Survival of *Salmonella* in Processed Chicken Products during Frozen Storage

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MS 09-179: Received 20 April 2009/Accepted 5 June 2009

**ABSTRACT**

Frozen chicken products have been identified recently as a cause of salmonellosis. At least eight salmonellosis outbreaks from 1998 to 2008 have implicated undercooked frozen chicken nuggets, strips, and entrees as infection vehicles. Thus, the presence of *Salmonella* in frozen products may pose an infection risk if the product is improperly cooked. The objective of the current study was to assess the survivability of *Salmonella* during frozen storage (−20°C) when inoculated in processed chicken products. Four *Salmonella* strains originally isolated from poultry were inoculated into frozen chicken nuggets (fully cooked) and frozen chicken strips (containing raw poultry) at initial populations of 10^6 to 10^7 CFU/g. Survival was assessed during storage at −20°C for 16 weeks by measuring bacterial growth on minimal, selective, and nonselective agars. Results indicate that cell populations measured in nonselective agars (plate count agar and plate count agar supplemented with tetracycline) and minimal (M9) agar remained relatively constant during the entire −20°C storage period studied (16 weeks) for both chicken nuggets and strips. However, cell populations were significantly (P < 0.05) lower when measured in selective agar (XLT4) during the 16 weeks of frozen storage for both chicken nuggets and strips, suggesting that these cells were structurally injured. The data presented in this study indicate that *Salmonella* can survive frozen storage when inoculated in frozen, processed chicken products and confirm that microbial counts on selective agar are not representative of the total population of samples subject to freezing.

Frozen, breaded chicken products containing raw poultry have been recently identified as risk factors for *Salmonella* infection (8, 16). Salmonellosis outbreaks in Australia (15), British Columbia (8, 16), and Minnesota (22) have implicated raw, frozen chicken nuggets, strips, and entrees as transmission vehicles of infection. The cooked appearance of frozen breaded chicken products containing raw poultry makes them potentially dangerous; consumers are likely to identify them as fully cooked and only reheat them before consumption, as suggested by epidemiological investigations of recent outbreaks (8, 22).

Previous research has studied the survival of *Salmonella* in meat and poultry products (mostly unprocessed) during frozen storage, with results ranging from complete to minimal survival, with varying degrees of injury. Georgala and Hurst (13) reported survival of *Salmonella* Typhimurium during 90 days of storage at −20°C when inoculated in comminuted beef. Foster and Mead (12) measured almost 100% survival of *Salmonella* (serotypes Agona, Cerro, Haardt, Livingstone, and Typhimurium) in nonsterile minced chicken breast during 100 days at −20°C and approximately 10% in nonsterile minced chicken leg. Barrell (2) demonstrated that *Salmonella* Typhimurium U285 was able to survive frozen storage (−18 to −20°C) when inoculated in cooked minced beef; structurally injured but not metabolically injured cells were detected. However, freeze-induced metabolic injury in *Salmonella* has been previously reported (23). Metabolic injury and structural injury differ only in the degree of cell damage; all injured cells have their permeability barriers damaged, but in metabolically injured cells, functional components related to their metabolic activities are affected as well (19). Escartin et al. (11) reported drastic reductions, as large as 3 log, of *Salmonella* populations in naturally contaminated raw pork during 42 and 78 weeks of storage at −15°C; structural injury was not assessed. Dykes and Moorhead (9) found no significant variation in *Salmonella* populations (serotypes Brandenberg, Dublin, and Typhimurium) during 9 months of storage at −18°C when inoculated in beef trimmings; structural injury was assessed but not detected.

Due to the variability in results reported in the published literature regarding the survival of *Salmonella* in different meat and poultry products during frozen storage, and in response to the recent association of frozen chicken products with salmonellosis outbreaks, the objective of this study was to assess the survival of *Salmonella* during frozen storage when inoculated in chicken nuggets and strips.

**MATERIALS AND METHODS**

**Processed chicken products.** Two kinds of frozen products were used: cooked chicken nuggets (each nugget, ~17 g) and raw chicken strips (each strip, ~25 g). The cooked nuggets were described on the product’s label as “Breast nuggets. Fully cooked. Breaded nugget-shaped chicken breast patties with rib meat”; the chicken strips were described as “Crunchy chicken strips.

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TABLE 1. Salmonella strains used*  

<table>
<thead>
<tr>
<th>S. enterica serovar</th>
<th>Resistant to:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kentucky Typhimurium</td>
<td>Streptomycin and tetracycline, ampicillin, cephalothin, cefllofur, cefoxitin, streptomycin, and tetracycline</td>
</tr>
<tr>
<td>Kentucky Typhimurium</td>
<td>Not antibiotic resistant</td>
</tr>
</tbody>
</table>

* Data provided by Dr. Jianghong Meng, University of Maryland (7).

Contains uncooked poultry. Breaded strips-shaped chicken patties. Chicken meat, water, sodium phosphates, and salt are listed as ingredients in both products, with variations in other components.

**Bacterial strains.** Two strains of *Salmonella* Kentucky and two strains of *Salmonella* Typhimurium isolated from chicken (7) with and without antibiotic resistance (Table 1) were used. The chicken strips used in this study contain raw poultry; therefore, antibiotic-resistant strains were considered in order to overcome potential interference with background microflora when monitoring *Salmonella* survival in XLT4 agar. For consistency, antibiotic-resistant strains were also considered when monitoring *Salmonella* survival in cooked chicken nuggets.

**Inoculation procedures.** Cells were grown to stationary phase on tryptic soy broth (Difco, Becton Dickinson, Sparks, MD) or tryptic soy broth (Difco, Becton Dickinson) with 0.01 g of tetracycline per liter for 18 h at 37°C. Stationary-phase cultures were used in order to realistically represent the state of natural contaminants of meat. After incubation, all strains were combined in one inoculation cocktail. The day before inoculation, the frozen chicken products were thawed overnight at 4°C. A total of 0.2 ml of the *Salmonella* cocktail was inoculated using a syringe in two spots (0.1 ml each) of the thawed chicken nuggets, and 0.3 ml (0.15 ml in each of two spots) was inoculated into the thawed chicken strips. A set of samples was tested immediately for initial population determination. All other samples were placed in their original package and stored in a laboratory freezer at −20°C immediately after inoculation. An inoculation level of ~10^5 CFU/g was targeted for both products. Inoculation spots were located on the center and edge of the products, at a depth equivalent to one-half of their width (total width, −1 cm for both nuggets and strips).

**Frozen storage.** A laboratory freezer set to −20°C (±1°C) was used to store samples during frozen storage. Frozen poultry products are commonly stored at temperatures close to −20°C (1). The temperature inside the freezer was monitored at every sample collection point with a mercury thermometer in glycerol. Freezing rate was determined for two separate sets of samples (nuggets and strips). Thawed samples (initial temperature, 3 ± 1°C) were placed in the freezer, and temperatures were monitored hourly with a calibrated infrared thermometer.

**Microbiological analysis.** Duplicate samples were analyzed for each set (i.e., cooked nuggets and raw strips) at frequencies ranging from weekly to every 3 weeks depending on the stage of the study. Frozen samples were stomached, serially diluted, plated on minimal, selective, and nonselective agars, and incubated at 37°C for 24 h. Metabolically injured cells are not able to produce colonies on minimal agar, nor are structurally injured cells able to produce colonies in selective agar; metabolically and structurally injured cells are able to produce colonies on nonselective agar (2, 19, 20). Controls for noninoculated nuggets and strips were determined. The detection limit of the method is 50 CFU/g.

**Microbiological media.** Nonselective agars used were plate count agar (PCA; Difco, Becton Dickinson) and PCA (Difco, Becton Dickinson) with 0.01 g of tetracycline per liter (PCA-Tet; Acros Organics, Pittsburgh, PA); the selective agar used was XLT4 agar (Difco, Becton Dickinson). Minimal agar (M9) consisted of 200 ml of M9 minimal salts 5X (Difco, Becton Dickinson), 800 ml of distilled water, and 15 g of agar powder (Fisher Scientific, Pittsburgh, PA), which were mixed and autoclaved (121°C for 20 min). When cooled to room temperature, 2 ml of autoclaved 1 M MgSO_4 solution, 0.1 ml of autoclaved 1 M CaCl_2 solution, and 20 ml of filter-sterilized (0.45-μm-pore-size syringe filter) 20% glucose solution were added.

**Statistical analysis.** The software SPSS for Windows version 16.0 (SPSS Inc., Chicago IL) was used in the statistical analysis of the data. Mean cell populations measured immediately after inoculation (week 0) were compared for the nuggets and strips data sets separately, using analysis of variance (P < 0.05) and Duncan’s multiple range test as post hoc test. Mean cell counts averaged over the 16 weeks studied measured on these four agars were compared for the nuggets and strips data sets separately, using analysis of variance (P < 0.05) and Duncan’s multiple range test (Table 2). In addition, mean cell counts measured on each agar over time (weeks 0 to 16) were compared for the nuggets and strips data sets separately, using analysis of variance (P < 0.05) and Duncan’s multiple range test (statistical analysis not shown).

**RESULTS**

As determined on PCA, PCA-Tet, and M9 agar, cell populations of ~10^5 CFU/g were achieved for both nuggets and strips sample sets (Table 3) after inoculation. Results indicate that cell counts immediately after inoculation on XLT4 agar for both nuggets and strips (Table 3) were significantly different (P < 0.05) from those on PCA, PCA-Tet, and M9 agar. Decreased bacterial counts on XLT4 agar may be a result of stress associated with microbial growth on selective media and have been previously reported in a similar study (2). No growth was observed on XLT4, PCA, PCA-Tet, or M9 agar for controls of noninoculated chicken nuggets (fully cooked). For controls of noninoculated chicken strips (containing raw poultry), no growth was observed.
observed on XLT4 or PCA-Tet but bacterial counts were detected on PCA and M9 agar at levels of $10^2$ to $10^5$ CFU/g.

After inoculation, samples were subjected to frozen storage ($-20 \pm 1^\circ C$). For both nuggets and strips, the average freezing rate was $10.5^\circ C/h$ until the freezing point was reached (about $-12^\circ C$) and $7^\circ C/h$ thereafter, until the final storage temperature of $-20^\circ C$ was reached. The total freezing process was completed in 4 to 5 h for samples starting at $-3^\circ C$. During the storage period studied (16 weeks), for both nuggets and strips data sets, a relatively constant population level of $\sim 10^5$ CFU/g was measured when monitoring growth on PCA, PCA-Tet, and M9 agar (Figs. 1 and 2). Mean cell counts on XLT4 agar were identified as significantly different from counts on PCA, PCA-Tet, and M9 agar in both nuggets and strips data sets and were the lowest measured of all four agars ($3.2 \pm 0.5$ and $3.1 \pm 0.6$ CFU/g for nuggets and strips, respectively). Mean cell counts on PCA, PCA-Tet, and M9 agar were considered to be equivalent ($P < 0.05$) from weeks 0 to 16 in the chicken strips data set. For the nuggets data set, significant differences were identified between counts on PCA, PCA-Tet, and M9 agar. These statistical differences, however, are considered to be of no practical significance, as can be deduced from Figure 1.

Cell counts on XLT4 agar measured immediately after inoculation (week 0) were identified as significantly different from counts during the frozen storage period (weeks 1 and 16) in both nuggets and strips data sets. As seen in Figures 1 and 2, after an initial decrease during the first week of frozen storage ($\sim 1$ log), counts on XLT4 agar remained consistently lower than those on PCA, PCA-Tet, and M9 agar during the 16 weeks of frozen storage studied for both nuggets and strips data sets. For the strips data set, statistical analysis of cell counts on XLT4 over time also revealed significant differences between counts from weeks 1 to 16, supporting the trend seen in Figure 2 of slight decrease followed by recovery after week 10. For PCA, PCA-Tet, and M9 in both nuggets and strips data sets, averaged cell counts for every time point measured throughout the storage period were found to be either equivalent or different, based on apparent statistically significant differences but with no clear chronological trend, as can be deduced from Figures 1 and 2.

### DISCUSSION

The results presented in this study demonstrate that *Salmonella* can readily survive frozen storage ($-20 \pm 1^\circ C$) for at least 16 weeks when inoculated in raw and cooked frozen, breaded chicken products, with structural injury as a consequence. These results are in close agreement with those of Barrell (2) involving cooked minced beef; however, Barrell reports a slight trend of decreasing population ($\sim 0.5$ log over 10 weeks of frozen storage). In our study, cell populations measured in complete and minimal agars remained relatively constant from week 0 to week 16. Also, Barrell observed a less pronounced difference between counts on selective and nonselective agars suggesting a lower proportion of structurally injured cells. Georgala and Hurst (13) reported a rapid decrease in *Salmonella* population during initial freezing when inoculated in comminuted beef, followed by constant levels during frozen storage. A similar decrease in population during initial freezing was observed in our study during the initial weeks of frozen storage.
freezing was also observed in our study when monitoring cell population in selective agar. Georgala and Hurst do not specify, however, which kind of microbiological media were used to measure Salmonella populations. High survival of Salmonella during frozen storage was also observed by Foster and Mead (12) in minced chicken breast; however, survival in minced chicken leg was lower. These results were attributed to the different pH of breast (5.8) and leg (6.4) samples. The pH of the samples used in our study was determined experimentally as ~6.3 for the chicken strips and ~6.6 for the chicken nuggets, suggesting predominance of chicken leg meat. Foster and Mead also found that polyphosphates had a negative effect on the survival of Salmonella during frozen storage when inoculated in both chicken breast and leg. Chicken nuggets and strips used in our study report sodium phosphates in the ingredients list; the concentration, however, is unknown.

Our results conflict with those of Dykes and Moorhead (9), who report complete survival of Salmonella in beef trimmings during frozen storage but no significant difference (P < 0.05) between counts on selective and nonselective media, indicating absence of structural injury. These results were attributed by Dykes and Moorhead to the high fat content of the samples used (10%), which may act as an insulating compound, to the fact that the pathogen was surface inoculated, and/or to factors intrinsic to the Salmonella serotypes used. The fat content of the samples used in our study, as deduced from information on the labels, were 19.7 and 19.6% for the chicken strips and nuggets, respectively, and may have contributed to protecting the cells during freezing. The results presented in our study also conflict with those of Escartin et al. (11), which indicate a significant decrease in Salmonella and total aerobes population in raw pork during frozen storage. Although the use of naturally contaminated pork may suggest high variability in the study of Escartin et al., a clear decreasing trend was observed in three different trials of 22, 42, and 78 weeks of frozen storage. The freezing rate of pork samples was not reported in their study; however, the fact that samples were frozen and stored at −15 ± 2°C may have negatively affected the survival of Salmonella.

Previous research has demonstrated that slow freezing (1 to 10°C/h), characterized by extracellular ice formation and osmotic dehydration of the cell, is more detrimental to the cell than rapid freezing (>50°C/h), characterized by intracellular ice formation (1, 4, 10, 20). Also, death rates during frozen storage are more pronounced at temperatures just below the freezing point and are usually slow below −20°C (12, 13). In our study, the freezing rate of chicken nuggets and strips qualifies as slow freezing (7 to 10.5°C/h), but no death or severe damage to the cells was observed, suggesting a protective effect of the chicken matrix.

A recent study conducted in Canada by Bucher et al. (5) identified Salmonella Heidelberg as the most commonly isolated serovar from chicken nuggets and strips. Salmonella Heidelberg has been previously implicated in a salmonellosis outbreak in Canada associated with chicken nuggets and strips (8, 16). The second most commonly isolated serovar in the study of Bucher et al. was serovar Kentucky, and it was reported that both Salmonella Heidelberg and Salmonella Kentucky are also the most commonly isolated serovars from chicken in Canada. Salmonella Heidelberg, Typhimurium, and Enteritidis have been identified in recent salmonellosis outbreaks associated with frozen chicken products in the United States (22). The Salmonella strains used in our study, Salmonella Typhimurium and Salmonella Kentucky (Table 1), were previously isolated from poultry (7). It is expected that Salmonella naturally found in raw poultry, the main ingredient of chicken nuggets and strips, may also be found in the final product. However, differential sensitivity to freezing, if any, may result in increased prevalence of certain serovars in the frozen product. The variability in results reported in the literature regarding the survival of Salmonella during frozen storage may be partially attributed to variability in serovars used; however, factors related to the meat or poultry product and storage temperature, among others, may have influenced the results as well.

This study, like those of Barrell (2), Georgala and Hurst (13), Foster and Mead (12), and Dykes and Moorhead (9), reports a high survival rate of Salmonella during frozen storage. Similar results have been obtained for Campylobacter spp. in meat packs (14) and broilers. It has been hypothesized that the meat matrix acts as a hydrocolloid, binding free water and thus reducing the harmful effect of freezing on bacterial cells (18). In addition, it can be inferred that storage at −20°C represents a mild stress for Salmonella, particularly when the bacterium is inoculated in a meat matrix, and such storage is only capable of temporarily injuring bacterial cells. Thus, in our study we report no death or metabolic injury of Salmonella during 16 weeks of frozen storage, only structural injury. Though not only concerning food processing conditions, extensive evidence points at damaged outer membranes as the major effect of freezing bacteria (17), as suggested by leakage of cellular components into the growth medium, penetration of surfactants, dyes, and enzymes into the cell (1, 3, 6, 20, 21), and sensitization to antibiotics (21) and bacteriocins (3). Sublethally damaged foodborne pathogens are able to rapidly repair the injury once environmental conditions become favorable and regain their capacity to cause disease (4, 19). Failure to detect these injured cells, when using selective microbiological media for example, may represent a significant risk of foodborne illness. As anticipated early by Speck and Ray (24), frozen foods contaminated with pathogenic bacteria can be an unsuspected health hazard for the consumer. This risk is currently of higher concern particularly for frozen, breaded chicken products containing raw poultry because of the protective effect of the chicken matrix for bacterial survival during freezing, as well as the likelihood of undercooking by the consumers.

REFERENCES


