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Comparison of Key Aroma Compounds in Five Different Types of Japanese Soy Sauces by Aroma Extract Dilution Analysis (AEDA)

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ABSTRACT: An investigation by the aroma extract dilution analysis (AEDA) technique of the aroma concentrate from five different types of Japanese soy sauces, categorized according to Japan Agricultural Standards as Koikuchi Shoyu (KS), Usukuchi Shoyu (US), Tamari Shoyu (TS), Sai-Shikomi Shoyu (SSS), and Shiro Shoyu (SS), revealed 25 key aroma compounds. Among them, 3-ethyl-1,2-cyclopentanedione and 2′-aminoacetophenone were identified in the soy sauces for the first time. Whereas 3-(methylthio)propanal (methional) and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon) were detected in all of the soy sauce aroma concentrates as having high flavor dilution (FD) factors, 4-ethyl-2-methoxyphenol was detected as having a high FD factor in only four of the soy sauces (KS, US, TS, and SSS). Furthermore, 5(or 2)-ethyl-4-hydroxy-2(or 5)-methyl-3(2*H*)-furanone (4-HEMF) and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (4-HDMF), which were thought to be the key odorants in KS, were detected in KS, US, TS, and SSS, but the FD factors widely varied among them. The sensory evaluations demonstrated that the aroma descriptions of a cooked potato-like note and a caramel-like/seasoning-like note were evaluated as high scores with no significant differences among the five soy sauces. On the other hand, a burnt/spicy note was evaluated as having high scores in KS, TS, and SSS, but it was evaluated as having a low score in SS. The comparative AEDA experiments and the auxiliary sensory experiments demonstrated that the five different types of Japanese soy sauces varied in their key aroma compounds and aroma characteristics, and the key aroma compounds in KS might not always be highly contributing in the other types of Japanese soy sauces.

KEYWORDS: *Japanese soy sauce, aroma extract dilution analysis, methional, sotolon, 4-HEMF*

INTRODUCTION

Traditionally brewed soy sauce has been one of the most popular seasonings in Japan for hundreds of years. Five typical varieties of Japanese soy sauce, categorized according to the Japan Agricultural Standards as Koikuchi Shoyu (KS), Usukuchi Shoyu (US), Tamari Shoyu (TS), Sai-Shikomi Shoyu (SSS), and Shiro Shoyu (SS), are provincially brewed for traditional dishes.[1,2] In recent years, KS, which accounts for about 85% of the total soy sauce consumption in Japan, has been widely consumed all over the world. KS is used not only as a sauce for food but also as a seasoning for cooking. Nearly equal amounts of soybeans boiled to denature the protein and wheat comminuted after roasting are used as the starting materials for brewing KS, followed by the addition of a small amount of seed mold of *Aspergillus oryzae* or *Aspergillus sojae* for cultivation over several days under high-humidity conditions.[1] This cultured mold (Koji) is then mixed in an approximate 20% saline solution, and the obtained mash (Moromi) is traditionally fermented for 6−8 months. First, a salt-tolerant lactic acid bacterium (*Tetragenococcus halophius*) and salt-tolerant yeasts (*Candida versatilis* and *Candida etchellsii*) grow in the mash, followed by growth of the main fermentation yeast (*Zygosaccharomyces rouxii*). After maturation, the brew is compressed to separate the filtrate and the residue. KS is produced as a dark-red liquid by heating the filtrate to deactivate the enzymes and to bring about a complex flavor.

US is a light-brown soy sauce mainly consumed in midwestern Japan. Although the starting materials are about the same as for KS, the product is obtained as a light-brown liquid using a slightly higher concentration of saline solution to gently age the mash. Saccharified rice malt is often added to mellow the salty taste. US is generally used as a seasoning in cooking to avoid deepening the original food color. TS is a dark-brown soy sauce brewed from >80% soybeans and a small amount of wheat. In general, TS does not undergo a heating process after fermentation. TS is mainly used as a sauce for sliced raw fish (Sashimi) in addition to its use as a seasoning for boiled fish (Tsukuda-ni) and rice crackers (Senbei), the surfaces of which are preferred to be browned. SSS is traditionally brewed and consumed in western Japan. SSS is produced by much the same brewing process as for KS. Instead of using the saline solution, however, raw KS, which is not heated after fermentation, is used for brewing. SSS is used as a seasoning for stewed food with sugar (Kanro-ni) and a sauce for sliced raw fish. SS is a pale-yellow soy sauce produced in central Japan. In contrast to TS, SS is brewed from >90% wheat and a small amount of soybeans at a low temperature for the short period of 3 months without heating after fermentation. SS is generally used as a seasoning to avoid deepening the original food color. The five different types of Japanese soy sauces have different characteristics due to the differences in their materials and brewing conditions.

Many investigations concerning the volatile compounds in soy sauce have already been reported. Over 300 compounds have already been identified as volatile components in soy sauce

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as well as their formation mechanisms such as fermentation by koji molds, yeasts, lactic acid bacteria, and thermal changes during fermentation and heat sterilization.[1,3] In particular, 5(or 2)-ethyl-4-hydroxy-2(or 5)-methyl-3(2*H*)-furanone (4-HEMF), exhibiting a caramel-like aroma, is reported as one of the most important aroma components in soy sauce.[4] Recently, another study suggested that (*R*)-(+)-4-HEMF exhibited the strongest roasted sweet aroma among the four isomers of two enantiomers and two chiral isomers.[5] Its generation mechanism is suggested as the Maillard reaction based on pentose sugar[6] or the Maillard reaction and successive yeast fermentation.[7,8]

In recent years, the key aroma compounds in KS were reported by combining the aroma extract dilution analysis (AEDA) of the soy sauce aroma concentrate, quantification of the key aroma compounds exhibiting high flavor dilution (FD) factors by a stable isotope dilution assay, and the sensory evaluation of their reconstituted solution.[9] This study suggested that 3-methylbutanal, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon), 4-HEMF, 2-methylbutanal, and 3-(methylthio)propanal (methional) were the most important aroma components in KS. On the other hand, the key aroma compounds in the other types of Japanese soy sauces have not yet been fully clarified due to the few previous studies.[10,11]

The objectives of the present investigation were to clarify the key aroma compounds in the five different types of Japanese soy sauces by AEDA. Moreover, the relationship between the key aroma compounds and their flavor characteristics in the whole soy sauce aroma of the respective soy sauces was investigated.

■ MATERIALS AND METHODS

Materials. *Soy Sauce Samples.* KS was purchased from Chiba Shoyu Co., Ltd. (Katori, Japan). US, TS, SSS, and SS were purchased from Oharahisakichi Shoten Co., Ltd. (Yuasa, Japan).

Chemicals. Ethyl acetate, 2-methylbutanal, 3-methylbutanal, ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, methional, 2-ethyl-3,5-dimethylpyrazine, phenylacetaldehyde, 3-methylbutanoic acid, 3-methyl-1,2-cyclopentanedione, 2-methoxyphenol, 3-ethyl-1,2-cyclopentanedione, 3-hydroxy-2-methyl-4-pyrone (maltol), 4-ethyl-2-methoxyphenol, 4-HEMF, 4-hydroxy-5-methyl-3(2*H*)-furanone, 2′-aminoacetophenone, 2,6-dimethoxyphenol, and 2-octanol were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (4-HDMF), 4-ethylphenol, 2-methoxy-4-vinylphenol, sotolon, and phenylacetic acid were purchased from Sigma-Aldrich Japan Co., Ltd. (Tokyo, Japan). *trans*-4,5-Epoxy-(*E*)-2-decenal was synthesized according to a literature procedure.[12]

Preparation of the Aroma Concentrates of the Five Different Varieties of Japanese Soy Sauces. The aroma concentrates of the respective soy sauces were obtained according to the concentrating method in a previous paper with a slight modification.[13] An aliquot (5 g) of the soy sauce was passed through a glass column (100 mm × 10 mm i.d.) filled with 5 mL of SP700 resin (Mitsubishi Chemical Co., Ltd.), which was conditioned with distilled water before use, followed by washing with distilled water (5 mL × 4) and eluting with 20 mL of dichloromethane. The dichloromethane fraction was dried with an excess amount of anhydrous sodium sulfate and then distilled by solvent-assisted flavor evaporation (SAFE) (40 °C, <5.0 × 10^{-3} Pa).[14] The soy sauce aroma concentrate was obtained by concentrating the dichloromethane layer by rotary evaporation, followed by nitrogen steam evaporation to about 100 μL. The respective aroma concentrates were applied to the gas chromatography−olfactometry (GC-O) and AEDA.

Identification and Semiquantitative Analysis of the Key Aroma Compounds in the Aroma Concentrates of the Five Different Types of Japanese Soy Sauces. An aliquot (100 g) of the soy sauce containing 0.200 μg/kg 2-octanol as an internal standard material was passed through a glass column (200 mm × 30 mm i.d.) filled with 100 mL of SP700 resin, followed by washing with distilled water (25 mL × 3) and eluting with 100 mL of dichloromethane. The dichloromethane fraction was dried using an excess amount of anhydrous sodium sulfate and then distilled by SAFE. The soy sauce aroma concentrate was obtained by concentrating the dichloromethane layer by rotary evaporation, followed by nitrogen steam evaporation to about 100 μL. Identification of each key aroma compound was performed by comparing its Kovats GC retention index (RI) and mass spectrum with those of the authentic compound by gas chromatography−mass spectrometry (GC-MS), in addition to comparison of its RI and odor quality with those of the authentic compound by GC-O. Semiquantitative data of the respective key aroma compounds were calculated on the basis of the internal standard material using an estimated response factor of 1 by GC-MS.

GC-O. An Agilent model 6850 series gas chromatograph equipped with a thermal conductivity detector (TCD) was used for the GC-O analysis. A fused silica column (30 m × 0.25 mm i.d. coated with a 0.25 μm film of DB-WAX, J&W Scientific; or 30 m × 0.25 mm i.d. coated with a 0.25 μm film of DB-1, J&W Scientific) was used with a 1.0 μL splitless injection. The purge valve time was 60 s. The column temperature was programmed from 40 to 210 °C at the rate of 5 °C/min. The injector and detector temperatures were 250 and 230 °C, respectively. Helium was used as the carrier gas at the flow rate of 1 mL/min. A glass sniffing port was connected to the outlet of the TCD and heated to >210 °C by a ribbon heater. Moist air was pumped into the sniffing port at 100 mL/min to quickly remove the odorant eluted from the TCD out of the sniffing port. The soy sauce aroma concentrates were applied to the GC-O analysis by two subjects. Determination of the odor qualities detected by sniffing was achieved by triplicate experiments for each subject.

AEDA. The original odor concentrate of the soy sauce aroma concentrate was stepwise diluted with dichloromethane to 1:4, 1:16, 1:64, 1:256, 1:1024, and 1:4096, and aliquots (1 μL) of each fraction were analyzed by capillary GC on a DB-WAX column. An AEDA was performed three times with respect to each sample by two subjects.[15] The detection of each compound was defined as not less than two detections by both subjects, and the FD factor of each compound was determined as the maximum dilution degree of detection.

GC-MS. An Agilent 7890A gas chromatograph coupled to an Agilent model 5975C inert XL series mass spectrometer was used. A fused silica column (60 m × 0.25 mm i.d. coated with a 0.25 μm film of DB-WAX, J&W Scientific; or 60 m × 0.25 mm i.d. coated with a 0.25 μm film of DB-1, J&W Scientific) was used. The column temperature was programmed from 80 or 40 °C to 210 °C at the rate of 3 °C/min. The injector temperature was 250 °C. Helium was used as the carrier gas at the flow rate of 1 mL/min. Aliquots (1 μL) of the sample were injected, and the split ratio was 1:30 or splitless. The mass spectrometer was used under the following conditions: ionization voltage, 70 eV (EI); ion source temperature, 150 °C.

Sensory Evaluation of Five Different Varieties of Japanese Soy Sauces. Eighteen subjects were recruited from Ogawa & Co., Ltd. They were trained for 6 months (1 h/day) with in-house programs of recognition, description, and discrimination tests involving about 300 odorants. The respective soy sauce samples (5 mL) were poured into a plastic cup at 25 °C, and the blind trials concerning their orthonasal aromas were carried out. The sensory panelists were asked to score the strength of caramel-like/seasoning-like, cooked potato-like, burnt/spicy, fruity, and sour note on a scale from 1 (weak) to 7 (strong). The evaluation terms were defined as the following aromas: sotolon for caramel-like/seasoning-like note, methional for cooked potato-like note, 4-ethyl-2-methoxyphenol for burnt/spicy note, ethyl 2-methylbutanoate for fruity note, and 3-methylbutanoic acid for sour note. A two-way layout analysis of variance without replication was applied to the sensory evaluation results performed by the seven-point scoring method.

■ RESULTS AND DISCUSSION

Identification of the Key Aroma Compounds in KS. KS had a burnt/spicy, sour, and caramel-like/seasoning-like aroma,

Table 1. Key Aroma Compounds in Five Different Types of Japanese Soy Sauces

	retention index				FD factor[a]					
no.	DB-WAX	DB-1	odor qualities	compound	KS	US	TS	SSS	SS	ref
1	937		stimulus	ethyl acetate	4	4	nd	nd	nd	31
2	950		malty	2- and 3-methylbutanal	nd	nd	4	4	nd	31, 32
3	975		fruity	ethyl 2-methylpropanoate	4	nd	nd	nd	nd	9
4	1005		fruity	ethyl 2-methylbutanoate	16	nd	nd	4	nd	33
5	1030		fruity	ethyl 3-methylbutanoate	4	nd	nd	4	nd	34
6	1449		cooked potato-like	3-(methylthio)propanal (methional)	256	64	256	256	256	35
7	1463		roasty	2-ethyl-3,5-dimethylpyraizine	4	nd	4	4	nd	35
8	1533		fruity	unknown	64	nd	nd	nd	nd	
9	1638		honey-like	phenylacetaldehyde	4	16	16	16	nd	32
10	1664		sour, cheese-like	3-methylbutanoic acid	16	4	64	16	nd	32
11	1837		caramel-like	3-methyl-1,2-cyclopentanedione	16	4	16	16	nd	36
12	1855		burnt	2-methoxyphenol	16	4	64	16	nd	32
13	1883		caramel-like	3-ethyl-1,2-cyclopentanedione	4	nd	4	4	nd	
14	1964		caramel-like	3-hydroxy-2-methyl-4-pyranone (maltol)	4	nd	16	4	nd	37
15	2000	1342	sweet, metallic	*trans*-4,5-epoxy-(*E*)-2-decenal[b]	16	nd	4	16	16	9
16	2025		spicy, burnt	4-ethyl-2-methoxyphenol	1024	256	256	1024	nd	38
17	2033		caramel-like, sweet	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (4-HDMF)	64	4	256	16	nd	36
18	2059		caramel-like, sweet	5(or 2)-ethyl-4-hydroxy-2(or 5)-methyl-3(2*H*)-furanone (4-HEMF)	256	16	4	64	nd	4
19	2129		caramel-like, sweet	4-hydroxy-5-methyl-3(2*H*)-furanone	16	4	nd	4	nd	39
20	2178		sweet, spicy	4-ethylphenol	16	16	16	16	nd	32
21	2191		spicy, burnt	2-methoxy-4-vinylphenol	64	16	16	16	nd	40
22	2198		caramel-like, seasoning-like	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon)	1024	1024	256	1024	1024	41
23	2218	1276	grape-like	2′-aminoacetophenone[a]	nd	nd	nd	4	nd	
24	2265		spicy, burnt	2,6-dimethoxyphenol	16	nd	16	4	nd	32
25	2553		honey-like	phenylacetic acid	16	nd	16	4	nd	36

[a]KS, Koikuchi; US, Usukuchi; TS, Tamari; SSS, Sai-Shikomi; SS, Shiro; nd, not detected. [b]The compound was tentatively identified by GC-O analysis using DB-WAX and DB-1 columns by comparison to the standard compounds.

and the aroma concentrate of KS had the same characteristics when a*mouillette*, which is a filter paper for sensory evaluation, was dipped into it and sniffed.

To evaluate the key aroma compounds in KS, the AEDA was applied to the aroma concentrate of KS, and 23 compounds were detected as having FD factors of not less than 4 (Table 1). Among them, 3-ethyl-1,2-cyclopentanedione (**13**) was identified as the key aroma compound in the soy sauce for the first time. 4-Ethyl-2-methoxyphenol (**16**), exhibiting the burnt/spicy note, and sotolon (**22**), exhibiting the caramel-like/seasoning-like note, were detected as having the highest FD factor of 1024, followed by methional (**6**), exhibiting the cooked potato-like note, 4-HEMF (**18**) and 4-HDMF (**17**), exhibiting the caramel-like note, and 2-methoxy-4-vinylphenol (**21**), exhibiting the spicy note, as having FD factors of not less than 64. Most of these compounds might be important aroma components of the KS aroma because they have been already reported as key aroma compounds in KS by Steinhaus et al.[9] On the contrary, the FD factors of 2-phenylethanol, 4-ethyl-2-methoxyphenol (**16**), and 2-methoxy-4-vinylphenol (**21**), which are thought to be generated by the yeast fermentation of *C. versatilis* or thermal degradation from ferulic acid,[16−18] were different from the previous study. Because the brewing process is unique in each soy sauce brewery, these differences might be due to the difference in the production conditions, such as the starting materials, varieties of yeasts, fermentation temperature and period, and heat sterilization condition.

Sotolon (**22**), exhibiting the highest FD factor of 1024, is suggested to be generated from L-threonine[19,20] or 2-oxobutyric acid and α-ketoglutaric acid by a condensation reaction.[21] The other key aroma compound, 4-ethyl-2-methoxyphenol (**16**), exhibiting the highest FD factor of 1024, as well as 2-methoxy-4-vinylphenol (**21**), is thought to be generated by yeast fermentation[16,17] or thermal degradation from ferulic acid.[18] 4-HEMF (**18**), which was detected as having the next highest FD factor of 256, is suggested to be formed by the Maillard reaction based on pentose sugar[6] or the Maillard reaction and successive yeast fermentation.[7,8] Methional (**6**) is well-known to be generated from methionine by the Maillard reaction and following Strecker degradation.[22] 4-HDMF might be generated by the Maillard reaction[23] or yeast fermentation.[24] Many previous studies concerning the formation mechanisms of the other key aroma compounds have also already been demonstrated.[3,12,23,25−27]

These studies suggest that various brewing conditions, such as the chemical compositions of the starting materials, varieties of molds and yeasts, fermentation temperature and period, and heat sterilization condition, influenced the formation of the key aroma components in the KS.

Comparison of the FD Factors and the Putative Quantitative Values of the Aroma Concentrates Made from Five Different Types of Japanese Soy Sauces. To clarify the key aroma compounds in the five types of Japanese soy sauces, AEDA experiments were performed (Table 1). The semiquantitative analyses were performed to support the AEDA data, and they were well in line with the AEDA data (Table 2). On the basis of the respective AEDA results, methional (**6**) and sotolon (**22**) were detected in all of the soy sauce aroma

Table 2. Semiquantitative Analysis of the Key Aroma Compounds in Five Different Types of Japanese Soy Sauces by GC-MS

no.	compound	concentation,[a] μg/kg (RSD,[b] %)				
		KS	US	TS	SSS	SS
1	ethyl acetate	3140 (4)	3120 (2)	555 (7)	2130 (6)	153 (7)
2a	3-methylbutanal	251 (8)	189 (9)	370 (9)	301 (4)	86.0 (6)
2b	2-methylbutanal	258 (3)	291 (9)	326 (7)	278 (4)	73.0 (4)
3	ethyl 2-methylpropanoate	29.2 (7)	nd	nd	nd	nd
4	ethyl 2-methylbutanoate	14.4 (6)	nd	1.83 (6)	3.52 (7)	nd
5	ethyl 3-methylbutanoate	9.21 (6)	1.14 (5)	3.06 (8)	4.22 (9)	nd
6	3-(methylthio)propanal (methional)	297 (5)	113 (8)	227 (6)	90.0 (7)	126 (2)
7	2-ethyl-3,5-dimethylpyraizine	6.61 (9)	3.08 (2)	8.32 (2)	5.25 (6)	nd
9	phenylacetaldehyde	5450 (2)	7350 (7)	11200 (2)	6850 (4)	1240 (4)
10	3-methylbutanoic acid	2610 (3)	321 (9)	5890 (5)	1410 (3)	30.5 (7)
11	3-methyl-1,2-cyclopentanedione	361 (2)	36.3 (9)	472 (3)	151 (2)	1.71 (3)
12	2-methoxyphenol	119 (2)	26.4 (5)	354 (3)	240 (6)	0.473 (4)
13	3-ethyl-1,2-cyclopentanedione	32.8 (5)	4.18 (5)	23.0 (5)	9.24 (8)	nd
14	3-hydroxy-2-methyl-4-pyranone (maltol)	9060 (1)	1680 (2)	16000 (2)	7910 (6)	3.04 (5)
16	4-ethyl-2-methoxyphenol	2450 (1)	1080 (6)	900 (3)	1930 (7)	0.605 (9)
17	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (4-HDMF)	2710 (2)	207 (3)	3690 (2)	653 (0)	2.02 (7)
18	5(or 2)-ethyl-4-hydroxy-2(or 5)-methyl-3(2*H*)-furanone (4-HEMF)	22000 (6)	3550 (4)	800 (1)	11500 (5)	16.5 (5)
19	4-hydroxy-5-methyl-3(2*H*)-furanone	18800 (2)	7740 (5)	2120 (3)	5510 (2)	954 (7)
20	4-ethylphenol	499 (6)	165 (5)	299 (3)	527 (6)	1.78 (5)
21	2-methoxy-4-vinylphenol	18.5 (1)	3.28 (5)	3.55 (4)	9.49 (7)	0.566 (8)
22	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon)	36.7 (2)	40.8 (5)	23.5 (3)	46.5 (5)	38.0 (5)
24	2,6-dimethoxyphenol	142 (1)	23.2 (6)	471 (3)	114 (8)	4.91 (6)
25	phenylacetic acid	3850 (6)	255 (7)	3970 (2)	3140 (5)	11.4 (5)

[a]The concentration of each compound was calculated on the basis of the ratio of the peak area of the respective compounds and that of 0.200 mg/kg of 2-octanol using an estimated response factor of 1. Data are the mean value of triplicate measurements. KS, Koikuchi; US, Usukuchi; TS, Tamari; SSS, Sai-Shikomi; SS, Shiro; nd, not detected. [b]Ratio of the standard deviation to the mean.

concentrates with high FD factors. These compounds might be some of the most important aroma compounds in every type of Japanese soy sauce. 4-Ethyl-2-methoxyphenol (**16**), detected as having FD factors of 256−1024 in the four soy sauces except for SS, might also be an important aroma compound. Because SS is brewed at a low temperature and for a short period, 4-ethyl-2-methoxyphenol (**16**), which is thought to be formed by *C. versatilis*,[16] might not be detected at all.

Although all of the key aroma compounds in US were common to KS, the FD factors of 4-HDMF (**17**) and 4-HEMF (**18**) in US were detected at a lower dilution degree than in KS (Table 1). Furthermore, ethyl 2-methylpropanoate (**3**), ethyl 2-methylbutanoate (**4**), ethyl 3-methylbutanoate (**5**), 2-ethyl-3,5-dimethylpyrazine (**7**), 3-ethyl-1,2-cyclopentanedione (**13**), maltol (**14**), *trans*-4,5-epoxy-(*E*)-2-decenal (**15**), 2,6-dimethoxyphenol (**24**), and phenylacetic acid (**25**) were not detected in US. In comparison to KS, a low fermentation degree due to the higher concentration of the saline solution might be a reason for this difference.[1]

Whereas most of the key aroma compounds were common in TS and KS, ethyl acetate (**1**), ethyl 2-methylpropanoate (**3**), ethyl 2-methylbutanoate (**4**), and ethyl 3-methylbutanoate (**5**) were not detected in TS. These differences might be influenced by the formation by the yeast metabolism, the decomposition by heat, by esterase in the mash, and the volatilization during brewing.[1,28] On the other hand, 2- and 3-methylbutanal (**2**), which are Strecker aldehydes from L-isoleucine and L-leucine,[22] were detected only in TS. 4-HEMF (**18**) was detected with an extremely lower FD factor in TS, whereas 4-HMF (**19**) was not detected in TS (Table 1). Because the previous studies concerning soy paste demonstrated that the generation of 4-HEMF (**18**) was affected by various factors during brewing, such as the heating condition of the starting materials, glucose contents in the mash, varieties of yeasts, fermentation temperature, and fermentation period,[6,8] the quantity of 4-HEMF (**18**) in the soy sauce might also be affected by these factors. Although many more samples should be examined, 4-HEMF (**18**), which is well-known as one of the key aroma components in KS, might not always be important in TS. Because TS is generally produced without heating after fermentation, 4-HMF (**19**), which is generated from pentoses by thermal treatment,[29] was not detected at all in TS.

Although almost the same key aroma compounds were detected in SSS and KS, ethyl acetate (**1**) and ethyl 2-methylpropanoate (**3**) were not detected in SSS. In contrast, 2- and 3-methylbutanal (**2**) and 2′-aminoacetophenone (**23**) were detected only in SSS. 2′-Aminoacetophenone (**23**), which might be generated from tryptophan,[30] was identified as the aroma-active compound in the soy sauce for the first time.

Only methional (**6**), *trans*-4,5-epoxy-(*E*)-2-decenal (**15**), and sotolon (**22**) were detected in SS in this experiment. The low fermentation temperature, short fermentation period, and lack of a heating process after fermentation might be reasons for this difference.

Sensory Evaluations of the Five Different Types of Soy Sauces. To verify the contribution of the aroma compounds detected by GC-O in the soy sauce aroma, the respective soy sauces were evaluated on the basis of the aroma descriptions of the key compounds (Table 3). A few differences detected in the cooked potato-like note among all of the soy sauces were well in line with the FD factors of methional (**6**), exhibiting the cooked potato-like note in the respective AEDA

Table 3. Sensory Evaluation of Five Different Types of Japanese Soy Sauces

	sensory score[a] (SD[b])				
variety[c]	caramel-like/seasoning-like	cooked potato-like	spicy/burnt	fruity	sour
KS	4.0 (0.7)	4.1 (0.9)	4.9ac (1.1)	3.6 (1.4)	4.3d (1.2)
US	3.6 (1.0)	4.3 (1.0)	3.9ab (1.1)	3.0 (1.3)	3.4de (0.8)
TS	4.2 (1.3)	4.8 (1.3)	4.7ac (1.2)	2.9 (1.3)	4.3d (1.7)
SSS	4.0 (1.0)	4.4 (1.3)	5.1c (1.3)	3.3 (0.8)	3.9de (1.2)
SS	3.9 (1.3)	5.1 (1.4)	3.1b (1.4)	3.1 (1.0)	2.9e (0.9)

[a]Different letters indicate significant differences within the column ($p < 0.05$). [b]Standard deviation. [c]KS, Koikuchi; US, Usukuchi; TS, Tamari; SSS, Sai-Shikomi; SS, Shiro.

results (Table 1). Despite the large differences in FD factors of 4-HDMF (**17**) and 4-HEMF (**18**) among the respective soy sauce aroma concentrates, there were no significant differences in the caramel-like/seasoning-like note due to sotolon (**22**), exhibiting the highest FD factor in the respective soy sauce aroma concentrates. The burnt/spicy notes in KS, TS, and SSS were significantly stronger than that in SS. This might be due to the different FD factors of 2-methoxyphenol (**12**), 4-ethyl-2-methoxyphenol (**16**), 4-ethylphenol (**20**), and 2-methoxy-4-vinylphenol (**21**) among them. The sour notes, scored significantly higher in KS and TS than in SS, might be caused by the different FD factors of 3-methylbutanoic acid (**10**). No significant difference in the fruity note was observed among all of the soy sauces due to the low FD factors of the corresponding compounds in the respective aroma concentrates.

In conclusion, the AEDA experiments of the five different types of Japanese soy sauces, categorized according to Japan Agricultural Standards, revealed a total of 25 key aroma compounds. Whereas some of the key aroma compounds, such as methional (**6**) and sotolon (**22**), were detected in common among all of the soy sauce aroma concentrates as having high FD factors, 4-HEMF (**18**) and 4-HDMF (**17**), which have often been investigated as the key odorants in KS, were not always detected as having high FD factors in every type of Japanese soy sauce. The sensory evaluations demonstrated that the strengths of the aroma characteristics of the respective soy sauces were well in line with those of the corresponding key aroma compounds exhibiting the high FD factors by AEDA. Especially, the cooked potato-like note might be one of the most important characteristics in Japanese soy sauce due to the high sensory scores in addition to the high FD factors in all of the soy sauces. On the contrary, the burnt/spicy note might not always be important in Japanese soy sauce because of the varied sensory scores among the samples.

The comparative AEDA experiments and the auxiliary sensory experiments demonstrated that some of the key aroma compounds screened by AEDA might be in common among the five types of Japanese soy sauces, but other compounds might exhibit widely differing contributions to them. Because each soy sauce brewery produces soy sauces using independent conditions, such as differences in starting materials (cultivar, seasonal fluctuation, ratio of soybeans and wheat), varieties of koji molds and yeasts, fermentation temperature and period, and heat sterilization condition, many more samples should be screened to characterize the five types of Japanese soy sauces in connection with the aroma-active compounds in them. Moreover, although the highly volatile compounds exhibited low FD factors in these experiments, there is the possibility that they were lost from the aroma concentrate during condensation. To confirm the contribution of these compounds to the soy sauce aroma, it might be better to discuss the key aroma compounds in the soy sauce in consideration of the other methods such as the dynamic head space method. Furthermore, because there are few quantitative data about some of the key aroma compounds, such as methional (**6**) and sotolon (**22**), due to their low concentrations in the soy sauce, further studies focusing on them are needed to clarify their influences on the respective soy sauce aromas in addition to the previous studies.

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Notes

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