Microbial Quality of Ground Beef after Simulated Freezer Failure

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ABSTRACT

Six lots of ground meat, obtained at intervals from a local supermarket, were frozen, and later held with other frozen foods in the freezer compartment of a refrigerator-freezer where power failure was simulated by unplugging the unit. Mean values for the counts (log_{10}) of the beef as purchased were as follows: aerobic and psychrotrophic plate counts 6.35 and 6.66, respectively; presumptive coliforms 4.48; coagulase-positive staphylococci 4.67; and presumptive Clostridium perfringens 1.43. Presumptive salmonellae were detected in three of the six lots. Counts of the same order of magnitude as above were obtained after 7 days in the freezers, complete defrost of the meat and 6 h thereafter. Between 6 and 24 h, aerobic and psychrotrophic plate counts and numbers of coliforms and coagulase-positive staphylococci increased approximately 10-fold. Forty-eight hours after complete defrost, further increases in counts occurred. The appearance and aroma of the meat were acceptable 24 h after defrost; after 48 h, it would have been discarded because of browning, slime and off-odors.

Extensive research on the bacterial populations of ground beef available on the retail market and on the effect of freezing and freezer storage on certain microorganisms has been reported. However, little attention has been given to the microbiology of frozen foods has been limited chiefly to precooked frozen foods containing packages of other frozen foods and ice cubes. It was approached until 12 h after complete defrost when the last samples were tested. The present investigation on the effect of freezer failure was planned to assay defrosted ground meat after longer intervals to determine when marked increases in bacterial loads and obvious changes in appearance and odor occurred.

MATERIALS AND METHODS

Six lots of fresh ground beef, weighing approximately 2.8 kg each, were purchased at intervals from January to June from a local supermarket. The ground beef was at least 80% lean. In preparation for freezing on the days of purchase, the packages comprising a lot were mixed for 2 min in a Kitchen-Aid K-5 mixer with a dough hook. A sample was removed to assay the initial bacteriological population. Portions, weighing 454 g on a dietetic scale, were molded in loaf pans, 13 by 5.5 by 5.5 cm, and wrapped in heavy duty aluminum foil. The mixer bowl, dough hook, pans, spoons, and foil were sterilized before use. Copper-constantan thermocouples attached to a Leeds and Northrup Speedomax H Recorder were inserted into the centers of five packages of meat to give a continuous record of the internal temperatures. The sixth thermocouple was suspended in the freezers to record ambient temperatures during freezing, holding and thawing.

The meat was frozen in a home-type chest freezer where the temperature ranged from −26.1 to −20.6°C. After 6 days in this unit, the packages of meat were transferred to a General Electric Refrigerator-Home Freezer NH 10 FB. The temperature in the separate freezer compartment varied between −20.6 and −13.3°C. To make the load representative of a home refrigerator-freezer, the compartment contained packages of other frozen foods and ice cubes. It was approximately two-thirds full and the total load was 9.1 kg. The contents of the freezer compartment were allowed to equilibrate for 1 day before power failure was simulated by unplugging the refrigerator-freezer and allowing the temperature to rise and the contents to thaw. The door was opened only to remove packages of meat for assay. Thawing was considered to be complete when the internal temperature of the beef reached 0.6°C. This required from 25 to 26 h in the six power failure simulations. Bacteriological assays were carried out when the appliance was disconnected (on a package of ground beef thawed in cold running water), after complete thaw in the unit, and at 6, 24, and 48 h after complete thaw.

Bacteriological examination

Media for the assays were obtained from Difco Laboratories, Detroit, Michigan unless otherwise noted. Fifty-gram samples were taken aseptically from the portions of ground beef. These were blended with 450 ml of 0.1% peptone water at low speed for 0.5 min and at high speed for 1.5 min. Further dilutions were made with 0.1% peptone water. Pour plates on Plate Count Agar were incubated at 35°C for 48 ± 2 h for aerobic plate counts (8) and at 7°C for 10 days for psychrotrophic counts (23).

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For enumeration of coliforms, portions of the dilutions of beef homogenates were mixed with tryptic soy broth (TSB) to recover injured coliforms (24), and were plated after 0.5 h of incubation or immediately. Pour plates with an overlay of Violet Red Bile Agar were incubated at 35 C for 48 ± 2 h. Confirmation of presumptive coliforms was based on formation of gas or effervescence by typical colonies inoculated into tubes of brilliant green bile broth (17). However, confirmation tests were done only on samples of the first lot of meat, and thus the results are reported as presumptive coliforms.

Nonselective enrichment in TSB was used for enumeration of staphylococci and portions were plated immediately or after incubation of 0.5 h at 35 C. Spread plates on Baird-Parker Agar and incubated for 48 ± 2 h at 35 C (6). Colonies suspected to be Staphylococcus aureus were transferred to brain-heart infusion broth (Gibco, Madison, Wisconsin) and incubated. Coagulase plasma with EDTA was used to test for coagulase-positive organisms.

Clostridium perfringens was isolated by pour plating with Sulfite-Polymixin-Sulfadiazine Agar (17) with an overlay and incubating anaerobically (BBL Gas Pak System) for 48 ± 2 h at 35 C. Black colonies were counted as presumptive C. perfringens.

To determine the incidence of salmonellae, procedures (8) including preenrichment with lactose broth, incubation in selenite cystine broth and tetrathionate broth, spread plates on Brilliant Green Agar and incubation anaerobically (BBL Gas Pak System) for 24 ± 2 h. Confirmation of presumptive salmonellae was done only on samples of the first lot of meat, and thus the results are reported as presumptive salmonellae.

Statistical analysis
Average plate counts were computed for each sample and logarithms (log<sub>10</sub> per gram) of the averages were compiled. Means and standard deviations of the means were calculated on these figures for bacterial counts. Analysis of variance and least significant differences between the counts at different times.

RESULTS AND DISCUSSION

Microorganisms in retail ground beef
Means, standard deviations of the means and ranges of bacterial counts (log<sub>10</sub> per gram) and figures for the incidence of presumptive salmonellae are given in Table 1. The aerobic plate counts for the six lots of fresh ground beef as purchased ranged from 5.05 to 7.85 with a mean of 6.35. Surveys of bacterial quality of ground beef available on retail markets have shown wide variations in aerobic plate counts. Foster et al. (9) analyzed 150 retail samples of ground beef and found that the counts varied from 4.84 to 7.92 with a mean of 6.81. These investigators also compiled the results of nine earlier surveys in which the means ranged from 6.28 to 7.89. In the present study, the psychrotrophic plate counts of the beef as purchased ranged from 5.52 to 8.05 with a mean of 6.66. This mean was slightly but significantly (P < 0.05) higher than that for the aerobic plate count. Duitschaever et al. (4) reported psychrotrophic counts which were twice as high as mesophilic counts for numerous samples of retail hamburger sold in bulk but comparable mesophilic and psychrotrophic counts for other retail types of ground beef.

Counts for coliforms and staphylococci presented in Table 1 are those for plates prepared from portions incubated with TSB for 0.5 h. These counts followed the same pattern, and were generally slightly higher than those from portions not incubated with TSB. The fresh ground beef had a mean coliform count of 4.48. Rogers and McCleskey (20) reported a slightly higher mean while Duitschaever et al. (4) found lower mean coliform counts for all types of ground beef. Goepfert (12) assayed 955 samples of ground beef; only 4% had presumptive coliform counts higher than 4.00. In the present study, the mean coagulase-positive staphylococci count was 4.67. These organisms were not detected in one lot, while another had a count of 7.94. Both of these figures, 4.67 and 7.94, are higher than many of those reported in the literature (4,6,9,10,18). The temperature of the ground beef at the time of purchase ranged from 4.4 to 7.8 C. Duitschaever et al. (5) found that higher temperatures in the display cases correlated with higher counts. Other possible contributing causes and conditions have been reviewed (4).

The mean C. perfringens count was 1.43. The microorganisms were not detected in two of the fresh retail lots, while the range of the other four was from 1.00 to 2.93. The mean count was of the same order of magnitude and only slightly lower than that found by Foster et al. (9). Presumptive salmonellae were detected in three of the six lots. Results of recent surveys indicated that a low incidence of salmonellae in ground beef at the retail level (18), or no confirmed salmonellae (4), no suspicious colonies (5), and no salmonellae isolates (9).

Effect of freezing and freezer storage
At the start of defrost after a week in the freezer units, the mean aerobic and psychrotrophic plate counts and the mean counts for coliforms, coagulase-positive staphylococci and C. perfringens (Table 1) did not differ significantly (P < 0.05) from those obtained on the ground beef at the time of purchase. C. perfringens organisms were detected in three of the six lots and presumptive salmonellae, in only one of six lots.

Temperature ranges in the freezers and in the ground beef in the six experiments are given in Table 2. During storage in the chest freezer and equilibration in the refrigerator-freezer and at the start of defrost, the temperatures ranged from −26.1 to −13.3 C in the freezer units and from −23.3 to −13.3 C in the ground beef.

Ladiese et al. (15) froze ground beef at −20 C and did not find any reduction in the number of viable C. perfringens organisms after 24 h, although viability was decreased after 1 to 4 months of freezer storage. Duitschaever et al. (5) stated that "counts in frozen meat are known to stabilize or even decrease during storage." Kraft et al. (14) found that mesophilic and psychrotrophic populations were greatly reduced by freezing and freezer storage. That cryogenic freezing was more destructive than mechanical freezing in an air blast and that coagulase-positive staphylococci were relatively


**TABLE 1. Bacterial counts (log$_{10}$/g) and incidence of salmonellae in ground beef.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Aerobic plate count</th>
<th>Psychrotrophic plate count</th>
<th>Coliforms$^1$</th>
<th>Coagulase positive staphylococci$^2$</th>
<th>C. perfringens</th>
<th>Means, standard deviations of the means and ranges for log$_{10}$ counts</th>
<th>No. lots positive for presumptive salmonellae</th>
</tr>
</thead>
<tbody>
<tr>
<td>As purchased</td>
<td>6.35 ± 0.41$^{ab}$</td>
<td>6.66 ± 0.41$^{ab}$</td>
<td>4.48 ± 0.24$^a$</td>
<td>4.67 ± 1.05$^{a}$</td>
<td>1.43 ± 0.53$^{ab}$</td>
<td>3 of 6</td>
<td></td>
</tr>
<tr>
<td>5.05 - 7.85</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At start of defrost</td>
<td>5.96 ± 0.43</td>
<td>6.45 ± 0.40$^a$</td>
<td>4.52 ± 0.31$^a$</td>
<td>4.71$^a$ ± 1.31$^a$</td>
<td>0.98 ± 0.51$^a$</td>
<td>1 of 6</td>
<td></td>
</tr>
<tr>
<td>4.05 - 6.97</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At complete defrost</td>
<td>6.12 ± 0.35$^a$</td>
<td>6.51 ± 0.39$^a$</td>
<td>4.62 ± 0.22$^{ab}$</td>
<td>.90$^a$ ± 1.35$^a$</td>
<td>1.16 ± 0.44$^a$</td>
<td>2 of 6</td>
<td></td>
</tr>
<tr>
<td>4.77 - 7.16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 h after complete defrost</td>
<td>6.13 ± 0.39$^a$</td>
<td>6.82 ± 0.43$^a$</td>
<td>4.86 ± 0.28$^{ab}$</td>
<td>4.93$^a$ ± 1.40$^a$</td>
<td>0.78 - 0.70$^a$</td>
<td>4 of 6</td>
<td></td>
</tr>
<tr>
<td>4.60 - 7.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>24 h after complete defrost</td>
<td>7.56 ± 0.36$^{bc}$</td>
<td>7.94 ± 0.37$^{bc}$</td>
<td>5.65 ± 0.47$^b$</td>
<td>5.62$^b$ ± 1.43$^a$</td>
<td>1.43 ± 0.51$^{ab}$</td>
<td>5 of 6</td>
<td></td>
</tr>
<tr>
<td>6.15 - 8.24</td>
<td>6.51 - 9.05</td>
<td>5.39 ± 7.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 h after complete defrost</td>
<td>8.41 ± 0.11$^c$</td>
<td>8.55 ± 0.36$^c$</td>
<td>7.28 ± 0.17$^c$</td>
<td>7.75$^a$ ± 0.35$^a$</td>
<td>3.18 ± 0.47$^a$</td>
<td>5 of 6</td>
<td></td>
</tr>
<tr>
<td>8.14 - 8.90</td>
<td>6.80 - 9.18</td>
<td>6.72 - 7.82</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD (P &lt; 0.05)</td>
<td>1.30</td>
<td>1.30</td>
<td>1.08</td>
<td>4.56</td>
<td>1.94</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^1$Counts of plates prepared after incubation for 0.5 h in tryptic soy broth.

$^2$Non detected in one or more lots.

$^3$Counts on 5 instead of 6 lots included in the mean.

$a,b,c$Significant differences (P < 0.05) exist between two or more means in a column not followed by a common superscript letter.

**TABLE 2. Ranges of temperature (C) in freezers and ground beef during six simulated freezer failure experiments.**

<table>
<thead>
<tr>
<th>Time</th>
<th>Freezer</th>
<th>Ground beef</th>
</tr>
</thead>
<tbody>
<tr>
<td>During storage in</td>
<td>-26.1 to -20.6</td>
<td>-23.3 to -17.8</td>
</tr>
<tr>
<td>chest freezer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>During 24-h equilibration</td>
<td>-20.6 to -13.3</td>
<td>-17.8 to -13.3</td>
</tr>
<tr>
<td>in refrigerator-freezer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At start of defrost</td>
<td>-17.8 to -13.3</td>
<td>-17.8 to -13.3</td>
</tr>
<tr>
<td>At complete defrost</td>
<td>2.2 to 5.6</td>
<td>0.6</td>
</tr>
<tr>
<td>6 h after complete</td>
<td>4.4 to 5.6</td>
<td>2.2 to 13.3</td>
</tr>
<tr>
<td>complete defrost</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h after complete</td>
<td>16.7 to 22.2</td>
<td>16.7 to 22.2</td>
</tr>
<tr>
<td>complete defrost</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 h after complete</td>
<td>23.3 to 27.8</td>
<td>22.2 to 26.7</td>
</tr>
<tr>
<td>complete defrost</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^1$Converted from temperatures recorded in (F).

Stable during frozen storage. In the present study, the slower rate of freezing and the shorter period of freezer storage may account for the lack of significant decreases in bacterial counts.

**Effects of simulated freezer failure**

When the ground beef was completely defrosted (25 to 26 h after disconnecting the refrigerator-freezer) and 6 h thereafter, the aerobic and psychrotrophic plate counts and the numbers of coagulase-positive staphylococci and C. perfringens organisms (Table 1) did not differ significantly (P < 0.05) from those obtained when the meat was purchased or after freezer storage at the start of defrost. Presumptive salmonellae were detected in two and four of the six lots, respectively. Increases in counts during these periods were not expected since the temperature in the ground beef at complete defrost was 0.6°C and only ranged from 2.2 to 13.3°C 6 h after complete defrost. Angelotti et al. (1) concluded that salmonellae and staphylococci did not grow at or below 5.6°C in refrigerated foods. According to Rey et al. (19), six strains of C. perfringens did not multiply in thioglycolate medium at 5°C. Earlier Sulzbacher (21) found that freezing and thawing usually lengthened the lag and generation times of psychrophilic and mesophilic organisms in ground meat.

Twenty-four hours after complete defrost, mean values for aerobic and psychrotrophic plate counts and for numbers of coliforms and coagulase-positive staphylococci were approximately 10-fold higher than the respective figures at the time of purchase. The difference in number of coliforms was significant (P < 0.05) and the differences in aerobic and psychrotrophic plate counts approached significance. The range of temperatures 24 h after complete defrost was from 16.7 to 22.2°C in the refrigerator-freezer and in the ground meat. The mean value for C. perfringens organisms was the same as at the time of purchase. Tuomi et al. (22) stated that rapid growth of C. perfringens occurred between 31 to 50°C. Although all the samples of meat were light brown on the surface, and slightly slimy to touch in four of the six power failure simulations, no off-odors were detected 24 h after complete defrost. Beck and Milone (3) reported discoloration in ground beef thawed in the original container at room temperature by the twenty-second hour of thawing.

Mean values for aerobic and psychrotrophic plate counts and for numbers of coliforms were significantly higher (P < 0.05) 48 h after complete defrost than they were at the time of purchase or 6 h after complete...
defrost. Coagulase-positive staphylococci and *C. perfringens* were detected in all lots and the mean counts were approximately 100-fold higher than 24 h after complete defrost. Presumptive salmonellae were detected in five of the six lots at both of these intervals. Temperatures in the ground beef ranged from 22.2 to 26.7 °C during the time between 24 and 48 h after complete defrost. The six samples of meat were very brown and slimy and had developed strong off-odors. Ayres (2) found that beef slices with low initial counts had off-odor by the second or third day when held at 25 °C. The slime point was set at 7.8 (log10) microorganisms per cm2. Kontu et al. (13) reported that rejection of ground beef patties occurred when the bacterial count reached log 8.8.

**Implications of the results**

Compared with various suggested guidelines and proposed standards (18, 26, 27), the bacterial loads of the ground beef as purchased were high. Two of the six lots had aerobic plate counts that exceeded the criteria established in 13 states and the numbers of presumptive coliforms in four of the six lots were higher than the criteria in eight states (25). Of greater concern was the finding that one of the lots had more than a million coagulase-positive staphylococci at the time of purchase. One day after complete defrost (49 or 50 h after the refrigerator-freezer was disconnected) three of the five lots had similar counts. Upwards of one million coagulase-positive staphylococci in meat products have been reported to cause illness (16). The counts for *C. perfringens*, even 48 h after complete defrost, were well below the numbers, 10⁸ and 10⁹ per gram, required to produce foodborne illness (17).

Under conditions of this experiment, marked increases in numbers of bacteria occurred between 6 and 24 h after complete defrost (31 and 49 h after disconnecting the appliance). Forty-eight hours after complete defrost, the meat would have been discarded because of off-odors and a brown slimy surface. However, at 24 h, the meat was still acceptable when judged on the basis of appearance and aroma. These results suggest (a) that multiplication of bacteria of public health significance may occur before overt signs of spoilage do, and (b) that ground beef should be cooked and consumed after freezer failure only if the time period does not exceed 30 h and the temperature in the meat is no higher than 15 °C.

**REFERENCES**