The formation of HEMF[2(or 5)-ethyl-5(or 2)-methyl-4-hydroxy-3(2H)-furanone], the aroma component specific to miso and soy sauce, was promoted by cultivating the halo-tolerant yeast, *Zygosaccharomyces rouxii*, in a medium including the amino-carbonyl reaction products based on ribose and glycine. The glucose concentration in the medium influenced the HEMF formation by *Z. rouxii*. 

Key words: 2(or 5)-ethyl-5(or 2)-methyl-4-hydroxy-3(2H)-furanone; aroma component; miso; yeast

HEMF is very important as the characteristic aroma component of soy sauce and miso, with a threshold value of less than 20 ppb in water. Sasaki et al. have reported that the pentose-phosphate cycle was necessary for HEMF biosynthesis by yeast in soy sauce. Blank and Fay have reported that the formation of HDMF[2,5-dimethyl-4-hydroxy-3(2H)-furanone] and HEMF progressed in a mixture of the amino acid, glycine or alanine, and pentose in a pH 6.0 buffer solution by a 1-hr reaction at 90°C. HEMF was detected in the mixture of pentose and alanine, and HDMF instead of HEMF was detected in the mixture of pentose and glycine. Blank et al. have reported that HEMF was formed by combining the Amadori compound of 5 carbons from pentose with acetaldehyde produced by the Strecker degradation of alanine, however, the amount of HEMF formed by the amino-carbonyl reaction was very small and did not reach the content level detected in miso or soy sauce.

In our previous study, HEMF formation was reported to have been promoted by cultivating yeast in a heat-sterilized medium which contained glucose, ribose and an amino acid. The authors assumed that HEMF containing 7 carbons had been formed by the combination of the amino-carbonyl reaction products of 5 carbons and the chemical compounds of 2 carbons which perhaps were provided by active glucose metabolism of the yeast.

This present study was carried out in order to clarify the role of yeast in the HEMF formation according to the mechanism involving the amino-carbonyl reaction proposed by Blank et al. We confirmed that HEMF formation was promoted by yeast cultivation in a medium including the amino-carbonyl reaction products based on ribose and glycine. The effects of the type of yeast and growth conditions on the formation of HEMF were investigated by yeast cultivation for 5 weeks. The rate of generation and amount of the precursor of HEMF containing 2 carbons are considered to be influenced by the type of yeast, and by the glucose concentration and NaCl concentration in the medium.

Three types of yeast were used in this study. *Z. rouxii* 061 was selected in order to produce the well-known red-brown Sendai miso by the Experimental Station of Miyagiken Miso-Shoyu Industry Cooperative (Miyagi Prefecture, Japan), *Candida versatilis* used for soy sauce brewing was the halo-tolerant yeast, and *Saccharomyces cerevisiae* K7 used for sake brewing was the halosensitive yeast. These latter two yeast strains were provided as stock cultures by Iwate Industrial Research Institute.

Each medium was prepared as is shown in Table 1. All the media were adjusted to pH 5.2, before putting 100 ml of each into a 200-ml flask and sterilizing at ca. 120°C for 15 min. During the heat-sterilization process, an amino-carbonyl reaction would have proceeded when the medium contained both sugar and amino acids. In
were incubated at 27°C. The yeast cells in each culture, pH value, residual amount of glucose, and NaCl concentration in the medium were measured as described in our previous paper. Aroma concentrates were prepared and analyzed, the experimental and analytical conditions having been described in detail in a previous report. The concentration of HEMF was based on the calibration curve for an authentic sample.

The HEMF formation in media of types I-1, II, III, IV and V is shown in Fig. 1. The results for the media of types I-1 and II show that HEMF formation was promoted by yeast cultivation in a medium containing amino-carbonyl reaction products based on ribose and glycine. The concentration of HEMF formed for the media of types I-1 and III indicate that glucose did not affect the formation of the precursor of HEMF containing 5 carbons. The results for the media of types I-1, IV and V suggest that the precursor of 5 carbons produced by the amino-carbonyl reaction of ribose with glycine had been formed more stably in the buffer solution containing KH₂PO₄ and MgSO₄·7H₂O.

Z. rouxii 061 was incubated for 1–5 weeks with three levels of glucose concentration in order to clarify the role of glucose in the medium. The amount of HEMF was negligible in the media of types I-1 (7.5% Glu), I-A (3.0% Glu) and I-B (no Glu) without yeast inoculation when incubated under the same conditions as those used for the yeast cultivation. As is shown in Fig. 2, the highest concentrations of HEMF were found after 3 weeks in the media of types I-1 (7.5% Glu) and I-A (3.0% Glu), the HEMF concentration in medium type I-A (3.0% Glu) being about 70% of that in medium type I-1 (7.5% Glu) after 3 weeks. The concentration of HEMF was very low in medium type I-B (no Glu).

Z. rouxii 061 was incubated for 1–5 weeks under four different concentrations of NaCl. HEMF could not be detected with the media prepared without yeast inoculation after 3 or 5 weeks. As is shown in Fig. 2, the different NaCl concentrations of 5%, 10% and 15% did not affect the level of HEMF concentration in the medium at the final stage. The medium excluding NaCl resulted in good growth of the yeast, but the activity of

### Table 1. Composition of the Media Used for HEMF Formation

<table>
<thead>
<tr>
<th>Media Type</th>
<th>Ribose (g)</th>
<th>Glycine (g)</th>
<th>Glucose (g)</th>
<th>NaCl (g)</th>
<th>KH₂PO₄ (g)</th>
<th>MgSO₄·7H₂O (g)</th>
<th>Yeast extract (g)</th>
<th>Water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-1, II, III, IV, V</td>
<td>2.5</td>
<td>1.0</td>
<td>7.5</td>
<td>10</td>
<td>1.0</td>
<td>0.5</td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td>I-2</td>
<td>2.5</td>
<td>1.0</td>
<td>7.5</td>
<td>0</td>
<td>1.0</td>
<td>0.5</td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td>I-3</td>
<td>2.5</td>
<td>1.0</td>
<td>7.5</td>
<td>5</td>
<td>1.0</td>
<td>0.5</td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td>I-4</td>
<td>2.5</td>
<td>1.0</td>
<td>7.5</td>
<td>15</td>
<td>1.0</td>
<td>0.5</td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td>I-A</td>
<td>3.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-B</td>
<td>3.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Medium type I: A 50-ml water solution containing ribose and glucose and that containing the other constituents were separately autoclaved and mixed.

Medium type II: A 50-ml water solution containing ribose and glucose and that containing the other constituents were separately autoclaved and mixed.

Medium type III: A 50-ml water solution containing only glucose and that containing the other constituents were separately autoclaved and mixed.

Medium type IV: A 50-ml water solution containing ribose and glycine and that containing the other constituents were separately autoclaved and mixed.

Medium type V: A 50-ml water solution containing ribose, glycine, KH₂PO₄ and MgSO₄·7H₂O and that containing the other constituents were separately autoclaved and mixed.

**Fig. 1.** Effect of Medium Preparation on the Formation of HEMF by Z. rouxii 061.

The compositions and preparation methods for the media are shown in Table 1.
HEMF formation by the yeast was low. *C. versatilis* in the media of types I-1 (10% NaCl) and I-2 (no NaCl), and *S. cerevisiae* K7 in medium type I-2 (no NaCl) were confirmed to form HEMF. The amounts formed by *C. versatilis* and *S. cerevisiae* K7 were lower than that by *Z. rouxii*. The HEMF formation by *S. cerevisiae* K7 in medium type I-1 (10% NaCl) was negligible because the yeast could not multiply with this medium.

These results suggest that the type of yeast and growth conditions, like the glucose and NaCl concentration in the medium, affected the generation rate and amount of the precursor of HEMF containing 2 carbons. One of the roles of yeast in HEMF formation is probably to provide the glucose metabolites containing 2 carbons. HEMF in miso and soy sauce is considered to be formed by the combination of the compound containing 5 carbons produced through the amino-carbonyl reaction under mild conditions of fermentation, and the chemical compounds of 2 carbons provided by the yeast.

**Acknowledgments**

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**References**


