Survival of Escherichia coli O157:H7 in Ground-Beef Patties during Storage at 2, −2, 15 and then −2°C, and −20°C†

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ABSTRACT

The survival of Escherichia coli O157:H7 and of a nonpathogenic control strain of E. coli was monitored in raw ground beef that was stored at 2°C for 4 weeks, −2°C for 4 weeks, 15°C for 4 h and then −2°C for 4 weeks, and −20°C. Irradiated ground beef was inoculated with one E. coli control strain or with a four-strain cocktail of E. coli O157:H7 (ca. 10⁵ CFU/g), formed into patties (30 to 45 g), and stored at the appropriate temperature. The numbers of the E. coli control strain decreased by 1.4 log₁₀ CFU/g, and pathogen numbers declined 1.9 log₁₀ CFU/g when patties were stored for 4 weeks at 2°C. When patties were stored at −2°C for 4 weeks, the numbers of the E. coli control strain and the serotype O157:H7 strains decreased 2.8 and 1.5 log₁₀ CFU/g, respectively. Patties stored at 15°C for 4 h prior to storage at −2°C for 4 weeks resulted in 1.6 and 2.7 log₁₀ CFU/g reduction in the numbers of E. coli and E. coli O157:H7, respectively. Storage of retail ground beef at 15°C for 4 h (tempering) did not result in increased numbers of colony forming units per gram, as determined with violet red bile, MRS lactobacilli, and plate-count agars. Frozen storage (−20°C) of ground-beef patties that had been inoculated with a single strain of E. coli resulted in approximately a 1 to 2 log₁₀-CFU/g reduction in the numbers of the control strain and individual serotype O157:H7 strains after 1 year. There was no significant difference between the survival of the control strain and the O157:H7 strains, nor was there a difference between O157:H7 strains. These data demonstrate that tempering of ground-beef patties prior to low-temperature storage accelerated the decline in the numbers of E. coli O157:H7.

Ground beef is most often implicated in foodborne outbreaks of Escherichia coli O157:H7 (12), although a variety of foods, including apple cider (3), fermented sausage (4), mayonnaise (24), and water (21), have been associated with human illness. The storage conditions used during distribution of ground-beef patties may contribute to the dissemination of E. coli O157:H7. Ground-beef patties are commonly shipped in a frozen state to fast-food restaurants, and E. coli O157:H7 survives in frozen ground beef for up to 9 months with little decline in cell numbers (6). In addition, Jackson et al. (14) reported that the storage temperature of ground-beef patties influences the subsequent heat tolerance of E. coli O157:H7. Cells in frozen patties had greater heat tolerance than did cells in ground beef stored at 15°C. Likewise, the acid tolerance of E. coli O157:H7 in ground beef is influenced by storage conditions, since incubation at 15°C for 4 h prior to storage at 4 or −20°C sensitized the surviving cells to synthetic gastric fluid (5). These data suggest that refrigerated rather than frozen storage of ground beef increases the susceptibility of the pathogen to unfavorable conditions.

With the exception of studies that describe a limited number of strains (5, 6, 14), there is little information on the effects of frozen and low-temperature storage on E. coli O157:H7 strains in ground beef. This study was conducted to determine if storage temperature and tempering influence the survival of E. coli O157:H7 in ground-beef patties. Additionally, the survival rates of three O157:H7 strains were compared with each other and with an E. coli strain in frozen ground-beef patties.

MATERIALS AND METHODS

Bacterial strains. Four E. coli O157:H7 strains (CDC 9490, ATCC 43889, ATCC 43894, ATCC 43895) from outbreaks involving contaminated ground beef were used. E. coli C FRIK 123 (Food Research Institute–Kaspar, culture collection strain 123) was used as a nonpathogenic control strain. The strains were grown in trypticase soy broth (BBL, Becton Dickinson and Co., Cockeysville, Md.) at 37°C for 24 h, with shaking (150 rpm). When ground beef was inoculated with a mixture of O157:H7 strains, equal volumes of 24-h cultures were combined.

Ground beef inoculation and storage. Ground beef (20% fat) chubs (size 4.5 kg) were treated with an electron beam linear accelerator (Thomson CCS Lin Ac, Saint-Aubin, France) at Iowa State University (total dosage, 2.2 to 2.4 kGy). The chubs were stored at −20°C. Prior to inoculation, ground beef was thawed at 4°C and ground using an AS-200 Hobart grinder (Hobart Manufacturing Company, Troy, Ohio) with a 1-cm plate. All grinder parts were cleaned with Micro soap (International Products Corporation, Burlington, N.J.) and bleached, wrapped in aluminum foil, and sterilized at 121°C at 15 psi for 25 min prior to use.

 Cultures were appropriately diluted in 0.1% Bacto peptone (Difco Laboratories, Detroit, Mich.), which was added to obtain a final concentration of ca. 10⁵ CFU/g of ground beef. Following

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inoculation, the ground beef was again passed through the grinder in order to distribute the inoculum. Ground-beef patties (30 to 45 g) were aseptically formed by hand and placed into individual Whirl-Pak bags (Nalco Company, Modesto, Calif.). The hamburger patties were then stored at 2, −2, or 15°C for 4 h followed by storage at −2, or −70°C for 1 h, followed by storage at −20°C.

**E. coli enumeration.** For each sample, two ground-beef patties were removed and analyzed for numbers of *E. coli*. Storage times were 0, 1, and 24 h, and then monthly times were used thereafter, until month 12. For the 4- to 5-week studies, ground-beef patties were tested at 0, 4 (for patties incubated at 15°C for 4 h only), and 24 h, after which they were tested weekly. In order to sample, ground-beef patties were thawed at room temperature (25°C) until they became pliable (ca. 0.5 h), cut with a sterile tongue depressor (Fisher Scientific, Pittsburgh, Pa.) and then one-half (15 to 22.5 g) of the patty was transferred to a stomacher bag (Seward Medical, London, UK). A 1:10 dilution of the ground-beef sample (wt/wt) was made with 0.1% Bacto peptone (Difco) and then stomached (model 400; Tekmar Co., Cincinnati, Ohio) for 1 min. If necessary, serial dilutions were made in 0.1% peptone, and the appropriate dilutions were plated on MacConkey sorbitol agar (Difco). The plates were incubated overnight at 42°C, and the number of sorbitol-positive colonies (FRIK 123) or sorbitol-negative colonies (O157:H7 strains) was determined. Suspect and representative sorbitol-negative colonies were tested for the O157 antigen using latex agglutination (Oxoid, Basingstoke, UK).

**Microbiological analyses of ground beef.** Ground chuck was obtained from a local grocery store. Patties (30 to 45 g) were aseptically formed and placed into individual Whirl-Pak bags (Nalco). The ground-beef patties were tested for numbers of indicator (coliform) and spoilage bacteria (lactic acid bacteria and total plate count) prior to storage, after 4 h at 15°C, after an additional 20 h at −2°C, and then weekly during storage at −2°C. At each sampling time, two patties were tested for coliforms (using violet red bile agar; Difco), lactic acid bacteria (using MRS lactobacilli agar; Difco), and total counts (using plate count agar; Difco), using standard methods (23).

**Statistical analyses.** A minimum of two and as many as nine trials were conducted for each storage temperature regimen. Results were analyzed for variance and statistical significance with the Statistical Analysis System (SAS Institute, Inc., Cary, N.C.).

**RESULTS**

**Microbiological testing of irradiated ground beef.** Analyses of the irradiated ground beef before inoculation with the serotype O157:H7 strains found no *E. coli* O157:H7. The total numbers of bacteria, as determined with plate count agar, in the ground-beef chubs ranged from $10^2$ to $10^3$ CFU/g. The residual microbial flora and the length of storage in these studies necessitated the use of MacConkey sorbitol agar for enumeration of *E. coli* O157:H7.

**Low-temperature storage.** Inoculation of ground beef with a mixture of four *E. coli* O157:H7 strains and storage of the patties at 2°C for 4 weeks resulted in a decrease of $1.9 \log_{10}$ CFU/g (Fig. 1). Likewise, the numbers of the *E. coli* control strain decreased by $1.4 \log_{10}$ CFU/g after 4 weeks of storage at 2°C.

Because cellular damage is increased and viability decreased near the freezing point (13), the survival of *E. coli* O157:H7 in ground-beef patties stored at −2°C was monitored. Numbers of the *E. coli* O157:H7 cocktail decreased 1.5 $\log_{10}$ CFU/g after 4 weeks of storage, whereas numbers of the *E. coli* control strain decreased 2.8 $\log_{10}$ CFU/g, a value that was significantly different ($P < 0.001$) from the decrease observed for the mixture of O157:H7 strains. There was no statistical difference in the survival of *E. coli* O157:H7 in ground-beef patties stored at 2 and −2°C (Figs. 1 and 2).

**Ground-beef patties tempered and stored at −2°C.** Preincubation of inoculated ground-beef patties at 15°C for 4 h prior to storage at −2°C was detrimental to the survival of *E. coli* O157:H7 (Fig. 3). The numbers of the *E. coli* O157:H7 cocktail decreased 2.7 $\log_{10}$ CFU/g, whereas the *E. coli* control strain decreased 1.6 $\log_{10}$ CFU/g after 4 weeks of storage. The decrease in *E. coli* O157:H7 numbers in patties stored at 15°C and −2°C was significantly different from the decrease in numbers during storage at 2 and −2°C ($P < 0.03$ at 2°C and $P < 0.001$ at −2°C).

**Microbiological analyses of tempered ground beef stored at −2°C.** The numbers of coliforms, lactic acid bacteria, and total aerobic bacteria were determined for retail ground beef after purchase and following tempering (15°C for 4 h) and storage at −2°C. The results of these analyses
are shown in Table 1. The average number of coliforms in the retail ground beef was ca. 3.7 log_{10} CFU/g, which remained essentially unchanged during preincubation (15°C for 4 h) and storage (−2°C). Plate counts with MRS lactobacilli agar were initially 5.4 log_{10} CFU/g of ground beef, a value that remained constant during tempering (Table 1) but that increased during storage for 1 week (6.3 log_{10} CFU/g, 1 week; 5.8 log_{10} CFU/g, 4 weeks; data not shown). Likewise, the total aerobic count of the ground beef remained unchanged during tempering (Table 1) but increased from 6.0 to 7.0 log_{10} CFU/g after 2 weeks of storage at −2°C (data not shown). These results indicate that the tempering process (15°C, 4 h) does not result in a significant increase in the number of indicator or spoilage bacteria.

Frozen storage. Ground beef inoculated with individual strains of E. coli O157:H7 (ATCC 43889, 43894, and 43895) and stored at −20°C for 12 months resulted in an approximate reduction of 1.0 log_{10} CFU/g (Fig. 4). Survival of the individual O157:H7 strains did not differ significantly from each other or from the E. coli control strain (FRIK 123). However, the E. coli control strain had the greatest reduction (ca. 2.0 log_{10} CFU/g) and the lowest number of viable cells (2.7 log_{10} CFU/g) after 12 months of storage.

**DISCUSSION**

Doyle and Schoeni (6) demonstrated that O157:H7 strain 932 is able to survive in frozen ground-beef patties for 9 months with little decline in numbers. This is significant because most ground-beef patties shipped to fast-food restaurants are transported frozen. It is unknown whether the ability to survive for several months in ground beef is a property unique to some or all strains of serotype O157: H7 or other non-O157 strains. In this study, there was no significant difference among the survival of an E. coli control strain and that of three serotype O157:H7 strains (ATCC 43894, 43895, 43889), nor was there a difference among the O157:H7 strains during frozen storage in ground beef. Numbers of E. coli O157:H7 declined approximately 1 log_{10} CFU/g during frozen storage in ground beef for 1 year, and the numbers of the E. coli control strain decreased approximately 2 log_{10} CFU/g, but the difference was not statistically different.

Microorganisms present in food that is stored frozen incur damage to DNA, RNA, proteins, and the cell wall (7, 9, 16–19). The damage to these essential elements and structures results from the freezing of water, which increases the molarity of cellular electrolytes and physical damage caused by ice-crystal formation. The susceptibility of an organism to freezing varies from strain to strain and depends upon several factors, including the composition of the suspending menstruum (i.e., food), the cooling rate, the final storage temperature, the length of time of frozen storage, and the rate of thawing. Ingram (13) noted that microbial inactivation occurred most rapidly at temperatures around the freezing point. The freezing point of fresh beef is around −2°C (2); therefore, we evaluated the survival of E. coli O157:H7 in ground-beef patties stored at 2 and −2°C. The numbers of E. coli O157:H7 in ground beef decreased 1.9 and 1.5 log_{10} CFU/g after 4 weeks of storage at 2 and −2°C, respectively. The decrease in viable numbers of the O157:H7 strains noted at these temperatures occurred in a much shorter period of time (4 weeks) and

<table>
<thead>
<tr>
<th>Sample point</th>
<th>VRBA(^a)</th>
<th>MRS(^b)</th>
<th>PCA(^c)</th>
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<td>Raw material</td>
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<td>5.8</td>
<td>6.3</td>
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<td>5.3</td>
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\(^a\) VRBA, violet-red bile agar used for coliform enumeration.
\(^b\) MRS, MRS lactobacilli agar used for enumeration of lactic acid bacteria.
\(^c\) PCA, plate count agar used for enumeration of total aerobic count.
\(^d\) Ground beef was purchased locally (raw material).
\(^e\) Sample was collected after the patties had been incubated at 15°C for 4 h.
\(^f\) Sample was collected after the patties had been incubated at 15°C for 4 h and then stored at −2°C for 20 h.

![Figure 3](http://example.com/fig3.png)

**FIGURE 3.** Survival of E. coli O157:H7 (○; n = 9) and E. coli control strain (FRIK 123; ■; n = 3) in ground-beef patties at 15°C for 4 h and then at −2°C; numbers reported are averages from two trials.

![Figure 4](http://example.com/fig4.png)

**FIGURE 4.** Survival of E. coli O157:H7 (43889, ▲; 43894, ○; and 43895, ◦) (n = 2) and E. coli control strain (FRIK 123; ■; n = 2) in ground-beef patties at −20°C.
was greater than the 1.0-log decrease in numbers of *E. coli* O157:H7 observed in frozen ground-beef patties stored for 12 months. However, the 1-log decrease in numbers of *E. coli* O157:H7 strains in ground beef incubated at refrigeration temperature (5) or lower (i.e., −2°C) is likely not achieved in retail nonfrozen ground beef because of its short shelf life (22).

Tempering and conditioning (15°C for 4 h) of ground beef or salami batter has been reported to render *E. coli* O157:H7 susceptible to acid and the intrinsic and extrinsic factors associated with salami production, respectively (5, 8). It is speculated that the shift from a nonpermissive growth temperature (i.e., refrigeration temperature) to a more favorable temperature for growth switches cell processes from protective functions to reproduction. Log-phase bacteria are generally more susceptible to environmental stresses (3, 15). Thus, tempering of ground-beef patties inoculated with strains of serotype O157:H7 followed by storage at −2°C was evaluated to determine if this process was detrimental to survival and to determine whether numbers decreased within a time frame that was compatible with the shelf-life of ground beef. Tempering (15°C, 4 h) of the ground-beef patties followed by storage at low temperature resulted in a 2.9-log_{10} CFU/g decrease in the number of *E. coli* O157:H7 after 5 weeks of storage (Fig. 4). Although the decrease in viable numbers of O157:H7 was greater and more rapid in ground-beef patties tempered and stored at low temperature than in patties stored directly at and −2°C, the process is not commercially feasible at this time. In contrast to the reduction in viable numbers that was dependent upon the length of storage, acid susceptibility in *E. coli* O157:H7, triggered by tempering, is independent of storage time (5). Furthermore, *E. coli* O157:H7 is more sensitive to heat in ground-beef patties stored at 15°C than in frozen patties (14). The ca. 2-log reduction in viable numbers of *E. coli* O157:H7 after 2 weeks of storage (tempering and storage at −2°C) is significant when one considers the numbers of this pathogen (ca. 700 CFU/g (11)) that have been found in ground beef involved in outbreaks.

The impact of tempering and low-temperature storage on the growth of coliforms, lactic acid bacteria, and total aerobic bacteria in ground-beef patties was negligible, as numbers remained essentially unchanged during tempering and storage. These results are similar to those of Sawaya et al. (20), who extended the shelf-life of fresh poultry carcasses using storage under supercooled conditions (e.g., −2°C).

The results of the present study (with ground beef) demonstrate that tempering was more effective in reducing the numbers of *E. coli* O157:H7 than was storage at refrigeration temperatures or lower. Tempering of ground beef did not result in appreciable increases in the number of lactic acid bacteria or in total plate counts. Additional studies are needed to optimize the temperatures and times required to temper ground beef and to evaluate tempering in combination with other antimicrobial practices, such as beef pasteurization (10). Future studies might also investigate tempering and low-temperature storage of carcasses or trim rather than of ground beef. The results of the present study further our understanding of the factors and conditions that influence the survival of *E. coli* O157:H7 in ground beef.

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**REFERENCES**


