Evaluate survival/growth during frozen, refrigerated, or retail type storage, and thermal resistance, following storage of *Escherichia coli* O157:H7 contamination on or in marinated, tenderized or restructured beef steaks and roasts which will minimize survival or enhance destruction of the pathogen

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Background

Escherichia coli O157:H7 is considered as an adulterant of raw ground beef and of otherwise non-intact beef products. Because of its inherent acid tolerance, and low infective dose (≥10 cells; Doyle et al., 1997), regulatory agencies and industry have been prompted to evaluate processes that may contribute to spread of disease through ground beef, non-intact beef, and beef products made from trimmings (NACMCF, 2002; Sporing, 1999; USDA-FSIS, 1999). The United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) conducts sampling and testing of ground beef for the presence of E. coli O157:H7 (USDA-FSIS, 2002) and, since 1999, has also tested non-intact beef products for the pathogen. USDA-FSIS (2002) defines non-intact beef products as those injected with solutions, mechanically tenderized by needling, cubing or pounding devices, or those reconstructed (or restructured) into formed entrees (i.e., scored to incorporate marinade, or formed, reconstructed products like roasts and gyros). The processes that may introduce pathogens below the surface include chopping, grinding, flaking or mincing; in fact, any process in which the integrity of an intact cut is modified or altered could allow for introduction of a pathogen.

It can be summarized that even though at low rates, mechanical tenderization and injection of flavoring and tenderizing ingredients could potentially, even though infrequently and at low levels, internalize foodborne pathogens from the surface to the interior of products like beef steaks and roasts. If such contaminated products were considered or mistaken as intact beef and intentionally or accidentally undercooked by consumers during traditional cooking processes such as grilling, broiling and frying, then the internalized pathogen might survive and pose a safety concern to the consumers. Furthermore, the risk of pathogen survival could be higher if chemical ingredients used in marination, tenderization or restructuring formulations were protective against thermal inactivation of pathogens, and could contribute to a higher risk of infection from such products. Therefore, in the present study, we evaluated the effect of certain marination, tenderization and restructuring ingredients, used in non-intact steaks and roasts, on thermal inactivation of E. coli O157:H7, when the pathogen was embedded into the meat, and when the cooking of such products by various methods was insufficient. Since internalization of pathogen and subsequent thermal inactivation could be unpredictable and non-uniform in such products, and because such pathogen internalization is established to occur, even though infrequently, we decided to examine the worst case scenario, and thus, developed a model system using ground beef to conduct the study. This allowed uniform internalization of the pathogen and thorough mixing of the ingredients into the product, and hence reliable and consistent evaluation of the effect(s) of the various ingredients and cooking processes on reduction or enhancement of thermal inactivation of inoculated E. coli O157:H7.

The stated objectives for this work were:

To develop modified formulations and processes for marination, tenderization and restructuring of beef steaks and roasts which will minimize risk of Escherichia coli O157:H7.
Methodology

Approach 1:

Preparation of model non-intact beef system

Beef knuckles (95% lean) were obtained from a local meat packing plant (Swift and Company, Greeley, Colorado), and stored at -23°C in the Meat Laboratory, Center for Red Meat Safety, Department of Animal Sciences at Colorado State University for no longer than 7 days. After thawing at 3°C for 24 h, the excess seam fat was removed, and the knuckles were cut into smaller pieces to facilitate grinding.

E. coli O157:H7 strains and preparation of the inoculum

A 5-strain composite culture, which included the strains ATCC 43895 (hamburger isolate), ATCC 43894 (human isolate), ATCC 43889 (human isolate), ATCC 43888 (human isolate), and EO139 (jerky isolate) was used to prepare the inoculum. The strain EO139 was kindly provided by Dr. M. P. Doyle (Univ. of Georgia, Griffin, Georgia).

Treatment, inoculation, and storage of samples

The individual marination ingredients, dissolved in 22 ml of sterile distilled water, were mixed with the samples to obtain the concentrations of treatments:

1. control,
2. water (sterile distilled water),
3. citric acid (Fisher Scientific, Fair Lawn, New Jersey, 0.2%; wt/wt),
4. acetic acid (Fisher Scientific, 0.3%; v/wt),
5. potassium lactate (PURAC America, Inc., Lincolshire, Illinois, 1.8%; v/wt),
6. calcium lactate (Mallinckrodt Baker, Inc., Phillipsburg, New Jersey, 0.63%; wt/wt),
7. calcium ascorbate (SIGMA-ALDRICH, Inc., St. Louis, Missouri, 0.86%; wt/wt),
8. calcium chloride (Fisher Scientific, 0.23%, wt/wt), and
9. sodium chloride (Fisher Scientific, 2.5%; wt/wt).

The marination ingredients tested in combination included:

1. control,
2. water (sterile distilled water),
3. calcium ascorbate (Ca-A, SIGMA-ALDRICH, Inc., 0.86%; wt/wt),
4. calcium chloride (Ca-C, Fisher Scientific, 0.23%; wt/wt),
5. acetic acid (AA, Fisher Scientific, 0.3%; v/wt),
6. citric acid (CA, Fisher Scientific, 0.2%; wt/wt),
7. sodium chloride (NA, Fisher Scientific, 0.5%; wt/wt), and mixtures;
8. Ca-A+NA,
9. Ca-C+NA,
10. AA+NA,
11. CA+NA,
12. Ca-A+Ca-C+NA,
13. Ca-A+AA+NA,
14. Ca-A+CA+NA,
15. Ca-C+AA+NA, and
16. Ca-C+CA+NA.

The 700 g of ground beef was thoroughly mixed with the treatments using a bowl-lift stand mixer (KitchenAid®, Professional 600, St. Joseph, Michigan) for 2 min at a speed setting of 2. The treated beef was extruded as sample of 30 g into tubes (2.5 cm diameter × 10 cm height) using a caulking gun (Facilities Maintenance, Colorado State University). Samples were inoculated (7 log CFU/g) with a pipette below the surface with E. coli O157:H7, and stored (4oC) overnight.

The following restructuring ingredients, dissolved in 20 ml of distilled water were also tested:
(1) control,
(2) water (sterile distilled water),
(3) Fibrimex® (FM, FNA Foods Inc., Calgary, Canada, 10%; wt/wt); mixture (10:1) of fibrinogen and thrombin,
(4) ActivaTMRM (A, Ajinomoto USA, Paramus, New Jersey, 0.75%; wt/wt); a commercial preparation of transglutaminase,
(5) algin/calcium meat binding system (AC); mixture of sodium alginate (TextureezeTM MT 200, ISP, Wayne, New Jersey, 0.4%; wt/wt), calcium carbonate (SIGMAALDRICH, Inc., 0.075%; wt/wt), and encapsulated lactic acid (Capshure® encapsulated lactic acid/calcium lactate, LCL-135-50, Balchem corporation, Slate Hill, New York, 0.6%; wt/wt),
(6) salt/phosphate meat binding system (SP); mixture of sodium chloride (Fisher Scientific, 1.4% wt/wt), sodium tripolyphosphate (FMC Corporation, Philadelphia, Pennsylvania, 0.32%; wt/wt),
(7) lactic acid (LA, PURAC America, Inc., 0.3%; wt/wt),
(8) FM+LA,
(9) A+LA,
(10) AC+LA, and
(11) SP+LA.
In addition, a meat tenderizer and other ingredients were tested:
(1) control and 39 ml of following treatments (concentrations in samples);
(2) water (sterile distilled water),
(3) flavoring agent (FA),
(4) calcium chloride (Ca-C, Fisher Scientific, 0.23%; wt/wt) + FA,
(5) (Ca-C, Fisher Scientific, 0.23%; wt/wt) + acetic acid (Fisher Scientific, 0.3%, v/wt) + FA,
(6) sodium chloride (NA, Fisher Scientific, 0.5%; wt/wt) + sodium tripolyphosphate (ST, FMC Corporation, 0.25%; wt/wt),
(7) NA + ST + FA,
(8) NA + ST + potassium lactate (PL, PURAC America, Inc., 1.8%; v/wt) (modified from Vote et al., 2000),
(9) NA + ST + PL + FA,
(10) NA + ST + PL + acetic acid (AA, Fisher Scientific, 0.3%; v/wt), and
(11) NA + ST + PL + AA + FA.

The flavoring agent was used to improve flavor because Scanga et al. (2000) found that addition of a beef-flavoring agent increased flavor of the beef treated with calcium chloride. Use of calcium chloride for tenderizing beef causes an adverse effect on palatability, imparting bitter, metallic and sour taste after cooking products (Scanga et al., 2000; Eilers et al., 1994; Morris et al., 1997). The flavoring agent, which was modified from a study of Calicioglu et al. (2003), was formulated with black pepper (Heller Seasoning and Ingredients, Inc., Chicago, Illinois, 0.42 g), garlic powder (Excalibur Seasoning Co. Ltd., Pekin, Illinois, 0.875 g), onion powder (Excalibur Seasoning Co. Ltd., 1.05 g), old hickory smoked salt (Tone Brothers inc., Ankeny, Iowa, 3.045 g), Worcestershire sauce (Heinz, Pittsburgh, Pennsylvania, 10.5 ml), and sterile distilled water (6.11 ml). The 700 g of ground beef was mixed with 7 ml of Escherichia coli O157:H7 inoculum in a bowl-lift stand mixer (KitchenAid®) for 2 min, and it was mixed with the treatments for 2 min. The samples were extruded into tubes and stored at 4°C as described above.
Cooking of samples and microbial analyses

In preliminary studies, samples were heated to 55, 60 or 65°C. The results, however, indicated no changes in counts when heating to 55°C. Therefore, subsequently, samples were cooked to 60° and 65°C. Sterile thermocouples (Pico Technology Limited, Cambridgeshire, United Kingdom) were inserted into the center of the tubes of ground beef to monitor temperature increases during cooking.

The samples for the evaluation of individual ingredients and their combinations were analyzed immediately after inoculation, after overnight refrigerated storage, and after heating of the samples in the circulating water bath. The 30 g sample was aseptically transferred to 24 oz (Whirl-Pak®, Nasco, Modesto, California), containing 70 ml of maximum recovery diluent (MRD; 0.85% NaCl, Fisher Scientific, and 0.1 % peptone, Difco).

pH and water activity measurement

The pH of the homogenate of each sample, after being plated, was measured using a digital pH meter (Denver Instruments, Arvada, Colorado) with a glass electrode. For the experiments on evaluation of marination, restructuring and tenderization ingredients, pH and water activity measurements were taken immediately after inoculation, after overnight storage of the treated and inoculated samples (before heating), and after heating (60° and 65°C). The water activity of the freshly ground beef batches were measured by an AquaLab (model series 3, Decagon Devices Inc., Pullman, Washington) water activity meter.

Measurements of fat, moisture and cooking loss

In the experiments for evaluating individual treatments and their combinations, fat and moisture content was measured in fresh ground beef, the treated and inoculated samples, and the samples after heating. Approximately 2 g of samples were used in these analyses. Treatments with individual marination, restructuring and tenderization ingredients and combinations were also evaluated for the resulting cooking-loss due to heating. The weights of the treated and inoculated samples were measured immediately before cooking in the circulating water bath. After heating, the liquid purge from the samples was collected in previously labeled tubes, and the samples were reweighed to determine the weight-loss due to expulsion of purge. The experiments were repeated twice with three samples in each replicate.

Approach 2:
Preparation of beef samples and inoculum of E. coli O157:H7

Inoculation and treatment of samples

The treatments selected from Approach 1 were used in the studies for Approach 2. Ground beef (2,000 g; 95% lean) was mixed with 20 ml of E. coli O157:H7 inoculum (8.0 log CFU/ml) in a bowl-lift stand mixer (KitchenAid®) for 2 min, and it was mixed for 2 min with:

1. control; and 20 ml of following restructuring ingredients:
2. Fibrimex® (FM, FNA Foods Inc., 10%; wt/wt); mixture (10:1) of fibrinogen and thrombin,
3. ActivaTMRM (A, Ajinomoto USA, 0.75%; wt/wt); a commercial preparation of transglutaminase,
4. salt/phosphate meat binding system (SP); mixture of sodium chloride (Fisher Scientific, 0.75% wt/wt), sodium tripolyphosphate (FMC Corporation, 0.12%; wt/wt), and
5. SP+lactic acid (SP + LA, PURAC America, Inc., 0.4%; wt/wt).

Also, the following ingredients were tested:
1. control,
(2) calcium chloride (Ca-C, Fisher Scientific, 0.23%; wt/wt) + flavoring agent (FA),
(3) Ca-C + FA + acetic acid (AA, Fisher Scientific, 0.3%; v/wt),
(4) sodium chloride (NA, Fisher Scientific, 0.5%; wt/wt) + sodium tripolyphosphate (ST, FMC Corporation, 0.25%; wt/wt) + potassium lactate (PT, PURAC America, Inc., 1.8%; v/wt) (modified from Vote et al., 2000), and
(5) NA + ST + PL + AA.

**Preparation and storage of non-intact beef product**

The treated ground beef portions (100 g) were used to make patties using a manual hamburger patty maker (10 cm diameter). A stack of seven patties were packaged aerobically (samples were placed on retail foam trays, 20.32 × 25.4 cm; Pactiv, Lake Forest, Illinois) by covering with air-permeable film (Omni-film; Pliant Corporation, Union town, Ohio) or were placed in vacuum bags (30.48 × 30.48 cm, 3 mil standard barrier, nylon/PE vacuum pouch; Koch, Kansas City, Missouri, USA), evacuated, and sealed using a vacuum packager (Hollymatic Corp., Countryside, Illinois, USA). Samples in both types of packaging were stored at -20 (only vacuum package), 4, or 12°C for 50, 14 (aerobic) and 40 (vacuum), or 2 (aerobic) and 10 days (vacuum), respectively.

**Analyses**

The samples were cooked by grilling, broiling, and frying using George Foreman® grill (Salton, Inc., Lake Forest, Illinois), Oster® stainless steel toaster oven (Sunbeam Products, Inc., Boca Raton, Florida), and Toastmaster Cool-Touch Griddle (Toastmaster Inc.®, Columbia, Missouri), respectively. The samples were cooked by placing them on the grilling or frying surface or in the oven for broiling. For broiling and frying, the samples were flipped over when the internal temperature reached 40 or 42.5°C, before they were cooked to the target internal temperature of 60 or 65°C, respectively. Sterile thermocouples (Pico Technology Limited) were inserted into the center of the sample to monitor the temperature increase during cooking to 60 or 65°C internal temperature. The samples were cooked after inoculation followed by treatment (day 0) and during storage.

Total bacterial (TSA) and *E. coli* O157:H7 (MEMB) populations, and pH were determined before and after cooking. Water activity, fat, and moisture contents of fresh beef were also determined. The analytical procedures were same as described in Approach 1. The experiments were repeated twice with two samples in each replicate.

**Findings**

**Approach 1:**

The water activity and the pH of the ground beef were 0.984 to 0.996 (Tables 1 to 5) and 5.49 to 5.59 (Tables 6 to 10), respectively. In general, water activity values of beef samples were not dramatically changed during the process (Tables 1 to 5). The samples treated with sodium lactate had slightly lower water activity (0.960 to 0.970) than other samples (0.970 to 0.983) (Tables 1 to 5). The pH values of samples were not changed dramatically after addition of ingredients, with the exception of citric and acetic acid, which reduced the pH from 5.20 to 5.01 and 5.28 to 4.98, respectively (Tables 6 to 10). The pH values of beef samples treated with acetic and citric acid combined with sodium chloride were also lower than those treated with other ingredients (Tables 6 to 10). The ground beef, made from 95% lean beef in the Meat Science Laboratory at the Department of Animal Sciences, Colorado State University, had 3.2 to 6.4% fat and 69.6 to 72.6% moisture. Acid treated samples had higher weight loss during cooking, but it was lowered once sodium tripolyphosphate was added to the samples (Tables 11 to 15). The control and the samples treated with ingredients, followed by heating to 60 and 65°C, resulted in injury to the inoculated
cells, as indicated by higher counts recovered with non-selective TSA and less selective MEMB, compared to more selective MSMAC agar plates (Table 16 to 24).

Citric and acetic acid, applied individually and in combination with sodium chloride, calcium ascorbate and calcium chloride increased thermal reduction (65°C) of total bacterial and *E. coli* O157:H7 populations compared to other treatments (Table 16 to 24). Sodium chloride showed a protective effect for *E. coli* O157:H7 during cooking (Tables 16 to 24), but when sodium chloride was combined with organic acids, the protective effect was not evident (Tables 19 to 24).

In general, total bacterial and *E. coli* O157:H7 counts were lower in 65°C than in 60°C cooked products, and no difference of total bacterial and *E. coli* O157:H7 counts was found among treatments after overnight marination (before heating) (Tables 25 to 30). Among the restructuring ingredients, treatment with ActivaTMRM showed 4.5-log reduction in total bacterial population (Table 25) due to heating to 65°C, while the *E. coli* O157:H7 counts were reduced by 4.7 log CFU g-1 (Tables 26 to 27) at the same temperature of heating. The treatment with the salt/phosphate meat binding system protected the pathogen from thermal inactivation. None of the individual or combined marination and restructuring ingredients showed any reduction in the total bacterial population or counts of *E. coli* O157:H7 after overnight storage of the treated samples (Tables 25 to 27). After cooking to 60°C, total bacterial populations and surviving counts of *E. coli* O157:H7, in samples containing calcium chloride, sodium chloride, sodium tripolyphosphate, or sodium lactate, were not different than those of control, water or flavoring agent treated samples, but once acetic acid was added to the treatments, surviving counts of *E. coli* O157:H7 became lower than those of water or flavoring agent treated samples (Tables 28 to 30). After cooking to 65°C, total bacterial populations and surviving counts of *E. coli* O157:H7 in samples containing sodium chloride and sodium tripolyphosphate were higher than those of other treatments (Tables 28 to 30). These results suggest that calcium chloride and other ingredients used in the meat industry may not be effective to destroy or may protect *E. coli* O157:H7 cells in nonintact meat during incomplete cooking. Therefore, an organic acid should be considered for inclusion in the formulation. Since addition of organic acid in the treatment containing calcium chloride increased weight loss during cooking of the reduced pH samples, use of sodium chloride and/or sodium tripolyphosphate may be useful in these formulations (Table 15).

**Approach 2:**

The beef used to make the non-intact products had 6 to 8% fat and 67 to 69% moisture content, while the water activity of the beef was 0.977. The pH values of untreated beef samples ranged from 5.16 to 5.62 (Tables 31 to 40). The samples treated with the salt/phosphate meat binding system in combination with lactic acid had lower pH than samples treated with other restructuring ingredients (Tables 31 to 35). In general, pH values of all samples were reduced during storage at 4 and 12°C. However, this reduction was more prominent in samples treated with ActivaTMRM (Table 31 to 35). The pH values of samples containing sodium chloride combined with sodium tripolyphosphate ranged from 5.60 to 5.97, and decreased to a range of 5.38 to 5.09 once acetic acid was added to the treatment (Tables 36 to 40).

Overall, *E. coli* O157:H7 populations were not changed during storage regardless of storage temperature, while the effect of cooking methods in decreasing surviving counts of *E. coli* O157:H7 was in the order broiling > frying ≥ grilling (Tables 41 to 60). Thermal inactivation of total bacterial populations and *E. coli* O157:H7 counts in the products, stored at -20°C in vacuum packages, did not reach 3.0 log CFU g-1, irrespective of the restructuring treatments, cooking methods, and internal temperatures to which the samples were cooked (Tables 41 and 42). The control samples, the samples treated with Fibrimex®, and those treated with ActivaTMRM showed approximately 2.0 log CFU g-1 thermal reduction in total bacterial counts when they were broiled to 65°C on day 50 of their frozen storage. The thermal inactivation of the pathogenic counts were also approximately 2.0
log CFU g⁻¹ when the control samples, Fibrinex® treated samples, and those treated with salt/phosphate meat binder with 0.4% lactic acid were broiled to 65°C, on day 50 of their frozen storage. The products stored at 4°C, when grilled to the internal temperature of 60°C, there was approximately 1-log or less reduction in total bacterial populations or in the pathogenic counts, irrespective of the restructuring ingredients used to treat the samples, duration of storage, and packaging atmosphere (Tables 43 to 46). The control and the Fibrinex® treated samples, stored in air-permeable packages at 4°C, showed almost 4.0 to 4.5 log reduction in total bacterial populations and \textit{E. coli} O157:H7 counts on day 14, when the samples were broiled to 65°C (Tables 43 and 44). The same level of reduction in total bacterial and pathogenic counts was observed in ActivaTMRM treated samples, stored in the same conditions for 7 days. When samples were stored in vacuum packages at 4°C for 40 days, broiling to 65°C reduced the total bacterial population and \textit{E. coli} O157:H7 counts by 3.0 to 4.0 log CFU g⁻¹ in the control samples, the samples treated Fibrinex® and those treated with the salt/phosphate meat binding system with or without lactic acid (Table 45 and 46). The samples treated with ActivaTMRM and stored in the same conditions showed 3-log reduction in the total bacterial and pathogenic counts when they were broiled to 65°C. Consistent with the results for samples stored at 4°C, grilling (60°C) of restructured samples stored at 12°C resulted in less than 1-log reduction in total bacterial population and \textit{E. coli} O157:H7 counts (Tables 47 to 50). At 12°C, after two days of aerobic storage, the ActivaTMRM and Fibrinex® treated products showed 3.3 to 3.4 log reduction in total bacterial and \textit{E. coli} O157:H7 counts, when the samples were broiled to 65°C (Tables 47 and 48). Products restructured with salt/phosphate meat binding system had protective effect on thermal inactivation of \textit{E. coli} O157:H7. However, when the samples treated with this restructuring system were stored in vacuum packages at 12°C, 5.2 and 4.3 log CFU g⁻¹ inactivation in \textit{E. coli} O157:H7 counts were achieved by broiling the samples to 65°C on day 5 and day 10 of storage, respectively (Tables 50). On these two days of storage, 4 and 3 log-reductions in total bacterial counts were observed when the restructured samples were broiled to 65°C. In vacuum-packed storage at 12°C, broiling the control samples, the samples treated with Fibrinex® and those treated with ActivaTMRM to 65°C resulted in 2.8, 3.0, 2.2 log reduction in total bacterial population, and 2.6, 3.0, 3.1 log reduction in \textit{E. coli} O157:H7 counts, respectively.

Overall, Fibrinex® and ActivaTMRM were the restructuring treatments that resulted in more effective thermal inactivation compared to other restructuring treatments. Compared to the control and other restructuring treatments, the products restructured with salt/phosphate meat binding system protected internalized \textit{E. coli} O157:H7 in the refrigerated (4°C) and frozen (-20°C) samples. Among the cooking methods, broiling at 65°C was found to be the most effective, while grilling at 60°C was least effective in thermal inactivation of the pathogenic bacteria. When samples were stored at 4° or 12°C, more days in storage resulted in more thermal reduction of total bacterial and \textit{E. coli} O157:H7 counts, when they were cooked. The difference in the surviving total bacterial and pathogenic counts between the two cooking temperatures is more prominent when the samples were broiled, compared to the other two cooking methods (Tables 51 to 60).

The treatments containing acetic acid reduced more total bacterial and \textit{E. coli} O157:H7 populations than other treatments when broiling to 65°C internal temperature (Tables 51 to 52). In aerobic storage at 4°C, the samples treated with calcium chloride, flavoring agent, and acetic acid and broiled to 65°C showed more effective thermal reduction of \textit{E. coli} O157:H7 counts, while such effectiveness was not evident when the treated samples were grilled or fried (Tables 53 to 54). The combined treatment of the samples with sodium chloride, sodium tripolyphosphate, sodium lactate and acetic acid was not effective in enhancing thermal reduction of \textit{E. coli} O157:H7, even though it contained acetic acid (Tables 53 to 54). As found in previous experiments, sodium chloride and sodium tripolyphosphate may have protected \textit{E. coli} O157:H7 cells during cooking. Consistent with the results for samples stored aerobically at 4°C, the samples treated with calcium chloride, flavoring
agent, and acetic acid, and stored in air-permeable packages at 12°C, had most effective thermal inactivation of *E. coli* O157:H7 when they were broiled to 65°C. However, the other treatments did not show such thermal reduction in pathogenic counts due to broiling (Tables 57 to 58). In vacuum storage at 12°C, the samples treated with sodium chloride, sodium tripolyphosphate, and sodium lactate had effective reduction in *E. coli* O157:H7 populations, when the samples were broiled to 65°C. When this treatment was applied in combination with acetic acid, the samples fried or broiled to 60 or 65°C showed effective thermal reduction of the pathogen. The results indicated that calcium chloride used in combination with acetic acid might be a useful treatment for tenderization of non-intact beef product, as such treatment showed effective thermal reduction of *E. coli* O157:H7 when the product was broiled to 65°C internal temperature. Treatment with sodium chloride and sodium tripolyphosphate minimized the weight-loss from the product due to expulsion of purge during cooking. In the products stored in vacuum packages at 12°C, these treatments did not show any protective effect against thermal inactivation of the pathogen inoculated in the products. Thus, these treatments may be good alternatives for products stored in vacuum storage at similar temperature.

**Implications**

Restructuring with salt/phosphate minimized weight-loss during cooking but had a protective effect on the pathogen during cooking; salt/phosphate in combination with 0.4% lactic acid may be a useful alternative for restructured beef products; among the commonly used meat-tenderizers, calcium chloride in combination with acetic acid showed greater thermal inactivation of *E. coli* O157:H7 in product broiled to 65°C; among the cooking methods, broiling non-intact beef products to an internal temperature of 65°C was most effective; and, cooking of frozen products from their frozen state was less effective in thermal inactivation of the pathogen, compared to products stored at 4° or 12°C.

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