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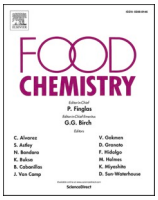

Solvent-assisted stir bar sorptive extraction and gas chromatography–mass spectrometry with simultaneous olfactometry for the characterization of aroma compounds in Japanese *Yamahai*-brewed sake

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ABSTRACT

Yamahai-brewed sake, a Japanese alcoholic beverage brewed from rice and produced via natural lactic acid fermentation, has a complex and rich flavor compared with *Sokujo*-brewed sake, brewed with a general method using pure lactic acid. This study aimed to characterize the aroma compounds in *Yamahai*-brewed sake using solvent-assisted stir bar sorptive extraction (SA-SBSE) with gas chromatography–olfactometry/mass spectrometry (GC-O/MS) and to confirm the enhanced sensitivity of GC-O/MS with SA-SBSE compared with SBSE alone. SA-SBSE with GC-O/MS increased the number of detected odor-active compounds and improved the FD factor sensitivity of *Yamahai*-brewed sake. SA-SBSE-GC–MS analysis of three pairs of *Yamahai*-brewed and *Sokujo*-brewed sake showed higher polar aroma compound content in *Yamahai*-brewed sake with a low rice polishing ratio. Quantification of 11 characteristic aroma compounds with a wide range of log K_{ow} values revealed that several compounds, including ethyl mandelate, ethyl 2-hydroxy-4-methylvalerate, and the newly identified γ-6-(*Z*)-dodecenolactone, were more abundant in *Yamahai*-brewed sake.

1. Introduction

Sake is a traditional Japanese alcoholic beverage brewed using rice, water, and koji mold. The popularity of this beverage has been increasing along with that of Japanese cuisine in recent years. *Yamahai* brewing is a traditional sake brewing method characterized by a fermentation process involving lactic acid bacteria-assisted spontaneous fermentation (Ohashi, 2007; Shirakami, Tsutsui, Nakajima, & Nomoto, 2016, Chap. 5). During sake production, yeasts are cultivated with koji, rice, and water to produce yeast mash, then koji, rice, and water are added to the yeast mash for the main fermentation process. In the *Sokujo* brewing method, applied in the production of most Japanese sakes, pure lactic acid is used during the yeast mash brewing process to lower the pH level and prevent bacterial growth. In contrast, in the *Yamahai* brewing method, naturally growing lactic acid bacteria are used instead of pure lactic acid to lower the pH and inhibit bacterial growth. Although *Yamahai*-brewed sake requires a longer and more careful production approach, it possesses a more complex and richer aroma and flavor than *Sokujo*-brewed sake (McGee, 2004; Ohashi, 2007). The *Kimoto* brewing method is virtually the same as the *Yamahai* brewing method, except for the yeast mash being pounded with poles in the case of the former. *Kimoto*-brewed sake reportedly contains more peptides than *Sokujo*-brewed sake (Tatsukami et al., 2018; Yamada, Furukawa, Hara, & Mizoguchi, 2005). However, no studies have so far focused on the aroma compounds in *Yamahai*- and *Kimoto*-brewed sakes.

Gas chromatography–mass spectrometry (GC–MS) is used for aroma compound analysis in alcoholic beverages with a variety of extraction techniques, including liquid–liquid extraction (Blanch, Reglero, & Herraiz, 1996; Tressl, Friese, Fendesack, & Koeppler, 1978), solid-phase extraction (López, Aznar, Cacho, & Ferreira, 2002), solvent-assisted flavor evaporation (SAFE) (Engel, Bahr, & Schieberle, 1999), solid-phase microextraction (SPME) (Lee, Paterson, Birkmyre, & Piggott, 2001), and stir bar sorptive extraction (SBSE) (Kishimoto, Wanikawa, Kagami, & Kawatsura, 2005). GC–MS with SBSE has been used to evaluate storage-related changes in sake aroma compounds (Isogai, Utsunomiya, Kanda, & Iwata, 2005), off-flavor aroma compounds in

Abbreviations: AEDA, Aroma extract dilution analysis; FD, Flavor dilution; GC, Gas chromatography; LVI, Large volume injection; RI, Retention index; SAFE, Solvent-assisted flavor evaporator; SBSE, Stir bar sorptive extraction; SIM, Selected ion monitoring; TDU, Thermal desorption unit.

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aged sake, spicy characteristic in competition sake (Isogai, Kanda, Iizuka, & Fujii, 2016; Isogai, Utsunomiya, Kanda, Iwata, & Nakano, 2006), and sensory evaluation score-associated aroma compounds (Mimura, Isogai, Iwashita, Bamba, & Fukusaki, 2014). SBSE, which uses polydimethylsiloxane (PDMS)-coated stir bars as an adsorbent, could significantly improve the extraction rate of compounds with moderate polarity (water–octanol partition coefficient (log K_{ow}) > 3). However, to evaluate aroma compounds that maintain the polar and nonpolar compound balance in the sample, the extraction rate of more polar compounds (log K_{ow} < 2) should be improved, which is inherently limited by the SBSE principle. In 2016, solvent-assisted stir bar sorptive extraction (SA-SBSE) was developed to swell PDMS-coated stir bars with certain solvents, which significantly improves the polar compound (log K_{ow} < 2) extraction rate (Ochiai, Sasamoto, David, & Sandra, 2016, David, Ochiai, & Sandra, 2019). SA-SBSE-GC–MS has been applied to aroma compound analysis in beer (Ochiai, Sasamoto, David, & Sandra, 2016), wine (Ochiai, Sasamoto, David, & Sandra, 2018; Ochiai, Sasamoto, David, & Sandra, 2022), sake (Isogai et al., 2022), and shochu (Li, Tsuta, Tanaka, Tsukahara, & Tsukahara, 2020). The use of SA-SBSE for aroma compound analysis, including polar compounds, is expected to be efficient for aromatic compound analysis in *Yamahai*-brewed sake.

Gas chromatography–olfactometry (GC–O), in which analysts sniff the compounds separated using gas chromatography (GC), allows the organoleptic evaluation of odor-active compounds (Grosch, 1993). GC–O with aroma extract dilution analysis (AEDA), in which analysts sniff stepwise diluted samples, is used for determining the odor activity of aroma compounds. Gas chromatography–olfactometry with mass spectrometry (GC–O/MS), therefore, has been used to analyze odor-active compounds in foods and beverages by combining extraction methods such as SAFE, SPME, and SBSE (Feng et al, 2015; Kumazawa, & Masuda, 1999; Sasaki et al., 2017; Song, & Liu, 2018). Odor-active compound evaluation using GC–O/MS would be more effective in SA-SBSE, which improves the extraction rate of polar aroma components.

This study aimed to characterize the aroma compounds in *Yamahai*-brewed sake using SA-SBSE–GC–O/MS and to confirm the enhanced sensitivity of GC–O with SA-SBSE compared with conventional SBSE. First, we used SA-SBSE–GC–O/MS to identify odor-active compounds in *Yamahai*-brewed sake. Second, we determined their flavor dilution (FD) factors, thus confirming the improved sensitivity of GC–O with SA-SBSE compared with conventional SBSE. Later, we evaluated the aroma compounds in *Yamahai*-brewed sake by comparing three pairs of *Yamahai*-brewed and *Sokujo*-brewed sakes for screening characteristic aroma compounds. Finally, quantitative analysis of the characteristic aroma compounds was performed for all *Yamahai*-brewed and *Sokujo*-brewed sake samples.

2. Materials and methods

2.1. Samples

Six *sake* samples were provided by Shata Shuzo Co., ltd (Ishikawa, Japan). Three samples (Y60, Y45, and Y35) were brewed using the *Yamahai* brewing method, and three (S60, S45, and S35) were brewed using the *Sokujo* brewing method.

Y60 and S60 were made from the same rice with a polishing ratio, called *Seimai-buai*, of 60 %. Rice polishing is the process by which the outer layer is polished using the rice polishing machine. The outer layers of unpolished rice contain large amounts of fats, minerals and proteins that spoil the flavor of sake. Highly polished rice is used to brew premium type of sake that has fruity aroma, such as apple and banana, derived from ethyl caproate and isoamyl acetate (Ohashi, K., 2007). The polishing ratio represents the weight of the polished grain as a percentage of the original unpolished grain weight. In the case of Y60 and S60, the outer 40 % of the grain is removed, leaving the central 60 %. A low polishing ratio means that the rice is highly polished. Y45 and S45 were produced from the same rice with a polishing ratio of 45 %. Similarly, Y35 and S35 were made from the same rice with a polishing ratio of 35 %. All samples were manufactured as commercial products.

2.2. Chemicals

Acetone, dichloromethane, diisopropyl ether, and sodium chloride (NaCl) were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Acetone and dichloromethane were of the grade used for pesticide residue and polychlorinated biphenyl tests. Diisopropyl ether and NaCl were of special reagent grade. The standard alkane mixture (C9-C40) was purchased from GL Science Inc. (Tokyo, Japan). Ethyl propanoate, ethyl butyrate, ethyl isovalerate, isobutanol, isoamyl acetate, 4-methyl-3-penten-2-one, isoamyl alcohol, ethyl hexanoate, dimethyltrisulfide, methional, benzaldehyde, ethyl 2-hydroxy-4-methylvalerate, diethyl succinate, methionol, ethyl phenylacetate, phenethyl acetate, guaiacol, phenethyl alcohol, 5-octanolide, 4-nonanolide, 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone (HEMF), 4-vinylguaiacol, ethyl mandelate, isoeugenol and vanillin (no. 1–9, 12, 15, 16, 21–24, 26, 27, 29, 31, 32, 34, 37, 38, 42, and 44 in Table 1) were purchased from Tokyo Chemical Industry Co., ltd. Acetic acid, isobutyric acid, butyric acid, isovaleric acid, hexanoic acid (no. 11, 17, 18, 20, 25), and sotolone were purchased from FUJIFILM Wako Pure Chemical Corporation. 2-Phenylethanal (no. 19) was purchased from Merck Corporation (Darmstadt, Germany). 2-Phenyl-2-butenal (no. 28) was purchased from Alfa Aesar (Ward Hill, MA, USA). 4-Methyl-5,6-dihydropyran-2-one (no. 30) was purchased from Angene International ltd. (Nanjing, China). Diethyl 2-hydroxypentanedioate and ethyl 2-hydroxy-3-phenylpropanoate (no. 36 and 41) were purchased from Toronto Research Chemicals (Toronto, ON, Canada). 4-Ethoxycarbonyl-γ-butanolactone (no. 39) was purchased from UkrOrgSyntez ltd. (Kyiv, Ukraine). γ-6-(*Z*)-dodecenolactone (no. 43) was procured from Soda Aromatic Co., ltd. (Tokyo, Japan). The purities of diethyl 2-hydroxypentanedioate and 4-ethoxycarbonyl-γ-butanolactone were over 90 % and 85 % respectively. The purities of other aroma compounds were over 95 %.

2.3. Aroma compound extraction using SA-SBSE and SBSE

Aroma compounds in the sake samples were extracted using SA-SBSE (Ochiai, Sasamoto, David, & Sandra, 2016). Stir bars (Flex Twister) coated with 63 μL of PDMS (10 mm length × 1.0 mm thickness) were obtained from GERSTEL GmbH & Co. KG (Mülheim an der Ruhr, Germany). For the solvent-swollen PDMS stir bar in SA-SBSE, 105 μL of the mixture with diisopropyl ether and dichloromethane (1:1) were added to a PDMS stir bar in a 2-mL glass vial, and the PDMS stir bar was then laid down and left for 30 min in the sealed vial before extraction. Sake samples were twofold diluted with ultrapure water before extraction. Subsequently, 5 μL of the twofold diluted sample were transferred into a 10-mL glass vial, and 30 % NaCl was added to the sample and dissolved. The (solvent-swollen) PDMS stir bar was added to the sample vial and extracted for 1 h at room temperature (approximately 25 °C) with a constant stirring rate of 800 rpm. The stir bar was then removed from the sake sample and dried on a filter paper. Next, the PDMS stir bar was added to 500 μL of acetone in a 10-mL glass vial and stirred for 30 min at a constant stirring rate of 350 rpm for aroma compound back extraction into acetone. SBSE was performed using the same method but without the solvent swelling process of the PDMS stir bar.

2.4. Large volume injection using a thermal desorption unit

Large volume injection (LVI) of the acetone extract (obtained from solvent back extraction) was performed using a thermal desorption unit (TDU, GERSTEL) connected to the cold injection system (CIS4, GERSTEL) inlet of an Agilent 7890A GC system (Agilent Technologies Inc., Palo Alto, CA, USA) equipped with an Agilent 5975C MS. After speed-controlled injection (at 0.85 μL/s) into a glass microvial in the TDU

Table 1
Aroma compounds in *Yamahai*-brewed sake detected by SA-SBSE-GC-O/MS.

No.	Compound	log K_{ow}	RI[A]	Ave-RI[B]	Score[C]	m/z[D]	odor quality[E]	FD-factor	Id[F]	MS peak ratio of SA-SBSE/SBSE[G]
1	ethyl propanoate	1.36	956	954	93	57	sweet, fruity	1	RI, MS, Std	3.7
2	ethyl butyrate	1.85	1038	1035	99	71	fruity	16	RI, MS, Std	2.7
3	ethyl isovalerate	2.26	1069	1069	80	85	fruity	16	RI, MS, Std	2.7
4	isobutanol	0.76	1096	1096	100	43	fusel	4	RI, MS, Std	28
5	isoamyl acetate	2.25	1126	1125	99	43	estery	16	RI, MS, Std	2.2
6	4-methyl-3-penten-2-one	1.37	1136	1128	72	83	sweet	4	RI, MS, Std	1.5
7	isoamyl alcohol	1.16	1222	1225	90	55	fusel	4096	RI, MS, Std	–
8	ethyl hexanoate	2.83	1241	1239	100	88	fruity	4096	RI, MS, Std	1.1
9	dimethyltrisulfide	1.87	1388	1379	72	126	sulfureous	4	RI, MS, Std	1.5
10	unknown 1	–	1434	–	–	117	fusel	64	–	2.4
11	acetic acid	−0.17	1454	1451	99	43	acid	4	RI, MS, Std	18
12	methional	0.41	1469	1455	78	104	sulfureous, fermented	4096	RI, MS, Std	SA-SBSE only
13	unknown 2	–	1474	–	–	75	fruity	16	–	11
14	unknown 3	–	1488	–	–	105	burnt	1024	–	6.4
15	benzaldehyde	1.48	1532	1523	99	106	chemical, almond	64	RI, MS, Std	3.2
16	ethyl 2-hydroxy-4-methylvalerate [H]	1.22	1551	1551	88	69	fruity	64	RI, MS, Std	6.2
17	isobutyric acid	1.00	1573	1569	98	73	acid	1	RI, MS, Std	52
18	butyric acid	0.79	1634	1632	98	60	acid	64	RI, MS, Std	SA-SBSE only
19	2-phenylethanal	0.79	1654	1642	94	91	floral, rose	16	RI, MS, Std	4.9
20	isovaleric acid	1.16	1676	1675	94	60	acid	256	RI, MS, Std	57
21	diethyl succinate	1.20	1684	1678	98	101	fruity	4	RI, MS, Std	3.5
22	methionol	0.44	1728	1721	95	106	sulfureous	16	RI, MS, Std	SA-SBSE only
23	ethyl phenylacetate	2.28	1798	1783	94	91	floral, rose	4	RI, MS, Std	1.3
24	phenethyl acetate	2.30	1830	1823	97	104	floral, rose	256	RI, MS, Std	1.3
25	hexanoic acid	1.92	1861	1852	99	60	acid	64	RI, MS, Std	41
26	guaiacol	1.34	1870	1864	94	109	phenolic, smoky	256	RI, MS, Std	19
27	phenethyl alcohol	1.36	1928	1924	96	122	floral, rose	4096	RI, MS, Std	–
28	2-phenyl-2-butenal	2.37	1958	1941	87	146	floral	16	RI, MS, Std	2.2
29	5-octanolide	1.59	1991	1975	70	99	cream	1	RI, MS, Std	SA-SBSE only
30	4-methyl-5,6-dihydropyran-2-one	0.52	2045	2034	90	112	sweet, creamy	256	RI, MS, Std	22
31	4-nonanolide	2.08	2069	2033	81	85	sweet, coconuts	1024	RI, MS, Std	2.3
32	HEMF isomer [H]	1.31	2078	2070	–	142	sweet, caramellic	4096	RI, MS, Std	SA-SBSE only
33	unknown 4	–	2085	–	–	114	sweet, coconuts	1024	–	28
34	HEMF isomer [H]	1.31	2108	2101	–	142	sweet, caramellic	64	RI, MS, Std	SA-SBSE only
35	unknown 5	–	2167	–	–	72	sweet	1024	–	46
36	diethyl 2-hydroxypentanedioate [H]	0.34	2176	2171	86	85	fruity, sweet	16	RI, MS, Std	75
37	4-vinylguaiacol	2.24	2206	2197	97	150	spicy	unclear	RI, MS, Std	3.9
38	ethyl mandelate [H]	1.03	2219	–	90	107	phenolic, fruity	4096	RI, MS, Std	14
39	4-ethoxycarbonyl-γ-butanolactone	−0.35	2245	2241	94	85	sweet	16	RI, MS, Std	27
40	3-hydroxy-4-phenyl-2-butanone	1.34	2277	2258	91	121	sweet, creamy	64	RI, MS	20
41	ethyl 2-hydroxy-3-phenylpropanoate	1.52	2294	2279	87	176	fruity, floral	1	RI, MS, Std	6.1
42	isoeugenol	2.65	2360	2355	76	164	spicy, clove	4	RI, MS, Std	SA-SBSE only
43	γ-6-(*Z*)-dodecenolactone	3.33	2415	2405	63	85	sweet, wax, green	256	RI, MS, Std	1.3
44	vanillin	1.05	2566	2566	94	152	sweet, creamy	4096	RI, MS, Std	6.6
45	unknown 6	–	2948	–	–	120	phenolic, sweet	16	–	35

[A] Measured RI on DB-WaxUI column(30 m × 0.25 mm i.d.; coated with a 0.25 μm film).
[B] Average RI in the database (Aroma Office [2]D). The average was calculated from RIs of several literatures.
[C] Mass spectral library match score.
[D] Selected ion for relative peak ratio and quantitation.
[E] Odor quality assigned during AEDA.
[F] Method of Identification: RI, comparison with the RI in the database(Aroma Office [2]D). MS, comparison with mass spectra stored in the NIST and Wiley libraries. Std, comparison with the authentic standard compound.
[G] Relative peak ratio of SA-SBSE to SBSE (the selected ion area).
[H] Optical isomer is unclear.

liner at 30 °C, the TDU was programmed from 30 °C (held for 0.5 min) at 140 °C/min to 80 °C (held for 7 min) with a 100 mL/min desorption flow. Desorbed compounds were focused on a Tenax TA packed liner in the CIS4 at 20 °C using liquid N_2. After desorption, the CIS4 was programmed from 20 °C to 240 °C (held for the total GC run time) to inject the trapped compounds into the analytical column. The injection was performed in splitless mode.

2.5. GC–O/MS analysis and AEDA

For the GC–O/MS analyses, 50, 200, or 800 μL LVI of the original acetone extract, i.e., nondiluted extract, was performed. The acetone extract was diluted to 1:4, 1:16, 1:64, and 1:256 with acetone, and 50 μL LVI of the diluted acetone extract was performed for the diluted extracts. Because the size of the microsyringe was 250 μL, the 800 μL LVI of the acetone extract was performed by repeating the 200 μL LVI four times while maintaining the CIS4 at 20 °C.

Separations were performed on a 60 m × 0.25 mm i.d. × 0.25 μm film thickness DB-HeavyWax column (Agilent). The column temperature was programmed from 40 °C (3-min hold) to 250 °C (15-min hold) at a rate of 5 °C/min. Helium was used as carrier gas at a flow rate of 1.2 mL/min. After 60 min of GC–O/MS analysis, the capillary column was back-flushed at a flow rate of 3.1 mL/min from the outlet to the inlet at 250 °C (10-min hold). The flow gas from the column exit was split to the MS and a sniffing port system (ODP3, GERSTEL) with a split ratio of 1:1. The MS was operated in scan mode using electron ionization at 70 eV. The scan range was set from m/z 33–300 and a sampling rate of three, thereby resulting in a scan rate of 2.72 scan/s. The aroma compounds

were identified based on retention index (RI) and mass spectra. The RIs were compared with those of the authentic compounds and Aroma Office ^{2}D database ver. 7 (Gerstel KK, Tokyo, Japan). Aroma Office ^{2}D is the most comprehensive database for aroma compounds (>116,000 entries). This software has a function to search RIs of aroma compounds from many literature references. The mass spectra were compared with those of authentic compounds and the Wiley/NIST database.

The FD factor and odor quality were confirmed by two trained panelists. Each panelist sniffed every sample twice with GC–O. The two detections by both panelists were determined as odor detection of the aroma compounds in each sample. Odor detections in the 800, 200, and 50 μL analyses of the original acetone extracts and 50 μL of the diluted acetone extract (1:4, 1:16, 1:64, and 1:256) correspond to the FD factors of 1, 4, 16, 64, 256, 1024, and 4096, respectively. The FD factor of each compound signifies the maximum dilution ratio of the odor detection sample diluted from 800 μL of the original acetone extract. If no odor was detected in the 800 μL analysis of the original extract, the FD factor was 0.

2.6. GC–MS analysis

For the GC–MS analyses, we performed 200 μL LVI of the acetone extract. The separations were performed on a 30 m × 0.25 mm i.d. × 0.25 μm film thickness DB-Wax UI column (Agilent). The column temperature was programmed from 40 °C (3-min hold) to 240 °C (17-min hold) at a rate of 5 °C/min. Helium was used as carrier gas at a flow rate of 1.2 mL/min. After 60 min of the GC–MS analysis, the capillary column was back-flushed at a flow rate of 3.1 mL/min from the outlet to the inlet at 240 °C (10-min hold). The MS was operated in the simultaneous selected ion monitoring (SIM)/scan mode using electron ionization at 70 eV. The scan range was set from *m/z* 33–300 and a sampling rate of three, thus resulting in a scan rate of 2.72 scan/s. Each sample was analyzed in triplicates. The peak areas of the identified compounds in the GC–MS data were extracted using MassHunter Quantitative Analysis software ver. 10.0 (Agilent Technologies). The SIM data were used to extract the peaks of dimethyltrisulfide (no. 9), methional (no. 12), HEMF (no. 32 and 34), unknown 4 (no. 33), and γ-6-(*Z*)-dodecenolactone (no. 43). The scan data were used to extract the peaks of the other compounds. The monitoring ions of each compound are listed in Table 1.

2.7. Aroma compound quantification

For the quantification of the 11 aroma compounds (1, 8, 12, 15, 16, 28, 29, 30, 31, 38, and 43), a standard addition calibration was performed as described previously (Ochiai, Sasamoto, David, & Sandra, 2016). Several standard solutions containing different amounts of aroma compounds in ethanol were prepared. Subsequently, 8 μL of the standard solution and 5 μL of the two fold diluted sake sample were transferred into a 10-mL glass vial. NaCl (30 %) was added to the mixture, and dissolved. The amounts of the aroma compounds in the standard solutions were adjusted to spike the concentrations in the sake samples. The estimated concentrations in sake after spiking the aroma compounds are listed Table S1. Five points in standard addition calibration curves between 0 and 320 μg/L (compound 1), 0 and 800 μg/L (compounds 8 and 15), 0 and 32 μg/L (compound 12), 0 and 160 μg/L (compound 16), 0 and 8000 μg/L (compound 30), or 0 and 8 μg/L (compounds 28, 29, 31, 38, and 43) were used for the quantitation. The solvent swollen PDMS stir bar was added to the sample vial, and SA-SBSE was performed as mentioned above. Acetone extracts were obtained with the back extraction of SA-SBSE, and 100 μL LVI of the acetone extract was performed. The separation conditions were the same as those for GC–MS analysis using a 30 m × 0.25 mm i.d. × 0.25 μm film thickness DB-Wax UI column (Agilent). The monitoring ions, listed in Table 1, were extracted using MassHunter Quantitative Analysis software ver. 10.0 (Agilent Technologies). The peak areas of compounds 12 and 43 were obtained from the SIM data. The peak areas of other compounds were extracted from the scan data. Calibration curves with a determination coefficient > 0.99 were created using the peak areas. The concentrations of aroma compounds in sake were calculated using the intersection of the calibration curve and the GC–MS peak intensity axis.

2.8. Orthogonal partial least squares discriminant analysis

Orthogonal partial least squares discriminant analysis (OPLS-DA) was performed using SIMCA v.14.0 (Umetrics, Umeå, Sweden) after the data were mean-centered and unit variant-scaled.

3. Results and discussion

3.1. Identification of odor-active compounds and determination of their FD factors in Yamahai-brewed sake using SA-SBSE–GC–O/MS

The aroma compounds in Y60 detected using SA-SBSE–GC–O/MS are shown in Table 1. We detected 45 compounds using GC–O/MS and identified 39 of them. The compounds with the highest FD factor of 4096 were as follows: isoamyl alcohol (no. 7), ethyl hexanoate (no. 8), methional (no. 12), phenethyl alcohol (no. 27), HEMF (no. 32), ethyl mandelate (no. 38), and vanillin (no. 44). The compounds with the second highest FD factor of 1024 were as follows: 4-nonanolide (no. 34), unknown-3, 4, and 5 (no. 14, 33, 35, respectively). Ethyl hexanoate and isoamyl alcohol are well-known aroma compounds that are responsible for the fruity aroma, called *ginjo-ka*, and off-flavor in sake (Utsunomiya, 2007). In a previous study on aroma compounds in sake using SBSE–GC–O/MS with AEDA, several aroma compounds, such as isoamyl acetate and ethyl isovalerate, were reported as odor-active compounds with high FD factors (Isogai, Utsunomiya, Kanda, & Iwata, 2005). In addition to these aroma compounds, we identified several others with high FD factors. The result indicates that methional, phenethyl alcohol, HEMF, ethyl mandelate, and vanillin can also strongly influence the aroma of sake. Moreover, 4-methyl-3-penten-2-one (no. 6), 2-phenyl-2-butenal (no. 28), diethyl 2-hydroxypentanedioate (no. 36), 4-ethoxycarbonyl-gamma-butanolactone (no. 39), 3-hydroxy-4-phenyl-2-butanone (no. 40), ethyl 2-hydroxy-3-phenylpropanoate (no. 41), isoeugenol (no. 42), and γ-6-(*Z*)-dodecenolactone (no. 43) were the first reported aroma compounds in sake. The 4-vinylguaiacol (no. 37) FD factor was unclear as it was difficult to distinguish its odor from that of an unknown sweet-odor compound that eluted very close. Although the unknown compound was only detected with olfactometry (not detected with MS), it was inferred to be sotolone by comparing it with the RI of the authentic standard.

3.2. Comparison of FD factors of aroma compounds in SA-SBSE and SBSE analysis of Yamahai-brewed sake

Compared with conventional SBSE, SA-SBSE enhances the recovery of polar compounds while maintaining or even improving that of apolar compounds (Ochiai, Sasamoto, David, & Sandra, 2016; Ochiai, Sasamoto, David, & Sandra, 2018). Table 1 shows the ratios of each peak area in SA-SBSE–GC–MS divided by SBSE-GC–MS. We observed a peak area increase for all compounds. Specifically, the peak areas of polar compounds with a log K_{ow} of <2 increased by factors of 1.5–75, and seven compounds (no. 12, 18, 22, 29, 32, 34, and 42) were detected only with SA-SBSE–GC–MS and not with SBSE–GC–MS.

Fig. 1 and Fig. 2 show a comparison of the FD factors obtained with SA-SBSE–GC–O/MS and SBSE–GC–O/MS for the identified and unidentified compounds. In Fig. 1, the compounds are sorted in the order of their log K_{ow} values. 4-Vinylguaiacol is not shown in Fig. 1 because of its unclear FD factor. Of the 38 compounds listed in Fig. 1, 10 were organoleptically detected only with SA-SBSE–GC–O (/MS), and 20 exhibited higher FD factors in SA-SBSE than in SBSE. Moreover, 9 of the former 10 compounds and 15 of the latter 20 compounds had low log K_{ow} values of <2 and were classified as polar aroma compounds. As for

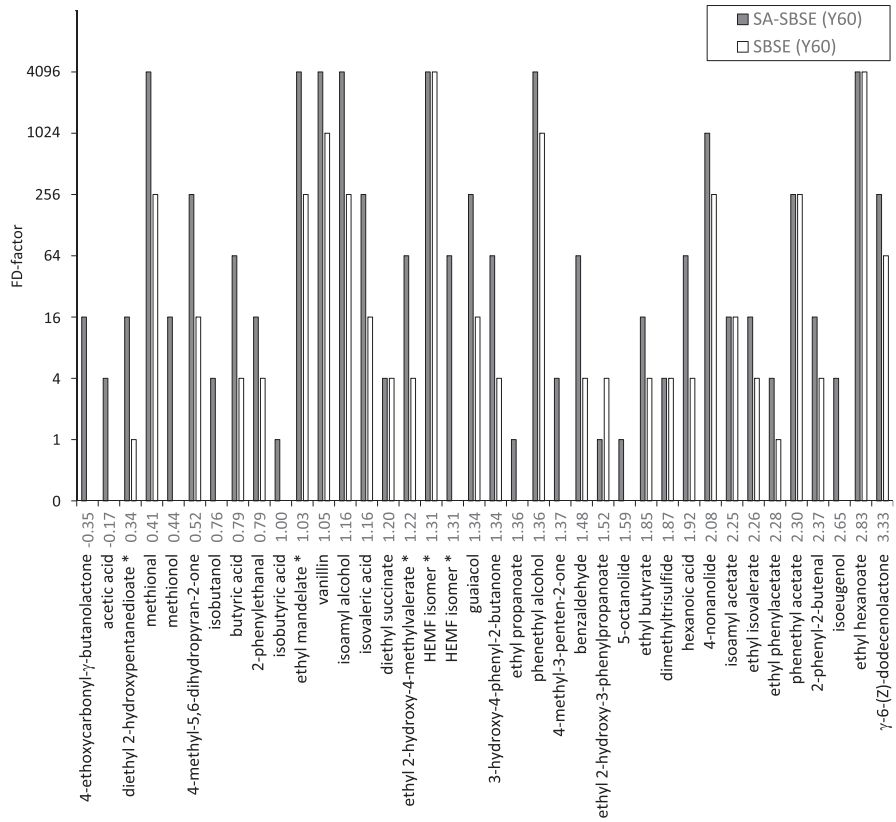

Fig. 1. A comparison of the identified compound FD factors between SBSE and SA-SBSE in the Y60 sample. The values in the x-axis show the log K_{ow} of the compounds. The compounds are sorted in the order of their log K_{ow}. The asterisk indicates that the optical isomer is unclear.

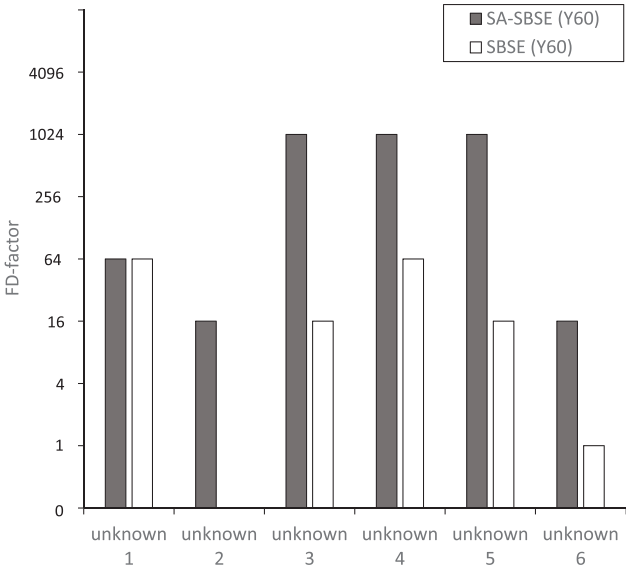

Fig. 2. A comparison of the unknown compound FD factors between SBSE and SA-SBSE in the Y60 sample.

the six unidentified compounds, one was detected only with SA-SBSE, and for four compounds, higher FD factors were reported with SA-SBSE than with SBSE (Fig. 2). SA-SBSE–GC–O (/MS) was more effective than SBSE–GC–O (/MS) in AEDA especially for polar compounds, and improved the target and dynamic ranges of odor-active compounds.

It has been reported that the aroma extracts obtained with SA-SBSE retain the aroma characteristics of the sample beverages better than those obtained with SBSE (Ochiai, Sasamoto, Sasaki, David, & Sandra, 2020). Specifically, when the stout beer acetone back-extract with SA-SBSE and SBSE were organoleptically evaluated with a mouillette, the fruity, floral aroma and roasted sensation were only slightly perceived with short-finish odors (light and airy flavor) using SBSE. In contrast, additional complex characteristics, such as caramel, chocolate, smoky, bitter, sour, and sweet, were perceived using SA-SBSE, thus leaving strong and long-finish odors (long-lasting flavor). In this study, the SBSE extract possessed short-finish odors, such as floral and fruity, whereas the SA-SBSE extract possessed showed long-finish odors with additional characteristics, such as caramel, yogurt, and fermented, which more closely resembled the aroma characteristics of the *Yamahai*-brewed sake sample. This difference is probably due to the enhanced recoveries of polar compounds with SA-SBSE. Therefore, the FD factor obtained with SA-SBSE–GC–O/MS is useful for accurately understanding the aroma characteristics of sample beverages.

3.3. Characteristic aroma compound specification with OPLS-DA

The aroma compounds detected with SA-SBSE–GC–O/MS (Table 1) are considered to contribute to the aroma of *Yamahai*-brewed sake. We focused on these compounds to evaluate the differences between *Yamahai*- and *Sokujo*-brewed sakes. Fig. 3 shows the OPLS-DA analysis results of three pairs of *Yamahai*-brewed and *Sokujo*-brewed sake samples (Y60, Y40, and Y30 and S60, S40, and S30, respectively) using *Yamahai*- or *Sokujo*-brewed sake as discriminant models. Isoamyl alcohol (no. 7) and phenethyl alcohol (no. 27) with saturated MS peaks, were excluded from the analysis. In the score plot (Fig. 3A), three *Yamahai*-brewed sake samples were located on the negative and three *Sokujo*-brewed sake samples were located on the positive side of the

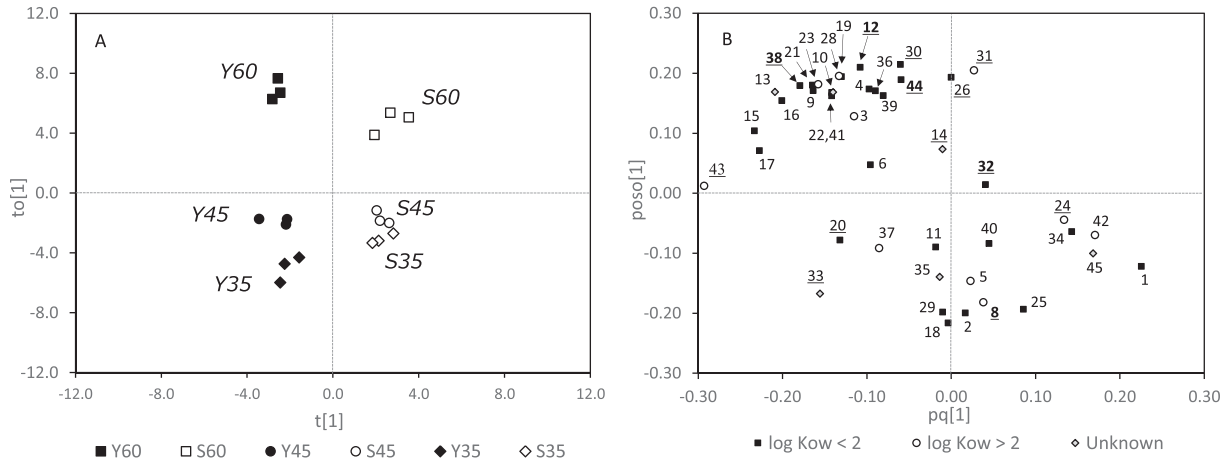

Fig. 3. OPLS-DA of the aroma compounds: (A) score plot and (B) loading plot. *Yamahai*-brewed and *Sokujo*-brewed sake are represented by black and open symbols, respectively. R^2X (cum): 0.844, R^2Y (cum): 0.96, Q^2 (cum): 0.919. Compounds that are classified as log $K_{ow} < 2$, log $K_{ow} > 2$, and unknown are represented by black squares, open circles and gray diamonds, respectively. Aroma compound numbers listed in Table 1 are indicated alongside the symbols. Aroma compounds of FD factor = 4096 and FD factor between 256 and 1024 are highlighted in bold underlined and underlined numbers, respectively. (1) ethyl propanoate, (2) ethyl butyrate,(3) ethyl isovalerate, (4) isobutanol, (5) isoamyl acetate, (6) 4-methyl-3-penten-2-one, **(8) ethyl hexanoate**, (9) dimethyltrisulfide, (10) unknown-1, (11) acetic acid, **(12) methional**, (13) unknown-2, (14) unknown 3, (15) benzaldehyde,(16) Ethyl 2-hydroxy-4-methylvalerate, (17) isobutyric acid, (18) butanoic acid, (19) 2-phenylethanal, (20) isovaleric acid, (21) diethyl succinate, (22) methionol, (23) ethyl phenylacetate, (24) phenethyl acetate, (25) hexanoic acid, (26) guaiacol, (28) 2-phenyl-2-butenal, (29) 5-octanolide, (30) 4-methyl-5,6-dihydropyran-2-one, (31) 4-nonanolide, **(32) HEMF isomer (RI 2078)**, (33) unknown-4, (34) HEMF isomer (RI 2101), (35) unknown 5, (36) diethyl 2-hydroxypentanedioate, **(37) 4-vinylguaiacol**, **(38) Ethyl mandelate**, (39) 4-ethoxycarbonyl-γ-butanolactone, (40) 3-hydroxy-4-phenyl-2-butanone, (41) ethyl 2-hydroxy-3-phenylpropanoate, (42) isoeugenol, (43) g-6-(Z)-dodecenolactone, **(44) vanillin**, (45) unknown-6.

predictive horizontal axis. This finding indicates that the compounds on the negative side of this axis in the loading plot (Fig. 3B) are those with a higher peak area in the *Yamahai*-brewed sake and that the compounds on the positive side of the axis are those with a higher peak area in the *Sokujo*-brewed sake. We observed 19 compounds with a value of ≤ -0.10 on the negative side of the predictive horizontal axis (*Yamahai*-brewed sake side) and 5 with a value of ≥ 0.10 on the positive side the axis (*Sokujo*-brewed sake side). Thus, indicating that more compounds were present on the *Yamahai*-brewed sake side. In other words, the *Yamahai*-brewed sake exhibited a higher variety of aroma compounds than the *Sokujo*-brewed sake. In addition, most compounds on the negative side of the predictive horizontal axis were located on the positive side of the orthogonal vertical axis. In the score plot, the rice polishing ratio was higher on the positive side of the orthogonal vertical axis. Thus, aroma compounds in *Yamahai*-brewed sake were more prevalent in the sake with higher rice polishing ratios. This result is consistent with the fact that the *Yamahai*-brewed sake characteristics tend to appear with a higher rice polishing ratio. Furthermore, sake with a low rice polishing ratio was located on the negative side of the orthogonal vertical axis.

Focusing on the aroma compound polarity in Fig. 3B, most compounds located on the *Yamahai*-brewed sake side, the negative side of the predictive horizontal axis, were polar compounds with log K_{ow} values of ≤ 2. It was observed that 11 of the 15 identified compounds located below -0.10 in the axis were polar compounds with a log K_{ow} value of ≤ 2.

On the *Yamahai*-brewed sake side in the loading plot (Fig. 3B), γ-6-(*Z*)-dodecenolactone, benzaldehyde, isobutyric acid, unknown 2, ethyl 2-hydroxy-4-methylvalerate, ethyl mandelate, and diethyl succinate were located in the high negative area of the predictive horizontal axis. The compounds contributed to the discrimination of *Yamahai*-brewed sake in the OPLS-DA model, because the VIP values of these compounds were ≥ 1 in Table 2. Compounds with a VIP value of ≥ 1 are generally considered to contribute largely to the discrimination in the OPLS-DA model. In particular, γ-6-(*Z*)-dodecenolactone exhibited the highest VIP value and a relatively high FD factor of 256, which suggests that it could potentially influence the aroma formation in *Yamahai*-brewed sake. Ethyl mandelate exhibited a high VIP value and the highest FD factor of 4096, the maximum value in the analysis, which signifies that it could potentially strongly influence the aroma formation in *Yamahai*-brewed sake. In contrast, on the *Sokujo*-brewed sake side in the loading plot (Fig. 3B), ethyl propanoate, isoeugenol, and unknown 6 were located in the high positive area of the predictive horizontal axis, and the VIP values of these compounds were ≥ 1 in Table 2. These compounds could potentially influence the aroma formation in *Sokujo*-brewed sake.

Next, looking at the orthogonal vertical axis in the loading plot, 4-methyl-5,6-dihydropyran-2-one, methional, 4-nonanolide, 2-

Table 2
VIP values and coefficients of selected aroma compounds in the orthogonal partial least squares discriminant analysis (OPLS-DA).

VIP	Coefficient	Compound
Positive direction		
1.72	0.23	γ-6-(*Z*)-dodecenolactone
1.41	0.16	benzaldehyde
1.39	0.15	isobutyric acid
1.35	0.08	unknown 2
1.30	0.05	ethyl 2-hydroxy-4-methylvalerate *
1.22	0.09	**ethyl mandelate** *
1.16	0.00	diethyl succinate
1.12	0.09	ethyl phenylacetate
1.12	0.07	dimethyltrisulfide
1.08	0.15	unknown-4
1.04	0.05	methionol
1.03	0.13	isovaleric acid
1.03	0.04	2-phenyl-2-butenal
1.03	0.01	ethyl 2-hydroxy-3-phenylpropanoate
1.01	0.02	2-phenylethanal
0.95	0.01	**methional**
Negative direction		
1.40	−0.15	ethyl propanoate
1.07	−0.11	isoeugenol
1.06	−0.12	unknown 6
1.03	−0.05	phenethyl acetate
1.03	−0.03	unknown 1
0.96	−0.06	ethyl isovalerate

Compounds in bold and underline showed FD factor > 4096.
Compounds in underline showed FD factor between 256 and 1024.
* Optical isomer is unclear.

phenylethanal, 2-phenyl-2-butenal, and guaiacol were located in the high positive area of the axis, the sake side with a high rice polishing ratio. These compounds could potentially affect the aroma formation in sake with a high rice polishing ratio. In particular, the FD factors of methional, 4-methyl-5,6-dihydropyran-2-one, and 4-nonanolide are high (4096, 256, and 1024, respectively), and their influence on aroma formation are expected to be high. In contrast, butyric acid, ethyl butyrate, 5-octanolide hexanoic acid, and ethyl hexanoate were located in the high negative area of the orthogonal vertical axis, the sake side with a low rice polishing ratio. These compounds could potentially affect the aroma formation in sake with a low rice polishing ratio. Specifically, the FD factor for ethyl hexanoate was as high as 4096, the maximum value in the analysis, which implies that it could potentially strongly influence the aroma formation in the sake with a low rice polishing ratio. Hexanoic acid and butyric acid, the precursors of ethyl hexanoate and ethyl butyrate (Saerens et al. 2006), were also located in the high negative area of the orthogonal vertical axis.

3.4. Characteristic aroma compound quantification

For the quantitative analysis of aroma compounds using SA-SBSE–GC–MS, we selected the aroma compounds that could affect the differences in sake types, such as *Yamahai*-brewed sake. The compounds were selected based on the following criteria: 1) compounds with OPLS-DA results showing large differences among the samples owing to the orthogonal vertical and the predictive horizontal axis, 2) compounds with relatively high FD factors, 3) polar and apolar compounds with a wide log K_{ow} value range, 4) compounds rarely reported as aroma compounds in sake, 5) compounds with MS peak areas detected only in SA-SBSE or increased more in SA-SBSE than in SBSE.

Table 3 shows the concentrations of γ-6-(*Z*)-dodecenolactone, ethyl mandelate, benzaldehyde, ethyl 2-hydroxy-4-methylvalerate, methional, 4-methyl-5,6-dihydropyran-2-one, 4-nonanolide, 2-phenyl-2-butenal, ethyl propanoate, ethyl hexanoate, and 5-octanolide. The determination coefficients of the linear regression calibration curve with standard addition method were higher than 0.99 for all compounds.

We observed that γ-6-(*Z*)-dodecenolactone, ethyl mandelate, and benzaldehyde, suggested by OPLS-DA to be prevalent in *Yamahai*-brewed sake, were more abundant in *Yamahai*-brewed sake when paired samples with the same rice polishing ratio were compared. Ethyl 2-hydroxy-4-methylvalerate was also abundant in *Yamahai*-brewed sake in 60 % and 40 % of the paired samples. Moreover, independent of the rice polishing ratio, γ-6-(*Z*)-dodecenolactone was 1.6–3.5 times more abundant in *Yamahai*-brewed sake. Siebert et al. (2018) reported that γ-6-(*Z*)-dodecenolactone was detected in white wine with SPME–GC–MS/MS and was present at a concentration of 5–147 ng/L, which is lower than the odor threshold of 700 ng/L in white wine. In this study, 547 ng/L of γ-6-(*Z*)-dodecenolactone was detected in Y60, which is 3.7 times higher than the maximum concentration detected in white wine. Ethyl mandelate showed a high FD factor of 4096 with phenolic and fruity aroma, thus indicating that the compound could highly influence aroma formation. Ethyl mandelate is assumed to be derived from aromatic compounds, such as phenolic acid and phenylalanine, distributed in the rice surface layer. Phenylalanine is reportedly converted to mandelic acid, a candidate precursor of ethyl mandelate, via cinnamic acid by fungal metabolism (Lapadatescu, Giniès, Le Quéré, & Bonnarme, 2000; Valera et al., 2020). Therefore, it is possible that mandelic acid production is accelerated during the lactic acid fermentation process in *Yamahai* brewing. Benzaldehyde, which is also abundant in *Yamahai*-brewed sake, is thought to be derived from aromatic compounds. Y60 contained benzaldehyde, which reportedly increases after several years of storage (Isogai, Utsunomiya, Kanda, & Iwata, 2005) and exhibits an odor threshold value of 990 μg/L (Utsunomiya, Isogai, & Iwata, 2004) at 377 μg/L (odor activity value of 0.4). Ethyl 2-hydroxy-4-methylvalerate, called ethyl leucate, is reportedly formed from leucine via leucic acid by the action of koji mold and yeast (Suzuki, Yoneyama, & Koizumi, 1982; Shimizu et al., 2016). The increase in ethyl 2-hydroxy-4-methylvalerate might be influenced by the use of high leucine-containing rice with high rice polishing ratio and *Yamahai* brewing.

The quantitative values of methional, 4-methyl-5,6-dihydropyran-2-one, 4-nonanolide, and 2-phenyl-2-butenal, suggested by OPLS-DA to be prevalent in the sake with a high rice polishing ratio, we reexamined.

Table 3
Concentrations of 11 aroma compounds by SA-SBSE-GC–MS in the *Yamahai*- and *Sokujo*-brewed sake samples.

No.	Compound	Concentration (μg/L)[A]						Concentration ratio[B]			*p*-Value[C]		
		Y60	S60	Y45	S45	Y35	S35	Y60/S60	Y45/S45	Y35/S35	Y60/S60	Y45/S45	Y35/S35
1	ethyl propanoate	50 ± 9.9^{a}	97 ± 3.2^{b}	120 ± 2.3^{c}	220 ± 9.8^{d}	120 ± 11^{c}	150 ± 7.7^{e}	0.5	0.5	0.8	0.019	0.005	0.004
8	ethyl hexanoate	230 ± 8.9^{a}	390 ± 27^{b}	670 ± 40^{c}	480 ± 5.0^{d}	830 ± 35^{c}	930 ± 24^{e}	0.6	1.4	0.9	0.016	0.012	0.038
12	methional	8.2 ± 1.2^{a}	3.6 ± 0.08^{b}	1.7 ± 0.10^{c}	1.2 ± 0.24^{cd}	1.1 ± 0.17^{d}	0.60 ± 0.14^{e}	2.3	1.4	1.8	0.027	0.075	0.007
15	benzaldehyde	380 ± 8.4^{a}	330 ± 1.6^{b}	410 ± 13^{a}	43 ± 0.46^{c}	250 ± 7.9^{d}	130 ± 6.1^{e}	1.2	9.5	1.9	0.015	0.0004	0.001
16	ethyl 2-hydroxy-4-methylvalerate[D]	39 ± 0.81^{a}	17 ± 0.02^{b}	25 ± 0.89^{c}	6.8 ± 0.18^{d}	13 ± 0.86^{e}	15 ± 1.3^{b}	2.3	3.7	0.9	0.0005	0.001	0.017
28	2-phenyl-2-butenal	4.1 ± 0.06^{a}	3.3 ± 0.09^{b}	0.97 ± 0.06^{c}	0.55 ± 0.11^{d}	0.56 ± 0.07^{de}	0.50 ± 0.12^{e}	1.2	1.8	1.1	0.005	0.012	0.179
29	5-octanolide	1.2 ± 0.04^{a}	0.95 ± 0.09^{b}	2.8 ± 0.03^{c}	3.0 ± 0.04^{d}	3.2 ± 0.38^{cd}	2.8 ± 0.20^{cd}	1.3	0.9	1.1	0.010	0.002	0.328
30	4-methyl-5,6-dihydropyran-2-one	1400 ± 37^{a}	960 ± 21^{b}	140 ± 1.7^{c}	55 ± 0.84^{d}	130 ± 9.0^{c}	80 ± 1.6^{e}	1.5	2.5	1.6	0.005	0.0001	0.011
31	4-nonanolide	1.8 ± 0.03^{a}	1.8 ± 0.08^{a}	0.86 ± 0.04^{b}	0.64 ± 0.002^{c}	0.58 ± 0.09^{bc}	0.47 ± 0.073^{c}	1.0	1.3	1.2	0.313	0.007	0.184
38	ethyl mandelate[D]	4.2 ± 0.02^{a}	2.2 ± 0.10^{b}	2.5 ± 0.12^{bc}	0.22 ± 0.01^{d}	1.4 ± 0.36^{ce}	0.62 ± 0.08^{e}	1.9	11	2.3	0.001	0.001	0.056
43	γ-6-(*Z*)-dodecenolactone	0.55 ± 0.02^{a}	0.35 ± 0.04^{b}	0.68 ± 0.04^{a}	0.19 ± 0.01^{c}	0.66 ± 0.12^{ab}	0.26 ± 0.08^{bc}	1.6	3.6	2.5	0.009	0.002	0.063

[A] The values represent the means ± standard deviation obtained from three independent samples. Different superscript lowercase letters (a-f) indicate significant difference according to the paired-samples *t*-test ($p < 0.05$).
[B] Concentration ratio of *Yamahai* and *Sokujyo*-brewed sake with the same rice polishing ratio.
[C] *p*-Value of *Yamahai*- and *Sokujyo*-brewed sake with the same rice polishing ratio.
[D] Optical isomer is unclear.

The results showed that higher the rice polishing ratio, the higher the contents of these compounds in the sake (Table 3). Methional and 4-methyl-5,6-dihydropyran-2-one were more abundant in *Yamahai*-brewed sake at 60 % polishing ratio of the paired samples. Methional and 4-nonanolide displayed high FD factors of 4096 and 1024, respectively, and presumably affected the sake quality. Methional, produced by Strecker degradation of methionine, reportedly exhibits a low odor threshold of 1 μg/L in sake (Isogai et al., 2022); thus, it showed a high odor activity value of 8 in Y60. 4-Nonanolide displays a coconut aroma and is known to be produced from linoleic acid (Romero-Guido et al., 2011). 2-Phenyl-2-butenal has a floral aroma and is assumed to be derived from aromatic compounds based on its structure. The precursors of each compound, such as methionine, linoleic acid, and aromatic compounds, are abundantly contained in the epithelial layer of sake rice, thus suggesting that the higher the rice polishing ratio, the higher the content of each aroma compound in the sake. The 4-methyl-5,6-dihydropyran-2-one formation pathway has not yet been clarified, although it has reportedly been detected in sake (Isogai, Kanda, Iizuka, & Fujii, 2016).

Ethyl propanoate, ethyl hexanoate, and 5-octanolide were more abundant in sake with a low rice polishing ratio. Sake with a low rice polishing ratio is suitable for making fruity sake containing a high amount of hexanoate. In addition, ethyl propanoate was more abundant in *Sokujo*- than in *Yamahai*-brewed sake.

In SA-SBSE–GC–MS, quantitative data were obtained for compounds with a wide range of log K_{ow} values, from 0.41 of methional to 3.33 of γ-6-(*Z*)-dodecenolactone. We also quantified compounds with a wide concentration range, from a few μg/L of γ-6-(*Z*)-dodecenolactone to 1400 μg/L of 4-methyl-5,6-dihydropyran-2-one.

4. Conclusions

We applied SA-SBSE–GC–O/MS to AEDA for the first time to determine the sensitivity and coverage improvement of the polar compounds in sake. Forty-five odor-active compounds were detected with SA-SBSE–GC–O/MS, which is 11 more than those detected with SBSE-GC–O/MS. We observed FD factor sensitivity improvement in the case of 24 compounds. SA-SBSE combined with GC–O/MS is an effective method for the accurate and comprehensive analysis of odor-active compounds in aroma science using GC analysis with green extraction technique, where highly efficient recovery of polar compounds is required.

The odor-active compounds detected with SA-SBSE–GC–O/MS were compared in *Yamahai*-brewed sake and *Sokujo*-brewed sake from the same brewery with SA-SBSE-GC–MS, and the aroma compound characteristics of *Yamahai*-brewed sake were investigated for the first time. OPLS-DA of 43 odor-active compounds and the quantitative analysis of 11 selected compounds aided in ascertaining that *Yamahai*-brewed sake contains several polar and apolar compounds such as ethyl mandelate, ethyl 2-hydroxy-4-methylvalerate, and γ-6-(*Z*)-dodecenolactone. In addition, quantitative analysis with SA-SBSE–GC–MS yielded concentration data for compounds with a wide polarity range (log K_{ow} values of 0.41–3.33) and concentration range (few μg/L to 1400 μg/L). Thus, the application of SA-SBSE–GC–O/MS holds potential for the analysis of sake and other alcoholic beverages.

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CRediT authorship contribution statement

Tetsuya Sasaki: Conceptualization, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition. **Nobuo Ochiai:** Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization. **Yuya Yamazaki:** Investigation, Validation, Writing – review & editing. **Kikuo Sasamoto:** Methodology, Resources, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.134640.

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