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Determination of SCFAs in Food Wastewater using HS-SPME

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In this study, we identified short chain volatile fatty acids (SCFAs) in wastewater using headspace solid-phase micro-extraction (HS-SPME) coupled to gas chromatography (GC) analysis. Among three different SPME coatings, (i.e., 85 μm Polyacrylate (PA), 75 μm Carboxen/Polydimethylsiloxane (CAR/PDMS) and 30/50 μm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS)), 75 μm CAR/PDMS fiber was chosen since it showed the most successful extraction of Acetic acid (AA) and Propionic acid (PA). The optimal extraction and quantification conditions for the fiber such as extraction time and temperature, desorption time and temperature, agitation speed, sample volume and headspace/aqueous ratio also have been determined. The resulting calibration curve for each analyte showed good linearity (R^2: 0.997~0.999) over the range from several ppbv to ppmv except for AA (R^2: 0.986), low method detection limit, and good reproducibility in the quantification of the SCFAs. The developed method was applied to analyze SCFAs in extracted liquid from food wastes. The results obtained from the developed method were comparable to those from the method using GC with direct liquid injection. However, the distillation method underestimated the total SCFAs (C_2~C_6 acids) especially when C_2~C_6 acids were at high levels.

Key words: SCFAs, SPME, Food wastewater, CAR/PDMS fiber, The optimal analysis conditions

1. Introduction

The so-called “short chain volatile fatty acids (SCFAs)” are linear organic acids with 2-to-6 carbon molecules: Acetic acid (AA), Propionic acid (PA), n-Butyric acid (n-BA), iso-Butyric acid (i-BA), n-Valeric acid (n-VA), iso-Valeric acid (i-VA), n-Caproic acid (n-CA), and iso-Caproic acid (i-CA). SCFAs are low-molecular mass organic acids with a strong hydrophilic character and high volatile compounds. They are produced largely as a result of the breakdown of dietary carbohydrates in the gut by anaerobic bacterial fermentation. The SCFAs present in landfill leachates,[1-3] animal manures,[4-5] wastewater thickened sludges[6] and anaerobic digesters.[7-8]

SCFAs serve various functions in the environment. As a product of anaerobic bacteria activity, they are used as indicators of anaerobic bacterial activity and play an important role in the biological removal of phosphorus and denitrification in wastewater and activated sludge.[9-12] Yo et al. (1999)[7] presented that SCFAs are important in the development of a sensitive monitoring method for evaluation of the quality of the discharged water from a three-stage swine wastewater treatment system (basin for separation of the solid/anaerobic reactor/aerobic reactor). The SVFA contents of immature leachates provide an indication of the maturity of the leachate and potential of the site to produce methane, a factor which needs to be considered in redevelopment.[2] Also, SCFAs are one of the odorous compounds in the mixture of animal manures and wastes including indoles and phenols, ammonia and volatile amines, and volatile sulfur containing compounds. Butyric acid, valeric and caproic are primarily responsible for the offensive odor because they have a lower odor detection threshold.[13-15]

As noted above, the methods available for the

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analysis of SCFAs, that have important functions in the environment. They are:

• Distillation methods[17]: The standard method of distilling VFAs by adding H_2SO_4 and titrates with NaOH to the distilled sample is a relatively simple and cost-effective procedure. However, this technique cannot determine particular species of VFAs and results are commonly reported as total VFA content containing up to six carbon atoms.

• Titrimetric method[10,18,19]: Mostly very simple procedures that can measure VFAs in water with minimum time and effort using pH change and predicted concentrations of AA, PA and BA by the difference in each -logKa (pKa) value.[19] This technique is often applicable to the on-line monitoring[16] of VFAs in digestion sludge,[20] sewage inflow,[21] and upflow anaerobic sludge blanket (UASB) reactor outflow.[22] However, phosphate, ammonium, sulfide weak acid subsystems and sulfide are liberated during pH titration and often this method is said to be not fit for low VFAs concentrations such as those present in domestic wastewater.[23]

• Ion chromatography method[2]: Formate can be determined and the reproducibility of the analysis is relatively good (% relative standard deviation (RSD); formate: 0.58 ~ valerate: 2.07). However, detection limits for the VFA species are rather high at approximately 5 mg/L, and this method requires multiple dilutions and varies in sample pretreatment to prevent column contaminations.

• GC with direct liquid injection without derivatization[24] : The most widely used method of VFA analysis injects tiny liquid samples without derivatization into GC-flame ionization detection (FID). Although this method is rapid, it can lead to contamination of the GC injection port, column and detecter; interfere with analysis; and degrade chromatographic performance.[25]

• GC[3,26] or High performance liquid chromatography (HPLC)[27,28] with derivatization method: After the transfer of VFAs from the aqueous phase into fatty acid methyl esters (FAMEs), FAMEs are extracted with solvent and determined using GC or HPLC. This method has the advantage of non-aqueous injection and in the case of HPLC with a derivatization method, VFAs of lower concentration can be determined including formate (acetate: 0.012 mg/L, formate: 0.0093 mg/L). However, this procedure suffers from a number of disadvantages such as the extended sample preparation times and costly disposal of used solvent. The highlighted potential problems associated with ester preparation include[29]: (1) incomplete conversion of lipids into FAMEs; (2) changes in the original fatty acid composition during transesterification; (3) incomplete extraction of FAMEs after transesterification; and (4) losses of highly volatile short-chain FAMEs, which could affect the reproducibility of extractions and the calibration of the procedure.

• Headspace gas chromatography (HS-GC)[30]: This method offers relatively rapid and solvent-free analyses and has sufficient detection limits for the determination of VFAs in food wastewater (AA : 3.7 mg/L ~ VA : 0.3 mg/L).

In this study, SCFAs (C_2~C_7 acids) in the food wastewater samples were analyzed using head-space SPME (HS-SPME) coupled to GC. SPME utilizing a small, coated fiber equilibrates with the gas phase prior to direct analysis by gas chromatography. The fibers are relatively inexpensive and reusable, and can be used in ambient and laboratory experiments for analysis of multiple volatile and semi-volatile compounds. SPME is based on an equilibrium process and the mass of analyte adsorbed by the fiber is proportional to its concentration in the sample matrix. Unlike other conventional methods, where extensive sample preparation is required, SPME is a one-step extraction procedure without solvent.[31,32] SPME has been the subject of extensive studies including research on its automation and optimization and the dynamics of absorption in a recent book, as well as a number of applications, including an analysis of volatile contaminants in foods,[33] volatile odorants in wastewater,[34] trace pesticides,[35] and volatile organic compounds in soil.[36]

The purpose of this study is to determine SCFAs in an aqueous solution using headspace solid-phase microextraction (HS-SPME) coupled to GC and determine the optimal parameters affecting extraction and desorption of SCFAs from an SPME fiber, such as extraction

time and temperature, desorption time and temperature, agitation speed, and the headspace/aqueous ratio. After the method was optimized it was applied to the analysis of SCFAs in real samples. The result was then compared with those in the distillation method and GC with liquid direct injection with the same samples, which are the most frequently used.

2. Experimental Procedures

2.1. Chemicals and materials

The volatile acids' standard mixtures containing 10 components ($C_1 \sim C_7$ acids), 10 mM each in deionized water were obtained from Supelco (Bellefonte, PA, USA). The HPLC analytical-grade dilution water and HCl used in this experiment were purchased from J. T. Baker (Phillipsburg, NJ, USA). NaCl was from SHINYO CHEMICALS (Osaka, Japan) and heated to 500°C overnight and then stored in a desiccator at room temperature. 2-ethylbutyric acid (2-EB), 2-methylburyric acid (2-MB), and 2-ethylvaleric acid (2-EV) used as the internal standards were obtained from Aldrich (Steinheim, Germany).

2.2. Instruments analysis

The SPME manual holder and coating fibers (85 PA; 75 CAR/PDMS; 30/50 DVB/CAR/PDMS) were purchased from Supelco (Bellefonte, PA, USA). The fibers were conditioned in the GC injector port according to the manufacturer's instructions. Analyses of SCFAs using SPME were carried out in a Hewlett Packard-5890 GC system (Hewlett-Packard, Avondale, PA, USA) coupled to FID. GC systems were equipped with a Merlin microseal septum and 0.75 mm i.d glass liner (Supelco, Bellefonte, PA, USA) designed for SPME to insure reproducibility between injections. A 30 m × 0.25 mm i.d. (0.25 μm film thickness) DB-Wax capillary column (J & W Scientific, Folsom, CA, USA) was used. The carrier gas was nitrogen of a purity 99.999% and kept at a 1 mL/min constant flow. The oven temperature program was held at 70°C for 2 min, raised to 170°C at 4.5°C/min, and held at 170°C for 2 min, then raised to 200°C at 10°C/min, and kept at 200°C for 1 min. The injection port and detector temperature were maintained at 250°C and 280°C each and splitless injection mode (1 min) was used.

Analyses of SCFAs using GC with liquid direct injection were carried out in a Simadzu GC-17A-FID system (Simadzu, Kyoto, Japan) equipped with auto sample injector (Shimadzu AOC-20i). A Nukol fused silica capillary column (30 m × 0.25 mm i.d.) with 0.25 μm film thickness (Supelco, Bellefonte, BA, USA) was used. The carrier gas was nitrogen of purity 99.999% and kept at a 1 mL/min constant flow. The oven temperature program was 130°C (held for 10 min) to 160°C (held for 5 min) at 4°C/min, and finally up to 200 °C (held for 2 min) at a rate of 20°C/min and the total analysis time was 26 min. The GC injection port and detector temperature were maintained at 200°C and 280°C each and the split injection mode (1 min) was used (Split ratio: 50:1). The injection volume, 1.0 μL, was injected into the GC injection port directly using an auto sample injector.

For determination of SCFAs with the same samples, the distillation sets were equipped with a 76 cm condenser proposed the standard method.[17]

2.3. SPME Procedure

Samples for method development were prepared by adding 40 mL of Milli-Q water and a stir bar saturated with NaCl 35% (w/v%) into a 120 mL vial, and pH was adjusted to 1.5 by injection HCl (0.5 M) through the vial. Then, the vial was sealed with polytetrafluoroethylene (PTFE) septum, spiked with the VFAs standard mixture and internal standard (2-EB) by injection through the septum using syringe and placed in a water bath at 30°C. The samples were stirred under magnetic stirring 400 revolution per minute (rpm) ± 5 rpm while an SPME fiber was exposed to the headspace of the vial for 30 min. The extraction temperature was controlled at 30°C. After 30 min of exposure, the fiber was immediately inserted into the GC injection port for desorption. If the analysis was delayed, the fibers were stored in the freezer at - 40°C and all analyses were conducted within 4 hours.

For the determination of SCFAs in real samples, the

aliquot of food wastewater samples was taken and centrifuged at 3,000 rpm for 5 min, and the supernatant was filtered through Whatman 0.45 µm GF/C filter papers. The filtered sample was diluted multiply times and then split to follow three routes for the HS-SPME method, GC-liquid direct injection method and distillation method. The analytical procedures were presented in Fig. 1.

3. Results and Discussion

3.1. SPME fiber selection

The affinity of the coating for an analyte is the most important factor in the successful use of SPME. Selection of the coating is based primarily on the polarity and volatility of the analyte. Three commercially available SPME fibers (PA, CAR/PDMS, DVB/CAR/PDMS) were investigated for their extraction properties for SCFAs in food wastewater in this study. Fig. 2. shows the relative extraction efficiencies of SCFAs (expressed by peak areas of each compound).

As shown in Fig. 2. the relative GC peak area (extraction property) for SCFAs was generally inversely proportional to its molecular weight (C_4~C_7 acids) and the most suitable fiber for the extraction of the compounds studied was the CAR/PDMS-coated fiber, which extracted all of the C_2~C_6 acids with the best efficiency. Detection time reproducibility of PA, CAR/PDMS, DVB/CAR/PDMS fiber GC peak was [% RSD (mean)] 0.09~0.36 (0.20), 0.07~0.49 (0.21) and 0.02~0.11 (0.05), respectively and the DVB/CAR/PDMS fiber was good. However, as the species of SCFAs in the environmenta are AA, PA, and BA, the CAR/PDMS fiber was chosen for further optimization.

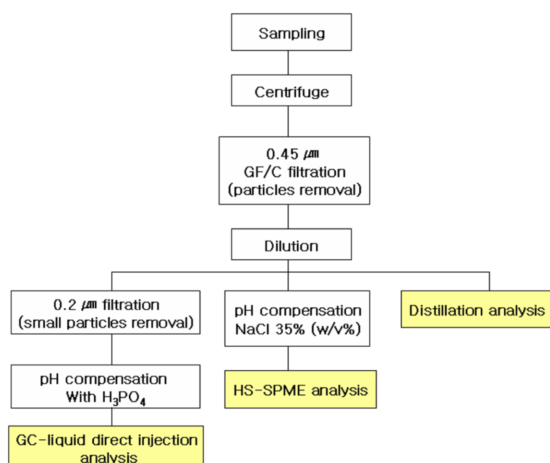
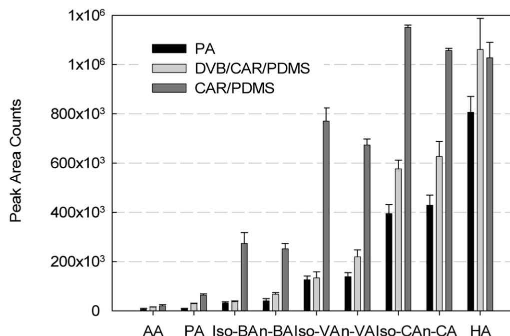


Fig. 1. Different procedures for SCFAs analysis in real samples.



[Acetic acid (AA), Propionic acid (PA), n-Butyric acid (n-BA), iso-Butyric acid (i-BA), n-Valeric acid (n-VA), iso-Valeric acid (i-VA), n-Caproic acid (n-CA), iso-Caproic acid (i-CA), Heptanoic acid (HA)]

Fig. 2. Extractability of different SPME fibers. (Concentration, 0.1 mM each; extraction temp., 25°C; extraction time, 30 min; desorption temp., 250°C; desorption time, 3 min; no stirring).

3.2. pH effect on analyte's absorption on fiber

The use of salt or pH adjustments reduces solubility of the analytes in the solution. SPME can extract only neutral (nonionic) species from the matrix. By properly adjusting the pH, weak acids and bases can be converted to their neutral forms, which enables them to be extracted by the SPME fiber. Since free C_2~C_7 acids are polar, at neutral pH, many of them are still in the ionic form and more soluble in water. And as the pKa of C_2~C_7 acids are 4.75~4.96,[37] upon lowering the pH, the acid-based equilibrium shifts toward the neutral forms of the acids which have a greater affinity for the fiber, and the amount extracted increases.

To fit the optimal pH condition, a 0.5 M HCl solution was injected and adjusted to the pH ranging from 1.0~3.0. As shown in Table 1, no significant variation in extraction of the C_2~C_7 acids was observed in the pH range 1.0~2.0. However, the extraction efficiency of fiber decreased at pH 3.0. The pH of the sample solutions 1.5 was used for further study because of the

Table 1. The matrix effects enhancement of the extraction of SCFAs with CAR/PDMS fiber

Compounds	Factor increase with pH effect				Factor increase with salt effect (w/v%)		
	pH 1.0	pH 1.5	pH 2.0	pH 3.0	0%	25%	35%
AA	1	0.9	0.8	0.6	0.6	0.8	1
PA	1	1	0.8	0.8	0.4	0.8	1
Iso-BA	1	0.9	0.9	0.7	0.5	0.8	1
n-BA	1	0.9	0.9	0.8	0.5	0.9	1
Iso-VA	1	1	0.9	0.8	0.5	0.7	1
n-VA	1	1	1	0.9	0.4	0.6	1
Iso-CA	1	1	0.9	0.9	0.3	0.6	1
n-CA	1	1	1	0.8	0.3	0.5	1
HA	1	1	0.9	0.9	0.2	0.5	1

[Acetic acid (AA), Propionic acid (PA), n-Butyric acid (n-BA), iso-Butyric acid (i-BA), n-Valeric acid (n-VA), iso-Valeric acid (i-VA), n-Caproic acid (n-CA), iso-Caproic acid (i-CA), Heptanoic acid (HA)]

recommended pH guidelines of the CAR/PDMS fiber ranging from pH 2.0~11.0 but in consideration of head-space exposure.

3.3. Salt effect on absorption of analyte on fiber

Altering the salt concentration in the solution can affect the analyte's adsorption on fiber. The suitability of the headspace SPME technique for the extraction of compounds in water depends on the transfer of analyte from the aqueous phase to the gaseous phase. The salt addition could significantly decrease their solubility in water, resulting in a higher concentration of these compounds in the headspace. Conversely, the higher the salt saturation concentration in the sample matrix, the greater the increase in the amount extracted on the fiber. The effect on ionic strength was determined by preparing standards with salt concentrations ranging from 0 to 35% (w/v). After adjusting 0.1 mM standard solution, CAR/PDMS fiber was exposed to the head-space of the vial for 30 min. As a result, a salt addition of 35%, compared to no salt added offers an improvement in the extraction efficiency of about 1.7~5.0 times. 35% of the salt, saturated salt solution, was added to all samples in further experiments.

3.4. Extraction-time profile

The extraction efficiencies of analytes increased with extraction time untill it reached equilibrium between the sample matrix, gaseous headspace, and fiber coating. Fig. 3. shows the effect of the equilibrium time (10, 20, 30, and 60 min) on the extraction efficiency of C_2~C_7 acids using a CAR/PDMS fiber. Since equilibrium times

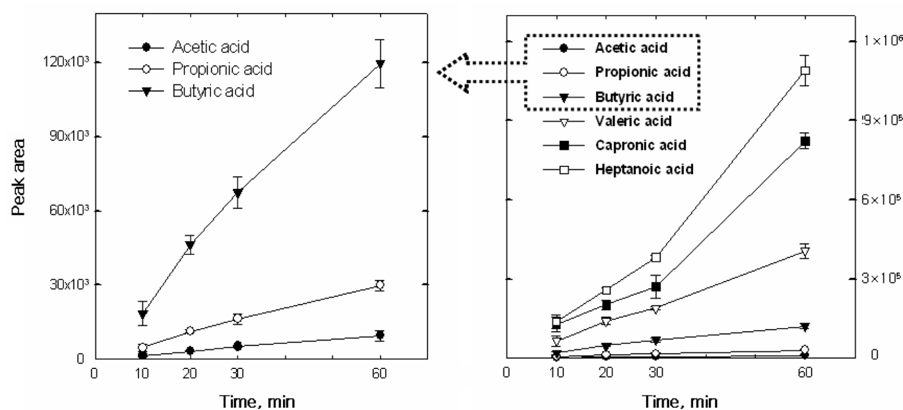


Fig. 3. Extraction time profiles of C_2~C_7 acids using a CAR/PDMS fiber. (Concentration, each 0.1 mM, desorption time and temp., 5 min and 250°C, pH 1.5, 35% salt concentration (w/v), no stirring).

for these fibers were 60 min more. when equilibration times are excessively long, shorter extraction times can be used. However, in such cases, the extraction time and mass transfer conditions must be controlled strictly to assure good precision. In this study, as the extraction time was shorter, the reproducibility of the fiber extraction efficiency was decreased [% RSD; 37.7(10 min) to 8.5(30 min) for AA, 33.5(10 min) to 7.0(60 min) for PA and 26.4(10 min) to 8.1(60 min) for BA]. The extraction time of 30 min was selected for further optimization. This provides sufficient extraction efficiency and allows the headspace SPME procedure to be performed approximately in the same time as that required for GC analysis (30 min).

3.5. Effect of extraction temperature

Extraction temperature plays an important role on the extraction of analytes because it influences the mass transfer rates and the partition coefficients of analytes. Extractions were performed at 25, 30, 40, and 50°C. Fig. 4 shows that there was not a noticeable difference in the peak areas of AA and PA, according to temperature. The peak areas of C_4~C_5 acids obtained at 30°C were a little higher than those achieved at 40 and 50°C (factors ranged from 1.0 to 4.3 for C_4~C_5 acids, respectively). On the contrary, when the temperature was raised from 30 to 50°C, a decrease is observed. This is most likely due to the heating of the fiber, as reported by Zhang and Pawliszyn,[38] resulting in decreased absorption and enhanced redesorption of the analyte onto the fibers. In consideration of the error bar range, the peak areas of 30 and 40°C did not show a noticeable difference. However, the relative standard deviations at 40°C were particularly high for isovaleric, isocaproic and caproic acids, 58%, 50%, and 28%, respectively. Thus, a 30°C extraction temperature was selected for further experiments.

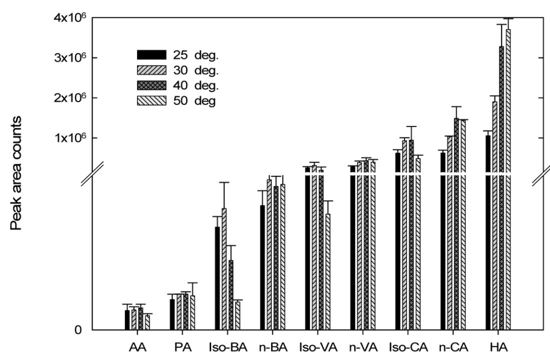


Fig. 4. The relative extraction efficiencies according to different extraction temperature on the CAR/PDMS fiber. (Concentration, each 0.1 mM; extraction time and temperature, 30 min and 30°C; desorption temp., 250°C; desorption time, 5 min; no stirring).

3.6. Effect of desorption temperature and time

Efficient thermal desorption of the analyte with SPME in a GC injection port is usually dependent on the injector temperature and exposure time. The optimal desorption temperature and time are needed to enhance the efficiency of detection and minimize a carryover effect (which is a condition in which a small peak in the residue analytes is detected).[39] The desorption temperature 200~280°C profile is studied. Peak areas of C_2~C_5 acids did not show a noticeable difference according to temperature and low percentages of carryover were found for these compounds (1~5%) but peak areas of C_6~C_7 acids were relatively increased with a desorption temperature from 200 to 280°C (absorption efficiency ranged from 25 to 43% for n-CA, HA, respectively). The highest temperature had to be used in order to obtain the maximum peak area with the minimum carryover effect within a range of 10~20°C lower than the temperature limit of the fiber. The desorption time of 3 min and 5 min were studied at a desorption temperature of 280°C. Similar peak areas were obtained along all the experiments for C_2~C_7 acids. Nevertheless, a desorption time of 5 min was selected for the complete desorption of real samples with high concentrations.

3.7. Effect of stirring rate

Sample agitation enhances extraction efficiency and decreases extraction time since as the sample was agitated, volatile analytes in solution could be easily transferd to headspace and absorbed on fiber coating materials.[31] The studied stirring rate (0, 400, 800, 1,200 rpm) shows that the extraction efficiencies increased with the stirring rate but it was not expected. The peak

areas obtained when at 1,200 rpm agitation was a high factor of 1.4~2.7 compared to no agitation; however it was only a high factor of 0.9~1.5 compared to 400 rpm. This fact could be explained by the volatility of C_2~C_7 acids, which allow a large amount of the analytes in the headspace even when no agitation is used. Poor precision (% RSD.; 1~18, 2~20, 2~27) was obtained as the stirring rate was increased from 400 to 1,200 rpm. Thus, 400 rpm was chosen as the proper stirring rate.

3.8. Headspace/aqueous ratio

The optimal sample volume and ratio between the headspace and the aqueous phase were tested. A 20~80 mL of sample was added at 120 mL vial (headspace/aqueous ratio: 1/6, 1/3, 1/2, and 3/4, respectively). In view of the error range, there was not a noticeable difference in absorption efficiency along the headspace/aqueous ratio. It means that a tiny amount of analytes is absorbed on the SPME fiber and a change of headspace/aqueous ratio has a small effect on absorption efficiency. However, when the 40 mL was added in a 120 mL vial, the extraction efficiency was a little higher than the others (Fig. 5.)

3.9. Internal standard selection

As the internal standard, 2-MB, 2-EB and 2-MV was studied. A GC peak of 2-EB and 2-MV was detected between n-VA and iso-CA, which did not overlap with the C_2~C_7 acids except 2-MB. 2-EB and 2-MV could be used as the internal standard; however, reproducibility of 2-EB was a little higher than 2-MV for the same concentrations [4 (% RSD, n=4) for 2-EB, 5 (% RSD, n=4) for 2-MV]. 2-EB was spiked with internal standard for further experiments.

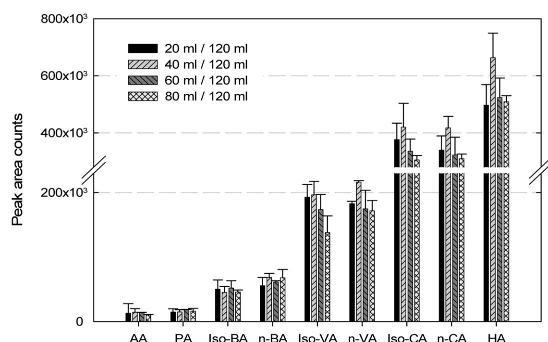


Fig. 5. Relative extraction efficiencies of different sample volume and ratio between headspace/aqueous phase on the CAR/PDMS fiber. (Concentration, each 0.1 mM; extraction temp., 30°C; extraction time, 30 min; desorption temp., 280°C; desorption time, 5 min; stirring rate, 400 rpm).

3.10. Linearity, precision and method detection limit

Method detection limits, precision and linearity for the analysis of C_2~C_7 acids used HS-SPME at the optimal conditions obtained through experiment (75 CAR/PDMS fiber; 40 mL sample in 120 mL vial; sample pH 1.5; salt concentration, 35% w/v; stirring rate, 400 rpm; 30 min absorption time at 30°C; 5 min desorption time at 280 GC injection port; internal standard, 2-EB). The resulting calibration curves were linear (R^2 = 0.996~0.999) (Table 2) over the range of 0.03~6.70 (PA), 0.08~7.50 (iso-BA), 0.07~7.40 (n-BA), 0.09~8.50 (iso-VA), 0.09~8.50 (n-VA), 0.10~10.00 (iso-CA), 0.05~10.20 (n-CA), and 0.06~11.10 (HA) mg/L except 0.03~5.10 mg/L for AA. R^2 for AA was slightly lower than other compounds because of substantial tailing of the chromatographic peak.

The method detection limits (MDL) for the compounds ranged from 100 and 13 μg/L for AA and PA to 11.1 μg/L for HA. MDLs from this study are lower than published for other analysis methods for determination of SCFAs in water. The reproducibility between fibers was measured to determine the error associated with the procedure. The average error produced between 75 CAR/PDMS fibers ranged from 4.1 to 12.0% for AA to HA.

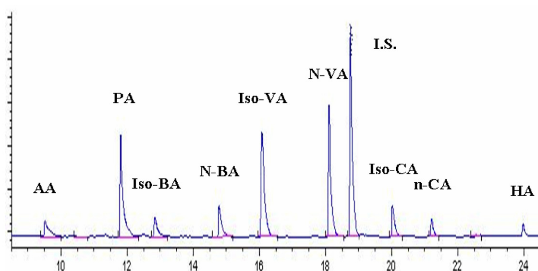
3.11. Analysis of environmental samples

The developed procedure was applied to the extraction of food wastes in the fermentation reactor. The chromatogram of the real sample is shown in Fig. 6. All the C_2~C_7 acids were detected from 8.5 min for AA to 24 min for HA within 25 min and 2-EB was used with the internal standard detected after n-VA. Substantial tailing of the chromatographic peak of AA and PA, with small molecular weight, was found.

Table 2. The R^2 of the calibration curve; % RSD and MDL of the method with HS-SPME for the SCFAs analysis

Compound	Linear range (mg/L)	Coefficient of determination (R^2)	MDL[a)] (μg/l)	% RSD[b)] (n=6)
Acetic acid (AA)	0.03~5.10	0.986	100	10.7
Propionic acid (PA)	0.03~6.70	0.996	13	7.2
Iso-Butyric acid (iso-BA)	0.08~7.50	0.998	7.5	7.6
n-Butyric acid (n-BA)	0.07~7.40	0.999	15	8.3
Iso-Valeric acid (iso-VA)	0.09~8.50	0.998	17	9.1
n-Valeric acid (n-VA)	0.09~8.50	0.999	43	5.2
Iso-Caproic acid (iso-CA)	0.10~10.00	0.999	10	4.1
n-Caproic acid (n-CA)	0.05~10.20	0.997	10.2	6.7
Heptanoic acid (HA)	0.06~11.10	0.997	11.1	12.0

[a)]Method detection limit calculated according to EPA[40)]
[b)]For the 75 CAR/PDMS fiber



[Acetic acid (AA), Propionic acid (PA), n-Butyric acid (n-BA), iso-Butyric acid (i-BA), n-Valeric acid (n-VA), iso-Valeric acid (i-VA), n-Caproic acid (n-CA), iso-Caproic acid (i-CA), Heptanoic acid (HA)]

Fig. 6. HS-SPME/GC-FID chromatograms of real samples (extraction of food wastes) with CAR/PDMS fiber. (extraction temp., 30°C; extraction time, 30 min; desorption temp., 280°C; desorption time, 5 min; stirring rate, 400 rpm), I.S. (internal standard: 2-ethylbutyric acid).

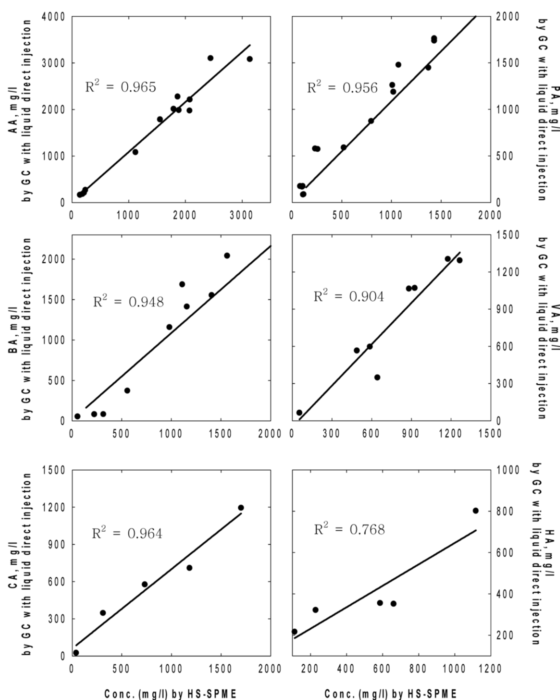


Fig. 7. Comparison of HS-SPME coupled to GC and GC with liquid direct injection of SCFAs in real food wastewater samples.

3.12. Comparison of results of determination for SCFAs in real same samples among HS-SPME, titration and GC with a liquid direct injection method

The results of the comparison analysis between HS-SPME and GC with liquid direct injection for determination of VFAs in extraction of food wastes are shown in Fig. 7. The resulting comparable curves were linear (R^2 = 0.904~0.965); 0.965 for AA, 0.956 for PA, 0.923 for iso-BA, 0.948 for BA, 0.904 for VA, and 0.964 for CA; except 0.835 for iso-VA and 0.768 for HA. Both methods showed statically smaller discrepancies (p-value; <0.0001 for AA, <0.0001 for PA, <0.0001 for BA, 0.0023 for iso-BA, 0.0003 for VA, 0.004 for iso-VA, 0.003 for CA, and 0.05 for HA). The iso-CA was not detected with GC with liquid direct injection analysis; however, it was detected with the HS-SPME method. This means that the iso-CA concentration in the samples was too low (4~10 mg/L) to detect GC with the liquid direct injection method; however, the HS-SPME method has a low MDL for iso-CA (10 μg/L for iso-CA) and could determine the iso-CA of low concentration in water.

The Fig. 8. shows comparison among three analytical

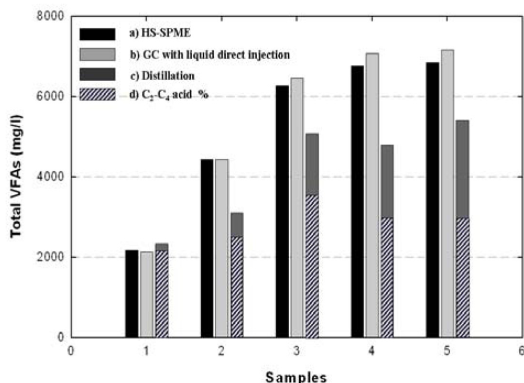


Fig. 8. Comparison among three analytical methods (HS-SPME coupled to GC, GC with liquid direct injection and the distillation method) for determination of Total SCFAs.

methods (HS-SPME coupled to GC, GC with liquid direct injection and the distillation method) for determination of Total SCFAs. from same food wastewater samples. The Total SCFAs concentration was highest when analyzed by GC with a liquid direct injection method and HS-SPME was similar but slightly lower concentrations detected. Further research is needed to verify this results. The distillation method tended to underestimate total SCFAs especially when C_5~C_6 acids are at a high level (Fig. 8.) Inferentially, it seems that the recovery rate of C_5~C_6 acids for the distillation method is low; however, further research is also needed to verify this results.

4. Conclusions

In this study, the HS-SPME coupled to GC for the analysis of SCFAs was developed. In short, the method showed high sensitivity and reproducibility. The developed method was applied to analyze SCFAs in extracted liquid of food wastes. the results obtained from the developed method were comparable with that from the method using GC with liquid direct injection. However, the distillation method would underestimate total SCFAs expecially when C_5~C_6 acids were at high levels. In fact, general, the GC with direct liquid injection GC method is also can analyze SCFAs rapidly and conveniently as HS-SPME for SCFAs analysis. However, GC with liquid direct linjection can lead to contamination of a GC column and a detector, and cause frequent maintenance of the instrument. and interfere with analysis and degrade chromatographic performance. The method with advantage of HS-SPME, however, does not require the frequent instrument maintenance while it can quantify the SCFAs accurately.

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