Oscillatory High Hydrostatic Pressure Inactivation of Zygosaccharomyces bailii

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ABSTRACT

Zygosaccharomyces bailii inactivation was evaluated in oscillatory high hydrostatic pressure (HHP) treatments at sublethal pressures (207, 241, or 276 MPa) and compared with continuous HHP treatments in laboratory model systems with a water activity (aw) of 0.98 and pH 3.5. The yeast was inoculated into laboratory model systems and subjected to HHP in sterile bags. Two HHP treatments were conducted: continuous (holding times of 5, 10, 15, 20, 30, 60, or 90 min) and oscillatory (two, three, or four cycles with holding times of 5 min and two cycles with holding times of 10 min). Oscillatory pressure treatments increased the effectiveness of HHP processing. For equal holding times, Z. bailii counts decreased as the number of cycles increased. Holding times of 20 min in HHP oscillatory treatments at 276 MPa assured inactivation (<10 CFU/ml) of Z. bailii initial inoculum. Oscillatory pressurization could be useful to decrease Z. bailii inactivation time.

High hydrostatic pressure (HHP) microbial inactivation depends on a number of interacting factors including type and number of microorganisms, magnitude and duration of treatment, temperature, and composition of the suspension media (2, 9–11). Other experimental variables that must be taken into account include compression and decompression rates (9, 11).

Pressure processing cost is a function of time and pressure. A low maximum operating pressure can cause drastic reductions in the fabrication costs. High-pressure processing may be combined with moderately high temperatures or other variables so the operating pressures required are not extremely high (2, 7, 9). The combination of pressure, time, and temperature at which the product is processed must therefore be evaluated carefully (9). The pressure required for microbial inactivation can be reduced if pulsed, successive, or oscillatory HHP treatments are applied. Information on oscillatory HHP use in foods is scarce. Hayakawa et al. (4, 5) reported successful inactivation of Bacillus stearothermophilus spores when oscillatory pressure treatments were combined with 70°C. Alemán et al. (1) on survivor S. cerevisiae counts during pulsed pressurization of inoculated pineapple juice. Successive, pulsed, or oscillatory HHP treatments need to be investigated further with other representative microorganisms. The objective of this research was to evaluate the effect of continuous and oscillatory HHP treatments at 207, 241, and 276 MPa on Zygosaccharomyces bailii suspended in laboratory model systems with a water activity of 0.98 and pH 3.5.

MATERIALS AND METHODS

Culture preparation. A strain of Z. bailii obtained from the Food Laboratory Culture Collection (Universidad de las Americas-Puebla, Mexico) was used. Initial inoculum was prepared by transferring a loopful of a stock culture maintained on potato dextrose agar slants to sterile Sabouraud glucose 2% broth. The inoculated broth was incubated in a rotatory shaker for 48 h at 27 ± 0.5°C. Bacteriological media were purchased from Difco (Difco Laboratories, Detroit, Mich.).

Laboratory model system preparation. Laboratory model system was prepared with Sabouraud glucose 2% broth with sucrose to adjust the aw to 0.98. The required quantity of sucrose needed to reach an aw of 0.98 was determined using Ross (12) and Norrish (8) equations. The 2% glucose in the broth was included in the calculations. The final sucrose concentration was 24.0%. The model system was prepared by dissolving broth and sucrose in deionized water and sterilized at 121°C for 15 min. The sterilized aw-adjusted medium was cooled and acidified to pH 3.5 by addition of filter-sterilized (0.45 μm) citric acid (50%) solution. Water activity was determined with an Aqua Lab CX-2 (Decagon Devices, Inc., Pullman, Wash.) calibrated and operated as described by López-Malo et al. (6). The pH and aw of the model

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Pressure treatments. The model system was poured (50 ml) in sterile bags and inoculated with 0.1 ml of yeast inoculum, giving initial counts of \( \approx 1.6 \times 10^6 \) CFU/ml. The inoculated bags were overwrapped in an outer polyethylene bag containing water and exposed to high hydrostatic pressure treatments at 21°C. The HHP treatments were applied in a warm isostatic pressing system (Engineered Pressure Systems, Inc., Andover, Mass.) with a cylindrical pressure chamber (height = 0.25 m, diameter = 0.10 m). A 5% Mobil Hydrosol 78 water solution was used as pressure medium. The rate of pressure increase and decrease was measured in every experimental trial. A smooth pressure rise of 2.4 MPa/s after an initial delay of 45 s was observed during compression, giving come-up times of 2.2, 2.4, 2.7, 3.1, 4.3, or 5.5 min for working pressures of 207, 241, 276, 345, 517, or 689 MPa, respectively. The decompression time was less than 15 s.

Preliminary HHP treatments were conducted at 207, 241, 276, 345, 517, and 689 MPa for 5 and 10 min. For pressures of 207, 241, and 276 MPa, two HHP treatments were also evaluated: continuous with holding times of 5, 10, 15, 20, 30, 60, or 90 min and oscillatory with one, two, three, or four cycles and holding times of 5 min each, or two cycles with holding times of 10 min each. Three trials with duplicate samples were performed for each treatment. Duration of HHP treatment and cycle were defined as holding time at the desired operating pressure.

Enumeration procedure. Surviving viable yeast counts were determined immediately after the HHP treatment by surface plating onto potato dextrose agar. Two plates were used for each dilution and were incubated at 27°C for 3 to 5 days.

Statistical analysis. Analysis of variance of the effect of HHP treatments on Z. bailii decimal reductions was performed (3). Statistica software (Statsoft, Tulsa, Okla.) was used to analyze the experimental results.

RESULTS AND DISCUSSION

In preliminary HHP experiments \(< 10 \) Z. bailii CFU/ml were observed from an initial inoculum of \( \approx 1.6 \times 10^6 \) CFU/ml when a 5-min or 10-min pressure treatment at 345, 517, or 689 MPa was applied. Treatments at these pressures can be considered lethal for Z. bailii. Therefore, the effect of oscillatory pressurization was investigated at lower pressures, 207, 241, or 276 MPa.

Continuous and oscillatory (a 5-min holding time for each cycle) pressurizations have an important effect on survivor counts (Fig. 1), which was more noticeable with increasing pressure. Lower Z. bailii counts were obtained at 276 MPa in comparison with the other pressure treatments. Lower counts were achieved with oscillatory pressurization; the survivor counts difference between continuous and oscillatory treatments was more noticeable after three or four cycles at 241 or 276 MPa. Hayakawa et al. (4, 5) reported successful inactivation of \( 10^4 \) to \( 10^6 \) B. steatothemophilus spores with HHP treatments at 600 MPa and 70°C when oscillatory pressure treatments of four to six compression–decompression cycles with 5-min holding times in each cycle were applied. Aleman et al. (1) reported greater decimal reductions of inoculated S. cerevisiae after pulsed pressure treatments at 270 MPa in comparison with static or continuous pressurization of pineapple juice. The pulsed high-pressure treatments evaluated by Aleman et al. (1) are quite different from the HHP treatments tested in our work. The holding time of Aleman et al. (1) was varied from 0.5 to 10 s, which permitted a greater number of cycles in a reduced total treatment time.

Greater decimal reductions of Z. bailii initial inocula were obtained with oscillatory HHP treatments, increasing pressure, and pressurization cycles (Table 1). Oscillatory pressurization was tested in an attempt to reduce the process time necessary to achieve a 6-log-cycle reduction of Z. bailii.

<table>
<thead>
<tr>
<th>Pressure (MPa)</th>
<th>207</th>
<th>241</th>
<th>276</th>
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</thead>
<tbody>
<tr>
<td>Continuous, 10 min</td>
<td>1.8 &lt;sup&gt;C&lt;/sup&gt;</td>
<td>3.6 &lt;sup&gt;C&lt;/sup&gt;</td>
<td>4.7 &lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>Continuous, 15 min</td>
<td>2.2 &lt;sup&gt;D&lt;/sup&gt;</td>
<td>4.0 &lt;sup&gt;D&lt;/sup&gt;</td>
<td>5.0 &lt;sup&gt;D&lt;/sup&gt;</td>
</tr>
<tr>
<td>Continuous, 20 min</td>
<td>3.0 &lt;sup&gt;E&lt;/sup&gt;</td>
<td>4.2 &lt;sup&gt;E&lt;/sup&gt;</td>
<td>5.2 &lt;sup&gt;E&lt;/sup&gt;</td>
</tr>
<tr>
<td>Continuous, 30 min</td>
<td>3.7 &lt;sup&gt;F&lt;/sup&gt;</td>
<td>5.3 &lt;sup&gt;F&lt;/sup&gt;</td>
<td>6.2 &lt;sup&gt;F&lt;/sup&gt;</td>
</tr>
<tr>
<td>Continuous, 60 min</td>
<td>4.5 &lt;sup&gt;G&lt;/sup&gt;</td>
<td>6.2 &lt;sup&gt;G&lt;/sup&gt;</td>
<td>6.2 &lt;sup&gt;G&lt;/sup&gt;</td>
</tr>
<tr>
<td>Continuous, 90 min</td>
<td>5.7 &lt;sup&gt;H&lt;/sup&gt;</td>
<td>6.2 &lt;sup&gt;G&lt;/sup&gt;</td>
<td>6.2 &lt;sup&gt;F&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oscillatory, two cycles—5 min each</td>
<td>2.0 &lt;sup&gt;C&lt;/sup&gt;</td>
<td>3.9 &lt;sup&gt;B&lt;/sup&gt;</td>
<td>5.1 &lt;sup&gt;D,F&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oscillatory, three cycles—5 min each</td>
<td>2.3 &lt;sup&gt;D&lt;/sup&gt;</td>
<td>4.2 &lt;sup&gt;E&lt;/sup&gt;</td>
<td>6.2 &lt;sup&gt;F&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oscillatory, four cycles—5 min each</td>
<td>3.2 &lt;sup&gt;E&lt;/sup&gt;</td>
<td>4.6 &lt;sup&gt;H&lt;/sup&gt;</td>
<td>6.2 &lt;sup&gt;F&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oscillatory, two cycles—10 min each</td>
<td>3.0 &lt;sup&gt;E&lt;/sup&gt;</td>
<td>4.3 &lt;sup&gt;E&lt;/sup&gt;</td>
<td>6.2 &lt;sup&gt;F&lt;/sup&gt;</td>
</tr>
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</table>

<sup>a</sup> Average values of six replicates. Treatments with different letters are significantly different \((P < 0.05)\).

<sup>b</sup> The come-up time was 2.2, 2.4, or 2.7 min for 207, 241, or 276 MPa, respectively.
initial inocula. At 276 MPa, after three or four pressure cycles of 5 min each or two cycles of 10 min each, 10^6 inactivation was obtained; however, reductions obtained with oscillatory treatments with a 10-min holding time at 207 or 241 MPa were not significantly different (P < 0.05) from those obtained in continuous or oscillatory treatments with a 5-min holding time. Apparently the decompression step was important for microbial inactivation, at 241 MPa four cycles of 5 min resulted in a significantly greater (P < 0.05) decimal reduction than that obtained with two cycles of 10 min. A pressure treatment at 276 MPa reduced the treatment time to assure a 6-log-cycle reduction. Three cycles with holding times of 5 min each were necessary to achieve a 10^6 reduction in comparison with a 30-min treatment in the continuous HHP experiment (Table 1).

The observed decimal reductions may be attributed to the effect of the holding time on the yeast viability as well as to the cycles of compression-decompression that also favor cell injury. When high pressure is instantly released, it could be assumed that either adiabatic expansion rate of water or aqueous solutions in the cells exceeds the expansion rate of the membranes, or the adiabatic expansion power exceeds the strength of the cell membrane (4, 5). Oscillatory pressure treatments increased the effectiveness of HHP processing; however, the characteristics of the oscillatory HHP treatments need to be optimized further, and it will be necessary to conduct in-depth studies to determine commercial feasibility.

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REFERENCES