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Development of gas chromatography–mass spectrometry with microwave distillation and simultaneous solid-phase microextraction for rapid determination of volatile constituents in ginger

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Abstract

In this study, gas chromatography–mass spectrometry (GC–MS) following microwave distillation and solid-phase microextraction (MD–SPME) was developed for the analysis of essential oil compounds in fresh ginger. In the proposed method, the isolation, extraction and concentration of volatile components in ginger were carried out in one single step, using the MD–SPME technique, and the analytes on the SPME fiber were analyzed by GC–MS. Some parameters, including SPME fiber coating, microwave power and irradiation time, were optimized. The optimal experiment parameters obtained were: 65 μm PDMS/DVB SPME fiber, a microwave power of 400 W and an irradiation time of 2 min. To demonstrate its feasibility, MD–SPME was compared with conventional SPME for the extraction of essential oil compounds in fresh ginger. Using MD–SPME followed by GC–MS, 54 compounds were separated and identified in ginger, which mainly included geranial (5.25%), zingiberene (15.48%), β-sesquiphellandrene (5.54%) and β-phellandrene (22.84%), whereas only 39 compounds were separated and identified by conventional SPME followed by GC–MS. The relative standard deviation (R.S.D.) values of less than 10% show that the proposed method has good repeatability. The result show that MD–SPME, followed by GC–MS, is a simple, rapid, solvent-free method for the determination of volatile compounds in ginger.

Keywords: Ginger; Essential oil; Microwave distillation; Solid-phase microextraction; Gas chromatography–mass spectrometry

1. Introduction

Ginger (*Zingiber officinale*) has a long history of being used as a spice and as a medicinal plant. It is cultivated on a large scale worldwide, including India, China, Jamaica and Nigeria. In China, ginger is a common traditional Chinese medicine (TCM), which is used for the treatment of many diseases, such as the common cold. In recent years, more and more pharmaceutical effects have been found about ginger. It can act as an aphrodisiac, a carminative, a rubifacient, an anti-asthmatic and as a stimulant to the gastrointestinal tract [1]. Ginger is often used for the treatment of stomachache, and cardiovascular and motor diseases. It also possesses anti-inflammatory activity and regulates bacterial growth, as well as providing protection for immune-depressed patients, such as individuals who are HIV positive [2,3]. Many active components, such as Zingiberene, have been found in the essential oil of ginger [4–6].

Various methods can be used for the isolation and extraction of essential oils from plant materials, including TCMs, which mainly include solvent extraction [7], supercritical fluid extraction (SFE) [8,9] and liquid-phase microwave-assisted process (MAP) extraction [10]. However, these methods always lead to the loss of some volatile compounds, low extraction efficiency, toxic solvent residues and are time-consuming. So, the development of simple, rapid and solvent-free methods for the analysis of essential oils is highly desirable.

Headspace solid-phase microextraction (HS–SPME) is a relatively new sampling and concentration technique for the extraction of plant essential oils [11–15]. In our previous studies [16–22], this technique has successfully been developed for the analysis of essential oils in TCMs. HS–SPME followed by gas chromatography–mass spectrometry (GC–MS) has been proven to be a simple, sensitive and solvent-free method for the analysis of TCM essential oils. However, conventional HS–SPME still

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requires about 30 min for the extraction of essential oil compounds in TCMs. Moreover, HS–SPME is not suitable for some semi-volatile compounds in TCMs [20–22].

The microwave-assisted extraction (MAE) technique was developed and applied to the isolation of volatile and active compounds from plant materials [23–26]. The main advantage of MAE is the reduction of extraction time and organic solvent production. Recently, a solvent-free technique-microwave distillation (SFMD)—was developed for the rapid extraction of essential oils in fresh plant material [27,28]. More recently, microwave distillation with concurrent solid-phase microextraction (MD–SPME) was first introduced for the successful isolation and concentration of essential oil components from *Artemisia selengensis* Turcz [29]. The MD–SPME technique combines the advantages of MAP and SPME, so it has a high extraction efficiency, no need for organic solvent, a small amount of sample and short extraction times.

In this work, MD–SPME was developed for the analysis of volatile compounds in fresh ginger, a TCM. Essential oils in ginger were isolated, extracted and concentrated by using MD–SPME, and analyzed by GC–MS. MD–SPME parameters, including SPME fiber coating, microwave power, and irradiation time, were studied. To demonstrate its feasibility, conventional HS–SPME was also applied to the analysis of essential oils in this TCM.

2. Materials and methods

2.1. Plant material, SPME fibers and MD–SPME apparatus

Fresh ginger rhizome samples were obtained from Yunnan (China) and used for MD–SPME. The following SPME fibers were used: 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 65 μm carbowax/divinylbenzene (CW/DVB), 75 μm carboxen poly(dimethylsiloxane) (CAR/PDMS) and 85 μm polyacrylate (PA); all were purchased from Supelco (Bellefonte, PA, USA). The microwave oven with a maximum delivery power of 700 W was purchased from Sanyo Company (Japan).

2.2. Optimization of the MD–SPME parameters

The three parameters of microwave power, SPME fiber coating and irradiation time can affect extraction efficiency. So, these parameters were studied. A mass of 1.0 g fresh ginger was ground to fine powder, and then introduced into a 25-ml glass bottle. First, selection of the optimum fiber was performed. The five fibers of PDMS, PDMS/DVB, CW/DVB, CAR/PDMS and PA were tested using the same microwave parameters: power of 400 W and irradiation time of 2.0 min. Then, microwave power (200, 400 and 700 W) and irradiation time (1, 2, 4 and 6 min) were investigated.

2.3. Analysis of essential oil in ginger by MD–SPME and conventional HS–SPME

The optimal parameters of PDMS/DVB fiber, microwave power of 400 W and irradiation time of 2 min were used for MD–SPME of the essential oils in fresh ginger (1.0 g). The analytes extracted on the fiber were desorbed at GC injector (250 °C for 3 min) and then analyzed by GC–MS.

To demonstrate this method's feasibility, 1 g ginger was ground to fine powder, and then put into a 25-ml glass bottle. Extraction of essential oil in fresh ginger was performed using

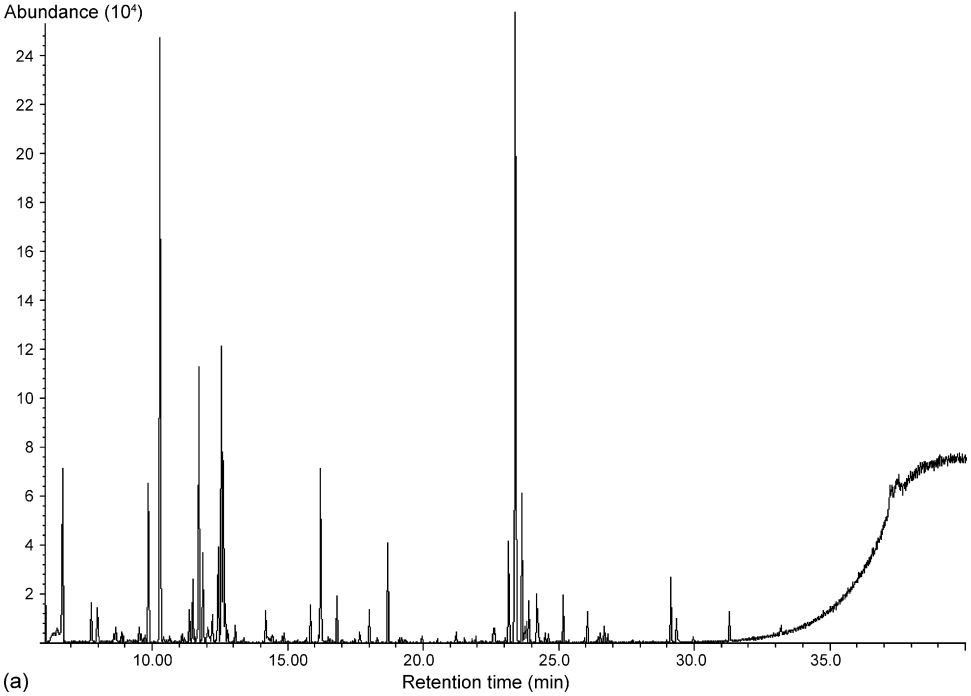
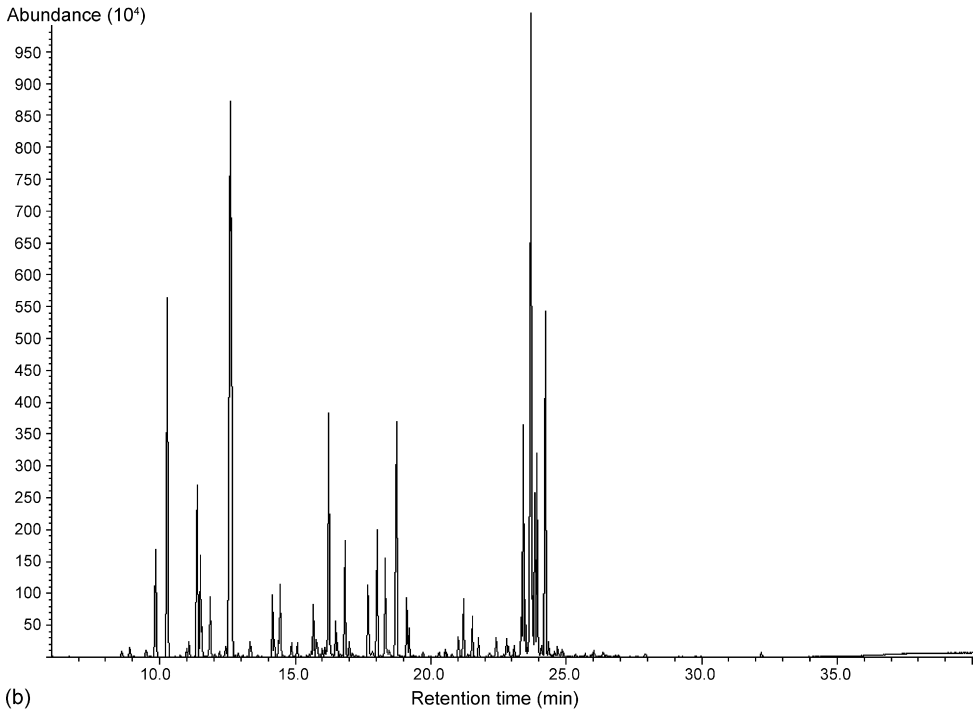


Fig. 1. Typical GC–MS total ion chromatograms of volatile compounds in ginger by MD–SPME/GC–MS using five different fibers of: (a) CAR/PDMS, (b) PDMS/DVB, (c) CW/DVB, (d) PDMS and (e) PA. Extraction conditions: sample mass of 1.0 g, a microwave power of 400 W and an irritation time of 2 min.



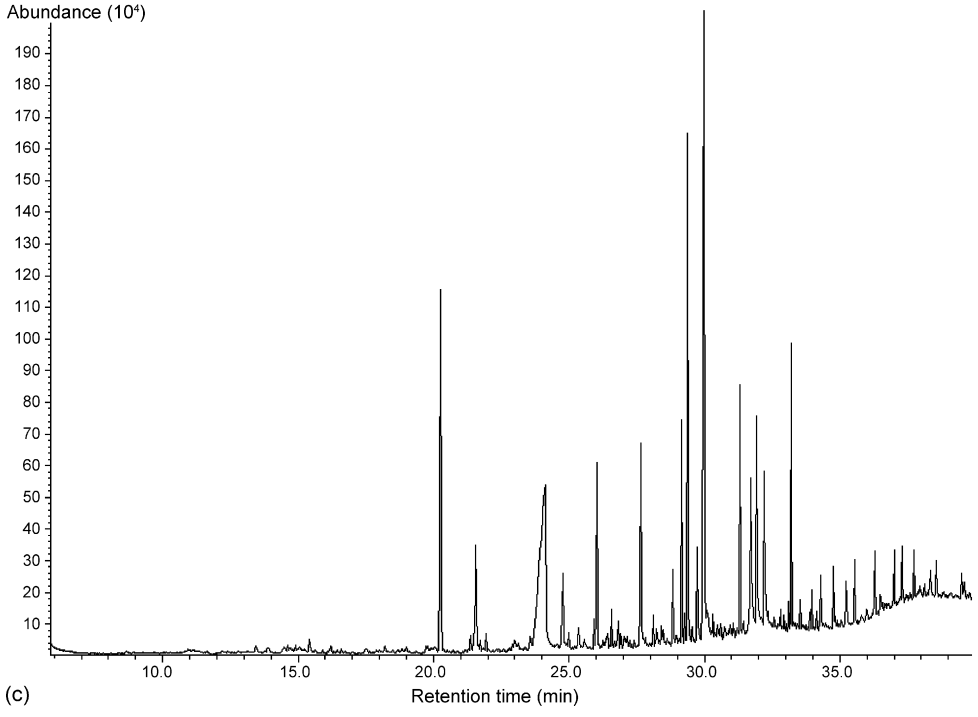


Fig. 1. (*Continued*)

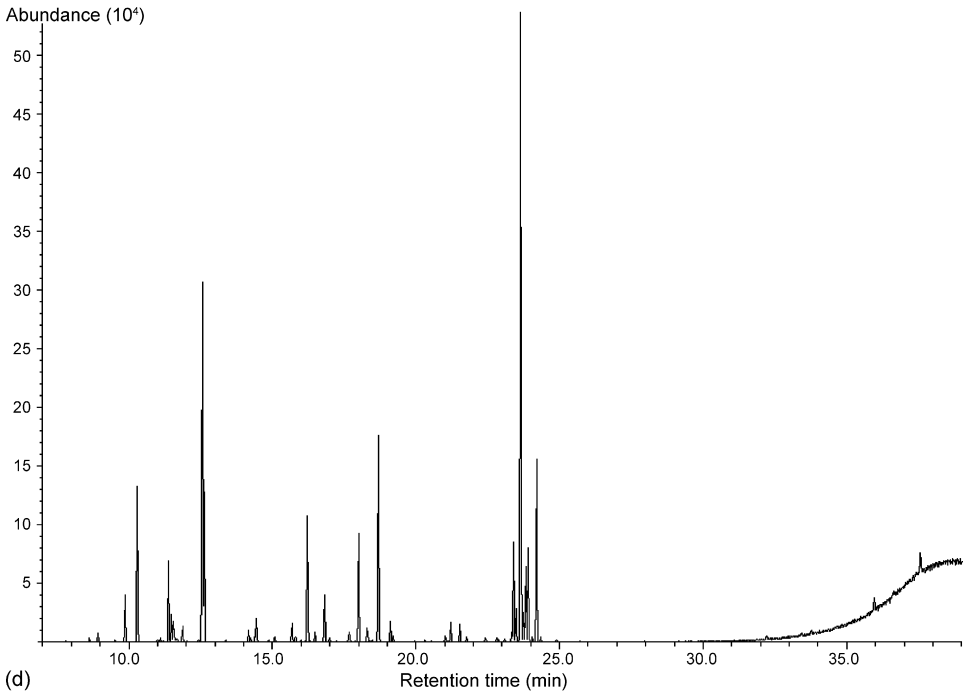
conventional HS–SPME with the conditions of PDMS/DVB fiber and an extraction time of 30 min. The analytes on the fiber were determined by GC–MS.

2.4. The precision of MD–SPME

The precision method was studied. Triplicate analyses of the essential oils in ginger were carried out under the optimum conditions, and the obtained peak areas of the essential oil compounds were used for the calculation of relative standard deviation (R.S.D.) values.

2.5. GC–MS analysis

Volatile compound analyses were carried out on a HP 6890 GC system, coupled with an HP MD5973 quadrupole



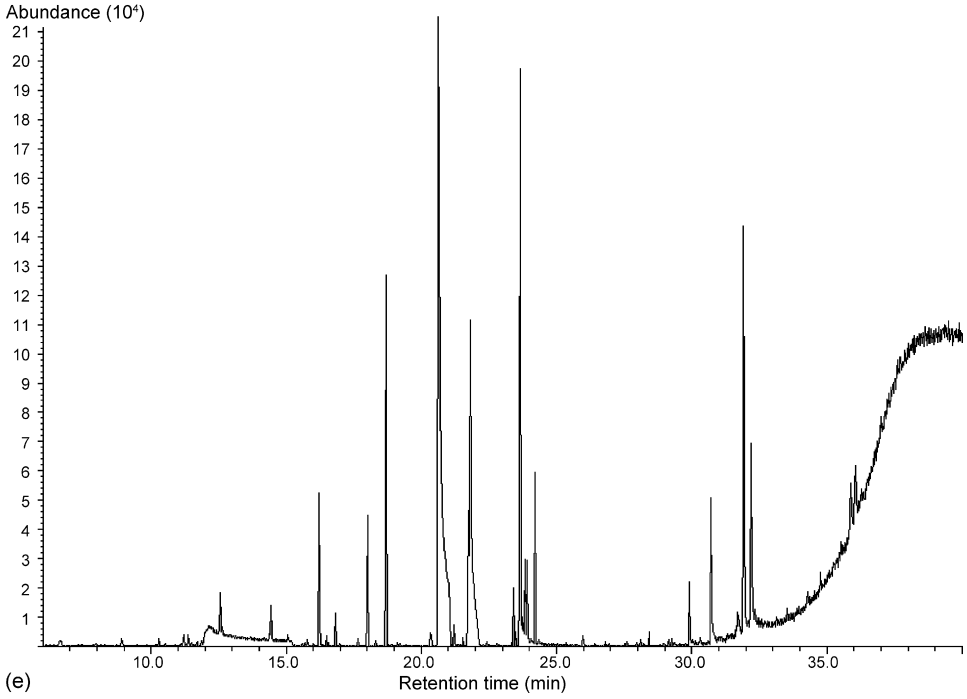


Fig. 1. (*Continued*).

mass spectrometer. The compounds were separated on an HP-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm film). Splitless injection was used for both conventional HS–SPME and MD–SPME samples. The column oven temperature was programmed to rise to an initial temperature of 40 °C (3 min) to 160 °C at 6 °C min^{-1}, then to 300 °C (10 min) at 10 °C min^{-1}. The injection temperature and ion source temperature were 250 and 280 °C, respectively. Helium was used as the carrier gas with a flow rate of 1 mL min^{-1}. The ionizing energy was 70 eV. All data were obtained by collecting the full-scan mass spectra within the scan range 45–550 amu. Compounds were identified using the Wiley 6.0 (Wiley, New York, NY, USA) mass spectral library and retention indices.

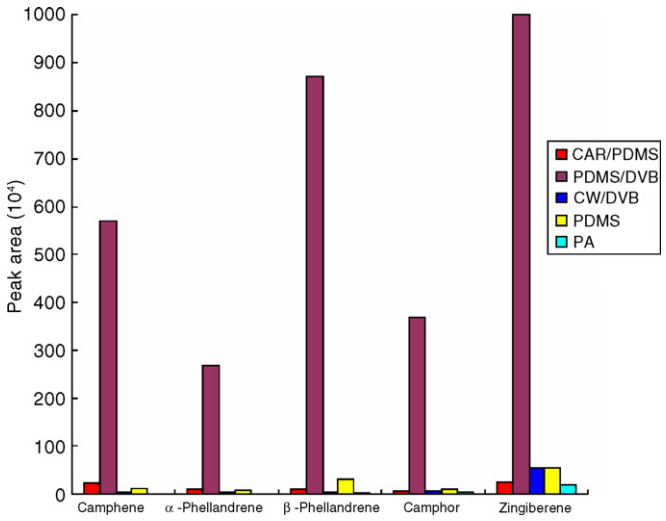


Fig. 2. Extraction profile obtained with different fibers for five active compounds in ginger. Extraction conditions: sample mass of 1.0 g, a microwave power of 400 W and an irritation time 2 min.

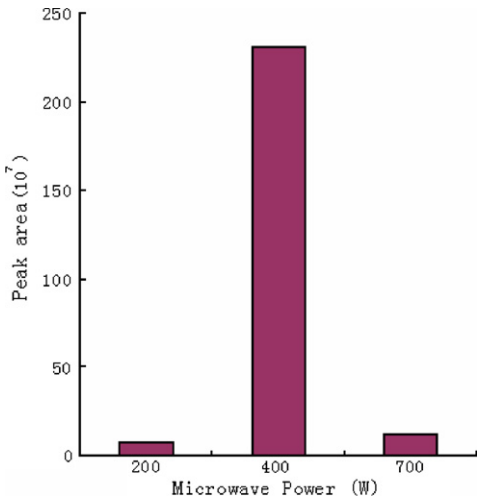


Fig. 3. Effect of the microwave power on the sum of peak areas of all volatile compounds in ginger. Extraction conditions: sample mass of 1.0 g, PDMS/DVB SPME fiber and an irradiation time of 2 min.

3. Results and discussion

3.1. Optimization of the MD–SPME parameters

Three MD–SPME parameters, including SPME fiber coating, microwave power and irradiation time, were studied. A total of 1 g of ginger was extracted by the five different fibers (PDMS, PDMS/DVB, CW/DVB, CAR/PDMS and PA) under the same microwave conditions (microwave power of 400 W and irradiation time of 2 min). Fig. 1 shows the total ion chromatograms of essential oil compounds in ginger by MD–SPME with these five fibers. As seen from the total ion strength of Fig. 1a–e, the PDMS/DVB fiber has a much better extraction efficiency than the other four fibers. (blank) In Fig. 2, the five main components of camphene, α-phellandrene, β-phellandrene, camphor and zingiberene in ginger (Table 1) were selected to compare the extraction efficiencies of the five fibers. As seen from Fig. 2, the PDMS/DVB fiber has the best extraction efficiency. As a result, PDMS/DVB was regarded as the optimal fiber for the extraction of the ginger. Second, microwave power and irradiation time were studied, using the fiber of PDMS/DVB. Figs. 3 and 4 indicate the sums of peak area at different microwave powers and irradiation times. It can be seen from Figs. 3 and 4 that the best extraction efficiency was achieved at 400 W and 2 min.

Based on the experimental results, the optimal MD–SPME conditions are: PDMS/DVB fiber, a microwave power of 400 W and an irradiation time of 2 min.

3.2. Determination of essential oils in ginger by MD–SPME

The optimized MD–SPME conditions were applied to the extraction and concentration of the volatile constituents in fresh ginger. The essential oil compounds in the ginger were isolated and concentrated by MD–SPME, and then the analytes that were extracted on the PDMS/DVB fiber were desorbed and analyzed by GC–MS. Fig. 5a is the GC–MS total ion chromatogram of the essential oils in ginger by MD–SPME. The chemical components in the ginger essential oils were identified by mass spectra library and retention indices. Fifty-four components in the essential oils were identified, and are listed in Table 1. Their relative contents were calculated in relation to the extracts. They mainly included geranial (5.25%), zingiberene (15.48%), β-sesquiphellandrene (5.54%) and β-phellandrene (22.84%).

3.3. Comparison of MD–SPME and conventional HS–SPME for the extraction of essential oils in ginger

As seen from Fig. 5a and b, more components were isolated and extracted from ginger by MD–SPME than by conventional HS–SPME. Table 1 shows all the compounds that were identified by these two extraction methods coupled with GC–MS. The relative contents of the identified compounds by the two methods are also listed in Table 1. The relative contents by conventional HS–SPME were much close to those described in the literature [30]. From Table 1, the proposed MD–SPME/GC–MS

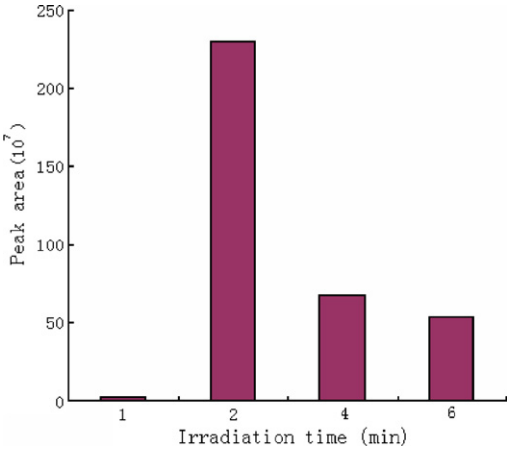


Fig. 4. Effect of the irradiation time on the sum of peak areas of all volatile compounds in ginger. Extraction conditions: sample mass of 1.0 g, PDMS/DVB SPME fiber and a microwave power of 400 W.

Table 1
Identification of chemical components in the essential oils of ginger

No.	Retention time (min)	Compounds	Retention indices	Relative contents (%)		R.S.D. of MD–SPME (%)
				MD–SPME	SPME	
1	8.60	2-Heptanone	890	0.11	ND	3.3
2	8.89	2-Heptanol	902	0.21	0.11	3.6
3	9.49	Tricyclene	913	0.14	ND	5.8
4	9.66	Thujene	928	0.02	ND	3.6
5	9.85	α-Pinene	941	2.13	0.41	4.6
6	10.29	Camphene	953	7.30	1.70	4.7
7	11.09	β-Pinene	980	0.26	0.05	2.4
8	11.39	6-Methyl-5-hepten-2-one	987	3.52	1.68	6.3
9	11.50	β-Myrcene	991	1.56	0.27	7.4
10	11.55	2-Methyl-6-hepten-1-ol	994	0.74	0.39	3.8
11	11.87	α-Phellandrene	998	1.11	0.27	4.6
12	12.03	Octanal	1001	0.04	ND	3.1
13	12.21	Terpinene	1020	0.09	ND	6.2
14	12.44	*p*-Cymene	1026	0.19	0.05	4.4
15	12.61	β-Phellandrene	1030	22.84	ND	5.8
16	13.07	β-Ocimene	1040	0.03	ND	3.9
17	13.32	Terpinene	1052	0.33	0.12	2.7
18	14.17	Linalool	1088	0.94	0.39	4.7
19	14.23	2-Nonanone	1104	0.24	ND	5.4
20	14.46	*p*-Meth-2-en-1-ol	1124	1.17	0.94	5.8
21	15.68	Camphor	1144	0.82	0.48	6.7
22	15.78	Camphene hydrate	1150	0.11	0.35	5.2
23	16.00	Isoborneol	1163	0.12	0.09	7.3
24	16.25	Borneol	1167	4.81	4.91	5.2
25	16.50	Terpinen-4-ol	1177	0.53	0.28	3.7
26	16.84	α-Terpineol	1189	1.92	ND	5.4
27	16.99	2-Thujenal	1208	0.23	0.23	4.1
28	17.69	Nerol	1230	1.47	1.47	6.3
29	18.03	Neral	1244	2.03	ND	7.4
30	18.33	Geraniol	1253	1.75	1.70	6.7
31	18.75	Geranial	1273	5.25	7.96	5.7
32	19.13	Bornyl acetate	1288	0.86	0.79	3.5
33	19.21	2-Undecanone	1294	0.41	0.32	5.5
34	19.72	2-Methoxy-4-vinylphenol	1314	0.06	0.06	8.3
35	20.32	Δ-Elemene	1340	0.07	0.12	4.3
36	20.59	α-Cubebene	1357	0.05	ND	6.3
37	21.03	Cycloisosativene	1370	0.43	0.52	6.5
38	21.22	Copaene	1381	0.91	1.23	5.8
39	21.54	β-Elemene	1395	0.66	1.14	2.4
40	21.78	α-Caryophellene	1420	0.28	0.33	6.4
41	22.43	γ-Elemene	1433	0.44	0.55	5.7
42	22.81	β-Farnesene	1460	0.22	0.31	4.3
43	23.09	Gurjuene	1475	0.19	ND	4.3
44	23.36	Germacrene D	1480	0.64	ND	3.7
45	23.43	Curcumene	1482	3.59	5.13	4.4
46	23.49	Valencene	1493	0.86	1.95	7.4
47	23.72	Zingiberene	1498	15.48	26.27	2.4
48	23.86	α-Bisabololene	1508	2.68	4.78	6.4
49	23.93	α-Farnesene	1510	2.51	4.19	7.3
50	24.09	(+)-Epi-bicyclosesquiphell-andrene	1521	0.16	0.25	5.5
51	24.23	β-Sesquiphellandrene	1526	5.54	8.19	6.3
52	24.36	Germacrene B	1557	0.19	ND	5.8
53	24.69	Zingiberenol	1583	0.14	0.14	3.5
54	24.90	Eudesmol	1614	0.07	ND	6.3

ND means no detection.

method identified the larger number of volatile compounds in ginger. The other 15 components in ginger cannot be identified by conventional SPME/GC–MS. It has been demonstrated that microwaves can significantly improve the extraction efficiencies of plant essential oil compounds.

Moreover, rapidity is another important feature of the proposed MD–SPME extraction method. Conventional HS–SPME required more than 30 min to isolate the volatile compounds to perform further extraction. In the proposed method, microwave irradiation, dry distillation and headspace extraction were com-

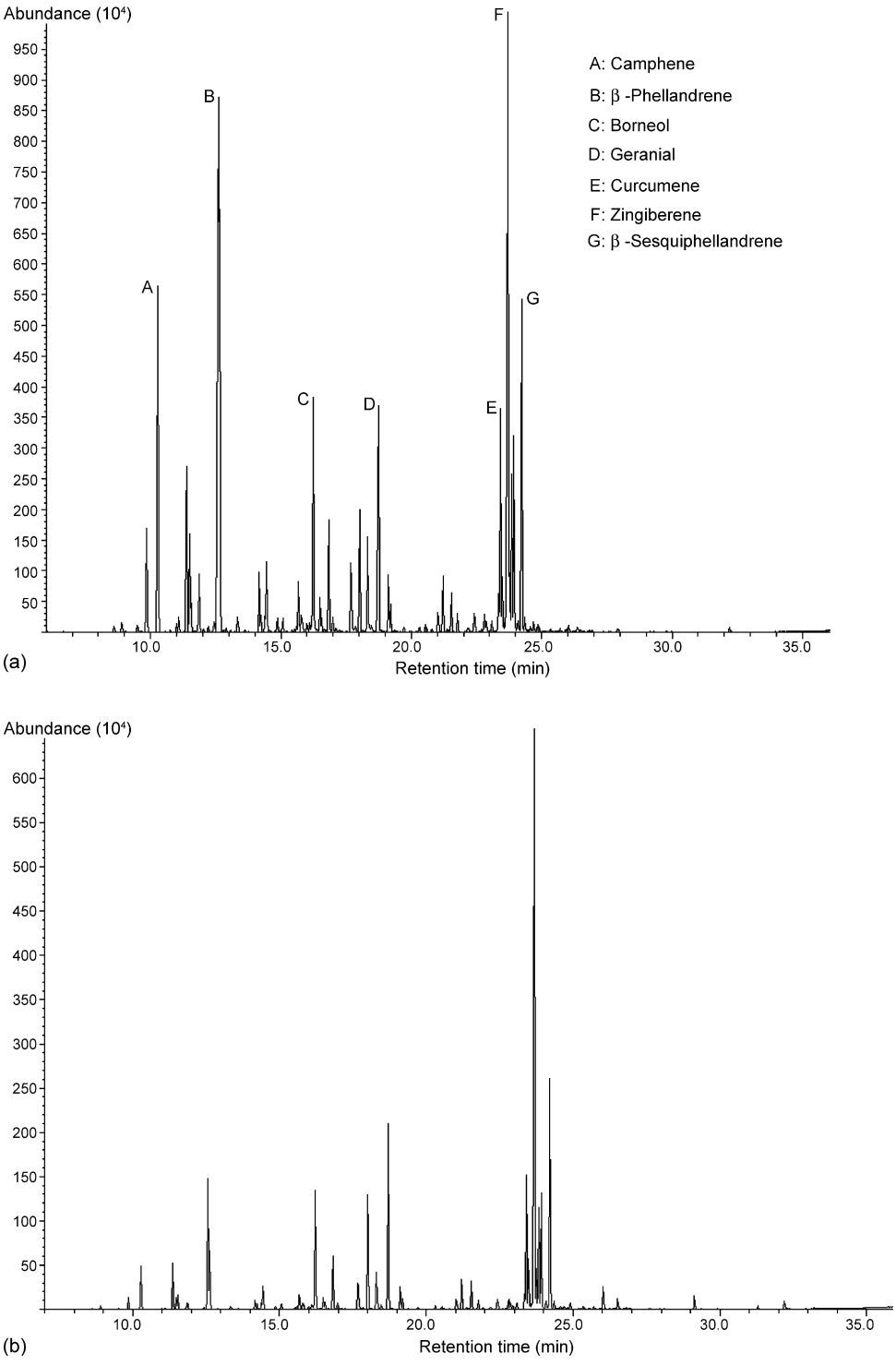


Fig. 5. The GC–MS total ion chromatograms of volatile compounds in ginger by: (a) MD–SPME and (b) conventional HS–SPME.

bined. This leads to a sample preparation time only 5 min. In MD–SPME, the extraction of volatile oils from plant materials was rapidly completed by dry distillation, and then the isolated volatile oil was simultaneously absorbed and concentrated by using SPME. On the other hand, MD–SPME required a small sample amount, no organic solvent and water for the determination of essential oil compounds in ginger.

Compared with conventional HS–SPME, MD–SPME is a simple, rapid, solvent-free and efficient method for the extraction of volatile components in ginger.

3.4. Precision of MD–SPME

The precision of this method was determined by replicating three analyses of the essential oils in ginger under the optimized

MD–SPME conditions. The R.S.D. values were calculated by the peak areas that were obtained by replicate analyses (Table 1). As seen from Table 1, all R.S.D. values are less than 10%, which shows that the developed MD–SPME/GC–MS method for the identification of essential oils in ginger has good precision.

4. Conclusions

In this study, an MD–SPME/GC–MS technique was successfully performed for the determination of volatile compounds in ginger. Fifty-four compounds were identified in ginger using the proposed method. Compared with conventional HS–SPME methods, MD–SPME/GC–MS is a simple, rapid, solvent-free and efficient method for the analysis of essential oils in ginger and other fresh plant tissues.

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