

Aroma Impact Components in Commercial Plain Sufu

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Volatile components of three commercial plain sufus or fermented soybean (*Glycine max*) curds (A–C) were extracted by a supercritical fluid extraction apparatus and analyzed by gas chromatography–mass spectrometry. A total of 83 compounds were found, of which 68 compounds were common among the three samples. Samples A, B, and C had 76, 75, and 74 components, respectively. Alcohols (17), acids (15), and esters (16) were the major classes. The rest of the classes were miscellaneous compounds (9), aldehydes (7), alkanes (5), aromatic compounds (5), ketones (3), furans (2), S-containing compounds (2), and other N-containing compounds (2). Gas chromatography–flame ionization detection–olfactometry analyses identified 17 potent odorants with different odor descriptions such as sour, sweet, fruity, coconut-like, and meaty. Fourteen odorants were further pinpointed by omission experiments among the 17 to be critical to the odor of commercial plain sufu. Acetic acid, methional, ethyl (*Z,Z*)-9,12-octadecadienoate, ethyl (*Z*)-9-octadecenoate, and 3-methylbutanoic acid were some of the potent odorants found.

KEYWORDS: Sufu; supercritical fluid extraction; flavor; Asia

INTRODUCTION

Plain fermented soybean (*Glycine max*) curd (FSC), which is also known as plain or white sufu, Chinese cheese, preserved bean curd, bean cured cheese, tofuru, etc., is a highly flavored, creamy cheese-like traditional fermented soybean food (1). It has been widely consumed as an appetizer and a side dish in China. Currently, over 300 000 metric tons of sufus are produced in China annually (2). Sufus are prepared by fermenting fresh tofu with mold from the genus *Mucor* or *Actinomucor* (2). The main processing steps to prepare sufus are (1) preparation of tofu, (2) inoculation of chosen mold species, (3) brining, and (4) aging (2). Tofu cubes inoculated with a mold will produce a dense mycelium on their surfaces. Extracellular enzymes from the mycelium such as proteases and lipases are released to the aging solution during brining. Macromolecules of the tofu are broken down into small-chained peptides, free amino acids, and fatty acids. They further react with the alcohol in the aging solution to form aromatic esters, which contribute to the distinct sufu flavor. Besides, sufu is also popular for its nutritive value as a good protein and calcium source (2).

Previous investigations on the volatile compounds of sufus made use of extraction techniques such as simultaneous distillation–extraction (SDE) or direct headspace injection (3–5).

However, the former may produce thermally generated artifacts, whereas the latter extract collects only low quantities of volatile compounds. Supercritical fluid extraction (SFE) may be an alternative method to minimize thermally generated products and to facilitate higher amount of compounds to be collected. SFE has been an important sample extraction tool in separation science. It is an alternative method to the more conventional extraction procedures making use of the special dissolving power of the supercritical fluid, which can be achieved by regulating its pressure and the temperature conditions (6). Carbon dioxide is the most widely used medium because its supercritical conditions are relatively easy to attain. Although volatile constituents of sufu have been reported, a more representative profile could be obtained by this extraction method (7). Therefore, the objectives of this investigation were to determine the volatile components in commercial plain sufus using the SFE technique, to determine their odor-active components using gas chromatography–olfactometry, and to further evaluate the potent components by omission experiments.

MATERIALS AND METHODS

Samples. Three samples of plain sufus (A–C) commonly consumed in Hong Kong were chosen and were picked randomly from the market shelves. Sample B was manufactured by a local tofu manufacturer. Samples A and C were products of Sichuan, China. Samples were kept in their own containers and stored in a laboratory refrigerator (4 °C) at The Chinese University of Hong Kong until sample extractions were ready. Due to their differences in size, original curds were cut into cubic shapes of 1 cm³ for sample extractions.

The majority of the chemical standards were purchased from Aldrich Chemical Co. (Milwaukee, WI). Chemical standards purchased from

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Acros Organics (Geel, Belgium) included propanoic acid (**2**), 2-ethylhexanoic acid (**9**), (*E*)-heptenal (**34**), and ethyl dodecanoate (**52**). 1,2-Cyclohexanediol (**28**) and pencyclohexane (**42**) were from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Ethyl pentadecanoate (**55**) was purchased from Fluka Chemical Co. (Ronkonkoma, NY).

Supercritical Fluid Extraction. Three grams of sample was used for each extraction. 2,4,6-Trimethylpyridine (0.5 mL) was used as an internal standard (I.S., 0.5 $\mu\text{g/mL}$ in boiled double-distilled water) and was added to the sample before extraction. Three grams of inert filler WetSupport (Isco, Inc., Lincoln, NE) was used to fill the space in the sample cartridge (Isco, Inc.) in the SFE system. An automated SFE system (model ISCO SFX 3560, Isco, Inc.) was used for the extraction. The extraction pressure and temperature for the carbon dioxide were 7500 psi and 60 °C, respectively. Fifteen milliliters of redistilled dichloromethane was used as the collection solvent with a 0.5 mL/min solvent replenishment rate during the operation. The restrictor flow rate was at 3.0 mL/min, and the dynamic extraction time was 30 min. Extracts were dried with 2.3 g of anhydrous sodium sulfate and then concentrated to 0.1 mL with a gentle stream of prepurified nitrogen gas (99.995%) (Hong Kong Oxygen and Acetylene Co. Ltd., Hong Kong). Triplicate extractions were done for each sample. Extracts were kept in a freezer (−70 °C) until further analyses were ready.

Gas Chromatography–Mass Spectrometry (GC-MS) Conditions. A GC-MS system consisting of a Hewlett-Packard 6890 gas chromatograph coupled with a Hewlett-Packard 5973 mass selective detector (MSD) was used for qualitative and quantitative analyses. Separations of the volatile compounds were performed on a fused silica open tubular column (Supelcowax-10, 60 m length \times 0.25 mm i.d. \times 0.25 μm film thickness, nominal; Supelco, Inc., Bellefonte, PA). One microliter of extract was injected at splitless mode with an injector temperature of 200 °C. Helium gas (ultrahigh-purity grade, 99.999%) was used as carrier gas. GC oven conditions were initially at 35 °C, programmed at 6 °C/min until 195 °C, and held for 90 min. The MS conditions were as follows: ion source temperature, 230 °C; MS quadrupoles temperature, 106 °C; electron multiplier, 1494 V; mass range, 33–550 amu; and scan rate, 2.94 scans/s.

Qualification and Quantification of Volatile Compounds. Tentative identification of compounds was performed by matching each mass spectrum of an unknown with that suggested by the Wiley Chemical database (7th ed., Wiley, New York, NY). Positive identification of each compound was done by comparing the retention index (RI) (**8**) and mass spectrum of an unknown with those of the authentic standard under the same analytical conditions as described by Chung (**4**).

Calculations of retention indices were done according to the method of van den Dool and Kratz (**8**). For compound quantification, a three-point calibration curve was developed for each positively identified compound. Briefly, a calibration curve was drawn by plotting the concentration ratio of a standard compound to the internal standard against its corresponding area ratio from a specific mass/charge fragment of the standard compound to that of the internal standard (**4**). The concentration of a compound was calculated on the basis of the response factor of the standard curve for the compound.

Gas Chromatography–Flame Ionization Detection–Olfactometry (GC-FID-O). A Shimadzu gas chromatography system (model GC-14B, Shimadzu, Kyoto, Japan) coupled with a sniff port and a flame ionization detector and equipped with a fused silica open tubular column (Supelcowax-10, 60 m length \times 0.25 mm i.d. \times 0.25 μm film thickness, nominal; Supelco, Inc.) was used to conduct the gas chromatography–olfactometry (GC-O) analysis. Ultrahigh-purity helium gas (99.999%) was employed as the carrier gas with a constant linear velocity of 30 cm/s. The effluent from the analytical column was split into two identical, 1-m columns with a Y-shaped connector (Restek, Bellefonte, PA) and directed to a FID and a sniff port. Oven temperature was initially maintained at 35 °C for 5 min and ramped at 6 °C/min to 195 °C, which was kept for 1 h. One microliter of extract was injected at splitless mode with an injector temperature at 200 °C. Two trained panelists were used to evaluate each sample three times. Each of them would note both the time and the descriptors when an odor was eluted at the sniff port. Initial tentative identification of an odorous compound from GC-O was made by comparing its retention index with that already positively identified by the GC-MS. Confirmation of the compounds

was based on matches of both its retention index and odor descriptions between the authentic standards and the tentatively identified compounds from the GC-O operated under the same experimental conditions.

Odor Activity Value (OAV). The OAV of a compound was calculated by dividing the concentration of a compound by its corresponding threshold value (**9**).

Statistical Analysis. One-way analysis of variance (ANOVA) and Tukey's studentized range test at the $p = 0.05$ level of significance (**10**) were used to evaluate the differences in the quantities of the compounds among the three brands of sufus.

Omission Experiments. Twelve panelists were recruited from the Food and Nutritional Science Programme and the Biology Department at The Chinese University of Hong Kong to perform the omission experiments (**11**). Each panelist performed a total of 19 sets of triangle tests in an air-conditioned room under fluorescent light. The test series were repeated two times. In each triangle test, the set of samples was arranged in a random order with one sample differed from the other two samples. The samples included a mixture of the standard chemical compounds and a mixture of the three brands of commercial plain sufu samples (**12**). Seventeen compounds chosen for the omission tests were based on the results of GC-O, and their concentrations used for the preparation of the mixture were derived from the mean quantities of the three brands of sufu in **Table 1**. Pure sunflower seed oil (Bontaste) was used as a base for the preparation of the model mixtures. In each model, one compound was omitted from the 17 chosen compounds. Therefore, 16 compounds were mixed in the sunflower seed oil in the models and were assigned the numbers 1–17. One preparation (model 0) contained all 17 compounds, whereas another one (model 18) contained only the oil. The latter was designed to evaluate if the sunflower seed oil would contribute odor to the models when used as a base. Both the mixed authentic sufu samples and the artificial mixtures were adjusted to final weights of 7 g and were placed in capped test tubes (Pyrex, disposable screw-cap culture tubes, 13 mm external diameter \times 100 mm height) for sensory evaluation by sniffing. To prevent sight judgment by panelists, all of the test tubes were wrapped with aluminum foil. Before distribution for panel evaluation, samples were mixed by a vortex for 5 min. Data from the three replicates of each model were pooled according to the criteria set by Smith (**13**). The total number of correct answers in each triangle test (maximum 36) was compared to the critical value required for a significance difference for the triangle test at $p < 0.05$ (**14**, **15**).

RESULTS AND DISCUSSION

The volatile components identified in the commercial plain sufu samples are shown in **Table 1**. A total of 83 compounds were found, of which 11 were tentatively identified. Samples A, B, and C have totals of 76, 75, and 74 compounds, respectively. Sixty-eight common compounds were distributed in various chemical classes. The majority of the common compounds belonged to alcohols (15), acids (13), and esters (12). This agreed well with the results that alcohols and esters were found in the greatest amounts in previous studies (**16**). The rest of the classes were miscellaneous compounds (7), aldehydes (6), aromatic compounds (4), alkanes (3), ketones (3), furans (2), S-containing compounds (2), and other N-containing compound (1). Thirty-one compounds identified were previously found in commercial plain sufus (**4**) using the SDE method (**Table 1**).

The acid class contained 15 compounds. Thirteen acids were common among the three samples. None of them was reported before in the commercial plain sufus investigation by Chung (**4**). Only acetic acid (**1**) in red sufus was reported before (**5**). The absence of different types of acid in plain sufus extracted by the SDE method might be due to the degradation of acid when subjected to a 2-h, high-temperature (100 °C) extraction (**4**). Among the acids, only acetic acid (**1**) and hexadecanoic acid (**15**) were found to be at high concentrations ($> 1.0 \times 10^5$

Table 1. (Continued)

no. ^a	compound ^b	RI ^c	ref ^d	(m/z) ^e	ID ^f	sample A		sample B		sample C	
						concn ^g ($\mu\text{g}/\text{kg}$)	range ($\mu\text{g}/\text{kg}$) ^h	concn ^g ($\mu\text{g}/\text{kg}$)	range ($\mu\text{g}/\text{kg}$) ^h	concn ^g ($\mu\text{g}/\text{kg}$)	range ($\mu\text{g}/\text{kg}$) ^h
furans (2)											
66	2-pentylfuran	1203	1, 2, 6ab	81	RI, MS	4.6×10^3	$3.6\text{--}6.2 (\times 10^3)$	4.0×10^3	$3.7\text{--}4.5 (\times 10^3)$	5.8×10^3	$4.7\text{--}7.0 (\times 10^3)$
67	2-furanmethanol [*]	1585	1	98	RI, MS	5.8×10^2 ^A	$4.8\text{--}6.4 (\times 10^2)$	3.3×10^2 ^B	$2.6\text{--}4.0 (\times 10^2)$	4.2×10^2 ^{AB}	$3.3\text{--}4.9 (\times 10^2)$
ketones (3)											
68	3-hydroxy-2-butanone [*]	1248	1, 2, 7b	45	RI, MS	5.5×10^4 ^A	$4.1\text{--}7.0 (\times 10^4)$	2.1×10^5 ^B	$1.3\text{--}2.8 (\times 10^5)$	1.7×10^5 ^{AB}	$1.3\text{--}2.4 (\times 10^5)$
69	2-methyl-2-cyclopenten-1-one	1281		67	RI, MS	2.1×10^3	$1.8\text{--}2.6 (\times 10^3)$	1.7×10^3	$1.1\text{--}2.1 (\times 10^3)$	4.3×10^3	$2.2\text{--}5.4 (\times 10^3)$
70	2-cyclohexen-1-one	1413		68	RI, MS	5.5×10^3	$3.7\text{--}6.7 (\times 10^3)$	4.7×10^3	$3.0\text{--}5.6 (\times 10^3)$	6.1×10^3	$4.6\text{--}8.1 (\times 10^3)$
miscellaneous compounds (9)											
71	camphor [*]	1501		108	RI, MS	4.1×10^2 ^{AB}	$4.0\text{--}4.2 (\times 10^2)$	2.7×10^2 ^A	$2.4\text{--}2.9 (\times 10^2)$	5.4×10^2 ^B	$4.1\text{--}6.2 (\times 10^2)$
72	naphthalene	1731	1, 2, 3, 6ab	128	RI, MS	2.8×10^2	$2.1\text{--}3.3 (\times 10^2)$	2.1×10^2	$1.2\text{--}2.7 (\times 10^2)$	3.3×10^2	$1.9\text{--}4.1 (\times 10^2)$
73	3-hydroxy-2-methyl-4H-pyran-4-one	1928	1, 2, 7b	126	RI, MS	1.9×10^4	$1.6\text{--}2.1 (\times 10^4)$	1.3×10^4	$8.4\text{--}18.0 (\times 10^3)$	1.0×10^4	$6.0\text{--}12.0 (\times 10^3)$
74	2-pyrrolidinone	2002		42	RI, MS	1.1×10^3	$8.5\text{--}14.0 (\times 10^2)$	2.0×10^3	$1.4\text{--}2.7 (\times 10^3)$	1.3×10^3	$9.1\text{--}16.0 (\times 10^2)$
75	2,6-di(<i>tert</i> -butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one ^k	2049		165	MS	3.0×10^2	$2.3\text{--}3.7 (\times 10^2)$	2.5×10^2	$1.7\text{--}3.4 (\times 10^2)$	2.1×10^2	$1.4\text{--}3.3 (\times 10^2)$
76	piperidinone [*] ^k	2095	1	99	MS	1.8×10^3 ^A	$1.7\text{--}2.0 (\times 10^3)$	6.4×10^3 ^B	$4.5\text{--}8.6 (\times 10^3)$	3.0×10^3 ^A	$2.0\text{--}3.6 (\times 10^3)$
77	1H-indole	2405	1, 2	117	RI, MS	1.2×10^3	$8.7\text{--}14.0 (\times 10^2)$	—	—	—	—
78	2,5-pyrrolidinedione [*]	2417		99	RI, MS	2.0×10^3 ^A	$1.7\text{--}2.5 (\times 10^3)$	5.0×10^3 ^B	$3.7\text{--}6.5 (\times 10^3)$	3.8×10^3 ^{AB}	$2.7\text{--}4.9 (\times 10^3)$
79	3-tridecen-1-yne ^k	2536		67	MS	—	—	3.5×10^3	$2.1\text{--}5.5 (\times 10^3)$	—	—
other N-containing compounds (2)											
80	<i>N,N</i> -dibutylformamide	1742		72	RI, MS	1.1×10^3	$1.0\text{--}1.2 (\times 10^3)$	6.4×10^2	$3.8\text{--}9.7 (\times 10^2)$	1.1×10^3	$3.5\text{--}18.0 (\times 10^2)$
81	3-methylbutanamide ^k	1857		59	MS	2.0×10^2	$1.5\text{--}2.7 (\times 10^2)$	—	—	4.6×10^2	$3.6\text{--}5.7 (\times 10^2)$
S-containing compounds (2)											
82	methional [*]	1423	2	104	RI, MS	6.4×10^2 ^A	$2.0\text{--}9.4 (\times 10^2)$	3.6×10^3 ^B	$3.1\text{--}4.3 (\times 10^3)$	9.8×10^2 ^A	$6.6\text{--}14.0 (\times 10^2)$
83	3-(methylthio)propanoic acid ^k	2207		61	MS	2.0×10^2	$1.7\text{--}2.3 (\times 10^2)$	3.0×10^2	$1.9\text{--}4.0 (\times 10^2)$	3.0×10^2	$1.9\text{--}4.0 (\times 10^2)$

^a Compound number. ^b Compounds in order of their retention indices in a chemical class. ^{*}, statistically significant difference at $p < 0.05$. Values in the same row with different letters are significantly different (Tukey, $p < 0.05$). ^c Linear retention indices calculated with reference to the method of van den Dool and Kratz (8). ^d Articles in which the compounds were reported: 1, Chung (4); 2, Chung (5); 3, Ames and Macleod (21); 4, Wilkens and Lin (19); 5, Hwan and Chou (16); 6, del Rosario et al., a, raw soybean, b, heated soybean (18); 7, Ho et al., a, plain sufu, b, red sufu (23). ^e Ion fragment selected used to calculate the concentration of a particular compound. ^f Positive identification of a compound was based on the comparison of the mass spectrum (MS) and retention index (RI) between the tentative compound with that of the authentic standard under the same experimental conditions; tentative identification was based on mass spectrum only. ^g Mean concentration from triplicate dry weight samples; —, not determined. ^h Concentration range ($\mu\text{g}/\text{kg}$). ^k Tentatively identified compound.

$\mu\text{g}/\text{kg}$). The majority of the acids found belonged to the free fatty acids released during extracellular digestion by lipases (2). These acids were described as having a cheesy note in general, including butanoic acid (5) (cheesy, sharp, rancid, sweaty, sour), 3-methylbutanoic acid (6) (cheese, rancid, sweaty), hexanoic acid (8) (cheesy, fatty, sweaty, sour, rancid, pungent), and nonanoic acid (11) (cheesy, waxy) (17). Others mainly contributed to an oily note including 2-methylpropanoic acid (3) (rancid butter), pentanoic acid (7) (sweaty, rancid), octanoic acid (10) (oily, fatty, rancid), dodecanoic acid (13) (fatty), and tetradecanoic acid (14) (waxy, oily) (17).

Alcohols were another major class found, which contained 17 compounds. Fifteen of them were found in all three samples. Alcohols were suggested to be an important flavor-contributing class because of their unique odors and high concentrations found in a previous investigation (4). Except for 2-cyclohexen-1-ol (21), 2-chlorocyclohexanol (23), 2-(2-butoxyethoxy)ethanol (24), 1,2-cyclohexanediol (28), 2,4-decadien-1-ol (29), and 2-phenoxyethanol (32), all alcohols were reported before in sufus and other soybean-based products (4, 5, 16, 18, 19). Ethanol (16) was purposely added to the mold-infested tofu during the aging period. Other alcohols could be produced during the fermentation by the action of various fungal enzymes (20). The origin of 2-chlorocyclohexanol (23) was unknown in the samples, although it is considered to be an environmental contaminant.

Seven aldehydes were identified, and six common ones were found in the samples. These compounds mostly contributed to green notes [hexanal (33), (*E*)-2-heptenal (34)], cinnamon notes [(*E,E*)-2,4-heptadienal (35)], and almond, aromatic, and sweet notes [benzaldehyde (36)] (17). Nearly all of them, including

compounds 33, 35, 36, 38, and 39, were previously identified in the commercial plain sufus (4). (*E*)-2-Heptenal (34) was detected in soybean milk, and benzeneacetaldehyde (37) was reported in both the commercial red sufus and the textured soy protein (5, 19, 21). Aldehydes could be generated by lipid oxidation, degradation, and fermentation (20, 21).

The only two other nitrogen-containing compounds found were *N,N*-dibutylformamide (80) and 3-methylbutanamide (81). They were both newly reported in sufu. *N,N*-Dibutylformamide (80) was reported in kombu, a Japanese seaweed (*Laminaria* spp.) (22). 3-Methylbutanamide (81) was present in Tilsit (a Danish cheese).

Esters were the third largest class found in the current samples. Among the 16 compounds found, 12 of them were found in all three samples. Twelve of them were detected in plain sufus before (4, 23). Ethyl 3-hydroxybutyrate (50), ethyl tetradecanoate (54), ethyl hexadecanoate (57), ethyl (*Z*)-9-octadecenoate (61), ethyl (*Z,Z*)-9,12-octadecadienoate (62), and ethyl (*Z,Z,Z*)-9,12,15-octadecatrienoate (65) were the esters that were found in both plain and red sufus (4, 5). Ethyl heptadecanoate (58), methyl (*E,E*)-9,12-octadecadienoate (59), methyl 5,8,11-heptadecatrienoate (63), and methyl 11,13-eicosadienoate (64) were tentatively identified in this experiment. The high molecular weight fatty acid esters were probably produced by the action of fungal lipases on the soybean lipid (24). They all give desirable aromas. For example, compounds 50–53 were all described as having fruity, grape-like, and rum-like aromas (17). Ethyl esters were derived from the esterification of free fatty acids and ethanol. As a result, ethyl esters of the corresponding fatty acids were expected to be present. However, the current results showed that some ethyl esters were absent

Table 2. Gas Chromatography–Flame Ionization Detection–Olfactometry Evaluations of Commercial Plain Sufu Samples

no. ^a	compound ^b	RI ^c	ID ^d	odor descriptor(s) ^e
1	acetic acid	1387	RI, odor	sour
6	3-methylbutanoic acid	1621	RI, odor	sweaty
19	1-hexanol	1303	RI, odor	herb-like, sweet
25	2-methoxyphenol	1823	RI, odor	alcoholic ⁱ
30	phenol	1962	RI, odor	woody
33	hexanal	1059	RI, odor	tea leaf-like
34	(<i>E</i>)-2-heptenal	1292	RI, odor	sweet, green
35	(<i>E,E</i>)-2,4-heptadienal	1463	RI, odor	moldy, mushroom-like
37	benzeneacetaldehyde	1618	RI, odor	floral
52	ethyl dodecanoate	1811	RI, odor	dried seaweed-like ⁱⁱ
61	ethyl (<i>Z</i>)-9-octadecenoate	2461	RI, odor	coconut
62	ethyl (<i>Z,Z</i>)-9,12-octadecadienoate	2519	RI, odor	sweet
65	ethyl (<i>Z,Z,Z</i>)-9,12,15-octadecatrienoate	2567	RI, odor	pungent
66	2-pentylfuran	1203	RI, odor	green
68	3-hydroxy-2-butanone	1248	RI, odor	buttery
72	naphthalene	1731	RI, odor	paper-like, dried seaweed-like
82	methional	1423	RI, odor	meaty

^a Compound number. ^b Compounds in order of their compound numbers. ^c Retention indices from **Table 1**. ^d Positive identification of a component by comparison of its retention index (RI) and odor characteristics with its authentic standard. ^e Odor descriptor(s) used for all samples unless indicated as follows: i, odor descriptor used for samples A and C; ii, odor descriptor used for sample B only.

even though their corresponding fatty acids were found. This could be an indication of either their presence in minute amount, which was below the detection limit of the analytical instrument, or their absence in the process.

Two furans, 2-pentylfuran (**66**) and 2-furanmethanol (**67**), were previously found in plain sufus (**4**). 2-Pentylfuran (**66**) was found to have a green bean, metallic, and vegetable aroma (**17**, **25**). Furans could be formed by sugar dehydration or fragmentation from the Maillard reaction (**25**). Furans were odor-contributing compounds and were mainly associated with sweet, fruity, nutty, and caramel-like odors (**25**).

Two of three ketones were newly identified in commercial plain sufu samples including 2-methyl-2-cyclopenten-1-one (**69**)- and 2-cyclohexen-1-one (**70**). 2-Methyl-2-cyclopenten-1-one (**69**) was detected in liquid smokes used in seasoned–dried Pacific saury (*Cololabis saira*) preparation (**26**). Pacific saury is a kind of marine fish found in Baja California, Alaska, and Japan (**26**). 2-Cyclohexen-1-one (**70**) was found in cashew (*Anacardium occidentale*) and marmeleiro (*Croton* species) honeys (**27**). Compound (**68**) was identified in plain sufus before (**4**) and had a buttery odor for 3-hydroxy-2-butanone (**17**). Ketones could be produced by fungal enzymatic actions during fermentation or by Maillard reaction (**25**, **28**).

Camphor (**71**), naphthalene (**72**), 3-hydroxy-2-methyl-4*H*-pyran-4-one (**73**), 2-pyrrolidinone (**74**), 2,6-di(*tert*-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one (**75**), piperidinone (**76**), and 2,5-pyrrolidinedione (**78**) were the common miscellaneous compounds found in the three brands. Camphor (**71**) was distinguished in honey (**29**). Naphthalene (**72**) had a mothball-like odor and was suspected as a contaminant from the environment (**30**). 3-Hydroxy-2-methyl-4*H*-pyran-4-one (**73**) has a malt, toasted-like flavor. 2-Pyrrolidinone (**74**) was noted in fermented fish sauce. 1*H*-Indole (**77**) was described as unpleasant, cadaverous, fecal, and putrid in high concentration but floral at low concentration (**17**). Compound **77** could be produced by microbial degradation (**31**).

Sulfur-containing compounds had a significant contribution to the aroma in foods (**4**, **25**). One possible way to produce sulfur-containing compounds was by the degradation of simple amino acids during the Maillard reaction (**25**). Methional (**82**) had an onion–meat-like odor and was described as a powerful flavor compound (**17**). In the GC-O analysis, it was described as meaty in odor. Methional (**82**) could be produced from

methionine, which underwent the enzymatic degradation (**2**) or the Maillard reaction (**25**) during the aging process. 3-(Methylthio)propanoic acid (**83**) was another sulfur-containing compound identified. It was found in many white and red wines and was considered to be an essential aroma-contributing component (**32**).

Among the many compounds found that could be originated at high-temperature conditions such as the Maillard reaction or lipid oxidation, some of them might be produced in the high-temperature injector. This was because the supercritical fluid extract might contain not only low molecular weight components but also some high molecular weight substrates as well. Nevertheless, data from the subsequent analyses have shown that the deduced character impact compounds were similar to the mixture of the three commercial samples. This suggested that the influences of the artifacts generated in the hot injector were at the minimum and were not critical to the authentic odor of the products.

In GC-FID-O analysis of the three samples (A–C), hexanal (**33**) was perceived at a retention index (RI) of <1100 in all three samples (**Table 2**) and was described as having a tea leaf-like aroma. Similar to other soybean-based products, hexanal could be produced by the autoxidation of soybean lipids (**2**). Between RIs of 1100 and 2000, 12 compounds were detected in all samples, of which 11 of them were common components among the three samples including 2-pentylfuran (**66**), 3-hydroxy-2-butanone (**68**), (*E*)-2-heptenal (**34**), 1-hexanol (**19**), acetic acid (**1**), methional (**82**), (*E,E*)-2,4-heptadienal (**35**), benzeneacetaldehyde (**37**), 3-methylbutanoic acid (**6**), naphthalene (**72**), and phenol (**30**).

2-Pentylfuran (**66**) was perceived with a green aroma at RI 1203, but it was identified to produce a green, beany flavor in soybean products (**33**). 3-Hydroxy-2-butanone (**68**) at RI 1248 was described as a buttery aroma in samples A and C, whereas it was reported as floral in sample B by panelists. The compound could come from the oxidation of 2,3-butanediol, which might be a product of the Maillard reaction (**34**). (*E*)-2-Heptenal (**34**) at RI 1292 had a combined note containing sweet and green aromas, which was formed by lipid oxidation and degradation of the unsaturated fat (**21**). 1-Hexanol (**19**) at RI 1303 contributed a prominent herb-like and sweet aroma, whereas acetic acid (**1**) at RI 1387 provided a significant sour note. They could be products from fermentation (**2**). Methional (**82**) at RI

Table 3. Threshold Values from Literature References and Calculated Odor Activity Values (OAVs) of Volatile Compounds Found in Commercial Plain Sufu Samples (A–C)

no. ^a	compound	RI ^b	threshold value (ng/mL) ^c	calcd OAV ^d		
				sample A	sample B	sample C
1	acetic acid	1387	$3.63 \times 10^{-1}/\text{air}$, 1	3.36×10^7	7.63×10^7	1.26×10^8
6	3-methylbutanoic acid	1621	$1.047 \times 10^{-2}/\text{air}$, 1	1.44×10^5	3.03×10^5	7.96×10^5
19	1-hexanol	1303	$1.862 \times 10^{-1}/\text{air}$, 1	6.37×10^3	8.12×10^3	1.89×10^4
25	2-methoxyphenol	1823	$5.25 \times 10^{-2}/\text{air}$, 1	2.95×10^3	nc	2.63×10^3
30	phenol	1962	$4.27 \times 10^{-1}/\text{air}$, 1	3.84×10^3	1.07×10^3	3.61×10^3
33	hexanal	1059	$5.754 \times 10^{-2}/\text{air}$, 1	2.55×10^3	4.21×10^3	9.47×10^3
34	(E)-2-heptenal	1292	$6.31 \times 10^{-2}/\text{air}$, 1	4.40×10^3	3.47×10^3	2.11×10^4
35	(E,E)-2,4-heptadienal	1463	$1.905 \times 10^{-2}/\text{air}$, 3	1.30×10^4	5.90×10^3	4.96×10^4
37	benzeneacetaldehyde	1618	$4 \times 10^0/\text{water}$, 4	3.49×10^1	9.79×10^1	1.10×10^2
52	ethyl dodecanoate	1811	$1.995 \times 10^{-2}/\text{air}$, 1	nc	1.96×10^4	nc
61	ethyl (Z)-9-octadecenoate	2461	$8.7 \times 10^{-1}/\text{liquid}$, 4	2.56×10^5	7.53×10^5	1.11×10^6
62	ethyl (Z,Z)-9,12-octadecadienoate	2519	$4.5 \times 10^{-1}/\text{liquid}$, 4	1.54×10^6	3.41×10^6	4.91×10^6
65	ethyl (Z,Z,Z)-9,12,15-octadecatrienoate	2567	na	nc	nc	nc
66	2-pentylfuran	1203	$9.12 \times 10^{-2}/\text{air}$, 1	1.35×10^4	1.50×10^4	6.38×10^4
68	3-hydroxy-2-butanone	1248	$8 \times 10^0/\text{water}$, 2	1.86×10^1	9.03×10^1	2.15×10^2
72	naphthalene	1731	$7.94 \times 10^{-2}/\text{air}$, 1	9.57×10^2	8.89×10^2	4.18×10^3
82	methional	1423	$2 \times 10^{-4}/\text{air}$, 1	8.68×10^5	6.01×10^6	4.91×10^6

^a Compound number. ^b Linear retention index. ^c Threshold values from the following references: 1, Devos et al. (36); 2, Buttery (37); 3, Rychlik et al. (38); 4, Fazzalari (39); na, threshold value not available. ^d Concentration of a compound/threshold value of the same compound (9); nc, not calculated. Solid content (w/total w %) and density of samples A, B, and C were 26.52%, 1.016 g/mL; 31.09%, 1.086 g/mL; and 28.11%, 1.042 g/mL, respectively.

1423 was perceived as meaty by the panelists. (E,E)-2,4-Heptadienal (35) at RI 1463 was described as moldy in samples A and C, whereas it was noted as mushroom-like in sample B. A desirable floral aroma was perceived and was due to the presence of benzeneacetaldehyde (37) at RI 1618, but an undesirable sweaty aroma was perceived at RI 1621, which was due to the presence of 3-methylbutanoic acid (6). A paper-like aroma was noted at RI 1731 for both samples A and B corresponding to the compound naphthalene (72), which was also described as dried seaweed-like in sample C. A woody aroma was perceived at RI 1962, which was contributed by phenol (30). 2-Methoxyphenol (25) (RI 1962) was reported in samples A and C, with an alcohol-like sensation. Ethyl dodecanoate (52) (RI 1811) with a dried seaweed-like aroma was found only in sample B.

For RI > 2000, three common compounds were found in all three samples. Ethyl (Z)-9-octadecenoate (61) (RI 2461) was perceived with a coconut aroma. The remaining two esters were ethyl (Z,Z)-9,12-octadecadienoate (62) (RI 2519) and ethyl (Z,Z,Z)-9,12,15-octadecatrienoate (65) (RI 2567). They had sweet and irritating–pungent aromas, respectively. Their precursors were apparently 9,12-octadecadienoic acid and 9,12,15-octadecatrienoic acid because both were major free fatty acids found in soybean (35). In the presence of an ample amount of ethanol, the corresponding ethyl esters were formed.

Overall, esters (4), aldehydes (4), and alcohols (3) were the dominant classes with respect to the high number of individual compounds containing odor. Their aroma descriptors include tea leaf-like, sweet, green, herb-like, meaty, moldy, buttery, floral, alcohol-like, woody, coconut-like, sweet, and pungent (Table 3). This observation reiterates the importance of the presence of both esters and alcohols in plain sufus (4, 23).

Further evaluation of the odorants by calculation of their OAV was carried out. In Table 3, acetic acid (1) had the highest OAV ($> 1 \times 10^5$) among all samples, followed by methional (82) or ethyl (Z,Z)-9,12-octadecadienoate (62), then ethyl (Z)-9-octadecenoate (61) and 3-methylbutanoic acid (6). As a result, the odor impact of the corresponding descriptors should be in the following order: sour > sweet or meaty > coconut-like > sweaty. Ethyl dodecanoate (52), which was described as a dried seaweed-like aroma in the GC-O experiment, was found only

Table 4. Results of the Omission Experiments on the Model Plain Sufu Mixtures

model	compound(s) omitted in a model plain sufu mixture ^a	signif ^b
0	none	–
1	acetic acid (1)	*
2	3-methylbutanoic acid (6)	*
3	1-hexanol (19)	*
4	2-methoxyphenol (25)	*
5	phenol (30)	*
6	hexanal (33)	*
7	(E)-2-heptenal (34)	*
8	(E,E)-2,4-heptadienal (35)	–
9	benzeneacetaldehyde (37)	*
10	ethyl dodecanoate (52)	*
11	ethyl (Z)-9-octadecenoate (61)	*
12	ethyl (Z,Z)-9,12-octadecadienoate (62)	*
13	ethyl (Z,Z,Z)-9,12,15-octadecatrienoate (65)	–
14	2-pentylfuran (66)	*
15	3-hydroxy-2-butanone (68)	*
16	naphthalene (72)	–
17	methional (82)	*
18	double-distilled water vs pure sunflower seed oil	–

^a Model mixture (models 1–17) with one less standard compound was compared to a mixture of three authentic commercial plain sufu samples. Model 0 contained all 17 standard compounds. Model 18 did not contain any standard compounds.

^b *, statistically significant difference at $p < 0.05$, –: statistically insignificant difference at $p > 0.05$ (5, 13).

in sample B and was absent in both samples A and C. Its OAV was ~200 times stronger than that of benzeneacetaldehyde (37) in the same sample B.

To reconfirm the importance of the odorants to commercial plain sufus, omission experiments were carried out (Table 4). With 12 panelists evaluating the series of samples, a total of 36 trials per panelist were carried out. The minimum number of correct judgments for each set of triangle test to conclude a significant difference for a trial was 18 (15). Because there was no significant difference ($p > 0.05$) in odor between the sunflower seed oil (the base) and the double-distilled water (model 18, Table 4), sunflower seed oil was concluded to be a suitable base to reconstitute the sufu model mixtures. Similarly, the lack of a significant difference between the model mixture

prepared from standard chemicals and the homogenized mixture of the three commercial plain sufu brands (model 0) was an indication of the similarity of the aroma model in mimicking the odor of the authentic commercial plain sufu. Evaluations of the model mixtures identified 14 key odorants of 17 potent ones from the original aroma model mixture (14) having significant difference in the omission experiments (Table 4). These odorants include hexanal (33), 2-pentylfuran (66), 3-hydroxy-2-butanone (68), (*E*)-2-heptenal (34), 1-hexanol (19), acetic acid (1), methional (82), benzeneacetaldehyde (37), 3-methylbutanoic acid (6), ethyl dodecanoate (52), 2-methoxyphenol (25), phenol (30), ethyl (*Z*)-9-octadecenoate (61), and ethyl (*Z,Z*)-9,12-octadecadienoate (62). On the other hand, (*E,E*)-2,4-heptadienal (35), naphthalene (72), and ethyl (*Z,Z,Z*)-9,12,15-octadecatrienoate (65) were the remaining three components showing no significant differences in the test, although their corresponding OAVs were >1.

In conclusion, 68 common volatile components were identified in the supercritical fluid extracts of commercial plain sufus. Quantitatively, esters, alcohols, and acids were found as the majority classes of compounds. In terms of aroma contribution to commercial plain sufus, 17 compounds were more prominent based on the GC-FID-O evaluation. Esters, aldehydes, and acids were the three new dominant classes. Calculations of the OAVs revealed that acetic acid (1), methional (82), ethyl (*Z,Z*)-9,12-octadecadienoate (62), ethyl (*Z*)-9-octadecenoate (61), and 3-methylbutanoic acid (6) were important and dominant odor contributors in the commercial plain sufus. Sour, sweet, meaty, coconut-like, and sweaty were some of the more important descriptors generated during the evaluation. The rest of the compounds were also important in contributing to the overall background flavor of the commercial plain sufus. Results from omission experiments short-listed 14 compounds from the original 17 GC-O compounds to be the key odorants in commercial plain sufus including compounds 1, 6, 19, 25, 30, 33, 34, 37, 52, 61, 62, 66, 68, and 82. It is hoped that these compounds could serve as markers for the evaluation and further improvement of the sufu products, particularly in the area of salt reduction as traditional products often contain high salt content, which may cause a health hazard if consumed frequently.

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Received for review August 19, 2004. Revised manuscript received December 6, 2004. Accepted December 12, 2004.

JF048617D