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Poly(dimethylsiloxane) Mini-disk Extraction

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A novel sampling method of the headspace poly(dimethylsiloxane) (PDMS) mini-disk extraction (HS-PDE) was developed, optimized, validated and applied for the GC/MS analysis of spices flavors. A prototype PDMS mini-disk (8 mm outer diameter, 0.157 mm thickness, 9.4 mg weight) has been designed and fabricated as a sorption device. The technique uses a small PDMS mini-disk and very small volume of organic solvent and less sample size than the solvent extraction. This new HS-PDE method is very simple to use, inexpensive, rapid, requires less labor. Linearities of calibration curves for α-pinene, β-pinene, limonene and γ-terpinene by HS-PDE combined with GC/MS were excellent having r^2 values greater than 0.99 at the dynamic range of 6.06 ~3500 ng/mL. The limit of detection (LOD) and the limit of quantitation (LOQ) showed very low values. This method exhibited good precision and accuracy. The overall extraction efficiency of this method was evaluated by using partition coefficients (K_p) and concentration factors (CF) for several characteristic components from nutmeg and mace. Partition coefficients were in the range from 2.04×10^4 to 4.42×10^5, while CF values were 0.88-15.03. HS-PDE was applied successfully for the analysis of flavors compositions from nutmeg, mace and cumin. The HS-PDE method is a very promising sampling technique for the characterization of volatile flavors.

Key Words : Poly(dimethylsiloxane), Mini-disk extraction, Gas chromatography-mass spectrometry, Nutmeg and mace, Cumin

Introduction

Poly(dimethylsiloxane) (PDMS) has a repeating $(CH_3)_2$ SiO unit. PDMS is a non-polar polymer. It has been recognized as the state of art materials used very importantly in the many industries. The chemists, Hyde at Corning Glass Works and Rochow et al. at General Electric Company were investigating heat-resistant materials for use as resinous binders in 1940's when they synthesized the first silicone polymers, demonstrated that they worked well and found a satisfactory chemical route to produce PDMS commercially.[1,2] PDMS is now commonly used as a stationary phase of gas chromatography (GC) columns and a component of preconcentration devices used in solid phase microextraction (SPME), stir bar sorptive extraction (SBSE), and thin film microextraction.

SPME invented by Pawliszyn and his colleagues in 1989 is a solvent-free technique of sample preparation.[3,4] SPME device looks like modified syringe consisting of a fiber holder and a thin 1-2 cm long retractable fused silica fiber coated on the outside with an appropriate sorbent such as PDMS. The amount of PDMS used in SPME fiber is typically in the order of 0.6 mL or less. This fiber is mounted concentric with the syringe needle which protects the fiber and delivers it from sample to injector. Few limitations of SPME includes the fragility of fibers, which can be easily broken or damaged during agitation or injection and contamination of the coating after exposure to dirty samples.[5] A wide variety of applications of SPME has been reported by many researchers,[6-12] well-documented reviews,[13-16] and books published.[17,18]

The solventless SBSE technique introduced by Sandra and coworkers in 1999 uses a stir bar coated with PDMS.[19] The method is based on the same principles as SPME where a magnetic iron stir rod is incorporated into a glass jacket, and the outer surface is coated with 50-300 mL PDMS layer. The sensitivity of this technique is increased by a factor of 100 to 1000 in comparison to SPME.[5] In most of the studies done to date on SBSE process, the thermal desorption equipment is generally used for desorption of analyte from the PDMS stir bar and analysis is carried out by GC/MS.[5,20] Despite of the few limitations in SPME and SBSE which are increasingly becoming the most frequently applied extraction techniques.

Since the amount of analyte extracted in SPME is proportional to the volume of the extraction phase, the sensitivity of a method can be improved by increasing the volume of the extraction phase.[17] Recently, Bruheim *et al.* introduced a thin film microextraction, and compared it to a SPME PDMS coated fiber and SBSE for application to semi-volatile analytes such as polycyclic aromatic hydrocarbons.[21,22] This extraction approach exhibited much higher extraction rates than SPME fiber due to the higher surface area to extraction phase volume of the thin film. In this technique, they used a thin sheet of PDMS membrane attached to a deactivated stainless steel rod like a flag. After the extraction, the cubic film (1 cm × 1 cm or 2 cm × 2 cm) was rolled around the stainless steel rod and finally fitted inside the glass liner of a GC injection port for thermal desorption. This fitting step consumes a non-negligible time with labor and the glass liner changing increases expense.

Recent approaches in sample preparation process prior to

GC/MS have been directed towards high performance technique, which is fast, simple, less labor and cheap, minimizes the consumption of organic solvent and produces little waste.[23,24] Miniaturization includes substantial scale down of sample sizes as well as analytical devices. Very recently we reported headspace mulberry paper bag micro solid phase extraction (HS-MPB-SPE) as a new sampling method alternative to traditional methods.[24-26] This method used poly(2,6-diphenyl-*p*-phenylene oxide) adsorbent (Tenax TA) enclosed in a mulberry paper, and 0.6 mL of petroleum ether for extraction. Further reduction of solvent consumption is highly desirable in this method.

In the present study, we fabricated a prototype PDMS mini-disk (8 mm outer diameter, 0.157 mm thickness, 9.4 mg weight) as a new alternative sorption device. It can be easily processed by molding and acquired for low cost. And headspace PDMS mini-disk extraction (HS-PDE) was established, optimized, and validated. The major goal of this study is to develop a novel technique of sample preparation based on PDMS to overcome above mentioned limitations such as the fragility of high cost fibers or the needs of the expensive thermal desorption equipment. Investigation of the practical application of this new technique for the GC/MS analysis of spices flavors from nutmeg, mace, and cumin is another objective of this study.

Both nutmeg and mace are spices came from the same nutmeg tree (*Myristica fragrans* Houtt) native to Indonesian Spice Islands. Nutmeg is the roughly egg-shaped brown seed kernel inside the fruit, while mace is the surrounding reddish lacy aril on the seed kernel. Nutmeg is usually used in powdered form to flavor. Nutmeg has analgesic, antispasmodic, antidontalgic, antiemetic, stomachic, stimulant, narcotic, carminative, astringent, aphrodisiac, hypolipidemic, antithrombotic, anti-platelet aggregation, antifungal, antidysenteric, and anti-inflammatory activities.[27,28]

Cumin is the dried seed of the herb *Cuminum cyminum* L., native from the east Mediterranean to East India. Cumin is one of the most popular spices throughout the world. Whole seeds or ground powder of cumin are used as a spice for their distinctive aroma. Cumin has antiseptic, antispasmodic, aphrodisiac, carminative, depurative, digestive, emmenagogue, parasiticide, stimulant, and tonic properties.[27]

With the growth in the use of spices, there has been continued research into the active components of spices not only from a flavor standpoint but also from functional perspective to explore the antioxidant properties of spices.[29] Since the nature and the relative amount of the aroma compounds present in the volatile fraction are the most distinctive features of a spice, the characterization of the aromatic profile can represent a useful tool to evaluate the organoleptic quality and it could be used to guarantee its authenticity.[30]

Different techniques have been proposed for the extraction of the volatile aroma compounds of plant samples or foods. Various methods such as steam distillation,[31-35] supercritical fluid extraction,[33,36,37] microwave extraction,[38] SPME,[39,40] combined with GC/MS or GC-olfactometry were reported. In addition, constituents of nutmeg were also analyzed directly without any extraction and characterized via MS/MS by using solid probe.[41]

This study presents development, optimization, validation and application of HS-PDE technique prior to GC/MS. Additionally, the relative extraction efficiencies of proposed method have been investigated for the analysis of spices such as nutmeg, mace and cumin.

Experimental

Spice Materials and Reagents. Both nutmeg ground powder and mace ground powder were obtained from McCormick & CO. Inc. (Hunt valley, MD, USA). Cumin powder was purchased from a local supermarket in Wuhan, China. Spice samples were used without any further pretreatment.

All working reference standards were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Tokyo Kasei (Nihonbashi, Tokyo, Japan). Organic solvents of chromatographic grade were obtained from Mallinckrodt Baker (Phillisburg, NJ, USA).

Fabrication of PDMS Mini-disk. PDMS solution kit (Sylgar 184 Silicone Elastomer Kit) was purchased from Dow Corning Korea Ltd. (Seoul, Korea). This kit includes a base solution A (Sylgard 184A) and a curing agent solution B (184B). Base solution A was mixed with curing agent solution B at ratio (A:B = 90:10, wt %) on a glass plate of Petri dish, the mixture was shaken for 10 min and kept in a desiccator for 24 hr. Then about 30 pieces of PDMS mini-disk (8 mm outer diameter, 0.157 mm thickness, 9.4 mg weight) were simultaneously fabricated using a mold. Prior to use, PDMS mini-disk was conditioned in the oven at 250 ^{o}C for 20 min in order to remove any impurities or contaminants, and blank run was performed.

Headspace PDMS Mini-disk Extraction (HS-PDE). A prototype PDMS mini-disk was pierced into a clean capillary glass tubing (0.35 mm o.d., 6 cm length) at the fixed position (about 1 cm) distant from top end side of a tubing. Then, a capillary glass tubing with a PDMS mini-disk was placed in the headspace of a 50 mL vial containing the spice powder sample (0.1 g) and the vial was sealed hermetically with a polytetrafluoroethylene (Teflon) mini-nut cap. Then this vial was kept in a sand bath for 30 min at 40 ^{o}C, volatile flavor compounds were exposed and adsorbed to both side surfaces of a PDMS mini-disk. After adsorption, a PDMS mini-disk was removed and immediately placed on an 1 mL microtube. Then analytes were extracted with 30 μL acetonitrile. After extraction, 1.0 μL of extract was injected to the GC or GC/MS for the analysis. The schematic illustration of HS-PDE procedure is depicted in Figure 1.

GC/MS and GC. GC/MS analyses were carried out by using a Trace GC 2000 and a GC-Q plus ion trap MS^{n} (Thermoquest-Finnigan, Austin, TX, USA) with electron impact (EI) ionization mode. In GC/MS, chromatographic separations were performed on a 6% cyanopropylphenyl-

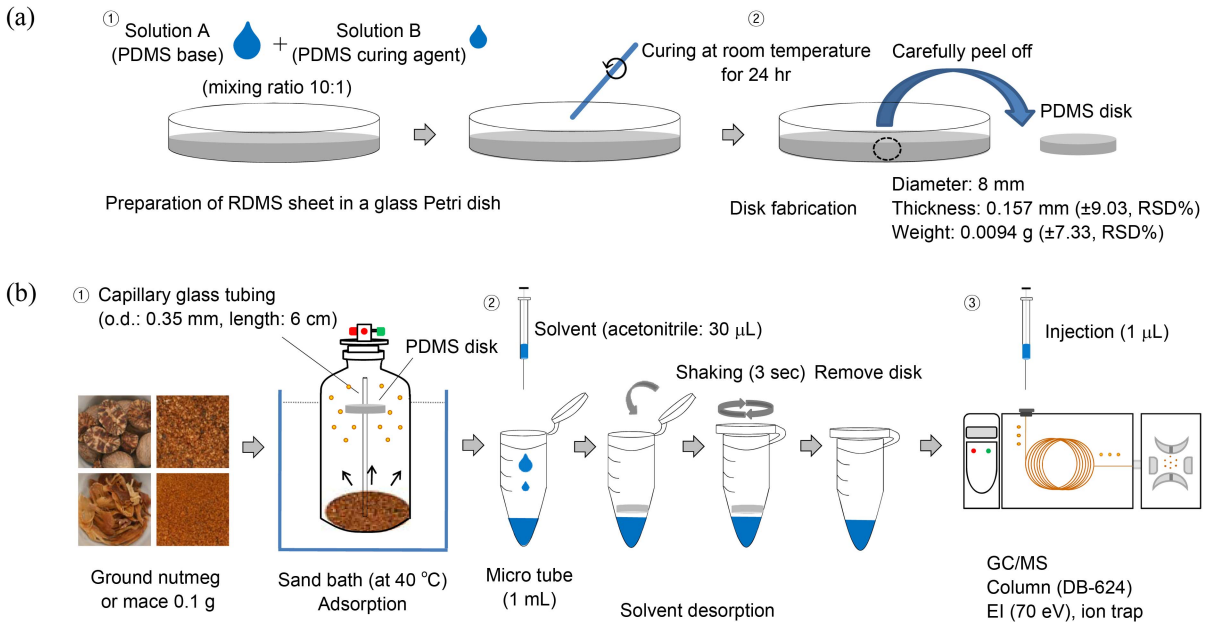

Figure 1. Schematic illustration showing how to fabricate a PDMS mini-disk and procedure of the headspace PDMS mini-disk extraction. (a) Fabricating method of PDMS mini-disk, (b) Procedure of HS-PDE and GC-MS.

94%-dimethylsiloxane copolymer (DB-624, 30 m length × 0.25 mm I.D. × 1.4 μm film thickness, J & W Scientific, Folsom, CA, USA) capillary column. Flow rate of carrier gas (He, 99.9995%) was 1.0 mL/min. The injector temperature was 240 °C. The oven temperature program was 50 °C (3 min) - 5 °C/min - 220 °C (10 min). A split injection with a ratio of 1:30 was used. The injected sample volume was 1.0 μL. The ion trap mass spectrometer was operated as follows; ionization voltage, 70 eV; ion source temperature, 200 °C. Transfer line temperature was 230 °C. The measuring mode was scanned from 50 to 500 mass ranges. The analytes were identified tentatively by comparison of their retention times and mass spectra with those of authentic standards. The tentative identification was also performed by comparing of the obtained mass spectra of relevant chromatographic peaks with those of corresponding spectra of the NIST and Wiley libraries.

Gas chromatographic analysis was performed using a Hewlett-Packard HP 5890 gas chromatograph (GC) equipped with flame ionization detector (FID). A DB-624 (30 m length × 0.25 mm I.D. × 1.4 μm film thickness, J & W Scientific, Folsom, CA, USA) capillary column was used under the following conditions. Oven temperature was kept at 50 °C and programmed to 220 °C at a rate of 5 °C/min, and then held steady for 10 min. Injector and FID temperatures were kept at 240 °C and 250 °C, respectively. Samples (1.0 μL) were injected in the split mode and the split ratio was adjusted at 1:30. Nitrogen (99.9%) was used as carrier gas at a constant flow rate of 1.0 mL/min. Flow rates of hydrogen and air were kept at 30 mL/min and 300 mL/min, respectively. Data were recorded using a HP 3396A integrator.

Results and Discussion

Optimization of HS-PDE. When volatile compounds in headspace above samples have been obtained on an elastic solid of PDMS mini-disk, it is necessary to desorb them with a liquid solvent compatible with PDMS and suitable for injection into the GC. Ethanol, methanol, and petroleum ether were considered, however, none of them was compatible due to swelling.[42] Dimethyl sulfoxide and tetrahydrofuran were also unsuitable to extract and separation. Then, acetonitrile was selected as the extraction solvent.

HS-PDE was optimized using α-pinene, β-pinene, limonene and γ-terpinene as model compounds. These compounds are considered as major characteristic components of nutmeg, mace, and cumin. Stock solutions at a concentration of each 1.0 mg/mL in acetonitrile were prepared, respectively. When HS-PDE was completed, then chromatographic analysis was conducted by using a GC with FID. The chromatographic peak areas of model compounds by 1.0 μL injection of extracts were considered as analytical signals for optimization. When a variable is optimized, its optimum value is fixed in subsequent steps.

The influence of the extraction temperature on the efficiency of HS-PDE was investigated in the range from 30 °C to 50 °C. As shown in Figure 2(a), the highest peak areas for model compounds were obtained at 40 °C. And Figure 2(b) shows the extraction time profile varied from 30 min to 90 min at 40 °C. The equilibrium condition for the adsorption processes of α-pinene, β-pinene, limonene and γ-terpinene was obtained after 30 min but 60 min for β-pinene. In subsequent steps, 40 °C extraction temperature and 30 min extraction time were used.

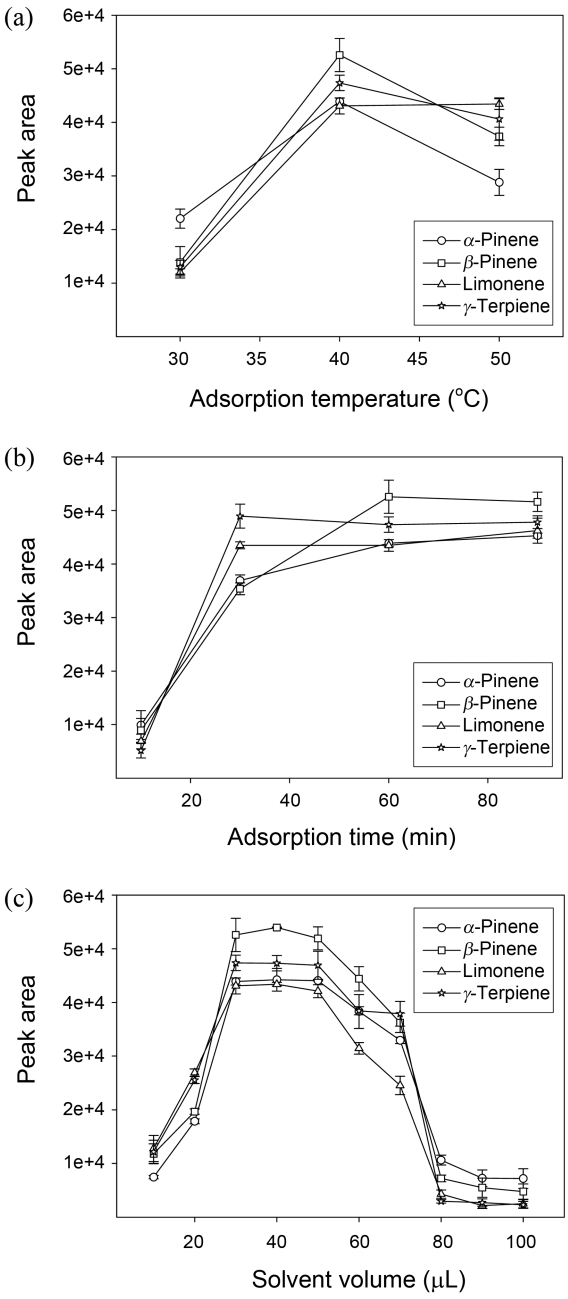

Figure 2. Influences of adsorption (a) temperature, (b) time, and (c) solvent volume on the efficiencies of HS-PDE and GC-FID.

The influence of different volumes of acetonitrile in the range of 10 μL - 100 μL was evaluated. As shown in Figure 2(c), the cleaner extraction of model compounds was obtained when a 30 μL volume of acetonitrile were employed. The results showed decreasing of peak area when the volume of acetonitrile is increased over 60 μL to 100 μL. This result indicates the signal could be decreased due to the dilution of analyte. Compared with conventional solvent extraction or solid phase extraction, HS-PDE can reduce significantly solvent consumption in the sample preparation. Since only 30 μL of solvent is used, there is minimal waste or exposure to toxic organic solvent. This method allowed combining of extraction, enrichment, and clean-up in a single step.

Method Validation of HS-PDE. The results of the validation experiments for HS-PDE and GC/MS are summarized in Table 1. Linearities for the six-point calibration curves were excellent having r^2 values greater than 0.99 at the range of 6.06 ng/mL - 3500 ng/mL. The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated by LOD = 3 s/m, and LOQ = 10 s/m, respectively. Where, s is the standard deviation of the blank and m is the slope obtained in the calibration curve for model compounds. The obtained LOD values were in the rage from 0.96 ng/μL to 1.80 ng/μL. Recovery test was performed analyzing by the proposed method using nutmeg or mace powder sample spiked the model compounds standard solutions. Recoveries showed satisfactory values, varied from 83.17% to 98.11%. The precision of the corresponding area of total ion chromatogram (TIC) expressed as relative standard deviation (%), varied between 1.97% and 8.52%.

Extraction Efficiency of HS-PDE. The overall extraction efficiency of HS-PDE was evaluated by the partition coefficient (K_p) and the relative concentration factor (CF) of the characteristic components of nutmeg and mace. The K_p and the CF values were calculated by the following forms.

$$K_p = (A_1\ V_0) / (A_0\ V_1)$$
$$CF = A_1 / A_0$$

where, A_1 is the peak area of the analytes by HS-PDE-GC/FID, A_0 is the corresponding peak area obtained by static HS-GC-FID using a 10 mL Hamilton 1010RN gas tight syringe (Supelco), V_0 is the volume of the gas sample

Table 1. LOD, LOQ, dynamic range, recovery, and reproducibility

		α-Pinene	β-Pinene	Limonene	γ-Terpinene
Linearity of calibration					
Equation	Slope	34.85	34.71	35.57	64.31
	Intercept	975.65	236.91	2148.86	2062.76
Determination coefficient (r^2)		0.9939	0.9948	0.9949	0.9908
Dynamic range		6.00 ng - 3500 ng	6.00 ng - 3500 ng	6.00 ng - 3500 ng	6.00 ng - 3500 ng
LOD (ng/μL)		1.80	1.80	1.80	0.96
LOQ (ng/μL)		6.00	6.00	6.00	6.00
Recovery	Nutmeg	96.60	94.19	83.17	90.54
	Mace	98.11	91.08	96.30	95.24
Reproducibility	Nutmeg	6.03%	8.52%	2.62%	2.65%
(RSD%)	Mace	2.39%	1.97%	3.45%	2.93%

Table 2. Partition coefficient (K_p) and concentration factor (CF) of nutmeg and mace samples by HS-PDE method (unit = mean ± RSD%)

Compounds	Nutmeg		Mace	
	K_p	CF	K_p	CF
α-Thujene	4.89×10^4(± 6.88)	1.66 (± 6.40)	1.28×10^5(± 5.22)	4.35 (± 3.64)
α-Pinene	4.28×10^4(± 3.28)	1.46 (± 6.24)	1.12×10^5(± 8.20)	3.81 (± 2.30)
Sabinene	7.25×10^4(± 20.93)	2.47 (± 8.34)	1.63×10^5(± 7.87)	5.55 (± 2.61)
β-Pinene	5.56×10^4(± 11.10)	1.89 (± 8.52)	1.46×10^5(± 4.41)	4.95 (± 1.95)
β-Myrcene	8.70×10^4(± 15.29)	2.96 (± 6.75)	2.13×10^5(± 13.43)	7.25 (± 4.32)
α-Phellandrene	6.78×10^4(± 7.26)	2.33 (± 7.28)	1.79×10^5(± 5.46)	6.10 (± 5.09)
3-Carene	6.82×10^4(± 14.38)	2.32 (± 1.78)	1.58×10^5(± 5.06)	5.36 (± 1.90)
α-Terpinene	6.67×10^4(± 8.22)	2.27 (± 2.05)	1.75×10^5(± 7.80)	5.95 (± 3.08)
Limonene	8.73×10^4(± 10.36)	2.97 (± 16.10)	1.89×10^5(± 4.58)	6.44 (± 3.45)
ρ-Cymene	1.06×10^5(± 16.74)	3.59 (± 3.07)	2.34×10^5(± 5.06)	7.94 (± 4.64)
β-Phellandrene	8.52×10^4(± 6.68)	2.90 (± 0.24)	1.93×10^5(± 4.27)	6.55 (± 3.03)
γ-Terpinene	8.45×10^4(± 5.18)	2.87 (± 2.65)	2.11×10^5(± 7.29)	7.16 (± 2.93)
Terpinolene	7.47×10^4(± 16.92)	2.54 (± 3.01)	1.64×10^5(± 2.04)	5.57 (± 2.38)
α-Terpineol	2.22×10^5(± 9.80)	7.56 (± 5.11)	-	-
Bornyl acetate	-	-	2.59×10^5(± 13.41)	0.88 (± 2.87)
Safrole	-	-	4.42×10^5(± 26.94)	15.03 (± 3.93)
Myristicin	2.70×10^4(± 13.65)	0.92 (± 15.84)	2.04×10^4(± 35.59)	0.69 (± 12.55)
Elemicin	-	-	2.00×10^5(± 12.76)	6.79 (± 4.72)

Table 3. Comparison of flavor composition of nutmeg by different methods

Peak No.	Compounds	Normalized peak area (%)	
		HS-PDE	HS-MPB-SPE
1	α-Thujene	3.48	5.42
2	α-Pinene	7.52	5.71
4	Sabinene	21.41	22.72
5	β-Pinene	9.47	6.22
6	β-Myrcene	2.83	4.29
7	α-Phellandrene	0.66	0.88
8	3-Carene	0.66	1.26
9	α-Terpinene	3.79	5.39
10	Limonene	9.80	11.02
11	ρ-Cymene	4.18	4.80
12	β-Phellandrene	4.64	6.13
13	γ-Terpinene	10.59	11.57
14	Terpinolene	-	2.66
15	Linalool oxide	3.35	2.27
16	Linalool	-	0.51
17	Sabinene hydrate	3.27	2.20
18	(–)-Terpinen-4-ol	6.70	3.99
19	α-Terpineol	0.46	0.56
21	Safrole	0.92	0.53
23	Germacrene D	0.62	0.34
24	α-Copaene	1.96	0.90
25	Methyl eugenol	0.62	-
26	Aromadendrene	0.46	0.25
27	β-Selinene	-	tr
28	Myristicin	1.83	0.14
29	Elemicin	1.23	0.15

tr = trace

Table 4. Comparison of flavor composition of mace by different methods

Peak No.	Compounds	Normalized peak area (%)	
		HS-PDE	HS-MPB-SPE
1	α-Thujene	13.43	9.54
2	α-Pinene	37.47	11.64
3	*DL*-Camphene	-	0.08
4	Sabinene	-	1.12
5	β-Pinene	19.37	7.44
6	β-Myrcene	2.11	4.91
7	α-Phellandrene	1.26	3.16
8	3-Carene	2.71	2.56
9	α-Terpinene	2.89	6.29
10	Limonene	7.24	15.22
11	ρ-Cymene	3.42	8.26
12	β-Phellandrene	4.66	9.84
13	γ-Terpinene	3.80	11.60
14	Terpinolene	0.98	3.43
15	Linalool oxide	-	0.81
17	Sabinene hydrate	-	0.48
18	(–)-Terpinen-4-ol	-	0.44
21	Safrole	0.66	2.91
24	α-Copaene	-	0.11
28	Myristicin	-	0.16

tr = trace

injected by static HS-GC, and V_1 is the volume of PDMS mini-disk.

These experiments were carried out using nutmeg or mace sample (0.1 g) in a 50 mL vial instead of standards to ensure that matrix effects were identical to those encountered

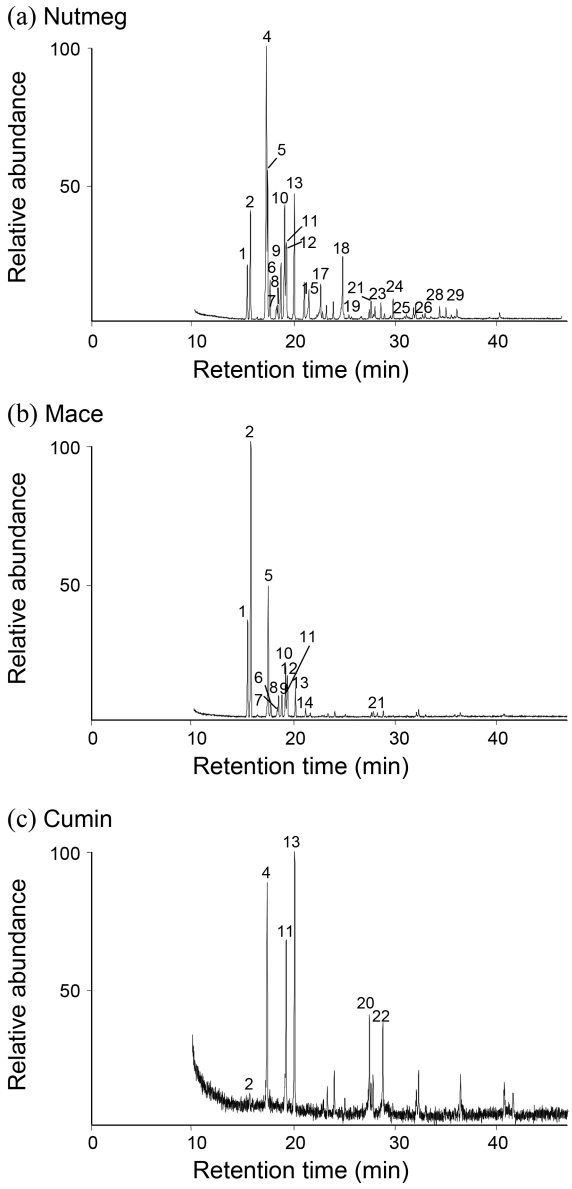

Figure 3. Typical TICs of flavor components of (a) nutmeg, (b) mace, and (c) cumin obtained by HS-PDE-GC/MS.

during actual sampling. Experimental K_p and CF values by HS-PDE for several characteristic compounds of nutmeg and mace sample showed good results (Table 2).

Application of HS-PDE for Analysis of Spices Flavors. The proposed method was applied to the analysis of flavor components of nutmeg, mace, and cumin powders. Typical total ion chromatograms (TIC) obtained by HS-PDE-GC/MS are shown in Figure 3. The peak numbers shown in Figure 3 correspond to those indicated in Table 3 (nutmeg), Table 4 (mace) and Table 5 (cumin). Flavor compositions by different extraction methods of HS-PDE and HS-MPB-SPE[24-26] were compared in Table 3, Table 4 and Table 5, respectively. HS-PDE using acetonitrile as a desorption solvent provided higher compositions of α-pinene, β-pinene, and (–)-terpinen-4-ol than HS-MPB using petroleum ether. In contrast, HS-MPB-SPE showed higher composition of limonene and γ-terpinene than HS-PDE. These results suggest that the detection of flavor compositions was affected by different solvents.

Table 5. Comparison of flavor composition of cumin by different methods

Peak No.	Compounds	Normalized peak area (%)	
		HS-PDE	HS-MPB-SPE
1	α-Thujene	-	0.37
2	α-Pinene	tr	0.47
3	Sabinene	-	0.65
4	β-Pinene	27.94	10.78
5	β-Myrcene	-	0.80
8	3-Carene	-	0.52
10	Limonene	-	0.57
11	ρ-Cymene	24.00	20.65
13	γ-Terpinene	29.38	37.49
20	Cuminaldehyde	9.25	14.08
22	Perillaldehyde	9.43	13.62

tr = trace

Conclusion

Headspace PDMS mini-disk extraction was newly developed, optimized, validated and applied for the determination of volatile aroma components of nutmeg, mace and cumin by GC/MS. The technique uses a small PDMS mini-disk, very small volume of organic solvent and less sample size. Linearities of calibration curves were generally excellent. HS-PDE was applied successfully for the analysis of flavors compositions from nutmeg, mace and cumin. It was found that the new HS-PDE method is very simple to use, requires less labor, inexpensive, rapid, significantly minimized the sample size and the solvent consumption relative to conventional solvent extraction. The HS-PDE method is a very promising sampling technique for the characterization of volatile flavors.

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