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Technical Report

Analysis of Aroma Compounds in Fatty Acid Containing Foods Using SPME Arrow-GC-MS

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Abstract:

SPME-GC-MS analysis (solid phase microextraction-gas chromatography-mass spectrometry) of aroma components is often used to evaluate the quality of food flavors. Furthermore, in recent years, SPME Arrow developed with larger loading capacity is commonly used. GC columns with a polyethylene glycol (PEG) stationary phase are the first choice for analyzing volatile components, but they can cause peak tailing problems due to carboxyl groups contained in acids interacting with hydroxyl groups contained in the stationary phase. This article describes an example of determining analytical conditions that do not result in extracting acid from Japanese sake, which contains large amounts of fatty acids. It also compares the aroma component profiles of sake before and after being stored in sherry casks.

Keywords: SPME Arrow, gas chromatograph mass spectrometer, tris (hydroxymethyl) aminomethane, sake stored in sherry casks

1. Introduction

Gas chromatograph-mass spectrometer (GC-MS) systems are commonly used to analyze the aroma components of foods as a technique for analyzing food flavor quality. Solid phase microextraction (SPME) enables easy extraction of volatile components with minimal use of solvents. The use of multifunctional autosamplers facilitates high reproducibility by providing strict control over extraction conditions such as temperature and time and is suited to the quantitative comparative analysis of multiple samples. Compared to conventional SPME fibers, SPME Arrow is coated with a larger volume of adsorbent, which allows for more sensitive analysis of components. In addition, its larger diameter provides higher durability. For the reasons above, the SPME Arrow technology is expected to become one of the most commonly used component extraction methods for analyzing aroma components in food.

This article describes the analysis of sake samples by SPME Arrow-GC-MS before and after being stored in sherry casks to determine the unique flavors derived from aging in such casks. Sake, whiskey, wine, and various other alcoholic beverages are stored in casks to impart them with unique flavors. Sherry casks are oak casks previously used to store sherry. Sake stored in sherry casks exhibits a unique color and flavor derived from storage in the casks. A sensory analysis performed by a panel of experts engaged in sake manufacturing reported strong notes of coconut, vanilla, oak, and whiskey. However, the unique characteristics derived from storing sake in sherry casks had not been analyzed by metabolomic analysis.

GC columns with a polyethylene glycol (PEG) stationary phase are the first choice for analyzing volatile components by GC. However, such columns can cause peak tailing problems due to the carboxyl groups in acids interacting with hydroxyl groups in the stationary phase. When analyzing sake samples, major tailing of fatty acid peaks can prevent detection and analysis of other co-eluting components, which can make it difficult to comprehensively analyze all aroma components in sake. Therefore, in order to analyze the aroma components that characterize sake aged in sherry casks, this research examined the conditions that result in not extracting acids from the sake.

Table 1 SPME Arrow-GC-MS Analysis Conditions

System Configuration	
GCMS	: GCMS-TQ™8050 NX
Autosampler	: AOC-6000
Column	: Supelcowax 10 (length 30 m, internal diameter 0.25 mm, film thickness 0.25 µm)
SPME Arrow Conditions	
SPME Arrow	: PDMS (length 20 mm, outer diameter 1.1 mm, film thickness 100 µm)
Conditioning temp.	: 250 °C
Pre Conditioning Time	: 15 min
Incubation Temp.	: 30 °C
Incubation Time	: 10 min
Stirrer Speed	: 1500 rpm
Sample Extract Time	: 60 min
Sample Desorb Time	: 2 min
GC Conditions	
Injection Temp.	: 250 °C
Injection Mode	: Splitless
Control Mode	: Constant Linear Velocity (30 cm/sec)
Column Oven Temp.	: 40 °C (5 min), 3 °C/min, 240 °C (20 min)
MS Conditions	
Interface Temp.	: 250 °C
Ion Source Temp.	: 200 °C
Ionization Method	: EI
Measurement Mode	: Scan (35-350 *m/z*)
Event Time	: 0.1 sec

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2. Experimental Method

Sherry casks were washed with water and filled with pasteurized sake ("Daiginjo" grade from Hyogo prefecture in Japan) and stored at room temperature for two months. Sake samples were collected before and after storage. For SPME-GC-MS analysis, samples were diluted with ultrapure water to an alcohol concentration of 10 %. Tris (hydroxymethyl) aminomethane (Tris) solution (4 mol/L) was also added to samples at the same time to adjust their pH level. 15 mL of the samples were transferred to 20 mL vials, 3 g of sodium chloride was added, and the samples were mixed in a vortex mixer. SPME Arrow polydimethylsiloxane (PMDS) fiber (outer diameter 1.1 mm, film thickness 100 µm, length 20 mm) was used to extract the volatile components. The fiber was preconditioned for 15 minutes at 250 °C before sample extraction. The samples were incubated for 10 minutes at 30 °C while shaking. Then the fiber was immersed directly into the samples for 60 minutes of extraction while stirring at 30 °C and 1500 rpm. After extraction, the fiber was inserted into the GC injection port for 2 minutes of thermal desorption of analytes at 250 °C. A GCMS-TQ8050 NX gas chromatograph mass spectrometer system was used with a Supelcowax 10 capillary GC column (length 30 m, internal diameter 0.25 mm, film thickness 0.25 µm). Analytes thermally desorbed from the SPME fiber were injected in splitless mode. Helium was used as the carrier gas. The following column oven temperature program was used. The temperature was held at 40 °C for 5 minutes, then increased to 240 °C at a rate of 3 °C/min, and finally held at 240 °C for 20 minutes. The MS unit was operated in scan mode from *m/z* 35 to 350. The ion source temperature was set to 200 °C and the interface temperature to 250 °C. GCMSsolution™ software was used for peak-picking and peak integration. Compounds were annotated based on mass spectral similarity searches of the NIST library and FFNSC3 library.

3. Results

Sake samples collected before and after sherry cask storage were analyzed using SPME Arrow-GC-MS. The obtained total ion chromatogram is shown in Fig.1. There were huge peaks of fatty acids (hexanoate, octanoate, decanoate, and dodecanoate), and their peak tailing was observed. Selecting peaks is difficult for other components that co-elute with fatty acids, even if they can be extracted and detected. In the case of target analysis focusing on only a few components of interest, by acquiring the retention time and mass spectrum of the standard solution and using the extracted ion chromatogram (EIC), even the data analysis of components that cannot be chromatographically separated from fatty acids is theoretically possible. However, this tactic cannot be used for non-targeted analysis. Deconvolution may be effective, but it often requires special software. Therefore, it was expected that a method in which the acid was not extracted by SPME would be of value. The PDMS fiber used for SPME is non-polar, and non-dissociative acids are more likely to be retained than dissociative acids. The pKa of the detected fatty acid was 4.8-5.3 (see PubChem). We attempted to suppress the retention of fatty acids in PDMS by raising the original pH of sake, which is roughly 4, and increasing the amount of dissociative fatty acids. Tris is highly soluble in water and is odorless. For this research, Tris was used to adjust the sample pH level. Total ion chromatograms obtained by SPME Arrow-GC-MS analysis of Tris-spiked sake samples are shown in Fig. 2. The peaks derived from fatty acids were reduced as the pH increased, and analyzing the peaks of components co-eluting with fatty acids became possible. Adjusting the pH level enabled comprehensive profiling of all aroma components in the sake samples and detailed analysis based on comparing the component profiles measured before and after being stored in sherry casks.

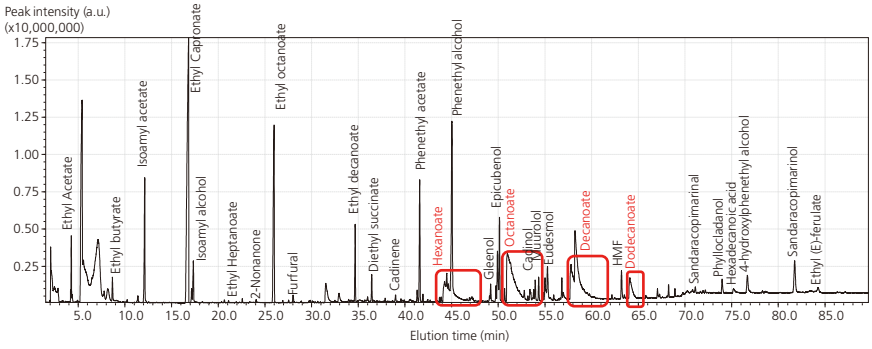


Fig. 1 TIC of Sake Stored in Sherry Casks Measured by SPME Arrow-GC-MS Analysis

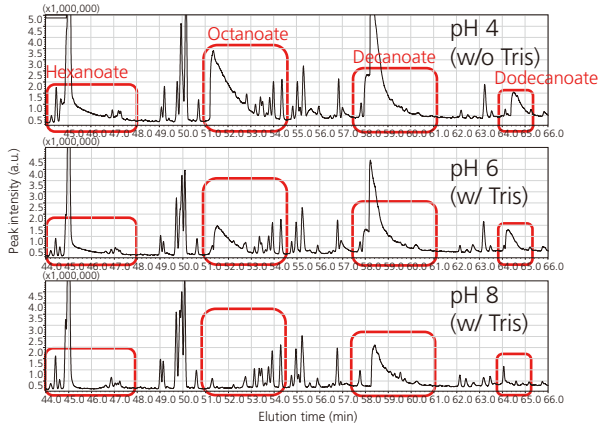


Fig. 2 Chromatogram of Sake Stored in Sherry Casks Measured Near Fatty Acid Elution Times by SPME Arrow-GC-MS Analysis

Because no fatty acid peaks were observed at pH 8, as shown in Fig. 2, samples with pH 4 (without Tris added) and adjusted to pH 8 were used for data analysis and comparison of volatile component profiles in sake before and after being stored in sherry casks. Data analysis was carried out with a sample adjusted to pH 8 for the components co-eluting with fatty acids. The components significantly elevated or diminished after sherry barrel storage are shown in Tables 1 and 2, respectively ($n = 5$, $p < 0.05$). Various aroma components, including fatty alcohols, organic acid esters, fatty acid esters, terpenes, and terpene alcohols, increased significantly. The aliphatic alcohols, 1-hexanol and 1-decanol, increased significantly after storage in sherry barrels. It is known that 1-hexanol was raised by storing beverages in oak barrels and was extracted from oak sawdust.[1][2] The aliphatic esters such as diethyl succinate were often detected in aged sake.[3] Phenylethyl acetate is also known to increase when wine is stored in oak barrels.[4] Whiskey lactone is a component derived from oak chips and is a component detected in wine and whiskey stored in oak barrels.[5] Aged alcoholic products are known to contain large quantities of terpenes and terpene alcohols, such as cadinene, calamenene, gleenol, epicubemol, elemol, eudesmol, sandaracopimarinal, phyllocladanol, and sandaracopimarinol. These originate from the wood materials used in barrels and are known to provide a wood-like aroma.[6][7][8] In sake, terpene provides a wood-like aroma and has been shown to have an antimicrobial effect.[8] There was a significant increase in such components after storing the sake in sherry casks, which suggests that these components might be imbuing the sake with unique flavors during storage.

Table 2 Aroma Components that Increased after Storing Sake in Sherry Casks

Category	Compound	RI	Target Mass	Similarity (%)	Comments
Aliphatic Alcohols	1-Hexanol	722	56	91	Grassy green-like aroma
	1-Decanol	1118	70	96	Like unripe fruit
Organic acid esters	Diethyl-succinate	1035	101	98	Increases with storage (aging aroma components) Fruit aroma and honey-like aroma
	2-phenylethyl-acetate	1167	104	94	Rose, peach, and honey-like aroma
	Ethyl 3-methylbutyl-butanedioate	1255	101	96	Co-eluted with fatty acids
	Diethyl azelate	1561	152	91	
	Ethyl ferulate	2569	222	91	
Fatty Acid Esters	Ethyl-nonanoate	888	88	92	Sweet aroma like oily nuts or wine lees
Other	2-Nonanone/2-Decanone	740	43	96	Increases with storage (aging aroma components) Ketone-like aroma, fruity aroma
	(2,2-diethoxyethyl)-benzene	1071	103	95	
	1,1,5-Trimethyl-1,2-dihydronaphthalene	1092	157	94	
	Whiskey lactone	1315	99	89	Coconut-like aroma
	3-(octadecyloxy)-1-Propanol	1871	77	83	Co-eluted with fatty acids
	Sesquirose furan	1626	69	79	
Terpenes	delta-Cadinene	1104	161	93	Contained in barrel-aged sake
	trans-Calamenene	1180	159	93	Contained in barrel-aged sake
	cis-Calamenene	1335	159	93	Contained in barrel-aged sake
	Gleenol	1386	121	93	Contained in barrel-aged sake
	Epicubenol	1415	119	93	Contained in barrel-aged sake
	alpha-Elemol	1430	93	94	Contained in barrel-aged sake
	Muurola-4,10(14)-dien-1-beta-ol	1500	159	86	
	gamma-Eudesmol/ 10-epi-gamma-Eudesmol	1519	161	92	Contained in barrel-aged sake
	Cadin-4-en-10-ol/T-Muurolol	1533	95	92	
	alpha-Muurolol/delta-Cadinol	1544	161	92	
	alpha-Eudesmol	1570	161	91	Contained in barrel-aged sake
	beta-Eudesmol	1577	59	90	Contained in barrel-aged sake
	Sandaracopimarinal	2153	271	91	Contained in barrel-aged sake
	Phyllocladanol	2240	232	81	Contained in barrel-aged sake
	Sandaracopimarinol	2493	257	92	Contained in barrel-aged sake
	alfa-/beta-Calacorene/ alpha-dehydro-ar-Himachalene	1250	157	85	Co-eluted with fatty acids
	alpha-/beta-Calacorene	1257	157	96	Co-eluted with fatty acids
Unknown	RT:29.952	875	177		
	RT:40.827	1146	101		
	RT:53.257	1510	57		
	RT:53.493	1518	104		
	RT:53.883	1527	109		
	RT:62.442	1812	59		
	RT:72.293	2194	103		

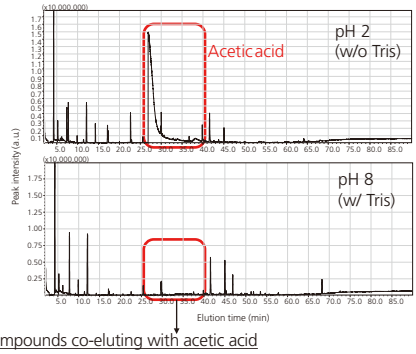
Table 3 Aroma Components that Decreased after Storing Sake in Sherry Casks

Category	Compound	RI	Target Mass	Similarity (%)	Comments
Organic acid esters	Ethyl butyrate	404	71	96	
	Ethyl hexanoate	583	88	97	Fruity aroma (apples)
	(Iso)propyl hexanoate	651	43	93	
	Isopentyl hexanoate	805	70	97	

4. Conclusion

In summary, this research showed that SPME Arrow-GC-MS can be used to comprehensively analyze volatile components in sake by adding a non-volatile water soluble primary amine (Tris) solution to samples, thus enabling an investigation into how aging sake in sherry casks affects its component profile. When no Tris was added, peak tailing from the large amounts of fatty acids contained in sake made it difficult to analyze data about components co-eluted with the fatty acids. When Tris was added to increase sample pH, the PDMS fiber did not retain dissociated fatty acids. As an example of applying this method, Fig. 3 shows the effects of adding Tris to apple cider vinegar analyzed by SPME Arrow-GC-MS. Massive tailing of peaks from the large amounts of acetic acid in the apple cider vinegar makes it difficult to analyze co-eluted components. Sake was analyzed by extracting components using direct immersion, whereas apple cider vinegar was analyzed by extracting components using the headspace method. Increasing the pH by adding Tris decreased the acetic acid peaks, which enabled peak-picking for co-eluted components.

The above results suggest that SPME-GC-MS with extraction using Tris can be used to analyze volatile components in a wide variety of foods that contain large amounts of acid.



Compounds co-eluting with acetic acid

Furfural	2,3-butandiol	Alfa-terpineol
5-methyl 5-nonanol	Undecanal	Dodecanal
Decanal	Beta-cyclocitral	Ethyl benzeneaceticacid
Benzaldehyde	Furfuryl alcohol	

Fig. 3 TIC of Apple Cider Vinegar Measured by SPME Arrow-GC-MS Analysis

For more details about this application, refer to the following article.
Taniguchi, M., Furuno, M., Yamada, T., Kawamura, K., Fukusaki, E.: Profiling of volatile compounds in Japanese sake stored in sherry casks using solid phase microextraction/gas chromatography/mass spectrometry analysis, J. Biosci. Bioeng, (2021) (e-pub) https://doi.org/10.1016/j.jbiosc.2021.03.014

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Multiple GCMS Sample Injection Methods in One Device

Perform liquid sample injection, headspace injection, solid phase micro extraction (SPME) and more, all in one device. In addition, by using the tool-switching feature, all the syringe tools for various injection methods installed on the park station can be swapped automatically.

Increased Data Reliability

Recording of syringe and fiber usage history on a chip improves the reliability of acquired data. Automated sample adjustment also reduces the burden on the operator and contributes to analysis accuracy.

High-Sensitivity Analysis Achieved by Latest Concentration Technology

Compared to previous SPME methods, the SPME Arrow achieves enhanced sensitivity and durability, and the ITEX DHS (In-tube Extraction Dynamic Headspace) offers higher sensitivity compared with previous HS, which makes analyses that employ the latest concentration technology possible.

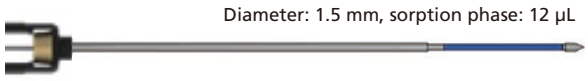
Accommodates a Wide Range of Sample Forms

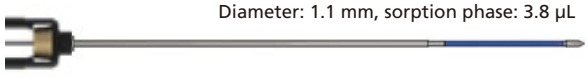
By using the AOC-6000 Plus with the OPTIC-4 multimode inlet, with its wealth of injection modes, pyrolysis analysis of solid samples, thermal desorption analysis of gaseous components, and a wide variety of other samples and analyses can be handled.

SPME Arrow Provides High Sensitivity and High Durability

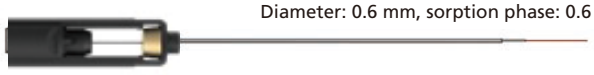
SPME Arrow, cutting-edge SPME technology, is coated with more adsorbent than conventional SPME fibers, enabling high-sensitivity analysis. Further, the thick and sturdy construction provides high durability.

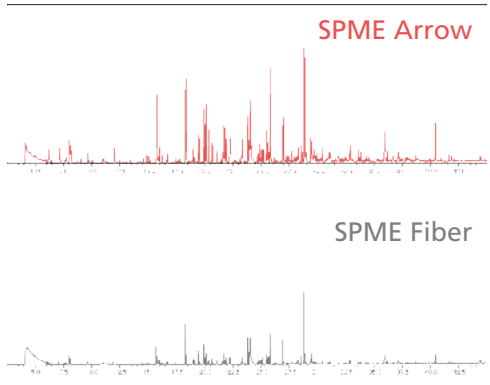
SPME Arrow





SPME Fiber





Analysis of Aroma Compounds in Coffee
(The PDMS 100 µm type SPME Arrow and SPME fibers are used.)

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