Comparison of simultaneous distillation extraction and solid-phase microextraction for the determination of volatile flavor components

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Abstract

Traditional simultaneous distillation extraction (SDE) and solid-phase microextraction (SPME) techniques were compared for their effectiveness in the extraction of volatile flavor compounds from various mustard paste samples. Each method was used to evaluate the responses of some analytes from real samples and calibration standards in order to provide sensitivity comparisons between the two techniques. Experimental results showed traditional SDE lacked the sensitivity needed to evaluate certain flavor volatiles, such as 1,2-propanediol. Dramatic improvements in the extraction ability of the SPME fibers over the traditional SDE method were noted. Different SPME fibers were investigated to determine the selectivity of the various fibers to the different flavor compounds present in the mustard paste samples. Parameters that might affect the SPME, such as the duration of absorption and desorption, temperature of extraction, and the polarity and structure of the fiber were investigated. Of the various fibers investigated, the PDMS–DVB fiber proved to be the most desirable for these analytes. © 2001 Elsevier Science B.V. All rights reserved.

1. Introduction

The determination of volatile components in a mixture is a process widely used in many disciplines, such as environmental, food, forensic, fragrance, oil, pharmaceutical and polymer analysis. The method of choice for many of these analyses is simultaneous distillation extraction (SDE) [1] followed by GC or GC–MS analysis. Extraction and concentration are usually necessary before analysis by GC is per-

formed. Several extraction and concentration methods have been used; among them are liquid–liquid extraction [2], liquid–liquid extraction with ultrasound [3], simultaneous stream distillation extraction [4], solid-phase extraction [5], and other techniques [6,7]. The main reason for extraction is to obtain a more concentrated samples, to eliminate interfering substances and to improve detection limits for specific compounds.

Solid-phase microextraction (SPME) [13] is a relatively new technique that is able to address the need for concentrating the analytes in the headspace [8]. SPME uses a small (1-cm long) piece of fused silica, on which a liquid phase, similar to a GC

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stationary phase, has been coated to absorb the desired analytes and concentrate them on the fiber. The selectivity of the extraction of target analytes in the gaseous phase can be significantly altered through the use of different liquid phase on the fiber [9].

Mustard paste, which is usually served as a spice for food flavoring, has become increasingly popular. The main ingredients of mustard paste are *Brassica nigra* and *Brassica alba* seeds. The most predominant constituent of *Brassica nigra* is allyl isothiocyanate, which accounts for more than 90% of the total volatile compounds. The most predominant constituent of *Brassica alba* is sinalbin disulfide. Consequently, the analysis of the volatile flavor compounds in mustard paste can identify the mustard varieties.

2. Materials and methods

2.1. Materials

Mustard pastes (made in Japan) were purchased from a local supermarket. The mustard paste is composed of mustard, sorbitol, corn oil, salt, water, artificial flavor, xanthan gum, turmeric, and artificial color (FD&C Yellow no. 5, FD&C Blue no. 1). The components of the standard solutions were all purchased from Sigma (St. Louis, MO, USA). Standard solutions were used to optimize GC–MS and SPME conditions. All solutions were stored at 4°C.

2.2. Sampling conditions of SPME

For the SPME determinations, a SPME holder and three fibers (100-μm PDMS, 65-μm PDMS–DVB and 65-μm CW–DVB) were used (Supelco, Bellefonte, PA, USA). The fibers were conditioned under helium at 290°C for 4–5 h prior to use. Between uses, fibers were kept sealed from ambient air by inserting the tip of the SPME needle into a small piece of septum to prevent accidental contamination. The sampling procedure involved placing 2–3 g of sample into a 20-ml vial and sealing with a screwtop septum-containing cap. The SPME needle was then inserted through the septum and suspended in the headspace of the vial. The vial was placed in a

waterbath maintained at 30, 50 and 70°C, respectively, to optimize temperature of extraction. The vial was submerged only as far as necessary to submerge the solid phase of the sample, to help keep the SPME fiber cool, which is a desired condition for SPME. This is because as the temperature of the fiber increases, the partition coefficient decreases [10]. The SPME holder was secured and the fiber extended into the headspace, and the fiber was equilibrated for 20, 40 and 60 min, respectively, to optimize the time of extraction. The fiber was then retracted, removed from the vial, and placed immediately into the injector of the GC at 250°C. Injection was accomplished by extending the fiber in the heated inlet for 3, 5 and 7 min, to optimize the time of desorption, while the injector operated in the splitless mode for 2 min. The additional time of exposure time in the injector port allowed the fiber to be cleaned of any compounds that may not be desorbed in the initial minute. Preliminary studies indicated that the above procedure allowed for reproducible, quantitative transfer of target analytes into the injector port of the GC–MS.

2.3. Sampling conditions of SDE

Simultaneous distillation–solvent extraction was carried out in a microversion apparatus, as described elsewhere [11]. Dichloromethane (chromatography-grade reagent, Merck) and *n*-tetradecane were used as solvent and internal standard, respectively. For each extraction, 10 g of mustard paste and 250 ml distilled water were placed in a 500-ml flask, 30 ml dichloromethane was placed in a 50-ml flask, stream distillation was stopped after 2 h, while the solvent extraction was continued for a further 15 min. The extract was concentrated to 1.0 ml at 55°C by Kuderna-Danish apparatus (NE-1, Japan). The injection volume was 2.0 μl with a split ratio of 20:1. A series of three consecutive extractions was performed on different aliquots of mustard pastes in order to evaluate the repeatability of SDE method.

2.4. Condition of GC–MS

Autosystem TurboMass GC–MS (Perkin-Elmer, USA) was used. A 30 m×0.25 mm Supelco-wax

quartz capillary column (Supelco, Bellefonte, PA, USA) with 0.25-µm film thickness was used to resolve the volatiles with the following temperature programming: initial oven temperature was 60°C, kept for 2 min; then was raised to 240°C at 4°C/min, and kept at 240°C for 15 min. Helium was used as carrier gas with column head pressure at 10 kPa. Programming split/splitless (PSS) injector temperature was at 250°C. In SPME analysis, the I.D. of the injection liner was 1.5 mm; the desorption time was 5 min in splitless mode; and the time of the splitless was 2 min. In the analysis of SDE extract, the I.D. of the injection liner was 4.0 mm; the split ratio was 20:1, and the amount of injection was 2 µl. The temperature of the GC–MS transfer line was 250°C. The MS was operated at 170°C in the electron impact mode (70 eV), scanning from m/z 33 to 350 in 0.3 s with an 0.2-s interval time of the scan; the voltage of the photoelectric multiplier tube (PMT) was 230 V. The mass spectral identifications of the volatiles were carried out by comparing to the NIST98 (National Institute of Standards and Technology, Gaithersburg, MD, USA) mass spectral library as well as to the Wiley 6.0 (Wiley, New York, NY, USA) mass spectral library.

3. Results and discussion

3.1. Comparison of SDE and headspace SPME technique

As shown in Table 1, traditional SDE technique could extract all the volatile flavor compounds of mustard paste, except for 1,2-propanediol. Compared with SPME, SDE could also extract high-molecular-mass and low volatility compounds such as oleic acid, 9-hexadecenoic acid and palmitic acid in volatile flavor compounds of mustard paste. So the traditional SDE technique seemed more comprehensive but less sensitive to trace components. Large amounts of furfural and furfural alcohol were found in the SDE extracts, perhaps arising from pyrolysis or hydrolysis during the SDE process. In fact, if these compounds were mustard paste volatile flavor components, they should easily be extracted by SPME. However, they were found only in SDE extracts. Representative TIC chromatograms of volatile flavor compounds from mustard pastes are shown in Fig. 1. Compared with SDE, SPME showed enormous advantages: simplicity, rapid solvent-free extraction, low cost, little interference, no apparent

Table 1
GC–MS identification of mustard paste volatiles and peak area percentages

| Peak no.	t_R (min)	Compound name	Peak area (%)			
			100-µm PDMS	65-µm CW–DVB	65-µm PDMS–DVB	SDE
1	5.08	1-Propene,3,3-thiobis	0.022	0.013	0.017	0.009
2	8.10	Thiocyanic acid, methyl ester	0.016	0.027	0.021	0.011
3	13.56	Allyl isothiocyanate	98.58	63.62	93.24	98.84
4	13.83	Furfural	ND	ND	ND	0.081
5	14.20	Diallyl disulfide	0.022	0.008	0.013	0.012
6	17.50	Methyl allyl trisulfide	0.014	0.012	0.013	0.006
7	17.88	1,2-Propanediol	1.128	35.77	3.125	ND
8	19.87	Furfural alcohol	ND	ND	ND	0.125
9	23.37	Diallyl trisulfide	0.015	0.014	0.014	0.005
10	26.93	Butylated hydroxyl toluene	0.070	0.013	0.041	0.011
11	28.47	5-Methyl-tetrahydrothiophen-2-one	0.051	0.330	0.195	0.014
12	31.25	Ethanol, 1-methoxy-,benzoate	0.009	0.041	0.027	0.011
13	34.72	2-Phenylethyl isothiocyanate	0.014	0.021	0.016	0.012
14	49.67	Palmitic acid	ND	ND	ND	0.508
15	50.61	9-Hexadecenoic acid	ND	ND	ND	0.799
16	50.90	Oleic acid	ND	ND	ND	1.522

ND, not determined.
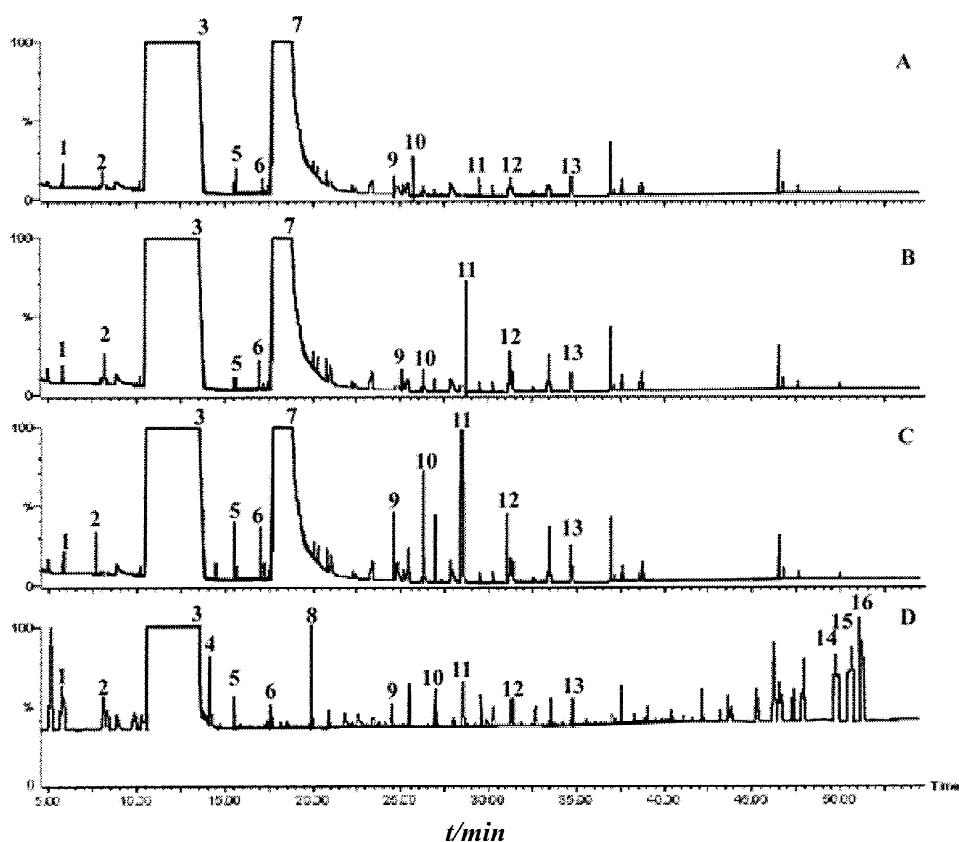
Fig. 1. Representative TIC chromatograms of volatile flavor compounds from mustard pastes extracted by SPME with 100-μm PDMS (A), 65-μm CW–DVB (B), 65-μm PDMS–DVB (C) and SDE (D). Identified peaks: 1 = 1-Propene, 3,3-thiobis(PT); 2 = thiocyanic acid, methyl ester (TAME); 3 = allyl isothiocyanate (AI); 5 = diallyl disulfide (DADS); 6 = methylallyl trisulfide (MAT); 7 = 1,2-propanediol (PP); 9 = diallyl trisulfide (DATS); 10 = butylatedhydroxyl toluene (BHT); 11 = 5-methyltetrahydrothiophen-2-one (MTHO); 12 = ethanol,1-methoxy-,benzoate (EMB); 13 = 2-phenylethylisothiocyanate (PLI).

sample hydrolysis or pyrolysis, the possibility of automation [12,16], and suitability for routinely screening. As shown in Table 2, the repeatability of SDE was apparently better than SPME. So for the quantitative analysis of volatiles the choice should be SDE. Compared with SPME, the main disadvantages of SDE were: time consuming, possibility of solvent contamination, and laborious manipulation

SDE and SPME had their own fundamental and practical trade-offs. SPME was suitable for simple, rapid routinely screening, while SDE was suitable for proper quantitative analysis.

3.2. Comparison of SPME conditions

In this study, the analysis of volatile flavor com-

pounds from mustard paste was originally undertaken through the use of headspace SPME technique. It was found that the ability of the SPME fiber to concentrate the analytes provided a dramatic improvement in the amount of analytes, and produced a method capable of analyzing volatile flavor compounds. Different fibers were experimented with to determine an optimum fiber for the detection and quantification of the flavor volatiles. The effectiveness of composition of the fiber coating, duration of absorption and desorption, temperature of absorption were investigated. Much higher sensitivity and lower detection limits were achieved using 65-μm polydimethylsiloxane–divinylbenzene (PDMS–DVB) fiber than with 100-μm PDMS fiber or 65-μm Carbowax–divinylbenzene (CW–DVB). The detection limits is

Table 2
Repeatability of SPME ($n=5$) and SDE ($n=3$)

Peak no.	Compound name	RSD (%)			
		100 μm CW–DVB	65 μm PDMS	65 μm PDMS–DVB	SDE
1	1-Propene,3,3-thiobis	6.23	3.71	6.74	2.03
2	Thiocyanic acid,methyl ester	5.76	8.73	4.73	3.74
3	Allyl isothiocyanate	8.64	3.52	2..22	1.61
4	Furfural	–	–	–	3.49
5	Diallyl disulfide	5.66	5.75	8.08	2.53
6	Methyl allyl trisulfide	–	8.77	9.24	3.68
7	1,2-Propanediol	4.60	4.20	7.99	–
8	Furfural alcohol	–	–	–	1.36
9	Allyl trisulfide	7.91	9.72	3.29	2.27
10	Butylated hydroxyl toluene	5.81	7.55	5.82	3.52
11	5-Methyl-tetrahydrothiophen-2-one	9.55	7.44	4.29	2.45
12	Ethanol,1-methoxy-,benzoate	7.91	4.75	6.48	1.45
13	2-Phenylethyl isothiocyanate	6.68	9.14	8.89	2.37
14	Palmitic acid	–	–	–	3.09
15	9-Hexadecenoic acid	–	–	–	1.34
16	Oleic acid	–	–	–	2.15

–, not determined.

less than 0.3 μg l^{-1} for most of analytes. The relative standard deviation (RSD) is better than 9%. This method was applied to a food sample (mustard paste) using an external calibration.

3.2.1. Extraction temperature

The extraction temperature profile obtained using a PDMS–DVB fiber is shown in Fig. 2. Optimum extraction efficiency was achieved at 50°C. The lower absorption of most analytes at 30°C was due to the decreased rate of diffusion of the analytes. The rate of diffusion of the analytes through the static
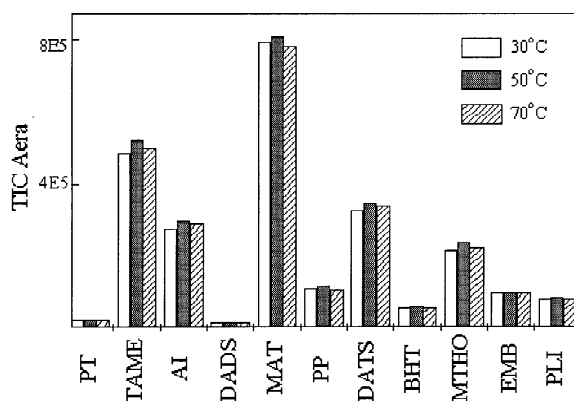

Fig. 2. Extraction temperature profile for 65-μm PDMS–DVB fiber. Extraction time, 40 min; desorption time, 5 min.

aqueous layer at the fiber-gas interface increased with increasing temperature, so that more analytes were absorbed at higher temperature if equilibrium had not been reached. The decreasing absorption with increasing temperature at 70°C was presumably due to the distribution constant decreasing with increasing temperature. The absorption process was exothermic, thus lowing the temperature increased the distribution constant at equilibrium. In practical applications when the extraction was stopped before reaching the equilibrium, not only thermodynamic but also kinetic aspects became important. An extraction temperature of 50°C was selected for this study using the three fibers, because this temperature was relatively easily maintained, and the improvement in sensitivity at higher temperature was not necessary.

3.2.2. Extraction time

The extraction time profile obtained using PDMS–DVB fiber is shown in Fig. 3. For the PDMS–DVB fiber, the equilibrium condition for the absorption of the most analytes was almost reached after 40 min. Factors that influenced the equilibration period were investigated by Pawliszyn and co-workers [13–15]. The equilibration rate was limited by (1) the mass transfer rate of the analytes through a thin static aqueous layer at the fiber-gas interface, (2) the
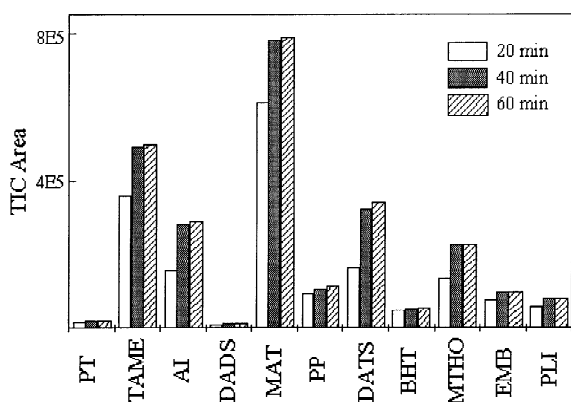
Fig. 3. Extraction time profile for 65-μm PDMS–DVB fiber. Extraction temperature, 50°C; desorption time, 5 min.

distribution constant of the fiber coating and (3) the thickness and kinds of the fiber coating. Extraction periods of 40 min were chosen for the three fibers since it was approximately equivalent to the time required to run GC in this experiment.

3.2.3. Desorption time

The desorption time profile obtained using the PDMS–DVB fiber is shown in Fig. 4. A desorption period of 5 min was enough to desorb the analytes from the PDMS–DVB fiber (temperature of the GC injection port, 250°C). So a desorption period of 5 min was used for the three fibers (temperature of the GC injection port, 250°C). No carryover of any volatile flavor component was observed.
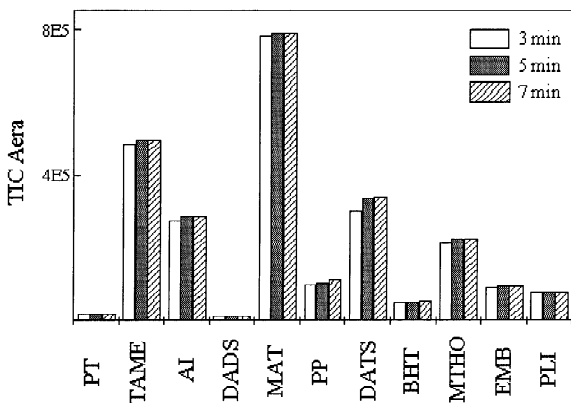
3.2.4. Comparison of different fibers

Three different fibers were evaluated to determine which fiber most effectively extracted flavor volatiles from mustard paste samples. The fibers that were used to extract analytes from the headspace of aliquots of the same sample for comparison of the relative extraction effectiveness were 100-μm PDMS, 65-μm CW–DVB and 65-μm PDMS–DVB, respectively. The results of the experiments on these three fibers are summarized in Fig. 5. These results show that, of the fibers evaluated, the PDMS–DVB fiber proved to be the most effective in extracting flavor volatiles overall, followed by the PDMS fiber, then the CW–DVB fiber. Therefore, the 65-μm PDMS–DVB fiber was used for all subsequent comparison experiments. The PDMS–DVB fiber was chosen as a representative to investigate the duration of absorption and desorption, temperature of absorption, detection of limits, and the precision of SPME in this investigation.

The PDMS–DVB fiber performed the most effective extractions, for this analysis, because the fiber coating was composed of a mixed coating containing PDMS, a liquid phase that favored the absorption of nonpolar analytes, as well as DVB, a porous solid that favored the adsorption of the more polar analytes. There was little difference between the PDMS fiber and the PDMS–DVB fiber in extracting the nonpolar analytes (butylated hydroxytoluene), but the more polar disulfide and trisulfide were extracted, on average, three times better by the PDMS–DVB
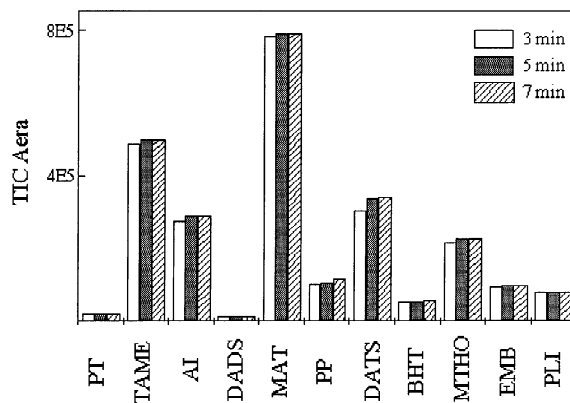

Fig. 4. Desorption time profile for 65-μm PDMS–DVB fiber. Extraction temperature, 50°C; extraction time, 40 min.

Fig. 5. Comparison of 100-μm PDMS, 65-μm PDMS–DVB and 65-μm CW–DVB fibers.

fiber as measured by peak area. The CW–DVB fiber, which was more selective towards polar analytes, did show enhanced extraction effectiveness of the polar analytes, but was less effective with the nonpolar analytes.

3.2.5. Repeatability

A series of five consecutive extractions were performed on different aliquots of mustard pastes in order to evaluate the repeatability of the headspace SPME (HS-SPME) method. The precision of the HS-SPME method was good and the RSD values were between 2.22 and 9.72% for all the 11 volatile flavor compounds in mustard paste (Table 2).

3.3. Mustard pastes determined by SPME–GC–MS

In total, 11 volatile compounds in mustard paste were identified, which accounts for 99% of TIC peak area as shown in Table 1. The four methods made up one another and validated mutually. Since allyl isothiocyanate was the main volatile constituents of the mustard pastes (Table 1), the main ingredient of the mustard pastes was *Brassica nigra* seeds.

4. Conclusions

Traditional SDE analysis of volatile compounds is a widely used technique. However, for many analyses, the SDE method lacked the sensitivity and convenience needed to perform adequately. SPME had the ability to perform these analyses where SDE fell short. Comparison of SDE and SPME showed that SPME determinations of flavor compounds were, on average, more sensitive under the conditions employed in this study. The increased sen-

sitivity allowed fast, accurate determinations of flavor compounds and easy performance of analyses. Consequently, SPME was suitable for simple, rapid, routine screening, while SDE was used for proper quantitative analysis.

Profiling of different mustard paste samples were performed. Different fibers were investigated with the best fiber found to be 65-μm PDMS–DVB. The optimal parameters for SPME sampling were also investigated.

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