Volatile Compounds of Headspace Gas in the Japanese Fish Sauce Ishiru

Toshihide Michihata,1 Toshihiro Yano,2 and Toshiki Enomoto2,†

1Food Processing Laboratory, Industrial Research Institute of Ishikawa, Kanazawa, Ishikawa 920-0223, Japan
2Department of Food Science, Ishikawa Agricultural College, Nonoichi, Ishikawa 921-8836, Japan

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Volatile compounds from the headspace gas of ten brands of the Japanese fish sauce ishiru were analyzed by GC-MS with a thermal-desorption cold-trap system. Many volatile peaks were detected and 51 compounds were identified. The major volatile compounds in ishiru included aldehydes (such as 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, and benzaldehyde), nitrogen-containing compounds (such as pyrazine derivatives and trimethylamine), sulfur-containing compounds (such as dimethyl disulfide), and ketones (such as 2-butanone and 3-methyl-2-butanone). On the other hand, volatile fatty acids were nearly absent in the headspace gas of ishiru.

Key words: ishiru; fish sauce; headspace gas; volatile compounds; GC-MS with thermal-desorption cold-trap system

Fish sauce is a brown liquid condiment with a characteristic odor, widely consumed in many areas of Southeast Asia. Two kinds of fish sauce ishiru made from squid livers and sardines have been traditionally produced on the Noto Peninsula of Ishikawa Prefecture.1) This ishiru, along with shottsuru in Akita and ikanago-shoyu in Kagawa, are the three major fish sauces in Japan. Ishiru is produced from a mixture of fish and salt (4:1) fermented for 1 to 2 years. Volatile compounds in fish sauces of various origins have been identified by several researchers using different techniques of sample preparation and methods of analysis.5–8) We have reported ingredients other than volatile compounds; GC-MS with thermal-desorption cold-trap system. In addition, the compounds of shottsuru and ikanago-shoyu were measured, and compared with those of ishiru.

All ishiru samples, five brands of ika-ishiru (SQ-1~SQ-5) made from squid livers and five brands of iwashi-ishiru (SA-1~SA-5) made from sardines, were purchased from local markets in the Noto Peninsula. Two brands of shottsuru (A, B) were purchased from a department store in Akita Prefecture, and one brand of ikanago-shoyu was obtained from Marukin Shoyu Co., Ltd., Kagawa Prefecture. A Tenax TA column (160 mm × 3 mm i.d. 20/35 mesh, 100 mg) was from GL Sciences Inc. (Tokyo). Authentic chemicals of the volatile compounds were from Wako Pure Chemicals Industries (Osaka).

The total nitrogen values were measured with a KJEL-Auto (Mitamura Riken Kogyo Co., Ltd., Tokyo). The pH and soluble solids were measured by a pH meter and a hand refractometer, respectively.

The collection of headspace volatiles from ishiru was done by a modified method of Shimoda et al.5,6) and Peralta et al.9) Ishiru (25 ml), in which 5 μl of 0.25% cyclohexanol had been added as the internal standard, was placed in a flask kept at 50°C. Dry N2 gas (purity 99.999%) was passed through the flask at a flow rate of 50 ml/min, and the volatile compounds were introduced and trapped in a Tenax TA column for 60 min. Then dry N2 gas was passed through the Tenax TA column for 30 min to remove water. The column was placed in a heating block of the thermal-desorption cold-trap system (CP-4010, GL Sciences) and heated to 250°C to free the volatile compounds, which were then cryofocused and injected into the GC column.

Separation was done on a GC-17A gas chromatograph (Shimadzu, Kyoto) with a 60 m × 0.25 mm i.d. silica capillary column with fused TC-WAX film 0.25 μm thick (GL Sciences) and a flame ionization detector. The oven temperature was held at 50°C for 5 min and programmed to rise from 50°C to 230°C at 3°C/min. Injector and detector temperatures were set at 150°C and 230°C, respectively. A QP-5050A model mass spectrometer (Shimadzu) attached to a GC-17A gas chromatograph programmed under the same conditions was used for identification of the volatile compounds. The mass spectra were obtained by electron-impact ionization at 70 eV. Mass spectral data and retention times were compared with authentic compounds and reported data (NIST database, '98 edition). The values of identified volatile compounds were calculated from peak areas in relation to the internal standard corresponding to 0.5 ppm. Results are means of triplicate samples.

† To whom correspondence should be addressed. Fax: +81-76-248-8402; E-mail: enomoto@ishikawa-c.ac.jp
Table 1. Total Nitrogen, pH Values, and Soluble Solids of Ishiru, Shottsuru, and Ikanago-shoyu

<table>
<thead>
<tr>
<th></th>
<th>Ika-Ishiru</th>
<th>Iwashi-Ishiru</th>
<th>Shottsuru</th>
<th>Ikanago-shoyu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SQ-1</td>
<td>SQ-2</td>
<td>SQ-3</td>
<td>SQ-4</td>
</tr>
<tr>
<td>Total nitrogen (g/100 ml)</td>
<td>2.12</td>
<td>2.01</td>
<td>2.05</td>
<td>2.49</td>
</tr>
<tr>
<td>pH</td>
<td>4.82</td>
<td>4.77</td>
<td>5.04</td>
<td>6.25</td>
</tr>
<tr>
<td>Soluble solids</td>
<td>39.2</td>
<td>40.0</td>
<td>40.0</td>
<td>37.0</td>
</tr>
</tbody>
</table>

Fig. 1. Typical Chromatograms of Headspace Volatiles of SQ-1 and SA-3.

The total nitrogen, pH, and soluble solids of ishiru, shottsuru, and ikanago-shoyu are shown in Table 1. The total nitrogen of ishiru and ikanago-shoyu was higher than that of shottsuru, and with one exception, the same was found for soluble solids. The pH of ishiru was from 4.77 to 6.53, the pHs of shottsuru and ikanago-shoyu were similar.

Typical GC-MS chromatograms of the SQ-1 and SA-3 samples of ishiru are in Fig. 1. A list of the principal volatile compounds in the headspace gas of three fish sauces and the relative amounts of volatile compounds that were identified are given in Table 2. In a study done by the same technique, 2-methylpropanoic acid and 2,2-dimethylpropanoic acid were found to be abundant in Taiwanese and Philippine fish sauce (patis). Butanoic acid was identified as the major acid in patis treated by steam distillation under reduced pressure. 3-Methylbutanoic acid is the most abundant acid in shottsuru, and acetic acid is abundant in Thai fish sauce (nampla). In this
Table 2. Volatile Compounds Identified in Ishiru, Shottsuru, and Ikanago-shoyu

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Compound name</th>
<th>Acids</th>
<th>Aldehydes</th>
<th>Ketones</th>
<th>Alcohols</th>
<th>Nitrogen-containing compounds</th>
<th>Sulfur-containing compounds</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ika-Ishiru</td>
<td>Iwashi-Ishiru</td>
<td>Shottsuru</td>
<td>Ikanago-shoyu</td>
<td>Ika-Ishiru</td>
<td>Iwashi-Ishiru</td>
<td>Shottsuru</td>
</tr>
<tr>
<td>41</td>
<td>2,2-Dimethylpropanoic</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.01</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>43</td>
<td>Acetic</td>
<td>TR</td>
<td>0.27</td>
<td>0.06</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>0.11</td>
</tr>
<tr>
<td>46</td>
<td>Propanoic</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>48</td>
<td>Butanoic</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>49</td>
<td>2-Methylpropanoic</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>51</td>
<td>3-Methylbutanoic</td>
<td>ND</td>
<td>ND</td>
<td>0.02</td>
<td>0.01</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

1) Peak area ratio of the samples to the internal standard.
2) Peak nos. refer to peaks in Fig. 1.
3) ND, not detected.
4) TR (trace), concentration of less than 0.01 peak area ratio.
study, amounts of acetic acid in the three ishiru samples SQ-3, SQ-5, and SA-4 were less than the amount of the internal standard, and amounts of other acids still less among the headspace volatiles of all ishiru samples. A trace of acetic acid was detected in shottsuru and ikanago-shoyu samples, and other acids were limited to small quantities. Acids seemed not to be associated with the characteristic ishiru odor.

All samples of fish sauces had high levels of 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, benzaldehyde, 2-butanone, and 3-methyl-2-butane. These aldehydes and ketones were similar to those found in other fish sauces by the same technique.\textsuperscript{6,8} Aldehydes have been derived from lipid oxidation during fermentation, and branched, short-chain aldehydes or aromatic aldehydes might have resulted from deamination of amino acids.\textsuperscript{10} These aldehydes are generally considered to cause unpleasant oxidation flavors in foods,\textsuperscript{11} and their odor threshold values are low.\textsuperscript{12} Therefore, the detected aldehydes are important in the ishiru odor. Ketones seem to be responsible for the cheesy note in fish sauce odor.\textsuperscript{8} However, such compounds seem not to affect ishiru odor because of their low concentrations and high odor threshold values.\textsuperscript{12}

A large number of normal and branched alcohols were detected in ishiru. Nevertheless, alcohols probably affected the odor of ishiru little except for the SQ-5 samples, because the odor threshold values were high. In SQ-5, 2-methyl-1-butanol and 3-methyl-1-butanol were remarkably present in the headspace gas. As SQ-5 is about 4.7% lactic acid,\textsuperscript{1,4} these alcohols might be obtained by microbial fermentation such as that of lactic acid bacteria. Large amounts of pyrazine derivatives were detected in all ishiru samples. Sanceda et al.\textsuperscript{5} found that pyrazines could be responsible for the burnt and sweet odors of Vietnamese fish sauce (noucmam). The burnt and sweet odors of ishiru could be attributed to its level of pyrazines, which was higher than in shottsuru or ikanago-shoyu. Trimethylamine, together with aldehydes, seem to be the source of fishy, ammonia-like odors.\textsuperscript{13} Trimethylamine had a large peak area compared with the peak of the internal standard in SQ-5, SA-3, -4, -5, shottsuru, and ikanago-shoyu. The pH values of these samples were higher than those of other ishiru samples. Shimoda et al.\textsuperscript{6,8} reported that the volatility of trimethylamine increases when the sample pH is increased, suggesting that the amount of trimethylamine in the headspace gas of fish sauce might be affected by the pH of the sauce.

Among the sulfur-containing compounds, dimethyl disulfide had the largest peak area in all ishiru samples. Judging from its low odor threshold value\textsuperscript{13} and the concentration in the headspace gas of ishiru, dimethyl disulfide together with trimethylamine seemed to be important in ishiru odors, especially the unpleasant ones.

Several esters and 2-furanmethanol were detected in ishiru. Esters are found in most fermented seafood, and might be products of esterification of alcohols with carboxylic acids formed by microbial or enzymatic decomposition of lipids.\textsuperscript{14} These esters and 2-furanmethanol could not contribute to the ishiru odor because of their low concentrations and high odor threshold values.\textsuperscript{12}

In conclusion, high concentrations of volatile aldehydes, pyrazine derivatives, dimethyl disulfide, and trimethylamine seem to be the most potent contributors to the characteristic ishiru odor. Relationship between the volatile compounds and the sensory evaluations in ishiru remains to be examined.

References


12) Devos, M., Patte, F., Roualt, J., Laffort, P., and
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