Inactivation of *Bacillus stearothermophilus* Spores in Soybean Water Extracts at Ultra-High Temperatures in a Scraped-Surface Heat Exchanger

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ABSTRACT

The kinetics of thermal inactivation of *Bacillus stearothermophilus* spores in water extracts of soybeans ("soymilk") was studied using a pilot scale scraped-surface heat exchanger. Survivor curves followed typical first-order inactivation reactions, with D-values ranging from 21.9 sec at 259°F to 5.3 sec at 268°F. The z-value was 15°F, corresponding to an activation energy of 88.6 kcal/mole. Lethal effects in the heating and cooling cylinders accounted for more than 50% of the inactivation above a heater outlet (holding) temperature of 265°F.

Thermal processing is one of the most important methods for preserving food materials. However, concomitant with the inactivation of pathogenic organisms and/or spores may be a reduction in the level of certain nutrients and other quality factors. As processing temperature is increased, however, the rate of microbial inactivation becomes much higher than the rate of destruction of most nutrients or other quality factors. This is the basis for high temperature/short time (HTST) and ultra-high temperature (UHT) processing of foods. Thus, UHT processes, generally in the range of 250-300°F (with the appropriate low holding time) can result in a very high sterilizing effect with relatively little change in chemical, nutritional and organoleptic quality of food.

The sterilizing efficiency of UHT processes is assessed primarily in the laboratory using small volumes of spore suspensions sealed in capillary tubes, heating by immersion in a fluid and determining the proportion of surviving spores. Unfortunately, data obtained in this manner do not always match that obtained in actual large-scale plant situations (5,7). Burton et al. (1) reported that practical sterilization may show a greater rate of change of thermal death at ultra high temperature than would be expected from capillary tube experiments. In addition, because of the very short heating times involved at temperatures exceeding 250°F, the results may have to be extrapolated to estimate sterilization efficiencies in the UHT range. Thus small inaccuracies in the thermal death data obtained at lower temperatures will lead to substantial errors in the UHT region. Therefore it is necessary to evaluate UHT processes with experiments and operating conditions as close to practice as possible rather than by extrapolation from laboratory results.

One method particularly suited for obtaining the ultra-high temperatures and short times required to produce a satisfactory product, especially for proteinaceous liquid foods containing particulates, is the scraped-surface heat exchanger (SSHE). In these units, a concentrically mounted shaft, fitted with blades, rotates within a cylinder. The product to be processed is pumped into one end of the SSHE cylinder and is continuously agitated and scraped from the heat transfer surface by the blades. This minimizes fouling and burn-on problems on the cylinder wall. In addition to the agitation and scraping effects of the blades, the turbulence induced in the bulk of the fluid near the heat transfer surface makes heat transfer more efficient (3).

This paper presents results of an investigation of the efficacy of a vertical, liquid-full SSHE for sterilization of a model, low-acid proteinaceous liquid food (water extracts of soybeans or "soymilk"). In low acid foods with pH greater than 4.5, spores of *Clostridium botulinum* are generally used as the criterion for evaluating the success of a thermal processing treatment (12,20). However, because of the hazards involved with the large-scale experimental use of *C. botulinum*, *B. stearothermophilus* spores were used instead.

MATERIALS AND METHODS

UHT processing system

A schematic process flow diagram of the experimental equipment is shown in Fig. 1. Two feed tanks were used, one filled with water and the
other with the model liquid food ("soymilk"), both kept at a
temperature of 120-122 F. Prior to the experiments, the equipment was
sterilized by circulating hot water from one feed tank throughout the
system. The soymilk was at a pH of 5.9 and was inoculated with spores of
B. stearothermophilus to give a count of \(10^8\) spores/ml. The
inoculated raw soymilk was constantly agitated with a mechanical
stirrer.

Figure 1. Schematic flow diagram of the ultra-high tempera-
ture continuous sterilization of liquid foods. 1 = soy milk
supply tank, 2 = water supply tank, 3 = three-way valve, 4 =
pump system, 5 = mass flow meter, 6 = SSHE heater, 7 =
holding tube, 8 = SSHE cooler, 9 = motor controlling blade
rotation, 10 = back-pressure valve, 11 = sampling point | S =
steam, CW = cold water.

The soymilk or water was pumped through a 3-way valve connecting
these two feed tanks by a high-pressure reciprocating pump with a
variable speed drive (Manton-Gaulin Mfg. Co., Everett, MA), which
served as a timing pump. Mass flow rate was maintained at 10.8 ± 0.1
lb/min for all experiments and was measured with a mass flow sensor
(Model LSOA, Micro-Motion, Inc. Boulder, CO). The soymilk was
heated to ultra-high temperatures (250-280 F) in the SSHE heater using
high pressure steam of 8.1 psig. The

Analytical methods
Total solids and ash content were measured by gravimetric
procedures (9). Protein content was measured by the microbriquet
method (4) using bovine serum albumin as the standard. Total lipid
was measured by a Soxhlet method modified specifically for soybean
samples using chloroform:methanol (2:1 v/v) as solvent (6). Carbohydrate is expressed as the difference between total solids and
protein, fat and ash.

Preparation of test organism
Bacillus stearothermophilus NCA 1518 was a gift from the late Dr. Z.
J. Ordal, University of Illinois. It was maintained on nutrient agar
slants. Actively growing vegetative cells were prepared for inoculating the
surface of the sporulating medium. Cells from slants were used to
inoculate a flask containing 50 ml of nutrient broth (Difco). After 12 h
of growth on a shaker-incubator at 55 C, cells were removed from the
incubator. One hundred ml portions of nutrient agar (Difco)
supplemented with 0.03% of MnSO<sub>4</sub> (6) were poured into 10-oz
prescription bottles. After the medium was autoclaved it was allowed to
solidify laying on its side. Agar in each bottle was then inoculated by
spreading 0.5 ml of the cell suspension evenly over the entire surface.
The spore crops were grown on the surface of agar for 3 days at 55 C.
Then 10 ml of sterile, distilled water were added to each bottle, and the
surface of each bottle was gently scraped with a sterilized tooth. The liquid
from the bottles were pooled, centrifuged and washed three times with
sterile, distilled water. The washed spores were treated with 0.1 mg/ml
lysozyme, 3x crystalline Sigma Chemical Company, St. Louis, MO),
and placed in a shaker at 37 C for 3 h, then washed 6 times by
centrifugation with sterile, distilled water. The spore slurry was suspended in water and stored at 4 C for approximately one month.

Recovery and counting
Dextrose tryptone agar (DTA, Difco) plus 0.5% soluble starch was used
as the recovery medium. The plates were inverted and incubated
at 55 C for 72 h and then counted. Duplicate or triplicate readings on
plates were taken for each sample. Sterile, deionized water was used as the
diluent in preparing dilutions for plating.

Activation of spores
Five 10-ml portions of spore-inoculated raw soymilk were taken from
the feed tank and dispensed into screw-capped tubes. These suspensions were heated in water at 100 C for five different time periods
and then cooled immediately in an ice bath. Plate counts were made from
each tube, using DTA plus 0.5% soluble starch as the plating medium.
The plates were incubated at 55 C for 72 h.

RESULTS AND DISCUSSION
Heat activation for spore germination
In any thermal resistance determination, it is essential to
obtain an accurate estimate of the initial spore
population before the application of heat. Many freshly
prepared spore suspensions will not germinate unless
activated. Heat is an effective method of breaking the
dormant state of spores (10), although the exact
time/temperature combination to use depends on the
species, composition of the media on which the spores
have been grown, age of the spore suspension and other
factors (7). The results of heat activation experiments for
our system are shown in Fig. 2. Our optimum
conditions were 100 C for 40 min which can be compared to
similar studies of Mikolajcik and Rajkowski (13) who
obtained 100 C for 10 min for B. stearothermophilus
ATCC 7953 in a commercial soy protein infant formula at
pH 6.5, and to 100 C for 60 min in deionized water at
pH 6.5 (18).
UHT inactivation of Bacillus Spores

The selection of a recovery medium was based on the relative ability of various media to support growth of severely heated cells as well as their ability to support growth of unheated cells. Various types of media may give equal estimates of the number of viable unheated spores but divergent estimates of the number of spores surviving heat treatment.

For the initial (control) counts, the spores suspended in raw soymilk (not processed in the UHT system) were heat shocked at 100°C for 40 min and then enumerated in various media. The results in Table 1 show that the addition of soluble starch to DTA affects the spore count significantly. Similarly, spores suspended in soymilk processed at 262°F for 4.19 sec in the SSHE-UHT system also showed better recovery with starch in the recovery medium. Hence DTA plus 0.5% soluble starch was used as the recovery medium in all subsequent experiments.

**TABLE 1. The effect of the addition of soluble starch to plating medium (DTA) on the recovery and growth of B. stearothermophilus spores.**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Heat shocked&lt;sup&gt;a&lt;/sup&gt;</th>
<th>UHT Processed&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTA</td>
<td>$8.0 \times 10^3$</td>
<td>$3.8 \times 10^3$</td>
</tr>
<tr>
<td>DTA + 0.1% Starch</td>
<td>$9.1 \times 10^3$</td>
<td>$5.2 \times 10^3$</td>
</tr>
<tr>
<td>DTA + 0.3% Starch</td>
<td>$12.5 \times 10^3$</td>
<td>$6.4 \times 10^3$</td>
</tr>
<tr>
<td>DTA + 0.5% Starch</td>
<td>$19.3 \times 10^3$</td>
<td>$8.1 \times 10^3$</td>
</tr>
<tr>
<td>DTA + 0.7% Starch</td>
<td>$16.1 \times 10^3$</td>
<td>$7.4 \times 10^3$</td>
</tr>
<tr>
<td>DTA + 0.9% Starch</td>
<td>$18.3 \times 10^3$</td>
<td>$7.4 \times 10^3$</td>
</tr>
</tbody>
</table>

<sup>a</sup>Sporous were heat shocked at 212°F for 40 min prior to counting; average count per 3 plates.

<sup>b</sup>Sporous seeded in raw soymilk, UHT processed as described in text at 262°F for 4.19 sec and counted; average per 2 plates.

**Figure 2. Heat activation of B. stearothermophilus spores by heating at 100°C for various times.**

Thermal processing treatments: Survivor curves

Ideally such a study should encompass a range of heating temperatures as wide as possible, with a minimum of 4-6 temperatures spaced about ±2 units apart (11). Accordingly, preliminary experiments were done with spore-inoculated raw soymilk at 6 temperatures from 250°F to 280°F at intervals of 6°F. However, there was no significant inactivation at heater outlet (and holding) temperature of 250°F up to the maximum holding time studied. When the heater outlet temperature was 256°F, colony counts increased with increased holding times up to 6.78 sec and then decreased with longer holding time. This is probably due to the acceleration of germination of spores by sub-lethal heating (4), i.e., samples processed at this temperature for shorter holding times were being inactivated at a rate slower than the rate of activation, thus manifesting itself as an increase in the number of survivors. At higher temperatures, heat activation occurred at higher rates and is completed in a shorter period of time, so the above effect was masked and could not be detected.

At the higher end of the temperature range (greater than 268°F), however, the rate of inactivation was so rapid that enumeration of the survivors was difficult. At 274°F, more than 90% of the spores were inactivated in the SSHE heater and cooler alone. It was impossible in practice to overcome this problem by increasing the initial load of spores in the raw soymilk. For each experiment, over 150 gal of soymilk were processed; even with the maximum inoculation of $10^9,10^{11}$ spores, it resulted in a final count of only $10^8-10^9$ spores/ml. Apart from the effort involved in producing such a large quantity of spores, problems of spore-aging and batch inconsistency could occur (6).

Owing to these practical constraints in our experiments, the data were obtained at temperatures of 259-268°F at holding times of 1.56-10.89 sec. Figure 3 shows the survivor curves under these conditions, obtained by plotting the logarithms of the surviving fraction ($N\lbrack t\rbrack /N_0$) versus holding time, where $N$ is the spore count after a particular treatment and $N_0$ is the initial spore count. Survivor curves at each temperature were obtained by a linear regression analysis using the least squares method. All survivor curves in the 259-268°F temperature range followed a first-order rate of inactivation. Reaction rate constants ($k$) and corresponding D-values are listed in Table 2.

Significant lethal effects were observed in the heating and cooling cylinders also. When the survivor curves in Fig. 3 were extrapolated back to zero holding time, none of the curves intercepted the surviving fraction at the initial count ($N\lbrack t\rbrack =1$). This implied that a certain degree of lethality was obtained in the come-up (in the SSHE heater) and cool-down (in the SSHE cooler) periods. At the flow rate used here, the mean residence time was about 51 sec in the heater and about 75 sec in the cooler. At 262°F, approximately 8% of the spores were killed in the heater and cooler, 50% at 265°F and...
67% at 268 F; preliminary experiments had earlier indicated about 90% were inactivated at a heater outlet temperature of 274 F. The interrelationship between lethal effects, heat transfer and residence time distribution in the SSHE heater have been discussed by Cuevas et al. (2).

**Temperature effects**

Figure 4 shows an Arrhenius plot of the survivor data. The activation energy obtained was 88,645 cal/mole corresponding to a z-value of 14.98 F. Table 3 compares our data to some found in the published literature. Other z-values that have been reported are 16.9 F for TH24 spores (7) and 15 F (21) and 12.6 F (12) for FS7954 spores. The wide variation is obviously due to factors such as the strain of the test organism, sporulation conditions, enumeration methods, medium or product used, type of equipment used for heat treatment and others. However, our data obtained under conditions simulating actual commercial processing conditions in a continuous flow scraped-surface heat exchanger falls close to data obtained by others workers using different techniques and should prove particularly useful in designing thermal processes for proteinaceous low-acid liquid foods.

![Figure 4: Effect of temperature on reaction rate constant for B. stearothermophilus in soymilk. ΔE = activation energy, R = correlation coefficient.](image)

**TABLE 2. Thermal death rate constants (k) and decimal reduction times (D) of B. stearothermophilus in soymilk.**

<table>
<thead>
<tr>
<th>Temperature (F)</th>
<th>D-value (sec)</th>
<th>k (sec⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>259</td>
<td>21.85</td>
<td>0.1054</td>
</tr>
<tr>
<td>262</td>
<td>14.10</td>
<td>0.1633</td>
</tr>
<tr>
<td>265</td>
<td>9.80</td>
<td>0.2351</td>
</tr>
<tr>
<td>268</td>
<td>5.31</td>
<td>0.4340</td>
</tr>
</tbody>
</table>

**TABLE 3. D- and z-values for B. stearothermophilus in liquid products reported in the literature.**

<table>
<thead>
<tr>
<th>Product or suspending medium</th>
<th>Temperature range (F)</th>
<th>Heating equipment</th>
<th>Spore type</th>
<th>Dₜ₉₅₅</th>
<th>z-value</th>
<th>Source of data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk protein base formula</td>
<td>239-257</td>
<td>Screw-capped tubes</td>
<td>ATCC 7953</td>
<td>3.56</td>
<td>13.94</td>
<td>Ref. 13</td>
</tr>
<tr>
<td>Soy protein base formula</td>
<td>239-257</td>
<td>Screw-capped tubes</td>
<td>ATCC 7953</td>
<td>3.64</td>
<td>13.70</td>
<td>Ref. 13</td>
</tr>
<tr>
<td>Distilled water</td>
<td>239-298</td>
<td>Stirred vessel</td>
<td>Not specified</td>
<td>0.17</td>
<td>11.70</td>
<td>Ref. 19</td>
</tr>
<tr>
<td>Distilled water</td>
<td>248-320</td>
<td>Capillary tubes</td>
<td>TH 24</td>
<td>13.10</td>
<td>13.5</td>
<td>Ref. 5</td>
</tr>
<tr>
<td>Milk</td>
<td>248-320</td>
<td>Capillary tubes</td>
<td>TH 24</td>
<td>6.45</td>
<td>13.8</td>
<td>Ref. 5</td>
</tr>
<tr>
<td>Soy extracts</td>
<td>259-268</td>
<td>Scraped-surface heat exchanger</td>
<td>NCA 1518</td>
<td>1.63</td>
<td>14.9</td>
<td>This work</td>
</tr>
</tbody>
</table>

**ACKNOWLEDGMENTS**

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REFERENCES


Figure 4. Thin layer chromatogram of polycyclic hydrocarbons including benzo(a)pyrene. Absorbent=Acetyl cellulose and solvent system=Methanol-ether-water (4: 4: 1).