ABSTRACT

Three batches of butter made in small experimental lots from commercial cream were contaminated with Salmonella typhimurium var. copenhagen by inoculating the cream and wash water. Contaminated butter was held 10 weeks at 77, 40, 32, 0, and -10 F. Salmonella increased at 77 F and decreased at ≤ 40 F. Most significant decline in viable Salmonella was at 0 or -10 F, in unsalted butter followed in order by lightly salted butter and moderately salted butter.

Salmonellosis is considered one of the most important zoonotic diseases; it affects as many as 2 million persons annually in the United States (7). The most important reservoirs of human salmonellosis are livestock and poultry. It therefore follows that the most important vehicle of Salmonella is human or animal food, the most suspect foods being those lightly cooked and subjected to much handling.

While butter is not commonly considered a source of salmonellosis, little concern for its potential suggested that it be studied. Experimentally, sweet cream butter has been reported to support survival of Salmonella at room temperature and at 0 °C (9). Zagaevski reports Salmonella viability of up to 9 months in butter (10).

Variations in salt content as well as commercial utilization of lower storage temperatures (0 to -10 °F) that have been studied, made it appear feasible to attempt to determine effects of salt content and time and temperature in storage in butter inoculated with Salmonella.

MATERIALS AND METHODS

Bacterial cultures

A Salmonella species which is commonly incriminated in food-borne salmonellosis, was used. The culture (Salmonella typhimurium var. copenhagen) was procured from the Division of Biology, Kansas State University, and maintained on tryptose soy agar slants.

The inoculum was prepared by washing the cells from 24- hr slants with sterile 0.1% tryptone solution (8). The suspension was centrifuged at 500 × g for 30 min and the supernatant fluid discarded. Cells were resuspended in sterile 0.1% tryptone solution and adjusted to an optical density of 0.4 at 520 ms on a spectrophotometer (Bausch & Lomb Spectronic 20). This resulted in a cell concentration of 6.5 × 10⁷/ml (variance of 34.8) as predetermed by the least squares regression technique of Fryer (1). The inoculum for batches 1 and 2 consisted of 5.0 ml of the suspension. Batch 3 was inoculated using 500 ml water-tryptone cell suspension (O.D. 0.4) as wash water. The concentration was confirmed by plating on Brilliant Green sulfadiazine (BGS) agar (Difco) using the spreader technique.

Manufacture and sampling of butter

Butter was manufactured in a sterile glass electric churn. Pasteurized grade A cream, 4.5 pints per churning, was used. Fat content, standard plate count, and coliform count were determined on the cream as described in Standard Methods (6). All cream was confirmed by BGS agar plating to contain no Salmonella prior to use.

Batch 1 (unsalted butter, cream inoculated). To 4.5 pints of 36.5% fat cream (45 F) was added 5.0 ml tryptone cell suspension of S. typhimurium var. copenhagen (O.D. 0.4). The inoculated cream was churned approximately 45 min and the butter placed in a sterilized pyrex bowl after discarding the buttermilk. The butter was washed with 300 ml sterile deionized distilled water (40 F) and worked with a sterile wooden paddle to distribute the moisture evenly. The butter was placed in sterile screw-top jars of 130 ml capacity, each jar receiving 10 g of butter. Five lots were formed (13 jars/lot) and one lot placed at each of these temperatures: 77, 40, 32, 0, and -10 F (25, 4.44, 0, -17.77, and -33.33 C). Samples were withdrawn and placed in jars for initial Salmonella count and chemical analysis. Chemical analysis was performed by the Kohman method (3).

Batch 2 (salted butter, cream inoculated). To 4.5 pints of 37.5% fat cream (45 F) was added 5.0 ml of tryptone cell suspension of S. typhimurium var. copenhagen (O.D. 0.4). Working, churning, and washing were accomplished as in batch 1 except that 20 g of sterile NaCl was added to the butter. The butter was divided, stored, and sampled as batch 1.

Batch 3 (salted, washed water inoculated). Churning was accomplished in 45 min using 4.5 pints of 30.5% fat cream. Buttermilk was discarded and the inoculated wash water (500 ml) prepared by adding 75 ml tryptone cell suspension to 425 ml sterile deionized distilled water (40 F). The resulting cell suspension (O.D. 0.4) was used to wash the butter and to act as another possible contamination route. Nineteen grams of sterile NaCl was worked into the butter mass after it was washed. The butter was divided, stored, and sampled as with batches 1 and 2.

Table 1 summarizes composition of the cream, the resulting butter, and inoculum used.
tergit DI mixture was then serially diluted and prewarmed. Dilutions were small, the plates were spread on agar plates; otherwise even spreading necessary for uniform colony distribution was hampered. The beaker containing the butter-tergitol mixture was then placed in a 45 C (113 F) magnetically agitated water bath until the butter had completely melted. Concentration 8.5 x 10⁶/ml 1.0 x 10⁶/ml 6.4 x 10⁶/ml Volume added (ml) 5.0 5.0 500

Salmonella cell suspension
Concentration 8.5 x 10⁶/ml 1.0 x 10⁶/ml 6.4 x 10⁶/ml

Butter
% Fat 85.8 82.0 84.1
% Moisture 13.3 15.3 14.1
% Salt 0.0 2.2 1.7
% Curd 0.9 0.5 0.1
Initial salmonellae 1.1 x 10⁶/g 2.7 x 10⁶/g 2.2 x 10⁶/g

Table 1. Composition of batches of experimental butter

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Batch 1</th>
<th>Batch 2</th>
<th>Batch 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cream</td>
<td>36.5</td>
<td>37.5</td>
<td>30.0</td>
</tr>
<tr>
<td>Standard plate count/ml</td>
<td>6000</td>
<td>3300</td>
<td>3000</td>
</tr>
<tr>
<td>Salmonella cell suspension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration</td>
<td>8.5 x 10⁶</td>
<td>1.0 x 10⁶</td>
<td>6.4 x 10⁶</td>
</tr>
<tr>
<td>Volume added (ml)</td>
<td>5.0</td>
<td>5.0</td>
<td>500</td>
</tr>
<tr>
<td>Butter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Fat</td>
<td>85.8</td>
<td>82.0</td>
<td>84.1</td>
</tr>
<tr>
<td>% Moisture</td>
<td>13.3</td>
<td>15.3</td>
<td>14.1</td>
</tr>
<tr>
<td>% Salt</td>
<td>0.0</td>
<td>2.2</td>
<td>1.7</td>
</tr>
<tr>
<td>% Curd</td>
<td>0.9</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Initial salmonellae</td>
<td>1.1 x 10⁶</td>
<td>2.7 x 10⁶</td>
<td>2.2 x 10⁶</td>
</tr>
</tbody>
</table>

Table 2. Analysis of variance table for butter stored at 40 F.

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>Degrees of freedom</th>
<th>Mean square of variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>12</td>
<td>0.52477**</td>
</tr>
<tr>
<td>Batch</td>
<td>2</td>
<td>1.21540**</td>
</tr>
<tr>
<td>Temp.</td>
<td>3</td>
<td>0.11278**</td>
</tr>
<tr>
<td>Time x batch</td>
<td>24</td>
<td>0.02654**</td>
</tr>
<tr>
<td>Time x temp.</td>
<td>36</td>
<td>0.01823**</td>
</tr>
<tr>
<td>Batch x temp.</td>
<td>6</td>
<td>0.10608**</td>
</tr>
<tr>
<td>Error</td>
<td>72</td>
<td>0.01104</td>
</tr>
</tbody>
</table>

*Mean square significant at .05 level
**Mean square significant at .01 level

Enumeration of Salmonella
Salmonella were enumerated by the BGS-spreader-plate technique. All lots were tested for Salmonella initially and at these intervals: 3, 6, 9, 14, 21, 28, 35, 42, 56, 63, and 70 days.

Samples were removed from storage at stated intervals. A 5-g sample was immediately aseptically removed by a heat-sterilized spatula and placed in a sterile 50 ml beaker. One milliliter of 10% tergitol No. 7 solution was added by sterile pipette to be emulsified (2). Tergitol No. 7 solution used per sample was increased to 3 ml in instances where undiluted butter was spread on BCS agar plates; otherwise even spreading necessary for uniform colony distribution was hampered. The beaker containing the butter-tergitol mixture was then placed in a 45 C (113 F) magnetically agitated water bath until the butter had completely melted (3). Concurrently warming in the same water bath were 9 and 99 ml sterile dilution blanks containing 0.1% tryptone solution. The blanks were warmed to prevent the melted butter from solidifying when dilution was in progress. The butter-tergitol mixture was then serially diluted and 0.1 ml from each dilution spread on BCS agar plates with sterile 3 mm bent glass rods. Duplicate plates were utilized. When undiluted butter was spread on BCS agar plates, the plates had been prewarmed to 39 C to enhance spreading.

Plates were incubated at 37 C for 24 hr; when colonies were small, the plates were allowed to incubate an additional 24 hr before counting. A Quebec dark field colony counter was utilized counting only plates with 30-300 colonies of typical Salmonella.

Typical Salmonella colonies were streaked and stabbed on triple sugar iron and lysine iron agar slants and streaked on trypticase soy agar slants. The slants were incubated at 37 C for 24 hr and results recorded. Confirmation of isolates as S. typhimurium var. copenhagen was accomplished by slide
agglutination with group B antisera (Difco) and by flagellar agglutination with Salmonella H antiserum, Poly (Difco).

RESULTS AND DISCUSSION

Salmonella typhimurium var, copenhagen was recovered from all butter samples at all time intervals and at all temperatures. At no time did the recovery level at temperatures ≤40 F exceed the initial count of the same batch. All batches supported the growth of Salmonella when stored at 77 F as illustrated in Fig. 1.

Viable Salmonella of batch 1 (unsalted, cream inoculated) decreased to 5.26 to 0.71% of the original at temperatures ≤40 F by the end of 10 weeks storage. The same butter stored at 77 F became rancid and had nearly a 3.35 log increase in viable Salmonella within 3 days; then viable Salmonella gradually began to decline to 1.3 logs below the initial count at the end of 10 weeks storage.

In batch 2 (salted, cream inoculated) the Salmonella count declined to 22.52 to 13.04% of original at temperatures ≤40 F by the end of 10 weeks storage. A portion of the same butter stored at 77 F became rancid later than batch 1 and had a 3.1 log increase in viable Salmonella within 3 days, followed by a fluctuating recovery level, then declining to 0.2 log below the initial count at 56 days, followed by a slight increase of about 0.6 log by the end of 10 weeks storage.

Viable Salmonella in batch 3 (salted, wash water inoculated) decreased to 5.02 to 14.06% of original at temperatures ≤40 F by the end of 10 weeks storage. Salmonella in a portion of the same butter stored at 77 F increased by about 1.6 logs within 3 days and to 2.4 logs above original at 35 days. The recovery level then declined to about 0.8 log above the initial count at the end of 10 weeks storage.

The data were converted to percentages of original

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**Figure 3.** Percentages of Salmonella survival in butter batches 1, 2, and 3 stored at 32 F for 10 weeks.

**Figure 4.** Percentages of Salmonella survival in butter batches 1, 2, and 3 stored at 0 F for 10 weeks.

**Figure 5.** Percentages of Salmonella survival in butter batches 1, 2, and 3 stored at −10 F for 10 weeks.
count for all batches stored at ≤40 F. All counts from butter samples stored at 77 F were converted to log deviations from initial counts. Transformations were necessary to compare different original populations. Figures 1 through 5 graphically represent ranges of transformed populations for times, temperatures, and batches involved. We recognize some differences, because of manufacturing methods, among batches and between experimental butter and commercial butter. There were, theoretically, differences in moisture—droplet size and in dispersion as well as in salt distribution. Contamination levels—to enable observation of trends—were necessarily higher than probable under commercial conditions.

A three-way analysis of variance was computed on all counts derived from butter stored at ≤40 F. The data were converted to a square root of the log of the percentage for programming. Table 2 presents the analysis of variance.

Computer analyses (Table 2) showed that all variables significantly affected Salmonella population means. In general, the longer a sample was held at temperatures ≤40 F, the fewer Salmonella survived. Salmonella in batch 2 declined less than in batch 3, where they declined significantly less than in batch 1.

Batch 2 had slightly more salt (2.2%) and lower initial count than batches 1 or 3 (Table 1). Theoretically, salt, being hydrophilic, can contribute to larger moisture droplets, which enhance Salmonella survival. Further, the salt (2.2% overall and approximately 11.5% in brine of batch 2) may have inhibited the natural flora and somewhat affected Salmonella. Batch 2 had more curd available for nutrients than batch 3 but less than batch 1. Batch 3 was significantly different from the other batches, having a mean survival rate between batches 2 and 1. Batch 3 was higher in salt (1.7%) than was batch 1; its original count differed only slightly. Apparently the low curd (0.1%) of batch 3 was not important, as batch 1 had 0.9% curd. Batch 1 was significantly less able to support Salmonella than were batches 2 or 3. Apparently the lack of salt may have allowed more natural flora to survive.

Temperature, as a variable, was significant; lower storage temperatures (0 and −10 F) significantly reduced survival means, compared with 40 and 32 F temperatures. Mean survival rate as it applies to temperature is listed here from high to low:

<table>
<thead>
<tr>
<th>Temperature (°F)</th>
<th>Means (^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>0.448</td>
</tr>
<tr>
<td>32</td>
<td>0.430</td>
</tr>
<tr>
<td>0</td>
<td>0.367</td>
</tr>
<tr>
<td>−10</td>
<td>0.333</td>
</tr>
</tbody>
</table>

\(^1\) LSD = 0.04732 (Differences >0.047 are significant.)

Differences in survival means between 40 and 32 F and between 0 and −10 F were not significant. Combinations of variables were significant as time, temperature, and composition combined were significant.

Wide disagreement exists regarding what constitutes an infective dose of Salmonella organisms. Much depends on the host’s general health. The U. S. Public Health Service recommends a 0 tolerance, but no experimental butter sampled during the 10 weeks storage met this standard.

Evidently, salt content, as usually employed by the butter industry (1–4%), is not significantly bactericidal to S. typhimurium var. copenhagen. Butter will readily support Salmonella growth at room temperature, whereas refrigeration or freezing for short periods offers no promise of eliminating Salmonella from butter.

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**REFERENCES**


