacrene D	0.05	tr
18	β-thujone	tr	0.01	**47**	α-terpineol	0.15	0.09
19	(*Z*)-linalool oxide	0.02	0.11	**48**	piperitone	1.66	1.82
20	hexyl *n*-valerate	0.01	0.02	**49**	germacrene B	0.39	0.07
21	menthone	4.96	5.85	**50**	α-farnesene	0.02	0.05
22	(*E*)-sabinene hydrate	0.01	0.04	**51**	(*E*)-verbenyl acetate	0.14	0.12
23	(*E*)-linalool oxide	tr	0.03	**52**	geranyl acetone	0.02	0.02
24	isomenthone	2.88	6.43	**53**	perilla alcohol	0.06	–
25	(*Z*)-3-hexenyl isovalerate	0.02	0.06	**54**	caryophyllene oxide	0.16	0.03
26	(*E*)-2-decenol	–	0.02	**55**	(*Z*)-nerolidol	0.02	–
27	β-pinene oxide	tr	0.02	**56**	spathulenol	0.10	0.01
28	β-bourbonene	0.02	0.05	**57**	thymol	0.02	tr
29	isopinocamphone	0.01	0.07	**58**	tetradecanoic acid	–	0.02

[a] Values for DIST and SPME ($n = 3$; mass range *m/z* 40–300) indicate the distribution of the identified compounds (98.18 and 96.88%, respectively) from leaves and flowers (plant height = 60–80 cm). [b] tr, trace compounds. [c] –, not detected.

Table 2. Characteristic Terpenic Compounds from Sachalinmint Leaves (and Flowers) from Different Stem Positions (A, 0–20 cm; B, 20–40 cm; C, 40–60 cm; D, 60–80 cm) Detected by Solvent-Based Essential Oil Analysis (DIST) and HS-SPME Sampling ($n = 3$)

compound	method	A	B	C	D
menthol	DIST	87.88 ± 1.01	87.72 ± 0.27	87.93 ± 0.11	88.06 ± 0.44
	SPME	58.90 ± 3.25	48.54 ± 2.44	65.28 ± 2.42	71.46 ± 1.93
menthone	DIST	5.29 ± 0.38	5.28 ± 0.48	3.86 ± 0.13	2.46 ± 0.12
	SPME	12.90 ± 1.06	13.34 ± 1.91	10.98 ± 0.64	6.21 ± 0.43
isomenthone	DIST	3.06 ± 0.20	3.19 ± 0.03	3.28 ± 0.09	3.66 ± 0.09
	SPME	5.32 ± 0.50	6.58 ± 0.21	6.50 ± 0.71	6.83 ± 0.39
menthyl acetate	DIST	0.00 ± 0.00	0.49 ± 0.04	1.55 ± 0.06	2.00 ± 0.08
	SPME	0.68 ± 0.03	0.74 ± 0.03	1.14 ± 0.25	2.15 ± 0.13
neomenthol	DIST	1.70 ± 0.07	1.80 ± 0.04	1.89 ± 0.12	2.01 ± 0.02
	SPME	1.42 ± 0.11	1.55 ± 0.12	1.88 ± 0.12	2.21 ± 0.08
piperitone	DIST	1.78 ± 0.05	1.88 ± 0.02	1.91 ± 0.07	1.85 ± 0.15
	SPME	1.31 ± 0.10	1.43 ± 0.35	1.68 ± 0.04	1.89 ± 0.12

components, independent of the sampling method, are menthol, menthone, and isomenthone. Almost all of the 58 identified compounds could be detected by both analysis methods, and, with the exception of menthol, most of the monoterpene compounds were detected at higher levels by using HS-SPME with a 30 s extraction time (**Table 1**). To avoid fiber-discriminating effects with regard to the high menthol concentrations (see menthol/neomenthol relationship in **Figure 3**), HS-SPME exposure times in a range between 10 s and 5 min were tested. Although the linearity of SPME response for terpenic compounds under short fiber exposure times, as reported by Coleman and Lawrence (*20*), could not be found for *M. sachalinensis*, an unequilibrated extraction time of 30 s was found to be sufficient to detect both low- and high-eluting compounds, similar to earlier investigations with *M.* × *piperita* using a 1 min exposure (*2*). This is in contrast to plant samples such as yarrow with a relatively high content of sesquiterpene compounds, which required an exposure time of 10 min (*1*). The average relative standard deviation (RSD) for all tested compounds from HS-SPME sampling (**Table 2**) was on average 7.7% and ranged from 1 to 24.5% (average RSD for distillation samples = 3.7%). Although the reproducibility of HS-SPME may restrict this extraction technique to screening tests, it is applicable as a semiquantitative analysis tool for essential oil matrices from different sources.

Detailed analyses of the menthol/menthone ratio and other monoterpenes in various plant parts of sachalinmint as detected by common solvent-based essential oil analysis and HS-SPME sampling have not been reported before (**Tables 1** and **2**). The steam-distilled samples showed a minor complex matrix with a very high menthol and a much lower menthone content, averaging 87.9 and 4.2%, respectively, in contrast to earlier reported results with a menthol and menthone content ranging from 70 to 80% and from 8 to 12%, respectively (*4*, *5*). From comparison of the HS-SPME and steam-distilled samples, the same tendencies of increasing menthol/menthone ratio in the

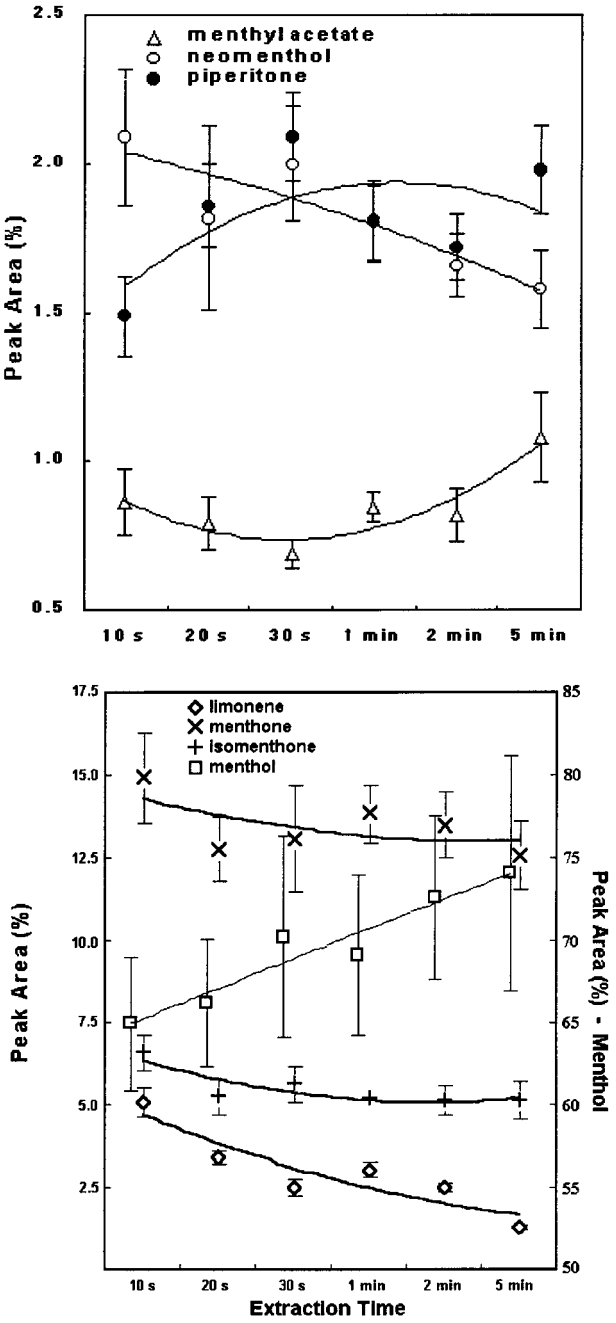

Figure 3. Extraction efficiency of the PDMS fiber using HS-SPME sampling under unequilibrated conditions ($n = 5$) with focus on important terpenoids from sachalinmint leaves and flowers (plant height = 60–80 cm; vertical bars, ± SD).

basipetal direction could be detected by both analysis methods. Previous publications on analyses of *M.* × *piperita*, however, showed the same pattern of increasing menthol content as an effect of leaf maturation (*9*, *11*, *26*).

In **Table 2** are shown characteristic terpenic compounds, besides menthol and menthone, in the *p*-menthane group from sachalinmint, as obtained by distillation and HS-SPME sampling. As noted with regard to the extraction efficiency, using HS-SPME might give more detailed information about less important terpenic compounds (**Table 1**). Isomenthone, menthyl acetate, neomenthol, and partly piperitone increased during leaf maturation in contrast to results for *M.* × *piperita* with decreasing isomenthone content in older leaves (*2*). Dimandja et al. (*27*) described a method for essential oil analysis to separate and qualitatively identify moderately complex samples of peppermint and spearmint using modifications of the GC-MS technique (one- and two-dimensional) and, in this way, tried to extend the detection limits of GC-MS. The present results underscore the applicability of HS-SPME for both screening and semiquantitative analyses of essential oil samples with respect to plant physiology.

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