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
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 (MF3), 15 (MF15), 30 (MF30), 60 (MF60), 90 (MF90), 120 (MF120), and 150 (MF150), then 2 fractions of MF150 were heated for 30 (MF-HT30) and 60 min (MF-HT60) 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 H2O 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

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

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