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# Changes in the Aroma Compounds of Sake during Aging

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Changes in the aroma of sake during aging were investigated by aroma extract dilution analysis (AEDA) and quantitative analysis using the stir bar sorptive extraction method. In AEDA, more odor zones were detected in aged sake than in fresh sake. The dilution factors of aldehydes, polysulfides, and some esters were greater in the aged sake, and their increase during aging was confirmed through a quantitative analysis of sake stored for 0−35 years. Among these compounds, 3-methylbutanal, methional, and dimethyltrisulfide (DMTS) were present in aged sake at concentrations exceeding their odor thresholds, and the highest odor active value was observed for DMTS. Sensory tests showed that supplementation with DMTS contributed to both the total odor intensity and the sulfury odor of aged sake aroma.

**KEYWORDS: Aged sake aroma; dimethyltrisulfide; 3-methylbutanal; methional; stir bar sorptive extraction**

## INTRODUCTION

Storing sake for several years changes its color, taste, and aroma. The aroma of aged sake is typically described as having "caramel", "burnt", "heavy", and "complicated" characteristics. In general, such sake is regarded as stale. Recently, however, sake aged for three to a few dozen years, called "jyukusei-syu", has attracted the attention of some consumers for its unique aroma and mellow taste.

Several studies have been published concerning the volatile compounds of aged sake. Takahashi isolated 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon) as a burnt flavoring compound (*1*). We also confirmed the contribution of sotolon to the aroma of aged sake by comparing its concentration in aged sake with its odor threshold value (*2*). Other compounds, such as esters (*3*), acids (*4*), sulfur compounds (*5*, *6*), and carbonyl compounds (*7*), were reported to increase during aging. However, their sensory contributions to the aroma are not clear.

In the previous work, we carried out gas chromatography/olfactometry analysis of sake extracts and detected not only sotolon but also furfural, dimethyltrisulfide (DMTS), benzaldehyde, and diethyl succinate in aged sake extracts (*2*). DMTS was found in sake for the first time, but its peak on a gas chromatogram was too small to quantify by the solvent extraction method.

In this work, we applied stir bar sorptive extraction (SBSE) to analyze the aroma of aged sake. SBSE, which uses a stir bar coated with poly(dimethylsiloxane) (PDMS), has been widely used for its versatility and sensitivity. Many SBSE applications have been reported, for example, in the analysis of volatile compounds in coffee brew (*8*) and whisky (*9*) and of off-flavor compounds in drinking water (*10*) and wine (*11*).

The aim of this work was to investigate the change in the aroma of sake during aging by aroma extract dilution analysis (AEDA) and quantitative analysis using the SBSE method. The contributions of volatile compounds to the aroma of aged sake are also discussed.

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## MATERIALS AND METHODS

**Sake Samples.** The sake samples were produced by our institute from 1967 to 2002 and stored in a cellar. The storage temperature was between 10 and 20 °C. Profiles of these samples are shown in **Table 1**.

**Reagents.** 2-Methylbutanal, 3-methylbutanal, ethyl 2-methylbutyrate, ethyl 3-methylbutyrate, dimethyl disulfide (DMDS), benzaldehyde, and phenylacetaldehyde were supplied by Sigma-Aldrich. Isoamyl acetate, methional, diethyl succinate, ethyl phenylacetate, phenethyl acetate, and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone were from Tokyo Kasei Kogyo (Tokyo, Japan). DMTS was purchased from Acros Organics (Fairlawn, NJ). *n*-Alkanes ($C_6$−$C_{20}$) were from GL Science (Tokyo, Japan).

**AEDA Using SBSE.** *SBSE.* A sake sample was adjusted to a 10% (v/v) ethanol concentration with pure water. Then it was diluted to ratios of 1:5, 1:25, and 1:125 with 10% ethanol. The sample and a stir bar (Twister from Gerstel, Mulheim an der Ruhr, Germany) were placed in a 10 mL glass headspace vial. After the addition of 2 g of NaCl, the vial was sealed with a Teflon-coated crimp cap. The stir bar was stirred at 700 rpm for 30 min. It was then removed from the sample, dried with a lint-free tissue, and transferred to a glass thermal desorption tube for GC/MS (gas chromatography/mass spectrometry) analysis.

*Thermodesorption.* A Gerstel TDS 2 thermodesorption system was used. The desorption tube was introduced into a thermodesorption unit. Then, the stir bar was thermally desorbed by heating the TDS 2 from 20 (for 1 min) to 230 (for 4 min) °C at 60 °C/min. The desorbed compounds were cryofocused in the CIS4 at −150 °C. After that, the CIS4 temperature was increased from −150 to 230 °C at 12 °C/s and held for 4 min. The trapped compounds were injected onto a GC column. Injection was performed in the splitless mode.

*GC/Olfactometry.* An Agilent Technologies (Palo Alto, CA) 6890/5973 GC/MS system was used. The column used was an Agilent Technologies HP-INNOWax (30 m × 0.25 mm, 0.25 μm). The GC oven temperature was held at 40 °C for 5 min and then increased at a rate of 5 °C/min to 240 °C and held for 15 min. Helium was used as

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**Table 1.** Profiles of Samples

| production year | storage length (years) | polishing rate of rice (%) | alcohol (%, v/v) | pH | absorbance at 430 nm | 3-deoxy glucosone (mM) |
|---|---|---|---|---|---|---|
| 2002 | 0 | 40 | 16.3 | 4.2 | 0.02 | 0.11 |
| 2000 | 2 | 50 | 17.3 | 4.2 | 0.03 | 0.07 |
| 1997 | 5 | 50 | 18.7 | 4.4 | 0.04 | 0.10 |
| 1994 | 8 | 40 | 18.0 | 4.1 | 0.06 | 0.20 |
| 1991 | 11 | 50 | 19.1 | 4.4 | 0.09 | 0.34 |
| 1988 | 14 | 50 | 18.0 | 4.1 | 0.08 | 0.25 |
| 1985 | 17 | 50 | 18.1 | 4.2 | 0.10 | 0.29 |
| 1982 | 20 | 50 | 17.8 | 4.1 | 0.12 | 0.39 |
| 1980 | 22 | 50 | 17.3 | 4.2 | 0.15 | 0.46 |
| 1976 | 26 | 50 | 16.9 | 4.3 | 0.15 | 0.42 |
| 1974 | 28 | 50 | 19.4 | 4.5 | 0.67 | 1.37 |
| 1973 | 29 | 70 | 17.9 | 4.3 | 0.64 | 1.76 |
| 1967 | 35 | 50 | 16.9 | 4.4 | 0.78 | 1.09 |

a carrier gas with a flow rate of 1 mL/min. A flow splitter was connected to the column exit, and the column flow was split 2:1 between the sniffing port and the mass selective detector (MSD). Sniffing was conducted by two trained panelists. Each panelist sniffed a sample twice. Odor zones detected at least twice out of the four times were regarded as odor active. The compounds were identified by comparing their odor properties, their chromatographic retention properties, and the MS spectra with those of standard compounds. The Kovats indices were calculated according to the literature (*12*) using *n*-alkanes ($C_6-C_{20}$) as reference compounds. The mass spectrometer was operated with an ionization voltage of 70 eV and scanned from *m*/*z* 30 to 450 at 0.29 s/scan.

**Quantification of Volatile Compounds.** The compounds identified above were quantified by SBSE coupled with GC/MS. SBSE was carried out as described above except that 3-octanol (5 mg/L) was added as an internal standard. The injection was performed in the split mode with a split rate of 50:1. The splitless mode was used for the analysis of methional. As for quantitative analysis, the column exit was directly connected to the MSD. The detection mode was selected ion monitoring, and the ions listed in **Table 3** were used for quantification of the volatile compounds. The other analytical conditions were the same as AEDA.

**Sensory Analysis.** DMTS's impact on the aroma of aged sake was investigated by sensory analysis using a trained panel of 10 members. DMTS and sotolon were added to sake produced in 2000 (base sake). The amounts added are shown in **Table 5**. These compounds were not detected in the base sake.

Samples were codified using three digit random numbers. To avoid bias caused by the order of presentation, each panelist evaluated the samples in a different order. The panelists were instructed to smell each sample and evaluate it for three aspects—overall odor intensity, caramel/burnt odor, and sulfury odor on a scale of 0−5. The data obtained were submitted to analysis of variance (ANOVA).

**General Analysis.** The color of the sake was determined by measuring the absorbance at 430 nm in a 10 mm cell after centrifugation. 3-Deoxyglucosone (3-DG) was measured according to the method of Iwano (*13*). We added 0.5 mL of sake to 5 mL of water and 1 mL of 2,4-dinitrophenylhdrazine (0.2% in 2 N HCl) and incubated the mixture for 30 min at 30 °C. Then, 10 mL of 0.3 N NaOH was added and the mixture was incubated for 30 min at room temperature. Absorbance at 530 nm was measured in a 10 mm cell. The influence of pyruvic acid on the measurement was eliminated by the method of Otsuka (*14*). The concentration of pyruvic acid was measured using F-kit (Roche Diagnostics, Tokyo, Japan), and the reduced value of pyruvic acid to 3-DG was calculated from a standard curve of pyruvic acid vs absorbance at 530 nm. 3-DG concentration was corrected by subtracting the reduced value.

## RESULTS

**AEDA Results.** The aromas of an aged sake (made in 1967) and that of a fresh sake (made in 2002) were compared by AEDA. **Table 2** lists the odorants whose dilution factors (FD) differed between the two samples. Greater numbers of odorants were detected in the aged sake. The FDs of 2-methylbutanal/3-methylbutanal, ethyl 2-methylbutyrate, 3-methyl-2-butene-1-thiol, ethyl 4-methylpentanoate, DMTS, and methional were 25−125 times higher in the aged sake than in the fresh sake. It is the first to suggest the presence of 3-methyl-2-butene-1-thiol in sake, which gives beer a lightstruck character (*15*), but we cannot detect its peak by MSD. The FDs of ethyl 3-methylbutyrate and phenylacetaldehyde were five times higher in aged sake. DMDS, furfural, and ethyl phenylacetate were detected only in aged sake, although their FDs were not very high (FD = 5). We previously showed that furfural increased during storage but that the concentration was below the detection threshold (*2*). On the other hand, the FDs of isoamyl acetate and phenethyl acetate were higher in the fresh sake than in the aged sake. Sotolon was not detected in this experiment, possibly due to PDMS's low affinity to hydrophilic compounds.

**Table 2.** Difference of Odors between Aged Sake and Fresh Sake by AEDA

| | | | FD | |
|---|---|---|---|---|
| RI | odor property | compound | aged | fresh |
| 906 | aldehyde, nut | 2-methylbutanal + 3-methylbutanal[a] | 25 | |
| 993 | resin | unknown | 5 | |
| 1036 | roasted, earthy | unknown | 5 | |
| 1041 | sweet, ester | ethyl 2-methylbutyrate[a] | 125 | |
| 1055 | sweet, ester | ethyl 3-methylbutyrate[a] | 125 | 15 |
| 1061 | resin | DMDS[a] | 5 | |
| 1087 | sulfur, garlic | 3-methyl-2-butene-1-thiol[b] | 25 | 1 |
| 1103 | banana | isoamyl acetate[a] | 5 | 125 |
| 1116 | garlic, metal | unknown | 1 | 5 |
| 1154 | powder, roasted | unknown | 5 | |
| 1173 | sweet, ester | ethyl 4-methylpentanoate[a] | 25 | |
| 1323 | nut, powder | 2,3-dimethylpyrazine[b] + unknown | 5 | |
| 1369 | sulfur | DMTS[a] | 125 | 1 |
| 1407 | sweet, ester | unknown | 5 | |
| 1445 | potato, roased | methional[b] | 25 | 1 |
| 1473 | burnt, chemical | furfural[a] | 5 | |
| 1512 | grass | unknown | | 25 |
| 1617 | burnt | unknown | 25 | 5 |
| 1635 | flower | phenylacetaldehyde[a] | 25 | 5 |
| 1663 | nut, powder | unknown | 5 | 1 |
| 1721 | honey, caramel | unknown | 5 | 1 |
| 1720 | plastic, sulfur | unknown | 1 | |
| 1760 | sulfur | unknown | 1 | |
| 1771 | flower | ethyl phenylacetate[a] | 5 | |
| 1808 | flower | phenethyl acetate[a] | | |
| 1821 | curry | unknown | 1 | |
| 1958 | honey, caramel | unknown | 1 | 5 |
| 2012 | roasted, wax | unknown | 1 | |
| 2097 | medicinal | unknown | 1 | |
| 2199 | cereal | unknown | 1 | |

[a] Compound identified by comparison of RI value, odor property, and mass spectra with those of standard compound. [b] Compound tentatively identified by comparison of RI value and odor property of standard compound.

**Precision and Recovery of the SBSE Method.** To confirm the changes in the concentrations of those compounds during storage, their concentrations in aged sake were determined using the SBSE method. We also analyzed benzaldehyde and diethyl succinate, which we detected by GC/olfactometry in our previous work (*2*). **Table 3** shows the coefficients of variation (CV) and the recovery rates of the assays. The CVs of the tested compounds were less than 6%. Recovery rates of 87−103% were calculated via the analysis of standard solutions, sake samples, and spiked sake samples with standard solution. It was considered that the SBSE method was able to quantify these volatile compounds.

**Table 3.** Monitor Ions, CVs, and Recovery Rates

| | monitor ion | CV[a] (%) | recovery rate (%) |
|---|---|---|---|
| 2-methylbutanal | 58 | 1.9 | 98 |
| 3-methylbutanal | 58 | 5.8 | 99 |
| methional | 104 | 2.1 | 103 |
| benzaldehyde | 106 | 2.5 | 87 |
| phenylacetaldehyde | 120 | 5.7 | 89 |
| DMDS | 94 | 2.5 | 93 |
| DMTS | 126 | 2.9 | 96 |
| ethyl 2-methylbutyrate | 102 | 1.4 | 95 |
| ethyl 3-methylbutyrate | 88 | 3.1 | 94 |
| diethyl succinate | 101 | 4.9 | 98 |
| ethyl phenylacetate | 91 | 2.5 | 96 |
| isoamyl acetate | 70 | 1.8 | 100 |
| phenethyl acetate | 104 | 2.7 | 90 |

[a] CV was calculated from five injections of standard solution.

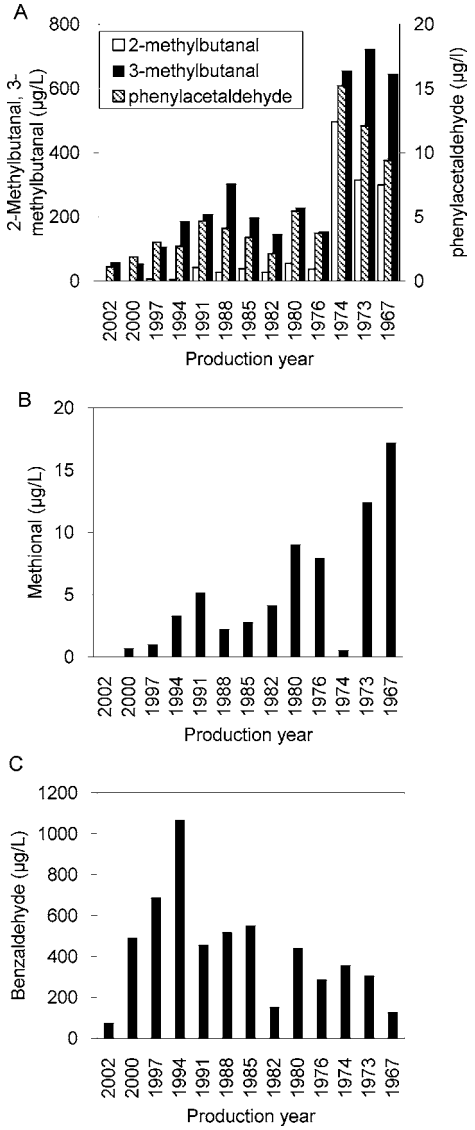


**Figure 1.** Concentrations of aldehydes in stored sake.

**Changes in the Concentrations of Volatile Compounds during Aging. Figures 1−3** show the concentrations of the volatile compounds in the stored sake. The quantified results generally coincided with the AEDA results.

**Figure 1A** shows the concentrations of aldehydes, such as 2-methylbutanal, 3-methylbutanal, and phenylacetaldehyde, all of which increased during storage. Methional (**Figure 1B**) also increased during storage, but an exceptionally low concentration was observed in the sample from 1974. The concentration of benzaldehyde peaked after 8 years of storage and then decreased (**Figure 1C**).

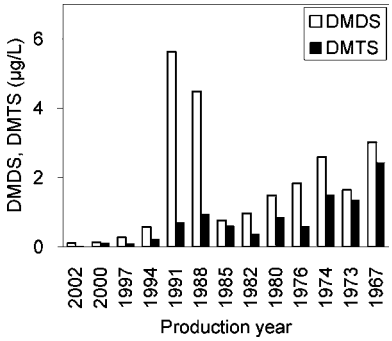


**Figure 2.** Concentrations of polysulfides in stored sake.

**Figure 2** shows the concentrations of DMDS and DMTS. The fresh sake contained only trace amounts of these polysulfides. They tended to increase with the storage period, although especially high concentrations of DMDS were observed in the samples from 1991 and 1988.

The concentrations of ethyl esters such as ethyl 2-methylbutyrate, ethyl 3-methylbutyrate, ethyl phenylacetate, and diethyl succinate also increased during storage (**Figure 3A**,**B**). On the other hand, acetate esters such as isoamyl acetate and phenethyl acetate decreased (**Figure 3C**). The concentration of ethyl 4-methylpentanoate could not be determined due to the absence of a commercial standard compound.

**Contribution of DMTS to the Aged Sake Aroma. Table 4** shows the relationships between the concentrations in the tested sake and the threshold values of the aroma compounds named above. The odor active values (OAV) of 3-methylbutanal, methional, benzaldehyde, and DMTS exceeded 1. Among them, that of DMTS was the highest.

To further investigate DMTS's contribution to the aroma of aged sake, it was added to a base sake and sensory tests were carried out. In combination with DMTS, sotolon was added to simulate aged sake, and the interaction between the two compounds was also studied. The concentration of added sotolon was the average concentration found in commercial aged sake stored for 10−30 years and was five times higher than its recognition threshold. The added concentrations of DMTS were in the range found in the samples investigated in this study.

**Figure 4A**,**B** shows that not only the sulfury odor but the total odor intensity increased with the concentration of added DMTS. For both attributes, the scores of the samples supplemented with 1 and 2 $\mu$g/L of DMTS were significantly higher than the scores of the samples with no additions (5% level).

Furthermore, the interaction between DMTS and sotolon was investigated. **Figure 4C** shows that the caramel/burnt odor, a typical character of sotolon, increased when the sample was supplemented with 1 $\mu$g/L of DMTS in addition to 15 $\mu$g/L of sotolon, as compared to the sample with no DMTS supplementation. However, the score decreased when 2 $\mu$g/L of DMTS was added.

The ability of sotolon to mask the sulfury odor was also investigated (**Figure 4D**). The intensity of the sulfury odor was almost the same whether sotolon was added or not. Thus, at the levels used here, sotolon did not seem to mask the sulfury odor.

## DISCUSSION

We investigated the change in the aroma of sake during storage by analyzing sake made and stored in our institute. The

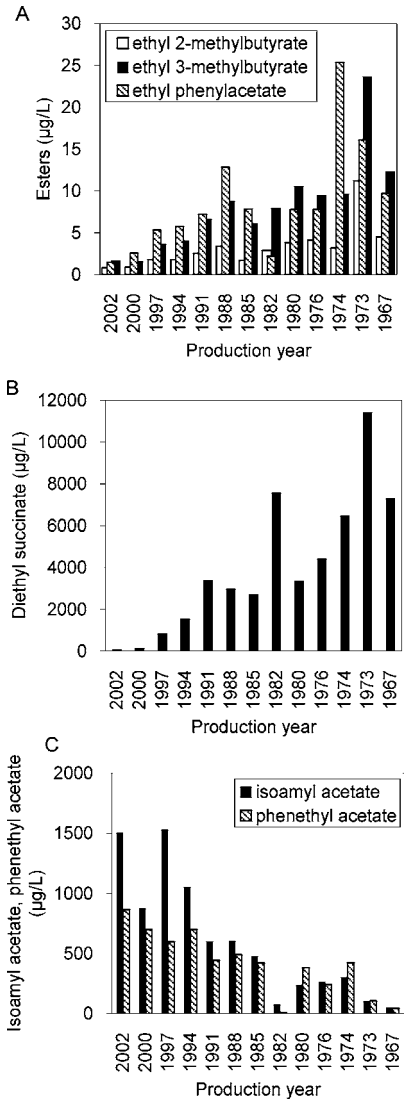


**Figure 3.** Concentrations of esters in stored sake.

**Table 4.** Concentrations of Volatile Compounds in Stored Sake, Threshold Values, and OAV

| compound | detection threshold ($\mu$g/L) | concentration in stored sake ($\mu$g/L) | | OAV | |
|---|---|---|---|---|---|
| | | min | max | min | max |
| 2-methylpropanal | 1000[d] | 8 | 364 | <0.1 | 0.4 |
| 2-methylbutanal | 1500[d] | n.d. | 996 | <0.1 | 0.3 |
| 3-methylbutanal | 120[a] | 55 | 722 | 0.5 | 6.0 |
| methional | 10[c] | n.d. | 17 | <0.1 | 1.7 |
| benzaldehyde | 990[a] | 77 | 1067 | <0.1 | 1.1 |
| phenylacetaldehyde | 25[f] | 1.1 | 15 | <0.1 | 0.6 |
| DMDS | 7[d] | 0.11 | 5.6 | <0.1 | 0.8 |
| DMTS | 0.18[a] | 0.04 | 2.4 | 0.2 | 14 |
| ethyl 2-methylbutyrate | 7200[e] | 0.8 | 11 | <0.1 | 0.1 |
| ethyl 3-methylbutyrate | 18200[e] | 1.6 | 24 | <0.1 | 0.2 |
| diethyl succinate | 100000[c] | 76 | 11424 | <0.1 | 0.1 |
| ethyl phenylacetate | 100[c] | 1.5 | 25 | <0.1 | 0.3 |
| isoamyl acetate | 300[b] | 50 | 1531 | 0.2 | 5.7 |
| phenethyl acetate | 3000, 3800[d] | 10 | 865 | <0.1 | 0.3 |

[a−c] Odor threshold in sake according to refs *31* (*a*), *32* (*b*), determined for this work in our laboratory (*c*). [d,e] Odor threshold in beer according to refs *33* (*d*) and *34* (*e*). [f] Odor threshold in wine accoding to ref *21*.

samples were made in a similar way (to maintain high quality), but the storage periods ranged from 0 to 35 years. We applied the SBSE method for AEDA and quantitative analysis of the

**Table 5.** Added Concentrations of DMTS and Sotolon for Sensory Tests

| sample | A | B | C | D | E |
|---|---|---|---|---|---|
| DMTS ($\mu$g/L) | 0 | 0.5 | 1 | 2 | 1 |
| sotolon ($\mu$g/L) | 15 | 15 | 15 | 15 | 0 |

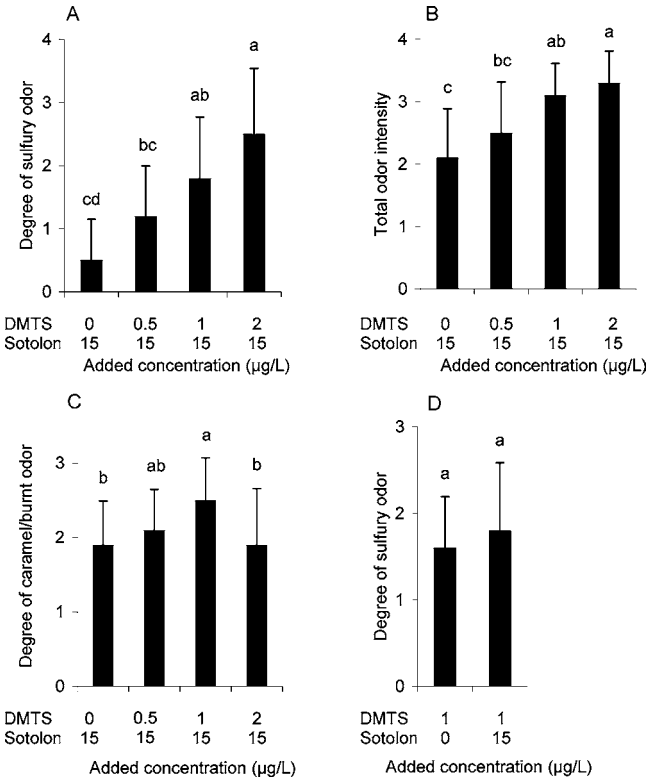


**Figure 4.** Effect of DMTS on sensory evaluation and interaction with sotolon. The results are shown using letters to indicate differences; any values not followed by the same letter are significantly different at the 5% level.

compounds related to the aroma of aged sake. In AEDA, aldehydes, esters, and polysulfides were more strongly detected in sake stored for 35 years than in fresh sake (**Table 2**). Their increase with age was confirmed by quantification of the compounds in the stored sake (**Figures 1−3**).

Among these compounds, 3-methylbutanal, methional, benzaldehyde, and DMTS were contained at concentrations exceeding their odor thresholds in aged sake, as shown in **Table 4**. The sensory contributions of these compounds to the aroma of aged sake were demonstrated for the first time.

3-Methylbutanal is known to be an off-flavor compound in nonpasteurized sake. The formation mechanism of 3-methylbutanal from isoamyl alcohol through enzymatic oxidation is well-known (*16*−*18*). However, its formation after pasteurization in sake has not been fully studied. On the other hand, increases in aldehydes such as 2-methylbutanal, 3-methylbutanal, and phenylacetaladehyde have been observed during the storage of beer (*19*). In our data, these aldehydes increased in manners similar to each other and correlated well with 3-DG ($R = 0.92-0.95$), an intermediate of the Maillard reaction, and they also correlated with absorbance at 430 nm ($R = 0.85-0.90$). On the basis of these observations, these aldehydes are considered to form through the Strecker degradation of their corresponding amino acids.

The OAV of 3-methylbutanal in the oldest sake was 6.0, whereas that in the fresh sake was 0.5. The odor of 3-meth-

ylbutanal is described as "aldehyde", "green", or "nutty". It may contribute to the pungent attribute of aged sake's aroma.

The presence of methional in sake was confirmed here for the first time, although its presence was estimated by Oba (*6*). The aroma does not seem very strong (FD < 1.7), but it contributes to the overall aroma to some extent. It is also related to the aroma of oxidation-spoiled white wines (*20*−*22*).

It is reported that the stability of the compound is affected by various parameters such as pH and sulfite in beer (*28*). One of the reasons for the low methional concentration in the sample from 1974 might be slightly high pH of the sample (**Table 1**), although other unknown factors might also be related.

The OAV of benzaldehyde exceeded 1 in one sample (that from 1994). It was reported that the compound increased during the storage of beer for 6 months (*18*). In the case of sake, it seemed to increase up to a certain age and then decrease (**Figure 1C**). The contribution of the compound to the aroma of aged sake may be only temporary. It is not clear why benzaldehyde began to decrease at a certain age, but we observed the increase in ethyl benzoate during storage (data not shown), which might be formed through oxidation and esterification of benzaldehyde.

DMDS was found in aged sake by Sato (*5*), and we confirmed its increase during storage (**Figure 2**). However, the concentration of DMDS was below the detection threshold. On the other hand, the OAV of DMTS exceeded 1 in the sake stored for 8 years and reached 14 in that stored for 35 years. Therefore, the sulfury attribute of the aroma of aged sake is ascribed to DMTS rather than DMDS.

The addition of DMTS to fresh sake at the level detected in the aged sake actually enhanced the sulfury odor (**Figure 4A**). It also increased the total odor intensity (**Figure 4B**). The sum of the OAV logarithm of DMTS and that of sotolon correlated well with the total odor intensity ($R^2 = 0.99$), which suggests that both compounds contribute independently to odor intensity.

The odor of DMTS by itself is "sulfury", "oniony", or like cooked vegetables. However, when DMTS was added in combination with sotolon, the caramel/burnt odor, which is characteristic of sotolon, increased as compared to the sample with no addition of DMTS. It is not clear at present whether this enhancement resulted from the change in the volatility by coexisting compounds (*23*) or by a synergistic interaction at the olfactory sensory neuron level (*24*). On the other hand, the enhancement was not observed when the sulfury odor of DMTS was too strong. Because the caramel/burnt odor is a desirable characteristic for aged sake, the balance of the two compounds seems to be important.

The effect of sotolon to mask the sulfury odor was not observed here (15 $\mu$g/L of sotolon and 1 $\mu$g/L of DMTS; **Figure 4D**). However, some very old sake contained an excess amount of sotolon as compared to DMTS; for example, the sample from 1974 contained 140 $\mu$g/L of sotolon and 1.6 $\mu$g/L of DMTS. In such a case, the masking effect of sotolon might be expected.

The odor of overaged sake is called "hineka" by sake brewers. Utsunomiya reported that when DMTS was added to sake, the odor was described as "hineka" by about 30% of sake brewery technicians (*25*), thus implying that DMTS is an important odorant in the aroma of aged sake.

Although the formation of DMTS in sake has not been studied, that in beer has been well-studied (*26*−*30*). In beer, there are considered to be two formation pathways of DMTS: the reaction between methanesulfenic acid and hydrogen sulfide (*26*) and the oxidation of methanethiol derived from the degradation of methional (*28*−*30*). Because methanesulfenic acid is formed from S-methylcysteine sulfoxide in hops, the latter pathway is plausible in the case of sake. The increase of methional during storage (**Figure 1B**) also supports the latter pathway. The formation mechanism of DMTS in sake is under investigation.

Among esters, ethyl esters such as ethyl 2-methylbutyrate, ethyl 3-methylbutyrate, ethyl phenylacetate, and diethyl succinate increased during storage (**Figure 3A**,**B**). Increases in these ethyl esters were also reported in beer (*19*). However, these compounds do not contribute to the aged sake aroma, at least individually, because their OAVs are below 1 even in 35 year old sake.

The OAV of isoamyl acetate decreased from 6 in the fresh sake to 0.2 in the oldest one. The OAV of ethyl caproate also decreased, from 30 to 3 (data not shown). The decreases in these esters greatly affected the change in the aroma of sake during aging.

Although the contributions of aldehydes and polysulfides to the aroma of aged sake were demonstrated, other compounds may have contributed too. We tried to reconstruct the aroma of aged sake by adding these aldehydes, esters, polysulfides, and sotolon to a nonaged sake. The similarity of the reconstructed sample to real aged sake was about 50% (data not shown). On the other hand, several odorants were not identified through AEDA. A roasted odor at RI = 1036 and 1154, a honeylike odor at RI = 1721, and a sulfury odor (3-methyl-2-butene-1-thiol) at RI = 1087 may be related to the aroma of aged sake since these attributes are constituents of the aroma of aged sake. Further study will be needed to elucidate the overall aroma of aged sake.

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