Monoterpene Composition of Essential Oil from Peppermint (*Mentha* × *piperita* L.) with Regard to Leaf Position Using Solid-Phase Microextraction and Gas Chromatography/Mass Spectrometry Analysis

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Monoterpene compounds of leaf pairs and flowers of *Mentha* × *piperita* have been studied by direct headspace sampling using solid-phase microextraction coupled with gas chromatography/mass spectrometry (SPME−GC/MS). The content of peppermint-characteristic compounds such as menthol, menthyl acetate, and neomenthol increased in a basipetal direction (older plant parts), whereas menthone and isomenthone showed higher levels in the acropetal direction (younger plant parts). Higher levels of menthofuran were found in peppermint flowers in contrast to the leaves. SPME sampling resulted in relatively higher amounts of high-volatile monoterpenes and lower detection of less volatile compounds such as menthol and menthone, compared to solvent-based samples from essential oil distillation.

Keywords: *Mentha* × *piperita L.; headspace; SPME−GC/MS; basipetal; acropetal; menthol; menthone*

INTRODUCTION

Solid-phase microextraction (SPME) offers an alternative technique for the extraction of organic volatiles from different sample sources, compared with conventional methods such as steam distillation, solvent and supercritical CO_2 extraction, or headspace trapping. Since the commercial access of SPME equipment in 1993, a continually increasing number of applications coupled with gas chromatography has been recorded in biological and chemical sciences.

SPME represents a reliable method for the screening of simple but also very complex mixtures of organic volatiles. The technique is widely applied in environmental research for the detection of air pollutants (Zhang and Pawliszyn, 1993; Chai and Pawliszyn, 1995), water impurities (Arthur et al., 1992; Santos et al., 1996; Thomas et al., 1996), and pesticides (Eisert and Levsen, 1995; Magdic et al., 1996). Especially in food chemistry and food quality control have a considerable number of applications been established (Yang and Peppard, 1994; Steffen and Pawliszyn, 1996; Penton, 1997). Flavors from both food, for example, apple (Matich et al., 1996; Song et al., 1997), strawberry (Ulrich et al., 1995), cheese (Chin et al., 1996), and onions (Jarvenpaa et al., 1998) and beverages such as milk (Stevenson and Chen, 1996), malt products (Constant and Collier, 1997), orange juice (Jia et al., 1998), and vodka (Ng et al., 1996) have been screened by SPME headspace sampling. In contrast to traditional and more time-consuming methods such as steam distillation extraction (SDE) and organic solvent extraction, SPME also provides a simplified extraction procedure for gas chromatographic (GC) analysis of essential oil from different sources, for example, hops (Field et al., 1996), anise and peppermint (Czerwinski et al., 1996), sweet fennel, nutmeg, and mandarin orange (Clark and Bunch, 1997), and cedar wood (Coleman and Lawrence, 1997).

The aim of this study was to determine semiquantitative differences in essential oil quality between flowers and leaves of various positions of *Mentha* × *piperita* L., clone Hardanger, by using headspace SPME−GC/MS analysis. As already described by many authors, the content and composition of peppermint essential oil are strongly influenced by harvest date and plant age (Court et al., 1993; Chalchat et al., 1997), light regime (Voirin et al., 1990; Fahlen et al., 1997), and fertilization and planting time (Marotti et al., 1994; Dragland and Aslaksen, 1997). Additionally, the oil composition is also related to leaf position (Vaverkova et al., 1987; Maffei et al., 1989; Voirin et al., 1990; Brun et al., 1991) with increasing menthol and decreasing menthone content in the basipetal direction. The morphologically dependent biochemical variation was the starting point for the present study of SPME of characteristic peppermint oil compounds with regard to harvest date and plant organ.

MATERIALS AND METHODS

Plant Material. Leaves and flowers of blooming *Mentha* × *piperita* plants (clone Hardanger) were harvested from trial plots at the Plant Biocentre field research area, Trondheim, Norway, in early September 1997. Samples for SPME were taken from 2 × 50 plants (pooled) with regard to leaf position in the basipetal direction (three replicates each): a, flowers; b, leaf pairs 1−3; c, leaf pairs 4−5; d, leaf pairs 6−7; and e, leaf pairs 8−9. Additionally, both flowers and leaves (positions 1−9) were collected from 2 × 50 plants for distillation of the essential oil by hydrodistillation (three replicates from pooled sample). All plant 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 February 1998.

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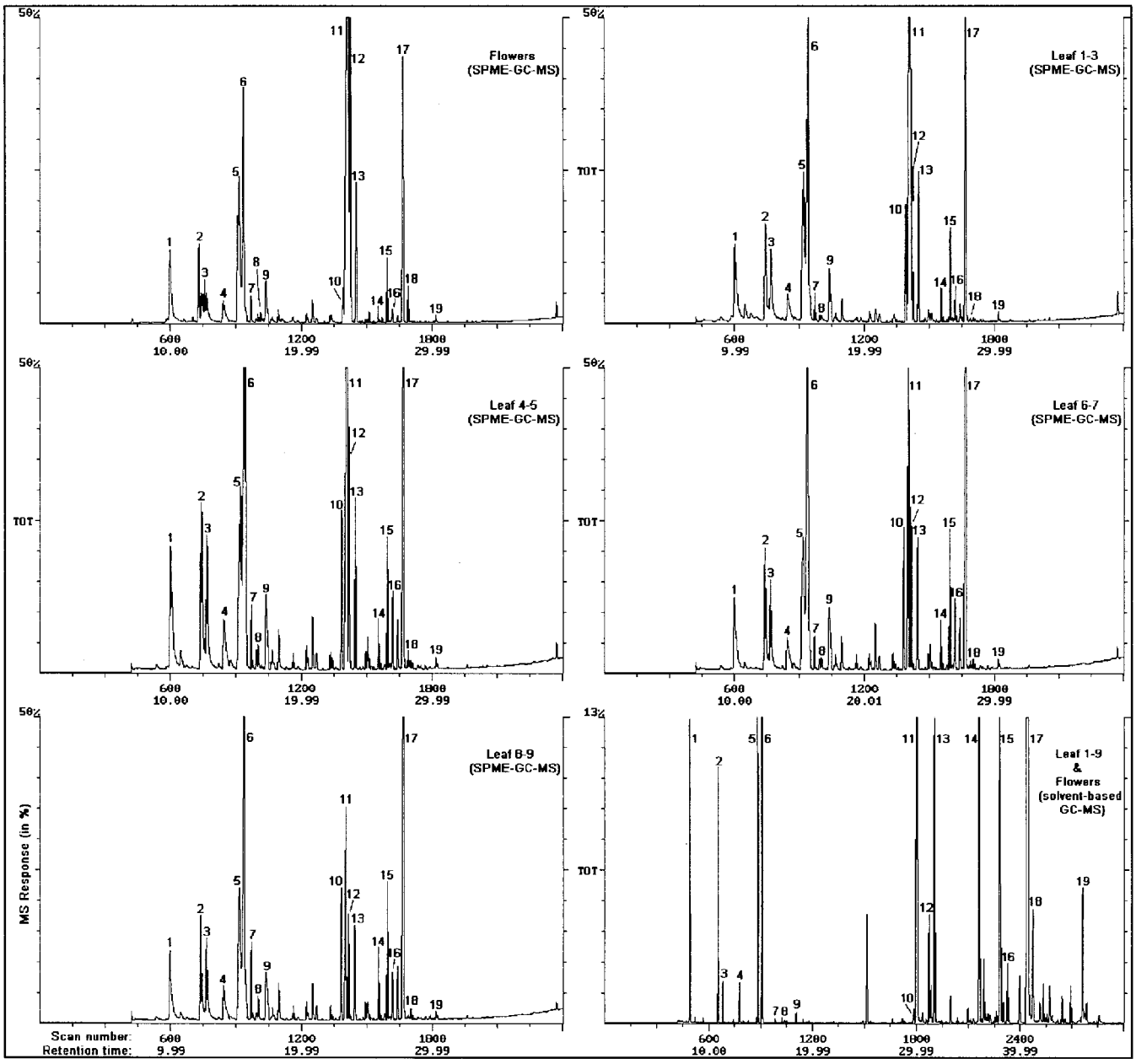

Figure 1. GC/MS profiles of headspace SPME of flowers and leaf pairs of peppermint compared with solvent-based GC/MS analysis (flower *and* leaf essential oil). Main peaks are marked, which correspond with the numbers given in Table 2.

Table 1. GC/MS Conditions for Headspace SPME and Solvent-Based Analysis

extraction method	injection	column	temperature program	GC	MS detector	carrier gas
headspace SPME	split/splitless, 2 min, 250 °C	Supelcowax (fused silica), 60 m × 0.25 mm i.d. (0.25 μm film thickness)	35 °C for 2 min, 35−250 °C at 5 °C/min, 10 min hold at 250 °C	Varian Star 3400CX	Varian Saturn 3, 170 °C	He (15 psi) at 100 mL/min
hydrodistillation	splitless, 250 °C, 1 μL	Supelcowax (fused silica), 60 m × 0.25 mm i.d. (0.25 μm film thickness)	60 °C for 1.1 min, 60−200 °C at 2 °C/min, 15 min hold at 200 °C	Varian Star 3400CX	Varian Saturn 3, 120 °C	He (15 psi) at 50 mL/min

Hydrodistillation. Leaves and flowers were separated from the stems, pooled, and crushed before distillation. The distillation apparatus consisted of a heating cap, a 3 L extraction flask, a 3 mL graduated receiver (Dean and Stark), and a condenser (jacketed coil). Fifty grams of dried plant material and 1.5 L of H_2O were used, and the distillation was carried out for 2 h after the mixture had reached the boiling point at 100 °C (oil recovery = 0.83 mL/50 g). The collected oil was stored in brown glass flasks at 4 °C prior to analysis. The GC samples were prepared by diluting 10 μL of oil in 1 mL of ethanol in brown autosampler flasks.

SPME and GC/MS Analysis. A PDMS coated fiber (100 μm) and a manual SPME holder (Supelco Inc.) were used in this study. In a blank run, the fiber was exposed to 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 phenolic cap and PTFE/silicone septum (Supelco) and stored in a drying cabinet at 25 °C for 24 h. The SPME fiber was exposed to each sample for 1 min at 25 °C (in the cabinet) by manually penetrating the septum (0.25 cm depth). Finally, the SPME fiber was inserted into the injection

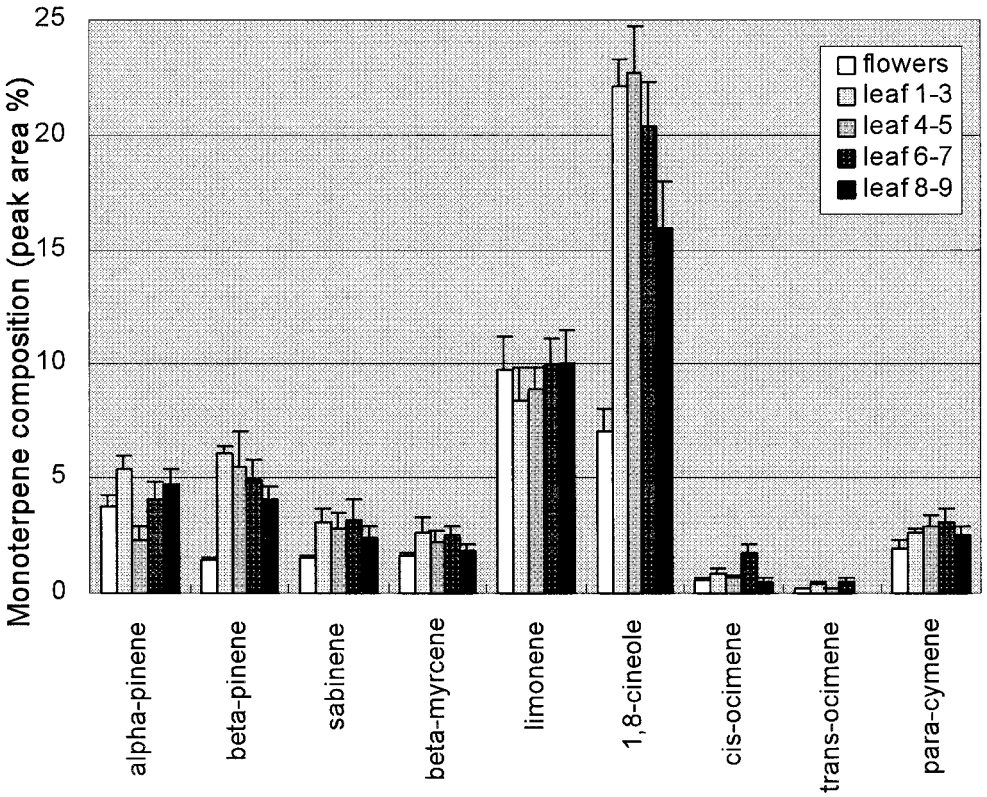

Figure 2. Headspace SPME of essential oil components of high volatility of flowers and leaf pairs of *Mentha* × *piperita*.

port of the GC for 3 min for sample desorption (see Table 1 for GC/MS conditions).

All mass spectra were aquired in EI mode (SPME samples, scan range = *m/z* 25−300, multiplier 1300 V; solvent-based samples, scan range = *m/z* 25−300, multiplier 1650 V). The essential oil compounds were identified by mass spectrum database search (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, 1997). Quantitative analysis (in percent) was performed by peak area measurement (TIC).

RESULTS AND DISCUSSION

Four character impact compounds in peppermint essential oil (menthol, menthyl acetate, menthone, and menthofuran; Maarse, 1991) could be detected by both SPME−GC/MS and solvent-based GC/MS analysis. Although headspace SPME is a discriminating method, that is, high-volatile compounds are favored, which results in higher fiber extraction and detection of monoterpenes such as α- and β-pinene, limonene, and 1,8-cineole, SPME covered the spectrum of main compounds of interest, which are detected in essential oil samples obtained by conventional distillation (Table 2).

As an equilibrium method, GC/MS profiles from SPME are strongly influenced by experimental conditions. In contrast to other studies, the extraction was carried out regardless of the equilibrium phase between the fiber coating and the headspace. The aim was to apply a method with a short and fixed extraction time after an equilibrium was established in the headpace gas (24 h) and, simultaneously, take advantage of an SPME method that could reflect the composition of essential oil volatiles by extracting both low- and high-eluting compounds at sufficient concentrations.

Having finished the preliminary tests with SPME on leaf samples of *Mentha* × *piperita* (fiber exposure times of 30, 60, 120, and 300 s), an appropriate exposure time of 1 min was chosen for all SPME samples. The linearity of SPME response of several terpenoids under short fiber exposure times has been demonstrated by Coleman and Lawrence (1997). In fact, works by other authors on essential oil containing preparations show that extraction times of even <1 min may be practical for SPME (Czerwinski et al., 1996).

The gas chromatograms obtained are shown in Figure 1 and describe the variation of oil composition between different plant organs and analysis methods. In general, the number of compounds detected by SPME−GC/MS is comparable to that detected by the conventional

Table 2. Essential Oil Composition of *Mentha* × *piperita* in SPME or Solvent-Based Samples [Average Amounts (in Percent) Determined by Peak Area Measurement plus Standard Deviation][a]

	compound	SPME sample	solvent-based sample
1	α-pinene	4.12 ± 0.65	1.63 ± 0.09
2	β-pinene	5.14 ± 0.66	1.74 ± 0.06
3	sabinene	2.88 ± 0.52	0.26 ± 0.03
4	β-myrcene	2.30 ± 0.39	0.26 ± 0.07
5	limonene	9.32 ± 1.30	2.29 ± 0.32
6	1,8-cineole	20.29 ± 1.60	5.79 ± 0.59
7	(*Z*)-ocimene	0.95 ± 0.18	trace ± 0.59
8	(*E*)-ocimene	0.36 ± 0.06	trace ± 0.59
9	*p*-cymene	2.78 ± 0.40	trace ± 0.59
10	(*E*)-*p*-menth-2-en-1-ol	2.72 ± 0.44	0.15 ± 0.02
11	menthone	16.08 ± 1.65	15.34 ± 1.67
12	menthofuran	3.33 ± 0.63	1.10 ± 0,74
13	isomenthone	2.98 ± 0.39	4.87 ± 0.57
14	menthyl acetate	0.96 ± 0.11	8.28 ± 0.76
15	neomenthol	2.42 ± 0.38	4.44 ± 0.34
16	β-caryophyllene	1.06 ± 0.12	0.88 ± 0.10
17	menthol	16.65 ± 1.25	46.63 ± 2.51
18	pulegone	0.23 ± 0.05	1.20 ± 0.25
19	piperitone	0.19 ± 0.02	1.43 ± 0.33

[a] SPME data represent average values from summarized results (leaf positions 1−9 and flowers).

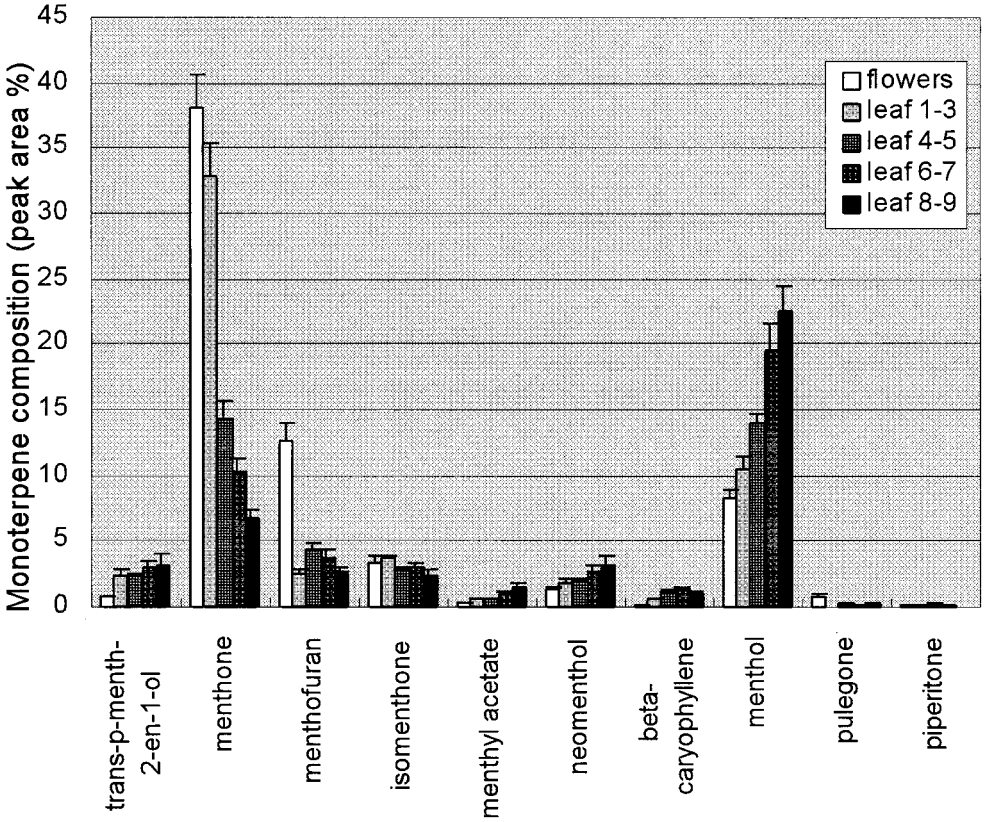

Figure 3. Headspace SPME of characteristic essential oil components of flowers and leaf pairs of *Mentha* × *piperita*.

solvent-based analysis. On the other hand, compounds detected by SPME−GC/MS show an average relative standard deviation that ranks from 8 to 23% (data not shown), but even though SPME achieves only limited reproducibility, it is applicable as a screening method for such semiquantitative analyses (Figures 2 and 3).

In contrast to Maffei et al. (1989), high-volatile terpenoids such as β-pinene and 1,8-cineole showed decreasing concentrations from apical to basal leaves and increasing limonene and *p*-cymene amounts, whereas quality-impact compounds within the *p*-menthane group such as menthone, isomenthone, menthol, menthyl acetate, and neomenthol showed almost the same tendencies as reported by Maffei et al. (1989) and Voirin et al. (1990). The increase in the content of menthone and the decline in menthol in the acropetal direction for leaves could be detected by SPME−GC/MS. This trend was consistent with previous studies (Brun et al., 1991; Voirin and Bayet, 1996). Higher contents of menthyl acetate and neomenthol were found in basal leaves, which agrees with the results of Vaverkova et al. (1987) and Maffei et al. (1989).

Viewed from the perspective of ontogenetic development and oil quality demand, the results indicate that peppermint plants should be harvested at a growth stage with a relatively high number of basal leaves to obtain an essential oil quality with a high concentration of menthol and a low content of menthone. On the other hand, undesired components such as menthofuran will increase from the vegetative growth stage to full bloom (Court et al., 1993). Peppermint flowers contained 4 times as much menthofuran as the leaves from different nodes (Figure 3).

In summary, headspace SPME−GC/MS analysis of peppermint essential oil volatiles from different plant organs gave reliable results that could be confirmed by earlier investigations with conventional extraction and analysis methods.

CONCLUSIONS

On the basis of this study, it can be concluded that headspace SPME shows high applicability to the extraction of essential oil volatiles from leaves and flowers of *Mentha* × *piperita*. Although the SPME−GC/MS profiles differ greatly from solvent-based GC/MS chromatograms, SPME offers a reliable and time-sparing method for the comparison of essential oil compositions of different plant samples with varying analyte concentrations.

In conformity with earlier investigations by other authors, GC/MS data show that the highest amounts of menthol can be found in basipetal leaves of peppermint plants, whereas the concentration of menthone is relatively higher in acropetal parts and flowers. These results underscore the conclusion that the harvest of peppermint plants in bloom and not in the vegetative stage will probably result in an essential oil quality with higher amounts of menthol.

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