Note

Sterilization of Plasma Powder by Treatment with Supercritical Carbon Dioxide

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Animal blood has received much attention as an unutilized protein source of high quality in terms of nutritive and functional properties.1,2) However, since plasma powder derived from animal blood contains a large number of microorganisms, sterilization is essential before use as a starting material for food. Powdery products such as spices are generally difficult to sterilize by heat treatment without undesirable changes in quality. Sterilization with ethylene oxide or radial rays is possible, but is limited to applicable materials by the regulations in Japan. New sterilization methods such as sterilization with superheated steam3) and extrusion cooking4) have been recently studied, but deterioration of the quality due to heat is inevitable. We reported previously the preparation of defatted rice-Koji for brewing sake5,6) and enzyme preparations7) free from microorganisms by supercritical carbon dioxide (SC-CO₂) treatment without decreases in any enzymatic activities. This paper describes sterilization of plasma powder by treatment with SC-CO₂.

Sterilization was carried out using the SC-CO₂ extraction apparatus under the conditions reported previously.5–8) When ethanol or acetic acid was added to CO₂ as an entrainer, CO₂ alone was further passed through the sample for 20 min after the sterilization treatment to remove the entrainer. Porcine plasma powder was used in this study, which was obtained by separation from blood cells followed by vacuum-drying at low temperature. The number of general living microorganisms in the plasma powder was determined by the standard method9) to be 7.5 × 10⁷ per gram of the plasma powder. The number of bacterial endospores which could grow after heat treatment at 60°C for 60 min or at 100°C for 10 min was less than 10² per gram of the plasma powder. In order to determine the water solubility of the plasma powder as an indication of deterioration caused by SC-CO₂ treatment, the weight of the insoluble fraction was determined as follows: 40 grams of water was added to one gram of the plasma powder. After stirring the mixture thoroughly, insoluble matter was recovered by centrifugation at 3,500 rpm for 10 min. Fresh water was again added to the precipitate, and the same procedure was repeated three times. The weight of the residue was determined as the insoluble fraction after drying at 105°C for 6 hr.

The number of living cells in the dry plasma powder with a water content of 6% did not decrease much with an increase in the treatment time for sterilization with SC-CO₂ alone or with several increases and decreases in the CO₂ pressure, from 1 atm to 200 atm. Table I shows

<table>
<thead>
<tr>
<th>Run No.</th>
<th>Addition to SC-CO₂</th>
<th>Initial water contenta (%)</th>
<th>Final water content (%)</th>
<th>Ratio of living cells (%), Solubility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>—</td>
<td>6.8</td>
<td>5.3</td>
<td>94.0</td>
</tr>
<tr>
<td>2</td>
<td>—</td>
<td>16.5</td>
<td>7.8</td>
<td>5.2 × 10⁻², —</td>
</tr>
<tr>
<td>3</td>
<td>—</td>
<td>30.7</td>
<td>8.9</td>
<td>4.9 × 10⁻³, 91.0</td>
</tr>
<tr>
<td>4</td>
<td>2.3</td>
<td>6.6</td>
<td>4.0</td>
<td>1, 93.2</td>
</tr>
<tr>
<td>5</td>
<td>1.9</td>
<td>16.5</td>
<td>4.5</td>
<td>6.2 × 10⁻², 90.6</td>
</tr>
<tr>
<td>6</td>
<td>4.5</td>
<td>29.1</td>
<td>7.6</td>
<td>2.7 × 10⁻³, 74.2</td>
</tr>
<tr>
<td>7</td>
<td>0.72</td>
<td>6.8</td>
<td>4.0</td>
<td>0.20, —</td>
</tr>
<tr>
<td>8</td>
<td>0.41</td>
<td>16.7</td>
<td>2.9</td>
<td>4.1 × 10⁻³, 89.2</td>
</tr>
</tbody>
</table>

a) The water content of the dry plasma powder was 6.8%.
b) The treatment time was 2 hr.
c) The initial solubility of the dry plasma powder was 95.5%.

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TABLE I. STERILIZATION OF PLASMA POWDER WITH SC-CO₂ AT 35°C AND 200 atm
the effects of the water content of the plasma powder and
the addition of ethanol or acetic acid to SC-CO$_2$ on the
sterilizing action of SC-CO$_2$ at 200 atm and 35 ℃. The
initial water content of the plasma powder was adjusted by
adding sterile water to the dry sample. As SC-CO$_2$

solves water, the water content of the powder decreased
with the treatment. The ratio of living cells decreased
remarkably with an increase in the initial water content
(Runs 1, 2 and 3) without a decrease in the solubility of the
plasma powder in the SC-CO$_2$ treatment. However, the
water content of the treated powder (Runs 2 and 3) was
higher than that of the dry sample (Run 1). A high water
content is not suitable for long storage of plasma powder.

By adding ethanol to SC-CO$_2$, the water content of the
treated powder decreased whereas the sterilizing effect was
not increased (Runs 4, 5 and 6). An increase in the weight
ratio of ethanol to SC-CO$_2$ also caused a decrease in the
solubility of the powder (Run 6). With the addition of
acetic acid to SC-CO$_2$, the sterilizing effect was enhanced
in the case of the dry powder (Run 7). With the wet
powder, the addition of acetic acid to SC-CO$_2$ resulted in
a great decrease in the ratio of living cells as well as in the
final water content without a decrease in the solubility
(Run 8). It seems that the solubility of water in SC-CO$_2$
was enhanced by the addition of ethanol or acetic acid as
an entrainer. Acetic acid is harmless even if it remains in
the plasma. Thus, SC-CO$_2$ containing acetic acid is ex-
pected to be utilizable as a safe sterilizing reagent for
thermally unstable materials, instead of the currently
used methods.

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