JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. 2003, 51, 7841−7847 7841

Microwave-Assisted Headspace Solid-Phase Microextraction for the Analysis of Bioemissions from *Eucalyptus citriodora* Leaves

GUOHUA XIONG, CAROLYN GOODRIDGE, LIMEI WANG, YONG CHEN, AND JANUSZ PAWLISZYN*

Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

Microwave-assisted headspace solid-phase microextraction (MA-HS-SPME) was developed as a simple and effective method for fast sampling of volatile organic compounds (VOCs) from *Eucalyptus citriodora* Hook (*E. citriodora*) leaves. During microwave heating, a simple shielding device made of aluminum foil was used to protect the SPME fiber from microwave irradiation while allowing the sample to be heated. A room temperature water bath was also used to allow microwave heating to be conducted in a more controlled manner. The inner heating caused by microwave irradiation dramatically accelerated the emission of VOCs from the sample, but no marked change in headspace temperature in the sample vial was found. Under optimum conditions, the extraction efficiencies obtained with microwave heating were much higher than those obtained without microwave heating for all fibers used, namely, 7-μm polydimethylsiloxane (PDMS), 100-μm polydimethylsiloxane (PDMS), 65-μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), and 75-μm carboxen/polydimethylsiloxane (CAR/PDMS). The improvement of extraction efficiency using MA-HS-SPME allowed more VOC events to be detected, with more balanced extraction of VOCs of lower and higher molecular masses. Moreover, a good linear relationship was found between sample size and GC-FID response (total peak area of VOCs), indicating the usefulness of MA-HS-SPME for quantitative analysis of individual volatile compounds in *E. citriodora* leaves.

KEYWORDS: Microwave-assisted headspace solid-phase microextraction (MA-HS-SPME); volatile organic compounds (VOCs); *Eucalyptus citriodora* (*E. citriodora*)

INTRODUCTION

Over the past years, headspace solid-phase microextraction (HS-SPME) has been widely employed for the analysis of volatile organic compounds (VOCs) in a variety of complicated matrices such as environmental, food, and biomedical samples (*1*−*7*). To facilitate HS-SPME, it is essential to have target analytes transferred from the sample matrices into the headspace. Usually, adequate agitation allows good recovery of VOCs from aqueous samples, as does an increase in sample salt concentration or proper adjustment of pH (*8*). However, when HS-SPME is used for samples for which a strong association between native analytes and sample matrix exists, heating may be required to enhance the release of analytes into the headspace phase. Moreover, an increase in the extraction temperature is generally beneficial in speeding the achievement of extraction equilibrium. However, high-temperature extraction may also cause significant deterioration of the coating/sample distribution coefficient, resulting in a decrease in the equilibrium amount of analytes extracted.

To prevent loss of sensitivity during heating, an internally cooled SPME device has been previously developed in our laboratory, which was found to be effective for the quantitative extraction of many volatile analytes from a range of matrices (*9*). However, it involved the use of liquid carbon dioxide and was not compatible with commercial SPME fibers. Recently, Page and Lacroix (*10*) have designed a heating/cooling jacket device for HS-SPME volatile detection in vegetable oils, in which HS-SPME was first conducted for 45 min at 100 °C by heating with steam and then continued for 10 min at 0 °C when ice water was used to cool the SPME fiber. Whereas the use of cooling devices helps to increase the sensitivity of analysis, the use of SPME fibers with new coatings has allowed for extraction at higher temperatures. With the development of strongly adsorptive SPME fibers/coatings such as carboxen/polydimethylsiloxane (CAR/PDMS) and divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), it has been reported that SPME fibers retain their function at relatively high temperatures (40−90 °C) for the extraction of some VOCs (*10*−*12*).

Microwave heating was studied in this work to facilitate HS-SPME of VOCs from solid samples. Specifically, we explored a method to accelerate the evaporation of VOCs from *Eucalyptus*

* Corresponding author [telephone (519) 885-1211; fax (519) 746-0435; e-mail janusz@uwaterloo.ca].

10.1021/jf0346105 CCC: $25.00 © 2003 American Chemical Society
Published on Web 12/06/2003

citriodora Hook (*E. citriodora*) leaves while allowing SPME of the VOCs to be carried out at low temperatures in the headspace and avoiding the use of complicated devices for conditions control. Microwave heating involves internal heating based on conduction and dielectric polarization caused by microwave irradiation (*13*). It therefore not only is more efficient when compared to traditional heating but also may result in an external temperature much lower than that of the sample with the control of the time and output power of microwave irradiation. Recently, microwave-assisted techniques have been described as time saving, energy saving, and highly efficient and have been widely used for sample preparation and chemical syntheses (*14*−*19*). We have developed a method for the generation of standard gas mixtures of VOCs or semi-VOCs based on the use of microwave heating (*20*). Moreover, researchers have combined SPME with microwave-assisted extraction (MAE) for sample preparation and sampling as the combination utilizes the powerful ability of MAE for releasing analytes from solid sample matrices and the rapidity, sensitivity, and selectivity of SPME for the extraction of analytes (*21*−*25*). No studies have been reported previously that involve the combination of microwave heating with HS-SPME.

E. citriodora leaves were selected as model samples in this work on the basis of the following considerations: (1) *Eucalyptus* is among the important vegetations for biogenic emissions in wide regions of the world. Biogenic emissions from plants are among the main sources of VOCs in the atmospheric environment and play an important role in the carbon cycle between the biosphere and atmosphere (*26*). (2) Bioemissions from *E. citriodora* leaves contain a wide range of VOCs. *Eucalyptus* essential oils may be categorized into three classes of commercial importance: the medical oils, which contain substantial amounts of eucalyptol; the industrial oils, containing terpenes, which are used for flotation purposes in mining operations; and the aromatic oils such as *E. citriodora*, which are characterized by their aroma (*27*). (3) Traditional procedures for the preparation of *Eucalyptus* leaf powders for fragrance compound analysis are quite complicated and tedious. (4) SPME has been shown to be a useful tool to monitor VOCs emitted by living leaves of *E. citriodora* trees and has also been used for the analysis of fragrance compounds from leaves of different species of *Eucalyptus* trees (*28*−*33*).

EXPERIMENTAL PROCEDURES

SPME Fibers. Four kinds of commercial SPME fibers provided by Supelco (Bellefonte, PA), namely, 7-μm polydimethylsiloxane (PDMS), 100-μm polydimethylsiloxane (PDMS), 65-μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), and 75-μm carboxen/polydimethylsiloxane (CAR/PDMS), were used.

Sample Collection and Storage. The leaves of *E. citriodora* were freshly collected from living trees in a greenhouse of the University of Waterloo (20−25 leaves were collected at each sample time). Leaves were immediately cut into small pieces (~5 × 5 mm^2) and homogenized. Controls were analyzed without storage, and the remaining samples were tested following storage at 4 °C. To avoid release of bio-VOCs and to protect the sample from contamination during storage, the small pieces of *E. citriodora* leaves were carefully wrapped in clean aluminum foil sealed with Teflon tape and were placed in 100-mL capped glass bottles.

Microwave-Assisted HS-SPME (MA-HS-SPME) Setup. A domestic microwave oven (Samsung model MW5490W, 1000 W) was used as the microwave source. The power output ranged from level 1 (lowest) to level 10 (highest). A 40-mL glass vial (~8.5-cm high) was used to hold *E. citriodora* leaves, which was capped with an open top with a Teflon-faced septum. When HS-SPME was conducted, the SPME fiber was exposed to the headspace above the leaves. As the SPME holder was not required for this work, the tensing spring was removed from the fiber assembly. A simple device was designed to shield the SPME fiber from microwave irradiation (**Figure 1**).

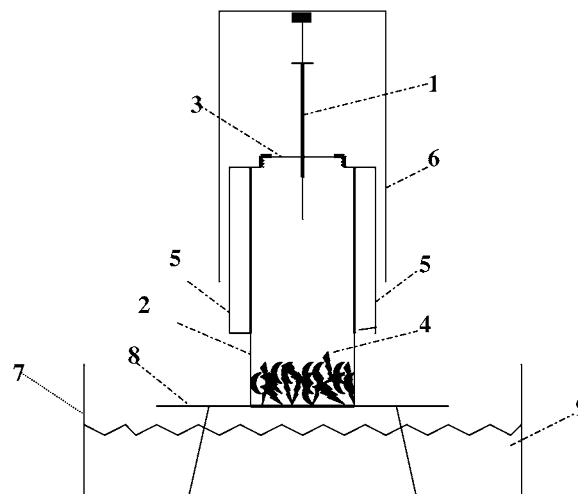

Figure 1. Shielding device for MA-HS-SPME used in domestic microwave oven: 1, commercial SPME fiber; 2, 40-mL glass vial; 3, open top with a Teflon-faced septum; 4, *E. citriodora* leaves (small pieces via cutting); 5, bilayer shelter; 6, aluminum foil shelter; 7, water bath; 8, ceramic holder; 9, tap water.

Measurement of Headspace Temperature Inside the Sample Vial. A thermocouple (Omega, Stamford, CT) was employed to measure the temperature change caused by microwave heating in the headspace inside the sample vial. Two small holes with a diameter of ~1.5 mm were made on the Teflon-faced septum in the sample vial. Two layers of Teflon tape were used to seal the holes and then fixed during microwave heating. Immediately following microwave heating, the thermocouple was inserted into the sample vial through the small holes on the Teflon-faced septum by piecing the Teflon tape. The tips of the thermocouple were positioned at a height in the sample vial similar to the height of SPME fiber. Finally, the thermometer reading was recorded when the thermocouple was stable. The whole procedure for each temperature measurement lasted 30−40 s.

GC-FID and GC-MS Analysis. For general analysis, a Varian model 3400 GC equipped with a flame ionization detector (FID) was employed, using an SPB-5 capillary column (30 m × 0.25 mm × 1 μm) from Supelco (Bellefonte, PA). Hydrogen was used as carrier gas at 30 psi. The column was programmed from 50 to 250 °C at 10 °C/min. The detector was maintained at 280 °C. For PDMS and PDMS/DVB fibers, the injector was controlled at 250 °C and the desorption time was 1 min, whereas the CAR/PDMS fiber was desorbed for 2 min at 300 °C.

For compound identification, a 6890 series GC system equipped with a 5973 mass selective detector (MSD) was used (Agilent, Palo Alto, CA). The conditions for the GC system were as follows: HP-MS5 column (30 m × 0.25 mm × 0.25 μm); helium at 10 psi as carrier gas (constant pressure mode); splitless injection; inlet temperature at 300 °C; and the column/oven at 50 °C initially and ramped to 250 °C at 10 °C/min. The conditions for the MSD were as follows: TIC mode; EM voltage at 1600 V; Aux-2 temperature at 280 °C; quadruple temperature at 150 °C; and MS source temperature at 230 °C.

RESULTS AND DISCUSSION

Protecting the SPME Fiber from Microwave Irradiation. The major challenge in coupling microwave heating with HS-SPME was prevention of damage to the SPME fiber by microwave irradiation. Microwaves are unable to penetrate or be absorbed by metal materials, but both the septum-piercing needle and the fiber attachment tubing of the commercial SPME fiber were composed of steel and had sharp shapes. Point discharge would therefore occur when the fiber was put under microwave irradiation, leading to fiber damage.

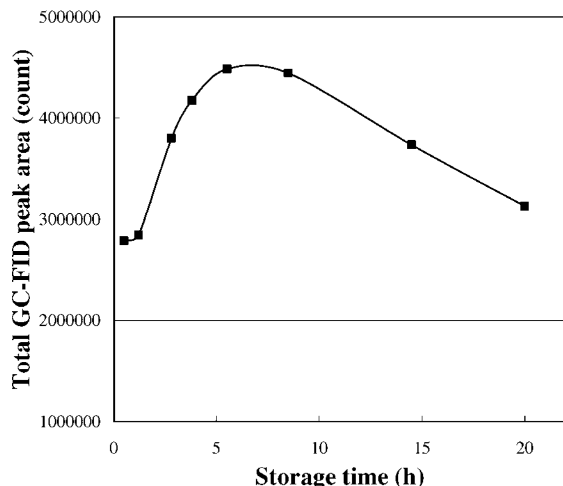

Figure 2. Change in bio-VOC concentration in the sample vial during storage of *E. citriodora* leaves at room temperature. 0.500 g *E. citriodora* leaves was stored in a 40-mL vial at room temperature (24.5 °C). The SPME fiber used was 100-μm PDMS, and sampling was conducted using HS-SPME and a 3-min extraction time.

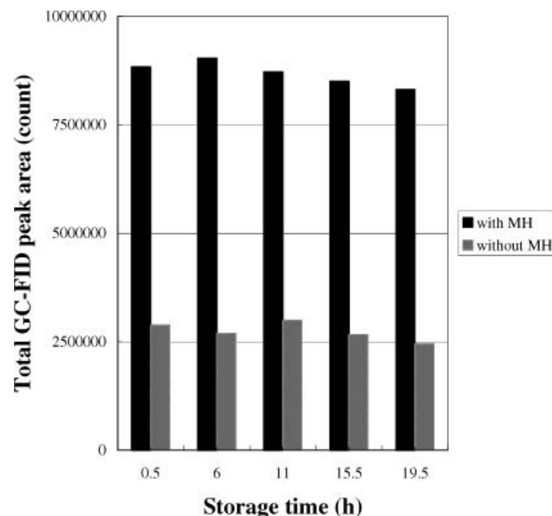

Figure 3. Bioemission of *E. citriodora* leaves after storage at low temperature. After storage in a refrigerator at 4 °C, a 0.500-g aliquot of *E. citriodora* leaves was weighed and transferred into a 40-mL vial. The sample was warmed for 40 min at room temperature (24.5 °C) prior to HS-SPME or MA-HS-SPME. The SPME fiber used was 100-μm PDMS, and HS-SPME extraction was for 3 min. For cases with microwave heating (MH), the power output was at level 4 (medium-low), the heating time was 2 min, and a shielding device was used as shown in **Figure 1**.

Figure 1 illustrates a simple shielding device designed to protect the SPME fiber from microwave irradiation. A thin layer of Styrofoam covered with aluminum foil formed a removable bilayer shelter (*5*), which embraced the upper part of the glass vial but allowed the bottom part with a height of ~2.5 cm to accept microwave irradiation. Another shelter (*6*) was a one-end-capped tube made of aluminum foil and was easily removed from the SPME fiber. A circular glass container (diameter = 15 cm; height = 6.5 cm) containing 300 mL of tap water was used to protect the fiber from the vertical reflection of microwave from the bottom face inside the oven. The water was at room temperature initially and was replenished for each run. When microwave heating coupled with SPME was conducted, the vial loaded with sample was placed over the water (*7*) supported by a ceramic holder (*8*). The timing of the SPME procedure commenced from exposure of the SPME fiber to the headspace in the sample vial. It typically took ~10 s from starting timing to the beginning of microwave heating. SPME generally continued for an additional 50 s after the end of microwave heating.

It was demonstrated that no point discharge or damage to SPME fibers occurred with the use of the simple shielding device during MA-HS-SPME.

Sample Behavior. The amount of VOCs emitted from *E. citriodora* leaves varied among leaves. To gather representative results of VOCs, at least 20 pieces of *E. citriodora* leaves, including young and old ones, were picked each time. As not all of the sample could be analyzed immediately after collection, some of the samples had to be stored prior to analysis. However, bioemission activity does not stop during sample storage. **Figure 2** shows that the VOC concentration in the headspace phase above 0.5 g of ground leaves of *E. citriodora* (cut into ca. 5 × 5 mm^2 per piece) changed dramatically during sample storage at room temperature. This variation was attributed to bioemission as well as sorption−desorption of VOCs between the gas phase, the *E. citriodora* leaf matrix, and the inner wall of the vial.

To minimize VOC emission from *E. citriodora* leaves during storage, the sample was stored at 4 °C. **Figure 3** shows that storage at 4 °C for 0.5−19.5 h did not cause a significant increase in VOC emissions from *E. citriodora* leaves. The relative standard deviation (RSD) of the total GC-FID area of the VOCs for five tests corresponding to different sample and storage times was 7.7%.

Table 1. Changes in Headspace Phase Temperature in the Sample Vial with Microwave Heating[a]

output level of microwave irradiation	temperature of headspace phase (°C)			
	with water bath		without water bath	
	2-min MH	3-min MH	2-min MH	3-min MH
4 (medium-low)	26.5 ± 1.3	38.3 ± 3.8	41.8 ± 2.6	84.5 ± 3.3
7 (medium-high)	35.1 ± 2.2	67.6 ± 3.1	62.0 ± 1.8	91.5 ± 3.7

[a] 0.500 g of *E. citriodora* leaves in a 40 mL sample vial; water bath, 300 mL; initial temperature, 24.5 °C. The shielding device as shown in **Figure 1** was used, but the one-end-capped shelter (part 6 in **Figure 1**) was avoided. Number of parallel tests: $n = 3$.

According to a preliminary experiment, the fresh *E. citriodora* leaves decreased by 58.0% in weight following exposure to air for 48 h at room temperature. It was believed that the lost materials comprised mainly moisture and small quantities of VOCs. The high moisture in *E. citriodora* leaves make them effective at absorbing microwave energy. Low-temperature storage of *E. citriodora* leaves did not significantly affect microwave-assisted evaporation of VOCs (**Figure 3**). The RSD of total the GC-FID area of these tests was 3.2%, lower than that obtained without microwave heating.

Temperature Control and Microwave-Assisted Evaporation of VOCs. During microwave heating, the temperature of the headspace is generally lower than that of the sample matrix before the thermal transfer equilibrium between sample matrix and its headspace phase is reached. For traditional heating systems, it is difficult to produce such a temperature difference without the use of a special cooling system.

The temperatures of the headspace above *E. citriodora* leaves were tested immediately following a microwave irradiation for 2 or 3 min in a commercial microwave oven. **Table 1** indicates that microwave heating yielded a much lower headspace temperature inside the sample vial when the water bath was

used compared to when the water bath was not used. The water bath absorbed excessive microwave irradiation inside the oven, thus allowing microwave heating to be conducted in a more controlled manner. It is interesting to note that a 2-min microwave heating at medium-low output caused a slight increase of the headspace temperature (∼2 °C) when the water bath was employed, whereas the temperature increase was 17 °C without the water bath. In contrast, microwave heating at medium-high output yielded an 11 °C increase in the headspace phase temperature when the water bath was used. A 3-min microwave heating introduced a marked change of the headspace temperature for all cases studied. For example, when the microwave output was set at medium-low and the water bath was used, a 14 °C increase in headspace temperature was observed.

HS-SPME of VOCs was found to be compatible with microwave heating through the use of a simple device designed to protect the SPME fiber. To avoid a marked increase of the headspace phase temperature, a 2-min microwave heating at medium-low output and a 3-min total extraction time (i.e., 10 s prior to microwave heating, 2 min while microwave heating, and 50 s after microwave heating) were employed. **Figure 3** demonstrates that with the use of a 100-μm PDMS fiber for the extraction of VOCs from *E. citriodora* leaves, the total peak area (based on GC-FID results) of the extracted VOCs obtained with microwave heating was 3.1 times greater than that obtained without microwave heating. The results indicate that it is possible to dramatically increase the sensitivity of VOC analysis using MA-HS-SPME.

HS-SPME of VOCs with Different Fibers. Four kinds of SPME fibers, 7-μm PDMS, 100-μm PDMS, 65-μm PDMS/DVB, and 75-μm CAR/PDMS, were investigated for HS-SPME analysis of VOCs from *E. citriodora* leaves. When the extraction time was set as 3 min without microwave heating, the extraction amounts of VOCs were very similar whether a 100-μm PDMS, 65-μm PDMS/DVB, or a 75-μm CAR/PDMS fiber was used. However, a 7-μm PDMS fiber yielded a much lower extraction amount, only 8.8−10.3% compared with those obtained by using other fibers. With 2 min of microwave heating at medium-low during a 3-min HS-SPME extraction time, the extraction amounts increased to 4.4, 3.1, 2.9, and 2.9 times as much as those obtained without microwave heating for the 7-μm PDMS, 100-μm PDMS, 65-μm PDMS/DVB, and 75-μm CAR/PDMS fibers, respectively (**Figure 4**). Microwave heating was found to be beneficial for extraction efficiency using all four SPME fibers when they were used for VOC extraction, demonstrating the suitability of all the fibers for MA-HS-SPME.

The emissions from *E. citriodora* leaves were composed of two separate groups (**Figure 5**). Group 1 (G1) contained compounds with retention times of <10 min, corresponding to compounds with lower molecular masses, and group 2 (G2) contained compounds with retention times of >10 min, corresponding to compounds with higher molecular masses. The ratio of total GC-FID peak area of the two groups was constant and closely related to SPME fiber type. The ratios of total peak area of G1 compounds to G2 compounds were found to be 1.1, 9.1, 9.5, and 15.3 for 7-μm PDMS, 100-μm PDMS, 65-μm PDMS/DVB, and 75-μm CAR/PDMS fibers, respectively, when 3-min SPME of VOCs from *E. citriodora* leaves was carried out at room temperature (**Figure 6**). Among these fibers, the 7-μm PDMS fiber yielded the most balanced extraction of G1 and G2 VOCs. It is understandable due to its thin coating film. The equilibrium time of VOC extraction with the 7-μm PDMS fiber was much shorter compared to other kinds of fibers, although

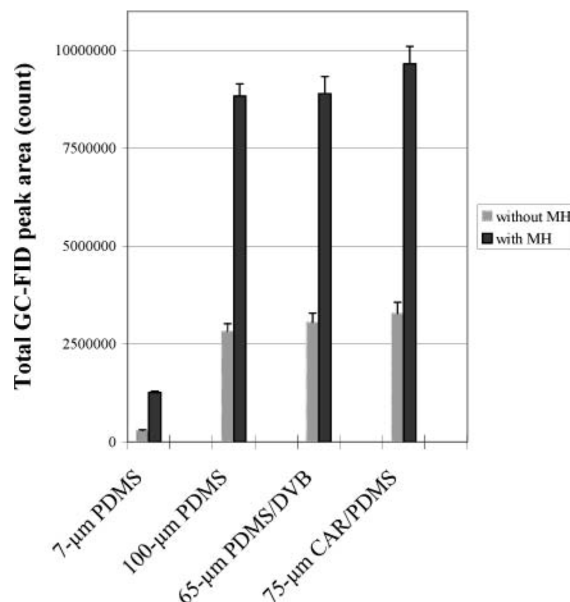

Figure 4. Efficiency of HS-SPME of VOCs from *E. citriodora* leaves with different SPME fibers. 0.500 g of *E. citriodora* leaves in a 40-mL vial was subjected to HS-SPME extraction for 3 min. For cases with microwave heating (MH), the power output was at level 4 (medium-low), the heating time was 2 min, and a shielding device was used as shown in **Figure 1**.

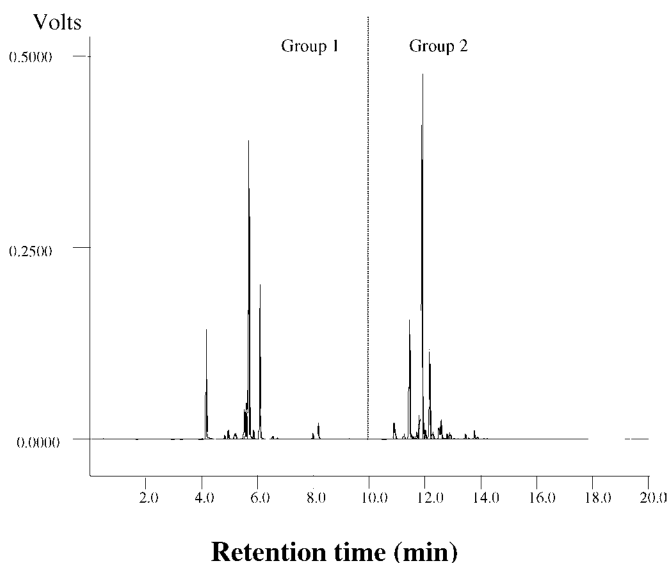

Figure 5. GC-FID chromatograms of bio-VOCs from *E. citriodora* leaves. 0.500 g of *E. citriodora* leaves in a 40-mL vial was used. The SPME fiber used was 7-μm PDMS, and HS-SPME extraction was for 3 min. For cases with microwave heating (MH), the power output was at level 4 (medium-low), the heating time was 2 min, and a shielding device was used as shown in **Figure 1**.

a 3-min SPME at room temperature is likely nonequilibrium extraction. Thus, this fiber is especially beneficial for high molecular weight compound extraction as these compounds typically take a longer time to reach equilibrium status when other kinds of SPME fibers are used. The ratio (G1/G2) was as high as 15 when the 75-μm CAR/PDMS fiber was employed as the molecular sieve, Carboxen coating is reported to be highly efficient in trapping volatiles.

In addition to its dramatic increase in VOC total extraction amount, microwave heating was also highly efficient at improving the balance of extracted compounds of the two groups. The ratios of total peak area of G1 compounds to G2 compounds

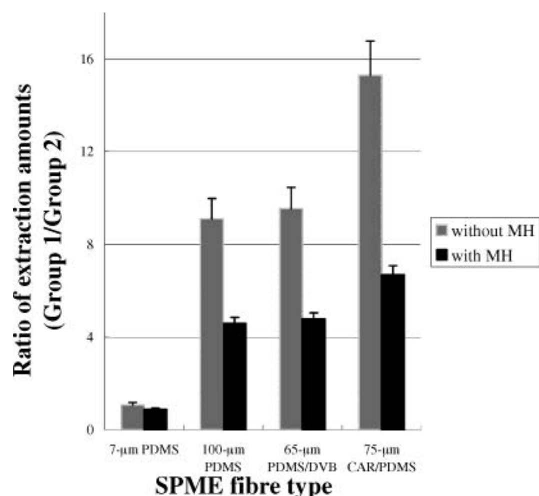

Figure 6. Effect of microwave heating on the ratio of HS-SPME efficiency (group 1 VOCs to group 2 VOCs). 0.500 g of *E. citriodora* leaves in a 40-mL vial was subjected to HS-SPME extraction for 3 min. For cases with microwave heating (MH), the power output was at level 4 (medium-low), the heating time was 2 min, and a shielding device was used as shown in **Figure 1**.

(G1/G2) changed to 0.89, 4.6, 4.8, and 6.7 for the 7-μm PDMS, 100-μm PDMS, 65-μm PDMS/DVB, and 75-μm CAR/PDMS fibers, respectively, with 2 min of microwave heating at microwave output level 4 (medium-low) during a 3-min HS-SPME extraction of VOCs from *E. citriodora* leaves (**Figure 6**). This may be due to the well-distributed evaporation by inner heating with microwave irradiation, achieved by all VOCs investigated.

Comparison of HS-SPME of VOCs with Microwave Heating and with Traditional Heating. Jia et al. (*2*) clearly demonstrated that the amount of flavor compounds extracted from orange juice by HS-SPME using a 100-μm PDMS fiber decreased with the increase of extraction temperature from 25 to 80 °C. It was also reported that the higher the extraction temperature, the shorter the time to reach equilibrium. However, recent studies have demonstrated that CAR/PDMS and DVB/CAR/PDMS fibers, two relatively new commercial fibers having SPME extraction mechanisms classified as adsorption, possess an unusual ability to resist the negative effects of increasing extraction temperature (*10*−*12*). Thus, it is meaningful to make a comparison of HS-SPME of VOCs from *E. citriodora* leaves with microwave or traditional heating by using the 75-μm CAR/PDMS fiber.

MA-HS-SPME was carried out in same way as described above. HS-SPME efficiency, measured by total GC-FID peak area, increased with the increase in the power output of microwave heating (**Figure 7**). When microwave heating was at its highest level, the efficiency of HS-SPME of VOCs was 5.6 times greater than that obtained without heating. Traditional heating also markedly improved the HS-SPME efficiency, but no significant difference was observed between water bath temperatures of 70 and 90 °C. The extraction efficiencies obtained in these cases were slightly higher than those values obtained with microwave heating at medium-low output level, ~3.2 times greater than those obtained without heating. In short, the efficiency of HS-SPME of VOCs with microwave heating at the highest output level was >170% more efficient when compared to traditional heating at a water bath temperature of 70 or 90 °C. This is understandable because, as discussed above, microwave heating is highly beneficial to HS-SPME due to the high efficiency of inner heating and the temperature difference between sample matrix and headspace phase.

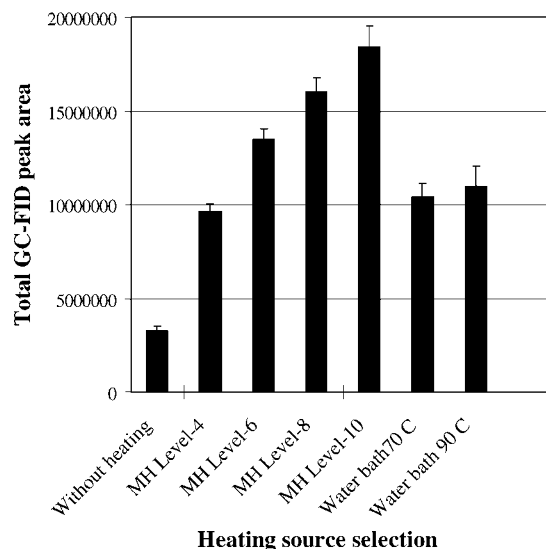

Figure 7. Comparison of HS-SPME efficiencies obtained with microwave heating and with water-bath heating. 0.500 g of *E. citriodora* leaves in a 40-mL vial was used. The SPME fiber used was 75-μm CAR/PDMS, and HS-SPME extraction was for 3 min. For microwave heating (MH), the power output was at level 4 (medium-low), the heating time was 2 min, and a shielding device was used as shown in **Figure 1**. For cases with water-bath heating, the heating time was 2 min.

Table 2. GC-FID Peaks of Bio-VOCs from *E. citriodora* Leaves Extracted by HS-SPME with and without Microwave Heating[a]

HS-SPME conditions		GC-FID peaks of group 1	GC-FID peaks of group 2	total GC-FID peaks
7-μm PDMS	without MH	15 ± 0	22 ± 1	37 ± 1
	with MH	18 ± 1	32 ± 1	50 ± 1
100-μm PDMS	without MH	30 ± 1	23 ± 0	53 ± 1
	with MH	64 ± 0	36 ± 1	100 ± 1
65-μm PDMS/DVB	without MH	38 ± 1	24 ± 1	62 ± 1
	with MH	65 ± 1	36 ± 0	101 ± 1
75-μm CAR/PDMS	without MH	43 ± 1	23 ± 0	65 ± 1
	with MH	68 ± 1	36 ± 1	104 ± 1

[a] *E. citriodora* leaves (0.500 g) were stored in a 40-mL vial for 40 min prior to HS-SPME or MA-HS-SPME. Extraction time = 3 min. Microwave heating (MH): 2 min at power output level 4 (for cases MH applied). Threshold value of GC-FID peak = 500 (counts). Number of parallel tests: $n = 3$.

Qualitative and Quantitative Analysis of VOCs. Qualitative analysis of VOCs from *E. citriodora* leaves was conducted. For all four kinds of SPME fibers investigated, MA-HS-SPME detected more VOCs compared to HS-SPME without microwave heating (**Table 2**). For example, 100 VOC events were detected using a 100-μm PDMS fiber coupled with MA-HS-SPME, whereas only 53 VOC events were detected without the use of microwave heating (the threshold value for GC-FID detection was set as 500 counts). This result could be attributed to increased bioemissions caused by microwave-assisted evaporation.

Sample size is generally an important factor that should be carefully controlled during quantitative analysis. To ensure all *E. citriodora* leaves inside the sample vial were exposed to microwave irradiation, the maximum sample size was 1 g. **Figure 8** shows a strong linear relationship between a sample size of 0.1−0.8 g and GC-FID response when a 100-μm PDMS fiber was used with the selected extraction conditions. This

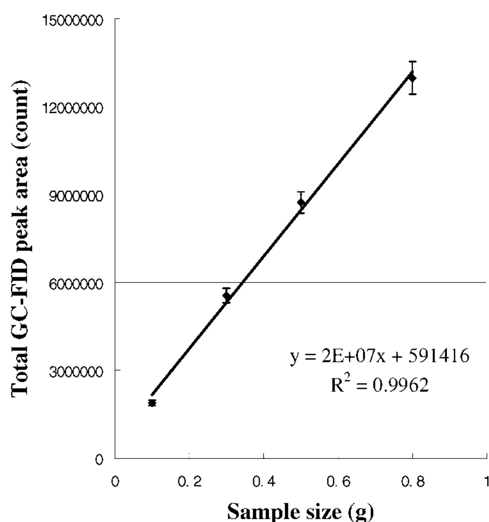

Figure 8. GC-FID signal versus sample size for MA-HS-SPME. 0.1–0.8 g of *E. citriodora* leaves in a 40-mL vial was subjected to HS-SPME extraction for 3 min using a 100-μm PDMS fiber. The power output of microwave heating was at level 4 (medium-low); the heating time was 2 min, and a shielding device was used as shown in **Figure 1**.

implies that MA-HS-SPME is potentially useful for quantitative analysis of individual fragrance compounds in *E. citriodora* leaves.

Due to natural emission and microwave-assisted evaporation of VOCs from *E. citriodora* leaves, their concentrations in the headspace inside the sample vial vary between time points during MA-HS-SPME extraction. Inner standards spiked into the samples are necessary for quantitative analysis of the VOCs. In this work, a standard solution of α-pinene, eucalyptol, and γ-terpinene in methanol was spiked into the *E. citriodora* leaves. A syringe was used to spray the standard solution onto the leaves. The recovery of the spiked standards had a RSD of 45% ($n = 3$) (data not given here). Because MA-HS-SPME demonstrated strong reproducibility (described above), this variation was likely caused by the nonhomogeneous nature of the standard spiking. Therefore, a focus in future work will be to define a method in which standards can be more homogeneously distributed in *E. citriodora* leaves. Also, standardization of incubation time of standards with samples will need to be carefully considered in order to achieve representative results. Most recently, Ezquerro et al. developed a multiple HS-SPME for the quantitative determination of VOCs in multilayer packagings (*34*), which might be adapted to MA-HS-SPME with some modification.

Conclusion. MA-HS-SPME has been developed via on-line combination of microwave heating and SPME for extraction of VOCs from *E. citriodora* leaves. The devices employed were simple in design and easily obtainable. No complicated heating/cooling devices were involved for temperature control. Increasing extraction efficiency of MA-HS-SPME was obtained compared to HS-SPME without microwave heating. MA-HS-SPME also yielded a more balanced extraction for VOCs with lower and higher molecular masses. This method was shown to be fast and effective for VOC sampling of *E. citriodora* and shows promise for use in more diverse samples.

LITERATURE CITED

(1) Zhang, Z.; Pawliszyn, J. Headspace solid-phase microextraction. *Anal. Chem.* **1993**, *65*, 1843−1852.

(2) Jia, M.; Zhang, Q. H.; Min, D. B. Optimization of solid-phase microextraction analysis for headspace flavor compounds of orange juice. *J. Agric. Food Chem.* **1998**, *46*, 2744−2747.

(3) Zygmunt, B.; Jastrzebska, A.; Namiesnik, J. Solid-phase microextraction—a convenient tool for the determination of organic pollutants in environmental matrices. *Crit. Rev. Anal. Chem.* **2001**, *31*, 1−18.

(4) Ulrich, S. Solid-phase microextraction in biomedical analysis. *J. Chromatogr. A* **2000**, *902*, 167−194.

(5) Mills, G. A.; Walker, V. Headspace solid-phase microextraction procedures for gas chromatographic analysis of biological fluids and materials. *J. Chromatogr. A* **2000**, *902*, 267−287.

(6) Pawliszyn, J., Ed. *Applications of Solid-phase Microextraction*; The Royal Society of Chemistry: Cambridge, U.K., 1999.

(7) Wercinski, S. C. S., Ed. *Solid-Phase Microextraction—A Practical Guide*; Dekker: New York, 1999.

(8) Pawliszyn, J. *Solid-phase Microextraction: Theory and Practice*; Wiley-VCH: New York, 1997.

(9) Zhang, Z.; Pawliszyn, J. Quantitative extraction using an internally cooled solid-phase microextraction device. *Anal. Chem.* **1995**, *67*, 34−43.

(10) Page, B. D.; Lacroix, G. Analysis of volatile contaminants in vegetable oils by headspace solid-phase microextraction with Carboxen-based fibres. *J. Chromatogr. A* **2000**, *873*, 79−94.

(11) Brunton, N. P.; Cronin, D. A.; Monahan, F. J. The effects of temperature and pressure on the performance of Carboxen/PDMS fibres during solid-phase microextraction (SPME) of headspace volatiles from cooked and raw turkey breast. *Flavour Fragrance J.* **2001**, *16*, 294−302.

(12) Lee, J.-H.; Diono, R.; Kim, G.-Y.; Min, D. B. Optimization of solid-phase microextraction analysis for the headspace volatile compounds of Parmesan cheese. *J. Agric. Food Chem.* **2003**, *51*, 1136−1140.

(13) Mingos, D. M. P.; Baghurst, D. R. Applications of microwave dielectric heating effects to synthetic problems in chemistry. *Chem. Soc. Rev.* **1991**, *20*, 1−47.

(14) Kingston, H. M., Haswell, S. J., Eds. *Microwave-Enhanced Chemistry—Fundamentals, Sample Preparation and Applications*; American Chemical Society: Washington, DC, 1997.

(15) Richter, R. C.; Link, D.; Kingston, H. M. Microwave-enhanced chemistry. *Anal. Chem.* **2000**, *73*, 30A−37A.

(16) Smith, F.; Xiong, G. The use of microwave-assisted techniques for samples preparation in environmental organic analysis. In *Encyclopedia of Analytical Chemistry*; Meyers, R. A., Ed.; Wiley: Chichester, U.K., 2000; pp 2903−2914.

(17) Lopez-Avila, V. Sample preparation for environmental analysis. *Crit. Rev. Anal. Chem.* **1999**, *29* (3), 195−230.

(18) Ericsson, M.; Colmsjö, A. Dynamic microwave-assisted extraction coupled on-line with solid-phase extraction and large-volume injection gas chromatography: determination of organophosphate esters in air samples. *Anal. Chem.* **2003**, *75*, 1713−1719.

(19) Prevot, A. B.; Gulmini, M.; Zelano, V.; Pramauro, E. Microwave-assisted extraction of polycyclic aromatic hydrocarbons from marine sediments using nonionic surfactant solutions. *Anal. Chem.* **2001**, *73*, 3790−3795.

(20) Xiong, G.; Pawliszyn, J. Microwave-assisted generation of standard gas mixtures. *Anal. Chem.* **2002**, *74*, 2446−2449.

(21) Wang, Y.; Bonilla, M.; McNair, H. M.; Khaled, M. Solid-phase microextraction associated with microwave assisted extraction of food products. *J. High Resolut. Chromatogr.* **1997**, *20* (4), 213−216.

(22) Ho, W.-H.; Hsieh, S.-J. Solid-phase microextraction associated with microwave assisted extraction of organochlorine pesticides in medicinal plants. *Anal. Chim. Acta* **2001**, *428* (1), 111−120.

(23) Falqui-Cao, C.; Wang, Z.; Urruty, L.; Pommier, J.-J.; Montury, M. Focused microwave assistance for extracting some pesticide residues from strawberries into water before their determination by SPME/HPLC/DAD. *J. Agric. Food Chem.* **2001**, *49*, 5092−5097.

(24) Grimm, C. C.; LIoyd, S. W.; Zimba, P. V.; Palmer, M. Microwave distillation−solid-phase microextraction gas chromatographic analysis of geosmin and 2-methylisoborneol in catfish. *Am. Lab.* **2000**, *32* (3), 40, 42, 44, 46, 48.

(25) Barrero, M.; Bazemore, R. A.; Silva, J.; Grimm, C.; Yoon, Y. Extraction methods for determination of geosmin and 2-MIB in channel catfish flesh. *Abstracts of Papers*, 223rd National Meeting of the American Chemical Society, Orlando, FL; ACS: Washington, DC, 2002; AGFD-036.

(26) Kesselmeier, J.; Staudt, M. Biogenic volatile organic compounds (VOC): an overview on emission, physiology and ecology. *J. Atmos. Chem.* **1999**, *33* (1), 23−28.

(27) Boland, D. J., Brophy, J. J., House, A. P. N., Eds. *Eucalyptus Leaf Oils*−*Use, Chemistry, Distillation and Marketing*; Inkata Press: Melbourne, Australia, 1991.

(28) Wirthensohn, M. G.; Sedgley, M.; Jones, G. P. Epicuticular wax of juvenile Eucalyptus leaves and headspace analysis of leaf volatiles. *J. Essent. Oil Res.* **2000**, *12*, 401−411.

(29) Verzera, A.; Campisi, S.; Zappala, M.; Bonaccorsi, I. SPME-GC-MS analysis of honey volatile components for the characterization of different floral origin. *Am. Lab.* **2001**, *33* (15), 18, 20−21.

(30) Zini, C. A.; Augusto, F.; Christensen, E.; Smith, B. P.; Caramao, E. B.; Pawliszyn, J. Monitoring biogenic volatile compounds emitted by *Eucalyptus citriodora* using SPME. *Anal. Chem.* **2001**, *73*, 4729−4735.

(31) Zini, C. A.; Lord, H.; Christensen, E.; De Assis, T. F.; Caramao, E. B.; Pawliszyn, J. Automation of solid-phase microextraction−gas chromatography−mass spectrometry extraction of Eucalyptus volatiles. *J. Chromatogr. Sci.* **2002**, *40* (3), 140−146.

(32) Zini, C. A.; Zanin, K. D.; Christensen, E.; Caramao, E. B.; Pawliszyn, J. Solid-phase microextraction of volatile compounds from the chopped leaves of three species of *Eucalyptus*. *J. Agric. Food Chem.* **2003**, *51*, 2679−2686.

(33) Zini, C. A.; Augusto, F.; Christensen, E.; Caramao, E. B.; Pawliszyn, J. SPME applied to the study of volatile organic compounds emitted by three species of *Eucalyptus* in situ. *J. Agric. Food Chem.* **2002**, *50*, 7199−7205.

(34) Ezquerro, Ó; Pons, B.; Tena, M. T. Multiple headspace solid-phase microextraction for the quantitative determination of volatile organic compounds in multilayer packagings. *J. Chromatogr. A* **2003**, *999* (1−2), 155−164.

Received for review June 9, 2003. Revised manuscript received September 25, 2003. Accepted October 5, 2003. This project was financially supported by the Naturaal Science and Engineering Research Council (NSERC), Canada.

JF0346105