

# Identification and Characterization of Volatile Components Causing the Characteristic Flavor in Miso (Japanese Fermented Soybean Paste) and Heat-Processed Miso Products

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**ABSTRACT:** The aroma concentrates of two types of raw miso (traditional Japanese fermented soybean paste) were prepared by combining solid phase extraction (SPE) and solvent-assisted flavor evaporation (SAFE) techniques. The aroma extract dilution analysis (AEDA) applied to the volatile fraction revealed 39 odor-active peaks with FD factors between 4<sup>1</sup> and 4<sup>8</sup>. Among the perceived odorants, 32 odorants were identified or tentatively identified from the 39 odor-active peaks, and the newly identified odorants for the miso were half of them. Furthermore, by comparison of the FD factors between the raw miso and heat-processed miso, it was found that one increasing odorant (methional) and three decreasing odorants (1-octen-3-one, (Z)-1,5-octadien-3-one, and *trans*-4,5-epoxy-(*E*)-2-decenal) contributed to the flavor change during the heat processing. This finding suggested that the flavor change in the raw miso during heat processing is attributed to relatively few odorant changes. In addition, it was assumed that the amino acids included in the miso have a significant influence on the remarkable disappearance of the three decreasing odorants.

**KEYWORDS:** miso, Japanese fermented soybean paste, flavor, heat processing, AEDA

## INTRODUCTION

Miso (Japanese fermented soybean paste), which is indispensable for use in Japanese foods, is one of the fermented foods representing the food culture in Japan, and large amounts of miso are consumed in the form of miso soup in Japan. In addition, miso is interesting for not only its excellent nutritional value but also several functionality effects to help maintain one's health.<sup>1</sup> Miso is manufactured by a fermentation process under a high concentration of salt and with many different microbial activities such as shoyu (Japanese soy sauce); that is, miso is a fermented product that is made from cooked (boiled) soybeans and other cereals (miso koji) in the presence of a high salt content. Miso koji is made from a single cereal or bean, which is cooked (steamed) rice, barley, or soybean itself, by inoculation with a koji starter of *Aspergillus oryze*. The miso koji is added to the cooked soybean and salt and then generally fermented for a period of 3–12 months. During the fermenting process, yeast and lactic acid bacteria grow and hydrolyze the soybean protein and carbohydrate; as a result, the miso product becomes a paste, which remains solid. On the basis of the type of koji, which makes use of cereal, the miso products were classified into several types, and approximately 80% of the product in Japan is rice miso, which make use of rice koji. In addition, the manufacturing method of traditional miso does not include a sterilization process, and the product of this manufacturing method is said to be the so-called raw miso. Generally, raw miso has a rich flavor, but it is well-known to be delicate because the miso flavor is extremely unstable when heating.

For such a miso product, the flavor is one of the important quality factors determining the value of the miso product, and it is a significant problem for quality improvement of the products to have a high quality flavor with good acceptability. Therefore, there are many previous papers clarifying the miso flavor

components. The flavor components of miso have been already reported to have over 200 volatiles,<sup>2</sup> and it was believed that miso flavor was formed from the complex mixture of a large number of compounds. In particular, 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone (HEMF), which forms with the yeast, is a characteristic odorant of miso,<sup>3,4</sup> and the balance of HEMF, methionol, and 4-ethylguaiacol was considered to significantly explain the various types of miso flavor characters.<sup>5</sup> However, there are extremely few studies that have investigated the miso flavor in detail by gas chromatography–olfactometry (GC-O), such as an aroma extract dilution analysis (AEDA),<sup>6,7</sup> and the actual significance of the odorant in the miso flavor is therefore still mostly unknown.

On the other hand, in general, recent commercial miso products, made industrially, are packed and sealed in a container or film. Therefore, ethanol is often added and/or sterilized with heating to prevent expansion of the products by carbon dioxide, which the yeast produces. The sterilization by heating of the miso product was generally done under relatively mild heating conditions,<sup>8</sup> but there is a concern about its influence on the delicate miso flavor. However, the influence of heating during the manufacturing process on the miso flavor change is very unclear.

The objective of the present investigation was to elucidate the potent odorants of the raw miso (traditional products of fermented soybean paste) by AEDA. Furthermore, to clarify the flavor change in the industrially made miso products during heat processing, a comparison of the raw miso pastes and the heat-processed one was performed.

**Received:** September 13, 2013

**Revised:** November 19, 2013

**Accepted:** November 23, 2013

**Published:** November 23, 2013

## MATERIALS AND METHODS

**Materials.** Two types of raw rice misos were used. Thin colored salty rice miso (white miso) and red salty rice miso (red miso) products were purchased from Maruman Co., Ltd. (Nagano, Japan). The raw miso was packed in an aluminum laminated pouch and then sealed under vacuum. The packed miso was heat processed at 80 °C for 10 min in a water bath followed by immediate cooling in ice water.

**Chemicals.** The following compounds were synthesized according to literature procedures: (Z)-1,5-octadien-3-one;<sup>9</sup> (Z)-1,5-octadien-3-ol;<sup>9</sup> 3-methyl-2,4-nonanedione;<sup>10</sup> and *cis*- and *trans*-4,5-epoxy-(E)-2-decenals.<sup>11</sup> Compounds (Table 2) 1–3, 5, 7, 8, 10–15, 18, 20, 22, 25, 26, 29, 32, 33, 38, and 1-octen-3-ol were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan); compounds 21, 27, 28, 31, 37, and 39 were obtained from Sigma-Aldrich Japan (Tokyo, Japan); and compound 19 was obtained from Nihon Firmenich (Tokyo, Japan).

**Isolation of the Volatiles from Miso.** The internal standard solution (10  $\mu$ L) prepared from 2% 2-octanol in ethanol was added to the miso paste (20 g), and then the miso paste was suspended in 100 mL of distilled water at room temperature. After homogenization of the miso paste suspension, the residual substances were removed by centrifugation at 3000 rpm for 20 min at 5 °C and then filtered through filter paper. The filtrate (approximately 100 mL) was passed through a column packed with 5 g of a porous polymer adsorbed resin (SP700; Mitsubishi Chemical Co., Ltd., Tokyo, Japan).<sup>12</sup> The adsorbed compounds were eluted with 20 mL of methylene chloride and then dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. To remove the nonvolatile material, the eluate was distilled under reduced pressure (40 °C at  $5 \times 10^{-3}$  Pa) using solvent-assisted flavor evaporation (SAFE).<sup>13</sup> The distillate was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and the solvent was then removed by rotary evaporation (35 °C at 550 mmHg) to approximately 5 mL. Further concentration was conducted in a nitrogen stream to approximately 100  $\mu$ L. The resulting concentrate was used as the sample for the AEDA and gas chromatography–mass spectrometry (GC-MS) analyses.

**Enrichment of Odorants for Identification.** For the identification experiments, the miso volatiles were isolated from the suspended red salty rice miso (red miso) paste solution by combining the SPE and SAFE techniques as described above. These procedures were repeated, and all of the volatile fractions were combined (a total of 500 g of miso paste) and concentrated to approximately 1.5 mL. Part of the concentrated volatile fraction (500  $\mu$ L) was applied onto a glass column (15 °C, 20  $\times$  0.7 cm i.d.) filled with 5 g of silica gel (Wakogel C-200) in *n*-pentane. Elution was performed using the following solvents: *n*-pentane (30 mL, fraction I), *n*-pentane/diethyl ether (30 mL, 10 + 1, v/v, fraction II), *n*-pentane/diethyl ether (30 mL, 10 + 2, v/v, fraction III), *n*-pentane/diethyl ether (30 mL, 1 + 1, v/v, fraction IV), and diethyl ether (30 mL, fraction V). The solution was concentrated to approximately 100  $\mu$ L as already described.

**Model Experiments To Determine the Thermal Stability of 1-Octen-3-one and 1-Octen-3-ol.** At the concentration of 1 ppm each, 1-octen-3-one and 1-octen-3-ol were dissolved in a citric/Na<sub>2</sub>HPO<sub>4</sub> buffer solution of pH 5.2 (mixed 1/10 M citric acid and 1/5 M Na<sub>2</sub>HPO<sub>4</sub>). In addition, to part of this model aqueous solution (200 mL) was dissolved 1% of amino acids (2.50 g of L-lysine monohydrochloride and/or 2.54 g of sodium hydrogen L-(+)-glutamate monohydrate) or glucose. These model solutions were added to glass bottles without a headspace (ca. 60 mL), which were sealed with silicone-coated septa, and then treated by heat processing (80 °C for 10 min) in a water bath, followed by immediate cooling to about 10 °C in ice water. The analytes in the heat-treated and untreated solutions (50 mL) were isolated by extraction with methylene chloride (25 mL  $\times$  2). After drying over anhydrous sodium sulfate, the solvent was removed by rotary evaporation (35 °C at 550 mmHg) to approximately 5 mL. Further concentration was conducted in a nitrogen stream to approximately 100  $\mu$ L. The internal standard solution (5  $\mu$ L) prepared from 2% 2-octanol in methylene chloride was added to the concentrate before the solvent was removed by the evaporator. Quantification of the 1-octen-3-one and 1-octen-3-ol was

performed by GC-MS, and the residual ratios (%) were calculated from the measured values (the ratio of the peak area of each odorant to the peak area of internal standard) of the analytes in the thermally treated and nontreated solutions.

**Gas Chromatography–Olfactometry.** An Agilent 6850 series gas chromatograph equipped with a thermal conductivity detector (TCD) (Agilent Technologies, Santa Clara, CA, USA) and fused silica column (30 m  $\times$  0.25 mm i.d., coated with a 0.25  $\mu$ m film of DB-Wax; Agilent Technologies) was used in the splitless injection mode (splitless time = 1 min). The column temperature was programmed from 40 to 210 °C at the rate of 5 °C/min for all runs. The injector and detector temperatures were both 250 °C. Helium was used as the carrier gas at the flow rate of 1 mL/min. A glass sniffing port was connected to the outlet of the TCD and heated by a ribbon heater along with moist air being pumped into the sniffing port at about 100 mL/min to quickly remove the odorant from the sniffing port that had been eluted from the TCD.

**Aroma Extract Dilution Analysis.** The original odor concentrate of the miso paste was stepwise diluted with methylene chloride to 4<sup>n</sup> ( $n = 0$ –9), and aliquots (1  $\mu$ L) of each fraction were analyzed by capillary GC using a DB-Wax column. The odorants were then detected by GC eluate sniffing (GC-O). The flavor dilution (FD) factors of the odorants were determined by AEDA.<sup>14</sup> Before the FD factor measurement, two panelists repeatedly confirmed the retention time and the odor quality of the odorants using each diluted sample (4<sup>0</sup> and 4<sup>1</sup>), and then the FD factors of the odorants were defined as the dilution step that both panelists detected.

**Gas Chromatography–Mass Spectrometry.** An Agilent 7890 N gas chromatograph coupled to an Agilent 5975C inert XL series mass spectrometer (Agilent Technologies) was used. The column was a 60 m  $\times$  0.25 mm i.d. DB-Wax fused silica capillary type or 30 m  $\times$  0.25 mm i.d. DB-1 fused silica capillary type (Agilent Technologies) with a film thickness of 0.25  $\mu$ m. The column temperature was programmed from 80 to 230 °C or from 40 to 230 °C at the rate of 3 °C/min. The injector temperature was 250 °C, and the flow rate of the helium carrier gas was 1 mL/min. An injection volume of 1 or 0.2  $\mu$ L was applied using the split (the split ratio was 1:30) or splitless technique. The mass spectrometer was used with the ionization voltage of 70 eV (EI) and ion source temperature of 150 °C. The quantities of the selected odorants in each volatile fraction of the miso pastes were determined from the selected ion peak areas obtained by mass chromatography. To determine the odorants, the GC-MS was operated in the selected ion and scan (SIM-SCAN) mode, and the selected ions were monitored in the ranges listed in Table 1. The

**Table 1. Selected Ion and Conversion Factors for Mass Chromatography (SIM)**

compound	selected ion ( $m/z$ )	conversion factor <sup>a</sup>
1-octen-3-one	70	3.0
(Z)-1,5-octadien-3-one	55	2.0
methional	76	12.5
<i>trans</i> -4,5-epoxy-(E)-2-decenal	68	2.3

<sup>a</sup>The conversion factor was determined on the basis of the ratio of the selected ion against the overall ion of the mass spectra for each authentic sample (total ion peak area = selected ion peak area  $\times$  conversion factor).

semiquantitation amounts of odorants in the miso paste were calculated from the ratio of the total ion peak areas of the internal standard and that of the analytes. On the basis of the ratio of the selected ion versus the overall ions of the mass spectra for each authentic sample, the total ion peak area of each compound was converted from the selected ion peak area. The response factor of each odorant versus the internal standard was defined as 1.

**Identification of the Components.** Each component was identified by comparing its Kovats GC retention index (RI), mass spectrum, and odor quality to those of the authentic compounds.

Table 2. Potent Odorants Showing FD Factors (FD &gt; 1) in the Raw Miso (White and Red)

no.	RI <sup>a</sup>	fraction <sup>b</sup>	compound <sup>c</sup>	odor quality <sup>g</sup>	log <sub>4</sub> (FD factor)	
					white	red
1	975	II	ethyl isobutyrate <sup>e</sup>	fruity	2	2
2	1040	II	ethyl butyrate	fruity	2	1
3	1050	II	ethyl 2-methylbutyrate <sup>e</sup>	fruity	2	1
4	1194			yogurt-like	1	1
5	1301	II	1-octen-3-one <sup>e</sup>	mushroom-like	3	4
6	1375	II	(Z)-1,5-octadien-3-one <sup>e</sup>	metallic	3	4
7	1439	V	acetic acid	sour	1	1
8	1453	III	methional	potato-like	2	3
9	1463			sulfurous, putrid	1	1
10	1537	II	(E)-2-nonenal	green, sweet	1	1
11	1593	II	(E,Z)-2,6-nonadienal <sup>e,d</sup>	green, cucumber-like	1	1
12	1598	IV	2,5-dimethyl-4-methoxy-3(2H)-furanone <sup>e,d</sup>	sweet, caramel-like	2	2
13	1646	III	phenylacetaldehyde	sweet, honey-like	2	2
14	1674	IV	3-methylbutanoic acid	sweaty, rancid	3	3
15	1703	III	(E,E)-2,4-nonadienal	fatty	2	1
16	1722	III	3-methyl-2,4-nonanedione <sup>d,e</sup>	green, hay-like	1	<1
17	1781			putrid	1	1
18	1815	III	(E,E)-2,4-decadienal <sup>d</sup>	fatty	1	2
19	1825	II	β-damascenone <sup>e</sup>	sweet, honey-like	1	<1
20	1864	III	2-methoxyphenol	burnt	4	2
21	1894	IV	3-ethyl-1,2-cyclopentanedione <sup>e</sup>	sweet, sugar-like	1	1
22	1968	V	maltol	sweet	5	5
23	1989	III	cis-4,5-epoxy-(E)-2-decenal <sup>e</sup>	sweet, juicy, metallic	1	1
24	2004	III	trans-4,5-epoxy-(E)-2-decenal <sup>e</sup>	sweet, juicy, metallic	3	4
25	2026	III	4-ethyl-2-methoxyphenol <sup>d</sup>	spicy	2	1
26	2030	IV	4-hydroxy-2,5-dimethyl-3(2H)-furanone	sweet	5	6
27	2036	IV	4-nonanolide	sweet	2	2
28	2065	IV	4-hydrox-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone	sweet, sugar-like	8	7
29	2171	III	eugenol <sup>e</sup>	spicy	2	1
30	2185			spicy	1	1
31	2198	III	2-methoxy-4-vinylphenol	spicy	2	<1
32	2204	IV	3-hydroxy-4,5-dimethyl-2(5H)-furanone <sup>e,f</sup>	caramel-like	6	6
33	2227	III	o-aminoacetophenone <sup>d,e</sup>	grape-like	1	<1
34	2256			phenolic	1	1
35	2265			burnt, spicy	1	1
36	2534			burnt, powdery	<1	1
37	2544	V	phenylacetic acid <sup>e</sup>	sweet, honey-like	3	3
38	2555	IV	vanillin	sweet, vanilla-like	1	1
39	2613	V	3-phenylpropionic acid <sup>e</sup>	leather-like	1	1

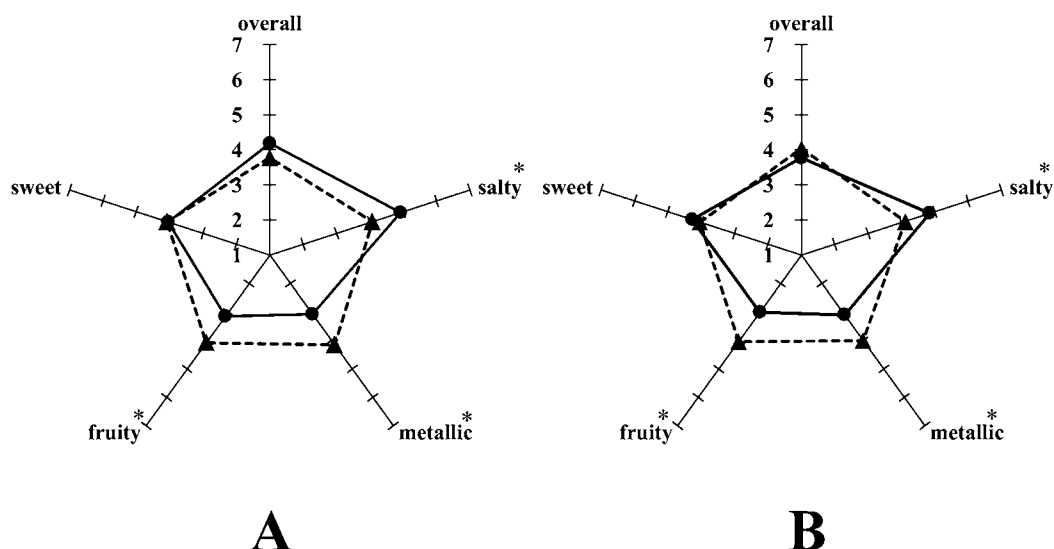
<sup>a</sup>Retention index on DB-Wax column (30 m × 0.25 mm i.d.; coated with a 0.25 μm film) observed for GC-O. <sup>b</sup>Fraction in which most of the compound appeared after column chromatography on silica gel of the aroma concentrate (I–V). <sup>c</sup>The compound was identified by comparison with the reference substance on the basis of the following criteria: retention index (RI) on stationary phases given in DB-Wax, mass spectra, and odor quality. <sup>d</sup>The MS signals were too weak for unequivocal interpretation. The compound was tentatively identified by comparison with the reference substance on the basis of the following criteria: retention index (RI) on stationary phases given in DB-Wax and odor quality. <sup>e</sup>Newly identified compounds in miso. <sup>f</sup>The compound was identified by comparison with the reference substance on the basis of the following criteria: retention index (RI) on stationary phases given in DB-1 (1068), mass spectra, and odor quality. <sup>g</sup>Odor quality assigned during AEDA.

**Sensory Evaluation.** Two types of rice miso (white and red) and those heat processed (80 °C for 10 min) were used. The miso paste (30 g) was suspended in distilled water (300 mL) at room temperature. Each miso (raw and heated miso) suspension (20 mL) was placed in a glass beaker at about 20 °C. The samples were immediately consumed by 13 panelists between the ages of 25 and 44. Each of these panelists had previously received extensive training in the descriptive sensory analysis of a miso suspension (paste) and had experience in the sensory profiling of various food samples. The intensity of each attribute of the tested miso (raw and heat processed) suspensions was scored on the scale of 1 (weak), 2, 3, ..., 7 (strong). The miso flavor of the tested samples was compared to the control (the corresponding raw miso suspension) flavor, and the attribute scales (fruity note, metallic note, sweet note, salty note as characteristic

for soy sauce, and total flavor intensity) of the control (raw miso suspension) flavor were defined as 4. The results obtained from all of the panelists were then averaged. The significant differences between the raw and heated miso samples were calculated by using Student's *t* test.

## RESULTS AND DISCUSSION

**Potent Odorants in Miso Flavor.** Two types of commercial raw misos, which contained no added ethanol, were used. These were the thin colored salty rice miso (white miso) and red salty rice misos (red miso) products made in the Nagano prefecture of Japan. The volatile concentrates of the raw miso pastes were prepared by combining SFE and SAFE



**Figure 1.** Flavor change in raw miso products during heat processing (80 °C, 10 min): nonheated miso suspensions (▲) and the corresponding heated miso suspensions (●) (A, white rice miso; B, red rice miso). For the control (raw miso suspension), all of the category scales were defined as 4. \*,  $P < 0.05$ .

**Table 3.** Potent Odorants in the Raw and Heated Miso (White and Red) That Showed FD Factor Changes

no.	RI <sup>a</sup>	compound	odor quality	log <sub>4</sub> (FD factor)			
				white		red	
				nonheated	heated	nonheated	heated
5	1301	1-octen-3-one	mushroom-like	3	<1	4	<1
6	1375	(Z)-1,5-octadien-3-one	metallic	3	<1	4	1
8	1453	methional	potato-like	2	3	3	4
24	2004	trans-4,5-epoxy-(E)-2-decenal	sweet, juicy, metallic	3	<1	4	2

<sup>a</sup>Retention index on DB-Wax column (30 m × 0.25 mm id.; coated with a 0.25 μm film).

techniques from a 20% suspension of miso. The volatile concentrate obtained with this sampling operation without excessive heating well-reproduced the characteristic aroma of the raw miso, and it was regarded as the most preferable analytical sample for the screening of the potent odorants using GC-O.

The AEDA technique was applied to the volatile concentrate, which had been prepared from the two types of raw miso (white and red), and revealed 39 odor-active peaks with FD factors between 4<sup>1</sup> and 4<sup>8</sup>; thus, it was confirmed that the flavor of the raw miso consisted of many potent odorants (Table 2). After enrichment of the volatile fraction, which was prepared from 500 g of the red miso by column chromatography, 32 of these odorants, which included 16 newly identified odorants from the miso, could be identified by GC-MS and GC-O analyses. HEMF (28) was given the highest FD factor in both of the tested miso samples, and these results substantiate previous knowledge<sup>3</sup> that 28 was an important odorant for the miso flavor. Because the odor thresholds of these newly identified compounds were extremely low, the major portion of the potent odorants in the miso can be assumed to be too low in concentration and thus difficult to clarify.

These results demonstrated that a small amount of odorant, which has an extremely low odor threshold, plays a significant role in miso flavor in addition to the major conventionally known volatiles such as 28.

**Flavor Change in Miso Products during Heat Processing.** To confirm the flavor change in the raw miso during heat processing, the 10% suspensions of the raw and

heated misos were compared by sensory evaluation (Figure 1). By heat processing of the raw miso, the soy sauce-like salty note increased, and the metallic and fruity notes markedly decreased. Because these flavor changes were observed for both tested miso samples, it can be expected that the raw misos undergo a universal flavor change during heat processing.

To clarify the odorants, which contributed to these flavor changes, the raw and heated miso aromas were analyzed by the comparative AEDA and GC-MS technique, and the odorants that underwent FD factor changes were identified (Table 3). However, there were extremely few odorants that had FD factor changes, and this finding indicated the possibility that the miso flavor change by heating was attributed to relatively few odorants. Among these odorants, the potent odorant, which had an increased FD factor by heating in both tested miso samples, was only methional (8). However, because the odor quality of methional gave a green and potato-like note, accordingly, it is difficult to explain the salty note in the heated miso aroma by only this single odorant. On the other hand, 1-octen-3-one (5), (Z)-1,5-octadien-3-one (6), and trans-4,5-epoxy-(E)-2-decenal (24) markedly decreased by the heat processing. Therefore, these odorants can be assumed to be responsible for the reduction in the metallic and fruity notes of the heat-processed raw miso. However, there is a limit in the comparison of individual odorants with odor attributes due to the synergistic and antagonistic effects between the odorants and/or other constituents in the miso. Therefore, it would be necessary to investigate in detail any flavor recombination, addition, and/or omission tests.



In addition, to elucidate the detailed compositional change during heat processing, the residual ratios of the three odorants in the red raw miso were measured. Consequently, the residual ratios of these odorants were remarkably low (the concentration of **6** was less than the detection limit), and these odorants hardly remained during this heating condition (Table 4). On the other hand, the vinyl alcohols (1-octen-3-ol and

**Table 4. Semiquantitation Amount and Residual Ratio<sup>a</sup> of Decreased Odorants in the Red Miso during Heat Processing**

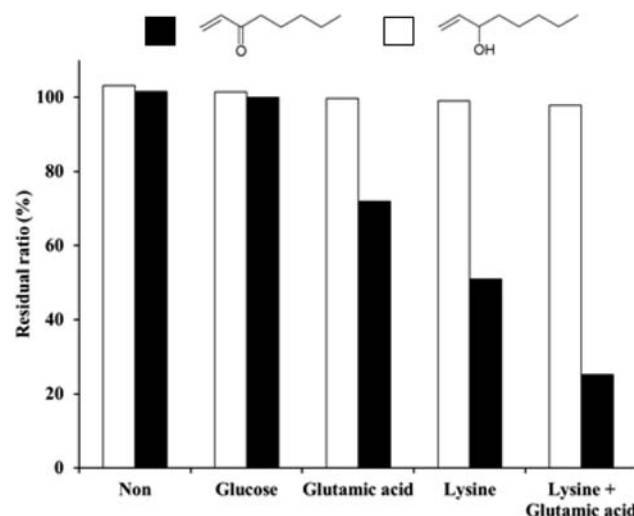
compound	semiquantitation amount (mg/kg)		residual ratio (%)
	nonheated	heated	
1-octen-3-one	1.7	0.008	0.5
methional	2.2	8.2	377
<i>trans</i> -4,5-epoxy-( <i>E</i> )-2-decenal	0.075	0.0005	0.6

<sup>a</sup>These amounts and residual ratio are triplicate results.

(*Z*)-1,5-octadien-3-ol), which are analogous and corresponded to these vinyl ketones (**5** and **6**), were included in the tested miso samples. However, these vinyl alcohols did not decrease at all under this heating condition. Accordingly, it can be considered that the difference in reactivity of the ketone and alcohol structure has an effect on the thermal stability of the odorants in the raw miso. The decreased odorants with heating have the characteristic structure of an  $\alpha,\beta$ -unsaturated ketone. Therefore, it can be presumed that the low thermal stability of the decreased odorants can allow an easy reaction with the nucleophilic reagents such as a nucleophilic attack on the carbonyl carbon or Michael addition to the vinyl moiety.

There are various nucleophilic reagents in miso. Because it was expected to have more of an effect on the reaction during heat processing, the major components were focused on. The main constituents of the miso, except for water and salts, are the protein and carbohydrate, and the major portion of them was broken down into small molecule compounds, such as amino acids and glucose, by fermentation.<sup>15</sup> In addition, the free amino acids in the miso have a specific characteristic that they include a large amount of the basic and acidic amino acids such as lysine, arginine, glutamic acid, and aspartic acid.<sup>15</sup> Therefore, the effect of the major components in the miso on the stability of the odorants, which decreased during the heat processing, was examined using **5** and 1-octen-3-ol with the model experiments (Figure 2). As a consequence, the stability of 1-octen-3-ol was slightly affected under this reaction condition. On the other hand, **5** evidently decreased in the presence of the amino acids, whereas the decrease of **5** was not observed under the present conditions of water or glucose. In addition, the type of amino acid was one of the important factors for the stability of **5**, and then the residual ratio of **5** for the model reaction including lysine (basic amino acid) was lower than that of the glutamic acid (acidic amino acid). These findings demonstrate that **5** was unstable under the heat-processing conditions in the presence of amino acids, and the stability of **5** has a close connection with the number of amino moieties of the amino acid. Therefore, it is considered that miso, which contains a high concentration of free amino acids, especially the basic amino acids, can easily lose **5** during heat processing.

On the basis of the results of the model experiments, it was expected that the low thermal stability of **5** in the miso was



**Figure 2.** Residual ratios of 1-octen-3-one and 1-octen-3-ol after model solutions were heated with dissolved amino acids (lysine and/or glutamic acid) or glucose. The residual ratio is the mean value of triplicate results.

affected by the molecular structure as the vinyl ketone, which seems to easily undergo the nucleophilic attack reaction or Michael addition. Furthermore, it was assumed that the characteristic of the miso that included a large amount of amino acids, in particular, the more reactive basic amino acids, causes the extremely poor stability of **5** under mild heating conditions such as 10 min at 80 °C. Because this decreasing mechanism of **5** was expected to also occur with **6** and **24**, which showed low residual ratios, it can be therefore considered that this mechanism is one of the most important factors for the flavor change during heat processing of raw miso.

On the basis of these results, to better clarify the characteristic miso flavor, it is also necessary to take into account not only the major odorant but also trace odorant amounts such as the newly identified ones. In addition, to understand the flavor change during the processing and cooking, it is necessary to take into consideration the reactivity of the major components in the miso. However, these results were obtained with only two types of miso products. Therefore, for further improvement of the flavor of the industrial miso product, it will be important to understand the potent odorants of various product types and the detailed deterioration mechanism. Therefore, a more detailed study of the various kinds of misos, such as the different raw materials, the manufacturing conditions, and/or the maturational degree, is needed.

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### Notes

The authors declare no competing financial interest.

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