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**RESEARCH ARTICLE**

# Changes in Volatile Aroma Compounds of Traditional Chinese-type Soy Sauce During Moromi Fermentation and Heat Treatment

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**Abstract** Considering the important influence of long-time (150 day) moromi fermentation and heat treatment on the aroma formation of traditional Chinese-type soy sauce (TCSS), volatile compounds in samples taken from different stages of moromi fermentation and heat treatment were analyzed by solid phase microextraction coupled with gas chromatography-mass spectrometry. Results showed that a total of 76 volatile compounds were identified in all the samples, and most of the volatile compounds were common. During 150 day of moromi fermentation, relative contents of acids, alcohols, aldehydes and ketones, esters, and furan(one)s along with all the sensory attributes of acidic, alcoholic, fruity, caramel-like, smoky, and malty changed greatly. Notably, relative contents of alcohols, aldehydes and ketones along with the sensory intensities of alcoholic, caramel-like, and smoky of heated sample (80ºC/60 min) decreased markedly, whereas there were slight increases in relative contents of furan(one)s, phenols, and sulfur-containing compounds of it. Long-time moromi fermentation and heat treatment have significant influence on the formation and relative contents of volatile compounds in TCSS, whereas changes in volatile compounds and their relative contents of the samples were responsible for the differences in sensory attributes.

**Keywords:** volatile compound, traditional Chinese-type soy sauce, gas chromatography-mass spectrometry, moromi fermentation, heat treatment

## Introduction

Soy sauce, which originated in China over 2,500 years ago, is one of the indispensable fermentation condiments in China, Korea, Japan, and Southeast Asia countries. The annual production of soy sauce in China is more than 5,000,000 tons, accounting for over 55% of the world production (1,2). However, Japanese-type soy sauce is more internationally competitive for its very characteristic aroma, a key quality index for soy sauce, when compared with traditional Chinese-type soy sauce (TCSS) (2,3). Hence, it's necessary for TCSS to improve its aroma quality. In general, the formation of aroma compounds in TCSS depends on raw materials, strains of microbiology, *koji* culturing, moromi fermentation, and pasteurization (1,3,4). Although formation mechanism of aroma compounds in TCSS is complex, *koji* culturing, moromi fermentation, and heat treatment are generally regarded as critical stages affecting the final aroma profile of TCSS (1,3-5).

There was long list of studies on volatile compounds in soy sauce and other fermented products (1-3,5-14), nearly 300 volatile compounds have been isolated and identified in soy sauce. However, to the best of our knowledge, there were no studies on changes in volatile compounds of TCSS during moromi fermentation and heat treatment so far. Whereas a detailed study on changes in volatile compounds and sensory attributes of TCSS during moromi fermentation and heat treatment is necessary, because results from this study would give some information on optimization of quality control and development of simultaneous (on-line) measurement for volatile compounds during the practical production of TCSS.

Therefore, one objective of the present study was to investigate differences in sensory attributes of the samples taken from different stages of moromi fermentation and heat treatment. The other objective was to try to explain

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reasons responsible for the sensory attribute differences by comparing their volatile compounds profiles and relative contents.

## Materials and Methods

**Raw materials and chemicals** Soybean, wheat starch, and edible salt were purchased from Guanghui Agricultural Products Co., Ltd. (Linkou, China), Runfon Flour Co., Ltd. (Guangzhou, China), and Zhongshan Salt Industrial Co., Ltd. (Zhongshan, China), respectively. *Aspergillus oryzae* HN3.042 was used in TCSS processing. Ethyl 2-methylpropanoate, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 3-methylbutanal, and phenol, 4-ethyl-2-methoxy- were purchased from Weibo Chemicals Co., Ltd. (Guangzhou, China). Acetic acid, ethanol, and other chemicals were of the highest commercial grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

**Preparation of TCSS and samples** Samples were directly obtained from a famous soy sauce manufacturing company (Meiweixian Flavoring Foods Co., Ltd., Zhongshan, China). The main manufacturing processes were as follows: (1) soybean was washed thrice and soaked thoroughly with tap water; (2) soaked soybean was steamed with high temperature and pressure to obtain moderately denatured soybean; (3) *A. oryzae* HN3.042 together with wheat starch was inoculated into the steamed soybean to make *koji*; (4) the resulting *koji* was mixed with brine to yield moromi; (5) the ripened moromi was pressed to obtain TCSS, which was finally pasteurized and bottled. The moromi fermentation liquids of TCSS were taken on day 5 ($MF_5$), 15 ($MF_{15}$), 30 ($MF_{30}$), 60 ($MF_{60}$), 90 ($MF_{90}$), 120 ($MF_{120}$), and 150 ($MF_{150}$), then 2 fractions of $MF_{150}$ were heated for 30 ($MF\text{-}HT_{30}$) and 60 min ($MF\text{-}HT_{60}$) at 80ºC in sealed stainless containers, respectively. All samples were filtered through filter papers, then kept in polyethylene vials and stored in a refrigerator at −20ºC until ready for analysis.

**Proximate analysis** ***Total nitrogen and reducing sugar analysis***: Contents of total nitrogen and reducing sugar in samples were measured according to AOAC methods (15). ***Formaldehyde nitrogen and total titratable acid analysis***: Formaldehyde nitrogen and total titratable acid were measured by titration method (16). Twenty mL of diluted samples were mixed with 60 mL $H_2O$ and titrated to pH 9.6 with 0.05 M NaOH before 10 mL formalin solution (37%) was added. The consumed volume was recorded to determine total titratable acids of samples. The samples were finally titrated to pH 9.6 with 0.05 M NaOH.

**Table 1. Sensory attributes and their corresponding reference compounds**

| Sensory attribute | Reference compound[1)] | Physical reference |
|---|---|---|
| Sour | Acetic acid | Vinegar |
| Alcoholic | Ethanol | Alcohol |
| Fruity | Ethyl 2-methylpropanoate | Apple/strawberry |
| Caramel-like | 4-Hydroxy-2,5-dimethyl-3(2H)-furanone | Burnt sugar |
| Malty | 3-Methylbutanal | Mashing barely |
| Smoky | 4-Ethyl-2-methoxy-phenol | Burnt wet wood |

[1)] 7 suprathreshold aqueous solutions of reference compounds (25-mL in Teflon vials).

**Sensory evaluation** Descriptive analysis (DA) was performed to determine the differences in sensory aroma characteristics among samples (3). Analysis was carried out with a panel of 9 flavorists (25 to 40 ages, 7 males and 2 females) in a professional Flavor & Fragrance Company. The flavorists selected as panelists were subjected to a ranking test with a series of 7 suprathreshold aqueous solutions of reference compounds (Table 1, 25-mL in Teflon vials) and were asked to score the aroma intensities on a 7-point linear scale from 0 (none) to 3 (strongest). Sensory evaluation was performed in sensory panel room at 23±2ºC at 3 different sessions. Results of sensory evaluation, the aroma intensities of chemicals, were discussed and should be obtained agreements by all the panelists eventually.

The 6 aroma intensities of samples were evaluated using a 7-point linear scale from 0 (none) to 3 (strongest). The results were plotted in a spider web diagram.

**Volatile compounds collection by solid phase microextraction** A solid phase microextraction fiber coated with 75 μm carboxen/polydimethylsiloxane (Supelco, Bellefonte, PA, USA) was selected to collect high volatile compounds for its high sensitivity and good selectivity to polar and non-polar compounds (2). Before sampling, the fiber was preconditioned for 1 hr and 30 min at 275 and 250ºC, respectively, in the gas chromatography (GC) injector port to eliminate possible residues on the coated fiber. Prior to analysis, samples (100 mL) were added with 10 μL methanolic solution of 2-methyl-3-heptanone as an internal standard at final concentration of 33.3 ng/g. Ten mL of samples saturated with NaCl were sealed in a dedicated bottle and preheated at 45ºC, stirred by a magnetic stirring bar with a speed of 200 rpm. The adsorption time was 40 min, and the concentrates were desorbed at 230ºC in the injection port of gas chromatograph (Trace GC-2000; Thermo Finnigan, Austin, TX, USA) by holding in the splitless mode for 3 min. The solid phase microextraction fiber was cleaned by keeping it in the GC injection port for additional 5 min.

**GC and GC-mass spectrometry (MS) analysis** GC-MS analysis was carried out using Finnigan TRACE GC-2000 GS-MS (Thermo Finnigan), equipped with a DB-5MS column (30 m length×0.25 mm i.d.×0.25 μm film thickness, J&W Scientific, Folsom, CA, USA). Volatile compounds adsorbed on the fibers were transferred into the GC injector with a splitless mode with an injection purge-off time of 3 min and injection temperature of 230ºC. The initial temperature of GC oven was held for 2 min at 50ºC, then raised up to 80ºC (held for 5 min) with a speed of 5ºC/min, finally, increased to 230ºC (held for 10 min) at a speed of 7ºC/min. Ultra high purity helium was used as carrier gas at a constant speed of 1.0 mL/min. Mass spectrometer conditions were as follows: mass selective detection (MSD) capillary direct-interface temperature was 250ºC. Ionization energy was 70 eV. Mass range was 35-335 a.m.u. electron-multiplier voltage was 1,800 V.

**Compound identification and quantification** Each compound was tentatively identified by comparing its mass spectral data with those of a NIST library (including Wiley and Mainlib) and published literatures (1,5). Compounds were reported on the basis of their similarity (>750). Quantitative analysis was based on the ratio between peak area of a particular compound and that of internal standard (2-methyl-3-heptanone) in sample (1).

**Statistical analysis** All tests were conducted in triplicate. Data are reported as means±standard deviation (SD). Analysis of variance (ANOVA) and significant differences among means were tested by one-way ANOVA using SPSS software (version 15.0 for Windows, SPSS Inc., Chicago, IL, USA).

## Results and Discussion

**Proximate analysis** As seen from Table 2, concentrations of total nitrogen, formaldehyde nitrogen, and reducing sugar increased from initial moromi fermentation stage to 60 (1.52%), 60 (0.91%), and 30 day (4.91%), respectively, and then decreased thereafter. As for total titratable acid, its content increased significantly during the whole moromi fermentation with values from 0.94 ($MF_5$) to 1.63% ($MF_{150}$). Moreover, heat treatment had no significant ($p>0.05$) influence on all the proximate indices mentioned above (Table 2). Similar change trends of total nitrogen, formaldehyde nitrogen, reducing sugar, and total titratable acid were observed in traditional Chinese fish sauce and soy sauces prepared with extruded and traditional raw materials (17,18). The decreases of formaldehyde nitrogen and reducing sugar in $MF_{60}$-$MF_{150}$ might be due to the energy utilization of yeasts and lactic acid bacteria and Maillard reaction between them. Maillard reaction might be the main reason responsible for decrease of formaldehyde nitrogen and reducing sugar in MF-$HT_{30}$ and MF-$HT_{60}$ because of high temperature employed (4,18,19). As expected, concentrations of total nitrogen, formaldehyde nitrogen, reducing sugar, and total titratable acid increased significantly ($p<0.05$) from 5 to 15 day because of high activities of protease and amylase in moromis (4,17,18).

**Sensory analysis** The sensory aroma profiles of samples based on DA test by 9 panelists are shown in Fig. 1. Results showed that aroma intensities of alcoholic, fruity, caramel-like, and smoky increased continually to 150 day. Otherwise, aroma intensity of acidic increased quickly from 0.27 ($MF_5$) to 1.77 ($MF_{90}$) and then decreased slightly to 1.63 ($MF_{150}$). The results were in line with the change of total titratable acid (Table 2). Conversely, aroma intensity of malty decreased slightly from 2.77 ($MF_5$) to 1.87 ($MF_{150}$). ANOVA showed that there were significant ($p<0.05$) differences in attribute intensities of acidic, alcoholic, fruity, caramel-like, smoky, and malty between $MF_5$ and $MF_{150}$, whereas the difference in sensory attribute intensities between $MF_{120}$ and $MF_{150}$ were not significantly ($p>0.05$). This indicated that 150 day of moromi fermentation was appropriate for TCSS production. $MF_{150}$ and MF-$HT_{60}$ were significantly ($p<0.05$) different in their attributes of alcoholic, caramel-like, and smoky, whereas there were no significant ($p>0.05$) differences in the 6 sensory attributes between $MF_{150}$ and MF-$HT_{30}$. All these results suggested that moromi fermentation time and heat treatment of 80ºC/60 min had significant influence on

**Table 2. Changes in proximate indices of samples during moromi fermentation and heat treatment**

| Proximate indice[1] | $MF_5$ | $MF_{15}$ | $MF_{30}$ | $MF_{60}$ | $MF_{90}$ | $MF_{120}$ | $MF_{150}$ | MF-$HT_{30}$ | MF-$HT_{60}$ |
|---|---|---|---|---|---|---|---|---|---|
| TN (g/100 g) | 0.96±0.05[2] | 1.38±0.06 | 1.45±0.04 | 1.52±0.05 | 1.51±0.06 | 1.48±0.06 | 1.46±0.02 | 1.47±0.03 | 1.48±0.02 |
| RS (g/100 g) | 3.10±0.12 | 4.01±0.14 | 4.91±0.16 | 4.48±0.12 | 3.96±0.14 | 3.48±0.17 | 3.33±0.13 | 3.22±0.13 | 3.06±0.09 |
| FN (g/100 mL) | 0.49±0.02 | 0.81±0.03 | 0.87±0.03 | 0.91±0.03 | 0.90±0.03 | 0.89±0.02 | 0.88±0.03 | 0.86±0.02 | 0.84±0.03 |
| TTA (g/100 mL) | 0.94±0.02 | 1.17±0.03 | 1.34±0.03 | 1.52±0.05 | 1.65±0.04 | 1.62±0.03 | 1.63±0.04 | 1.64±0.04 | 1.66±0.05 |

[1] TN, FN, RS, and TTA represent contents of total nitrogen, formaldehyde nitrogen, reducing sugar, and total titratable acid, respectively.

[2] Values are mean±SD ($n=3$).

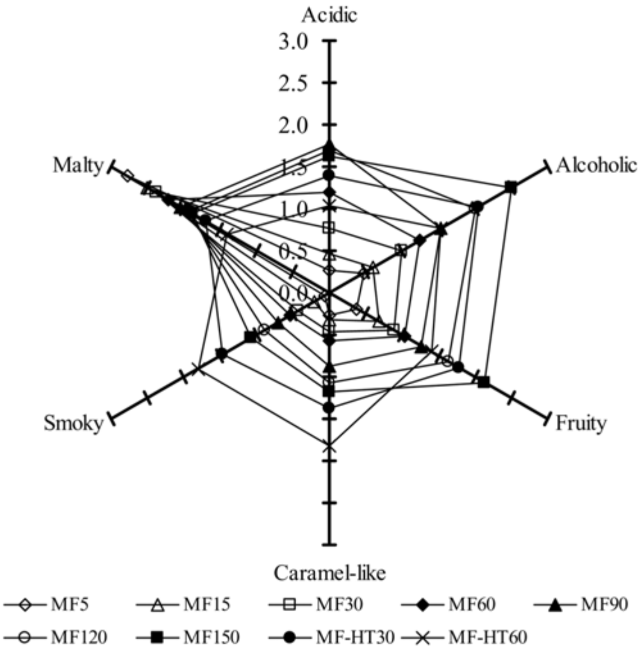

**Fig. 1. Changes in sensory attribute intensities of samples during moromi fermentation and heat treatment.**

sensory attributes of TCSS, but they were not significantly ($p$>0.05) affected by heat treatment of 80ºC/30 min. Similar changes in sensory attributes of alcoholic and fruity were observed in Japanese-type soy sauce (3), while changes in sensory attributes of acidic, caramel-like, and malty were different from the present study. The discrepancy in 2 studies could be explained by the difference of heat temperature used in this study (80ºC) and the previous study (145ºC). In the present study, heat treatment at 80ºC would have negative impacts on the aroma profile of TCSS. Therefore, adopting mild sterilization method was one of efficient measures for producing TCSS with good flavor. Results of sensory evaluation in the present study (Fig. 1) indicated that 80ºC/30 min might be a better method for TCSS sterilization, which was of importance for practical TCSS production. Notably, the different levels and varieties of volatile compounds might result in the differences in sensory attributes among samples.

**Changes in volatile compounds of samples during moromi fermentation and heat treatment** To clarify the reasons for different sensory attributes in samples, volatile compounds in different samples were identified and quantitated, and results were summarized in Table 3. Totally, 76 volatile compounds including 8 acids, 16 alcohols, 13 aldehydes, and ketones, 21 esters, 6 furan(one)s, 3 miscellaneous compounds, 5 phenols, 2 pyran(one)s, and 2 sulfur-containing compounds were identified in all samples. Alkanes and alkenes were not taken into consideration in the present study because of their neglectable aroma (20). Although most of volatile compounds identified in all samples were common, there were still part of volatile compounds (Table 3) belonging to certain samples alone. Moreover, the relative contents of volatile compounds (Table 3, Fig. 2) also changed with the proceeding of moromi fermentation and heat treatment.

As seen from Fig. 2, the relative contents of acids during moromi fermentation increased to 5.38 (MF$_{90}$), and then decreased slightly to 4.79 (MF$_{150}$). In contrast with MF$_{150}$, no significant ($p$>0.05) decreases of relative contents of acids in MF-HT$_{30}$ and MF-HT$_{60}$ was observed. Changes in relative content of acids during moromi fermentation and heat treatment were in accordance with those of total titratable acid and sensory attribute of acidic in DA test (Table 2, Fig. 1). Among the acids, acetic acid, a product of microbiology fermentation (1), was the major acid that existed in TCSS in the present study. Similar result was also observed in Japanese-type, Korean-type, and Thai-type soy sauces (1,2,5). Moreover, fatty acids are regarded as degradation products of soybean fat and contribute substantially to the flavor of soy sauce, because active lipase systems in most microorganisms present in moromi could degrade triglycerides to free fatty acids and glycerol, monoglycerides, diglycerides (5). However, only few fatty acids were identified in the preset study, the absence of some fatty acids but present in corresponding esters suggested that the most acids formed esters through esterification reaction during moromi fermentation (13).

The relative contents of alcohols in samples increased gradually during the whole moromi fermentation, while heat treatment resulted in significant decreases of the relative contents of alcohols in MF-HT$_{30}$ (11.38%) and MF-HT$_{60}$ (29.16%) (Fig. 2). Basically, changes in relative contents of alcohols in fermentation liquids were consistent with result of DA evaluation (Fig. 1). The results suggested that alcoholic fermentation happened in early moromi fermentation stage and continued to the end of that. Among the alcohols, relative contents of ethanol, 1-propanol, 2-methyl-, 1-butanol, 3-methyl-, 1-octen-3-ol, and phenylethyl alcohol were dominant and increased along with moromi fermentation (Table 3). Moreover, some alcohols, such as 1-hexanol, 4-hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, (R-), etc appeared at early stage and disappeared along with moromi fermentation (Table 3). It should be noted that ethanol (alcoholic), 1-butanol, 3-methyl- (medicinal/metallic), 1-octen-3-ol (mushroom), and phenylethyl alcohol (flowery/sweet) were reported as aroma-impact compounds in soy sauce (3,5). Hence, changes in the above aroma-impact compounds might be used as index for quality control during TCSS processing. In general, metabolism of microorganism, reduction of carbonyl, and degradation of unsaturated fatty acids are regarded as 3 pathways of alcohols formation during soy sauce fermentation

**Table 3. Changes in volatile compounds of traditional Chinese-type soy sauce (TCSS) during moromi fermentation and heat treatment$^{1)}$**

| RT$^{2)}$ (min) | Compound | $MF_5$ | $MF_{15}$ | $MF_{30}$ | $MF_{60}$ | $MF_{90}$ | $MF_{120}$ | $MF_{150}$ | $MF\text{-}HT_{30}$ | $MF\text{-}HT_{60}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Acids** | | | | | | | | | |
| 2.14 | Acetic acid$^{1,5)}$ | 0.07±0.03$^{3)}$ | 0.45±0.15 | 1.03±0.22 | 1.84±0.45 | 2.54±0.58 | 2.52±0.50 | 2.07±0.47 | 1.90±0.36 | 1.79±0.35 |
| 5.65 | Butanoic acid, 3-methyl-$^{1)}$ | 0.17±0.05 | 0.47±0.13 | 1.35±0.34 | 1.41±0.41 | 1.68±0.46 | 1.59±0.41 | 1.19±0.29 | 1.27±0.29 | 1.35±0.36 |
| 5.84 | Butanoic acid, 2-methyl-$^{5)}$ | ND | 0.10±0.03 | 0.37±0.08 | 0.53±0.13 | 0.64±0.24 | 0.56±0.15 | 0.57±0.13 | 0.60±0.14 | 0.65±0.18 |
| 13.16 | Hexanoic acid, 2-ethyl-$^{1)}$ | ND | 0.17±0.06 | 0.32±0.10 | 0.33±0.11 | 0.41±0.12 | 0.44±0.11 | 0.52±0.17 | 0.48±0.13 | 0.43±0.12 |
| 14.01 | Benzoic acid$^{1,5)}$ | ND | ND | ND | ND | 0.03±0.02 | 0.07±0.04 | 0.10±0.04 | 0.09±0.04 | 0.09±0.04 |
| 14.24 | Octanoic acid | ND | ND | ND | ND | 0.02±0.01 | 0.02±0.01 | 0.02±0.01 | 0.03±0.01 | 0.04±0.02 |
| 15.74 | Benzeneacetic acid$^{1)}$ | ND | ND | ND | 0.01±0.02 | 0.06±0.03 | 0.15±0.04 | 0.37±0.14 | 0.35±0.13 | 0.34±0.14 |
| 16.03 | Nonanoic acid | ND | ND | ND | 0.01±0.01 | 0.01±0.01 | 0.02±0.01 | 0.02±0.02 | 0.03±0.01 | 0.05±0.02 |
| | **Alcohols** | | | | | | | | | |
| 1.85 | Ethanol$^{1,5)}$ | 0.17±0.05 | 0.44±0.13 | 0.64±0.22 | 1.00±0.31 | 1.41±0.42 | 2.49±0.64 | 3.34±0.78 | 3.04±0.75 | 2.35±0.64 |
| 2.24 | 1-Propanol, 2-methyl-$^{1,5)}$ | ND | 0.15±0.06 | 0.43±0.13 | 0.74±0.21 | 1.24±0.39 | 1.77±0.58 | 1.82±0.68 | 1.64±0.52 | 1.21±0.40 |
| 3.35 | 1-Butanol, 3-methyl-$^{1,5)}$ | 0.19±0.07 | 0.43±0.16 | 0.71±0.21 | 1.19±0.37 | 1.45±0.41 | 2.03±0.55 | 2.22±0.51 | 1.99±0.47 | 1.48±0.24 |
| 4.36 | 2,3-Butanediol$^{1,5)}$ | ND | ND | ND | ND | 0.05±0.03 | 0.09±0.04 | 0.21±0.06 | 0.20±0.05 | 0.19±0.05 |
| 5.79 | 1-Hexanol | 0.17±0.06 | 0.03±0.03 | ND | ND | ND | ND | ND | ND | ND |
| 8.84 | 1-Octen-3-ol$^{5)}$ | 0.08±0.03 | 0.11±0.04 | 0.33±0.08 | 0.58±0.16 | 0.84±0.29 | 1.23±0.42 | 1.13±0.35 | 0.84±0.31 | 0.73±0.23 |
| 9.44 | 3-Octanol | 0.07±0.04 | 0.04±0.03 | 0.02±0.03 | ND | ND | ND | ND | ND | ND |
| 10.59 | Benzyl alcohol$^{1,5)}$ | 0.11±0.05 | 0.13±0.04 | 0.15±0.03 | 0.19±0.06 | 0.26±0.06 | 0.25±0.07 | 0.19±0.05 | 0.17±0.04 | 0.15±0.04 |
| 11.80 | 2-Octen-1-ol, (Z)- | 0.32±0.10 | 0.27±0.08 | 0.14±0.05 | 0.06±0.02 | 0.03±0.02 | 0.02±0.02 | 0.01±0.02 | 0.02±0.01 | ND |
| 11.900 | 1-Octenol | 0.31±0.08 | 0.26±0.09 | 0.22±0.06 | 0.18±0.06 | 0.17±0.05 | 0.16±0.04 | 0.13±0.05 | 0.11±0.04 | 0.09±0.03 |
| 13.00 | Phenylethyl alcohol$^{1,5)}$ | 0.15±0.04 | 0.27±0.08 | 0.39±0.09 | 0.40±0.11 | 0.45±0.10 | 0.68±0.21 | 1.21±0.36 | 1.17±0.36 | 1.13±0.34 |
| 14.18 | 4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, (R)- | 0.10±0.04 | 0.05±0.03 | 0.04±0.03 | ND | ND | ND | ND | ND | ND |
| 14.40 | Bicycleo(Z,Z)heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)- | 0.05±0.03 | 0.04±0.02 | 0.03±0.02 | 0.02±0.01 | ND | ND | ND | ND | ND |
| 20.95 | Cedrol | 0.02±0.02 | 0.02±0.02 | 0.04±0.02 | 0.02±0.01 | 0.03±0.02 | 0.02±0.02 | 0.01±0.01 | ND | ND |
| 21.03 | 1,4-Methanoazulen-9-ol, decahydro-1,5,5,8a-tertramethyl-, [1R-(1?-)] | 0.02±0.01 | 0.03±0.02 | 0.05±0.03 | 0.07±0.03 | 0.05±0.02 | 0.04±0.02 | ND | ND | ND |
| 21.11 | 8BetaH-cedran-8-ol | 0.40±0.09 | 0.44±0.09 | 0.54±0.15 | 0.56±0.16 | 0.61±0.19 | 0.53±0.15 | 0.36±0.10 | 0.24±0.08 | 0.19±0.09 |
| | **Aldehydes & ketones** | | | | | | | | | |
| 2.52 | Butanal, 3-methyl-$^{5)}$ | 0.15±0.03 | 0.31±0.09 | 0.33±0.11 | 0.47±0.10 | 0.52±0.11 | 0.60±0.13 | 0.65±0.16 | 0.63±0.16 | 0.54±0.18 |
| 3.15 | Butanal, 2-methyl-$^{1,5)}$ | 0.69±0.19 | 1.15±0.39 | 1.51±0.38 | 2.38±0.60 | 2.97±0.78 | 3.27±0.85 | 3.22±0.81 | 3.20±0.80 | 2.03±0.68 |
| 4.28 | 3-Penten-2-one, 4-methyl- | ND | ND | ND | ND | ND | ND | ND | ND | 0.47±0.15 |
| 8.30 | Benzaldehyde$^{1,5)}$ | 0.71±0.30 | 0.67±0.28 | 0.65±0.24 | 0.62±0.23 | 0.60±0.19 | 0.59±0.20 | 0.60±0.22 | 0.54±0.21 | 0.53±0.17 |
| 9.65 | Octanal$^{5)}$ | 0.03±0.02 | 0.05±0.02 | 0.06±0.02 | 0.06±0.03 | 0.06±0.02 | 0.07±0.03 | 0.08±0.03 | 0.07±0.02 | 0.06±0.02 |
| 11.11 | Benzeneacetaldehyde$^{1,5)}$ | 0.04±0.03 | 0.09±0.03 | 0.25±0.07 | 0.32±0.08 | 0.43±0.12 | 0.68±0.19 | 1.00±0.28 | 0.99±0.25 | 0.96±0.23 |
| 11.56 | 2-Octenal, (E)- | 1.97±0.58 | 1.44±0.47 | 1.40±0.50 | 0.82±0.30 | 0.34±0.10 | 0.14±0.05 | ND | ND | ND |
| 12.83 | Nonanal$^{5)}$ | 0.10±0.04 | 0.11±0.04 | 0.13±0.05 | 0.14±0.05 | 0.20±0.09 | 0.21±0.08 | 0.22±0.08 | 0.25±0.09 | 0.26±0.08 |
| 13.40 | 3,5-Dimethyl-4-heptanone | ND | ND | ND | ND | ND | ND | 0.03±0.02 | 0.02±0.02 | 0.01±0.01 |

**Table 3. Continued**

| RT2) (min) | Compound | MF$_5$ | MF$_{15}$ | MF$_{30}$ | MF$_{60}$ | MF$_{90}$ | MF$_{120}$ | MF$_{150}$ | MF-HT$_{30}$ | MF-HT$_{60}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 13.87 | Bicyclo(2,2,1)heptan-2-one, (1S) | 0.01±0.01 | 0.01±0.01 | 0.02±0.01 | 0.02±0.02 | 0.02±0.01 | 0.01±0.02 | ND | ND | ND |
| 15.04 | Decanal5) | 0.10±0.03 | 0.14±0.05 | 0.20±0.06 | 0.24±0.08 | 0.24±0.09 | 0.21±0.07 | 0.20±0.06 | 0.18±0.06 | 0.17±0.05 |
| 16.17 | Indane-4-carboxaldehyde | ND | ND | 0.05±0.03 | 0.07±0.03 | 0.20±0.08 | 0.21±0.06 | 0.20±0.07 | 0.19±0.08 | 0.17±0.07 |
| 20.57 | (+)-Longicamphenylone | ND | ND | ND | ND | 0.02±0.01 | 0.01±0.01 | ND | ND | ND |
|  | **Esters** |  |  |  |  |  |  |  |  |  |
| 2.38 | Ethyl acetate1,5) | 0.56±0.17 | 1.05±0.37 | 1.93±0.53 | 2.73±0.72 | 3.94±1.14 | 4.21±1.17 | 4.40±1.29 | 4.35±1.38 | 4.33±1.39 |
| 9.52 | Hexanoic acid, ethyl ester5) | ND | ND | ND | 0.01±0.02 | 0.05±0.03 | 0.19±0.08 | 0.31±0.08 | 0.31±0.11 | 0.30±0.10 |
| 12.62 | Benzoic acid, methyl ester | ND | ND | 0.03±0.02 | 0.05±0.03 | 0.07±0.03 | 0.12±0.05 | 0.20±0.06 | 0.25±0.08 | 0.22±0.07 |
| 14.33 | Benzoic acid, ethyl ester5) | 0.05±0.03 | 0.06±0.03 | 0.10±0.04 | 0.20±0.06 | 0.26±0.10 | 0.47±0.14 | 0.62±0.21 | 0.59±0.20 | 0.53±0.25 |
| 14.85 | Octanoic acid, ethyl ester5) | 0.01±0.02 | 0.02±0.02 | 0.03±0.02 | 0.13±0.04 | 0.15±0.06 | 0.17±0.06 | 0.19±0.08 | 0.19±0.07 | 0.16±0.05 |
| 15.19 | 2-Propenoic acid, 3-(2-hydroxyphenyl)-, (E)- | 0.03±0.02 | 0.04±0.02 | 0.05±0.02 | 0.05±0.03 | 0.06±0.04 | 0.08±0.03 | 0.09±0.04 | 0.07±0.02 | 0.06±0.02 |
| 15.70 | Benzeneacetic acid, ethyl ester5) | ND | ND | 0.03±0.02 | 0.06±0.03 | 0.16±0.05 | 0.29±0.10 | 0.45±0.15 | 0.45±0.13 | 0.44±0.15 |
| 15.92 | Acetic acid, 2-phenylethyl ester | 0.06±0.03 | 0.12±0.04 | 0.15±0.05 | 0.24±0.06 | 0.27±0.08 | 0.38±0.11 | 0.53±0.16 | 0.58±0.20 | 0.57±0.14 |
| 16.59 | Nonanoic acid, ethyl ester | ND | ND | ND | 0.10±0.03 | 0.12±0.04 | 0.13±0.04 | 0.16±0.05 | 0.18±0.07 | 0.19±0.06 |
| 17.47 | 2,2,4-Trimethyl-1,3-pentanediol, diisobutyrate | 0.05±0.03 | 0.06±0.03 | 0.12±0.04 | 0.15±0.05 | 0.19±0.07 | 0.28±0.08 | 0.34±0.11 | 0.36±0.12 | 0.38±0.13 |
| 17.82 | Propanoic acid, 2-methyl, 3-hydroxy-2,4,4-trimethyl pentyl ester | ND | ND | 0.01±0.02 | 0.02±0.02 | 0.04±0.02 | 0.22±0.07 | 0.24±0.08 | 0.23±0.06 | 0.21±0.06 |
| 18.15 | Decanoic acid, ethyl ester | 0.01±0.01 | 0.02±0.01 | 0.02±0.01 | 0.03±0.01 | 0.03±0.02 | 0.07±0.03 | 0.09±0.04 | 0.07±0.03 | 0.06±0.02 |
| 18.68 | 1-Butanol, 3-methyl, benzoate | 0.03±0.02 | 0.13±0.05 | 0.20±0.07 | 0.30±0.09 | 0.50±0.15 | 0.60±0.21 | 0.55±0.16 | 0.54±0.15 | 0.55±0.14 |
| 18.86 | Dimethyl phthalate | 0.02±0.02 | 0.09±0.03 | 0.12±0.04 | 0.13±0.04 | 0.15±0.05 | 0.17±0.05 | 0.25±0.08 | 0.22±0.07 | 0.21±0.07 |
| 20.67 | Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester | 0.01±0.01 | 0.06±0.03 | 0.07±0.03 | 0.14±0.04 | 0.15±0.05 | 0.20±0.06 | 0.25±0.08 | 0.23±0.07 | 0.23±0.08 |
| 23.00 | Tetradecanoic acid, ethyl ester5) | ND | ND | ND | ND | ND | 0.06±0.02 | 0.18±0.06 | 0.17±0.06 | 0.18±0.07 |
| 23.74 | 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester | 0.10±0.03 | 0.11±0.04 | 0.15±0.04 | 0.22±0.07 | 0.23±0.08 | 0.25±0.07 | 0.30±0.10 | 0.27±0.10 | 0.28±0.09 |
| 24.71 | Dibutyl phthalate | ND | ND | ND | 0.02±0.01 | 0.02±0.01 | 0.02±0.02 | 0.05±0.02 | 0.04±0.02 | 0.03±0.02 |
| 25.07 | Pentadecanoic acid, ethyl ester5) | ND | ND | ND | ND | ND | 0.04±0.03 | 0.15±0.05 | 0.17±0.06 | 0.15±0.04 |
| 27.05 | 9,12-Octadecadienoic acid, ethyl ester | ND | ND | ND | ND | ND | ND | 0.03±0.02 | 0.04±0.01 | 0.04±0.02 |
| 27.15 | Ethyl, 9-octadecenoate, (E)- | ND | ND | ND | ND | ND | ND | 0.02±0.02 | 0.03±0.02 | 0.03±0.02 |
|  | **Furan(one)s** |  |  |  |  |  |  |  |  |  |
| 5.00 | Furfural1,5) | ND | ND | ND | ND | ND | ND | 0.09±0.05 | 0.13±0.05 | 0.22±0.07 |
| 5.41 | 3-Furfuranmethanol | ND | 0.01±0.01 | 0.03±0.01 | 0.14±0.04 | 0.18±0.05 | 0.20±0.06 | 0.30±0.08 | 0.35±0.09 | 0.32±0.10 |
| 6.80 | 2(3H)-Furanone, dihydro-1) | 0.08±0.03 | 0.12±0.04 | 0.23±0.06 | 0.30±0.07 | 0.43±0.13 | 0.48±0.16 | 0.48±0.17 | 0.47±0.13 | 0.47±0.15 |
| 13.60 | 3(2H)-Furanone, 4-methoxy-2,5-dimethyl- | ND | ND | ND | ND | 0.03±0.02 | 0.03±0.02 | 0.09±0.03 | 0.10±0.04 | 0.12±0.05 |
| 15.36 | Benzofuran, 2-ethenyl- | ND | ND | ND | ND | ND | ND | ND | ND | 0.03±0.02 |
| 17.63 | 2(3H)-Furanone, dihydro-5-pentyl- | 0.02±0.01 | 0.02±0.01 | 0.02±0.02 | 0.03±0.02 | 0.04±0.02 | 0.05±0.03 | 0.05±0.03 | 0.05±0.02 | 0.05±0.02 |

**Table 3. Continued**

| RT [2] (min) | Compound | $MF_5$ | $MF_{15}$ | $MF_{30}$ | $MF_{60}$ | $MF_{90}$ | $MF_{120}$ | $MF_{150}$ | $MF\text{-}HT_{30}$ | $MF\text{-}HT_{60}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Miscellaneous compounds** | | | | | | | | | |
| 10.81 | 1.4-Dioxin,2,3-dihydro-5,6-dimetyl- | ND | ND | ND | ND | ND | 0.05±0.03 | 0.14±0.04 | 0.13±0.03 | 0.13±0.05 |
| 11.63 | Ethanone, 1-(1H-pyrrol-2-yl)-[5] | ND | ND | ND | ND | ND | 0.13±0.03 | 0.20±0.05 | 0.20±0.07 | 0.19±0.06 |
| 17.14 | Benzamide | ND | ND | ND | ND | ND | 0.01±0.01 | 0.01±0.01 | 0.02±0.01 | 0.02±0.02 |
| | **Phenols** | | | | | | | | | |
| 12.28 | Phenol, 2-methoxy-[1,5] | ND | ND | ND | ND | ND | 0.03±0.02 | 0.07±0.03 | 0.11±0.04 | 0.12±0.05 |
| 16.52 | Phenol, m-tert-butyl- | ND | ND | 0.01±0.01 | 0.01±0.01 | 0.02±0.02 | 0.04±0.02 | 0.03±0.01 | 0.03±0.02 | 0.03±0.02 |
| 16.86 | 2-Methoxy-4-vinylphenol[1,5] | 0.19±0.06 | 0.19±0.05 | 0.15±0.04 | 0.14±0.04 | 0.10±0.03 | 0.08±0.03 | 0.09±0.03 | 0.12±0.04 | 0.12±0.03 |
| 19.63 | Butylated hydroxytoluene | 0.26±0.08 | 0.33±0.08 | 0.41±0.11 | 0.45±0.12 | 0.57±0.19 | 0.52±0.20 | 0.46±0.16 | 0.45±0.12 | 0.46±0.14 |
| 27.28 | Phenol, 2-[1-(4-hydroxyphenyl)-1- methylethyl]- | ND | ND | ND | ND | 0.01±0.01 | 0.01±0.01 | 0.02±0.01 | 0.02±0.01 | 0.02±0.02 |
| | **Pyran(on)es** | | | | | | | | | |
| 14.64 | 2-Methyl-3-methoxy-4H-pyran-4-one | 0.06±0.03 | 0.07±0.03 | 0.10±0.03 | 0.12±0.03 | 0.13±0.04 | 0.14±0.05 | 0.13±0.05 | 0.13±0.04 | 0.12±0.04 |
| 15.26 | 4H-Pyran-4-one, 2-methoxy-6-methyl- | ND | ND | ND | ND | 0.05±0.04 | 0.03±0.01 | 0.03±0.02 | 0.02±0.02 | 0.01±0.02 |
| | **Sulfur-containing compounds** | | | | | | | | | |
| 6.74 | Propanal, 3-(methylthio)- [1,5] | ND | ND | 0.08±0.04 | 0.12±0.04 | 0.31±0.12 | 0.48±0.13 | 0.44±0.15 | 0.51±0.18 | 0.49±0.17 |
| 8.70 | 1-Propanol, 3-(methylthio)- [1,5] | ND | ND | ND | ND | ND | 0.10±0.04 | 0.18±0.07 | 0.43±0.14 | 0.52±0.16 |

[1] The ratio between peak area of a particular compound and that of internal standard (2-methyl-3-heptanone) in sample was used as relative content of a compound in this study.

[2] Represents retention time of corresponding compound on column DB-5 in the present study.

[3] Each value is expressed as mean±SD ($n$=3); ND, not detected

[1,5] Represent corresponding compounds were identified in references 1 and/or 5.

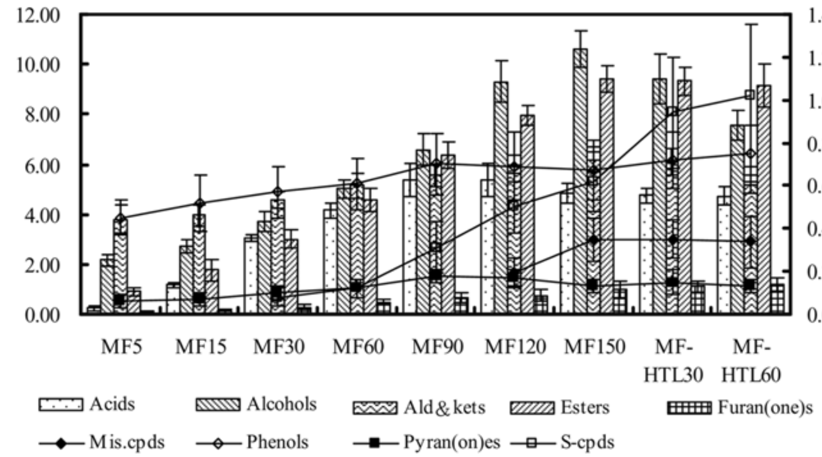

**Fig. 2. Changes in relative contents of different chemical classes of samples during moromi fermentation and heat treatment.** Vertical bars represent standard deviation ($n$=3) for each data point. Ald&kets, mis.cpds, and s-cpds represent chemical classes of aldehydes and ketones, miscellaneous compounds, and sulfur-containing compounds, respectively. Chemical classes of mis.cpds, phenols, pyran(on)es, and s-cpds correspond to the auxiliary axis and the left chemical classes correspond to the primary axis.

(5,21).

As shown in Fig. 2, similar to changes in alcohols, relative contents of aldehydes and ketones of samples increased continually during moromi fermentation and decreased slightly ($p>0.05$) when MF-HT$_{30}$ and MF-HT$_{60}$ suffered heat treatment. Among the aldehydes and ketones, relative contents of butanal, 2-methyl- (roasted cocoa), butanal, 3-methyl- (malty), benzaldehyde (burnt sugar/sweet/honey-like), and benzeneacetaldehyde (burnt sugar/sweet/honey-like) in samples were dominant and have been identified as aroma-impact compounds in soy sauce (1-3,5). Therefore, the relative contents of aldehydes and ketones might relate to formation of attributes of malty and caramel-like during moromi fermentation and heat treatment. Obviously, 3-penten-2-one and 4-methyl- were products of heat treatment and (+)-longicamphenylone was intermediate during moromi fermentation. The origins of some low-boiling aldehydes (i.e., 2-methylbutanal) detected in soy sauce might arise from amino acid degradation and microorganism transformation (5), while the origin of long-chain aldehydes (i.e., nonanal, decanal, etc) might come from the oxidation of lipid (22). Similar to the origin of long-chain aldehydes, 2-methylketones ($C_5$-$C_{15}$) might originate from the degradation of lipid, or were formed by beta oxidation and decarboxylation of fatty acids (23).

As seen from Table 3 and Fig. 2, there were 21 esters identified in all samples, the relative contents of them increased progressively with proceeding of moromi fermentation and decreased slightly ($p>0.05$) during subsequent heat treatment. For all samples, ethyl acetate, benzoic acid, ethyl ester, benzeneacetic acid, ethyl ester, acetic acid, 2-phenylethyl ester, 1-butanol, 3-methyl, and benzoate were found the dominant esters. In the present study, heat treatment caused a slightly ($p>0.05$) decrease in the relative contents of esters, which could explain deterioration of fruity attribute of MF-HT$_{30}$ and MF-HT$_{60}$ because most of esters, especially ethyl esters, are responsible for fruity note (Fig. 1). Moreover, esters could also contribute a good flavor by minimizing the sharpness and bitterness imparted by amines and fatty acids (24,25). The formation of esters was related to metabolism of lipids by yeast, which provided a large number of acids and alcohols that may undergo esterification to yield a variety of esters (5).

In the present study, the relative contents of furan(one)s increased gradually during moromi fermentation. Unlike changes in acids, alcohols, aldehydes and ketones, and esters, the relative contents of furan(on)es increased slightly ($p>0.05$) after heat treatment (Fig. 2). Among the furan(one)s, 2(3H)-furanone, dihydro- (sugar), 3(2H)-furanone, and 4-methoxy-2,5-dimethyl- (caramel-like) were reported as aroma-impact compounds in soy sauce and cheese (24,26), thus they could be important contributors to sensory attributes of malty and caramel-like, respectively. Moreover, it was obviously that furfural and benzofuran, 2-ethenyl- (Table 3) were products of heat treatment in MF-HT$_{30}$ and MF-HT$_{60}$.

Table 3 showed that only 3 miscellaneous compounds including 1.4-dioxin, 2,3-dihydro-5,6-dimetyl-, ethanone, 1-(1H-pyrrol-2-yl)-, and benzamide were detected in all samples, in which ethanone, 1-(1H-pyrrol-2-yl)- (roasty/popcorn-like) was regarded as aroma-impact compound in Japanese-type soy sauce (3), so the contribution of ethanone, 1-(1H-pyrrol-2-yl)- to sensory attribute of over-all should not be neglected. It should be noted that all the miscellaneous compounds were detected in later stage and the relative contents of them changed slightly during moromi fermentation and heat treatment.

Five phenols were detected in all samples and their relative contents increased gradually to 0.70 ($MF_{90}$) and then decreased slightly to 0.67 ($MF_{150}$) during moromi fermentation (Table 3, Fig. 2). Although heat treatment gave increases in the relative contents of phenols in MF-$HT_{30}$ and MF-$HT_{60}$, there was no significant ($p$>0.05) difference in the relative contents of phenols between $MF_{150}$ and MF-$HT_{30}$ or MF-$HT_{60}$. Among the phenols, methoxyphenols, i.e., phenol, 2-methoxy-, are components responsible for smoky flavor and significantly contribute to flavor of Japanese-type soy sauce (5,27). Therefore, heat treatment might be reasons for increase of the relative contents of phenols in MF-$HT_{30}$ and MF-$HT_{60}$ and caused enhancement of smoky attribute in DA evaluation (Fig. 1). As seen from Fig. 2, the relative contents of pyran(on)es increased from the initial moromi fermentation stage to 90 day ($MF_{90}$) and decreased slightly thereafter. There was no pronounced ($p$>0.05) influence of heat treatment on the relative contents of pyran(on)es in MF-$HT_{30}$ and MF-$HT_{60}$. The relative contents of sulfur-containing compounds in samples increased gradually during moromi fermentation and heat treatment, whereas there were no significant ($p$>0.05) differences between $MF_{150}$ and MF-$HT_{30}$ or MF-$HT_{60}$ (Fig. 2). Among the pyran(on)es and sulfur-containing compounds, only 2 pyran(on)es and 2 sulfur-containing compounds were identified in all samples (Table 3), in which 2-methyl-3-methoxy-4H-pyran-4-one (caramel-like) was regarded as important contributor to good aroma of soy sauce and propanal, 3-(methylthiol)- (cooked potato), and 1-propanol, 3-(methylthiol)- (potato/grassy) were identified as aroma-impact compounds in soy sauce (3,5). Hence, the differences in relative contents of pyran(on)es and sulfur-containing compounds of samples during moromi fermentation and heat treatment could result in the difference in caramel-like attribute in DA evaluation (Fig. 1).

In conclusion, the present study has clearly demonstrated the changes in volatile compounds and sensory attributes of TCSS during moromi fermentation and heat treatment. Long-time moromi fermentation is necessary for aroma formation of TCSS, and different heat treatment, especially manner of 80ºC/60 min, caused aroma deterioration of TCSS greatly. On the basis of results obtained from the current study, 150 day of moromi fermentation and heat treatment manner of 80ºC/30 min are suitable processing parameters to produce soy sauce with an ideal aroma profile. Moreover, based on the current results that most of the volatile compounds occurred at initial moromi fermentation stage, further work on optimizing *koji*-culturing process is of great importance and in process to improve the flavor of TCSS.

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