Heat Inactivation of *Staphylococcus epidermidis* at Various Water Activities

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Received 18 December 1980/Accepted 9 March 1981

Members of the family *Micrococccaeae* play an important role in food spoilage and even in food poisoning. In contrast to members of the family *Enterobacteriaceae*, these bacteria can grow in media with low water activities. Therefore, the heat resistance of *Staphylococcus epidermidis*, a rather resistant member of the family *Micrococccaeae*, was studied at water activities between 0.87 and 1.00. The heat inactivation curves were clearly biphasic at all temperatures and water activities tested. Especially at low water activities, the *D*-values of the tail phase were extremely high (at 0.87 water activity, a *D*-value at 70°C of 500 s was recorded).

Vegetative microorganisms such as staphylococci and micrococci can grow at water activities (*a*<sub>w</sub>) below 0.92 (2). Such low values normally inhibit growth of other bacteria such as members of the family *Enterobacteriaceae* (5) and aerobic and anaerobic sporeformers (6). To ensure that products with a low *a*<sub>w</sub> are microbiologically stable against staphylococci and micrococci, it is necessary to pasteurize these products.

The heat resistance of *Staphylococcus aureus* has been studied by several investigators (9) but rarely at low *a*<sub>w</sub>. To optimize these pasteurization processes, we investigated the heat resistance of *Staphylococcus epidermidis* in media with *a*<sub>w</sub> of between 1.00 and 0.87 and with sucrose as the *a*<sub>w</sub> -depressing agent at temperatures between 62 and 74°C.

**MATERIALS AND METHODS**

**Strain.** The *S. epidermidis* strain tested was originally isolated from an ambient-stable minced-meat product (sausage rolls) and classified according to the scheme of Baird-Parker (1).

Preparation of the inoculum and heating experiments. *S. epidermidis* was cultivated in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) for 48 h at 30°C. The cells were collected by centrifugation and washed twice with distilled water. Finally, the pellet was resuspended in the substrate to be tested (density, ~10<sup>8</sup> cells per ml) and left at room temperature for 30 min to reach a osmotic equilibrium with the substrate. The osmotic pressures of the heating media were regulated by varying the concentration of sucrose. The *a*<sub>w</sub> of the medium was measured with the Sina-scope (Sina A.G., Zurich, Switzerland). The pH was adjusted to 6.5 by adding HCl (0.1 mol/liter). Then, 0.3 ml of cell suspension was added to 30 ml of the heating medium, the temperature of which was controlled within 0.25°C. At preset time intervals, 1 ml of the heating menstruum was removed and suspended in 9 ml of diluent containing 1 g of peptone (Difco) and 8.5 g of NaCl per liter. The number of surviving microorganisms was determined in duplicate by dissemination of appropriate serial decimal dilutions into brain heart infusion agar. Colonies were counted after 3 days of incubation at 30°C.

**RESULTS AND DISCUSSION**

Most of the heat resistance curves of *S. epidermidis* are not linear but rather show a shoulder, a logarithmic phase, and a tail phase. In some cases, the *D*-values of the tail phase are extremely high, suggesting an effective process of stabilization of the target site(s) against heat inactivation.

Table 1 summarizes the *D*-values of the logarithmic and tail phases. It was difficult to establish accurately the low number of survivors in the tail phase, so the *D*-values of the tail phase must be interpreted with some reserve. The temperature dependence of the killing rate (*k*<sub>d</sub>) of the logarithmic inactivation phase at different *a*<sub>w</sub> is in good agreement with the Arrhenius equation (Fig. 1). As has been found earlier for *Citrobacter freundii* (8) and *Klebsiella pneumoniae* (7), the activation energy (*E*<sub>a</sub>) of the denaturation process decreases as the osmotic pressure of the heating medium increases (Table 2). Extrapolation of the Arrhenius curves obtained shows that these converging lines will meet at about 61°C at a *D*-value of about 800 s. This suggests that, at 60°C, the heat resistance is independent of the osmotic pressure. However, the experimental *D*-value of the inactivation at 60°C in water was only 12 s. This large difference between the extrapolated and the measured values indicates that there is no simple relation between the killing rate of *S. epidermi-
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dis and the osmotic pressure of the heating menstruum. By plotting the \( \ln k \)-values (determined at 66 and 68°C) against the \( a_w \) of the medium, we indeed observed a sharp bend at an \( a_w \) of about 0.94 (Fig. 2).

For *C. freundii* (8) we obtained \( \ln k = \ln k_0 - \alpha_T \) for the relation between the sucrose concentration (\( \alpha_T \)) and the inactivation rate.

With the same equation we calculated the \( \alpha_T \)-values for *S. epidermidis* (Table 3). Whereas for *C. freundii* \( \alpha_T \) depends strongly on the temperature, for *S. epidermidis* there is hardly any influence of the temperature on the \( \alpha_T \)-values. Figure 3 clearly shows that there is a difference in activation energy of the logarithmic and tail phases of inactivation. This difference suggests that the crucial inactivation mechanisms differ.

**Table 1.** D-values of the logarithmic and tail phases of heat inactivation curves of *S. epidermidis*

<table>
<thead>
<tr>
<th>Phase</th>
<th>( a_w )</th>
<th>D-value(s) at heating temp (°C) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log</td>
<td>1.00</td>
<td>64 66 68 70 72 74</td>
</tr>
<tr>
<td></td>
<td>0.99</td>
<td>22 24 26 28 30 32</td>
</tr>
<tr>
<td></td>
<td>0.97</td>
<td>54 56 58 60 62 64</td>
</tr>
<tr>
<td></td>
<td>0.95</td>
<td>324 340 356 372 388 404</td>
</tr>
<tr>
<td></td>
<td>0.93</td>
<td>240 256 272 288 304 320</td>
</tr>
<tr>
<td></td>
<td>0.91</td>
<td>228 244 260 276 292 308</td>
</tr>
<tr>
<td></td>
<td>0.87</td>
<td>204 220 236 252 268 284</td>
</tr>
<tr>
<td>Tail</td>
<td>1.00</td>
<td>- - - - - - - - - - - - - - - - -</td>
</tr>
<tr>
<td></td>
<td>0.99</td>
<td>- - - - - - - - - - - - - - - - -</td>
</tr>
<tr>
<td></td>
<td>0.97</td>
<td>- - - - - - - - - - - - - - - - -</td>
</tr>
<tr>
<td></td>
<td>0.95</td>
<td>1,200 1,300 1,400 1,500 1,600 1,700</td>
</tr>
<tr>
<td></td>
<td>0.93</td>
<td>1,500 1,600 1,700 1,800 1,900 2,000</td>
</tr>
<tr>
<td></td>
<td>0.93</td>
<td>1,800 1,900 2,000 2,100 2,200 2,300</td>
</tr>
<tr>
<td></td>
<td>0.91</td>
<td>1,600 1,700 1,800 1,900 2,000 2,100</td>
</tr>
<tr>
<td></td>
<td>0.87</td>
<td>1,400 1,500 1,600 1,700 1,800 1,900</td>
</tr>
</tbody>
</table>

* — Could not be determined accurately; number of survivors, 10 to 100.

**Table 2.** Activation energies (\( E_a \)) for heat inactivation of *S. epidermidis*

<table>
<thead>
<tr>
<th>( a_w )</th>
<th>Temp range (°C)</th>
<th>( E_a ) (MJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.91</td>
<td>66-72</td>
<td>0.210</td>
</tr>
<tr>
<td>0.93</td>
<td>66-74*</td>
<td>0.229</td>
</tr>
<tr>
<td>0.93</td>
<td>66-74†</td>
<td>0.351</td>
</tr>
<tr>
<td>0.95</td>
<td>64-70</td>
<td>0.262</td>
</tr>
</tbody>
</table>

* Log phase.
† Tail phase.

**Fig. 1.** Arrhenius plots of the inactivation of *S. epidermidis*. Symbols: (V) \( a_w = 0.98; \) (□) \( a_w = 0.93; \) (△) \( a_w = 0.91; T, Temperature.**
for the two phases.

Conclusions. *S. epidermidis* is a rather heat-resistant bacterium. Most probably due to an unknown heat adaptation mechanism, the *D*-values of the tail phases far exceed those for members of the family *Enterobacteriaceae* (3), including the heat-stable *Salmonella senftenberg* (4), and are of the same order of magnitude as the *D*-values for *Streptococcus faecalis* (10).

The rather high activation energy, especially for the tail phase of the inactivation reaction, makes a short-term, high-temperature pasteurization process most attractive for eliminating staphylococci and micrococci from foods with low *a*.<

**LITERATURE CITED**


