Title: Effect of lactic acid and commercial chilling processes on the survival of *Salmonella*, *Campylobacter coli* and *Yersinia* spp. in pork variety meats - **NPB #08-171**

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Industry Summary:
Previous research on pork variety meats reported a 2% lactic acid spray was the most effective antimicrobial of those tested with the least negative effect on product quality (Zerby et al., 1998). However, as there has been limited if any implementation of these interventions, the purpose of the proposed research was to validate the effect of current industry practices of chilling and freezing pork variety meats, with and without the application of lactic acid, on the survival of pathogens of concern to the pork industry. Four different variety meats (liver, heart, intestines and stomach) were inoculated with three different pathogens (*Salmonella, Campylobacter* and *Yersinia*) and subjected to four different chilling treatments (with and without the application of 2% lactic acid). Samples were taken before treatment, after treatment and throughout a six-month shelf-life to measure the level of reduction or survival of each organism. Additional testing was performed on uninoculated samples to evaluate the reduction and survival of aerobic plate count, total coliforms and *Escherichia coli* before and after treatment and throughout shelf-life. Although an extensive baseline was developed and intervention strategies were evaluated for pork variety meats in 1998, the majority of the pork industry continues to depend on conventional refrigeration and freezing as their primary methods for controlling microbial growth in these products.

With the reported increase in export of pork products, U.S. pork and pork variety meats are now more than ever before subject to the scrutiny and expectations of the countries we trade with. Some countries are more accepting of antimicrobial interventions such as organic acids than other countries. While other countries may approve of the application of water-based antimicrobial treatments, such as hot water, they may not approve of water-based antimicrobials containing additional levels of chlorine that might serve as an effective antimicrobial. In addition, due to the low relative monetary value of variety meats as compared to carcass meats, most decontamination interventions during the slaughter process are directed at carcass meats. Therefore, a company may be totally dependent on refrigeration and/or freezing for microbial control. Utilizing validated and published procedures, whether they are antimicrobial treatments of a chemical nature or commercial refrigeration and freezing, will greatly support processors of pork variety meats whether they do business on a domestic or an international level. Overall, the use of 2% lactic acid as a decontamination intervention in addition to good GMPs (employee hygiene, sanitation, and rapid chilling) during processing of pork variety meats results in significant reductions in levels of *Salmonella, Y. enterocolitica,* and *C. coli*, as well as indicator organisms (APC, ECC, and TCC). However, significant reductions were also observed on variety meats treated with only a water wash and subsequently frozen. Trends in levels of APC, ECC, and TCC are

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similar to trends in levels of pathogens and can thus be used as surrogates for pathogens to monitor and validate processes in plants. Results did vary between the different types of variety meats suggesting that one intervention may not be suitable for all products. The results of this study may be used to support industry best practices for reducing pathogens in pork variety meats destined for export.

Scientific Abstract:
Current industry chilling practices with and without the application of 2% L-lactic acid were compared for their effectiveness at reducing levels of *Salmonella, Yersinia enterocolitica, Campylobacter coli,* and common indicator organisms used in industry (aerobic plate count APC, *Escherichia coli,* and coliforms) on pork variety meats. Pork livers, hearts, intestines, and stomachs were either inoculated individually with 1 of the 3 pathogens or not inoculated and subjected to 1 of 5 treatments: 1 (water wash + lactic acid spray + freeze), 2 (freeze), 3 (water wash + lactic acid spray + chill + freeze), 4 (chill + freeze), and 5 (water wash + freeze). Samples were analyzed between treatment steps and after 2 months, 4 months, and 6 months of frozen storage. Results of effects of the steps within treatments showed that reductions in levels of pathogens after the water wash and lactic acid spray were significantly different ($P<0.05$) across variety meats. Treatment of variety meats with water wash and lactic acid before chilling resulted in ≥ 0.5 log CFU/sample ($P<0.05$) reductions when compared to chilling alone. Regardless of treatments, reductions in levels of *Salmonella* and *Y. enterocolitica* of 0.6-1.3 log CFU/sample were observed after freezing (0°C) overnight. Freezing reduced *C. coli* by ≥ 2.2 log CFU/sample regardless of previous treatment.
Throughout 6 months of frozen storage, reductions were observed in levels of all microorganisms equal to or greater than 1.3 log CFU/sample. The greatest reductions were observed on samples treated with lactic acid (Treatments 1 and 3) (1.3-5.0 log CFU/sample) while the smallest reductions were reported for samples without any spray treatment (Treatments 2 and 4) (0.7-4.5 log CFU/sample). Large reductions were observed in levels of *C. coli* (2.9-5.0 log CFU/sample) for all treatments. The results of this study suggest that, while the application of a water wash followed by freezing reduced levels of pathogens by approximately 1 log CFU/sample, the application of lactic acid before chilling and freezing variety meats results in significantly larger ($P<0.05$) reductions in microorganisms. Results also show that the indicator organisms, aerobic plate counts, *E. coli,* and coliforms, follow similar trends in reductions during treatment and survival during storage to the pathogen inoculum.

Introduction:
The U.S. Meat Export Federation reported that U.S. pork exports in February 2008 reached 346 pounds which is a 55% increase over the same period in February 2007. While China/Hong Kong was the largest market for U.S. pork and pork variety meats on a volume basis, Japan remained on top for percent of total pork and pork variety meat export value with Russia continuing to present a tremendous opportunity for growth. With the reported increase in export of pork products, U.S. products depend more heavily on the expectations and regulations in the countries we trade with – their food production practices, methods for distribution, cultural differences, available technology, and their food safety rules or lack thereof. As business and food safety interactions with countries outside the U.S. continue to increase, it must be recognized many countries have different food safety experiences as well as different levels of acceptable risk. Some countries may perceive a certain food safety risk as totally acceptable while others may place a low priority on addressing the same risk. Multiple options for controlling microorganisms on meat must be made available for processors since not all countries are accepting of the antimicrobial interventions currently in use in the U.S. Some countries are less accepting of chemical interventions such as organic acids therefore many plants are faced with turning antimicrobial interventions such as belt sprayers and carcass rinse cabinets on and off to suit their various international customers.
The primary method of limiting microbial growth and extending the shell life of pork variety meats is through conventional chilling and freezing of product. However, for the chilling of pork offal, there is little if any specific guidance or published best practices and no regulatory performance standard for chilling of pork offal. The only guidance recommendation from FSIS for chilling of carcasses and variety meats is that they begin

chilling within 1 hour from bleed-out for carcasses and within 1 hour from removal for variety meats. Additional guidance recommends that "parameters be defined, established and recorded so that carcasses reach a temperature of 40°F or less within 24 hours and maintained on all products." (USDA FSIS, 2002). Another point of consideration is that both pathogenic and spoilage bacteria will be present on raw meat and poultry. Restricting their growth through temperature control is important to minimize the risks associated with these microorganisms. Most pathogens are mesophiles and therefore grow best at temperatures between 20 to 45°C (68 to 113°F) with optima between 30 to 40°C (86 to 104°F). Therefore, most pathogens with few exceptions will not grow on chilled meat (Nottingham, 1985). Once the temperature of the surface falls below 10°C (50°F) growth of mesophiles will no longer occur and psychrotrophic spoilage flora will gradually displace the mesophiles which are common on freshly dressed carcasses. However, one pathogen of specific interest to the pork industry, *Yersinia* spp., is capable of growth at 5°C (41°F) and has been associated with outbreaks of foodborne illness and cross-contamination during the preparation of pork chitterlings (CDC, 2003).

In a Final Rule addressing the processing and handling of food, the FDA reviewed the growth of patterns of microorganisms in or on raw meat. The review states that the growth of organisms such as *Clostridium perfringens*, *Salmonella* and *Escherichia coli* are well controlled by cooling meat quickly after slaughter and maintaining refrigerated temperatures during storage and distribution. The review also refers specifically to *Salmonella* and *E. coli* O157:H7 as "capable of significant growth, however, in meat stored above refrigeration temperatures ('temperature abuse' conditions; above 50°F)". In addition, growth of these pathogens is further inhibited at refrigeration temperatures by competition from more numerous and faster-growing spoilage organisms (FDA, 1997).

Specifically referring to the growth of *Salmonella* on raw meat, the ARS (Agriculture Research Service) Pathogen Modeling Program does not even allow for modeling the growth of *Salmonella* at temperatures less than 50°F. At greater than 50°F, in sterile chicken breast, the model estimates approximately 2.5 days (60 hours) for one log growth of the organism with a lag phase of approximately 29.7 hours. Belk et al. (2000) observed bacterial populations of *Salmonella* on inoculated pork chops remained relatively constant during storage at 3.3, 6.7 or 10°C (40, 44 or 50°F) for 24 hours, with a slight increase (0.6 log CFU/cm^{2}) after 48 hours storage. Cutter et al. (2000) reported a reduction (1-3 log CFU/cm^{2}) in levels of APCs, *Listeria monocytogenes*, *Campylobacter coli* and *Salmonella* Typhimurium inoculated onto raw pork surfaces following either blast- or conventional-chilling treatments.

Proper chilling of animal carcasses and offal and maintaining proper refrigeration temperatures of meat products is important to limit the growth of both pathogenic and spoilage bacteria. Commonly used antimicrobial treatments, such as organic acids, hot water and acidified sodium chloride have been validated for pork variety meats (Zerby et al., 1998) however they have not been used as they are not often acceptable to the customer and consumer particularly in this ever increasing global market. Turning on and off antimicrobial systems is already a challenge for scheduling and export of pork carcasses and cuts. Although a product that already requires intensive cleaning and is of lower monetary value, such as variety meats, may not necessarily be afforded the capital expenditures necessary to purchase intervention cabinets or equipment, a comparison of these current practices with a commonly used antimicrobial such as lactic acid is of great interest to the pork industry. Building food safety into our processes and providing safe foods is the responsibility of all industry stakeholders so that we insure the same level of safety everywhere, every time and for everyone – no matter where they live or where they eat.

Objective(s):
To evaluate the effect of lactic acid and current industry practices for chilling and freezing on the survival of *Salmonella* spp.*,* *Campylobacter* spp. and *Yersinia* spp. on pork variety meats.

Materials & Methods:
Bacterial strains
Strains of *Salmonella* Hadar*, Y. enterocolitica,* and *C. coli* isolated from pork were obtained from the National Animal Disease Center (NADC, Ames, IA). *Salmonella* and *Y. enterocolitica* were grown on Tryptic Soy Agar plates (TSA, Difco, Sparks, MD) for 24 h at 37°C. Isolated colonies were transferred to TSA slants and stored at ambient temperature as stock cultures. *C. coli* was grown on Campy Line Agar (CLA) at 42°C for 48 h under microaerophilic conditions. Isolated colonies were transferred to cryocare vials (Key Scientific Products, Round Rock, TX) and frozen at -80°C as stock cultures. To allow differentiation of *Salmonella* and *Y. enterocolitica* inoculated on samples from naturally occurring flora, rifampicin-resistant strains of *Salmonella* and *Y. enterocolitica* were selected and stored on TSA slants at ambient temperature for use as stock cultures.

Collection of variety meats
Samples were collected on each sample day from a small pork processor in Texas. Following slaughter and after inspection, viscera from light weight market hogs (70-90 kg) were placed in a lug and taken to a work area for removal of specific organs. The heart was removed first by cutting the blood vessels within 5 cm of the heart muscle. The heart was then cut in half and blood clots were removed. The liver was removed by cutting the bile duct, with care not to spill excess bile onto the liver surface. The lobe with the gall bladder was not used to avoid inconsistency of the liver surface. The remaining liver was cut into 6 sections per animal. The stomach was removed from the digestive tract by cutting the digestive tract within 5 cm of the stomach. To simulate industry practice, a steel rod was placed through the esophageal tube to hang the stomach and a knife was used to cut the stomach open opposite the rod to allow contents to empty into an inedible barrel. Remaining visible digesta was removed by picking with a gloved hand, and the stomach was cut into 4 sections. Approximately 1.2 m of small intestine was removed from the digestive tract and sliced open to remove contents and to expose the interior surface of the intestine. Each section was cut into 4 pieces of approximately 30 cm in length. The sections and sample pieces were divided to be as equal as possible in size; however, the sizes of variety meat pieces varied based on the size of animals processed. The temperature of the variety meats when collected was approximately 35°C. Each type of variety meat was placed into a separate plastic bag, wrapped in shrouds, and placed in an insulated container to maintain temperature and transported to the Texas A&M Food Microbiology Laboratory.

Preparation of inoculum
The inoculum was prepared for each sampling day by transferring one loopful of *Salmonella* or *Y. enterocolitica* into each of 20-9 ml tubes of TSB and incubated for 18 h at 35°C. The inoculum for *C. coli* was prepared for each test day by removing a bead containing *C. coli* from the cryocare vial stored at -80°C and placed on a TSA plate containing 5% defibrinated sheep blood (BD). The plate with the bead was incubated under microaerophilic conditions at 42°C for 48 h. An isolated colony from the blood plate was inoculated into the tissue culture flask system, as described above, and the flask was incubated horizontally under microaerophilic conditions at 42°C for 48 h.

Initial wash
Upon arrival to the laboratory, a preliminary wash was applied to stomachs and intestines to remove excess digestive contents. Stomachs and intestines were placed in a lug with 12 liters of ambient temperature water (30°C) and swirled with a gloved hand for 30 s. This step was repeated once for both stomachs and intestines, after which the variety meats were returned to the insulated container in clean plastic bags, for later inoculation

and treatment. After transportation to the laboratory and this washing step, the temperature of the stomachs and the intestines was approximately 30°C.

Inoculation of samples
Individual variety meat samples were removed from the insulated container and placed on aluminum foil covered trays. Based on the results of preliminary studies, each sample was inoculated by dripping 0.5 ml (10^8 CFU/ml) of the respective inoculum (*Salmonella, Y. enterocolitica,* or *C. coli*) across the surface and spread with a sterile glass rod, which resulted in counts of approximately 10^6 CFU of *Salmonella* and *Y. enterocolitica* per sample, and approximately 10^5 CFU of *C. coli* per sample. Hearts and stomachs were inoculated on the internal surface of the organ, while liver and intestines were inoculated randomly on either the internal surface or the external surface. Based on preliminary data, samples inoculated with *Salmonella* or *Y. enterocolitica* were held at ambient temperature for 10-30 min, whereas samples inoculated with *C. coli* were held 30 min to 1 h to allow bacterial attachment to the surface. Non-inoculated samples (NI) of variety meats were sampled for aerobic plate count (APC), *E. coli* count (ECC), and total coliform count (TCC).

pH determination
On each test day, variety meat samples were monitored for pH. The initial pH of the surface of each type of variety meat was measured, and subsequently monitored throughout the treatments using a SympHony SB70P meter (VWR) and a flat bulb design SympHony electrode (VWR) by pressing the electrode bulb to the surface of each variety meat. The pH was obtained from two separate areas on each sample following each step in the treatments and averages were calculated for each variety meat at each point in the process.

Description of spraying equipment
Two hand held polyethylene compressed air sprayers (3.8 liter RL FloMaster, Root-Lowell Manufacturing, Lowell, MI) were used, one to apply potable water (25°C, 10 s, 50 ml) for the water wash and one to apply 2% L-lactic acid (40-50°C, 10 s, 50 ml). A cabinet was constructed using a Rubbermaid container (117.3 liters, Rubbermaid, Wooster, OH), steel framework, and 4 mm clear plastic sheeting (Husky, Poly-America, Grand Prairie, TX). The temperature was monitored in the sprayers using a digital thermometer (VWR Dual Thermometer, VWR) connected to Type K thermocouple sensors (Omega, Stamford, CT). Temperature was measured by placing the thermocouple sensors in the path of the spray to determine temperature of water and lactic acid solution at the spray nozzle.

Description of chilling equipment
Following spray treatments, variety meat samples designated for chilling were placed in individual sample bags (Sterile Sampling Bags, 178 x 305 mm, VWR) and arranged in a single layer on shelves in a refrigerator (General Electric Model TBX18HACHRWW, Louisville, KY). For samples designated to be frozen, bagged samples were arranged in a single layer on shelves in a freezer (Kenmore Model 253.28042801, Sears Roebuck and Company, Hoffman Estates, IL). Samples stored for frozen shelf life were held in one of three freezers (Kenmore Model 253.28042804, Sears Roebuck and Company, Hoffman Estates, IL; General Electric Model FCM7WMNAWW, General Electric Model FUM 21SVARWW, Louisville, KY). Temperatures of refrigerators and freezers were monitored using Type K thermocouple sensors attached to digital thermometers placed in the refrigerator and freezers. Sample temperatures were monitored during initial chilling, freezing, and throughout shelf life by inserting Type K thermocouple sensors into two samples of each type of variety meat each test day.

Description and application of treatments
On each test day, following inoculation, samples were randomly assigned to 1 of 5 treatments. Treatment 1: water wash (WW, 25°C, 10 s, 50 ml) + lactic acid spray (LA, 45°C, 10 s, 50 ml) + freeze (FR, 0°C), Treatment 2: freeze (FR, 0°C), Treatment 3: water wash (WW, 25°C, 10 s, 50 ml) + lactic acid spray (LA, 45°C, 10 s, 50 ml) + chill (CH, 4°C) + freeze (FR, 0°C) , Treatment 4: chill (CH, 4°C) + freeze (FR, 0°C), Treatment 5:

water wash (WW, 25°C, 10 s, 50 ml) + freeze (FR, 0°C). Spraying of both the water and lactic acid was done from a distance of approximately 15 cm from the surface of the samples. All treatments were stored for shelf life and sampled after 2 months, 4 months, and 6 months. Treatment 1, 3, and 5 samples were hung in the cabinet with wire S-hooks made from 14 gauge galvanized steel wire. The hooks were sanitized between samples by submersion in 95% ethanol.

Microbiological analysis

Following treatment of each sample piece, 100 ml of 0.1% peptone water was added to each sample bag and the sample was hand massaged for 1 min. Microorganisms were enumerated by plating 1 ml (0.25 ml on each of 4 plates) of the sample rinse, 0.1 ml of the rinse, and then 0.1 ml of appropriate 10-fold dilutions of the same on prepoured and dried rif-TSA plates for *Salmonella,* Rif-Yersinia Selective Agar plates for *Y. enterocolitica,* or CLA plates for *C. coli.* Aliquots of the sample rinse were spread over the surface of the plates with a sterile bent glass rod. Rif-TSA plates and Rif-Yersinia Selective Agar plates were incubated for 24 h at 35°C before counting and reporting the number of rif-resistant organisms per sample. CLA plates were incubated under microaerophilic conditions for 48 h at 42°C, as previously described, before counting and reporting the number of organisms per sample. Non-inoculated samples were analyzed for aerobic plate count using Aerobic Count Plate Petrifilm (3M Microbiology Products, St. Paul, MN) and total coliforms and *E. coli* using *E. coli*/Coliform Count Petrifilm (EC/CC) (3M). Counts were determined by plating 1 ml aliquots of appropriate decimal dilutions on Petrifilm plates. Plates were incubated for 48 h at 35°C for aerobic plate counts and for 24 h at 35°C for *E. coli* and total coliforms, before counting and reporting the counts per sample. To test for background levels of rifampicin resistant *Salmonella* or *Y. enterocolitica* and naturally occurring levels of *C. coli,* 3 samples of each type of variety meat were rinsed with 100 ml of 0.1% peptone each test day. One ml of the sample rinse was plated on 4 plates of the appropriate media for the organism used for that test day (rif-TSA, rif-Yersinia Selective Agar, or CLA). The plates were incubated and counted to ensure that background levels of the organisms were not interfering with tracking the inoculum level and reductions.

Statistical analysis

Microbiological data were calculated following the counting rules in the Compendium of Methods for the Microbiological Evaluation of Foods 4th ed. (2001) and transformed into logarithms before statistical analysis. Data were analyzed using analysis of variance (ANOVA) and the Mixed model procedures of the Statistical Analysis System (Version 9.2, SAS Institute, Inc., Cary, NC). Least squares means were separated using the pdiff option when significant ($P<0.05$).

Results:

Immediate treatment effects

The least squares means of the initial microbial levels on variety meats are presented in Table 1. The initial inoculum levels of *Salmonella* and *Y. enterocolitica* following inoculation and attachment were very consistent (6.1-6.3 log CFU/sample and 5.0-5.1 log CFU/sample across variety meats, respectively). There were significant (P<0.05) main effects caused by replicate for initial levels of *C. coli*, APC, *E. coli,* and coliforms. This was likely due to variation in initial levels of naturally occurring microorganisms.

Water washing resulted in slightly larger reductions of microorganisms on inoculated variety meats than on non inoculated variety meats (Tables 2-7). This was most likely because the inoculated bacteria were less firmly attached to the surface of intestines than the surfaces of the other variety meats. For *Salmonella* and *Y. enterocolitica,* both spraying steps (WW and LA) resulted in the lowest reductions on intestines when compared to other variety meats, as reported in Tables 2 and 3. It is also likely that the mucosal layer of the intestine, which aids in absorption, secretion, and digestion in the live animal, enhanced the attachment of bacteria and also prevented contact of the antimicrobial and the bacteria (Barrow et al., 1980; Rust, 1988). While water washing alone resulted in no reduction in levels of *Salmonella* on intestines, there were reductions of 0.1, 0.4, and 0.6 log CFU/sample on stomachs, hearts, and livers, respectively. When water washing was followed by a lactic acid spray, there were slightly greater reductions in levels of *Salmonella* (0.6 log CFU/sample) on livers, hearts, and stomachs, with the lowest reduction in levels of *Salmonella* on intestines (0.3 log CFU/sample). Results of samples analyzed after chilling alone showed mean reductions in levels of *Salmonella* of 0.1 log CFU/sample; however, when the water wash and lactic acid spray were applied before chilling, the reduction in levels was 0.6 log CFU/sample. Once all samples were frozen, there were significantly greater reductions (P<0.05) in levels of *Salmonella*, observed on stomachs than on other variety meats. The main effect of treatment was significant (P<0.05). Treatment 1 (WW+LA+FR) had higher reductions in levels of *Salmonella* when compared to other treatments (1.4 log CFU/sample). Samples from both Treatments 2 (FR) and 4 (CH + FR) had lower reductions in levels of *Salmonella* (0.6 and 0.5 log CFU/sample), and also had no spray treatments applied.

All treatment steps resulted in similar reductions in levels of both *Y. enterocolitica* and *Salmonella.* Reductions in levels of *Y. enterocolitica* (Table 3) after the water wash ranged from 0.2 to 0.8 log CFU/sample, and reductions in levels of *Y. enterocolitica* after the lactic acid spray ranged from 0.5 to 1.2 log CFU/sample. The main effect of variety meat was significant (P<0.05) for chilling and freezing. Significantly higher reductions (P<0.05) were observed on stomachs (0.7 log CFU/sample) when compared to other variety meats, regardless of treatment. Spraying variety meats with water and lactic acid before chilling resulted in a 0.7 log CFU/sample reduction in levels of *Y. enterocolitica*, which is a significantly larger reduction (P<0.05) than achieved by chilling alone. Treatment 1 (WW + LA + FR) was most effective, resulting in a reduction of 1.6 log CFU/sample of *Y. enterocolitica*. Treatment 3 (WW + LA + CH + FR), which also incorporated lactic acid as a decontamination step, resulted in a reduction in levels of *Y. enterocolitica* by 1.2 log CFU/sample. Overall, the treatments without spraying steps had significantly lower reductions (P <0.05) in levels of *Y. enterocolitica* than those with spraying steps.

The effects of the treatment steps on *C. coli* are presented in Table 4. After both chilling and freezing, larger reductions in levels of *C. coli* were observed than for *Salmonella* and *Y. enterocolitica.* It is important to note that the root mean square error for the *C. coli* ranged from 0.81 to 1.30 log CFU/sample, which shows that there was a larger range in the data with this organism than with the previous organisms. The water wash step was very effective in reducing levels of *C. coli* on livers, with a mean reduction of 2.1 log CFU/sample, which was significantly higher than for the other 3 variety meats. However, the mean reduction for stomachs showed that there was an increase in levels of *C. coli.* This may be due to increased variation in the range of microbial counts, as indicated by the root mean square error. In addition, the mean reductions on hearts, stomachs, and intestines are not statistically different (P>0.05). Reductions after lactic acid spraying ranged from 0.7 to 1.2 log CFU/sample. The application of lactic acid before chilling resulted in a significantly (P<0.05) higher reduction in levels of the organism (1.6 log CFU/sample) than chilling with no decontamination treatment (1.1 log CFU/sample). Freezing caused a minimum mean reduction in levels of *C. coli* of 2.1 log CFU/sample.

Treatment 1 (WW + LA + FR) resulted in significantly larger reductions than the other treatments (3.6 log CFU/sample). Similar to treatment effects on *Salmonella* and *Y. enterocolitica,* the treatments that did not include a decontamination step of water or lactic acid resulted in significantly lower reductions (P<0.05) that the other treatments. The reductions in levels of *C. coli* were 2.1 and 2.3 log CFU/sample for Treatment 4 (CH + FR) and 2 (FR), respectively, showing that reducing the temperature of the products by chilling and freezing alone had relatively large reductions in levels of *C. coli*.

Because plants do not introduce pathogens into their processes for validation, indicator organisms are such as APC, ECC and TCC are often used as surrogates. In this study, the reductions in levels of APC, ECC, and TCC were used to evaluate their use as indicator organisms in plants, and results are presented in Tables 5, 6 and 7. Reductions in levels of APC, ECC, and TCC on non inoculated variety meats were low for water wash, and some levels actually increased. However, there were no significant differences among variety meats. Lactic acid treatment reduced levels of APC, ECC, and TCC by 0.3-1.4 log CFU/sample. The highest reductions were observed on hearts. Treatments that included decontamination sprays (WW and LA) resulted in higher reductions after chilling and freezing. Growth of APC, ECC, and TCC occurred during chilling without any decontamination treatment before chilling. The mean growth for APC was 0.3 log CFU/sample, and the growth for ECC and TCC was 0.2 log CFU/sample. While Treatment 1 (WW + LA + FR)was more effective than all other treatments at reducing levels of pathogens tested, Treatment 1 (WW + LA + FR) and Treatment 3 (WW + LA + CH + FR) are not statistically different (P>0.05) in effectiveness at reducing APC, ECC, and TCC. Both treatments reduced APC by 1.3 log CFU/sample, and ECC and TCC by 1.3-1.5 log CFU/sample after freezing. Overall, the treatments that use lactic acid as a decontamination treatment before chilling and freezing had the greatest reductions. Treatment 1 (WW + LA +FR) was the most effective treatment for reducing levels of pathogens, but did not differ (P>0.05) from Treatment 3 (WW + LA + CH + FR) for reducing levels of APC, ECC, and TCC on non inoculated products. Treatment 5 (WW + FR) was more effective than the treatments that did not include a water wash or lactic acid spray (Treatments 2 (FR) and 4 (CH + FR)) at reducing levels of pathogens, but was not different for reductions in APC, ECC, and TCC on non inoculated samples. These data support the hypothesis that use of water wash and a lactic acid spray before chilling variety meats is more effective in reducing levels of microorganisms.

Effects of treatments during storage

There were significant (P<0.05) replicate interactions (replicate x storage time and replicate x treatment) reported during shelf life (data not shown). Significant replicate interactions (P<0.05) were reported for reductions in all microorganisms (*Salmonella, Y. enterocolitica, C. coli,* APC, ECC, and TCC) on livers. Reductions in levels of *C. coli* on stomachs and hearts also showed replicate interactions. Significant replicate interactions (P<0.05) were reported for reductions in both ECC and TCC on all variety meats. Overall, this shows that the treatments used do not result in consistent reductions.

As shown in Table 8, the main effects of storage time and treatment were significant (P<0.05) for log reductions of *Salmonella* over the frozen shelf life. The two way interactions of treatment by storage time are presented in Figure 1. For all variety meats, reductions in levels of *Salmonella* at 0 storage time were significantly smaller (P<0.05) than reductions after 2, 4, and 6 months of storage time. The largest decline in levels of *Salmonella* occurred within the first 2 months of frozen storage, while smaller reductions were observed after 4 months and 6 months, as displayed by the slopes of the lines in Figure 2. After freezing overnight (0 storage time) reductions in levels of *Salmonella* ranged from 0.6-1.3 log CFU/sample, while greater reductions in levels of *Salmonella* (1.2-2.8 log CFU/sample) were observed after storage for 2 months. At the end of frozen storage, there were significantly greater reductions (P<0.05) when compared to 0 storage time for intestines, stomachs, livers, and hearts (2.6, 3.3, 1.8, and 1.6 log CFU/sample, respectively) across all treatments. Across all storage times, the trend showed that the largest reductions in levels of *Salmonella* were on stomachs, followed by intestines, livers, and hearts, respectively.

For the reductions in levels of *Salmonella* due to the main effect of treatment within intestines, Treatments 1 (WW + LA + FR) and 3 (WW + LA + CH + FR) resulted in the greatest reductions (2.5 and 2.3 log CFU/sample, respectively). Treatments 1 and 3 were not different (P>0.05) from each other, but Treatment 3

was also not significantly different (P>0.05) from Treatment 5 (WW + FR) (2.2 log CFU/sample). This shows that spraying intestines with either water wash or a combination of water wash and lactic acid resulted in larger reductions in levels of *Salmonella* than chilling and freezing alone. Treatments 2 (FR) and 4 (CH + FR) resulted in reductions in levels of *Salmonella* of 1.9 and 1.8 log CFU/sample, respectively. Stomachs had the largest overall reductions in levels of *Salmonella* as compared to the other variety meats. Treatment 1 (WW + LA + FR) resulted in significantly larger reductions (P<0.05) in levels of *Salmonella* than the other treatments (3.2 log CFU/sample). Reductions in levels of *Salmonella* on stomachs during shelf life for Treatments 2, 3, 4, and 5 ranged from 2.2-2.8 log CFU/sample.

For the main effect of treatment within livers and hearts, reductions in levels of *Salmonella* following Treatments 1 (WW + LA + FR) and 3 (WW + LA + CH + FR) were greater than the other treatments, though not significantly different from each other (P>0.05) during shelf life. Least squares means of reductions in levels of the organism on livers and hearts following Treatments 1 and 3 were 1.8 log CFU/sample. Treatment 5 (WW + FR) was less effective than the treatments that included a lactic acid spray (Treatments 1 (WW + LA + FR) and 3(WW + LA + CH + FR)), though more effective than treatments that did not include any spray treatment (Treatments 2 (FR) and 4 (CH + FR). Reductions in levels of *Salmonella* following Treatment 5 (WW + FR) were 1.5 and 1.2 log CFU/sample for livers and hearts, respectively. Within each variety meat, Treatments 2 (FR) and 4 (CH + FR) resulted in lower reductions in levels of *Salmonella* (0.7-1.0 log CFU/sample) than treatments that included a water wash or lactic acid spray before storage.

The trends for reductions in levels of both *Salmonella* and *Y. enterocolitica* were similar. Results for main effects for *Y. enterocolitica* are shown in Table 9 and the two way interactions of treatment by storage time are shown in Figure 2. The intestines were the only variety meat that had a significant two way interaction of treatment by storage time (P=0.006) for reductions in levels of *Y. enterocolitica.* For intestines, livers, and hearts, the reductions in levels of *Y. enterocolitica* for the main effect of storage time were significantly lower (P<0.05) after 0 storage time (0.6-1.0 log CFU/sample) than 2, 4, or 6 months of storage. For stomachs, there was no difference (P>0.05) in reductions in levels of *Y. enterocolitica* between 0 storage time and 2 months storage time (1.2 and 1.9 log CFU/sample, respectively). Least squares means of reductions in levels of *Y. enterocolitica* at the end of shelf life (6 months) ranged from 1.7-2.5 log CFU/sample across all treatments. Across all variety meats and storage times, Treatment 1 (WW + LA + FR) resulted in the greatest reductions in levels of *Y. enterocolitica,* although Treatments 1 (WW + LA + FR) and 3 (WW + LA + CH + FR) were not different (P>0.05) within livers and hearts. Reductions in levels of *Y. enterocolitica* due to Treatment 1 (WW + LA + FR) ranged from 2.0-2.8 log CFU/sample across variety meats. The trend showed that Treatments 1 (WW + LA + FR) and 3 (WW + LA + CH + FR) were most effective, followed by Treatment 5 (WW + FR), and Treatments 2 (FR) and 4 (CH + FR) were least effective in reducing levels of *Y. enterocolitica*. However, the least squares means for all treatments over the storage time were equal to or greater than 1.0 log CFU/sample.

Results of reductions in levels of *C. coli* during shelf life are shown in Table 10, and two way interactions of treatment by storage time are shown in Figure 3. There were significant two way interactions (P<0.05) of treatment by storage time for both stomachs and hearts. Across all variety meats and treatments, 0 storage time resulted in the lowest (P<0.05) reductions in levels of *C. coli* when compared to the other storage times. The initial reduction in levels of the organism due to overnight freezing (0 storage time) ranged from 2.2-3.3 log CFU/sample. Levels of *C. coli* continued to decline over frozen storage, and the reductions after 6 months of storage ranged from 3.8-5.3 log CFU/sample. Across all storage times, the largest reductions in levels of *C. coli* were seen on intestines, followed by stomachs, livers, and hearts, respectively.

Across all storage times, there was less variation among the treatments in the reductions in levels of *C. coli* than in levels of *Salmonella* or *Y. enterocolitica*. This was likely due to the greater susceptibility of *C. coli* to drying that occurs during freezing (Oosterom, 1983). For all variety meats, the reductions in levels of *C. coli* due to the main effect of treatment ranged from 2.9-5.0 log CFU/sample. However, the rank of the treatments in order of effectiveness at reducing levels of *C. coli* varied across variety meats. Frozen storage most likely had the largest effect on the levels of the organism, regardless of the treatment applied before storage.

Results of reductions in levels of APC during frozen shelf life are shown in Table 11 and the two way interactions of treatment by storage time are shown in Figure 6. Across all treatments, the lowest reduction in levels of APC ($P<0.05$) was after 0 storage time as compared to 2, 4, or 6 months storage time. Reductions in levels of APC after overnight freezing (0 storage time) ranged from 0.5-1.3 log CFU/sample, while the range after 2 months of frozen storage was 1.1-2.4 log CFU/sample. At the end of shelf life (6 months) reductions in levels of APC ranged from 1.3-2.9 log CFU/sample.

For the main effect of treatment on intestines and liver, Treatment 1 (WW + LA + FR) resulted in significantly larger reductions ($P<0.05$) than all other treatments. For hearts, Treatment 1 (WW + LA + FR) and 3 (WW + LA + CH + FR) resulted in the largest reductions (2.3 and 2.7 log CFU/sample, respectively). The mean for Treatment 1 (WW + LA + FR) effects on stomachs showed the largest reduction, but was not different than Treatments 2 (FR) and 3 (WW + LA + CH + FR). Except for stomachs, reductions in levels of APC due to Treatments 2 (FR) and 4 (CH + FR) were significantly lower ($P<0.05$) than the other treatments. On intestines, hearts, and livers, Treatment 5 (WW + FR) was less effective than treatments that included lactic acid, but was more effective than the treatments that did not have a water wash or lactic acid spray.

Results of reductions in levels of ECC were similar to TCC and are shown in Tables 12 and 13. Two way interactions of treatment by storage time are shown in Figures 5 and 6. Significant interactions ($P<0.05$) were observed for reductions in levels of ECC on intestines and hearts and for reductions in levels of TCC on stomachs. For the main effect of storage time, the lowest reductions ($P<0.05$) in levels of ECC and TCC were observed after 0 storage time for all variety meats (0.6-1.2 and 0.7-1.2 log CFU/sample, respectively). At the end of shelf life storage (6 months), reductions in levels of ECC and TCC were 2.1-3.4 and 2.0-3.5 log CFU/sample, respectively. Reductions in levels of these microorganisms after 6 months of storage were significantly greater ($P<0.05$) than reductions at the beginning of shelf life for all variety meats.

For the main effect of treatment on reductions of levels of ECC and TCC, the largest reductions ($P<0.05$) were reported for Treatment 1 (WW + LA + FR) on intestines, stomachs, and livers. Reductions of levels of both ECC and TCC for Treatment 1 (WW + LA + FR) were 2.2-3.5 log CFU/sample. Treatment 3 (WW + LA + CH + FR) resulted in the largest mean reduction in levels of ECC and TCC on hearts, although it was not different ($P>0.05$) from Treatment 1 (WW + LA + FR) for reducing levels of ECC. Reductions in levels of ECC on hearts for Treatment 1 (WW + LA + FR) and 3 (WW + LA + CH + FR) were 2.9 and 3.2 log CFU/sample, respectively. Levels of TCC were reduced by 2.9 and 3.3 log CFU/sample for Treatments 1 (WW + LA + FR) and 3 (WW + LA + CH + FR), respectively. Treatment 5 (WW + FR) was more effective than Treatments 2 (FR) and 4 (CH + FR), but less effective than the treatments that included a lactic acid spray (Treatments 1 and 3). Across all treatments, least squares mean reductions of levels of ECC and TCC were equal to or greater than 1.0 log CFU/sample.

Across all variety meats, the pH was significantly higher after the water wash (Treatment 5 x Spray) (pH 6.0-6.8) when compared to the initial pH, which was expected due to the neutrality of water. Immediately after spraying the variety meats with a combination of water wash followed by lactic acid, the pH was significantly ($P<0.05$) lower (pH 3.8-4.0) than both initial pH and pH of samples only sprayed with water. However, mean pH for all treatments were not different ($P>0.05$) for stomachs and livers after 2 months and 4 months, respectively. The pH for Treatments 1 (WW + LA + FR) and 3 (WW + LA + CH + FR) was significantly lower ($P<0.05$) than the pH for Treatment 5 (WW + FR) throughout shelf life for intestines and hearts. The differences in the means after 6 months of frozen storage across treatments were within 0.8 and 0.3 pH units for intestines and hearts, respectively. It is likely that the pH effect due to lactic acid was buffered over storage time.

Discussion:

This study showed that a 2% lactic acid spray (45°C, 10 s, 50 ml) resulted in significant reductions in levels of microorganisms on variety meat surfaces. Lactic acid has previously been shown to effectively reduce microbial levels on beef, buffalo, and pork variety meats (Delmore et al., 2000; Patterson and Gibbs, 1979; Selvan et al., 2007; Woolthuis et al., 1984; Zerby et al., 1998). Patterson and Gibbs (1979) reported a 2 log CFU/cm^2 reduction in levels of APC on beef livers dipped in 1% lactic acid (7°C) for 15 min with agitation.

Woolthuis et al. (1984) reported that after pork livers were immersed in a 0.2% lactic acid solution for 5 min, were vacuum packaged, and stored for 1 day (3°C), levels of APCs were 2.2 log CFU/cm^2 lower than controls. After storage for 5 days (3°C), levels of APCs were approximately 3 log CFU/cm^2 lower when compared to controls. Delmore et al. (2000) treated beef variety meats with a 2% lactic acid spray (10 s) and reported reductions in levels of APC, ECC, and TCC (0.4-1.7, 0-1.8, -0.1-2.5 log CFU/g, respectively). Zerby et al. (1998) observed reductions of levels of APC, ECC, and TCC on pork variety meats after spraying with 2% lactic acid (10 s, 48-50°C). The reductions of the levels of APC ranged from 1.1-4.1 and 1.6-4.5 log CFU/g, respectively.

Zerby et al. (1998) observed reductions in levels of *Salmonella* on inoculated pork variety meats after spraying with 2% lactic acid (10 s, 48-50°C). Reductions in levels of *Salmonella* of approximately 2 log CFU/g were reported on livers, stomachs, and hearts, while reductions of approximately 3 log CFU/g were observed on chitterlings. In the same study, chitterlings were inoculated with *Y. enterocolitica* and immersed in 2% lactic acid (10 s, 48-50°C), which reduced levels of *Y. enterocolitica* below detectable levels. However, the overall effectiveness of lactic acid varied based on the variety meat surfaces. Water washing has been shown to be effective at reducing microbial levels of carcasses (Anderson et al., 1977). Hardin et al. (1995) showed that a water wash (35°C) followed by a 2% lactic acid wash (55°C) significantly reduced *E. coli* O157:H7 and *Salmonella* on beef surfaces. However, the treatment was less effective on beef inside rounds because of the surface variation where bacteria could become embedded or attached and not be contacted by the sprays. Similar results were reported by Ellebracht et al. (1999) when a 3 s hot water treatment was used to treat beef trimmings. These researchers noted that not all of the fat and lean surfaces were evenly exposed to the treatment because of overlap of the trim pieces. A similar effect may have occurred in the current study due to differences in the type and nature of the surfaces of the different variety meats. While the liver has a very smooth, regular surface, the intestine, heart, and stomach has more irregular, convoluted surfaces areas. These differences in surface types may have prevented even exposure of the surface and associated microorganisms to the water wash and lactic acid spray. In addition, the variation in variety meat surfaces, particularly the slimy mucosal layer of the intestine, could have similar compounding effects on all treatments. Woolthuis et al. (1984) reported that different sites of the liver surface were contaminated with different levels of blood or digestive contents, which may have contributed to variation within decontamination treatments. It is possible that a longer dwell time, increased temperature, increased volume, or a dip application method could increase effectiveness of these treatments. Patterson and Gibbs (1979) reported reductions in levels of APC by approximately 2 log CFU/cm^2 on chilled hearts and livers after immersion in 1% lactic acid for 15 min.

While it may be more efficient and more cost effective for plants to implement one antimicrobial intervention method to treat of all variety meats, one method may not be appropriate for all types of variety meats. Effects on quality would also need to be taken into consideration before determining treatments. Delmore et al. (2000) reported that immersion of livers in 2% acetic acid or 2% lactic acid resulted in a lighter color as compared to controls. Woolthuis et al. (1984) reported no effect on color when livers were immersed in 0.2% lactic acid for 5 min. However, Patterson and Gibbs (1979) reported that livers immersed in hot water (90°C) for 1 minute exhibited a cooked appearance. This suggests that concentration of the organic acid and temperature control of decontamination treatments is critical particularly for livers due to the denaturation of myoglobin and the impact on color.

Immediately after spraying with lactic acid, lower pH values were reported on all variety meats. Woolthuis et al. (1984) reported significantly lower ($P<0.05$) pH values on livers treated with 0.2% lactic acid for 5 min after 1 day of storage (2-4°C). However, pH values after 5 days of storage (2-4°C) were not different from controls. Similar results were found in the current study where the pH of samples treated with lactic acid was lower immediately after spraying when compared to the initial sample pH. Application of 1.25% lactic acid to hot calf carcasses reduced pH by over 3 units initially, but the pH returned to the original level after 72 h (Woolthuis and Smulders, 1985). After freezing and throughout shelf life, pH differences across treatments were quite small in this study. In this study, pH values after freezing and throughout shelf life storage did not differ between treatments with the exception of the intestines.

The reduction in levels of microorganisms due to freezing depends on the medium, as some media protect microorganisms while others enhance damage by freezing (Mazur, 1966). Nesbakken et al. (2008) reported that while blast chilling did not have an effect on the prevalence of *Y. enterocolitica* or the levels of APCs on pork carcasses, it did significantly reduce the prevalence of *Campylobacter* spp. and the levels of coliforms and *E. coli*. Stern and Kotula (1982) reported that levels of *Campylobacter* were reduced by 3 log CFU/g after 3 days of frozen storage (-15°C). Gill and Harrison (1985) reported variation in levels of *E. coli* on variety meats based not only on the length of the chilling curve, but also type of variety meat and batch of variety meat.

Epling et al. (1993) reported that a 2% lactic acid spray on pork carcasses reduced levels of both *Salmonella* and *Campylobacter* both 5 min after spraying and after 20 h of chilling at 4°C. The prevalence of *Campylobacter* spp. was reduced to a greater extent on carcasses that were chilled using conventional chilling as compared to a spray chilling system. This is likely due to the increased drying of the surface during conventional chilling. This was also observed when blast chilling was compared to conventional chilling for reducing *Campylobacter* spp. (Chang et al., 2003).

The results of this research showed that washing variety meats with water (25°C) for 10 s did not reduce levels of APC, ECC, or TCC. Data show higher levels of APC, ECC, and TCC following WW. Lactic acid treatment immediately reduced levels of all microorganisms (*Salmonella, Y. enterocolitica, C. coli,* APC, ECC, and TCC) by 0.3-1.4 log CFU/sample, similar to previous reports (Delmore et al., 2000; Zerby et al., 1998).

In the present study, higher levels of APC, ECC, and TCC were reported for non inoculated samples that were not sprayed with water or lactic acid before chilling (4°C) overnight. Hanna et al. (1982) also reported an increase in APC of pork livers, kidneys, and hearts over 5 days of aerobic storage at 2°C. Zerby et al. (1998) treated pork hearts with lactic acid (2%, 50°C, 10 s immersion) and stored treated and untreated controls for 63 days (vacuum packaged, 3°C). Lower levels of APC and TCC throughout the shelf life were reported for treated samples as compared to untreated controls. Zerby et al. (1998) also reported a minimum 2.5 log CFU/g reduction in levels of APC after treating pork variety meats with 2% lactic acid (50°C, 10 s), brine chilling (-12°C, 45 min), and freezing (0°C for 12 h, followed by blast freezing at -12°C for 6 h). They concluded that a multiple hurdle approach including a decontamination treatment such as lactic acid should be combined with GMPs for initial chilling of variety meats to reduce microbial contamination.

In general, during the shelf life storage time for this study (0°C, 6 months), treatments that included a lactic acid spray (45°C, 10 s, 50 ml) resulted in the greatest reductions of all microorganisms (*Salmonella, Y. enterocolitica, C. coli,* APC, ECC, and TCC) regardless of whether the samples were chilled before they were frozen (≥ 1.7 log CFU/sample). The treatment that included water wash only and freezing was intermediate in effectiveness (≥ 1.0 log CFU/sample) when compared to those treatments that included lactic acid and those treatments that had no water wash or lactic acid (≥ 0.7 log CFU/sample). For the treatments without the application of lactic acid or water wash, there was no trend that indicated whether chilling before freezing affected reductions in levels of any of the microorganisms.

In this study, it is important to recognize that there were significant effects ($P<0.05$) due to replication and replicate interactions, suggesting that the effectiveness of the treatments to reduce microbial levels on variety meats varied each sample day. It is reasonable that variation in background levels of microbial contamination each day likely affect the ability of treatments to reduce levels of microorganisms. There were also significant interactions between treatment and storage time, suggesting that treatment and storage do not always produce an additive effect when reducing levels of microorganisms.

Overall, the use of 2% lactic acid as a decontamination intervention in addition to good GMPs (employee hygiene, sanitation, and rapid chilling) during processing of pork variety meats will result in a significant reduction in levels of *Salmonella, Y. enterocolitica,* and *C. coli*, as well as indicator organisms (APC, ECC, and TCC). However, reductions of equal to or greater than 1 log CFU/sample of the pathogens were also observed on variety meats treated with a water wash followed by freezing. Trends in levels of APC, ECC, and TCC are similar to trends in levels of pathogens and therefore seem to be appropriate surrogates for pathogens to monitor and validate processes in processing plants.

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References

Anderson, M. E., R. T. Marshall, W. C. Stringer, and H. D. Naumann. 1977. Evaluation of a prototype beef carcass washer in a commercial plant. *J. Food Prot.* 44:35-38.

Barrow, P. A., B. E. Brooker, R. Fuller, and M. J. Newport. 1980. The attachment of bacteria to the gastric epithelium of the pig and its importance in the microecology of the intestine. *J. Appl. Bacteriol.* 48:147-154.

Belk, K.E., J.N. Sofos, G.C. Smith, J.A. Scanga, K. Segomelo, M.L. Kain. 2000. Growth of bacteria on pork carcasses and cuts following application of different chilling procedures and after being subjected to temperature abuse during distribution and by consumers. Final Report to National Pork Producers Council. NPPC #99-143.

Centers for Disease Control and Prevention. 2003. *Yersinia enterocolitica* gastroenteritis among infants exposed to chitterlings—Chicago, Illinois, 2002. *Morbid. and Mortal. Weekly Rep*. 52:956-958.

Chang, V. P., E. W. Mills, and C. N. Cutter. 2003. Reduction of bacteria on pork carcasses association with chilling method. *J. Food Prot.* 66:1019-1024.

Cutter, C. N. 2000. Effects of chilling methods for bacterial recovery and reducing bacteria on pork carcasses. Final Report to National Pork Producers Council. NPPC #00-015.

Delmore, R. J., J. N. Sofos, K. E. Belk, W. R. Lloyd, G. L. Bellinger, G. R. Schmidt, and G. C. Smith. 1999. Good manufacturing practices for improving the microbiological quality of beef variety meats. *Dairy, Food, and Environ. San.* 19:742-752.

Delmore, R. J., J. N. Sofos, G. R. Schmidt, K. E. Belk, W. R. Lloyd, and G. C. Smith. 2000. Interventions to reduce microbiological contamination of beef variety meats. *J. Food Prot.* 63:44-50.

Doyle, Ellin M. 2002. 2002. Survival and Growth of Bacterial Pathogens on Raw Meat During Chilling. Food Research Institute. University of Wisconsin-Madison.

Ellebracht, E. A., A. Castillo, L. M. Lucia, R. K. Miller, and G. R. Acuff. 1999. Reduction of pathogens using hot water and lactic acid on beef trimmings. *J. Food Sci.* 64:1094-1099.

Epling, L. K. M., J. A. Carpenter, and L. C. Blankenship. 1993. Prevalence of *Campylobacter* spp. and *Salmonella* spp. on pork carcasses and the reduction effected by spraying with lactic acid. *J. Food Prot.* 56:536-537.

FDA. Food and Drug Administration. 1997. 21CFR Part 179. Irradiation in the Production, Processing and Handling of Food. Final Rule.

Gill, C. O. 1998a. Microbiological contamination of meat during slaughter and butchering of cattle, sheep and pigs. p. 118-157. *In* Andrew Davies and Ron Board (ed.), the microbiology of meat and poultry. Blackie Academic & Professional, London, England.

Gill, C. O. 1988b. Microbiology of edible meat by-products, p. 179-224. *In* A. M. Pearson and T. R. Dutson (ed.), Edible meat by-products, advances in meat research volume 5. Elsevier Applied Science, Essex, England.

Gill, C. O., and J. C. L. Harrison. 1985. Evaluation of the hygienic efficiency of offal cooling procedures. *Food Microbiol.* 2:63-69.

Hanna, M. O., G. C. Smith, J. W. Savell, F. K. McKeith, and C. Vanderzant. 1982. Effects of packaging methods on the microbial flora of livers and kidneys from beef or pork. *J. Food Prot.* 45:74-81.

Hardin, M. D., G. R. Acuff, L. M. Lucia, J. S. Oman, and J. W. Savell. 1995. Comparison of methods for decontamination from beef carcass surfaces. *J. Food Prot.* 58:368-374.

Mazur, P. 1966. Physical and chemical basis of injury in single-celled microorganisms subjected to freezing and thawing. p. 213-315. *In* Meryman, H. T. (ed.) Cryobiology, Academic Press, New York.

Nesbakken, T., K. Eckner, and O. J. Rotterud. 2008. The effect of blast chilling on occurrence of human pathogenic *Yersinia enterocolitica* compared to *Campylobacter* spp. and numbers of hygienic indicators on pig carcasses. *Int. J. Food Microbiol.* 123:130-133.

Nottingham, P.M. 1985. Microbiology of Carcass Meats, p. 13-65. *In* M.H. Brown (ed.), Meat microbiology. Applied Science Publishers, LTD, New York, New York.

Oosterom, J. G., J. A. DeWilde, E. DeBoer, L. H. DeBlaauw, and H. Karman. 1983. Survival of *Campylobacter jejuni* during poultry processing and pig slaughtering. *J. Food Prot.* 46:702-706.

Patterson, J. T., and P. A. Gibbs. 1979. Vacuum-packaging of bovine edible offal. *Meat Sci.* 3:209-222.

Rust, R. E. 1988. Production of edible casings, p. 261-274. *In* A. M. Pearson and T. R. Dutson (ed.), Edible meat by-products, advances in meat research volume 5. Elsevier Applied Science, Essex, England.

Selvan, P., S. K. Mendiratta, K. Porteen, and K. N. Bhilegaonkar. 2007. Effects of lactic acid on quality of buffalo offals. *Int. J. Food Safety* 9:29-36.

Snijders, J. M. A., J. G. Van Logtestijn, D. A. A. Mossel, and F. J. M. Smulders. 1985. Lactic acid as a decontaminant in slaughter and processing procedures. *The Vet. Quar.* 4:277-282.

Stern, N. J., and A. W. Kotula. 1982. Survival of *Campylobacter jejuni* inoculated into ground beef. *Appl. Environ. Microbiol.* 44:1150-1153.

USDA FSIS. United States Department of Agriculture Food Safety Inspection Service. 2002. Guidance for minimizing the risk of *Escherichia coli* O157:H7 and *Salmonella* in beef slaughter operations.

Woolthuis, C. H. J., D. A. A. Mossel, J. G. Van Logtestijn, J. M. De Kruijf, and F. J. M. Smulders. 1984. Microbial decontamination of porcine liver with lactic acid and hot water. *J. Food Prot.* 47:220-226.

Woolthuis, C. H. J., and F. J. M. Smulders. 1985. Microbial decontamination of calf carcasses by lactic acid sprays. *J. Food Prot.* 48:832-837.

Zerby, H. N., R. J. Delmore Jr., R. Murphree, K. E. Belk, J. N. Sofos, G. R. Schmidt, G. L. Bellinger, A. Pape, M. Hardin, W. Lloyd, G. C. Smith. 1998. A microbiological profile of pork variety meats and intervention strategies for reducing microbiological contamination on pork variety meats. Final report to the U.S. Meat Export Federation.

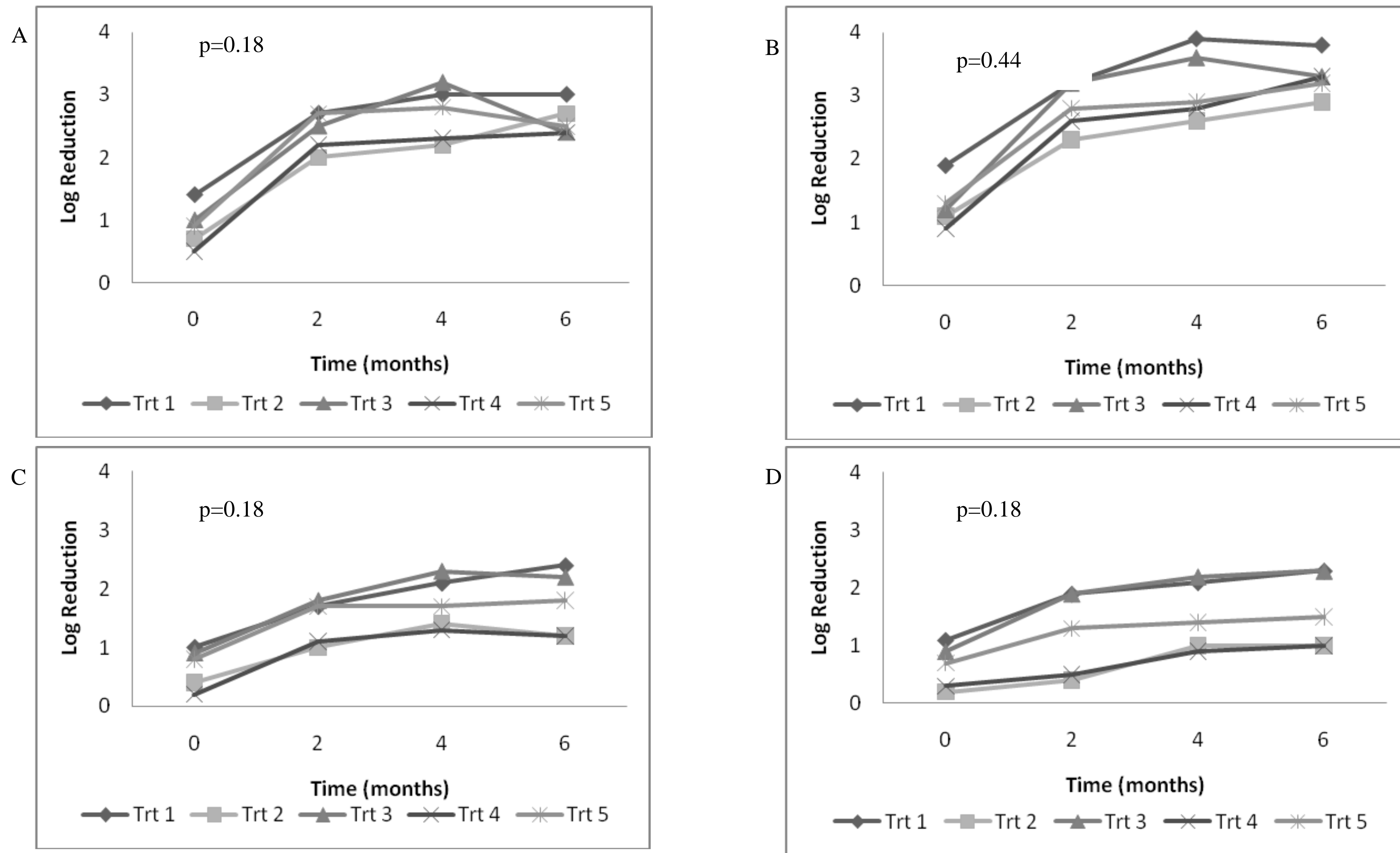

Figure 1. *Least squares means of the interaction between treatment and storage time of log reductions (log CFU/sample) of* Salmonella *on (A) intestine (B) stomach (C) liver (D) heart. Treatment 1: water wash + lactic acid + freeze; Treatment 2: freeze; Treatment 3: water wash + lactic acid + chill + freeze; Treatment 4: chill + freeze; Treatment 5: water wash + freeze.*

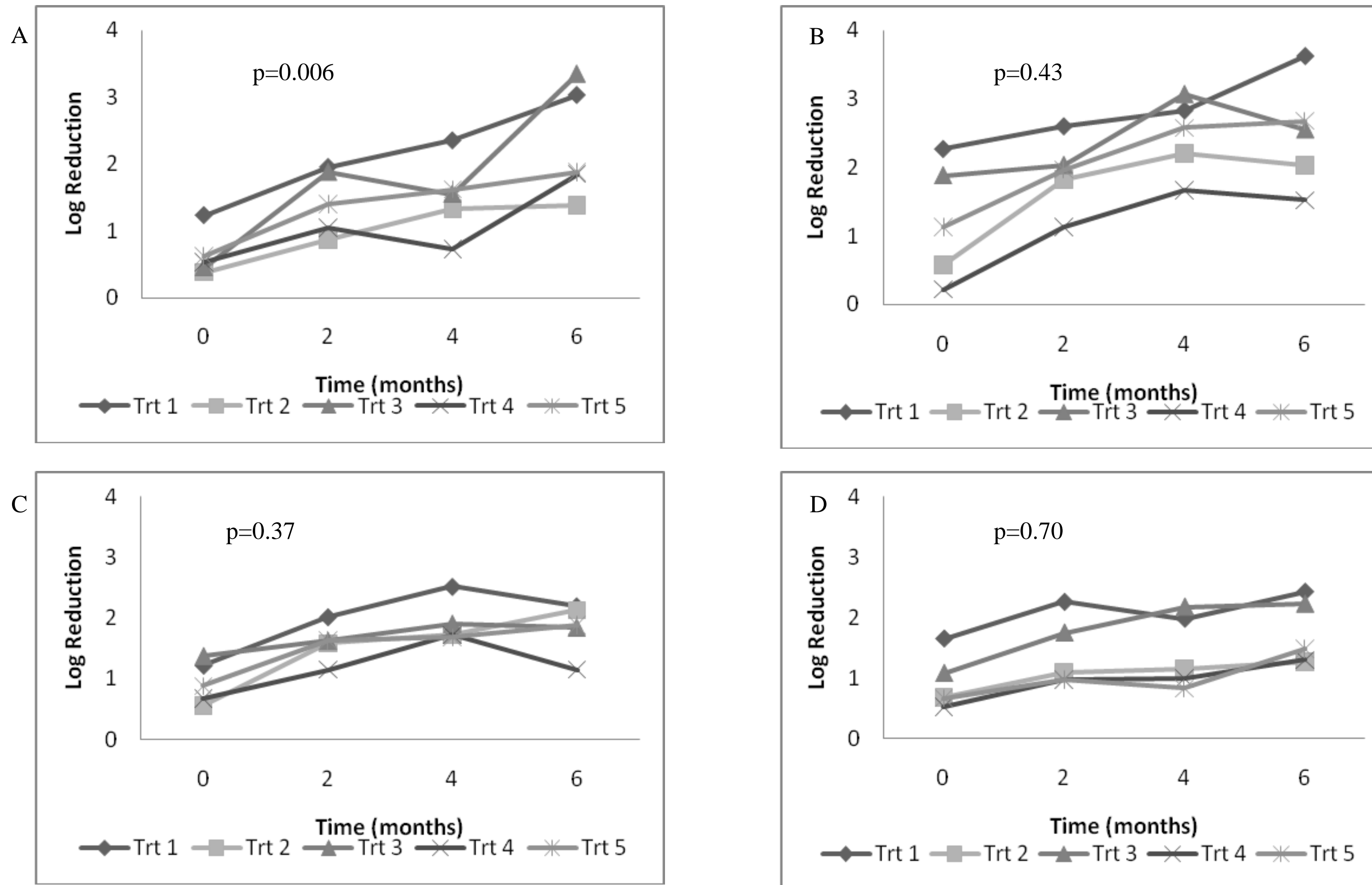

Figure 2. *Least squares means of the interaction between treatment and storage time of log reductions (log CFU/sample) of* Yersinia enterocolitica *on (A) intestine (B) stomach (C) liver (D) heart. Treatment 1: water wash + lactic acid + freeze; Treatment 2: freeze; Treatment 3: water wash + lactic acid + chill + freeze; Treatment 4: chill + freeze; Treatment 5: water wash + freeze.*

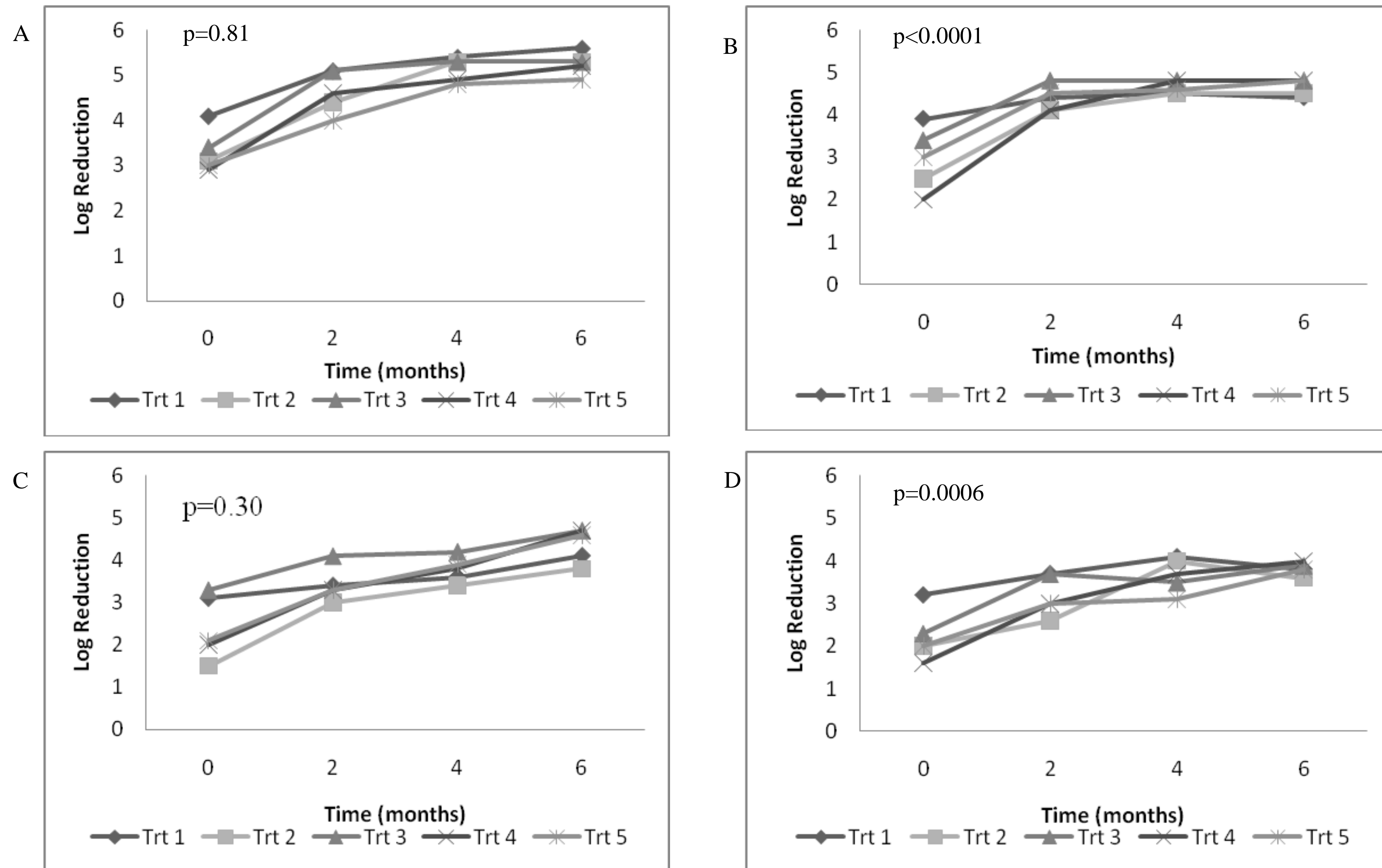

Figure 3. *Least squares means of the interaction between treatment and storage time of log reductions (log CFU/sample) of* Campylobacter coli *on (A) intestine (B) stomach (C) liver (D) heart. Treatment 1: water wash + lactic acid + freeze; Treatment 2: freeze; Treatment 3: water wash + lactic acid + chill + freeze; Treatment 4: chill + freeze; Treatment 5: water wash + freeze.*

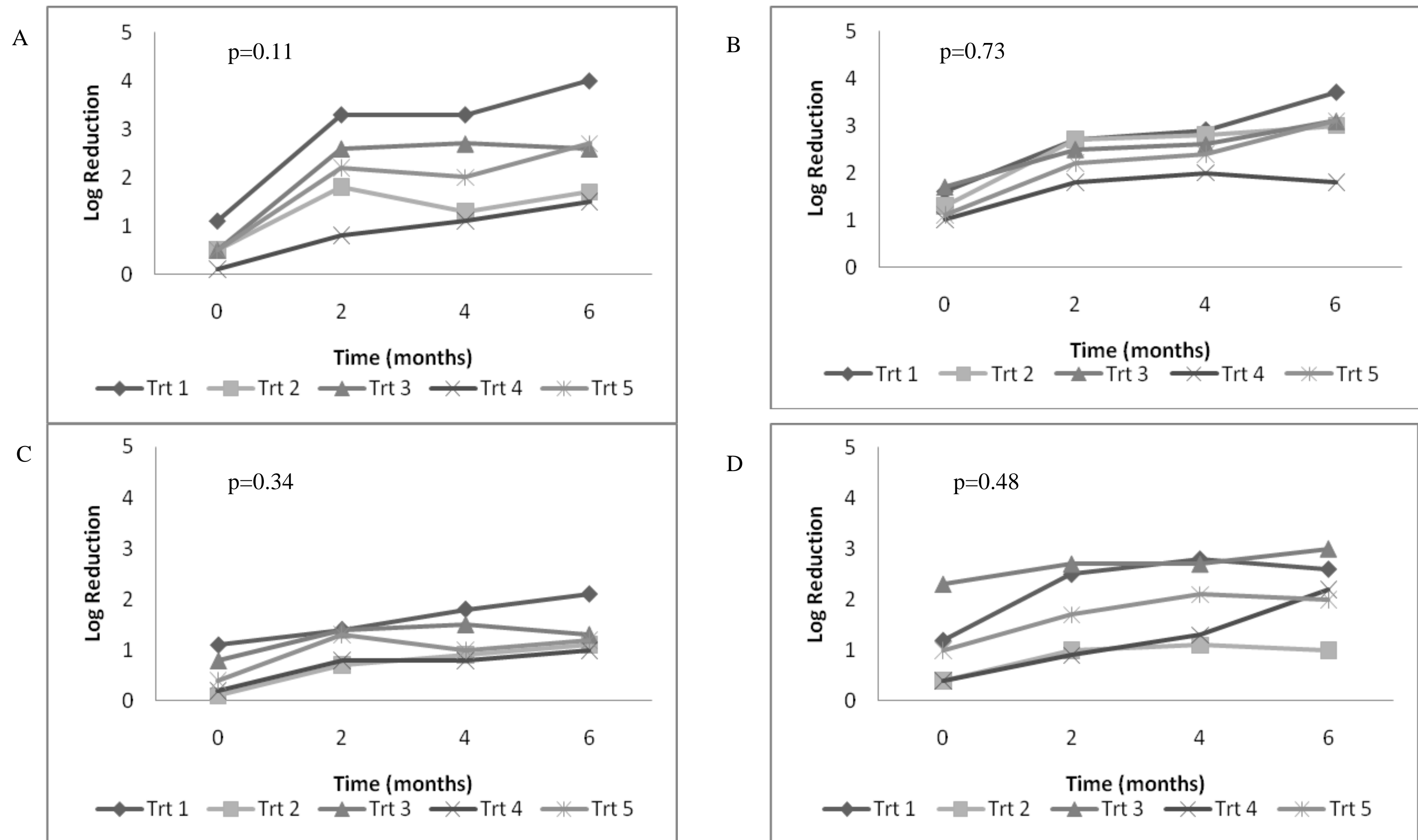

Figure 4. *Least squares means of the interaction between treatment and storage time of log reductions (log CFU/sample) of aerobic plate counts on (A) intestine (B) stomach (C) liver (D) heart. Treatment 1: water wash + lactic acid + freeze; Treatment 2: freeze; Treatment 3: water wash + lactic acid + chill + freeze; Treatment 4: chill + freeze; Treatment 5: water wash + freeze.*

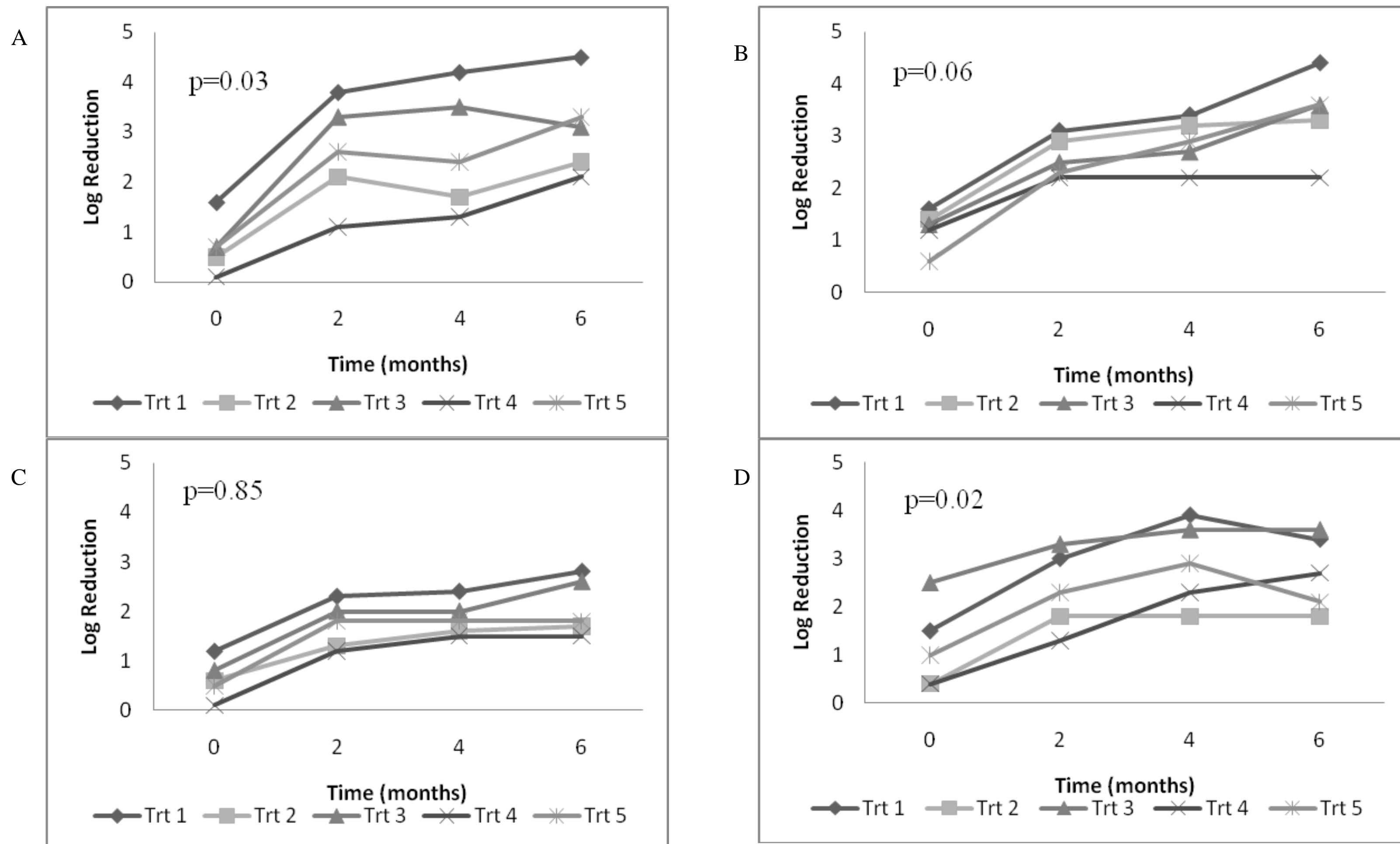

Figure 5. *Least squares means of the interaction between treatment and storage time of log reductions (log CFU/sample) of* Escherichia coli *on (A) intestine (B) stomach (C) liver (D) heart. Treatment 1: water wash + lactic acid + freeze; Treatment 2: freeze; Treatment 3: water wash + lactic acid + chill + freeze; Treatment 4: chill + freeze; Treatment 5: water wash + freeze.*

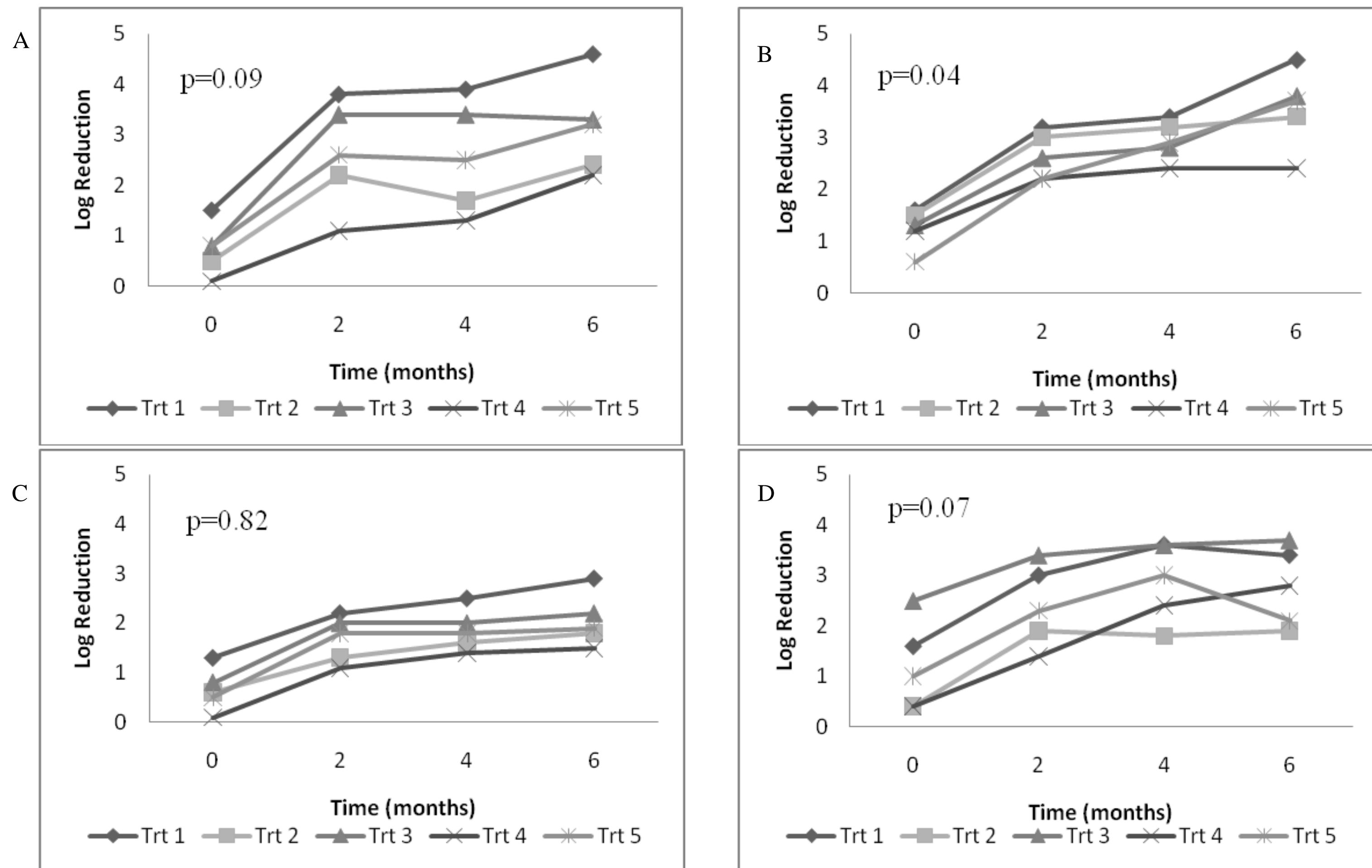

Figure 6. *Least squares means of the interaction between treatment and storage time of log reductions (log CFU/sample) of coliforms on (A) intestine (B) stomach (C) liver (D) heart. Treatment 1: water wash + lactic acid + freeze; Treatment 2: freeze; Treatment 3: water wash + lactic acid + chill + freeze; Treatment 4: chill + freeze; Treatment 5: water wash + freeze.*

Table 1. *Least squares means of initial microbial levels before application of treatments.*

Variety Meat	Log CFU/sample[a]					
	Salmonella	*Yersinia enterocolitica*	*Campylobacter coli*	Aerobic plate counts	*Escherichia coli*	Coliforms
Intestine	6.3 A	5.1	5.3 A	6.6 B	6.2 B	6.4 B
Stomach	6.3 A	5.0	4.7 B	7.1 A	6.6 A	6.8 A
Liver	6.1 B	5.0	4.6 B	6.0 C	5.6 C	5.8 C
Heart	6.2 B	5.0	4.2 C	5.6 D	5.0 D	5.1 D

[a]Means within each column bearing a common letter are not significantly different ($P > 0.05$).

Table 2. *Least squares means of log reductions of* Salmonella *after steps within treatments (log CFU/sample)*[a].

	WW[b]	LA[c]	CH[d]	FR[e]
Variety Meat				
Intestine	0.0 B	0.3 B	0.3	0.9 B
Stomach	0.1 AB	0.6 A	0.4	1.3 A
Liver	0.6 A	0.6 A	0.3	0.7 C
Heart	0.4 A	0.6 A	0.3	0.6 C
Treatment[f]				
1 WW+LA+FR	-	-	-	1.4 A
2 FR	-	-	-	0.6 C
3 WW+LA+CH+FR	-	-	0.6 A	1.0 B
4 CH+FR	-	-	0.1 B	0.5 C
5 WW+FR	-	-	-	1.0 B

[a]Initial inoculum level was 6.1-6.3 log CFU/sample.
[b]Sampled after water wash.
[c]Sampled after water wash followed by lactic acid spray.
[d] Sampled after internal temperature of samples reached 4°C with previous treatment components according to Treatment.
[e]Sampled after internal temperature of samples reached 0°C with previous treatment components according to Treatment.
[f]WW: water wash (10 s, 20-30°C); LA: lactic acid spray (10 s, 40-50°C); CH: chill (4°C); FR: freeze (0°C).

Table 3. *Least squares means of log reductions of* Yersinia enterocolitica *after steps within treatments (log CFU/sample)*[a].

	WW[b]	LA[c]	CH[d]	FR[e]
Variety Meat				
Intestine	0.2 B	0.5 B	0.2 B	0.6 C
Stomach	0.8 A	1.2 A	0.7 A	1.2 A
Liver	0.5 AB	0.8 AB	0.2 B	0.9 B
Heart	0.4 AB	0.8 AB	0.5 B	0.9 B
Treatment[f]				
1 WW+LA+FR	-	-	-	1.6 A
2 FR	-	-	-	0.6 D
3 WW+LA+CH+FR	-	-	0.7 A	1.2 B
4 CH+FR	-	-	0.1 B	0.5 D
5 WW+FR	-	-	-	0.8 C

[a]Initial inoculum level was 5.0-5.1 log CFU/sample.
[b]Sampled after water wash.
[c]Sampled after water wash followed by lactic acid spray.
[d]Sampled after internal temperature of samples reached 4°C with previous treatment components according to Treatment.
[e]Sampled after internal temperature of samples reached 0°C with previous treatment components according to Treatment.
[f]WW: water wash (10 s, 20-30°C); LA: lactic acid spray (10 s, 40-50°C); CH: chill (4°C); FR: freeze (0°C).

Table 4. *Least squares means of log reductions of* C. coli *after steps within treatments (log CFU/sample)*[a].

	WW[b]	LA[c]	CH[d]	FR[e]
Variety Meat				
Intestine	0.3 B	0.8	1.1 B	2.2 B
Stomach	-0.3 B	1.2	1.8 A	3.2 A
Liver	2.1 A	1.1	0.7 B	2.5 B
Heart	0.2 B	0.7	1.8 A	3.0 A
Treatment[f]				
1 WW+LA+FR	-	-	-	3.6 A
2 FR	-	-	-	2.3 C
3 WW+LA+CH+FR	-	-	1.6 A	3.1 B
4 CH+FR	-	-	1.1 B	2.1 C
5 WW+FR	-	-	-	2.6 B

[a]Initial inoculum level was 4.2-5.3 log CFU/sample.
[b]Sampled after water wash.
[c]Sampled after water wash followed by lactic acid spray.
[d]Sampled after internal temperature of samples reached 4°C with previous treatment components according to Treatment.
[e]Sampled after internal temperature of samples reached 0°C with previous treatment components according to Treatment.
[f]WW: water wash (10 s, 20-30°C); LA: lactic acid spray (10 s, 40-50°C); CH: chill (4°C); FR: freeze (0°C).

Table 5. *Least squares means of log reductions of aerobic plate counts (APCs) after steps within treatments (log CFU/sample)*[a].

	WW[b]	LA[c]	CH[d]	FR[e]
Variety Meat				
Intestine	0.0	0.3	-0.1	0.6 B
Stomach	-0.3	0.6	-0.1	1.3 A
Liver	0.0	0.3	0.0	0.5 B
Heart	0.1	0.7	0.4	1.1 A
Treatment[f]				
1 WW+LA+FR	-	-	-	1.3 A
2 FR	-	-	-	0.6 BC
3 WW+LA+CH+FR	-	-	0.4 A	1.3 A
4 CH+FR	-	-	-0.3 B	0.4 C
5 WW+FR	-	-	-	0.8 B

[a]Initial inoculum level was 5.6-7.1 log CFU/sample.
[b]Sampled after water wash.
[c]Sampled after water wash followed by lactic acid spray.
[d]Sampled after internal temperature of samples reached 4°C with previous treatment components according to Treatment.
[e]Sampled after internal temperature of samples reached 0°C with previous treatment components according to Treatment.
[f]WW: water wash (10 s, 20-30°C); LA: lactic acid spray (10 s, 40-50°C); CH: chill (4°C); FR: freeze (0°C).

Table 6. *Least squares means of log reductions of* Escherichia coli *after steps within treatments (log CFU/sample)*[a].

	WW[b]	LA[c]	CH[d]	FR[e]
Variety Meat				
Intestine	0.2	0.4 B	0.1	0.7 B
Stomach	-0.1	0.5 B	0.0	1.2 A
Liver	-0.1	0.4 B	0.0	0.6 B
Heart	-0.1	1.4 A	0.5	1.2 A
Treatment[f]				
1 WW+LA+FR	-	-	-	1.4 A
2 FR	-	-	-	0.7 B
3 WW+LA+CH+FR	-	-	0.5 A	1.3 A
4 CH+FR	-	-	-0.2 B	0.5 B
5 WW+FR	-	-	-	0.7 B

[a]Initial inoculum level was 5.0-6.6 log CFU/sample.
[b]Sampled after water wash.
[c]Sampled after water wash followed by lactic acid spray.
[d]Sampled after internal temperature of samples reached 4°C with previous treatment components according to Treatment.
[e]Sampled after internal temperature of samples reached 0°C with previous treatment components according to Treatment.
[f]WW: water wash (10 s, 20-30°C); LA: lactic acid spray (10 s, 40-50°C); CH: chill (4°C); FR: freeze (0°C).

Table 7. *Least squares means of log reductions of coliforms after steps within treatments (log CFU/sample)*[a].

	WW[b]	LA[c]	CH[d]	FR[e]
Variety Meat				
Intestine	0.2	0.3 B	0.1	0.8 B
Stomach	-0.1	0.6 B	0.0	1.2 A
Liver	0.1	0.3 B	0.0	0.7 B
Heart	-0.1	1.4 A	0.5	1.2 A
Treatment[f]				
1 WW+LA+FR	-	-	-	1.5 A
2 FR	-	-	-	0.8 B
3 WW+LA+CH+FR	-	-	0.5 A	1.4 A
4 CH+FR	-	-	-0.2 B	0.5 C
5 WW+FR	-	-	-	0.7 B

[a]Initial inoculum level was 5.1-6.8 log CFU/sample.
[b]Sampled after water wash.
[c]Sampled after water wash followed by lactic acid spray.
[d]Sampled after internal temperature of samples reached 4°C with previous treatment components according to Treatment.
[e]Sampled after internal temperature of samples reached 0°C with previous treatment components according to Treatment.
[f]WW: water wash (10 s, 20-30°C); LA: lactic acid spray (10 s, 40-50°C); CH: chill (4°C); FR: freeze (0°C).

Table 8. *Least squares means of log reductions of* Salmonella *(log CFU/sample) over 6 months of frozen storage*.

	Reductions (before-treatment minus after-treatment means) of *Salmonella* (log CFU/sample)[a]			
	Intestine	Stomach	Liver[b]	Heart
Storage time[c]				
0	0.9 C	1.3 C	0.7 C	0.6 C
2 months	2.4 B	2.8 B	1.4 B	1.2 B
4 months	2.7 A	3.2 A	1.7 A	1.5 A
6 months	2.6 AB	3.3 A	1.8 A	1.6 A
Treatment [d]				
1 WW+LA+FR	2.5 A	3.2 A	1.8 A	1.8 A
2 FR	1.9 C	2.2 D	1.0 C	0.7 C
3 WW+LA+CH+FR	2.3 AB	2.8 B	1.8 A	1.8 A
4 CH+FR	1.8 C	2.4 CD	1.0 C	0.7 C
5 WW+FR	2.2 B	2.6 BC	1.5 B	1.2 B

[a]Means within each column bearing a common letter are not significantly different (P>0.05).
[b]Interactions of replicate x storage time and replicate x treatment were significant (P<0.05) for livers (data not shown).
[c]0 storage time was measured when samples reached 0°C.
[d]WW: water wash (10 s, 20-30°C); LA: lactic acid spray (10 s, 40-50°C); CH: chill (4°C); FR: freeze (0°C).

Table 9. *Least squares means of log reductions of* Yersinia enterocolitica *(log CFU/sample) over 6 months of frozen storage.*

	Reductions (before-treatment minus after-treatment means) of *Y. enterocolitica* (log CFU/sample)[a]			
	Intestine	Stomach	Liver[b]	Heart
Storage Time[c]				
0	0.6 C	1.2 B	1.0 C	0.9 C
2 months	1.4 B	1.9 B	1.6 B	1.4 B
4 months	1.5 B	2.5 A	1.9 A	1.4 B
6 months	2.3 A	2.5 A	1.8 AB	1.7 A
Treatment [d]				
1 WW+LA+FR	2.1 A	2.8 A	2.0 A	2.1 A
2 FR	1.0 D	1.7 C	1.5 B	1.1 B
3 WW+LA+CH+FR	1.8 B	2.4 B	1.7 AB	1.8 A
4 CH+FR	1.0 D	1.1 D	1.2 C	1.0 B
5 WW+FR	1.4 C	2.1 B	1.5 B	1.0 B

[a]Means within each column bearing a common letter are not significantly different (P>0.05).
[b]Interactions of replicate x storage time and replicate x treatment were significant (P<0.05) for livers (data not shown).
[c]0 storage time was measured when samples reached 0°C.
[d]WW: water wash (10 s, 20-30°C); LA: lactic acid spray (10 s, 40-50°C); CH: chill (4°C); FR: freeze (0°C).

Table 10. *Least squares means of log reductions of* C. coli *(log CFU/sample) over 6 months of frozen storage.*

	Reductions (before-treatment minus after-treatment means) of *C. coli* (log CFU/sample)[a]			
	Intestine	Stomach[b]	Liver[b]	Heart[b]
Storage Time [c]				
0	3.3 C	3.0 C	2.5 C	2.2 C
2 months	4.6 B	4.4 B	3.5 B	3.2 B
4 months	5.1 A	4.6 A	3.7 B	3.7 A
6 months	5.3 A	4.6 A	4.4 A	3.8 A
Treatment [d]				
1 WW+LA+FR	5.0 A	4.3 A	3.5 B	3.7 A
2 FR	4.5 B	3.9 B	2.9 C	3.1 BC
3 WW+LA+CH+FR	4.8 AB	4.4 A	4.1 A	3.3 B
4 CH+FR	4.4 C	3.9 B	3.4 B	3.1 BC
5 WW+FR	4.2 C	4.2 A	3.6 B	3.0 C

[a]Means within each column bearing a common letter are not significantly different (P>0.05).
[b]Interactions of replicate x storage time and replicate x treatment were significant (P<0.05) for stomachs, livers, and hearts (data not shown).
[c]0 storage time was measured when samples reached 0°C.
[d]WW: water wash (10 s, 20-30°C); LA: lactic acid spray (10 s, 40-50°C); CH: chill (4°C); FR: freeze (0°C).

Table 11. *Least squares means of log reductions of aerobic plate counts (APCs) (log CFU/sample) over 6 months of frozen storage.*

	Reductions (before-treatment minus after-treatment means) of aerobic plate count (log CFU/sample)[a]			
	Intestine	Stomach	Liver	Heart
Storage Time[b]				
0	0.6 C	1.3 C	0.5 C	1.1 C
2 months	2.1 B	2.4 B	1.1 B	1.7 B
4 months	2.1 B	2.5 B	1.2 AB	2.0 AB
6 months	2.5 A	2.9 A	1.3 A	2.1 A
Treatment[c]				
1 WW+LA+FR	2.9 A	2.7 A	1.6 A	2.3 A
2 FR	1.3 C	2.4 AB	0.7 D	0.9 C
3 WW+LA+CH+FR	2.1 B	2.5 AB	1.3 B	2.7 A
4 CH+FR	0.8 D	1.6 C	0.7 D	1.2 C
5 WW+FR	1.9 B	2.2 B	1.0 C	1.7 B

[a]Means within each column bearing a common letter are not significantly different ($P>0.05$).
[b]0 storage time was measured when samples reached 0°C.
[c]WW: water wash (10 s, 20-30°C); LA: lactic acid spray (10 s, 40-50°C); CH: chill (4°C); FR: freeze (0°C).

Table 12. *Least squares means of log reductions of* E. coli *(log CFU/sample) over 6 months of frozen storage.*

	Reductions (before-treatment minus after-treatment means) of generic *E. coli* (log CFU/sample)[a]			
	Intestine	Stomach	Liver	Heart
Storage Time[b]				
0	0.7 C	1.2 C	0.6 C	1.2 C
2 months	2.6 B	2.6 B	1.7 B	2.3 B
4 months	2.6 B	2.9 B	1.8 B	2.9 A
6 months	3.1 A	3.4 A	2.1 A	2.7 A
Treatment[c]				
1 WW+LA+FR	3.5 A	3.1 A	2.2 A	2.9 A
2 FR	1.7 D	2.7 B	1.3 CD	1.5 C
3 WW+LA+CH+FR	2.6 B	2.5 BC	1.8 B	3.2 A
4 CH+FR	1.3 E	2.0 D	1.1 D	1.7 C
5 WW+FR	2.3 C	2.3 C	1.4 C	2.1 B

[a] Means within each column bearing a common letter are not significantly different (P>0.05).
[b] 0 storage time was measured when samples reached 0°C.
[c] WW: water wash (10 s, 20-30°C); LA: lactic acid spray (10 s, 40-50°C); CH: chill (4°C); FR: freeze (0°C).

Table 13. *Least squares means of log reductions of coliforms (log CFU/sample) over 6 months of frozen storage.*

	Reductions (before-treatment minus after-treatment means) of coliforms (log CFU/sample)[a]			
	Intestine	Stomach	Liver	Heart
Storage Time[b]				
0	0.8 C	1.2 C	0.7 C	1.2 C
2 months	2.6 B	2.6 B	1.7 B	2.4 B
4 months	2.6 B	2.9 B	1.8 AB	2.9 A
6 months	3.1 A	3.5 A	2.0 A	2.8 A
Treatment[c]				
WW+LA+FR	3.5 A	3.2 A	2.2 A	2.9 B
FR	1.7 D	2.8 B	1.3 C	1.5 D
WW+LA+CH+FR	2.7 B	2.6 BC	1.7 B	3.3 A
CH+FR	1.2 E	2.0 D	1.0 D	1.7 D
WW+FR	2.3 C	2.4 CD	1.5 C	2.1 C

[a] Means within each column bearing a common letter are not significantly different (P>0.05).
[b] 0 storage time was measured when samples reached 0°C.
[c] WW: water wash (10 s, 20-30°C); LA: lactic acid spray (10 s, 40-50°C); CH: chill (4°C); FR: freeze (0°C).