JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Quantification and Odor Contribution of 2-Furanmethanethiol in Different Types of Fermented Soybean Paste Miso

MOTOKO OHATA,[†,‡] TAKATOSHI TOMINAGA,[§] DENIS DUBOURDIEU,[§] KIKUE KUBOTA,[‡] AND ETSUKO SUGAWARA*[,†]

Faculty of Education, Iwate University, 3-18-33 Ueda Morioka, Iwate, Japan, Graduate School of Humanities and Laboratory of Food Chemistry, Ochanomizu University, 2-1-1 Otsuka Bunkyo-ku Tokyo, Japan, and Faculté d'Oenologie, Université de Victor Segalen Bordeaux 2, 351 Cours de la Libération, 33405 Talence, France

2-Furanmethanethiol, a compound contained in many kinds of food, was identified for the first time in five types of miso fermented soybean paste (red salty rice miso, thin-colored salty rice miso, weak salty rice miso, barley miso, and soy miso) by specific extraction of volatile thiols using *p*-hydroxymercuribenzoate. In the triangle test with red salty rice miso, which included a higher concentration of 2-furanmethanethiol, and thin-colored salty rice miso, which included a lower concentration of 2-furanmethanethiol, it was shown that the aroma of thin-colored salty rice miso was similar to that of red salty rice miso by adding 2-furanmethanethiol into thin-colored salty rice miso. In addition, a quantitative descriptive analysis (QDA) clearly shows that 2-furanmethanethiol contributed to the intensity of three odor qualities "thick, complex", "sweet", and "pleasant aroma like coffee beans" in six odor qualities and was a very important component of miso aroma.

KEYWORDS: Aroma; 2-furanmethanethiol; miso

INTRODUCTION

Miso is one of the traditional Japanese seasonings, and many Japanese drink miso soup every day. Miso is generally classified into five types: There are three kinds of rice miso (red salty rice miso, thin-colored salty rice miso, and weak salty rice miso), barley miso, and soy miso. Rice miso is made by mixing rice koji; koji is made from steamed rice with a starter culture of the fungus koji, salt, steamed soybeans, and yeast and then aged. Red salty rice miso and thin-colored salty rice miso are generally aged for about 12 and 6 months, respectively, but weak salty rice miso is aged for about 20 days. In barley miso and soy miso, the main material is soybeans, and barley koji or soybean koji, respectively, is mixed with the soybeans. The aging period of barley miso is about 12 months, and soy miso is aged for over 3 years (*1, 2*). About 80% of the manufactured miso is rice miso, and the greatest consumption in Japan is red salty rice miso (*1, 2*).

The taste of miso is governed by peptides, amino acids, and glucose, which are resolved from protein and starch by enzymes of the fungus koji and yeast. Melanoidine, which effects the brown color of miso, is generated during miso aging by the amino-carbonyl reaction, and the precursors in this reaction are peptides, amino acids, and glucose resolved from protein and starch. In addition, the aroma of miso is generated by the amino-carbonyl reaction.

The aroma of miso is one of the most important factors for its quality and for inspiring consumers to buy it. Studies (*3*−*5*) have reported that the aroma components in miso are formed by microbes, especially yeast, during the long period of aging and fermentation and that these components are the most important (*6, 7*). One such component is 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone, with a sweet caramel-like aroma (*6*−*9*). This aroma component is the key compound in red salty rice miso, thin-colored salty rice miso, and barley miso but has not been identified in weak salty rice miso and soy miso (*10*). We therefore consider that there is likely to be another important compound in the unidentified odorant formed by the amino-carbonyl reaction and/or yeast and that this compound would play an important common role in the aromas of all types of miso.

Thiol compounds have been identified in many kinds of food, and certain thiols have been important as the characteristic aroma components in coffee, Japanese green tea, yellow passion fruits, and Iberian ham especially (*11*−*14*). We anticipated that 2-furanmethanethiol in particular would be involved in the aroma of miso, because the aroma compounds of miso are formed by the amino-carbonyl reaction and fermentation of yeast. 2-Furanmethanethiol is a particularly odorous compound

* To whom correspondence should be addressed. Tel and Fax: +81-19-621-6609. E-mail: etsukos@iwate-u.ac.jp.
[†] Iwate University.
[‡] Ochanomizu University.
[§] Université de Victor Segalen Bordeaux 2.

10.1021/jf803258c CCC: $40.75
Published on Web 02/20/2009

involved in the aroma of roast coffee (*15*) and has already been reported to be generated by a heating process (the amino-carbonyl reaction) (*11, 16*−*18*). Additionally, Tominaga et al. have identified 2-furanmethanethiol as an odor component in certain samples of white wine and champagne and reported that 2-furanmethanethiol was generated by yeast during the fermentation of wine (*19, 20*).

The purposes of the present study were to confirm whether 2-furanmethanethiol was commonly present in the five types of miso and to clarify the contribution of 2-furanmethanethiol to the aroma of miso by a sensory evaluation. In addition, we investigated the contribution of 2-furanmethanethiol to the odor characteristics of miso by QDA by using reconstructed models of the miso aroma mixture.

MATERIALS AND METHODS

Materials. The three types of rice miso, barley miso, and soy miso were used in this study. Thin-colored salty rice miso (white miso), weak salty rice miso (sweet miso), barley miso, and soy miso were purchased at a local market in Tokyo. Red salty rice miso (red miso) was provided by the Experimental Station of Miyagi Miso-Shoyu Industry Cooperative (Miyagi, Japan).

Chemicals. Benzaldehyde, cysteamine, dichloromethane, 2-furanmethanethiol, *p*-hydroxymercuribenzoate (*p*-HMB), and 3-methylthiopropanal were obtained from Aldrich (Tokyo, Japan). Ethanol and diethyl ether were obtained from Kanto Chemical Co. (Tokyo, Japan). 1-Hexanol, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone, 3-hydroxy-2-methyl-4*H*-pyran-4-one, 2-methoxyphenol, 3-methylbutanoic acid, and 2-phenylethanol were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). 4-Methoxy-2-methyl-2-mercaptobutane was obtained from Oxford Chemicals (New Jersey).

Determination of the Value for *Y* (%) in Miso. A Minolta CR-200 Chroma-meter was used to determine the value for *Y* (%) of the five kinds of miso. The value for *Y* (%) shows the brown color of miso generated by the amino-carbonyl reaction during the aging process and indicates the degree of aging (*1*).

Extraction of 2-Furanmethanethiol in Miso. 2-Furanmethanethiol was extracted from 20 g of miso ground into a paste. The miso paste was diluted with a 15% (v/v) aqueous ethanol solution, and a 20% (w/w) miso suspension containing 335 ng/kg (final concentration) of 4-methoxy-2-methyl-2-mercaptobutane as the internal standard was prepared. This miso suspension was stirred by a magnetic stirrer for 30 min, kept at 4 °C for 24 h, and then centrifuged at 7000 rpm for 10 min at 4 °C.

The resulting supernatant was applied for the specific extraction of 2-furanmethanethiol (or volatile thiols), using *p*-HMB (*21*); 7.5 mL of 0.2 M *p*-HMB was added to the supernatant. The mixture was percolated into a strongly basic anion-exchange column (Dowex 1, Sigma; code 1 × 2−100), and the column was washed with 50 mL of Milli Q water. The volatile thiols were released from the thiol-*p*-HMB complex in the column by using a cysteamine solution (500 mg/50 mL) adjusted to pH 7 with 10 N NaOH. The eluate containing the volatile thiols released from the column was successively extracted with 4 and 2.5 mL of dichloromethane for 5 min each under magnetic stirring. The organic phases were combined, dried over anhydrous sodium sulfate, filtered, and concentrated.

Calibration of 2-Furanmethanethiol. Calibration standards were prepared by adding an increasing quantity (0−500 ng/kg of the 20% red miso suspension) of the 2-furanmethanethiol reference solution to the miso suspension. 2-Furanmethanethiol was extracted by using the method just described.

The standard curve for the 2-furanmethanethiol assay of miso was prepared by using a DB-XLB column [J&W, 60 m × 0.25 mm (i.d.), 0.25 μm film thickness] (**Figure 1**). The regression equation was linear as follows: 2-furanmethanethiol (ng/kg of the 20% miso suspension) = (2-furanmethanethiol height/IS height)/0.0033, where 2-furanmethanethiol height is the height of *m*/*z* 114 at the 2-furanmethanethiol peak and IS height is the height of *m*/*z* 134 at the IS peak. Two analyses were carried out on each of the 2-furanmethanethiol reference solutions.

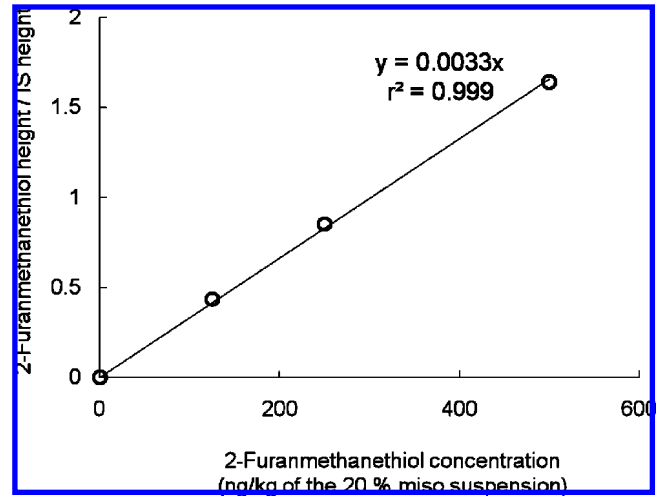

Figure 1. Standard curve for the 2-furanmethanethiol assay of the miso suspension. 2-Furanmethanethiol height indicated the height of *m*/*z* 114 at the peak for 2-furanmethanethiol, and IS height indicated the height of *m*/*z* 134 at the peak for the internal standard.

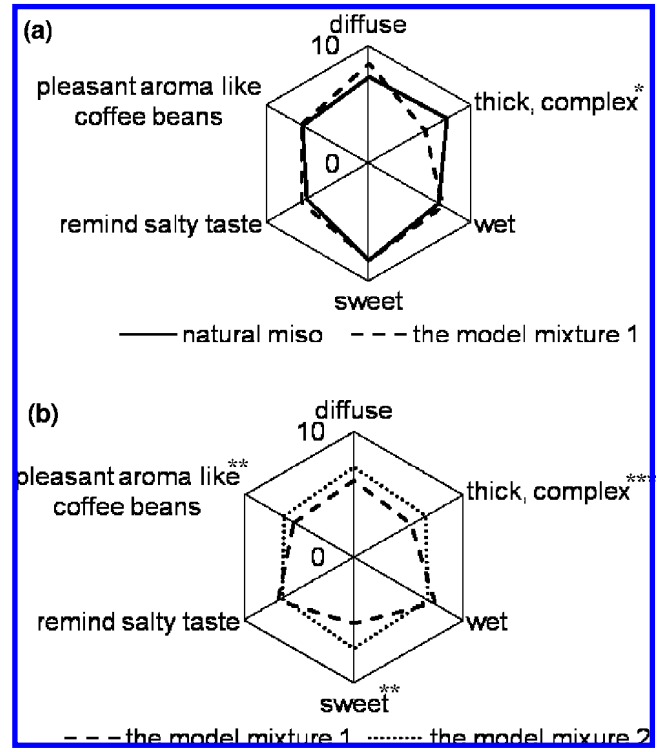

Figure 2. Odor profiles of the natural miso aroma and the reconstructed model aroma solution (**a**) and odor profiles of the reconstructed model aroma solution and that with added 2-furanmethanethiol (**b**). Model mixture 1 was a reconstructed model miso aroma solution composed of nine odor-active compounds, and model mixture 2 was a reconstructed model miso aroma solution with added 2-furanmethanethiol. *$p < 0.1$, **$p < 0.05$, and ***$p < 0.01$.

Gas Chromatography−**Mass Spectrometry (GC-MS) Conditions for the 2-Furanmethanethiol Assay.** 2-Furanmethanethiol was identified by a GC-MS analysis performed with a Shimadzu QP-2010 mass spectrometer combined with a Shimadzu GC-2010 gas chromatograph, using a fused silica capillary column (DB-XLB column). The oven temperature was held at 40 °C for the first 10 min, programmed to 220

Table 1. Color of Miso and Concentration and Odor Activity Value of 2-Furanmehtanethiol in Five Types of Miso

	red miso[b]	white miso[c]	sweet miso[d]	barley miso	soy miso
Y[a] (%)	9.1	22.9	31.7	9.9	5.1
2-furanmethanethiol (ng/kg miso paste)	215.1	25.0	45.1	89.7	409.1
OAV of 2-furanmethanethiol	538	62	113	224	1023

[a] Chromaticity value. [b] Red salty rice miso. [c] Thin-colored rice miso. [d] Weak salty rice miso.

at 3 °C/min, and then held at 220 °C for 1 min. The flow rate of carrier gas (He) was 1.5 mL/min, and the injection and detection temperatures were 210 and 230 °C, respectively. The mass spectrometer was operated in the electron impact (EI) mode with an ionization voltage of 70 eV, and the ion source temperature was set at 200 °C. The injection into GC-MS was carried out in splitless mode (splitless time, 60 min). 2-Furanmethanethiol and the internal standard (4-methoxy-2-methyl-2-mercaptobutane) were detected in the selected ion monitoring (SIM) mode, respectively selecting the ions of $m/z = 53$, 81, and 114 and $m/z = 53$, 75, and 134, and quantified with $m/z = 114$ and $m/z = 134$.

Triangle Test for Miso Aroma. Samples of red miso and white miso purchased at a local market were prepared. Each sample was ground, and 1.0 g of each miso paste was put into a brown vial. Two test sessions were performed. In the first session, normal red miso and normal white miso were used as samples, and in the second session, normal red miso and white miso with 200 ng/kg (final concentration) of 2-furanmethanethiol were used as samples. Three coded samples as one set in one session were presented at 25 °C to each panelist in a sensory booth. Two of these samples were identical, and one was different. Thirteen panelists (seven males and six females, aged 21−24 years) smelled each sample from left to right and selected the odd sample. A statistical analysis was performed by the binomial test (*22*).

Aroma Extract Dilution Analysis (AEDA). One hundred grams of red miso paste was diluted with Milli Q water, and 500 mL of a 20% (w/v) miso suspension was prepared. The supernatant by centrifugation (7000 rpm for 10 min at 4 °C) of this miso suspension was used to prepare an aroma concentrate by the Tenax TA resin adsorption method (*23*). The miso suspension was poured into a glass column filled with Tenax TA resin, and the aroma components were eliminated by pouring in 125 mL of ether. The eluate was concentrated at 40 °C under 1 atm. This procedure was conducted five times, and an aroma concentrate from a total 500 g of miso was obtained. The collected aroma concentrate was analyzed by gas chromatography/olfactometry (GC/O) and GC-MS; the experimental and analytical conditions were described in detail in a previous report (*23*). GC/O involves the effluent being split into equal parts at the end of the column, each part being respectively conveyed to a flame ionization detector (FID) and sniffing port. Serial dilutions of the miso aroma concentrate with diethyl ether were assessed by GC/O, and the flavor dilution (FD) factors of the odor-active compounds in miso were determined by AEDA (*24*). Concentrations of each compound were calculated based on the total yield of aroma concentration and percent of each peak area on the GC analysis. The total yield of aroma concentration was 367 mg (0.0734%).

QDA. *Preparation of Samples for QDA.* The paste of red miso was prepared. The model miso aroma (model mixture 1) was constructed with the nine compounds (**Table 3**) selected by AEDA, and the model aroma including 2-furanmethanethiol (model mixture 2) was also constructed with the 10 compounds listed in **Table 3**; that is to say, each compound was mixed at a determined concentration as described above in odorless water. The paste of miso (0.5 g) was placed in a brown vial (50 mL in vol), and 2 mL of model mixture 1 and model mixture 2 was poured into other brown vials, respectively. Each sample was coded by a random three-digit number.

Sensory Evaluation. Nine female panelists aged from 22 to 24 years were used for the evaluation. Before the evaluation sessions, the panelists selected a set of six qualities that aptly described the odor of miso (red miso) from the odor description proposed by Shimoda et al. (*25*). The panelists participated in evaluation sessions to share common perceptions about odor qualities many times.

The evaluation was performed by QDA in sensory booths at 25 °C. In the evaluation sessions, the panelists indicated the intensity of each of the six odor attributes by marking on a 10 cm visual line scale shown on a computer screen (maximum score of 10 and minimum of 0). First, miso and model mixture 1 were evaluated by the panelists, and it was confirmed that the odor profile of the model aroma was similar to that of the natural miso aroma. Then, model mixture 1 and model mixture 2 were evaluated. A statistical analysis was performed by Tukey's multiple-comparison test (*22*).

RESULTS AND DISCUSSION

Identification and Quantification of 2-Furanmethanethiol in Miso. 2-Furanmethanethiol was selectively extracted from miso by using *p*-HMB and analyzed by GC-MS in the SIM mode. The three selected ions shown by 2-furanmethanethiol ($m/z = 53$, 81, and 114) corresponded completely with the retention times shown by the 2-furanmethanethiol standard. The method developed by Tominaga et al. (*21*) to identify volatile thiols from many wines was applied to identify 2-furanmethanethiol from miso. This is the first time, to our knowledge, that this compound has been reported to be found in miso. Identifying thiol compounds is generally very difficult because of their trace amounts in food. In addition, 2-furanmethanethiol cannot be captured by the porous polymer resin adsorption method (*23*), which is generally used for preparing a miso aroma concentrate. We succeeded in this study in isolating and identifying 2-furanmethanethiol from five types of miso (red miso, white miso, sweet miso, barley miso, and soy miso) by using *p*-HMB.

The concentration and OAV of 2-furanmethanethiol in each of the five types of miso and the color of miso (the value of *Y*) are shown in **Table 1**. The 2-furanmethanethiol concentration in each type of miso was calculated by multiplying by the dilution rate of the 20% miso suspension and was about 20−400 ng/kg (of miso paste). We could not determine the perception threshold of 2-furanmethanethiol in miso. The threshold of 2-furanmethanethiol was reported in some papers; for example, Guadangni et al. determined at 5.0 ppt in water (*26*) and Guth et al. determined at 0.12 ppb in water (*27*). In miso, 2−3% of ethanol is generated by alcoholic fermentation during aging (*1*); therefore, the OAV of 2-furanmethanethiol in miso was calculated by using the threshold value, 0.4 ng/L in water/ethanol reported by Tominaga et al. (*28*). The OAV of 2-furanmethanethiol in miso was estimated to be in the range of 60−1000, and this value is very high. Especially, the 2-furanmethanethiol contents in red miso and a soy miso were completely higher than the perception threshold reported by some papers (*26, 27*). The results obtained in this study suggest that 2-furanmethanethiol is the aroma component common to all five types of miso and the important contributor to the aroma of miso. Because these results were found by using the threshold value in wine, it was necessary to investigate the contribution of 2-furanmethanethiol in miso by a sensory evaluation.

The 2-furanmethanethiol content of soy miso, which was the darkest and aged for the longest time, was the highest. When the color of miso was light, like white miso and sweet miso, the 2-furanmethanethiol content in these two types of miso was small (a concentration of 20−50 ng/kg of miso paste). The value for *Y* (%) in the chromaticity diagram is a barometer for the aging of miso (*1*). When the value for *Y* is low, the color of miso is dark, generally showing that the amino-carbonyl reaction has proceeded. 2-Furanmethanethiol has been identified in such

Table 2. Number Correctly Identifying Each Session of the Triangle Test[a]

	session 1[b]	session 2[c]
no. of panelists	13	13
no. correct	10[d]	5

[a] The p value was 0.01. [b] Prepared samples were normal red miso and normal white miso. [c] Prepared samples were normal red miso and white miso with added 2-furanmethanethiol. [d] This difference was significant with a probability of 99% ($p < 0.01$).

Table 3. Concentrations of the Odor-Active Compounds in Miso and Each Model Mixture for the Sensory Evaluation

	concentration		
compounds	miso (mg/kg)	model mixture 1 (mg/L)	model mixture 2 (mg/L)
4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone	78.86	78.0	78.0
3-hydroxy-2-methyl-4*H*-pyran-4-one	65.60	66.0	66.0
2-phenylethanol	64.99	65.0	65.0
3-methylbutanoic acid	2.356	2.4	2.4
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	2.274	2.2	2.2
3-methylthiopropanal	1.018	1.0	1.0
benzaldehyde	0.650	0.6	0.6
1-hexanol	0.546	0.5	0.5
2-methoxyphenol	0.358	0.4	0.4
2-furanmethanethiol	215.1 ng/kg[a]	[b]	200 ng/L

[a] Quantification by using *p*-HMB. [b] Not included.

foods as roasted meat, wheat bread, and popcorn (*29*) but has not been detected in raw coffee beans and raw meat. These results implied that 2-furanmethanethiol in miso was generated by the amino-carbonyl reaction during miso aging.

The precursors of 2-furanmethanethiol in miso by the amino-carbonyl reaction were presumed to be 2-furfural contained in steamed soybeans (result not published) and hydrogen sulfide generated from cysteine (and/or glutathione) by the Strecker degradation, but a detailed reaction pathway has not been investigated. Blanchard et al. assumed a formation mechanism of 2-futranmethanethiol in wine by yeast (*19*). We presumed that yeast participated in 2-furanmethanethiol formation in miso, too. It is necessary to clarify the formation mechanism of 2-furanmethanethiol in miso.

Distinction by the Triangle Test between Aroma of Red Salty Rice Miso and Aroma of Thin-Colored Salty Rice Miso with Added 2-Furanmethanethiol. To clarify the contribution of 2-furanmethanethiol to the aroma of miso, a triangle test was performed on samples of red miso, in which the 2-furanmethanethiol concentration was high, and white miso, in which 2-furanmethanethiol concentration was low. We examined whether the panelists could distinguish between two identical types of miso and odd types. The results are shown in **Table 2**.

In the first evaluation session, the triangle test was performed by using normal red miso and normal white miso. Thirteen panelists participated in this test, and 10 correct answers were identified as the odd sample difference. Therefore, the different samples could be significantly distinguished with a probability of 99% ($p < 0.01$). In the second evaluation session, the triangle test was performed by using normal red miso and white miso with added 2-furanmethanethiol, and only five correct answers were identified. These results showed that the aroma of white miso with added 2-furanmethanethiol was similar to that of red miso. In addition, the panelists evaluated that the aroma of white miso with added 2-furanmethanethiol was stronger than that of normal white miso.

Contribution of 2-Furanmethanethiol to the Miso Aroma. We investigated the contribution of 2-furanmethanethiol to the odor qualities of miso in detail by using QDA. To prepare the reconstructed mixtures used in the sensory evaluation, the most important aroma compounds, except for 2-furanmethanethiol, in the flavor of miso were first selected by the method of AEDA. 4-Hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone, 3-hydroxy-2-methyl-4*H*-pyran-4-one, 2-phenylethanol, 3-methylbutanoic acid, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 3-methylthiopropanal, benzaldehyde, 1-hexanol, and 2-methoxyphenol were selected as the aroma compounds with a high flavor dilution factor (FD factor of 50−5000). It has already been reported that these nine compounds were very important for the aroma of miso (*5*−*8*). The concentration of each of these compounds in red miso is shown in **Table 3**. The model mixtures were constructed on the basis of the concentrations of these odor-active compounds. We investigated the effect of adding 2-furanmethanethiol to the model mixture including these compounds by using QDA method.

Appropriate sensory attributes are needed for using the QDA method. In this study, a total of six sensory attributes for the aroma of miso had already been selected. These attributes were “diffuse”, “thick, complex”, “wet”, “sweet”, “remained salty taste”, and “pleasant aroma like coffee beans”. The odor profiles obtained in session 1, the natural miso aroma and model mixture 1 (without 2-furanmethanethiol), and session 2, the model mixture 1 and the model mixture 2 (including 2-furanmethanethiol), were drawn on a radar chart by using the six odor attributes, and their average scores were obtained for each session. As shown **Figure 2a**, the intensity of “thick, complex” in model mixture 1 was lower ($p < 0.1$) than the natural miso aroma. “Thick, complex” in the natural miso aroma had a high score (score, 7.61) to the second in odor attributes, and it was indicated that “thick, complex” was very important for the aroma of miso. Therefore, it was supposed that the aroma components that contributed to odor attribute “thick, complex” were absent in model mixture 1. While as shown **Figure 2b** for model mixture 2 (including 2-furanmethanethiol), the intensities of “thick, complex” ($p < 0.01$), “sweet” ($p < 0.05$), and “pleasant aroma like coffee beans” ($p < 0.05$) were significantly high, the intensities were heightened in “diffuse”. These results indicate that three attributes, “sweet”, “pleasant aroma like coffee beans”, and “thick, complex”, in six attributes were enhanced by adding 2-furanmethanethiol into the model mixture 1, and 2-furanmethanethiol contributed especially to the “thick, complex” of the potent odorant characteristics for miso aroma. It is suggested in this study that the aroma intensity of miso is enhanced by the presence of 2-furanmethanethiol and that 2-furanmethanethiol is a very important aroma component for miso.

ABBREVIATIONS USED

AEDA, aroma extract dilution analysis; OAV, odor activity value; FID, flame ionization detector; GC/O, gas chromatography−olfactometry; *p*-HMB, *p*-hydroxymercuribenzoate; SIM, selected ion monitoring; QDA, quantitative descriptive analysis.

LITERATURE CITED

(1) Yoshii, H. Miso. In *Encyclopedia of Brewing*, 2nd ed.; Noshiro, K., Yoshizawa, K., Kamata, K., Mizunuma, T., Tadenuma, M., Eds.; Asakura Shoten: Tokyo, 1991; pp 441−469.

(2) Kobayashi, A.; Sugawara, E. Flavor components between syoyu and miso Japanese fermented soybean seasonings. In *Flavor Chemistry of Ethnic Food*, 1st ed.; Shahidi, F., Ho, C.-T., Eds.;

Kluwer Academic Plenum Publisher: New York, 1999; pp 5−14.
(3) Tahara, Y. Analysis of soy sauce. *J. Pharm. Soc. Jpn.* **1887**, *61*, 80–89.
(4) Honma, N. Aroma and the aroma components of miso (1). *Nippon Jyozo Kyokaishi* **1987**, *82*, 480–487.
(5) Honma, N. Aroma and the aroma components of miso (2). *Nippon Jyozo Kyokaishi* **1987**, *82*, 548–553.
(6) Yokotsuka, T.; Sasaki, M.; Nunomura, N.; Asano, Y. The flavor of soy sauce (1). *Nippon Jyozo Kyokaishi* **1980**, *75*, 516–522.
(7) Sugawara, E. Identification of 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone as flavor component in miso. *Nippon Shokuhin Kogyo Gakkaishi* **1991**, *38*, 491–493.
(8) Nunomura, N.; Sasaki, M.; Asano, Y.; Yokotsuka, T. Isolation and identification of 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone, as a flavor component in *shoyu* (soy sauce). *Agric. Biol. Chem.* **1976**, *40*, 491–495.
(9) Sasaki, M.; Mori, S. The flavor of soy sauce. *Nippon Jyozo Kyokaishi* **1991**, *86*, 913–922.
(10) Sugawara, E.; Yonekura, Y. Comparison of aroma components in five types of miso. *Nippon Shokuhin Kogyo Gakkaishi* **1998**, *45*, 323–329.
(11) Tressl, R.; Silwer, R. Investigation of sulfur-containing components in roasted coffee. *J. Agric. Food Chem.* **1981**, *29*, 1078–1082.
(12) Kumazawa, K.; Kubota, K.; Masuda, H. Influence of manufacturing conditions and crop season on the formation of 4-mercapto-4-methyl-2-pentanone in Japanese green tea (sen-cha). *J. Agric. Food Chem.* **2005**, *53*, 5390–5396.
(13) Engel, K.; Tressl, P. Identification of new sulfur-containing volatiles in yellow passion fruits (*Passiflora edulis* f. *flavicarpa*). *J. Agric. Food Chem.* **1991**, *39*, 2249–2252.
(14) Carrapiso, A. I.; Ventanas, J.; Garcia, C. Characterization of the most odor-active compounds of Iberian ham headspace. *J. Agric. Food Chem.* **2002**, *50*, 1996–2000.
(15) Schieberle, P.; Hofmann, T. Influence of processing parameters on the aroma contribution of intense odorants in cysteine/carbohydrate reaction mixtures. *Lebensmittelchemie* **1998**, *50*, 105–108.
(16) Withycombe, D. A.; Mussinan, C. J. Identification of 2-methyl-3-furanthiol in the steam distillate from canned tuna fish. *J. Food Sci.* **1988**, *53*, 658–660.
(17) Farmer, L. J.; Patterson, R. L. Compounds contributing to meat flavour. *J. Sci. Food Agric.* **1990**, *40*, 201–205.
(18) Gasser, U.; Grosch, W. Primary odorants of chicken broth. *Z. Lebensm. Unters. Forsch.* **1990**, *190*, 3–8.
(19) Blanchard, L.; Tominaga, T.; Dobourdieu, D. Formation of furfurylthiol exhibiting a strong coffee aroma during oak barrel fermentation from furfural released by toasted staves. *J. Agric. Food Chem.* **2001**, *49*, 4833–4835.
(20) Tominaga, T.; Guimbertau, G.; Dubourdieu, D. Role of certain volatile thiols in the bouquet of aged champagne wines. *J. Agric. Food Chem.* **2003**, *51*, 1016–1020.
(21) Tominaga, T.; Dubourdieu, D. A novel method for quantification of 2-methyl-3-furanthiol and 2-furanmethanethiol in wine made from vitis vinifera grape varieties. *J. Agric. Food Chem.* **2006**, *54*, 29–33.
(22) Meilgaard, M.; Civille, G. V.; Carr, B. T. Basic statistical methods. In *Sensory Evaluation Techniques*, 4th ed.; Meilgaard, M., Civille, G. V., Carr, B. T., Eds.; CRC Press: Boca Raton, FL, 2006; pp 313−355.
(23) Sugawara, E.; Hashimoto, S.; Sakurai, Y.; Kobayashi, A. Formation by yeast of the HEMF (4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone) aroma component in miso with aging. *Biosci., Biotechnol., Biochem.* **1994**, *58*, 1134–1135.
(24) Grosch, W. Detection of potent odorants in foods by aroma extract dilution analysis. *Trends Food Sci. Technol.* **1993**, *4*, 68–73.
(25) Shimoda, M.; Tsukamoto, Y.; Ito, H.; Doi, Y.; Kameda, W.; Osajima, Y. Preparation and evaluation of odor reference solution for principal odor attributes of food. *Nippon Shokuhin Kogyo Gakkaishi* **1991**, *38*, 371–376.
(26) Guadagni, D. G.; Buttery, R. G.; Harris, J. Odor intensities of hop oil constituents. *J. Sci. Food Agric.* **1966**, *17*, 142–144.
(27) Guth, H.; Grosch, W. Identification of the character impact odorants of stewed beef juice by instrumental analysis and sensory studies. *J. Agric. Food Chem.* **1994**, *42*, 2862–2866.
(28) Tominaga, T.; Blanchard, L.; Darriet, P.; Dubourdieu, D. A powerful aromatic volatile thiol, 2-furanmethanethiol, exhibiting roast coffee aroma in wine made from several vitis vinifera grape varieties. *J. Agric. Food Chem.* **2000**, *48*, 1799–1802.
(29) Kerscher, R.; Grosch, W. Quantification of 2-methyl-3-furanthiol, 2-furfurylthiol, 3-mercapto-2-pentanone, and 2-mercapto-3-pentanone in heated meat. *J. Agric. Food Chem.* **1998**, *46*, 1954–1958.

Received for review October 20, 2008. Revised manuscript received January 21, 2009. Accepted January 24, 2009.

JF803258C