Characterization of White Particulates in Brine of Indigenous Fermented Foods

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ABSTRACT

White particulates were occasionally observed in the brine of indigenous fermented foods, including sufu, salt-preserved shell fish, oyster, finfish, and pork. Products with and without visible particulates were collected and brines were analyzed. The pH values, salinity, and alcohol content in brines containing particulates were slightly higher than those not containing particulates. Differences of amino acid profiles were not significant. When particulates were isolated and subjected to amino acid analysis, only tyrosine and phenylalanine were present and accounted for 34.82 ± 24.30% and 3.13 ± 2.93% on a dry weight basis, respectively. The particulates consisted largely of hollow particles as observed under scanning electron microscopy. When the particulates were dissolved in 0.4 N HCl, evaporated for crystallization, and examined using stereo microscopy and an X-ray diffractometer, L-tyrosine crystals were observed.

The addition of salt to indigenously fermented foods is primarily for the purpose of preserving against microbiological spoilage. Salt also imparts desirable flavor, aroma, and texture to fermented food products. The two principal advantages of food fermentation over other processes are to enhance flavor and to prevent spoilage (1,3).

Among the diverse brined fermented foods such as sufu, soy sauce moromi, soybean paste, salt-preserved shell fish, finfish, and pork available in local markets, the presence of visible white particulates, usually attached to the surface of solid particles (Fig. 1A), are occasionally observed. Most consumers and some producers believe that the appearance of white particulates is a defect of fermentation. However, some products containing white particulates are still consumed, because, in general, there are no obvious changes in sensory characteristics. The public health significance which may be associated with the presence of white particulates in fermented foods needs to be investigated.

In this study, the objectives were primarily to isolate white particulates from brined fermented foods available in the local markets and subjected to chemical and physical analyses. The pH, salt concentration, alcohol content, and amino acid composition in the broths of brined fermented foods with and without visible white particulates was also investigated.

Figure 1. Photographs of white particulates examined under a stereo microscope (A) and by SEM (B), and crystals of the particulates (C) and L-tyrosine (D).

MATERIALS AND METHODS

Sample collection

Brined fermented foods with or without visible white particulates were purchased from local grocery stores and markets. Products with particulates including 6 sufu with various brand names, 3 shell fish, 2 oysters, 1 small finfish, and 1 pork as well as products without particulates including 6 sufu, 2 oysters, and 1 small finfish were collected.

Analysis of broths

Broth of fermented products was drained from each product and analyzed for pH value (Suntek Digital pH meter, Model sp-7, Suntex Co., Taiwan), salinity, alcohol content, and amino acid composition. For salinity determination (5), 5 ml of broth was diluted to 250 ml with deionized water. Samples (5 ml) were withdrawn and diluted to 100 ml with deionized water. One milliliter of a 2% solution of potassium chromate was added, and the mixture was titrated with 0.02 N silver nitrate.

To determine alcohol content, 5 ml of broth was mixed with 20 ml of deionized water, and the mixture was distilled under mild boiling conditions. The distillate (10 ml) was filtered through a membrane (0.45-μm HV type Millipore membrane) and analyzed.
Isolation and characterization of particulates

White particulates in brined fermented products were photographed with a camera attached to a stereo microscope and with a scanning electron microscope (SEM). Particulates were manually isolated from broths, washed several times with deionized water, and dried in a forced-air oven at 65°C for 24 h. The dried particulates were ground with a mortar into powder and used for further analysis.

For amino acid analysis, 0.2 g of particulate powder was dissolved in 2 ml of 0.4 N HCl and diluted with 2 ml of citrate buffer (pH 2.2, Beckman buffer specified for amino acid analysis). Approximately 1 ml of the diluted solution was filtered (0.45 µm) and subjected to amino acid analysis as described above.

For X-ray diffraction analysis, approximately 50 mg of particulate powder was spread on a glass slide and two drops of 0.4 N HCl were added to dissolve the powder. The slide was evaporated for 24 h at ambient temperature to induce crystallization. The crystals formed on the slide were subjected to X-ray diffraction analysis with an X-ray diffractometer (Siemens D500 X-Ray Diffractometer, Germany). A pure L-form tyrosine (Sigma Co., St. Louis, MO) was spread on a slide and examined concurrently as a reference.

RESULTS AND DISCUSSION

Listed in Table 1 are pH values, salinity, and alcohol contents of brine from fermented foods containing and not containing visible white particulates. Mean values for pH and salt and alcohol contents were higher in foods with particulates than those without particulates. However, due to the diversity of products collected from local markets, mean values with large standard deviations were obtained.

The amino acid composition of brines from fermented products is also listed in Table 1. Large standard deviations were obtained for all samples. The difference was insignificant when comparisons were made on each amino acid content in foods containing and not containing white particulates.

When the white particulates were analyzed for amino acid composition (Table 1), only tyrosine and phenylalanine were detected. Tyrosine and phenylalanine accounted for 34.82 ± 24.30% and 3.13 ± 2.93% of the dry weight of particulates, respectively. During soy sauce fermentation, part of the tyrosine is precipitated from moromi mash liquid because of its low solubility (2). Tyrosine and phenylalanine are aromatic amino acids with comparatively low solubilities in water. Since products were not agitated during fermentation, particulates might be formed in localized areas as a result of specific conditions under which the concentrations of tyrosine and phenylalanine exceeded their saturation points. Yamashita et al. (6) detected a tyrosine sediment in fermented sauce and reported that tyrosine solubility is influenced by temperature, acidity, pH and ethanol, NaCl, and glucose concentrations.

Particulates were observed to be located on the surface of solid food particles (Fig. 1A). The size of each particulate varied greatly. Under SEM, tubular clusters were observed (Fig. 1B). Marshall (4) reported that tyrosine crusts or macroscopic crystals could be obtained during cooling of the concentrated solution consisting of pancreatic extract and

### Table 1. pH values, salinity, alcohol content, and amino acid composition of brine and particulates from fermented foods with and without visible white particulates.

<table>
<thead>
<tr>
<th>Item</th>
<th>With particulate</th>
<th>Without particulate</th>
<th>Particulate</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.04 ± 0.44</td>
<td>4.61 ± 0.44</td>
<td></td>
</tr>
<tr>
<td>Salinity, % NaCl</td>
<td>24.08 ± 10.65</td>
<td>17.35 ± 6.61</td>
<td></td>
</tr>
<tr>
<td>Alcohol, %</td>
<td>1.68 ± 1.96</td>
<td>1.15 ± 0.84</td>
<td></td>
</tr>
<tr>
<td>Amino acids, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asp</td>
<td>0.0122 ± 0.0161</td>
<td>0.0078 ± 0.0053</td>
<td></td>
</tr>
<tr>
<td>Thr</td>
<td>0.0169 ± 0.0161</td>
<td>0.0256 ± 0.0255</td>
<td></td>
</tr>
<tr>
<td>Ser</td>
<td>0.0048 ± 0.0051</td>
<td>0.0093 ± 0.0088</td>
<td></td>
</tr>
<tr>
<td>Glu</td>
<td>0.0340 ± 0.0364</td>
<td>0.0505 ± 0.0434</td>
<td></td>
</tr>
<tr>
<td>Pro</td>
<td>0.0238 ± 0.0208</td>
<td>0.0231 ± 0.0123</td>
<td></td>
</tr>
<tr>
<td>Gly</td>
<td>0.0056 ± 0.0047</td>
<td>0.0082 ± 0.0067</td>
<td></td>
</tr>
<tr>
<td>Ala</td>
<td>0.0529 ± 0.0423</td>
<td>0.0706 ± 0.0561</td>
<td></td>
</tr>
<tr>
<td>Val</td>
<td>0.2019 ± 0.0916</td>
<td>0.1726 ± 0.0722</td>
<td></td>
</tr>
<tr>
<td>Met</td>
<td>0.0320 ± 0.0444</td>
<td>0.0083 ± 0.0032</td>
<td></td>
</tr>
<tr>
<td>Ile</td>
<td>0.1999 ± 0.0987</td>
<td>0.1620 ± 0.0661</td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>0.3376 ± 0.1751</td>
<td>0.2905 ± 0.1264</td>
<td></td>
</tr>
<tr>
<td>Tyr</td>
<td>0.0550 ± 0.0314</td>
<td>0.0587 ± 0.0247</td>
<td>34.82 ± 24.30</td>
</tr>
<tr>
<td>Phe</td>
<td>0.1722 ± 0.1136</td>
<td>0.1646 ± 0.0869</td>
<td>3.13 ± 2.93</td>
</tr>
<tr>
<td>His</td>
<td>0.0017 ± 0.0014</td>
<td>0.0013 ± 0.0003</td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>0.0022 ± 0.0026</td>
<td>0.0016 ± 0.0004</td>
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</table>

* Means of all determinations with standard deviation.

casein which were incubated at 38°C for 3 to 7 d. When particulates were dissolved in 0.4 N HCl and evaporated for crystallization, the resulting crystals (Fig. IC) looked similar to L-tyrosine crystals (Fig. ID) which were prepared concurrently from a pure L-tyrosine. When the crystals from particulate powder were further subjected to X-ray diffraction (Fig. 2), the diffraction pattern was very similar to that of L-tyrosine. This confirmed that L-tyrosine was one of the principal constituents in the visible white particulates observed in brine of indigenous fermented foods.

ACKNOWLEDGMENTS

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REFERENCES