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Characterization of the Aroma of Salt-fermented Anchovy Sauce Using Solid Phase Microextraction-Gas Chromatography-Olfactometry Based on Sample Dilution Analysis

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Abstract Aroma-active compounds were evaluated from salt-fermented anchovy sauce by solid phase microextraction-gas chromatography-olfactometry (SPME-GC-O) based on sample dilution analysis (SDA). SPME extract from carboxen/polydimethylsiloxane (CAR/PDMS) fiber was the most similar to the original odor of salt-fermented anchovy sauce used for this experiment, followed by divinylbenzene/CAR/PDMS (DVB/CAR/PDMS) fiber. Because salt-fermented anchovy sauce contains 23% NaCl, NaCl concentration of diluent was considered when salt-fermented anchovy sauce was serially diluted. Linear relationship between GC response and sample concentration was observed when diluted with 23% NaCl solution, whereas not observed when diluted with deodorized distilled water. Eleven and 16 aroma-active compounds were detected by SPME-GC-O based on SDA using CAR/PDMS and DVB/CAR/PDMS fibers, respectively. Butanoic acid and 3-methyl butanoic acid showed the highest $\log_2$SD factors for CAR/PDMS and DVB/CAR/PDMS fibers. Dimethyl trisulfide, methional, trimethyl amine, 1-penten-3-ol, and acetic acid were also detected as potent aroma-active compounds.

Keywords: solid phase microextraction, gas chromatography-olfactometry, salt-fermented anchovy sauce, aroma-active compound, flavor

Introduction

Salt-fermented fish sauce, commonly produced in South-East Asia, is also one of the Korean traditional foods produced through microbial proteolysis (1, 2). Among the 54 salt-fermented fish sauces in Korea, salt-fermented anchovy and shrimp sauces are the most popular (3). Anchovy (*Engraulis japonicus*) is caught in all districts along the coast of Korea (4). Although anchovy is superior in taste and nutrition, more than 90% of the catch is preserved with salt or dried due to the fast decomposition speed (5). The unique and desirable flavor and taste of salt-fermented anchovy sauce, traditionally used as an ingredient for kimchi fermentation, are derived through the enzymatic reaction of microorganisms and autolysis brought on by adding 25-30% of salt to raw anchovy and maturing more than 6 months at room temperature (6, 7). Flavor is the most important factor in the quality control of salt-fermented anchovy sauce, which is achieved by instrumental analysis combined with sensory evaluation (8).

Solid phase microextraction (SPME) has been applied to many fields of food science including flavor chemistry and food analysis (9-11). Gas chromatography-olfactometry (GC-O) is a powerful tool for the screening and identification of aroma-active compounds in foods (12-14). However, GC-O methodology based on serial dilution of aroma extract, such as aroma extract dilution analysis (AEDA), CharmAnalysis, and headspace GC-O, cannot be directly employed into SPME-GC-O. Therefore, dilution analysis based on varying the fiber thickness and length has been developed for SPME-GC-O, even though this technique can be applied only to polydimethylsiloxane (PDMS) fiber (15). In addition, Kim *et al.* (16) used GC injector split ratio for aroma dilution analysis of SPME-GC-O.

Recently, new SPME-GC-O methodology based on sample dilution analysis (SDA) has been developed and applied to characterize aroma-active compounds of wine and soy sauce (17,18). Baek and Kim (18) mentioned that the amount of aroma-active compound absorbed by the SPME fiber was proportional to the initial concentration of aroma-active compound in the sample, and thus successive dilution of volatile extract in SPME, as in AEDA, could be achieved by varying the initial concentration of aroma-active compound in the sample, that is, through serial dilution of the sample. Sample dilution (SD) factor was defined as the highest sample dilution at which an odorant can be detected (18).

The objective of this study was to identify characteristic aroma-active compounds of salt-fermented anchovy sauce using SPME-GC-O based on SDA.

Materials and Methods

Assessment of the odor representativeness of salt-fermented anchovy sauce SPME apparatus (Supelco Co., Bellefonte, PA, USA) consisted of SPME fiber and holder. SPME fibers tested were polydimethylsiloxane (PDMS) 100 μm, polydimethylsiloxane/divinylbenzene (PDMS/DVB) 65 μm, polyacrylate (PA) 85 μm, carboxen/polydimethylsiloxane (CAR/PDMS) 75 μm, carbowax/divinylbenzene (CW/DVB) 65 μm, and divinylbenzene/carboxen/PDMS (DVB/CAR/PDMS) 50/30 μm. Salt-fermented anchovy sauce was purchased from a local market in Cheonan, Korea. To assess the odor representative-

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ness of salt-fermented anchovy sauce, a simple device was prepared as described previously (18). Salt-fermented anchovy sauce (2 mL) was put into 4 mL vial and allowed to reach equilibrium in a 40°C water bath for 40 min. SPME fiber was exposed to the headspace at full release (1 cm), and the volatiles were absorbed onto the fiber at 40 °C for 30 min based on the result of a preliminary experiment. Absorbed volatiles were desorbed in teflon-capped 20 mL vial wrapped with heating tape (250°C) for 1 min. After desorption, vial was immersed into ice to cool down. Six panelists were asked to perceive the reference salt-fermented anchovy sauce and odors inside vial, and evaluate odor similarity using a 10-cm scale ranging from 0 (far from the reference) to 10 (close to the reference). The result was analyzed statistically through one-way ANOVA using SAS program (19), and the significant difference was analyzed by Duncan's multiple range test ($p<0.05$).

SPME-GC-O of salt-fermented anchovy sauce Two milliliters of salt-fermented anchovy sauce was put into 4 mL vial and allowed to reach equilibrium at 40°C for 40 min. Volatile compounds of salt-fermented anchovy sauce were extracted at 40°C for 30 min. The fiber (CAR/PDMS or DVB/CAR/PDMS) was injected into the injection port for 1 min to desorb the volatiles. The GC-O system consisted of a Varian 3800 (Varian Instrument Group, Walnut Creek, CA, USA) equipped with a flame ionization detector and a sniffing port. The original salt-fermented anchovy sauce was serially diluted (1:2) using 23% NaCl solution. GC-O was carried out on a DB-WAX column (30 m length × 0.25 mm i.d × 0.25 μm film thickness: J & W Scientific, Folsom, CA, USA). Oven temperature was programmed from 40 to 200°C at a rate of 5°C/min with initial and final hold times of 5 and 10 min, respectively. Helium was used as carrier gas at a flow rate of 1.4 mL/min. Injector and detector temperatures were 280 and 250°C, respectively. SPME-GC-O was carried out in duplicate.

Gas chromatography-mass spectrometry (GC-MS) Volatile compounds were analyzed using an Agilent 6890N GC-Agilent 5973N mass selective detector (MSD) (Agilent Co., Palo Alto, CA, USA). GC separation was carried out on a DB-WAX (60 m length × 0.25 mm i.d × 0.25 μm film thickness: J&W Scientific, Folsom, CA, USA). Helium was used as the carrier gas at a flow rate of 2 mL/min. Oven temperature was programmed from 40 to 200°C at a rate of 5°C/min with initial and final hold times of 5 and 10 min, respectively. The other MSD conditions were as follows: injector temperature, 200°C; capillary direct interface temperature 250°C; ion source temperature 230 °C; ionization voltage 70 eV; mass range 33-330 a.m.u; scan rate 4.76 scan/sec.

Compound identification Positive identifications were made based on the comparison of retention indices (RI), Wiley 7N mass spectral database (Hewlett- Packard Co., Palo Alto, CA, USA), and aroma properties of unknowns with those of authentic standard compounds. RI was calculated using n-paraffins C_8-C_{22} as external references (20). Tentative identifications were made by matching mass spectra of unknowns with those of Wiley 7N mass spectral database and literatures or by matching RI values and aroma properties of unknowns with those of the authentic standard compounds. Authentic flavor compounds were supplied from Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA).

Results and Discussion

Extraction of volatile compounds by SPME is dependent on experimental parameters, such as ionic strength, heating temperature and time, sample volume, concentration, and stirring. Achieving odor quality of SPME extract close to the original sample odor is crucial. Rega *et al.* (21) assessed odor representativeness by direct gas chromatography-olfactometry for SPME. Because odor impressions emerging from SPME extracts generally do not resemble that of the original sample, it is necessary to evaluate the sensory quality of SPME extract prior to SPME-GC-O. Therefore, we used rapid and simple methodology for the selection of suitable SPME fiber for SPME-GC-O by evaluating the odor representativeness as described previously (18). Table 1 shows the results of similarity rates obtained from six fibers tested. SPME extract from CAR/PDMS fiber was the most similar to the original odor of salt-fermented anchovy sauce used for this experiment, followed by DVB/CAR/PDMS fiber. Carboxen-based fibers, such as CAR/PDMS and DVB/CAR/PDMS, are known to extract small polar compounds more efficiently. The primary sorption principle of these fibers is the surface phenomenon, which involves adsorption, not absorption, of volatiles on a porous carbon material (22). Therefore, we performed SPME-GC-O of salt-fermented anchovy sauce using CAR/PDMS and DVB/CAR/PDMS fibers.

NaCl concentration affects the absorption of volatiles during SPME (9). Because salt-fermented anchovy sauce contains approximately 23% NaCl, NaCl concentration of a diluent should be considered in performing SPME-GC-O based on SDA. When salt-fermented anchovy sauce is diluted with distilled water, NaCl concentrations of the diluted samples must be lowered upon serial dilution. To demonstrate the effect of NaCl concentration on absorption of volatiles during SPME, salt-fermented anchovy sauce was serially diluted (1:2) using either 23% NaCl solution or distilled water as a diluent. Linear relationship between GC response and concentrations of volatiles was

Table 1. Similarity rates obtained from SPME extract of salt-fermented anchovy sauce

SPME fiber	Similarity[1)]
CAR/PDMS	$7.1 \pm 0.9^{a,2)}$
DVB/CAR/PDMS	6.1 ± 1.6^{a}
CW/DVB	4.1 ± 1.6^{b}
PA	3.1 ± 0.9^{b}
PDMS/DVB	2.3 ± 1.3^{bc}
PDMS	1.9 ± 0.7^{c}

[1)]Mean ± standard deviation.
[2)]a-c: Values with different superscripts are significantly different ($p< 0.05$).

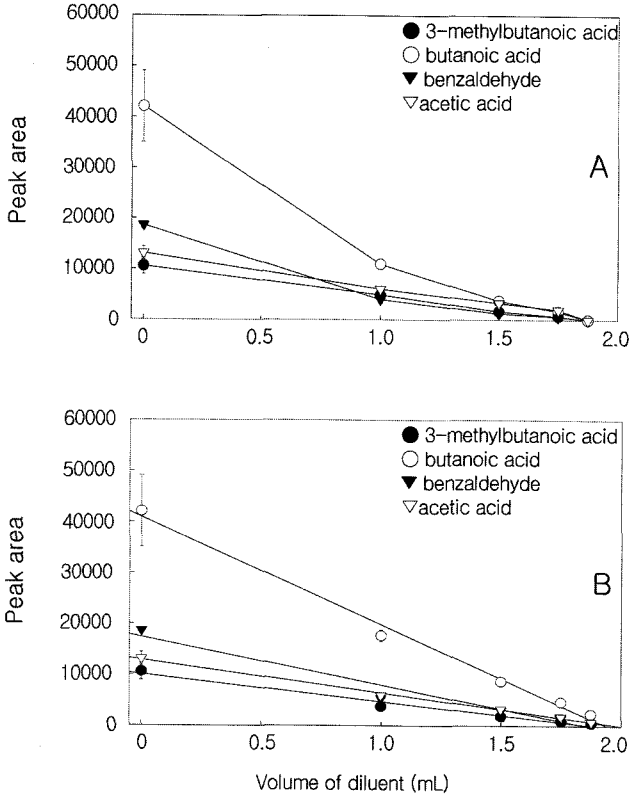

Fig. 1. Peak areas of volatiles versus different volumes of distilled water (A) and 23% NaCl solution (B)(serial dilution) for CAR/PDMS fiber in salt-fermented anchovy sauce.

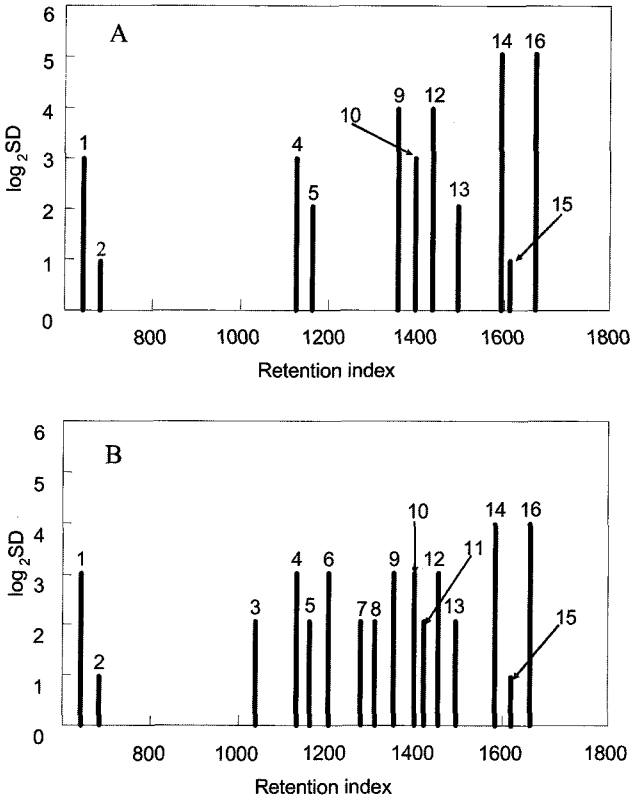

Fig. 2. Sample dilution chromatograms of volatiles isolated from salt-fermented anchovy sauce by SPME-GC-O based on SDA with CAR/PDMS (A) and DVB/CAR/PDMS (B) fibers.

not observed when diluted with distilled water (Fig. 1A), but observed when diluted with 23% NaCl solution (Fig. 1B). This result indicates that salt concentration of a diluent should be taken into consideration when performing SPME-GC-O based on sample dilution analysis (SDA). Therefore, we used 23% NaCl solution to serially dilute salt-fermented anchovy sauce for SPME-GC-O based on SDA.

Figure 2 shows SD chromatograms of volatiles isolated from salt-fermented anchovy sauce by SPME-GC-O based on SDA using CAR/PDMS and DVB/CAR/PDMS fibers. SD factor, like flavor dilution (FD) factor for AEDA, is defined as the highest sample dilution at which an odorant can be detected (18). Eleven and 16 aroma-active compounds were detected from salt-fermented anchovy sauce by SPME-GC-O based on SDA with CAR/PDMS and DVB/CAR/PDMS fibers, respectively (Fig. 2 and Table 2). Butanoic acid (no. 14, $\log_2$SD = 5 and 4) and 3-methyl butanoic acid (no. 16, $\log_2$SD = 5 and 4) were the most potent aroma-active compounds in salt-fermented anchovy sauce for CAR/PDMS and DVB/CAR/PDMS fibers. Butanoic acid and 3-methyl butanoic acid, having a sweaty odor, have been identified in some fermented foods (18, 23-26). Dimethyl trisulfide (no. 9), methional (no. 12), trimethyl amine (TMA) (no. 1), 1-penten-3-ol (no. 4), acetic acid (no. 10), and benzaldehyde (no. 13) were also detected as potent aroma-active compounds. 2,5-Dimethyl pyrazine (no. 7), 2-ethyl pyrazine (no. 8), and 2-ethyl-3-methyl pyrazine (no. 11) were detected only in the SPME extract by DVB/CAR/PDMS fiber. TMA, which is produced by the reduction of trimethyl amine oxide (TMAO) during microbial spoilage, has been associated with the odor of

Table 2. Aroma-active compounds from salt-fermented anchovy sauce by SPME-GC-O based on SDA

No[1)]	RI[2)]	Compound name	$\log_2$ SD		Aroma description
			A[3)]	B[4)]	
1	<800	trimethyl amine	3	3	fishy, sweaty
2	<800	dimethyl disulfide	1	1	disinfectant
3	1056	2-methyl-1-propanol	-[5)]	2	alcohol
4	1130	1-penten-3-ol	3	3	butter
5	1143	1-butanol	2	2	medicinal
6	1204	2-methyl-1-butanol	-	3	bitter
7	1324	2,5-dimethyl pyrazine	-	2	peanut
8	1335	2-ethyl pyrazine	-	2	musty
9	1372	dimethyl trisulfide	4	3	cooked cabbage
10	1401	acetic acid	3	3	sour
11	1408	2-ethyl-3-methyl pyrazine	-	2	earthy
12	1432	methional	4	3	baked potato
13	1497	benzaldehyde	2	2	bitter almond
14	1578	butanoic acid	5	4	sweaty
15	1625	furfuryl alcohol	1	1	cookie-like
16	1650	3-methyl butanoic acid	5	4	sweaty

[1)]Numbers correspond to those in Figure 2.
[2)]Retention indices were determined using C_8-C_{22} as external standards.
[3)]CAR/PDMS fiber.
[4)]DVB/CAR/PDMS fiber.
[5)]Not detected.

deteriorating fish (27). TMAO is involved in the regulation of the osmotic pressure in saltwater fishes (26) and is present in much smaller amounts in freshwater species (28). TMAO has no odor, but TMA is a very potent odor compound described as old-fishy or fish house-like (29). Sulfur-containing compounds, such as dimethyl disulfide and dimethyl trisulfide, are known to have sulfurous or cooked cabbage-like odor in marine products (30).

In conclusion, SPME-GC-O based on SDA has been successfully applied to the identification of potent aroma-active compounds in salt-fermented anchovy sauce. Results of this new SPME-GC-O methodology demonstrated that butanoic acid, 3-methyl butanoic acid, dimethyl trisulfide, methional, trimethyl amine, 1-penten-3-ol, and acetic acid play important roles in the salt-fermented anchovy sauce.

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