Production of Yarrow (*Achillea millefolium* L.) in Norway: Essential Oil Content and Quality

Jens Rohloff, Else Berit Skagen, Albert Haakon Steen, and Tor-Henning Iversen*

The Plant Biocentre, Department of Botany, Norwegian University of Science and Technology, 7491 Trondheim, Norway

In 1996, the production of *Achillea millefolium* L. at different locations in Norway was investigated with regard to the developmental stage. The oil content differed greatly between the vegetative stage (0.13%) and the stage of full bloom (0.34%). Changes in the composition of yarrow essential oil were found to be related to maturation of the plant, with increasing amounts of monoterpenes in relation to the sesquiterpene. However, a clear trend could be detected only for the monoterpenic compounds with increasing levels of α- and β-pinene and α-thujone and decreasing levels of sabinene, borneol, and bornyl acetate. Previously reported as major compounds, chamazulene and germacrene D could be found only in insignificant amounts. A solid-phase microextraction (SPME) procedure was applied for screening of the terpenic composition. Sesquiterpenic compounds such as β-bisabolene, α-bisabolol, and δ-cadinene were detected in substantial amounts by SPME in contrast to the steam-distilled samples.

Keywords: *Achillea millefolium L.; monoterpenes, sesquiterpenes; harvest date; plant organ; solid-phase microextraction (SPME); GC-MS analysis*

INTRODUCTION

Since the beginning of the 1990s there has been an increasing interest in using and growing medicinal plants and herbs in Norway. To promote commercial production and to meet the rising market demand, a major research project at different locations in Norway was started in 1995; the project focused on vegetative growth in the field. The goal of the project, which was called the Norwegian Herb Production, was to focus on cultivation, processing, marketing, and distribution of herbs. Of special interest were comparative analyses of essential oil composition in yarrow (*Achillea millefolium* L., Asteraceae) from cultivated plants. The results presented here are primarily from the project year 1996 but have been updated and adjusted by more recently obtained results.

A variety of medical applications, cosmetic properties, and insect repellent functions have been related to several classes of secondary metabolites found in *Achillea* species [see, e.g., Chandler et al. (1982) and Mitich (1990)]. The well-known medicinal use of these plants includes the prevention of infections and the treatment of wounds and fevers. They have also been found to have an effect against epilepsy and hemorrhage and in reducing hypertension. In plant growth studies inhibition of seed germination and insect repellment have been shown by using these species. The active constituents for most of the medicinal effects seem to be related to the volatile components in the essential oils and the sequiterpene lactones (Chandler et al., 1982). The composition of the essential oil isolated from the aerial part of yarrow has been extensively studied over the years [e.g.. Falk et al. (1975), Figueiredo et al. (1992a), Hofmann et al. (1992), and Afsharypour et al. (1996)]. More recently, the oil composition in yarrow roots, grown in tissue cultures and as plant roots, has been characterized in detail (Lourenço et al., 1999).

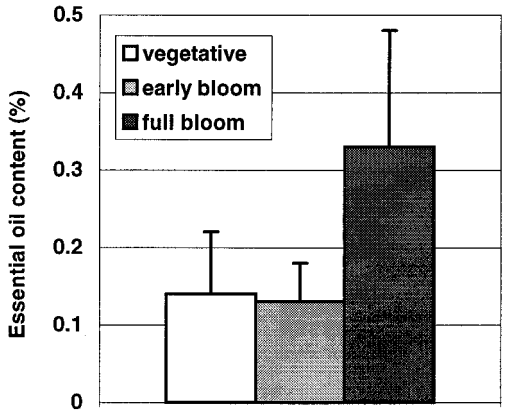

Figure 1. Essential oil production in yarrow at different growth stages (all locations).

The composition of the essential oil from aromatic plants is closely related to their developmental stage (Shu and Lawrence, 1997). Regarding the geographic origin, there seems to exist a relationship between environmental factors and oil composition in some Italian *Achillea* species (Maffei et al., 1989, 1993, 1994). In a previous paper from our group, semiquantitative differences in essential oil quality were determined between flowers and leaves located at various positions of the stem of peppermint that was grown in the field in Trondheim (Rohloff, 1999). In this context, direct headspace sampling using solid-phase microextraction coupled with gas chromatography−mass spectrometry (SPME-GC-MS) was adapted for the screening of terpenic compounds in yarrow (Yang and Pawliszyn, 1994; Czerwinski, 1996; Steffen and Pawliszyn, 1996; Field

* Corresponding author (e-mail Tor-Henning.Iversen@chembio.ntnu.no; fax 47 73 59 01 77; telephone 47 73 59 60 87).

10.1021/jf000720p CCC: $19.00
Published on Web 11/15/2000

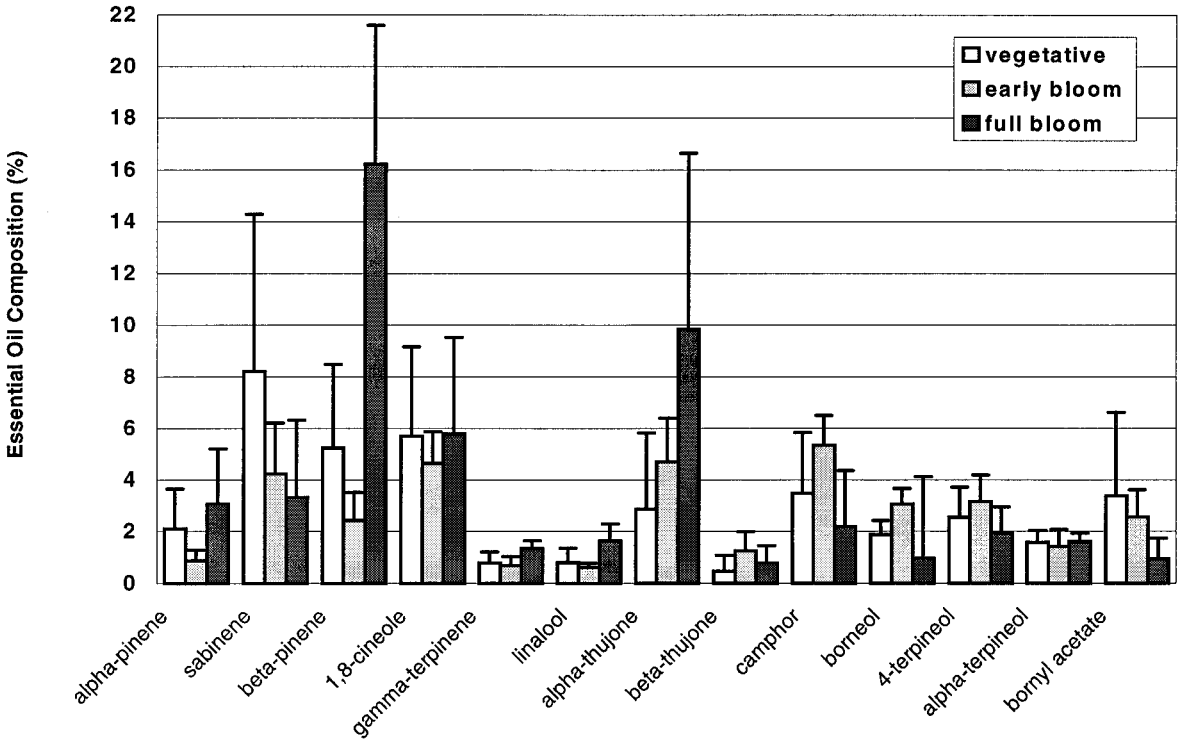

Figure 2. Composition of the essential oil (monoterpenes) from yarrow at different growth stages (all locations).

et al., 1996; Penton, 1997; Rohloff, 1999). The results obtained with SPME-GC-MS supplement the present study analysis using standard GC-MS of steam-distilled plant samples.

The overall aim of the present study was to investigate the growth conditions and especially the effect of the harvest date and developmental stage on the essential oil production and quality of yarrow.

MATERIALS AND METHODS

Plant Material. Plant material from cultivated plants was grown from seeds from wild populations (Stjørdal/Nord-Trøndelag) and purchased from Midtnorsk Blomsterengfrø in Nord-Trøndelag. The plants were distributed to seven farmers and the Plant Biocentre in Norway at various locations from Trøkstad, in Østfold County in the south (11° 20′; 59° 37′) to Løpsmarka in Nordland County in the northern part of Norway (14° 30′; 67° 18′). The plants were grown on plastic mulch in triple rows with 25−30 cm between plants and 50 cm between rows. Field locations were as follows: Brandbu/Oppland (10° 30′ 60° 25′); Flatråker/Hordaland (5° 30′; 60°); Løpsmarka/Nordland (14° 30′; 67° 18′); Råde/Østfold (10° 50′; 59° 21′); Snillfjord/Sør-Trøndelag (9° 30′; 63° 23′); Trøkstad/Østfold (11° 20′; 59° 37′); Uthaug/Sør-Trøndelag (9° 35′; 63° 44′); the Plant Biocentre/Sør-Trøndelag (10° 30′; 63° 15′). After harvest at different developmental stages, the plant material was oven-dried at temperatures between 30 and 40 °C at the different locations before the samples were sent to the Plant Biocentre. One pooled sample from each test field and the respective growth stage was submitted to steam distillation.

SPME samples were harvested from blooming yarrow at the Plant Biocentre in early August 1997 on the same day. Independent samples were taken from the test field established in 1996 from three trial plots (leaves, flower buds, and flowers with three replicates each), dried at 35 °C in a drying cabinet with a fan (Termaks TS 5410) for 48 h, and stored at room temperature.

Steam Distillation. The whole aerial part of the plant (leaves, flowers, and stems) was crushed and submitted to distillation. The glass apparatus consisted of a heating cap, a 5 L distillation flask, a 3 mL graduated receiver (Dean and Stark), and a condenser (jacketed coil). Exactly 100 g of dried plant material and 2.5 L of water were used, and each distillation was carried out for 2 h after the mixture had reached the boiling point. The collected oil and the immediately prepared samples were stored in brown flasks at 4 °C prior to analysis by gas chromatography.

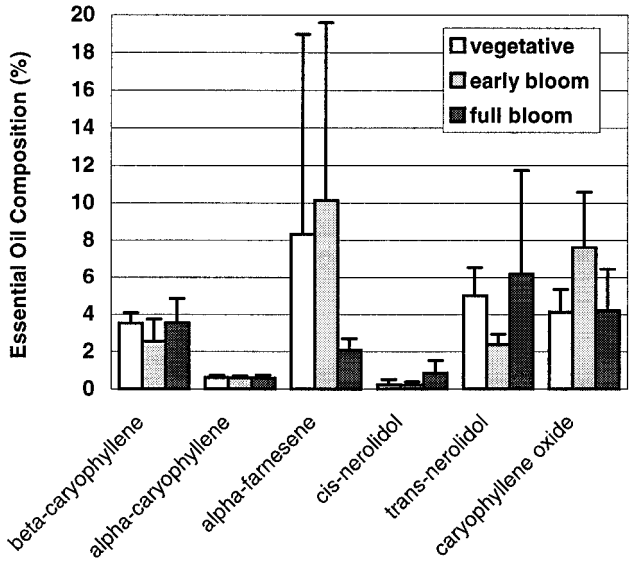

Figure 3. Composition of the essential oil (sesquiterpenes) from yarrow at different growth stages (all locations).

GC Analysis. The GC samples were prepared by diluting 25 μL of essential oil in 1 mL of ethanol for analysis in brown autosampler flasks. The essential oil constituents were analyzed by using a Varian Star 3400 CX gas chromatograph coupled with a Saturn 3 mass spectrometer.

Steam-Distilled Sample GC-MS Conditions: capillary column Supelcowax TM 10, 60 m, 0.25 mm inner diameter (0.25 μm film thickness); temperature raised from 35 to 200 °C at 2.9 °C/min (1.1 min hold at 200 °C); carrier gas, helium (23 psi); injector temperature, 250 °C; split, 1 min (50 mL/min); transfer line temperature, 200 °C; detector temperature, 200 °C.

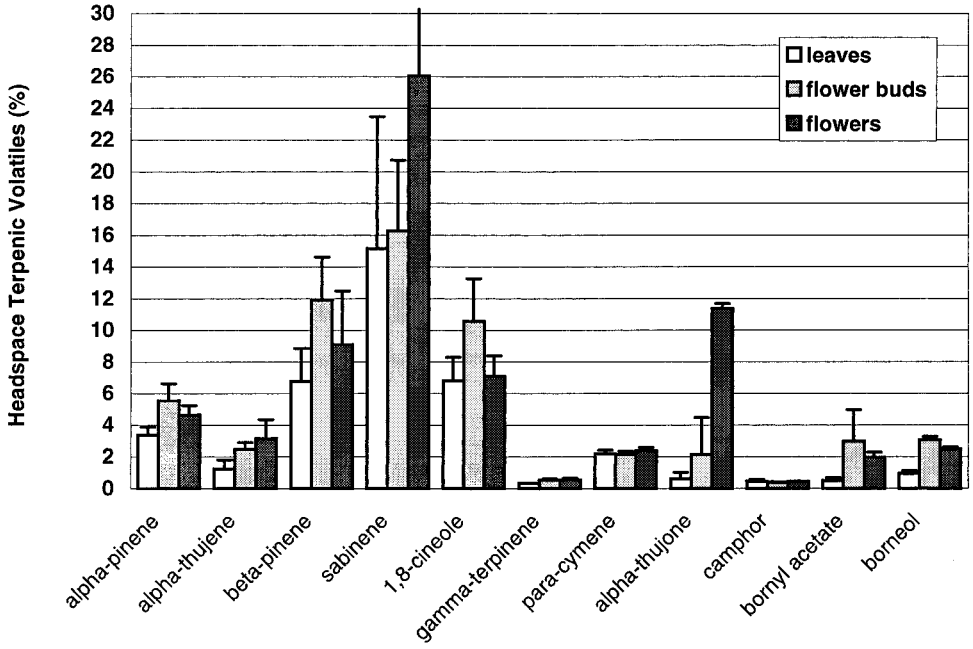

Figure 4. Composition of headspace terpenic volatiles (monoterpenes) of different plant organs from yarrow (the Plant Biocentre/S.-Trøndelag) detected by SPME-GC-MS technique.

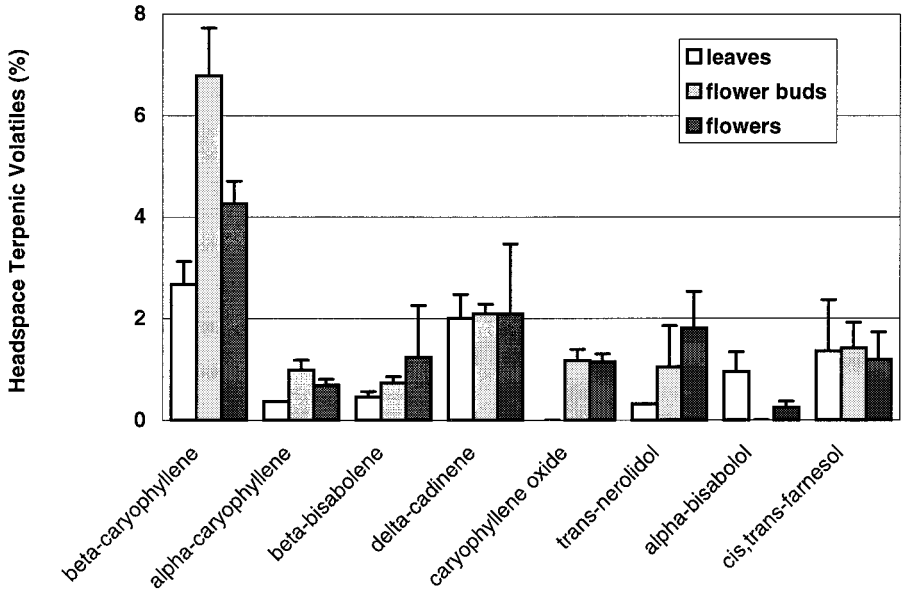

Figure 5. Composition of headspace terpenic volatiles (sesquiterpenes) of different plant organs from yarrow (the Plant Biocentre/S.-Trøndelag) detected by SPME-GC-MS technique.

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 phenolic cap and PTFE/silicone septum (Supelco Inc.) and stored in a drying cabinet at 50 °C for 50 min. The SPME fiber was exposed to each sample for 10 min by manually penetrating the septum (0.25 cm depth). 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, Chrompack CP Wax 52CB, 30 m, 0.32 mm inner diameter (0.25 μm film thickness); temperature held at 35 °C for 2 min, raised to 250 °C at 5 °C/min (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.

The active compounds from both essential oil samples and SPME sampling 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 normalization measurements (TIC = total ion count).

RESULTS AND DISCUSSION

Content and Composition of Essential Oils. In Figure 1 the content of essential oil is illustrated for analyses of all collected samples of yarrow combined, independent of the field locations. As shown, the average content at the vegetative stage is 0.13%, which slightly decreased when the plants started flowering, but this difference is not statistically significant. At the stage of full bloom, however, the content increased dramatically and reached an average of 0.34%.

The distribution of the major compounds in yarrow essential oil obtained by steam distillation is demonstrated in Figure 2 (monoterpenes) and Figure 3 (sesquiterpenes). In contrast to results from earlier investigations (Krupinska, 1986; Lamaison and Carnat, 1988; Kokkalou et al., 1992; Michler et al., 1992; Michler and Arnold, 1999), the plant material was found to be a chamazulene-free chemotype, as already indicated by the clear oil color of all samples. Regarding the greatly differing cultivation conditions (light, temperature, soil, etc.), it may be a factor that the samples were taken from hexaploid and not from tetraploid individuals, which normally produce a blue, chamazulene-containing oil (Michler et al., 1992; Michler and Arnold, 1999).

The monoterpene/sesquiterpene relationship seemed to be strongly affected by the harvest date, increasing from 2:1 to 3:1 from the vegetative stage to full bloom, respectively. This observation is supported by results obtained by Figueiredo et al. (1992a). As already described by other authors (Chialva and Gabri, 1987; Figueiredo et al., 1992a,b; Maffei et al., 1994; Wichtl, 1997), important monoterpenic (sabinene, β-pinene, 1,8-cineole, α-thujone, camphor, and 4-terpineol; see Figure 2) and sesquiterpenic compounds [β-caryophyllene, α-farnesene, (*E*)-nerolidol, caryophyllene oxide; see Figure 3] could be detected. Germacrene D, previously reported as a major compound of the sesquiterpene fraction (Hofmann et al., 1992; Figueiredo et al., 1992b; Maffei et al., 1994), could be found only in insignificant amounts.

With regard to the developmental stage, the content of monoterpenes especially was quite strongly affected with increasing amounts of α- and β-pinene and α-thujone detected from the vegetative stage to full bloom. The content of sabinene, camphor, borneol, and bornyl acetate, on the other hand, decreased throughout the season with the maturing of the plants (Figure 2).

When the SPME procedure was used on yarrow plant material cultivated at the Plant Biocentre in Mid-Norway (Figures 4 and 5), a dramatic quantitative difference was observed for the different compounds. In contrast to the distillation method, substantially higher contents of sabinene and 1,8-cineole were detected in SPME samples, whereas the concentration level of pinenes, α-thujone, and borneol seemed to be consistent with the results from the steam-distilled samples (Figures 2 and 4). Significant amounts of sesquiterpenes such as β-bisabolene, δ-cadinene, α-bisabolol, and (*Z*,*E*)-farnesol could be detected by using SPME-GC-MS (Figure 5) in contrast to the hydrodistilled samples. Additionally, monoterpenes such as α-thujene and *p*-cymene were extracted in substantial amounts by SPME (Figure 4). Because two analytical methods based on nonhomogeneous samples were applied, results from SPME analyses have therefore to be seen as a supplement to the results obtained from steam-distilled samples.

Regarding the varying essential oil content and quality throughout the season (steam-distilled samples) and the terpenic composition in different plant organs (SPME), it can be concluded that, due to commercial interests in the production of yarrow, harvesting should be carried out in the flowering stage with regard to a substantially higher plant yield with relatively higher contents of essential oil per plant weight unit. By using the SPME procedure on yarrow plant material, it can be illustrated that significant amounts of yarrow-characteristic sesquiterpenes could be detected in contrast to what was obtained using the steam-distilled samples. On the other hand, concentration levels of monoterpenes such as pinenes and α-thujone were reflected by using SPME. Thus, it can be concluded that SPME offers an applicable method for the screening of complex herb-based sample matrices with regard to headspace analysis of terpenic volatiles by GC-MS.

LITERATURE CITED

Afsharypour, S.; Asgary, S.; Lockwood, G. B. Volatile constituents of *Achillea millefolium* from Iran. *Flavour Fragrance J.* **1996**, *11*, 265−267.

Chandler, R. F.; Hooper, S. N.; Harvey, M. J. Ethnobotany and phytochemistry of yarrow, *Achillea millefolium*, Compositae. *Econ. Bot.* **1982**, *36*, 203−223.

Chialva, F.; Gabri, G. Headspace versus classical analysis. In *Capillary Gas Chromatography in Essential Oil Analysis*; Sandra, P., Bicchi, C., Eds.; Dr. Alfred Huethig Verlag: Heidelberg, Germany, 1987; pp 123−154.

Czerwinski, J.; Zygmunt, B.; Namiesnik, J. Headspace solid-phase microextraction for the GC-MS analysis of terpenoids in herb based formulations. *Fresenius' J. Anal. Chem.* **1996**, *356*, 80−83.

Falk, A. J.; Smolenski, S. J.; Bauer, L.; Bell, C. L. Isolation and identification of three new flavones from *Achillea millefolium* L. *J. Pharm. Sci.* **1975**, *64*, 1838−1842.

Field, J. A.; Nickerson, G.; James, D. D.; Heider, C. Determination of essential oils in hops by headspace solid-phase microextraction. *J. Agric. Food Chem.* **1996**, *44*, 1768−1772.

Figueiredo, A. C.; Barroso, J. G.; Pais, M. S. S.; Scheffer, J. J. C. Composition of the essential oil from leaves and flowers of *Achillea millefolium* ssp. millefolium. *Flavour Fragrance J.* **1992a**, *7*, 219−222.

Figueiredo, A. C.; Barroso, J. G.; Pais, M. S. S.; Scheffer, J. J. C. Composition of the essential oils from two populations of *Achillea millefolium* ssp. *millefolium*. *J. Chromatogr. Sci.* **1992b**, *30*, 392−395.

Hofmann, L.; Fritz, D.; Nitz, S.; Kollmannsberger, H.; Drawert, F. Essential oil composition of three polyploids in the *Achillea millefolium* "complex". *Phytochemistry* **1992**, *31*, 537−542.

Kokkalou, E.; Kokkini, S.; Hanlidou, E. Volatile constituents of *Achillea millefolium* in relation to their infraspecific variation. *Biochem. Syst. Ecol.* **1992**, *20*, 665−670.

Krupinska, A. Distribution of azulene-containing and azulene-free forms of yarrow (*Achillea millefolium*) sensu lato in north-western Poland. *Herba Polon.* **1986**, *31*, 39−46.

Lamaison, J. L.; Carnat, A. P. Search for azulene from the three subspecies of *Achillea millefolium* L. *Ann. Pharm. Fr.* **1988**, *46*, 139−143.

Lourenço, P. M. L.; Figueiredo, A. C.; Barroso, J. G.; Pedro, L. G.; Oliveira, M. M.; Deans, S. G.; Scheffer, J. J. C. Essential oils from hairy root cultures and from plant roots of *Achillea millefolium*. *Phytochemistry* **1999**, *51*, 637−642.

Maffei, M.; Chialva, F.; Codignola, A. Essential oils and chromosome numbers from Italian *Achillea* species. *J. Essent. Oil Res.* **1989**, *2*, 57−64.

Maffei, M.; Doglia, G.; Chialva, F.; Germano, F. Essential oils, chromosome numbers and karyotypes from Italian *Achillea* species. Part II. *J. Essent. Oil Res.* **1993**, *5*, 61−70.

Maffei, M.; Mucciarelli, M.; Scannerini, S. Essential oils from *Achillea* species of different geographic origin. *Biochem. Syst. Ecol.* **1994**, *22*, 679−687.

Michler, B.; Arnold, C. G. Predicting presence of proazulenes in the *Achillea millefolium* group. *Folia Geobot.* **1999**, *34*, 143−161.

Michler, B.; Preitschopf, A.; Erhard, P.; Arnold, C. G. *Achillea millefolium*: relationships among habitat factors, ploidy, occurrence of proazulene and the content of chamazulene in the essential oil. *Pharm. Ztg. Wiss.* **1992**, *137*, 23−29.

Mitich, L. W. Yarrow−the herb of achilles. *Weed Technol.* **1990**, *4*, 451−453.

Penton, Z. E. Sample preparation for gas chromatography with solid-phase extraction and solid-phase microextraction. *Adv. Chromatogr*. **1997**, *37*, 205−236.

Rohloff, J. 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. *J. Agric. Food Chem*. **1999**, *47*, 3782−3786.

Shu, C. K.; Lawrence, B. M. Reasons for the variation in composition of some commercial essential oils. In *Spices: Flavor Chemistry and Antioxidant Properties*; Risch, S. J., Ho, C. T., Eds.; ACS Symposium Series 660; American Chemical Society: Washington, DC, 1997; pp 138−159.

Steffen, A.; Pawliszyn, J. Analysis of flavor volatiles using headspace solid-phase microextraction. *J. Agric. Food Chem*. **1996**, *44*, 2187−2193.

Wichtl, M. *Teedrogen und Phytopharmaka—Ein Handbuch für die Praxis auf wissenschaftlicher Grundlage*; Wissenschaftliche Verlagsgesellschaft mbH (WVG): Stuttgart, Germany, 1997; pp 395−399.

Yang, X.; Pawliszyn, T. Solid-phase microextraction for flavor analysis. *J. Agric. Food Chem*. **1994**, *42*, 1925−1930.

Received for review June 9, 2000. Revised manuscript received September 28, 2000. Accepted September 29, 2000. This study was supported by the Norwegian Research Council (NFR) and the foundation “Norwegian Herb Production” (NUP).

JF000720P