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Ultrasonic nebulization extraction coupled with headspace single-drop microextraction of volatile and semivolatile compounds from the seed of *Cuminum cyminum* L.

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ABSTRACT

Ultrasonic nebulization extraction (UNE) coupled with headspace single-drop microextraction (HS-SDME) was developed. In the UNE process, the analytes were transferred from the aqueous phase to the gas phase. Then the analytes were transferred from the gas phase to the solvent phase by the carrier gas and extracted and enriched with suspended microdrop solvent. Finally, the microdrop solvent injected into GC–MS system. The parameters affecting extraction performance, such as type of suspended solvent, microdrop volume, flow rate of carrier gas, temperature of extraction vessel and extraction time were investigated and optimized. The proposed method can be applied for the extraction and enrichment of the volatile and semivolatile compounds simultaneously. The extraction efficiency of the proposed method was compared with that of ultrasonic extraction (UE) and UE-HS-SDME. Compared with UE-HS-SDME, the contents of constituents in the extract obtained by the proposed method were closer to those obtained by hydrodistillation (HD), which is a standard extraction method.

1. Introduction

Generally, spices are used for cooking in the kitchen and disease-treating in the healthcare [1]. *Cuminum cyminum* L. is a kind of spices and widely used as flavor food and pharmaceuticals [1–5]. The characteristic fragrance used as spices is resulted from the constituents of the essential oil in the plant. It is reported that the major components of the essential oil are β-pinene, p-cymene, γ-terpinene, cuminal and cuminalcohol [2,3]. Researches showed that the essential oil has the antimicrobial activity. *C. cyminum* L. has many medicinal properties, such as antibiosis, antifungal, anticarcinogenic properties, antidiabetic, antithrombotic properties and inoxidizability [5].

In the extraction of volatile constituents of plant materials, many methods were adopted, such as hydrodistillation (HD), solid-phase extraction (SPE), solid-phase microextraction (SPME) and single-drop microextraction (SDME). Among these methods, HD was affirmed as the standard one in Chinese Pharmacopoeia [6]. The method was time-consuming and a large amount of sample was required. Owing to these reasons, SPE [7], SPME [8–11] and SDME [12–14] have been developed. However, the fibers used in SPE and SPME were very expensive. SDME overcame the limitation and was used in many aspects. To satisfy various analytical requirements, SDME grows into direct immersion (DI)-SDME [15–18] and headspace (HS)-SDME [19–23]. The needle of microsyringe including solvent droplet is immersed in sample solution directly in (DI)-SDME, and the method is not applicable to solid samples insoluble in water. Compared with (DI)-SDME, (HS)-SDME, in which solvent droplet suspended above sample solution, is more suitable for various kinds of samples [19–21,23]. (HS)-SDME has the advantages of rapidity, portability, a small amount of sample and inexpensiveness.

Various extraction techniques related to headspace microextraction are developed in recent years. Headspace solid phase microextraction (HS-SPME) is commonly used. The fibers in SPME may be coated with liquid (polymer), solid (sorbent) or combination of both. When complex samples analyzed, high selectivity, low fiber contamination and matrix effect were achieved by HS-SPME. But the expensive fibers are not affordable in some laboratories. Continuous-flow micro-extraction (CFME) was introduced [24]. Because of the drop of solvent continuous contact with the sample solution, the method benefits from a higher enrichment factor than static liquid phase microextraction. Ultrasonic-assisted solvent extraction (USE) was coupled with HS-SPME for extraction of volatile and semivolatile compositions from the honey matrix [25].

The ultrasonic nebulization extraction (UNE) belongs to the ultrasonic extraction technique. It is an alternative method for the extraction of active constituents from the spices. In the UNE process [26–29], the ultrasonic fountain and aerosol were generated in the reactor. Along with the form of ultrasonic fountain, gaseous

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phase and aqueous phase exchanged each other continuously and reached the partition equilibrium quickly. The volatile components were transferred from sample solution into the gaseous phase. This method was suitable for the extraction of the volatile analytes and not satisfactory for the extraction of the semivolatile analytes of which the vapor pressure is between 1.33×10^{1} and 1.33×10^{-5} Pa at 20 °C.

In the work, UNE-HS-SDME was developed. In order to improve extraction performance, the extraction vessel was heated during the process of extracting target components from sample matrix. The extraction and concentration were carried out simultaneously. The volatile and semivolatile constituents in the spices can transferred into gaseous phase and the amount of target compounds concentrated in solvent microdrop increased significantly. For comparison, HD and UE-HS-SDME were applied.

2. Experimental

2.1. Materials and reagents

The seed of *C. cyminum* L. used in this experiment was purchased from a market in Changchun, Jilin Province, China. The seed of *C. cyminum* L. was crushed by a disintegrator (FW-100 Test Instrument Co. Ltd., Tianjin, China). Then the powders obtained were filtered with 60 mesh sieve and stored in the desiccator at room temperature prior to analysis.

The standards, p-cymene (99%) and γ-terpinene (98%) were purchased from Acros Company (NJ, USA). The standard β-pinene was purchased from Tokyo chemical industry (Tokyo, Japan). The extraction solvents, including n-decane (99%), n-dodecane (99%), n-pentadecane (99%) and n-heptadecane (99%), were purchased from Acros Company (NJ, USA). n-Dodecane was used as internal standard and purchased from Acros Company (NJ, USA). The mixture of n-alkanes (C-6 to C-19) was purchased from Accustandard (New Haven, USA). The deionized water was obtained with a Milli-Q water purification system (Millipore Co., USA). The other analytical reagents were purchased from Beijing Chemical Factory (Beijing, China). The stock solutions for the target analytes at the concentration of 4 mL L^{-1} were prepared by dissolving the analytes in n-hexane and stored in the dark place at 4 °C.

2.2. Instruments

The extraction and concentration system was assembled in our laboratory. The schematic diagram of the system is shown in Fig. 1. An ultrasonic humidifier (Beijing Branson Ultrasound Co. Ltd., China) was used for the purpose of ultrasonic nebulization. The maximum output power and the frequency of vibration are 35 W and 1.7 MHz, respectively. The extraction vessel was a self-made glass flask (100 mL, I.D. 10 cm) with three ports. One of the ports was on the bottom of extraction vessel sealed with the PVC film and the size of the port was the same as the piezocrstal. The other two ports were connected with gas inlet and gas outlet part, respectively. The gas outlet was connected with a globule of glass pipe. A heating tape connected with a thermocouple sensor (XMTD-2001 Xinghua AOTE temperature Instrument Co., Xinghua, China) was used for heating the extraction vessel and adjusting the heating temperature. The microextraction and sample injection were carried out with a 10 μL GC microsyringe (Zhenhaisan'ai Instrument Co. Ltd., Ningbo, China). An ultrasonic generator (KQ2200E Kunshan Ultrasonic Instrument Co. Ltd., Kunshan, China) with the maximum output power of 150 W and the frequency of 40 kHz was used.

All extracts obtained were analyzed with a GC–MS system (SHIMADZU, GC-MS-QP2010) equipped with the Rxi-5MS capillary column (30 m × 250 μm; 0.25 μm film thickness). The program of temperature was from 60 °C (holding for 3 min) to 120 °C at the rate of 5 °C/min, and then increased to 220 °C (holding for 5 min) at the rate of 15 °C/min. The other operating conditions are as follows: injection temperature was maintained at 260 °C; ion source temperature was maintained at 200 °C; ionization energy was 70 eV; injection volume was 1.0 μL; the flow rate of carrier gas (helium) was 1 mL min^{-1}. The split ratio representing the ratio of sample amount that goes on the column to that that is thrown away to the waste was 1:150 for the extracts obtained by HD and 1:50 for the extracts obtained by other extraction methods. The components in the extract were identified by computer matching their mass spectral fragmentation patterns with those in NIST27 Library and comparing with MS data reported in the literature [30].

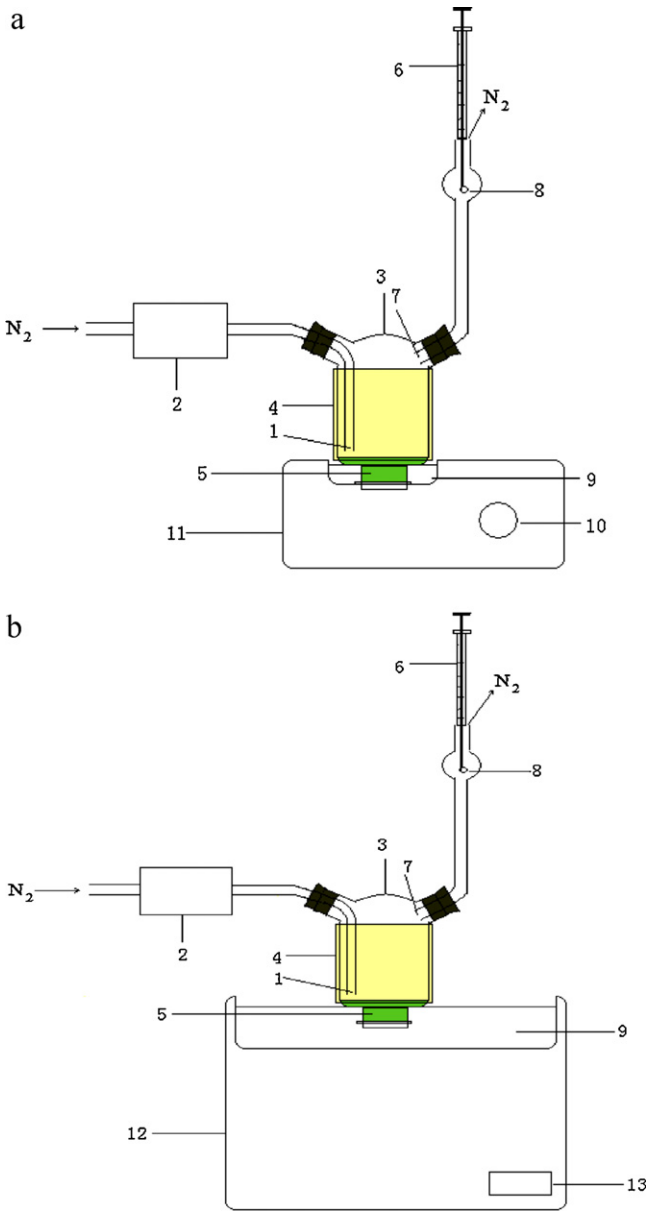

Fig. 1. The schematic diagram of UNE-HS-SDME system (a) and UE-HS-SDME system (b). (1) Gas inlet, (2) flow meter, (3) extraction vessel, (4) heating tape, (5) spices powder and purified water, (6) microsyringe, (7) gas outlet, (8) microdrop, (9) water, (10) switch 1, (11) ultrasonic nebulizer, (12) ultrasonic generator, and (13) switch 2.

2.3. Extraction procedure

2.3.1. Ultrasonic nebulization extraction-headspace-single drop microextraction

50 mg of sample powders and 5 mL of water were added into a 100 mL of extraction vessel. The schematic diagram of UNE-HS-SDME is shown in Fig. 1(a). N_2 was used as the carrier gas and flew through the extraction vessel continuously to carry the volatile and semivolatile compounds in the extraction vessel to the solvent microdrop. The needle of the 10 μL GC microsyringe was clamped at a fixed position in the globule of glass pipe and the solvent microdrop was suspended from the tip of the microsyringe. The extraction vessel was wrapped with a heating tape and controlled at 90 °C. Then the nebulizer was switched on and the carrier gas flew through the extraction vessel at the same time. 3 μL of n-heptadecane containing 0.01% n-dodecane (internal standard) was pushed out and suspended. The ultrasonic fountain was formed by ultrasonic vibration. The target analytes were transferred from the solid powders to the liquid phase (water) and then transferred from the liquid phase to the vapor phase. By the ultrasonic fountain, the gas–liquid distribution equilibrium of analyte will be rapidly reached. The volatile and semivolatile analytes evaporated and were transferred to the solvent microdrop by the carrier gas. While the water vapor cannot dissolve in the organic solvent and flowed from the gas outlet of the extraction vessel. The vapor pressure of the analytes increased at high temperature and the analytes can be efficiently transferred from the extraction vessel to the microdrop by the carrier gas. After extraction for 10 min, the extract was withdrawn into the microsyringe and 1.0 μL of the extract was injected into GC–MS system for analysis.

2.3.2. Ultrasonic extraction-headspace-single drop microextraction

The schematic diagram of UE-HS-SDME is shown in Fig. 1(b). The 100 mL of glass flask was used as extraction vessel and placed on the ultrasonic generator instead of the ultrasonic nebulizer. 50 mg of sample powders and 5 mL of water were put into the extraction vessel and the needle of the 10 μL GC microsyringe with the solvent microdrop was clamped at a fixed position in the globule of glass pipe. The carrier gas flowed through the extraction vessel when the ultrasonic generator was turned on. The extraction time was 10 min. The other extraction steps were the same as those mentioned in UNE-HS-SDME.

2.3.3. Ultrasonic extraction

50 mg of sample powders and 5 mL of ethanol were placed in the extraction vessel. After 10 min, the supernatant obtained was filtered through a 0.45 μm filter membrane and 1.0 μL of the extract was injected into the GC–MS system for analysis.

2.3.4. Hydrodistillation

According to the Chinese Pharmacopoeia [6], 20 g of sample and 300 mL of water were placed in a 500 mL round bottom flask and heated with heating jacket at 100 °C for 4 h. The essential oil obtained was collected, dried with anhydrous sodium sulphate and then stored at 4 °C in the refrigerator until analyzed.

3. Results and discussion

3.1. Optimization of extraction conditions

Some experimental parameters, including type of suspended solvent, microdrop volume, flow rate of carrier gas, temperature of extraction vessel and extraction time, were studied and optimized.

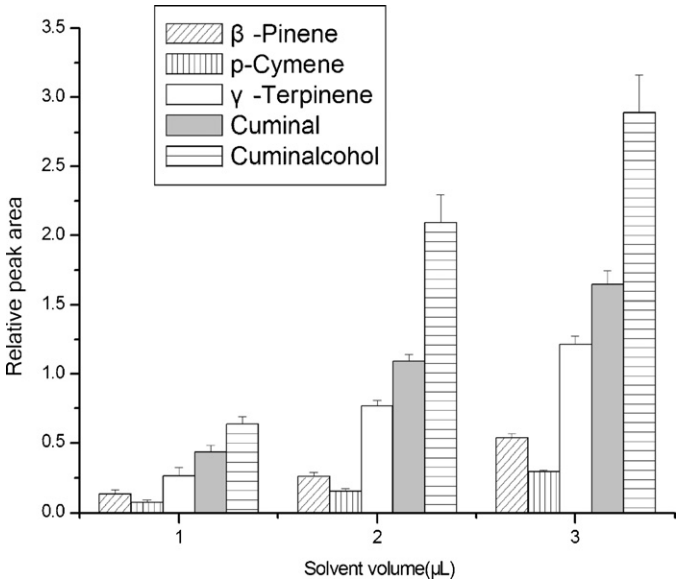

Fig. 2. Effect of solvent microdrop volume. Temperature of extraction vessel, 90 °C; flow rate of carrier gas, 200 mL min^{-1}; extraction time, 10 min.

3.1.1. Suspended solvent

The suspended solvents should have some properties, including high purity, low toxicity, high boiling point and low volatility. In addition, their gas chromatographic peaks should be separated from the target analyte ones. Three solvents, n-decane (99%), n-pentadecane (99%) and n-heptadecane (99%) were considered for the suitable suspended solvent. In the extraction process, the evaporation rate of suspended solvent should be examined. The boiling point of n-heptadecane was the highest. However, n-heptadecane easily solidified during the process of extraction. The n-decane had an obvious loss during the extraction because of the relatively fast evaporation rate and thus low extraction efficiency. The experimental results showed that n-pentadecane was suitable for the extraction. n-Pentadecane containing 0.01% n-dodecane (internal standard) was chosen as suspended solvent for subsequent work.

3.1.2. Solvent microdrop volume

The effect of solvent microdrop volume from 1 μL to 3 μL on the relative chromatographic peak area, which is the ratio of the gas chromatographic peak area of the target analytes to that of internal standard, is shown in Fig. 2. It can be seen that when the solvent microdrop volume increases, the peak areas of five target analytes increase. There are two main factors affecting the relative peak areas. On the one hand, the larger microdrop volume, the larger surface area of the microdrop which is beneficial to the enrichment of analytes in the microdrop. On the other hand, the concentrations of analytes can be diluted in the microdrop with the increase of solvent volume. The effect of the surface largeness should be predominant. When microdrop volume was too small, the precision for sampling was limited. When the volume exceeded 3 μL, the stability of solvent microdrop was limited. Based on the experimental results, 3 μL was chosen as the microdrop solvent volume in the work.

3.1.3. Temperature of extraction vessel

Temperature of extraction vessel has a significant effect on the mass transfer of the analytes from the aqueous phase to vapor phase during the process of extraction. The effect of temperature in the range of 0–120 °C was investigated and the experimental results are shown in Fig. 3. The relative peak areas of β-pinene, p-cymene, γ-terpinene decrease while those of cuminal and cumi-

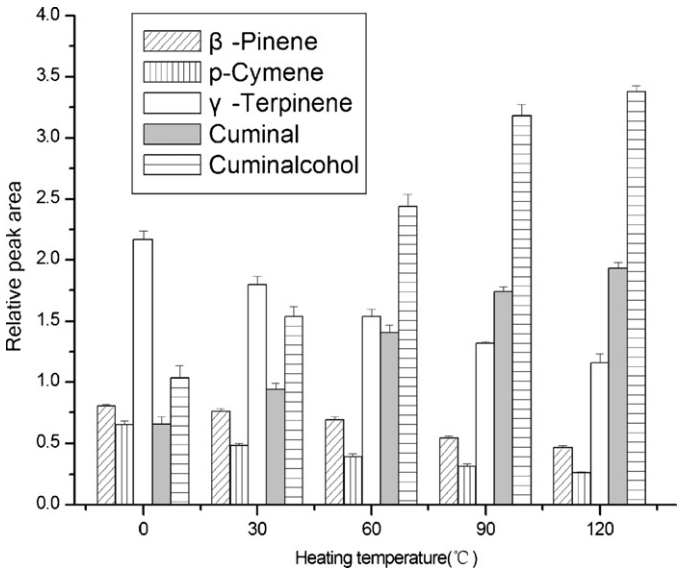

Fig. 3. Effect of the temperature of extraction vessel. Solvent microdrop volume, 3 μL; flow rate of carrier gas, 200 mL min^{-1}; extraction time, 10 min.

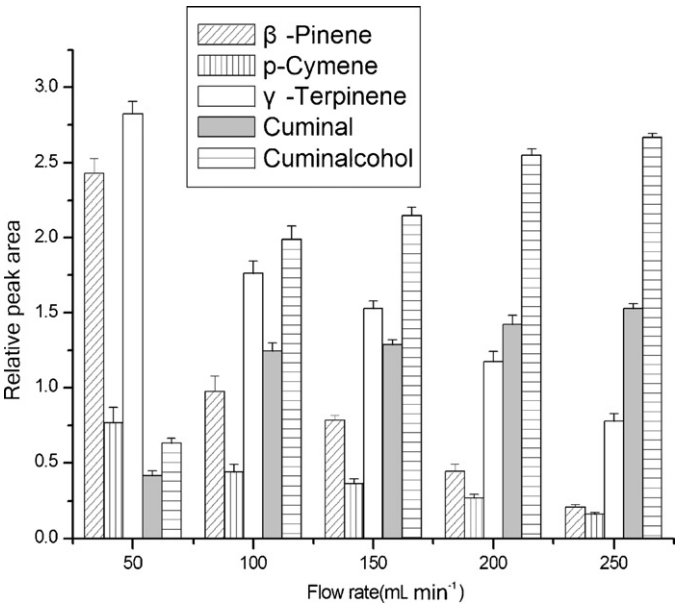

Fig. 4. Effect of flow rate of carrier gas. Solvent microdrop volume, 3 μL; temperature of extraction vessel, 90 °C; extraction time, 10 min.

nalcohol increase obviously with increase of the temperature. The mass transfer of analytes from aqueous phase to gas phase is easy and the mass transfer of analytes from gas phase to extraction solvent is difficult when the temperature increase. It is the result of the decrease of the partition coefficient of the analytes with the increase of the temperature. The signals of volatile analytes, such as β-pinene, p-cymene, γ-terpinene, decrease because the analytes tend to be in gas phase with the increase of temperature, and those of semivolatile analytes, such as cuminal and cuminalcohol, increase because the analytes tend to transfer to extraction solvent with the increase of temperature. As it was difficult to control too high temperature, 90 °C was chosen as the operation temperature.

3.1.4. Flow rate of carrier gas

Flow rate of carrier gas is crucial to ensure that the target analytes can be extracted and enriched effectively. The effect of the flow rate of carrier gas on the relative peak areas are shown in Fig. 4. The peak areas for β-pinene, p-cymene, γ-terpinene decrease with the increase of flow rate of carrier gas while the peak areas for cuminal and cuminalcohol increase with increase of the flow rate of carrier gas. On the one hand, the increase of the flow rate is beneficial to the transfer of the analytes from the extraction vessel to the microdrop, which is beneficial to the increase of the peak areas. On the other hand, the increase of the flow rate is not beneficial to the transfer of the analytes from the gas phase to the solvent phase, which is not beneficial to the increase of the peak areas. Because the volatility of semivolatile compounds, including cuminal and cuminalcohol, are lower than that of the volatile compounds, including β-pinene, p-cymene and γ-terpinene, semivolatile compounds are more easily transferred from gas phase into the organic phase and more difficultly from organic phase into gas phase than the volatile compounds at high flow rate. The flow rate of 200 mL min^{-1} was chosen by a compromise.

3.1.5. Extraction time

The effect of extraction time was tested in the range of 2–20 min. As shown in Fig. 5, the peak areas of β-pinene, p-cymene and γ-terpinene increase with the increase of the extraction time when the time was shorter than 5 min, and decrease with the increase of the extraction time ranging from 5 to 20 min. Fig. 5 shows that the relative peak areas for cuminal and cuminalcohol increase significantly with the increase of extraction time ranging from 2 min to 20 min. If the extraction time was too long, the concentration of the volatile analytes in gas phase decreased and the back extraction from the microdrop into the gas phase induced the decrease of the concentration of the analytes in microdrop. The semivolatile analytes are easily transferred into the solvent microdrop and the back extraction of the semivolatile analytes is more difficult than that of the volatile analytes. The extraction time of 10 min was selected in the following experiments.

3.2. Analysis of sample

The constituents in the extract from *C. cyminum* L. obtained by the proposed method were compared. The chromatograms of the extracts of *C. cyminum* L. are represented in Fig. 6 and the constituents identified by MS and their relative contents are listed in Table 1. In the proposed method, the RSD values for the major

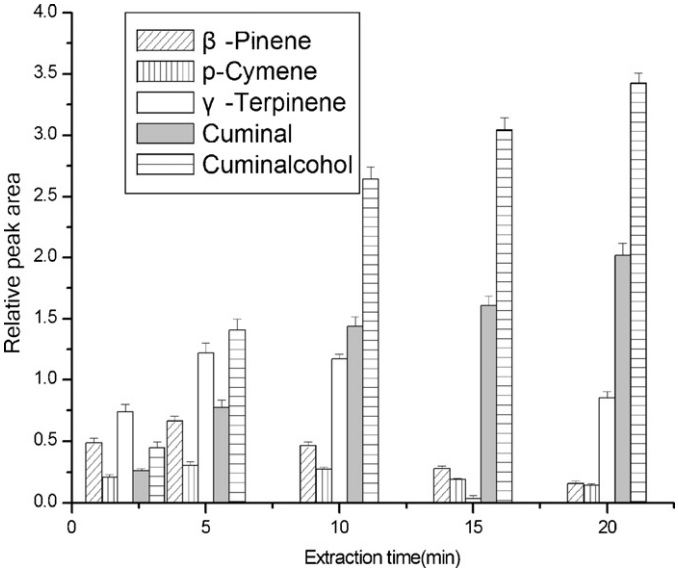

Fig. 5. Effect of extraction time. Solvent microdrop volume, 3 μL; temperature of extraction vessel, 90 °C; flow rate of carrier gas, 200 mL min^{-1}.

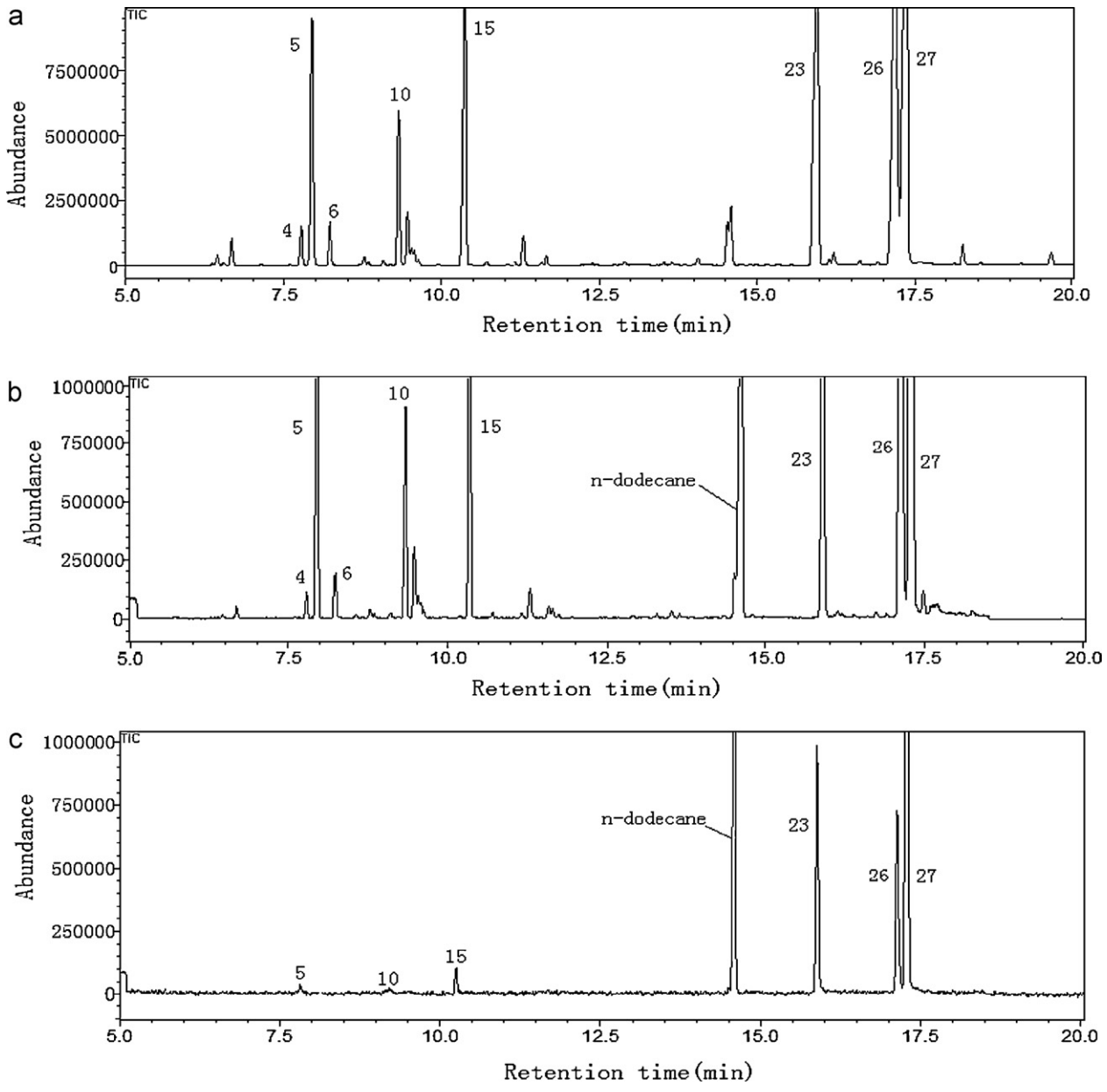

Fig. 6. Total ion chromatograms of extracts of the seed of *Cuminum cyminum* L. obtained by HD (a), UNE-HS-SDME (b) and UE-HS-SDME (c).

components, including β-pinene, p-cymene, γ-terpinene, cuminal and cuminalcohol are 1.9%, 4.3%, 5.6%, 1.0%, 2.5%, respectively. 28 compounds, 27 compounds and 7 compounds obtained by HD, UNE-HS-SDME and UE-HS-SDME were identified, respectively. The contents of β-pinene, p-cymene, γ-terpinene, cuminal and cuminalcohol obtained by the proposed method are 5.0%, 3.1%, 11.7%, 18.5% and 32.7%, respectively. The contents of β-pinene, p-cymene, γ-terpinene, cuminal and cuminalcohol obtained by HD are 8.4%, 4.8%, 10.6%, 18.4% and 24.4%, respectively. The experimental results indicate that compared with HD, the proposed method should be more beneficial to the extraction of the analytes with relative low volatility.

3.3. Comparison of different methods

The comparison of UE, UE-HS-SDME and UNE-HS-SDME was carried out and the results are given in Fig. 7. The relative content of the compounds and the total contents obtained by HD, UE-HS-SDME and UNE-HS-SDME are listed in Table 1. It can be found that

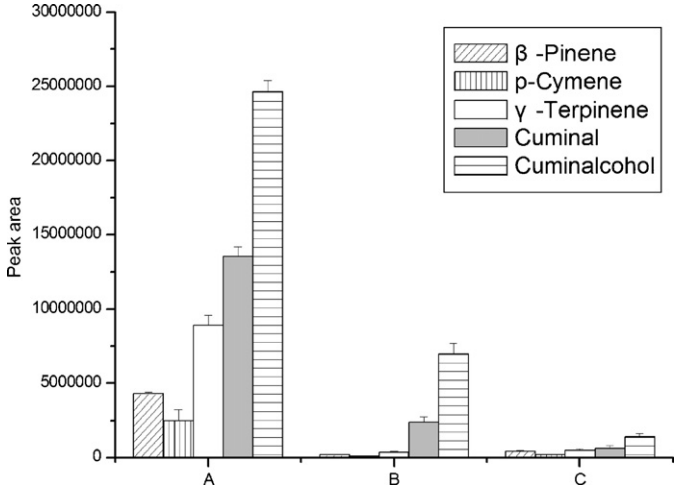

Fig. 7. Comparison of different methods. (A) UNE-HS-SDME, (B) UE-HS-SDME, and (C) UE.

Table 1
The composition analysis of compounds extracted from the seed of *Cuminum cyminum* L. by HD, UNE-HS-SDME and UE-HS-SDME.

Peak no.	Retention time (min)	Compounds	Volatility	Retention index[a]	Relative content (%)		
					HD	UNE-HS -SDME	UE-HS -SDME
1	6.447	2-Methyl-5-(1-methylethyl)-Bicyclo[3.1.0]hex-2-ene	Volatile	927	0.3	0.1	–
2	6.668	α-Pinene	Volatile	935	0.7	0.2	–
3	7.145	Camphene	Volatile	952	0.02	0.01	–
4	7.780	4-Methylene-1-(1-methylethyl)-Bicyclo[3.1.0]hexane	Volatile	974	1.0	0.4	–
5	7.951	β-Pinene	Volatile	980	8.4	5.0	1.5
6	8.225	β-Myrcene	Volatile	990	1.1	0.7	–
7	8.766	α-Phellandrene	Volatile	1008	0.2	0.2	–
8	8.831	3-Carene	Volatile	1010	0.1	0.1	–
9	9.081	4-Carene	Volatile	1019	0.1	0.1	–
10	9.319	p-Cymene	Volatile	1026	4.8	3.1	0.7
11	9.457	D-Limonene	Volatile	1031	1.5	1.1	–
12	9.516	β-Phellandrene	Volatile	1032	0.4	0.3	–
13	9.560	Eucalyptol	Volatile	1034	0.3	0.2	–
14	9.623	3,7-Dimethyl-,(E)-1,3,6-octatriene	Volatile	1036	0.1	0.1	–
15	10.355	γ-Terpinene	Volatile	1060	10.6	11.7	3.1
16	11.163	1-Methyl-4-(1-methylethylidene)-cyclohexene	Volatile	1087	0.1	0.1	–
17	11.297	L-Fenchone	Volatile	1091	0.8	0.4	–
18	11.652	Cis-β-Terpineol	Semivolatile	1130	0.2	0.3	–
19	12.897	trans-Pinocarveol	Semivolatile	1144	0.1	0.1	–
20	14.067	4-Methyl-1-(1-methylethyl)-3-Cyclohexen-1-ol	Semivolatile	1183	0.2	0.1	–
21	14.517	1,3,4-Trimethyl-3-Cyclohexen-1-Carboxaldehyde	Volatile	1198	1.2	1.0	0.4
22	14.568	Estragole	Volatile	1200	1.8	–	–
23	15.950	Cuminal	Semivolatile	1248	18.4	18.5	20.0
24	16.615	trans-p-Menth-2-en-7-ol	Semivolatile	1271	0.2	0.1	–
25	16.908	Phellandral	Semivolatile	1281	0.1	0.2	–
26	17.189	2-Caren-10-al	Volatile	1291	20.9	22.4	15.4
27	17.365	Cuminalcohol	Semivolatile	1297	24.4	32.7	58.7
28	18.266	p-Mentha-1,4-dien-7-ol	Semivolatile	1331	0.7	0.5	–
Total content fraction of determined compounds (%)					98.5	99.4	99.8

[a] According to the definition of the retention index, the value of the retention index was calculated based on the adjusted retention time of the analyte and the adjusted retention times of C6–C19 normal paraffin standards.

the peak areas of β-pinene, p-cymene, γ-terpinene, cuminal and cuminalcohol obtained by the proposed method are about 7–20 times larger than those obtained by UE. The peak areas of the analytes obtained by UNE-HS-SDME are about 4–26 times larger than those obtained by UE-HS-SDME. Table 1 shows that compared with UE-HS-SDME, the contents of the constituents obtained by the proposed method are closer to those obtained by HD. When UE-HS-SDME was applied, the extractability of the analyte was low and therefore most of the compounds were not quantifiable. The proposed method is available for extracting the volatile and semivolatile compounds from spices.

4. Conclusion

UNE-HS-SDME and heating were combined to extract the volatile and semivolatile compounds from seed of *C. cyminum* L. In the UNE process, the aerosol existed in the ultrasonic fountain of headspace of the extraction vessel. The analytes of spices in the aerosol were transferred from liquid phase to headspace. The volatile compounds were transferred easily from the headspace to the microdrop. The transfer of the semivolatile compounds from the headspace to the microdrop was improved by heating the vessel, which overcome the condensation of the semivolatile compounds in the wall of the tubes.

UNE-HS-SDME was developed and the conditions affecting the extraction performance were optimized. The constituents obtained by the proposed method were compared with those obtained by HD and UE-HS-SDME. The proposed method was compared with UE-HS-SDME and UE with the results indicated that the yields obtained by the proposed method were the highest. In the UNE process, the acoustic frequency is about 1.7 MHz. Because UNE is a gentle extraction method, the extraction is beneficial to compounds which are sensitive to temperature. The experimental results indicated that the nebulization should not affect the chemistry during extraction. The proposed method may be applied for the extraction of volatile and semivolatile compounds from plant materials.

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