Research Note

Survival after Cryogenic Freezing of Campylobacter Species in Ground Turkey Patties Treated with Polyphosphates†

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ABSTRACT

The use of polyphosphate-based marinades in the processing of poultry has been previously shown to increase the survival of Campylobacter species present in the exudates derived from these products. This study investigates the effects that some of the same polyphosphates have on the survival of Campylobacter species within a ground turkey product subjected to cryogenic freezing. Ground turkey patties with two different polyphosphate formulations added in two different concentrations were artificially contaminated with known concentrations of Campylobacter jejuni or Campylobacter coli. The patties were cryogenically frozen at −80°F (−62.2°C) with liquid nitrogen vapor and held at −20°C for 7 or 33 days, after which the number of Campylobacter surviving in the patties was determined. On average the cryogenic freezing resulted in a 2.5-log decrease in the survival of Campylobacter jejuni (19). The previously demonstrated undesirable effect that polyphosphates can have on the pH by the polyphosphates in the poultry exudates allowed the modulation of the pH in 2-phosphate-treated poultry products (28). Undercooked poultry and cross-contamination of other foods by raw poultry has been identified as a common method for Campylobacter transmission to humans (3). However, Campylobacter initially appears an unlikely candidate to persist within poultry processing conditions and cause such a significant amount of disease, given that it is nutritionally fastidious and sensitive to the oxygen levels present in a normal environment (19). Previous research attempting to understand the unexpected persistence of Campylobacter identified polyphosphate-based poultry marinades as enhancing Campylobacter survival within the exudate derived from processed poultry products (10). Additionally, it was determined that the added polyphosphates had little effect on the pH of the ground turkey meat; an effect which previously had been implicated in the enhancement of Campylobacter survival due to the presence of polyphosphates.

Foodborne pathogens are responsible for approximately 9.4 million cases of illness, 55,000 hospitalizations, and 1,350 deaths in the United States annually (25). Campylobacter species are responsible for ca. 9% of those illnesses and approximately 15% of hospitalizations (25). In the United States, the incidence of Campylobacter infection has increased 14% in 2012 over that in 2006 to 2008 (5). In addition to issues in the United States, Campylobacter spp. are responsible for the largest number of foodborne gastrointestinal bacterial infections in the developed world (4, 8, 19). Historically, poultry products have been identified as a primary avenue for the introduction of Campylobacter into the food supply (14, 15). Because of this, the USDA, Food Safety and Inspection Service (FSIS) has proposed new rules for sampling and performance standards for poultry products (27). Recent verification testing programs conducted by FSIS from 2011 to 2012 indicate that as many as 30% of young chicken and turkey carcasses test positive for Campylobacter spp. (28).

Undercooked poultry and cross-contamination of other foods by raw poultry has been identified as a common method for Campylobacter transmission to humans (3). However, Campylobacter initially appears an unlikely candidate to persist within poultry processing conditions and cause such a significant amount of disease, given that it is nutritionally fastidious and sensitive to the oxygen levels present in a normal environment (19). Previous research attempting to understand the unexpected persistence of Campylobacter identified polyphosphate-based poultry marinades as enhancing Campylobacter survival within the exudate derived from processed poultry products (10). Additionally, it was determined that the modulation of the pH by the polyphosphates in the poultry exudates allowed for the increased survival of Campylobacter in polyphosphate-treated poultry products (11). The previously demonstrated undesirable effect that polyphosphates can have on the pH raises the concern that polyphosphates may interfere with the action of intervention technologies (cryogenic freezing, high pressure, and UV light) designed to decrease the Campylobacter load present in poultry. Additional research is needed to determine the effect that polyphosphates may have on Campylobacter survival when applied to a poultry product undergoing an intervention designed to decrease bacterial numbers.

Cryogenic freezing involves the direct exposure of food to subfreezing temperatures to maintain the microbiological and physical quality, as well as the shelf life of foods (2, 13, 17, 20, 26). Despite the publication of many studies on the effect of freezing foods on foodborne pathogen survival, many of those studies used simple laboratory or retail freezers. There is little information regarding the effect of

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† Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.
cryogenic freezing on the viability of pathogenic microorganisms using pilot or industrial-scale equipment and conditions. Preliminary studies indicate that quick freezing of foods by using industrial equipment can result in the inactivation of foodborne pathogens (21). The effect of quick freezing on the survival of Campylobacter in ground poultry products has not yet been quantified. Furthermore, ground turkey patties are often supplied to and stored by the consumer in a frozen state to extend product freshness and shelf life. The purpose of this study was to determine the effect of polyphosphate type and concentration, in combination with quick freezing, on the survival of Campylobacter in a ground poultry product (ground turkey).

MATERIALS AND METHODS

Bacterial strains. Campylobacter jejuni (RM1221) and Campylobacter coli (RM1403) strains were obtained from Dr. Robert Mandrell (USDA, Agricultural Research Service, Western Regional Research Center, Albany, CA) and were maintained frozen (−80°C) until used. Three days before the procedure, each culture was grown on Brucella agar, prepared by adding 1.5% agarose to Brucella broth (BD, Sparks, MD), and incubated at 42°C in a microaerobic chamber (MACS-VA, Don Whitley, UK; 5% O2, 10% CO2, 85% N2) to obtain a 24-h culture. Two days before the procedure, the individual isolates were grown in Brucella broth (BD) and incubated at 42°C in a microaerobic chamber for 48 h.

Ground turkey patty production. The ground turkey was purchased from a local supermarket and frozen (−20°C). The frozen sample was irradiated to 20 kGy using a self-contained Cs gamma irradiator (Lockheed Georgia Company, Marietta, GA) at a dose of 0.073 kGy/min. The sample remained frozen until use. The irradiated frozen turkey was defrosted overnight in the refrigerator (4°C). Samples (130 g) were weighed aseptically and placed in sterile Whirl-Pak stomacher bags (Nasco, Salida, CA). Three milliliters of filter-sterilized sodium hexametaphosphate or pentasodium tripolyphosphate solutions (ICL Performance Products LP, St. Louis, MO) were added to obtain final polyphosphate concentrations of 0.25, 0.50, and 0%. The samples were mixed in a Stomacher Lab Blender 400 (Brinkmann, Seward, NY) for 1 min to ensure adequate distribution of the added polyphosphate. Thirteen milliliters of the 48-h Campylobacter inoculum was then added to the sample, which was pummeled again with a stomacher to ensure adequate distribution of the inoculum. Individual patties were formed by aseptically placing the uninoculated or the inoculated polyphosphate sample (0.25 and 0.50%) into a plastic circular cup (3.8-cm diameter and height of 1 cm). The resulting patties had an average mean weight of 13.38 ± 0.66 g. The individual patties were placed into Whirl-Pak bags (19 by 30 cm) before freezing. The pH of samples containing each polyphosphate concentration (0, 0.25, and 0.50%) and Brucella broth equal to the inoculum volume was measured using a stainless steel surface pH electrode attached to the pH meter (IQ Scientific Instruments, San Diego, CA).

Turkey patty flash freezing and storage. The turkey patties were flash frozen using a Cryo-Test Chamber (Air Products and Chemicals, Allentown, PA). The individually bagged patties were placed in the chamber and rapidly frozen to a temperature of −80°F (−62.2°C) by the application of liquid nitrogen vapor and held at that temperature for 4 min. Following the flash freezing treatment, the turkey patties were held at −20°C for 7 or 33 days.

Enumeration of surviving Campylobacter. After the designated frozen storage time, a 10× volume of Brucella broth (wt/vol) was added to each sample, pummeled in a stomacher for 1 min, and serially diluted using Brucella broth. Recovery of viable Campylobacter was done by plating the diluted samples onto Brucella agar in duplicate using the 6 by 6 drop plate method, with six replicates per dilution (6) and incubated overnight (42°C) in the microaerobic chamber (5% O2, 10% CO2, 85% N2).

Statistical analyses. An analysis of variance was performed using the Mixed procedure of the SAS Software System to determine the effects and interactions of species and conditions (24). Mean separations were performed using the Bonferroni least significant difference technique (18). Analyses were performed separately for C. jejuni and C. coli strains. All statistical tests of significance were performed at the P < 0.05 level.

RESULTS

Cryogenic freezing–mediated reduction of C. jejuni in ground turkey product is not influenced by polyphosphate usage. The 48-h C. jejuni RM1221 broth culture had a cell concentration of log 10^8 CFU/ml, and the concentration of C. coli RM1403 was log 10^9 CFU/ml. After addition to the polyphosphate-prepared ground turkey sample, the inoculated sample had cell densities of log 7 and log 8 CFU/g of C. jejuni and C. coli, respectively.

The recovery of the C. jejuni strain RM1221 from the flash frozen patties without polyphosphate additives resulted in an average reduction of 2.7 log after 7 days of storage at −20°C and 2.5 log after 33 days of storage (Fig. 1A). The addition of the hexametaphosphate at a final concentration (FC) of 0.25% in the ground turkey demonstrated C. jejuni reductions due to freezing of 2.8 log after 7 days of storage and 2.8 after 33 days of storage. Doubling the concentration of hexametaphosphate (0.5% FC) produced C. jejuni freezing reductions of 2.2 log after 7 days and 2.9 log after 33 days. The pentasodium tripolyphosphate-treated ground turkey produced similar results. Ground turkey patties with a FC of 0.25% tripolyphosphate had C. jejuni average flash freezing reduction of 2.1 log after 7 days and 2.4 log after 33 days. A 0.5% concentration of tripolyphosphate in the patties resulted in reductions due to freezing of 2.0 log after 7 days and 2.9 log after 33 days. For each of the different conditions, the culturable numbers of C. jejuni present in the patties at both 7 and 33 days were significantly different from the numbers of C. jejuni prior to freezing. However, the differences in C. jejuni numbers between samples with different polyphosphate treatments compared only within either the control (not treated), or 7 or 33 days postfreezing groups were not significant, with the exception of the 0.5% tripolyphosphate treatment at 7 days (Fig. 1A).

Cryogenic freezing–mediated reduction of C. coli in ground turkey product is not influenced by polyphosphate usage. The results observed for the patties containing C. coli strain RM1403 were similar to those for RM1221 (Fig. 1B). Patties without added polyphosphates exhibited
flash freezing reductions of 2.5 log after 7 days and 2.9 log after 33 days. Hexametaphosphate-containing patties at concentrations of 0.25 and 0.5% after freezing had reductions of 2.7 and 2.5 log after 7 days and 3.3 and 3.8 log after 33 days, respectively. Average flash freezing reductions in patties again containing 0.25% pentasodium tripolyphosphate were 2.4 log after 7 days and 3.2 log after 33 days; in patties containing 0.5% tripolyphosphate, the reductions were 2.6 log for 7 days and 3.6 log for 33 days. Also, similar to the C. jejuni results, the numbers of C. coli present in the patties with different concentrations of polyphosphate additives at both 7 and 33 days were significantly different from the numbers of C. coli prior to freezing. Likewise, when comparing samples with different polyphosphate treatments, the differences in viable C. coli numbers between those samples within either the control (not frozen), or 7 or 33 days postfreezing groups were not significant (Fig. 1B).

Polyphosphate additions do not affect the pH of ground poultry products. Adding different polyphosphate concentrations to the ground turkey meat produced only small changes to the pH of this product (Table 1). The pH of the ground turkey without additives was measured at 6.24. The addition of sodium hexametaphosphate at a FC of 0.25 and 0.5% produced pH values in the ground turkey of 6.26 and 6.20, respectively. Similarly, the addition of pentasodium tripolyphosphate at FCs of 0.25 and 0.5% resulted in ground turkey with pH values of 6.38 and 6.40.

### DISCUSSION

There has been considerable previous research into Campylobacter species survival in poultry after refrigeration or freezing (7, 9, 12, 23). This research has reported the reduction in the survival of Campylobacter in poultry products due to freezing temperatures (ca. -20°C) that range from 0.5 to 5 log reduction in CFU. The large variation in the range of reductions in Campylobacter numbers from freezing is caused by differences in the results reported from one experimental study to the next, which can be partially attributed to differences in the poultry material used in the experiments. Specifically, it has been observed that C. jejuni survives the freezing process and storage at significantly greater numbers within poultry meat versus on the surface of the poultry meat or on poultry skin (1, 22). Our study, which used a ground turkey product, resulted in an overall reduction of 2.4 to 2.9 log for C. jejuni and 2.9 to 3.8 log for the C. coli strain (Fig. 1A and 1B). This is a promising result, given that previous research involving Campylobacter survival in frozen ground chicken products demonstrated a maximum reduction of between 1.5 and 2 log when frozen at -20°C (1, 23). Note that the previous studies did not employ a rapid freezing technique, used in our study. The Cryo-Test Chamber reached a temperature of -62.2°C in less than 1 min after liquid nitrogen was first introduced. After 4 min at a temperature of -62.2°C, the turkey patties were completely frozen throughout. The completely frozen state of the turkey patties was achieved much more rapidly than with standard refrigeration at

### TABLE 1. pH of the ground turkey samples

<table>
<thead>
<tr>
<th>Polyphosphates level</th>
<th>pH of resulting combination</th>
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<tbody>
<tr>
<td>No polyphosphate added</td>
<td>6.24</td>
</tr>
<tr>
<td>Sodium hexametaphosphate added (0.25% FC)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.26</td>
</tr>
<tr>
<td>Sodium hexametaphosphate added (0.5% FC)</td>
<td>6.20</td>
</tr>
<tr>
<td>Pentasodium tripolyphosphate added (0.25% FC)</td>
<td>6.38</td>
</tr>
<tr>
<td>Pentasodium tripolyphosphate added (0.5% FC)</td>
<td>6.40</td>
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</tbody>
</table>

<sup>a</sup> FC, final concentration of polyphosphate in ground turkey (wt/wt).
—20°C, and this could account for the differences observed in Campylobacter survival between our research and previous studies. However, other researchers have suggested that rapid freezing to −20°C of poultry products containing Campylobacter can lead to a smaller reduction in Campylobacter numbers compared with a gradual freezing to −20°C in a commercial freezer (7). Although our flash freezing results provided greater reductions in Campylobacter numbers compared with previous studies using gradual freezing, note here that our experiments performed the rapid freezing at −62.2°C and storage at −20°C versus −20°C for both freezing and storage used in previous research. Additionally, note that the gradual freezing study used poultry skin as the food target contaminated with Campylobacter compared with the ground poultry used for the research reported in this study (7).

Another potential cause for the variation in Campylobacter freeze reductions is that the different freeze studies used different lengths of time for storage of samples at the freeze temperatures. Frozen storage times in previous studies ranged from as short as 10 to 14 days (1, 23) to as long as 220 days (9). However, several of the studies reported that the greatest reductions in Campylobacter numbers occurred during the first 7 days or even the first 24 h of freezing, with only limited reductions in numbers occurring after these time periods (1, 7, 9, 22, 23). Our results from flash freezing follow the observations of the previous studies, with on average, the largest percentage of reductions in Campylobacter numbers observed in our experiments occurring within the first 7 days after freezing, with smaller percentages of additional reductions occurring from 7 to 33 days (Fig. 1A and 1B). This trend appeared more pronounced in the C. jejuni strain compared with the C. coli strain. These results suggest that a significant reduction in Campylobacter can be achieved in a ground poultry product by flash freezing, without necessitating an additional long period of frozen storage.

Previous research had showed that the treatment of refrigerated poultry products with polyphosphates enhanced the survival of Campylobacter in the product by modulating the pH of the exudate of the product to a level preferred by the bacteria (10, 11). Therefore, based on previous research, it is not surprising that the different polyphosphate concentrations had no significant effects on Campylobacter survival in the flash frozen ground turkey patties. Ground meats have been shown to have a buffering effect when treated with an acidic or alkaline solution (16, 29). Therefore, the polyphosphate added to the ground turkey meat in our study had no significant effects on Campylobacter survival in the flash frozen ground turkey patties. Without a shift in pH toward a range more conducive to Campylobacter survival, the polyphosphates would have no effect on Campylobacter survival at cold storage temperatures.

In conclusion, our study suggests that flash freezing to −62.2°C provides a 2.5- to 3.7-log reduction in Campylobacter numbers in ground turkey meat. This level of reduction is superior to previous reported reductions of Campylobacter in ground poultry products after gradual freezing by using laboratory freezers at −20°C. Finally, the buffering ability of the ground meat allows the application of polyphosphate additives, without any detrimental effects on the reducing action of the flash freezing and frozen storage.

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REFERENCES


