Increased Heat Resistance of Salmonellae in Beef with Added Soy Proteins

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ABSTRACT

Raw beef inoculated with cells of a composite of five Salmonella strains was heated at 54 or 60°C. Survivors were enumerated by plating samples in plate count agar (PCA), XL agar or PCA followed by an XL agar overlay. Best differential recoveries of salmonellae were effected by incubation of PCA plates for 4 h at 37°C followed by overlay with XL agar and incubation for an additional 44 h. D-values of salmonellae at 54 and 60°C were increased significantly when ground beef was supplemented with 30% textured soy protein, soy protein concentrate or isolated soy protein. Increased heat resistance appeared to be caused by an increase in the pH of beef from 5.8-5.9 to 6.1 upon addition of the soy proteins. After adjusting the pH of mixtures of beef and soy proteins with hydrochloric or lactic acid to 5.8 to 5.9, survival of salmonellae to heat was reduced to the level of survival in beef alone. The pH of beef with added structured soy isolate was the same as beef, and heat resistance of salmonellae was not increased in this product. In the pH range 5.9 to 7.1, the maximum heat resistance of salmonellae in beef containing textured soy protein occurred at pH values of 6.5 to 6.8.

Prevention of salmonellae outbreaks in foods can be accomplished by limitation of contamination, inhibition of growth and destruction of the organism (6). Destruction of salmonellae, especially in fresh meats, is often accomplished by heating. However, some meats, such as beef, are often eaten after being cooked to temperatures that are not lethal for salmonellae. In the period 1973 to 1976, inadequate cooking or reheating contributed to 34% of the 61 outbreaks of salmonellosis (26). Outbreaks of salmonellosis have involved consumption of raw hamburger (8) and "rare" roast beef (9-12). The outbreaks in rare roast beef prompted studies to determine time-temperature processes for production of Salmonella-free roast beef (4,5,18) and federal regulations specifying commercial cooking procedures (1).

Soy proteins are increasingly being used in meat systems. Studies have shown that soy proteins can affect the growth of spoilage bacteria (3,16,20,22,31), enterotoxin formation by Staphylococcus aureus (15), growth (7,25) and sporulation (17) of Clostridium perfringens and neurotoxin formation by Clostridium botulinum (28,29).

The present study was undertaken to determine if cooking procedures adequate for destruction of salmonellae in ground beef would also apply to beef-soy formulations.

MATERIALS AND METHODS

Preparation of beef and soy products

A fresh, wholesale beef rib and beef kidney fat were purchased locally. Surface areas of the rib were singed with a Bunsen burner and trimmed. Bones were removed and the meat was ground aseptically. The ground beef and beef kidney fat were frozen until needed. Commercially prepared soy products used in the study were: (a) textured soy protein (TSPA) from Farmland Agriservices, Inc. (56% protein, dry basis), (b) textured soy protein (TSPB) from Archer Daniels Midland Co. (52% protein), (c) textured soy protein concentrate (TSC) from Staley Manufacturing Co. (72% protein), (d) isolated soy protein (ISP) from Ralston Purina Co. (92% protein), and (e) structured soy protein isolate (SSI) from Ralston Purina Co. (93% protein). The soy proteins were hydrated three to one (3.0 ml of sterile distilled water per g of soy product), unless otherwise stated, and combined with the ground beef to give a range of 5 to 50% (w/w) soy level in the final product. Kidney fat (95% fat) was added to give a final fat concentration equivalent to that of the ground beef control (21%). The final product was mixed in a meat grinder (Sears 400.8260).

In some experiments, the pH of the beef-soy product was adjusted by adding 5 N NaOH, 6 N HCl or 45% lactic acid to the water used to hydrate the soy products.

Inoculation

Salmonella newport, Salmonella manhattan, Salmonella lexington, Salmonella muenchen and Salmonella braenderup serotypes were obtained from N. A. Cox, Russell Research Center, Athens, GA. Stock cultures were maintained on slants of brain heart infusion (BHI) agar (Difco) at 4°C. From stock cultures, 10 ml of BHI broth (Difco) were inoculated and incubated at 37°C for 24 h. A 0.1-ml portion was transferred to each of 10 ml of BHI broth and incubated at 37°C for 48 h. Two 10-ml portions of the 48-h culture for each serotype of Salmonella were combined followed by centrifugation at 14,500 x g for 15 min. The pellet from each serotype was resuspended in 2.0 ml of saline, and equal portions from suspensions of each of the five serotypes were combined for the inoculum.

Beef or beef-soy formulation (20 to 30 g) was placed in a sterile plastic Stomacher ‘80’ bag (Seward) and inoculated with 0.1 ml of inoculum per 10 g of sample. Each 0.1 ml of inoculum was mixed by stomaching the sample for 30 s. Inoculated samples were regrind aseptically and loaded into 12-ml disposable Lancer liquid transfer pipets. Approximately 1.0-g quantities were dispensed into aluminum thermal death time tubes (25). Inoculated samples were held at 4°C for less than 3 h until tests were conducted. Mean salmonellae counts of beef and beef-soy samples before submersion in the water bath were 3.6 x 10^7 g.
Survival of salmonellae in beef or beef-soy

Heat-survival characteristics of the salmonellae inoculum were determined at 54 and 60°C. Aluminum tubes were completely submerged in a water bath preadjusted to the test temperature. The temperature was monitored with a Telethermometer (YSI Model 42SC) equipped with a thermistor probe inserted midway into an aluminum tube containing sample. The open end of this tube was sealed with caulking compound. When the temperature of the beef or beef-soy samples was equivalent to the water bath temperature (45 s at 54°C, 60 s at 60°C), triplicate samples were removed and immediately plunged into an ice-water bath. These samples were considered to be heated at the test temperature for "zero" minutes. At appropriate intervals, triplicate samples were removed from the water bath. Uninoculated samples were also heated and monitored for contaminants.

Recovery of heated cells

After cooling, the content of each tube was ejected into a Stomacher '80' bag (Seward) by using a steel rod of a diameter equal to the inside diameter of the aluminum tube. After weighing the samples, sterile 0.1% peptone was added to give an initial tenfold dilution. The sample was mixed for 30 s with a Colworth Stomacher 80, and serial dilutions were prepared with 0.1% peptone. Unless otherwise stated, portions of dilutions were mixed with 5 ml of plate count agar (PCA, Difco) in petri plates, and the plates were incubated at 37°C for 4 h to allow injured cells to recover. Then 10 ml of XL agar base (Difco) supplemented with sodium thiosulfate and ferric ammonium citrate were overlayed, and the plates were incubated for an additional 44 h.

Recovery on various media of a salmonellae composite from beef heated at 60°C. PCA = plate count agar (10 ml) incubated at 37°C for 36 h; PCA + 4 h + XL = plate count agar (5 ml) incubated at 37°C for 4 h followed by overlay of XL agar (10 ml) and incubated at 37°C for 44 h; PCA + XL = plate count agar (5 ml) followed by immediate overlay of XL agar (10 ml) and incubated at 37°C for 48 h; XL = XL agar (10 ml) incubated at 37°C for 48 h. Bars represent 95% confidence limits.

RESULTS

Recovery medium for heated salmonellae

Raw beef used in this study was trimmed to remove the majority of contaminants, and 10^2 to 10^5 colonies per g of uninoculated ground beef were detected on PCA incubated at 37°C. To differentiate salmonellae from other bacteria, XL agar was used for plating. Contaminants from raw beef did not form colonies typical of salmonellae on this medium. XL agar is not considered a selective medium, but our results (Fig. 1) showed that recoveries of heat-injured cells were low on XL agar. In preliminary experiments, salmonellae injured by heating in beef at 60°C were able to recover in pour plates of PCA after 4 h, but not completely in 2 h at 37°C (data not shown). Cells injured after heating at 54°C could recover in less than 4 h (data not shown). After 4 h of incubation, subsequent overlay with XL agar allowed the growth of repaired cells and formation of typical salmonellae colonies.

The D-values, as calculated from linear regression analysis of survivor curves, were not different (P < 0.05) when comparing recovery on PCA, PCA with immediate XL overlay, or PCA with incubation for 4 h at 37°C before the XL overlay was applied (Fig. 1). The D-value was lower for recovery on XL agar alone. The total number of cells recovered was similar with PCA and PCA + 4 h at 37°C + XL (P > 0.05), but was significantly lower on PCA with immediate XL overlay and lower still on direct pour plates of XL agar. For instance, after 2.5 min at 60°C, recovery of salmonellae on PCA was greater by 2 log_{10} than on XL agar. Similar results were observed with XL agar from another manufacturer. Recovery of salmonellae as shown in subsequent figures and tables was by plating on PCA + 4 h at 37°C + XL agar overlay.

Effect of soy additives in beef on heat resistance of salmonellae

Survival curves for the salmonellae inoculum in beef and in beef substituted with 30% TSPA are shown for samples heated at 54°C (Fig. 2) and 60°C (Fig. 3). Results of linear regression analysis of the data indicated D_{54} values of 16 min in beef and 23 min in beef + TSPA and a D_{60} value of 0.9 min in beef and 1.3 min in beef + TSPA. These differences were significant (P < 0.05) at both temperatures.

D-values in beef supplemented at the 30% level with two textured soy proteins, a textured soy concentrate and an isolated soy protein were significantly higher than D-values in beef alone (Table 1). The increased heat resistance was observed at both 54 and 60°C with added soy proteins hydrated with three parts water to one part soy. Similar results were observed at 54°C for heat resistance of
Heat resistance of salmonellae in beef with increasing concentration of soy protein

By increasing the concentration of TSPA in beef, the pH increased from 5.9 in beef alone to 6.3 in beef + 50% TSPA (Table 2). The number of cells surviving exposure to heating at 54°C for 45 min increased at higher concentrations of soy product. Greater survival than in beef (P = 0.05) was observed at soy concentrations of 20% and higher.

**Determination of moisture and pH**

The moisture content was 60% for raw ground beef and 58 to 60% in beef + 30% TSP, SPC, ISP or SSI. The water activity was 0.99 as determined for beef and beef + 30% TSP (data not shown).

The pH value for beef was 5.8 to 5.9 (Tables 2 and 3), and for beef + 30% TSP, SPC or ISP was 6.1 (Table 3). SSI fiber is sold at a pH of 5.2, a value which is not very functional in meats. According to the manufacturer, the pH was increased with sodium carbonate solution so that the final pH of beef + 30% SSI was 5.9.

**Effect of pH of soy proteins on heat resistance of salmonellae in beef**

The survival of salmonellae to heating at 54°C for 45 min in beef + 30% TSPA, TSPB, TSC or ISP was significantly higher than in beef (Table 3). These data support those in Table 1 in which D-values for salmonellae in beef alone (pH 5.8) and for beef + 30% TSP, SPC or ISP were higher than D-values in beef. When the pH of the beef-soy combinations was adjusted to pH 5.8 to 5.9 by hydration of soy products with water acidified with hydrochloric acid, the survival of salmonellae was not significantly different or was lower than in beef alone (pH 5.8). Similar results were observed when the pH values of beef-soy combinations were lowered with lactic acid to the pH of beef. When the pH of beef + 30% TSPA was adjusted to the range of 5.9 to 7.1, maximum survival of the salmonellae composite was in the range 6.6 to 6.8 for Trial 1, with approximately a 2-log$_{10}$ greater survival than at pH 5.9 (Table 4). For another batch of beef (Trial 2), a maximum survival was in the range of pH 6.5 to 6.8, with approximately a 2.5-log$_{10}$ greater survival than at pH 5.9.

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**Table 2. Survival of salmonellae after heating at 54°C for 45 min in beef or beef + textured soy protein A (hydrated 3 parts water to 1 part soy product).**

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>Survival (log$_{10}$)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td>5.87</td>
<td>4.54a</td>
</tr>
<tr>
<td>+ 5% TSPA</td>
<td>5.94</td>
<td>4.68c</td>
</tr>
<tr>
<td>+ 10% TSPA</td>
<td>6.00</td>
<td>4.69g</td>
</tr>
<tr>
<td>+ 20% TSPA</td>
<td>6.07</td>
<td>5.12a</td>
</tr>
<tr>
<td>+ 30% TSPA</td>
<td>6.17</td>
<td>5.72e</td>
</tr>
<tr>
<td>+ 40% TSPA</td>
<td>6.28</td>
<td>5.95f</td>
</tr>
<tr>
<td>+ 50% TSPA</td>
<td>6.31</td>
<td>6.30g</td>
</tr>
</tbody>
</table>

*Each pH value is the mean of five measurements.

*Values followed by different letters are significantly different (P = 0.05).

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**Table 1. D-values (min) at 54 and 60°C for salmonellae in beef or beef supplemented with 30% hydrated soy product.**

<table>
<thead>
<tr>
<th>Soy product</th>
<th>Temperature</th>
<th>54°C</th>
<th>60°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Textured soy protein A</td>
<td>23(16)a</td>
<td>1.3(0.9)a</td>
<td></td>
</tr>
<tr>
<td>Textured soy protein B</td>
<td>19(14)a</td>
<td>1.1(0.8)a</td>
<td></td>
</tr>
<tr>
<td>Textured soy concentrate</td>
<td>23(15)a</td>
<td>1.0(0.8)a</td>
<td></td>
</tr>
<tr>
<td>Isolated soy protein</td>
<td>23(18)a</td>
<td>1.0(0.8)a</td>
<td></td>
</tr>
<tr>
<td>Structured soy isolate</td>
<td>16(15)</td>
<td>0.7(0.7)</td>
<td></td>
</tr>
</tbody>
</table>

*Values followed by a letter are significantly different (P = 0.05) from the D-value of the beef control (in parentheses).
TABLE 3. Survival of salmonellae after heating at 54°C for 45 min in beef or beef supplemented with hydrated (3 to 1) soy products, with or without pH adjustment.

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Survival (log&lt;sub&gt;10&lt;/sub&gt; g&lt;sup&gt;d&lt;/sup&gt;)</th>
<th>pH&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Survival (log&lt;sub&gt;10&lt;/sub&gt; g&lt;sup&gt;d&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Trial 1</td>
<td>Trial 2</td>
<td>Trial 1</td>
</tr>
<tr>
<td>Beef</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ textured soy protein A (TSPA)</td>
<td>5.82</td>
<td>4.20&lt;sup&gt;g&lt;/sup&gt;</td>
<td>5.76</td>
<td>4.26&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>+ textured soy protein B (TSPB)</td>
<td>6.13</td>
<td>5.18&lt;sup&gt;h&lt;/sup&gt;</td>
<td>6.09</td>
<td>5.09&lt;sup&gt;l&lt;/sup&gt;</td>
</tr>
<tr>
<td>+ textured soy concentrate (TSC)</td>
<td>6.14</td>
<td>4.98&lt;sup&gt;h&lt;/sup&gt;</td>
<td>6.07</td>
<td>5.19&lt;sup&gt;l&lt;/sup&gt;</td>
</tr>
<tr>
<td>+ isolated soy protein (ISP)</td>
<td>6.11</td>
<td>5.09&lt;sup&gt;h&lt;/sup&gt;</td>
<td>6.05</td>
<td>5.07&lt;sup&gt;l&lt;/sup&gt;</td>
</tr>
<tr>
<td>+ TSPA adjusted</td>
<td>6.11</td>
<td>5.18&lt;sup&gt;h&lt;/sup&gt;</td>
<td>6.11</td>
<td>4.80&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>+ TSPB adjusted</td>
<td>5.85</td>
<td>4.23&lt;sup&gt;g&lt;/sup&gt;</td>
<td>5.78</td>
<td>3.94&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>+ TSC adjusted</td>
<td>5.86</td>
<td>4.13&lt;sup&gt;g&lt;/sup&gt;</td>
<td>5.77</td>
<td>3.54&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>+ ISP adjusted</td>
<td>5.78</td>
<td>3.26&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.76</td>
<td>3.00&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>pH adjusted by hydrating with water acidified with 6 N HCl.
<sup>b</sup>pH adjusted by hydrating with water acidified with 45% lactic acid.
<sup>c</sup>Each pH value is the mean of five measurements.
<sup>d</sup>Values followed by different letters are significantly different (P = 0.05).

**DISCUSSION**

To determine the heat resistance of salmonellae in raw beef, a recovery medium was needed that would differentiate salmonellae from other bacterial contaminants. Organisms injured by stresses, such as heat, are unlikely to recover on selective media because repair mechanisms are inhibited (10,24). XL agar, which is not considered to be a selective medium, allowed the differentiation of salmonellae colonies from the low numbers of colonies of other bacteria. However, XL agar did not allow the recovery of injured salmonellae cells. The “solid repair” method described by Ray (24) for recovery of injured bacterial cells was selected. In preliminary work, preincubation for 4 h at 37°C in a non-stress medium (PCA) before overlay with XL was found necessary for maximum recovery of cells injured by heating at 60°C for several minutes. Cells injured by heating at 54°C recovered in a shorter period of time.

The survival curve of salmonellae, plotted as a function of log<sub>10</sub> survivors versus time, indicated a good fit to linearity at 54 and 60°C. We did not observe the tailing effect reported by Thompson et al. (32) for the survival of *Salmonella typhimurium* TM-1 in beef heated at constant temperatures. The D-values calculated for salmonellae in beef were somewhat lower in this study than those reported by Goodfellow and Brown (18) for a six-strain composite of *Salmonella* in beef. D-values in this study were calculated for salmonellae in beef as 14 to 18 min at 54°C and 0.7 to 0.9 min at 60°C compared to 23 and 1.9 min, respectively as extrapolated from the thermal death curve of Goodfellow and Brown (18).

Heat resistance of the *Salmonella* composite was greater in beef containing 30% each of two textured soy proteins, a soy protein concentrate, and an isolated soy protein as compared to survival in beef. Significant differences in the survival of heated cells could be observed with addition of 10% soy product. Survival was even greater in beef supplemented with 40 and 50% soy product, and D-values could be expected to be higher with these levels of soy product. No significant differences were observed for heat resistance of salmonellae in beef after addition of structured soy isolate.

The lowering of water activity was considered as a possible explanation for increased survival of salmonellae in beef with added soy product. However, water activity measurements were equivalent for beef and beef + 30% TSP, and values for percentage moisture were similar for beef and beef-soy samples.

Hydrogen-ion concentration (pH) is known to affect the heat resistance of bacteria (13,14,19,21,30). The addition of soy products to meats has been shown to change the pH sufficiently to alter the amount of sporulation of *Clostridium perfringens* (17). At the 30% replacement level, soy products that increased the heat resistance of salmonellae also raised the pH of the product by 0.2 to 0.3 of a pH unit. Addition of the structured soy isolate did not increase...
heat-resistant properties nor the pH. Apparently this increase in pH was sufficient to account for increased heat resistance of soy-supplemented beef. When the pH of the beef-soy product was lowered with hydrochloric or lactic acid to near that of beef (pH 5.8 to 5.9), salmonellae survival was not significantly different or was less than survival in beef alone.

The optimum pH for heat resistance of *Salmonella typhimurium* was reported as 6.1 in phosphate buffer (21). Clark and Ordal (13) reported that the heat resistance of *S. typhimurium* in phosphate buffer becomes greater as the pH is lowered from 8.0 to below the neutral range. To our knowledge, no studies have reported the optimum pH in a beef product for survival of salmonellae. When the pH of beef + 30% TSPA was adjusted to a range from pH 5.9 to 7.1 with HCl or NaOH, optimum survival at 54°C was observed at pH values of 6.5 to 6.8.

Sodium chloride and other sodium salts have been reported to increase the heat resistance of *Staphylococcus aureus* (27). We considered the possibility that the increased heat resistance when pH was raised with NaOH may not be entirely a pH effect. However, when NaCl, at a molar concentration equivalent to the NaOH used to increase the pH to 6.8, was added to the beef-soy product, the heat resistance was not changed.

Our results indicate that minimal cooking procedures recommended for elimination of salmonellae from beef may not be sufficient to kill all salmonellae in some beef-soy products. Other formulated beef products where the pH is increased might also afford some protection to salmonellae contaminants.

**ACKNOWLEDGMENTS**

We thank N. A. Cox, C. E. Davis and R. L. Wilson for their advice and H. L. Hammond for technical assistance. Complimentary soybean products were provided by Farmland Agriservices, Inc., Archer Daniels Midland, Co., Staley Manufacturing Co., and Ralston Purina Co.

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