

FACTORS AFFECTING SALMONELLAE RECOVERY FROM BROILER CARCASSES
TREATED WITH TRISODIUM PHOSPHATE

by

DIANNA VIOLA BOURASSA

(Under the direction of Daniel L. Fletcher)

ABSTRACT

The effectiveness of trisodium phosphate application on the recovery of native salmonellae from broiler carcasses was investigated. Experiment 1 examined the residual effects of TSP following immersion chilling on salmonellae recovery when sampled on the day of processing and after refrigerated storage for 7 days. When carcasses were treated with TSP, salmonellae recovery was significantly lower than that of the control carcasses on both sampling days demonstrating a residual effect. Salmonellae recovery was also shown to decrease after 7 days of refrigeration for control groups. Experiment 2 examined the role of enrichment pH in TSP's effectiveness against salmonellae. However, in this experiment the alkaline TSP and the alkalized peptone enrichment carcasses (pH shock) did not have a lower incidence of salmonellae recovery than the neutralized-TSP and control carcasses. The inability of TSP to decrease salmonellae recovery in Experiment 2 was speculated to be due to lack of washing action, which would typically occur during immersion chilling.

INDEX WORDS: Trisodium Phosphate, Salmonellae, Broilers, Whole Carcass Enrichment

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DEDICATION

I would like to dedicate this thesis to my loving husband Jason for his never-ending patience and support in all that I do.

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CHAPTER 1

INTRODUCTION

The food supply in developed countries is relatively safe. However, pathogens are still a cause of food-borne gastroenteritis in the United States. For meat and poultry products, the pathogens most often implicated are *Salmonella*, *Campylobacter*, *Escherichia coli*, *Clostridium perfringens*, and *Listeria monocytogenes*. Interventions during production and processing are necessary to minimize the exposure of the consumer to these pathogens. Intervention strategies such as competitive exclusion, increased biosecurity, HACCP (Hazard Analysis Critical Control Point) programs, carcass washing, and application of chemicals have been used to attempt to minimize pathogenic bacterial loads on carcasses. Many food-borne pathogens associated with poultry are brought into processing plants with contaminated animals, and may be unavoidable. Contamination of meat is often due to pathogens being transferred from the skin or from the contents of the gastrointestinal tract of the contaminated carcasses. Once in the plant, pathogens can be transferred to other carcasses via direct contact or via common contact surfaces resulting in high levels of cross-contamination. Some pathogens, such as *Listeria monocytogenes*, thrive in the processing plant environment and can colonize locations such as in drains and on hoses.

Trisodium phosphate (TSP) was approved by the Food and Drug Administration as a generally recognized as safe (GRAS) food processing aid in August 1992. Since then TSP has been used in breakfast cereals, snack foods, artificially sweetened fruit jellies, and processed cheese spreads as antioxidants and emulsifiers. In high concentrations, TSP is also used as a

cleaning agent in many food processing equipment cleansers. In October 1992, TSP was also approved by the United States Department of Agriculture for post-chill application in poultry processing plants. TSP was approved in Canada in January 1994 for pre-chill and post-chill applications. In February 1994, pre-chill carcass TSP application was approved by the USDA. For this application method, poultry carcasses are flushed online with an 8 to 12 percent TSP solution between 65 and 85 F. The TSP is filtered and recirculated with automated systems maintaining the concentration and temperature. The purpose of this project was to examine treatment, storage, and enrichment pH effects of TSP on reducing salmonellae recovery on broiler carcasses.

CHAPTER 2

LITERATURE REVIEW

Trisodium phosphate (TSP) is used in poultry processing plants as a food processing aid to assist in pathogen reduction. TSP has historically been used as a cleanser and in the past 15 years has also been used in food processing. Salmonellae and other microorganisms have been reported to decrease following the use of TSP on poultry carcasses presumably with alkaline pH being the main mechanism of action. Organoleptic properties of poultry treated with TSP are not reported to be adversely affected. TSP has also been successfully used on other food products as an antimicrobial agent.

Pathogen Reduction

The food supply in developed countries is relatively safe. However, pathogens are still a cause of food-borne gastroenteritis in the United States. For meat and poultry products, the pathogens most often implicated are *Salmonella*, *Campylobacter*, *Escherichia coli*, *Clostridium perfringens*, and *Listeria monocytogenes*. Broiler carcasses in processing plants are tested for *Salmonella* by the United States Department of Agriculture, Food Safety and Inspection Service. Interventions to reduce salmonellae during production and processing are intended to reduce exposure to the consumer. Intervention strategies such as competitive exclusion, increased biosecurity, Hazard Analysis Critical Control Point (HACCP) programs, carcass washing, and application of chemicals (chlorine, ozone) have been used to attempt to minimize pathogenic

bacterial loads on carcasses. TSP has been approved by USDA as an intervention step to reduce salmonellae during processing (Giese, 1992).

History of Trisodium Phosphate

TSP has historically been used as a household cleanser. TSP was approved by the Food and Drug Administration as a GRAS (generally recognized as safe) substance for use in food processing in August 1992. Since then TSP has been used as an ingredient in food processing contact surface cleaners. TSP has also been used in breakfast cereals, snack foods, artificially sweetened fruit jellies, and processed cheese spreads as antioxidants and emulsifiers. In October 1992, TSP was approved by the United States Department of Agriculture for post-chill use in poultry processing plants (Bender and Brotsky, 1992). TSP has been approved for pre-chill, post-chill, and in air-chilling operations in Canada (Canadian Food Inspection Agency, 2004). In February 1994, pre-chill carcass TSP application was approved by the USDA (Brown, 1994).

Trisodium Phosphate Mechanisms

TSP's antimicrobial activity works in several ways; 1) high pH causes cell membrane disruption (Mendonca et al., 1994), 2) detachment of bacteria from the carcass surface (Lee et al., 1994), and 3) lipid removal or detergent-like activity (Bender and Brotsky, 1992; Giese, 1992; Kim and Slavik, 1994).

The cytoplasmic membrane in gram-negative bacteria is known to be more susceptible to high pH than in gram-positive bacteria (Murray et al., 1965). High pH has frequently been reported to have detrimental effects on *Salmonella*. The destruction of the gram-negative

pathogen *Salmonella* at high pH was demonstrated by Cotterill (1968). At 50° C, a pH of 11 was reported to eliminate *Salmonella* in culture broth in less than a minute (Kinner and Moats, 1981). *Salmonella* when attached to chicken skin was reported to be killed more rapidly at a pH 9 versus a pH 6 (Humphrey et al., 1981). On beef tissue, high pH treatments NaOH and KOH were also reported to reduce *Salmonella* (Dickson, 1988). The high pH (~12) works through disruption of gram-negative bacterial cytoplasmic membranes (Mendonca et al., 1994). Egg washwater with a pH 11.0 and 37.7° C was reported to prevent cross-contamination and egg shell penetration of *Salmonella* (Catalano and Knabel, 1994).

When using electron microscopy on TSP and high pH treated *Salmonella* cells, Sampathkumar et al. (2003) reported that cells showed a loss in their general shape, disorganized and loosely packed DNA and polyribosomes, distortions and collapse in membrane wall continuity, condensation and beading of cytoplasmic material, and vacuolization in comparison to control cells. It was concluded that TSP at its inherent pH reduced cell viability and osmotic response in a concentration-dependant manner and permeabilized cytoplasmic membranes. However, when the TSP was neutralized, these effects were negated.

High pH treatments have been shown to be effective against a variety of microorganisms. Teo, et al. (1996) demonstrated that a pH 11.0 can reduce *E. coli* O157:H7 by almost 3 logs and *Salmonella enteritidis* by 4.5 logs after 3 minutes at 35° C when observed in a NaHCO₃ stock solution titrated with NaOH.

When the pH of beef trimmings was increased to 9.6 by ammonia injection *E. coli* O157:H7 was significantly reduced in comparison to controls (Niebuhr and Dickson, 2003). However, this pH enhancement was only able to minimally reduce *Listeria* by 0.5 logs. It was

suggested that this minimal reduction was because alkaline treatments have less effect on gram-positive bacteria due to their thick peptidoglycan layers.

In a study reported by Capita et al.(2002a), *Listeria monocytogenes* was reported to be significantly inactivated and suppressed from growth for up to 5 days by both TSP and NaOH treatments of the same pH. Also, the pH decreased by 1 unit to pH 8 from day 0 to 5 days of storage at 2 C allowing for residual pH effects. This report indicated that pH plays a major role in *Listeria* reduction.

Lillard (1994) suggested that the large reductions in *Salmonella* numbers reported may be due to an artifact of high pH from TSP in recovery mediums. To overcome this artifact, Lillard attempted to reduce the residual pH by rinsing TSP off. However, with a 2 L sterile rinse the pH was only reduced from 12.1 to 11.6 in the recovery medium.

Changes in pH during refrigerated storage are limited due to the buffering capacity found in meat and meat products (Gibson, 1988). Residual high surface pH on beef tissue after alkaline (TSP) treatment was reported to occur at least after 24 hours of refrigerated storage with a minimal pH reduction from 10.94 to 9.23 (Dorsa et al., 1998b).

Trisodium Phosphate Application Methods

Variation by Concentration

The concentration of TSP is an important factor during TSP treatment. In a study by Li et al., (1995) chiller water from a commercial processing plant was collected and TSP was added to 0.1%, 0.2% and 0.3% concentrations. The chiller water was then inoculated with *Campylobacter jejuni* to yield 10^6 to 10^7 cells/mL. After 20 minutes at 4 C, the 0.1% and 0.2%

TSP concentrations did not significantly decrease *Campylobacter* in the chiller water. However, at 0.3%, *Campylobacter* recovery was eliminated within 5 minutes of treatment.

When a low concentration of 1% TSP was used to dip inoculated chicken skin for 30 minutes at 25° C, *Salmonella*, *Listeria monocytogenes*, and psychrotrophic bacteria were significantly reduced in comparison to water dipped chicken skin (Hwang and Beuchat, 1995).

Prechill broiler breast skin samples were inoculated with *Salmonella typhimurium* and spray treated with either 5% or 10% TSP for 30 seconds at 206 kPa at 20 C. Samples were then rinsed off by spraying with tap water for 30 seconds. After the skin was stomached and direct plated, no significant differences were reported in *Salmonella* counts due to TSP concentration, however TSP did significantly reduce recovery in comparison to both water sprayed and non-sprayed controls (Xiong et al. 1998b).

Capita et al. (2000a), sampled prechill broiler breast skin samples for psychrotrophic and mesophilic bacteria after being dipped in either 8%, 10%, or 12% TSP for 15 minutes at 20 C. For both psychrotrophs and mesophils, counts were reduced as concentrations increased. Both the 10% and 12% TSP treatment had significantly larger decreases of psychrotrophic counts than the 8% treatment. The 12% TSP treatment had significantly higher reductions of mesophilic counts than treatment at 8%. In comparison to non-treated controls, all concentrations significantly reduced counts.

Raw chicken wings inoculated with *Yarrowia lipolytica* (a predominant spoilage yeast in raw poultry) were dipped in 4%, 8%, or 12% TSP for 60 seconds at 21 C. The 8% and 12% TSP-treated wing had significantly fewer *Yarrowia* after treatment than wings treated with 4% TSP. However, all concentrations significantly reduced *Yarrowia* counts in comparison to

controls. All three concentration of TSP tested also significantly reduced aerobic microorganisms in comparison to controls, but aerobic counts were not different between concentrations (Ismail et al., 2001).

In a study reported by Capita et al. (2001), prechill breast and dorsal skin samples were inoculated with *Listeria monocytogenes* and dipped in 8%, 10%, or 12% TSP solutions for 15 minutes at 20 C. As TSP-treatment concentrations increased, *Listeria* counts were significantly decreased. Each concentration also significantly decreased counts in comparison to controls.

Listeria monocytogenes inoculated raw chicken legs were dipped either 8%, 10%, or 12% TSP for 15 minutes at 20° C. As TSP concentration increased *Listeria* counts were significantly decreased. All concentrations also significantly reduced numbers in comparison to water dipped controls (Capita et al. 2002a).

In a later study reported by Capita et al. (2003), whole chicken legs versus excised chicken skin samples were inoculated with *Listeria monocytogenes* and treated with 8%, 10%, or 12% TSP for 15 minutes at 20 C. The 12% TSP treatment was reported more effective on excised skin than whole legs samples due to higher accessibility to *Listeria* on the excised skin. *Listeria* reductions were not affected by sample type when treated with the 8% or 10% concentrations. On the whole leg samples, the 12% concentration reduced counts significantly more than 8% TSP. On the excised skin samples, the 12% TSP reduced significantly more counts than both the 8% and 10% concentrations. Application of any of these concentrations also significantly decreased *Listeria* counts in comparison to water dipped controls.

When *Listeria* inoculated fresh shrimp and rainbow trout filets were treated with 10% or 20% TSP both psychrotrophic and *Listeria* counts were not significantly reduced by either

concentration on shrimp. However, both psychrotrophic and *Listeria* counts were significantly decreased by the 20% TSP treatment of the rainbow trout filets (Mu et al., 1997).

Variation by Treatment Time

Length of TSP treatment time is an important factor in microbial reduction. Prechill broiler carcass were either sprayed for 10 or 20 seconds, or dipped for 6 seconds in 10% TSP. After a drip time of 5 minutes TSP was then rinsed off each carcass with water in the same manner each carcass was treated. Carcasses were sampled for aerobic microorganisms, Enterobacteriaceae, *Pseudomonas*, *Lactobacillus*, and salmonellae. Aerobic microorganisms and Enterobacteriaceae counts were significantly reduced by each treatment time. Both the 10 second spray and 6 second dip had significantly higher reductions than the 20 second spray treatment. *Pseudomonas* counts were significantly reduced by each treatment time and there were no significant differences between treatment times. However, when *Lactobacillus* were sampled, only the 10 second spray treatment significantly reduced counts. Salmonellae incidence was decreased by 10% after the 10 second treatment and by 29% after the 20 second treatment. The 6 second dip did not decrease salmonellae incidence (Ellerbroek et al., 1996).

In a study reported by Arritt et al. (2002), chicken breast skin samples were spray treated for 3 seconds with 10% TSP and given either 0.5, 3, or 10 minutes contact time. Skin samples were inoculated with *Campylobacter jejuni* either prior to or following the TSP treatments. Application of bacteria both prior to and following treatment yielded significant *Campylobacter* count reductions. TSP contact time did not significantly affect *Campylobacter* counts when

bacteria were applied prior to treatment. However, when bacteria were applied after treatment, *Campylobacter* count reductions increased as contact time increased.

Variation by Treatment Temperature

Treatment temperature is also a factor in TSP application. In a study reported by Kim et al. (1994a), chicken drumsticks were inoculated with *Salmonella typhimurium* and dipped in 10% TSP for 15 seconds at 10 or 50° C. Scanning electron microscopy was used to determine the effect of TSP on *Salmonella typhimurium*. At both temperatures TSP-treated skin samples had significantly fewer *Salmonella* and cleaner skin surfaces than control skin samples. It was concluded that TSP aided in the removal of both debris and *Salmonella* from skin surfaces.

Processing Location of TSP Application

Application of TSP at different points on the processing line have been examined. Breast skin samples were treated by either scalding in an 8% TSP solution at 50° C for 2 minutes, chilling in 8% TSP at 0° C for 60 minutes, or dipping in 8% TSP at 23° C for 15 seconds. All application methods were reported to be similarly effective in reducing *Salmonella typhimurium* on inoculated chicken breast skin pieces (Tamblyn et al., 1997).

Broiler carcasses inoculated with *Salmonella typhimurium* were either chilled in 10% TSP at 4 C for 45 minutes or sprayed with 10% TSP at 25 C for 15 seconds. Immersion chilling in 10% TSP significantly decreased aerobic, *Salmonella typhimurium*, *Escherichia coli*, and total coliform counts. The spray treatment significantly reduced *Salmonella* counts. However, there

were no significant reductions of aerobic, *Escherichia coli*, or total coliform counts (Fabrizio et al., 2002).

Variation by Multiple Treatment Factors

Other studies have examined multiple TSP application factors. Sliced beef tissue inoculated with *Salmonella typhimurium*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 was dipped in 8%, 10%, or 12% TSP for 15 seconds, 1, or 3 minutes at 25, 40, or 55 C on either lean or adipose tissue. TSP concentration was reported not to significantly effect reductions in *Salmonella*, *Listeria*, or *E. coli* counts. Application temperature did not affect *Salmonella* counts on lean tissue, but on adipose tissue as temperature increased reductions increased. *Salmonella* count reductions also increased as contact time increased. Application temperature was better able to affect *Listeria* counts on lean tissue where reductions increased at temperature increased. Increased exposure time also increased *Listeria* count reductions. *E. coli* counts were not significantly affected by TSP application temperature or contact time.

However, counts were significantly reduced in comparison to controls (Dickson et al., 1994).

In a study reported by Bautista et al. (1997) turkey carcasses inoculated with *Salmonella* were treated with 2.93%, 10%, 17.07%, or 20% TSP at pressures ranging from 276 to 621 kPa. It was reported that TSP was not significantly effective against total aerobe or coliform counts, or *Salmonella* prevalence regardless of application concentration or pressure in comparison to water sprayed controls.

Li et al. (1997) treated *Salmonella typhimurium* inoculated carcasses with 5% or 10% TSP at 207, 345, or 827 kPa for 30 or 90 seconds. The 10% TSP treatment lowered *Salmonella*

counts significantly more than the 5% concentration. At the 30 second exposure time the 827 kPa pressure lowered *Salmonella* significantly more than the 207 and 345 kPa treatments. When exposed for 90 seconds, pressure did not affect *Salmonella* recovery.

In a study reported by Wang et al. (1997), *Salmonella* inoculated chicken skin was treated with 10% TSP at 10, 35, or 60 C and at 206.8, 413.7, 620.5, 827.4 or 1034.2 kPa for 30 seconds. As treatment pressure and temperature increased, *Salmonella* counts decreased.

Temperature, pressure, treatment time, and exposure time were evaluated when applying TSP to *Salmonella typhimurium* inoculated chicken skin samples (Xiong et al., 1998a). Samples were treated with 10% TSP at 25, 40, 55, or 70 C, at 207, 414, 621, 828, or 1034 kPa, for 30, 60, 90, 120, or 180 seconds. After treatment TSP remained on the samples for 30, 60, 90, 120, or 180 seconds. The 40 and 55 C spray temperatures had a significantly larger reduction than the 25 and 70 C treatments. Application pressure did not significantly affect *Salmonella* count reductions. The 90 and 120 second treatment times reduced *Salmonella* counts significantly more than the 30, 60, and 120 second treatment times. Exposure time had little effect on *Salmonella* count reductions. All treatments except exposure time at 180 seconds significantly reduced *Salmonella* counts in comparison to both water sprayed and control samples.

Sampling Method Variation

Sampling methods appear to affect the efficacy of TSP. Li et al. (1994) immersion treated *Salmonella* inoculated chicken skins with 1% TSP for 10 minutes at 23 C and were able to reduce recovery using direct plating (0.75 log reduction) and scanning electron microscopy (0.375 log reduction) recovery methods.

When post-chill broiler carcasses were dipped in 10% TSP for 15 seconds at 50° C no reduction in *Campylobacter* counts was reported when sampled by nitrocellulose membrane lifting or culture methods immediately after treatment (Slavik et al., 1994).

Beef surfaces were treated with 10% TSP for 15 seconds at 10 C, and *Salmonella typhimurium* was not reduced on either fat or fascia tissue when using plating methods. However, when scanning electron microscopy was used, the TSP-treatment did significantly reduce *Salmonella*. *E. coli* was reduced on both fat and fascia tissue when using plating and scanning electron microscopy methods (Kim and Slavik, 1994).

In a study reported by Kim et al. (1994b), post-chill broiler carcasses were dipped in 10% TSP for 15 seconds at 50° C and either rinsed off with cold tap water immediately after treatment or following storage for 1 day. When rinsed immediately, *Salmonella* recovery was lowered from 3/25 (control) to 0/25 (TSP) positive carcasses. Without rinsing *Salmonella* recovery was lowered from 6/25 (control) to 2/25 (TSP) positive carcasses. In a second trial in the same Kim et al. (1994b) study, carcasses were inoculated and then treated as before. In this trial all carcasses, both control and treated, were positive for *Salmonella* in both treatment groups using whole carcass rinse culture methods. However, when using a nitrocellulose membrane lifting method there was 44% fewer *Salmonella* positive carcasses compared to controls when sampled immediately after treatment and 24% fewer positive carcasses when sampled after 1 day. There were 12% more positives for the TSP-treated carcasses that were not rinsed off than the ones immediately sampled.

Lillard (1994) dipped carcasses previously inoculated with 10^8 cfu *Salmonella* into a 10% TSP solution for 15 minutes at 10° C and sampled by whole carcass rinses and skin

homogenates. Before sampling, carcasses were either rinsed off with 2 L of water or the TSP was not rinsed off and either buffered or non-buffered peptone was used for sampling. Using whole carcass rinses and skin sampling, TSP-treatment significantly reduced *Salmonella* in comparison to controls both with and without rinsing off TSP as well as with buffered or non-buffered peptone. However, *Salmonella* recovery was higher for TSP-treated carcasses that were rinsed versus not rinsed and for TSP-treated samples enriched in buffered versus non-buffered peptone.

In a study reported by Ellerbroek et al. (1997), both neck skin sampling and whole carcass rinse methods were used. Salmonellae, total aerobic mesophilic counts, *E. coli*, Enterobacteriaceae, and *Pseudomonas* counts were reported to be significantly lower for both neck skin sampling and whole carcass rinse methods in comparison to controls when treated with TSP for 15 seconds. However, *Listeria* and *Campylobacter* counts were reduced only by the whole carcass rinse sampling method.

When *Salmonella enteritidis* biofilms were treated with 10% TSP for 15 seconds 71% of the biofilm at the surface and 25% at the base was removed on 48 hour biofilms. On a 72 hour biofilm 73% was removed from the surface, 27% was removed at a 5 µm depth, and 9% was removed at the base. This study indicated that biofilm age is an important factor in the efficacy of TSP on surfaces (Korber et al., 1997).

Salmonella typhimurium inoculated pre-chill broiler carcasses were spray treated with 10% TSP at 413 kPa for 17 seconds 35° C and after 1 minute of resident time TSP was rinsed off the carcasses by spraying with tap water at 551 kPa for 17 seconds in an inside outside bird

washer. Carcasses were sampled by a whole carcass rinse procedure and *Salmonella* counts were measured by a Most Probable Number method. Both *Salmonella* and total aerobic counts were significantly reduced by the TSP-treatment in comparison to controls (Yang et al., 1998).

Broiler and turkey carcasses were treated with TSP for 15 seconds in commercial trials and sampled for *Salmonella*, Enterobacteriaceae, coliforms, Pseudomonads, and total aerobic counts (Coppen et al., 1998). When sampled by whole carcass rinse significant count reductions were reported for each microorganism sampled. Samples were later analyzed by most probable number procedure (MPN) and control carcasses were reported to have significantly higher numbers of *Salmonella* than TSP-treated carcasses.

In a study reported by Whyte et al. (2001), neck skin samples were dipped in 10% TSP for 15 seconds at 20° C and *Salmonella*, *Campylobacter*, *E. coli*, Enterobacteriaceae, and total viable counts were found to be significantly reduced in comparison to controls.

Capita et al. (2002c) reported that TSP lowered counts of *Listeria monocytogenes* on breast skin significantly more on leg skin than on dorsal skin samples. The application of 6% TSP on catfish skin for 10 minutes at 21° C effectively reduced both *Salmonella typhimurium*, *Listeria monocytogenes*, and *Edwardsiella tarda* (Kim and Marshall, 2002).

Effects of Trisodium Phosphate on Storage of Treated Products

Mixed results have been reported on the antimicrobial effects of TSP on poultry carcasses after storage. When carcasses were dipped in TSP for 15s, salmonellae recovery was significantly decreased both one day and 6 days after treatment following refrigeration at 4° C (Kim et al., 1994a). The 6 day storage group showed a decrease in salmonellae recovery

compared to the 1 day storage group indicating residual TSP action. TSP was also reported to be effective after 8 days of refrigerated storage by Colin and Salvat (1996).

When immersion chilled or spray-treated with TSP, *Salmonella* was significantly reduced on the day of treatment, but after 7 days of refrigeration at 4° C there was a numerical but non-significant decrease in *Salmonella* when compared to control carcasses thus indicating no residual TSP action against *Salmonella* (Fabrizio et al., 2002).

Listeria has been reported to be reduced after storage time when previously treated with TSP. In a study reported by Colin and Salvat (1996), *Listeria* was not reduced on the day of treatment but was significantly decreased after 7 days of storage at 2° C. When chicken wings were stored overnight at 4 or 10° C there was a larger increase in growth in the 10° C stored group with TSP-treated carcasses than control carcasses for *Listeria* (Rodriguez de Ledesma et al., 1996).

However, *Listeria monocytogenes* was reported to be significantly reduced by TSP-treatments on the day of treatment and after 1, 3, and 5 days of storage at 2° C (Capita et al., 2001). *Listeria monocytogenes* was also reported to be reduced on the day of treatment and after 1, 3, and 5 days of storage at 2° C in a later study by Capita et al. (2002c).

TSP has also been reported to reduce *Campylobacter* on poultry carcasses after both 1 and 6 days of refrigeration at 4° C (Slavik et al., 1994). Also, after 1 day of storage TSP was able to reduce *Campylobacter* incidence when there was no reduction on the day of treatment (Slavik et al., 1994).

Other microorganisms such as aerobic bacteria, *E. coli*, Enterobacteriaceae, coliforms,

Pseudomonas, and yeasts have been reported to be reduced with storage after TSP-treatment.

Ellerbroek et al. (1996) reported total viable counts, Enterobacteriaceae, and *Pseudomonas* were significantly reduced on the day of treatment, after 3, and after 6 days of refrigeration at 4° C in comparison to controls when sprayed for 10 or 20 seconds or dipped for 6 seconds in TSP.

Results were found by Colin and Salvat (1996) that total mesophilic aerobic counts, *E. coli*, Enterobacteriaceae, and *Pseudomonas* counts were significantly reduced by TSP-treatment after 1, 6, 7, 8, and 11 days of refrigerated storage at 2° C. These researchers also found significant reductions in total aerobic mesophilic counts, Pseudomonads, and total aerobic psychrotrophic counts in a second trial after 1, 7, 10, and 15 days of storage.

Kim and Marshall (1999) measured aerobic plate counts on TSP-treated chicken legs and found that shelf-life could be extended up to 16 days when a 7.5 to 10% TSP concentration was used. When sampling TSP-treated chicken skin, mesophilic and psychrotrophic bacterial counts were decreased in comparison to controls on the day of treatment and after 1, 3, or 5 days of storage at 2° C (Capita et al., 2000a).

In a study reported by Ismail et al. (2001), aerobic microorganisms on chicken wings were significantly lowered in comparison to controls on the day of TSP-treatment and after storage for 3, 6, and 9 days at 5° C. Aerobic plate counts, *E. coli*, and coliform counts were significantly reduced by immersion chilling in TSP for 45 minutes but not with spray-treatment in comparison to control carcasses both on the day of treatment and after 7 days of refrigeration (Fabrizio et al., 2002). It was speculated that spray-treatment was not as effective as the immersion chilling treatment due to short treatment time for spraying.

Microorganisms such as lactobacillus and yeast were not affected after storage by the

TSP-treatment. *Lactobacillus* counts were not significantly decreased with a 20 second TSP spray after 3 days of storage (Ellerbroek et al., 1996). When sampling for *Yarrowia lipolytica*, TSP reduced populations on the day of treatment but not after any length of storage (Ismail et al., 2001).

TSP has been reported to be effective after storage time on beef. In a study reported by Dorsa et al. (1997), TSP-treatment resulted in significantly lowered mesophilic aerobic populations, *E. coli*, *Listeria innocua*, *Clostridium sporogenes*, *Pseudomonas*, and lactic acid bacteria on beef carcass tissue during 21 days of vacuum-packaged storage.

When beef carcass tissue was inoculated, treated, ground after 24 hours, and then stored at 4° C for 21 days, TSP-treatment effectively reduced *E. coli* O157:H7, *Listeria innocua*, *Salmonella typhimurium*, and *Clostridium sporogenes* (Dorsa et al., 1998a). Similarly, *Salmonella typhimurium* and *E. coli* were significantly reduced by TSP-treatment on beef surfaces on the day of treatment, after 2 days of storage at 4° C, and after 7, 21, and 35 days of vacuum-packaged storage at 4° C (Cutter and Rivera-Betancourt, 2000).

In a study reported by Pohlman et al. (2002), TSP-treatment was effective in reduction of *Salmonella typhimurium*, *Escherichia coli*, coliforms, and aerobic plate counts on beef tissue when sampled on the day of treatment, and after 1, 2, 3, and 7 days of refrigerated storage.

Organoleptic Properties Associated with Trisodium Phosphate Use

The effect of TSP treatment on organoleptic properties of food has also been reported. Hollender et al. (1993) reported an increase in purchase preference for TSP-treated chicken in comparison to controls after 8 days of refrigerated storage.

Hathcox et al. (1995) reported that there was no affect on consumer acceptance of raw and fried chicken pieces when a 12% TSP treatment was used. When sensory analysis was used to measure color there were no differences found.

Rodriguez de Ledesma et al. (1996) reported TSP-treated chicken wings being preferable to water dipped wings. It was speculated that this was due to an increase in water holding capacity therefor improving appearance (Capita et al., 2002b).

Ellerbroek, et al. (1997) also reported that there was no detrimental affect to organoleptic quality after TSP treatment. For frozen chicken breasts, TSP-treatment was reported to significantly reduce drip and cooking losses as well as decreasing crystal formation and shrinkage of myofibrils (Yoon, 2002).

Some reports in the literature demonstrate a negative affect on organoleptic properties. Using a sensory analysis panel, Kim and Marshall (1999) found that chicken legs treated with 10% TSP had a less favorable odor on 4 of 5 storage days. They also noted that appearance scores were not different than those of the controls after 0 and 4 days of storage, but were significantly different at 8, 12, and 16 days of storage.

In a study reported by Capita et al. (2000b), color, smell and overall acceptability were scored for raw chicken thighs on the day of treatment and after 7 days of refrigeration and color, smell, overall acceptability, texture, and flavor were scored for cooked chicken thighs. On the day of treatment, no differences were found in color, smell, or overall appearance for 8, 10, or 12% TSP concentrations. However, after refrigeration for 7 days, there was a decrease off odors measured by smell scores for the 12% TSP treatment. In addition, cooked chicken thighs had quality decreases in color, overall acceptability, and flavor for the 12% TSP-treatment group.

TSP treatment did not decrease quality and organoleptic properties in other foods. When fresh ground beef was extended with non-meat materials phosphates can increase redness therefore increasing color quality (Molins, 1991).

It was reported by Zhuang and Beuchat (1996) that color and brightness acceptance of ripened tomatoes was not decreased when treated with 5% TSP. Morris et al. (1997) reported no significant difference in the color, texture, and odor of pork when treated with 12% TSP. Pohlman et al. (2002) also reported an improved color extension and stability without negative odor scoring through 7 days of simulated display after TSP-treatment on beef trimmings made into ground beef.

The usefulness of TSP as an egg-washing agent was reported by Kim and Slavik (1996). They found that TSP would not be a practical egg-washing antimicrobial due to cuticle layer removal during treatment.

Trisodium Phosphate in Other Meats, Fruits, and Vegetables

Meat Products

The use of trisodium phosphate as an antimicrobial has been studied in other meat products. In a study reported by Fratamico et al. (1996), a 10% TSP treatment was able to reduce *E. coli* counts on beef adipose tissue but not on beef tenderloin. In a study reported by Dorsa et al. (1997), when beef tissue was treated with 12% TSP for 15 seconds at 32° C, *E. coli*, *Listeria*, *Clostridium sporogenes*, *Pseudomonas*, and lactic acid bacteria counts were all significantly reduced.

In a study reported by Dorsa et al. (1998a), treatment of beef tissue with 12% TSP for 15

seconds at 32° C did not significantly reduce *Salmonella typhimurium*, *E. coli* O157:H7, *Listeria innocua*, or *Clostridium sporogenes* counts when the tissue was inoculated at a low level (10^2). However, when a high inoculation level (10^8) was used, the TSP-treatment did significantly reduce *Salmonella typhimurium*, *E. coli* O157:H7, *Listeria innocua*, and *Clostridium sporogenes* counts. In this same study, TSP-treatment did not lead to a significant reduction of aerobic mesophils, lactic acid bacteria, or *Pseudomonas* when either low or high inoculation levels were used.

After treatment of beef carcass surfaces with 12% TSP for 15 seconds at 32° C *Salmonella typhimurium*, *E. coli* O157:H7, *Listeria innocua*, *Clostridium sporogenes*, aerobic bacteria, lactic acid bacteria, and *Pseudomonas* counts were significantly reduced in comparison to controls (Dorsa et al., 1998b). Cutter and Rivera-Betancourt (2000) also reported that the application of 10% TSP on beef surfaces for 15 seconds at 35° C significantly reduced *Salmonella typhimurium* and *E. coli* in comparison to control treatments.

Inoculated lamb breasts were treated with a 60 second, 12% TSP dip at 55° C leading to reductions in *E. coli* and aerobic plate counts (Ramirez et al., 2001). *Salmonella typhimurium*, *E. coli*, coliforms, and aerobic plate counts from ground beef were reported to be reduced by a 3 minute 10% TSP-treatment on pre-ground beef trimmings (Pohlman et al., 2002).

Fruits and Vegetables

TSP has also been used to treat fruits and vegetables. On lettuce and cabbage a 1% TSP-treatment was unable to reduce *Listeria* and concentration could not feasibly be increased due to decreases in organoleptic quality at higher concentrations (Zhang and Farber, 1996). Inoculated

alfalfa seeds treated with 5% TSP for 10 minutes had reductions in numbers of *Salmonella*. However, treatment with TSP significantly reduced germination (Weissinger and Beuchat, 2000). Inoculated apple disks were treated with 2% TSP for 5 minutes resulting in a reduction of *Salmonella chester* (Liao and Sapers, 2000). When strawberries were dipped in a 1% TSP solution for 2 minutes at 43° C *Salmonella montevideo*, *E. coli* O157:H7, poliovirus 1, and bacteriophages PRD1, X174, and MS2 were significantly reduced (Lukasik et al., 2003).

REFERENCES

- Arritt, F. M., J. D. Eifert, M. D. Pierson, and S. S. Sumner, 2002. Efficacy of antimicrobials against *Campylobacter jejuni* on chicken breast skin. J. Appl. Poult. Res. 11:358-366.
- Bautista, D. A., N. Sylvester, S. Barbut, and M. W. Griffiths, 1997. The determination of efficacy of antimicrobial rinses on turkey carcasses using response surface designs. Int. J. Food Microbiol. 34:279-292.
- Bender, F. G. and E. Brotsky, 1992. Process for treating poultry carcasses to control salmonellae growth. U.S. Patent 5,143,739.
- Brown, R., 1994. Rhone-Poulenc wins approval for TSP on pre-chill poultry. Feedstuffs. March 7.
- Canadian Food Inspection Agency. Subject: Microbial control agents reviewed by Health Canada (HC) and listed for use on raw poultry carcasses or parts.
<http://www.inspection.gc.ca/english/ anima/meavia/mmopmmhv/chap4/4.5e.shtml#4.5>. Accessed. March 2004.
- Capita, R., C. Alonso-Calleja, M. T. Garcia Arias, B. Moreno, and M. C. Garcia-Fernandez, 2000a. Note. Effect of trisodium phosphate on mesophilic and psychrotrophic bacterial flora attached to the skin of chicken carcasses during refrigerated storage. Food Sci. Tech. Int. 6:345-350.
- Capita, R., C. Alonso-Calleja, M. Sierra, B. Moreno, M. C. Garcia-Fernandez, 2000b. Effect of trisodium phosphate solutions washing on the sensory evaluation of poultry meat. Meat Sci. 55:471-474.
- Capita, R., C. Alonso-Calleja, C. Garcia-Fernandez, and B. Moreno, 2001. Efficacy of trisodium

- phosphate solutions in reducing *Listeria monocytogenes* populations on chicken skin during refrigerated storage. J. Food Prot. 64:1627-1630.
- Capita, R., C. Alonso-Calleja, M. C. Garcia-Fernandez, and B. Moreno, 2002a. Activity of trisodium phosphate compared with sodium hydroxide wash solutions against *Listeria monocytogenes* attached to chicken skin during refrigerated storage. Food Microbiol. 19:57-63.
- Capita, R., C. Alonso-Calleja, M. C. Garcia-Fernandez, and B. Moreno, 2002b. Review: Trisodium phosphate (TSP) treatment for decontamination of poultry. Food Sci. Tech. Int. 8:11-24.
- Capita, R., C. Alonso-Calleja, R. Rodriguez-Perez, B. Moreno, and M. C. Garcia-Fernandez, 2002c. Research Note: Influence of poultry carcass skin sample site on the effectiveness of trisodium phosphate against *Listeria monocytogenes*. J. Food Prot. 65:853-856.
- Capita, R., C. Alonso-Calleja, M. Prieto, M. C. Garcia-Fernandez, and B. Moreno, 2003. Effectiveness of trisodium phosphate against *Listeria monocytogenes* on excised and nonexcised chicken skin. J. Food Prot. 66:61-64.
- Catalano, C. R., and S. J. Knabel, 1994. Destruction of *Salmonella* in Pennsylvania egg processing plants and destruction by high pH. J. Food Prot.. 57:587-595.
- Colin, P. and G. Salvat, 1996. Decontamination of poultry carcasses using trisodium phosphate treatment. In: *Factors affecting the microbial quality of meat*. (eds. Hinton, M. H. and C. Rowlings), pp. 227-237. Bristol: University of Bristol Press.
- Coppen, P., S. Fenner, and G. Salvat, 1998. Antimicrobial efficacy of AvGard® carcase wash under industrial processing conditions. Br. Poult. Sci. 39:229-234.

- Cotterill, O. J., 1968. Equivalent pasteurization temperatures to kill *Salmonella* in liquid egg white at various pH levels. *Poult. Sci.* 47:354-365.
- Cutter, C. N. and M. Rivera-Betancourt, 2000. Interventions for the reduction of *Salmonella* typhimurium DT104 and non-O157:H7 enterohemorrhagic *Escherichia coli* on beef surfaces. *J. Food Prot.* 63:1326-1332.
- Dickson, J. S., 1988. Reduction of bacteria attached to meat surfaces by washing with selected compounds. *J. Food Prot.* 51:869-873.
- Dickson, J. S., C. G. Nettles Cutter, and G. R. Siragusa, 1994. Antimicrobial effects of trisodium phosphate against bacteria attached to beef tissue. *J. Food Prot.* 57:952-955.
- Dorsa, W. J., C. N. Cutter, and G. R. Siragusa, 1997. Effects of acetic acid, lactic acid and trisodium phosphate on the microflora of refrigerated beef carcass surface tissue inoculated with *Escherichia coli* O157:H7, *Listeria innocua*, and *Clostridium sporogenes*. *J. Food Prot.* 60:619-624.
- Dorsa, W. J., C. N. Cutter, and G. R. Siragusa, 1998a. Bacterial profile of ground beef made from carcass tissue experimentally contaminated with pathogenic and spoilage bacteria before being washed with hot water, alkaline solution, or organic acid and then stored at 4 or 12 C. *J. Food Prot.* 61:1109-1118.
- Dorsa, W. J., C. N. Cutter, and G. R. Siragusa, 1998b. Long-term bacterial profile of refrigerated ground beef made from carcass tissue, experimentally contaminated with pathogens and spoilage bacteria after hot water, alkaline, or organic acid washes. *J. Food Prot.* 61:1615-1622.

- Ellerbroek, L., E. M. Okolocha, and E. Weise, 1996. Lactic acid and trisodium phosphate for decontamination of poultry meat. In: *Factors affecting the microbial quality of meat*. (eds. M. H. Hinton and C. Rowlings) pp. 187-195. Bristol: University of Bristol Press.
- Ellerbroek, L., E. M. Okolocha, and E. Weise, 1997. Decontamination of poultry meat with trisodium phosphate and lactic acid. *Fleisch*. 77:1092.
- Fabrizio, K. A., R. R. Sharma, A. Demirci, and C. N. Cutter, 2002. Comparison of electrolyzed oxidizing water with various antimicrobial interventions to reduce *Salmonella* species on poultry. *Poult. Sci.* 81:1598-1605.
- Food Safety and Inspection Service. Subject: Progress report on *Salmonella* testing of raw meat and poultry products 1998-2002. <http://www.fsis.usda.gov/OPHS/haccp/salm5year.pdf>. Accessed March 2004.
- Fratamico, P. M., F. J. Schultz, R. C. Benedict, R. L. Buchanan, and P. H. Cooke, 1996. Factors influencing attachment of *Escherichia coli* O157:H7 to beef tissues and removal using selected sanitizing rinses. *J. Food Prot.* 59:453-459.
- Giese, J., 1992. Experimental process reduces salmonella on poultry. *Food Technol.* 46:112.
- Hathcox, A. K., C. A. Hwang, A. V. A. Resurreccion, and L. R. Beuchat, 1995. Consumer evaluation of raw and fried chicken after washing in trisodium phosphate or lactic acid-sodium benzoate solutions. *J. Food Sci.* 60:604-605, 610.
- Hollender, R., F. G. Bender, R. K. Jenkins, and C. L. Blak, 1993. Research note: Consumer evaluation of chicken treated with a trisodium phosphate application during processing. *Poul. Sci.* 72:755.
- Humphrey, T. J., D. G. Lanning, and D. Beresford, 1981. The effect of pH adjustment on the

- microbiology of chicken scald-tank water with particular reference to the death rate of *Salmonellas*. J. Appl. Bacteriol. 51:517-527.
- Hwang, C. A. and L. R. Beuchat, 1995. Efficacy of selected chemicals for killing pathogenic and spoilage microorganisms on chicken skin. J. Food Prot. 58:19-23.
- Ismail, S. A. S., T. Deak, H. A. Abd El-Rahman, M. A. M. Yassien, L. R. Beuchat, 2001. Effectiveness of immersion treatments with acids, trisodium phosphate, and herb decoctions in reducing populations of *Yarrowia lipolytica* and naturally occurring aerobic microorganisms on raw chicken. Int. J. Food Microiol. 64:13-19.
- Kim, C. R. and D. L. Marshall, 1999. Microbiological, colour and sensory changes of refrigerated chicken legs treated with selected phosphates. Food Res. Int. 32:209-215.
- Kim, J. and D. L. Marshall, 2002. Influence of catfish skin mucus on trisodium phosphate inactivation of attached *Salmonella* Typhimurium, *Edwardsiella tarda*, and *Listeria monocytogenes*. J. Food Prot. 65:1146-1151.
- Kim, J. and M. F. Slavik, 1994. Trisodium phosphate (TSP) treatment of beef surfaces to reduce *Escherichia coli* O157:H7 and *Salmonella typhimurium*. J. Food Sci. 59:20-22.
- Kim, J. W. and M. F. Slavik, 1996. Changes in eggshell surface microstructure after washing with cetylpyridinium chloride or trisodium phosphate. J. Food Prot. 59:859-863.
- Kim, J. W., M. F. Slavik, and F. G. Bender, 1994a. Removal of *Salmonella typhimurium* attached to chicken skin by rinsing with trisodium phosphate solution: scanning electron microscopic examination. J. Food Safety 14:77-84.
- Kim, J. W., M. F. Slavik, M. D. Pharr, D. P. Raben, C. M. Lobsinger, and S. Tsai, 1994b. Reduction of *Salmonella* on post-chill chicken carcasses by trisodium phosphate

- (Na_3PO_4) treatment. J. Food Safety 14:9-17.
- Kinner, J. A. and W. A. Moats, 1981. Effect os temperature, pH and detergent on survival of bacteria associated with shell eggs. Poult. Sci. 60:761-767.
- Korber, D. R., A. Choi, G. M. Wolfaardt, S. C. Ingham, and D. E. Caldwell, 1997. Substratum topography influences susceptibility of *Salmonella enteritidis* biofilms to trisodium phosphate. 63:3352-3358.
- Lee, R. M., P. A. Hartman, D. G. Olsen, and F. D. Williams, 1994. Metal ions reverse the inhibitory effects of selected food-grade phosphates in *Staphylococcus aureus*. J. Food Prot. 57:284-288.
- Li, Y., J. W. Kim, M. F. Slavik, C. L. Griffis, J. T. Walker, and H. Wang, 1994. *Salmonella typhimurium* attached to chicken skin reduced using electrical stimulation and inorganic salts. J. Food Sci. 59:23-25.
- Li, Y., J. T. Walker, M. F. Slavik, and H. Wang, 1995. Electrical treatment of poultry chiller water to destroy *Campylobacter jejuni*. J. Food Prot. 58:1330-1334.
- Li, Y., M. F. Slavik, J. T. Walker, and H. Xiong, 1997. Pre-chill spray of chicken carcasses to reduce *Salmonella typhimurium*. J. Food Sci. 62:605-607.
- Liao, C., and G. M. Sapers, 2000. Attachment and growth of *Salmonella* chester on apple fruits and in vivo response of attached bacteria to sanitizer treatments. J. Food Prot. 63:876-883.
- Lillard, H. S., 1994. Effect of trisodium phosphate on salmonellae attached to chicken skin. J. Food Prot. 57:465-469.
- Lukasik, J., M. L. Bradley, T. M. Scott, M. Dea, A. Koo, W. Y. Hsu, J. A. Bartz, and S. R.

- Farrah, 2003. Reduction of poliovirus 1, bacteriophages, *Salmonella montevideo*, and *Escherichia coli* O157:H7 on strawberries by physical and disinfectant washes. J. Food Prot. 66:188-193.
- Mendonca, A. F., T. L. Amoroso, and S. J. Knabel, 1994. Destruction of gram-negative food-borne pathogens by high pH involves disruption of the cytoplasmic membrane. Appl. Environ. Microbiol. 60:4009-4014.
- Molins, R. A., 1991. *Phosphates in Food*. Boca Raton, FL: CRC Press.
- Morris, C. A., L. M. Lucia, J. W. Savell, and G. R. Acuff, 1997. Trisodium phosphate treatment of pork carcasses. J. Food Sci. 62:402-403.
- Mu, D., Y. W. Huang, K. W. Gates, and W. H. Wu, 1997. Effect of trisodium phosphate on *Listeria monocytogenes* attached to rainbow trout (*Oncorhynchus mykiss*) and shrimp (*Penaeus* spp.) during refrigerated storage. J. Food Saf. 17:37-46.
- Murray, R. G., E. P. Steed, and H. E. Elson, 1965. The location of the mucopeptide in sections of the cell wall of *Escherichia coli* and other gram-negative bacteria. Can. J. Microbiol. 11:547-560.
- Niebuhr, S. E. and J. S. Dickson, 2003. Impact of pH enhancement on populations of *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in boneless lean beef trimmings. J. Food Prot. 66:874-877.
- Pohlman, F. W., M. R. Stivarum, K. S. McElyea, A. L. Waldroup, 2002. Reduction of *E. coli*, *Salmonella typhimurium*, coliforms, aerobic bacteria, and improvement of ground beef color using trisodium phosphate or cetylpyridinium chloride before grinding. Meat Sci. 60:349-356.

- Ramirez, A. J., G. R. Acuff, L. M. Lucia, and J. W. Savell, 2001. Lactic acid and trisodium phosphate treatment of lamb breast to reduce bacterial contamination. *J. Food Prot.* 64:1439-1441.
- Rodriguez de Ledesma, A. M., H. P. Riemann, and T. B. Farver, 1996. Short-time treatment with alkali and/or hot water to remove common pathogenic and spoilage bacteria from chicken wing skin. *J. Food Prot.* 59:746-750.
- Sampathkumar, B., G. G. Khachatourians, and D. R. Korber, 2003. High pH during trisodium phosphate treatment causes membrane damage and destruction of *Salmonella enterica* serovar enteritidis. *Appl. Environ. Microbiol.* 69:122-129.
- Slavik, M. F., J. W. Kim, M. D. Pharr, D. P. Raben, S. Tsai, and C. M. Lobsinger, 1994. Effect of trisodium phosphate on *Campylobacter* attached to post-chill chicken carcasses. *J. Food Prot.* 57:324-326.
- Tamblyn, K. C., D. E. Conner, and S. F. Bilgili, 1997. Utilization of the skin attachment model to determine the antibacterial efficacy of potential carcass treatments. *Poult. Sci.* 76:1318-1323.
- Teo, Y. L., T. J. Raynor, K. R. Ellajosyula, and S. J. Knabel, 1996. Synergistic effect of high temperature and high pH on the destruction of *Salmonella enteritidis* and *Escherichia coli* O157:H7. *J. Food Prot.* 59:1023-1030.
- Wang, W., Y. Li, M. F. Slavik, and H. Xiong, 1997. Trisodium phosphate and cetylpyridinium chloride spraying on chicken skin to reduce attached *Salmonella typhimurium*. *J. Food Prot.* 60:992-994.
- Weissinger, W. R., and L. R. Beuchat, 2000. Comparison of aqueous chemical treatments to

- eliminate *Salmonella* on alfalfa seeds. J. Food Prot. 63:1475-1482.
- Whyte, P., J. D. Collins, K. McGill, C. Monahan, and H. O'Mahony, 2001. Quantitative investigation of the effects of chemical decontamination procedures on the microbiological status of broiler carcasses during processing. J. Food Prot. 64:179-183.
- Xiong, H., Y. Li, M. Slavik, and J. Walker, 1998a. Chemical spray conditions for reducing bacteria on chicken skins. J. Food Sci. 63:699-701.
- Xiong, H., Y. Li, M. F. Slavik, and J. T. Walker, 1998b. Spraying chicken skin with selected chemicals to reduce attached *Salmonella typhimurium*. J. Food Prot. 61:272-275.
- Yang, Z., Y. Li, and M. Slavik, 1998. Use of antimicrobial spray applied with an inside-outside birdwasher to reduce bacterial contamination on prechilled chicken carcasses. J. Food Prot. 61:829-832.
- Yoon, K. S., 2002. Texture and microstructure properties of frozen chicken breasts pretreated with salt and phosphate solutions. Poult. Sci. 81:1910-1915.
- Zhang, S. and J. M. Farber, 1996. The effects of various disinfectants against *Listeria monocytogenes* on fresh-cut vegetables. Food Microbiol. 13:311-321.
- Zhuang, R. Y. and L. R. Beuchat, 1996. Effectiveness of trisodium phosphate for killing *Salmonella montevideo* on tomatoes. Lett. Appl. Microbiol. 22:97-100.

CHAPTER 3

**RECOVERY OF SALMONELLAE AFTER CHILLING AND AFTER SEVEN DAY
STORAGE FROM TSP-TREATED COMMERCIALY PROCESSED BROILER
CARCASSES¹**

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ABSTRACT Experiments were conducted to determine the effect of pre-chill trisodium phosphate (TSP) treatment on reducing salmonellae recovery from broiler carcasses immediately after chilling or following 7 d of storage. Carcasses were sampled for salmonellae using whole carcass enrichment for 24 h at 37 C. In each of seven trials, 40 carcasses were obtained pre-chill from a commercial processing plant. Batches of four carcasses were either subjected to a 5 s dip in 10% TSP (Treatment) or not dipped (Control). Two carcasses from each batch were sampled immediately after chilling (Day 0) and two after 7 d. For trials 1 and 2, TSP treatment and control groups were chilled in separate chill tanks for 45 min. For trials 3 through 7, carcasses were rinsed with water and individually bagged with ice and water prior to chilling. Half the carcasses in each treatment group were sampled immediately after chilling (Day 0) and half after 7 d. For trials 1 and 2, 85% (17/20) of control carcasses were salmonellae positive on Day 0 compared to 45% (9/20) of the TSP-treated carcasses, and after 7 d, 75% (15/20) of control carcasses were positive compared to 35% (7/20) for the TSP-treated carcasses. For trials 3 through 7, 46% (23/50) of control carcasses were salmonellae positive on Day 0 compared to 26% (13/50) of the TSP-treated carcasses, and after 7 d, 20% (10/50) of control carcasses were positive compared to 4% (2/50) of the TSP-treated carcasses. TSP treatment also resulted in significantly higher pH values for rinses. Salmonella recovery was decreased by refrigerated storage and treatment with TSP prior to immersion chilling.

Key Words: salmonellae, TSP, broilers, whole carcass enrichment, chilling

INTRODUCTION

Trisodium phosphate (TSP) has been shown to reduce the recovery of salmonellae from processed poultry (Giese, 1992; Kim et al., 1994a, 1994b; Li et al., 1994; Lillard, 1994; Somers et al., 1994). The use of TSP as a prechill carcass wash was patented by Bender and Brotsky (1991) and has been approved for the reduction of *Salmonella* in poultry processing plants by USDA (Giese, 1993). According to the Food Safety and Inspection Service (2003), 11.5% of broilers sampled from U.S. processing plants in 2002 were positive for *Salmonella*.

The decrease of salmonellae recovery from poultry carcasses immediately after treatment with TSP has been reported (Hwang and Beuchat, 1995; Colin and Salvat, 1996; Ellerbroek et al., 1996; Rodriguez de Ledesma et al., 1996). However, TSP's effectiveness on salmonellae recovery at the retail level (5-7 d post-processing) is less clear. Residual effects of TSP on *Salmonella* recovery (0.5 log reduction) after refrigerated storage for 6 d have been reported (Kim et al., 1994b). In these experiments excess TSP was not rinsed off the carcasses prior to placing the carcass in buffered peptone water for sampling. However, in a commercial application TSP would be rinsed off the carcass during immersion chilling prior to salmonellae sampling.

In most U.S. processing plants, carcasses treated with TSP drip for about one min prior to being dropped into (without rinsing) an immersion chiller of water and ice maintained under 40 C for 30 to 90 min. Residual TSP on the carcasses is washed off during immersion chilling raising the chiller water pH. Elevated pH has been shown to decrease chlorine efficiency in the chiller and chill water is often neutralized to optimize chlorine effectiveness.

The objective of the current study was to determine the efficacy of TSP both on the day of processing and after 7 d of storage at 2 C. Evaluation at 7 days was to determine whether TSP would effect salmonellae recovery on products as purchased by the consumer.

MATERIALS AND METHODS

Carcass Treatment

On each of 7 trial days, 40 broiler carcasses were obtained by removing them from the shackle line of a commercial processing plant immediately following the inside-outside bird washer before TSP treatment and chilling. Each carcass was individually placed in a clean plastic bag and transported to the pilot processing plant.

At 15 min intervals, groups of four carcasses were identified with wing bands and subjected to an inside-outside bird washer¹ at 80 psi for 10 s on shackles spaced every 30.5 cm (12 in). This second inside-outside carcass wash was done to rewet the carcass as it would have been in the commercial processing plant prior to TSP treatment. Tap water was used with no added chlorine to limit the decrease of salmonellae due to an additional chlorine wash. Alternating groups of four carcasses were either submerged for 5 s in a 10% TSP solution at 24° C or were used as non-treated controls. TSP-treated carcasses were allowed to drip for 1 min to simulate plant exposure time prior to immersion chilling. Control carcasses were not subjected to a 5 s water rinse because in a commercial situation carcasses are either treated with TSP or go directly into the chiller without an additional water dipping step.

¹Model MBW-16, Stork-Gamco Inc. Gainesville, GA.

Immersion Chilling Method

After treatment, carcasses in trials 1 and 2 were chilled in pilot scale immersion paddle chill tanks (228 L) filled with water and ice. One chill tank was used for TSP-treated carcasses and the other for control carcasses. Successive batches were added without emptying each chiller. All carcasses were chilled for 45 min with an approximate water overflow rate of 1 L/min to diminish chill water suspended solids and TSP concentration during the chill period (simulating commercial chilling). Chiller water pH was recorded at the end of trial 2. Due to higher chill water pH values in the TSP chiller, compared to the control chiller, the chilling procedure was modified.

Individual Bag Chilling Method

To reduce the possibility of salmonellae cross contamination and to better control chill water pH during chilling, an individual carcass bag chilling method was developed. Following treatment, carcasses in trials 3 through 7 were thoroughly rinsed off inside and outside with a hose four times by hand to minimize residual TSP entering chilling. Carcasses were immediately placed into clean 36 x 51 cm plastic bags. To each bag, for both control and TSP-treated carcasses, 300 mL of PBS at 20 times the normal concentration (1x PBS is 1.47 mM KH_2PO_4 , 10 mM Na_2HPO_4 , 2.7 mM KCl, 137 mM NaCl, pH = 7.4), 1.7 L of tap water, and 2 L of crushed ice were added. PBS was added to buffer and reduce the pH increase caused by TSP. Excess air was expelled and bags sealed with a cable tie. Bagged carcasses were placed into an immersion paddle chill tank filled with ice and water. Following 20 min in the chill tank, bagged carcasses were taken out of the ice water, aseptically removed from the bag, and placed

into a second bag. Two L of tap water and 2 L of ice without PBS were added and the bag sealed and chilled for an additional 20 min in a second chiller. The pH was recorded from the chill water in each bag after both the first and second chill. The pH of the chill tank water was measured before and after chilling to determine possible bag leakage.

Salmonellae Sampling

After chilling, carcasses were hung on sanitized shackles, allowed to drip for 5 min, individually placed into 41 x 41 cm polyethylene bags, and separated into two sampling groups. One group of 20 carcasses was sampled on the day of processing and the other (20 carcasses) was sampled following 7 d of storage at 2 C. Each carcass was sampled for salmonellae using whole carcass enrichment similar to the method described by Simmons et al. (2003). Carcasses were shaken for 1 min in 500 mL of buffered peptone water², and carcasses were incubated in the rinse solution 24h at 37 C. After incubation, 0.1 mL of the rinse solution was transferred to Rappaport-Vassiliadis broth² and 0.5 mL of the rinse solution was transferred to tetrathionate (Hanja)³ and incubated 24 h at 42 C. The broths were then streaked out onto modified lysine iron agar⁴ and brilliant green sulfa agar³ and incubated 24 h at 35 C. Suspect colonies were picked and triple sugar iron³ and lysine iron agar³ slants were stabbed and incubated 24h at 35 C. Poly O³ and Poly H⁵ agglutination tests were used to confirm presumptive positives.

Statistical Analysis

²Difco Laboratories, Detroit, MI.

³Becton Dickinson, Sparks, MD

⁴Oxoid, Basinstoke, Hampshire, UK

⁵Microgen, Camberly, Surrey, UK

Salmonellae incidence data were analyzed using the Chi-square test for independence. Chiller bag and tank water pH data were analyzed using the General Linear Models Procedure of SAS (SAS Institute, 1998). Sources of variation were treatment (2), first and second chilling steps (2), replication within trial (5), and trial (4), and the mean square error was the error test statistic.

RESULTS AND DISCUSSION

Trials 1 and 2

For trials 1 and 2, TSP significantly reduced the recovery of salmonellae on both the day of processing and after 7 d refrigerated storage when compared to non-treated control carcasses (Table 1.1). There were no significant differences in the prevalence of salmonellae recovered within either the control or TSP treatment groups from the day of processing to after 7 d of storage at 2 C.

The high incidence of recovery for the control group (85%) raised concerns about the potential for cross contamination in the immersion chill tanks. The high incidence, compared to commercial processing, may also be attributed to the lack of added chlorine to the chill water (typically 20 ppm). After chilling, the chill water pH in the TSP-treated chiller was 9.4 while the control chiller water was 7.0 despite an overflow rate of 1 L/min. Higher pH values may have independently influenced salmonellae recovery from the TSP-treated carcasses.

Trials 3 through 7

After modifying the chilling methods to prevent cross-contamination, with some exceptions there still was a significant decrease in salmonellae recovery from TSP-treated carcasses on both the day of processing and after 7 d of storage at 2 C in comparison to controls (Table 1.2). However, unlike trials 1 and 2, the recovery of salmonellae was significantly lower after 7 d of storage for both the TSP-treated and control carcasses, $P = 0.0004$ and $P = 0.0002$, respectively.

Similar results were reported in a study by Fabrizio et al. (2002) where *Salmonella* populations were significantly reduced on half carcasses both immediately following TSP treatment and after 7 d of storage at 4° C (reductions of 0.9 log₁₀ cfu/mL and 2.17 log₁₀ cfu/mL, respectively). Also, the residual effect of TSP was demonstrated with a minimal but significant reduction (0.19 log₁₀ cfu/mL) of *Salmonella* from sampling immediately following treatment to after 7 d of storage at 2 C.

The reduction in *Salmonella* incidence may have been due to TSP not being rinsed off or neutralized immediately following treatment. In a recent study by Simmons et al. (2003), salmonellae incidence was reported to be 33.9% for broiler carcasses obtained at the retail level. However, it was not known whether these carcasses were treated with TSP during processing. It was speculated that one possibility for this apparent increase in salmonellae recovery, when compared to the FSIS baseline (20% in the plant after chilling) may have been injured bacteria recovering during the time between processing and sale to the consumer. In the present study there was no increase, but in fact a decrease in salmonellae recovery detected after 7 d of storage at 2 C for both control and TSP-treated carcasses.

Salmonellae prevalence between trials was highly variable (Table 1.2). Control groups ranged from 1/10 to 10/10 and TSP-treated groups ranged from 0/10 to 6/10 on the day of processing. After 7 d of storage at 2 C, control groups varied from 0/10 to 9/10 positive carcasses and 0/10 to 4/10 positive carcasses for the TSP-treated groups. Trial 4 was the only trial in which there were more positive carcasses for the TSP-treated group (3/10) than the control (1/10) and in trial 6 there was only 1 positive carcass for the control and TSP groups combined. After 7 d of storage at 2 C, salmonellae were not recovered from the TSP-treated groups in three of the trials. In total for 6 of 14 days carcasses were sampled (2 sampling days per each of 7 trials), salmonellae recovery was not significantly different between TSP-treated and control carcasses. This variation in salmonellae recovery between trials is likely due to variation in flock salmonellae levels when entering the plant.

Chill Water pH

Chill water pH in trials 4 through 7 are presented in Table 1.3. The largest difference among individual chill water pH was 1.3 pH units for TSP carcasses and 1.9 for control carcasses. There were no significant treatment by trial interactions, so data were combined. Chill water pH was significantly lower in the second chill step compared to the first chill step for both control and TSP-treated carcasses. Although each carcass was thoroughly rinsed off with water four times and 20x PBS was added to each chiller bag in the first chill to help buffer pH, the pH readings for the TSP treatment group were still significantly higher than the control group for both the first and second chill (1.05 and 0.73 pH units, respectively).

Due to the higher bag chiller water pH in the TSP treatment, it is uncertain whether the reduction of salmonellae recovery was due exclusively to the TSP treatment or whether 40 min at this elevated pH may have also reduced recovery. Further experiments are necessary to differentiate the direct effects of TSP from secondary, long term pH effects.

This study determined the efficacy of TSP both on the day of processing and after 7 d of storage at 2 C. On both the day of processing and after 7 d of storage TSP decreased the salmonellae recovery in comparison to control carcasses. After 7 d of storage at 2 C salmonellae recovery decreased for both the control and TSP-treated carcasses.

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REFERENCES

- Bender, F. G., and E. Brotsky. 1991. Process for treating poultry carcasses to control salmonellae growth. U.S. Patent 5,069,922.
- Colin, P., and G. Salvat. 1996. Decontamination of poultry carcasses using trisodium phosphate treatment. Pages 227-237 in Factors Affecting the Microbial Quality of Meat. M. H. Hinton and C. Rowlings, eds., University of Bristol Press, Bristol.
- Ellerbroek, L., E. M. Okolocha, and E. Weise. 1996. Lactic acid and trisodium phosphate for decontamination of poultry meat. Pages 187-195 in Factors Affecting the Microbial Quality of Meat. M. H. Hinton and C. Rowlings, eds., University of Bristol Press, Bristol.
- Fabrizio, K. A., R. R. Sharma, A. Demirci, and C. N. Cutter. 2002. Comparison of electrolyzed oxidizing water with various antimicrobial interventions to reduce *Salmonella* species on poultry. Poult. Sci. 81:1598-1605.
- Food Safety and Inspection Service. Subject: Progress report on *Salmonella* testing of raw meat and poultry products 1998-2002. <http://www.fsis.usda.gov/OPHS/haccp/salm5year.pdf>. Accessed Aug. 2003.
- Giese, J. 1992. Experimental process reduces *Salmonella* on poultry. Food Technol. 46:112.
- Giese, J. 1993. *Salmonella* reduction process receives approval. Food Technol. 46:110.
- Hwang, C., and L. R. Beuchat. 1995. Efficacy of selected chemicals for killing pathogenic and spoilage microorganisms on chicken skin. J. Food Prot. 58:19-23.

- Kim, J. W., M. F. Slavik, and F. G. Bender. 1994a. Removal of *Salmonella typhimurium* attached to chicken skin by rinsing with trisodium phosphate solution: scanning electron microscopic examination. J. Food Safety 14:77-84.
- Kim, J. W., M. F. Slavik, M. D. Pharr, D. P. Raben, C. M. Lobsinger, and S. Tsai. 1994b. Reduction of *Salmonella* on post-chill chicken carcasses by trisodium phosphate (Na_3PO_4) treatment. J. Food Safety 14:9-17.
- Li, Y., J. W. Kim, M.F. Slavik, C. L. Griffis, J. T. Walker, and H. Wang. 1994. *Salmonella typhimurium* attached to chicken skin reduced using electrical stimulation and inorganic salts. J. Food Science 59:23-25.
- Lillard, H.S. 1994. Effect of trisodium phosphate on salmonellae attached to chicken skin. J. Food Prot. 57:465-469.
- Rodriguez de Ledesma, A. M., H. P. Riemann, and T. B. Farver. 1996. Short-time treatment with alkali and/or hot water to remove common pathogenic and spoilage bacteria from chicken wing skin. J. Food Prot. 59:746-750.
- SAS Institute. 1998. SAS User's Guide. Version 8. SAS Institute Inc., Cary, NC.
- Simmons, M., D. L. Fletcher, J. A. Cason, and M. E. Berrang. 2003. Recovery of *Salmonella* from retail broilers using a whole carcass enrichment procedure. J. Food Prot. 66:446-450.
- Somers, E. B., J. L. Schoeni, and C. L. Wong. 1994. Effect of trisodium phosphate on biofilm and planktonic cells of *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella typhimurium*. Int. J. Food Microbiol. 22:269-276.

TABLE 3.1. Recovery of salmonellae from TSP-treated and control carcasses on the day of processing and after 7 d of storage at 2 C for trials 1 and 2 (positive carcasses/number of carcasses sampled)

Trial	Day of processing			After 7 d of storage		
	Control	TSP	<i>P</i>	Control	TSP	<i>P</i>
1	9/10	3/10	0.0001	6/10	4/10	0.0308
2		8/10	6/10 0.1138	9/10	3/10	0.0001
Total	17/20	9/20	0.0001	15/20	7/20	0.0001
Percentage	85	45			75	35

TABLE 3.2. Recovery of salmonellae from TSP-treated and control carcasses on the day of processing and after 7 d of storage at 2 C for trials 3 through 7 (positive carcasses/number of carcasses sampled)

Trial	Day of processing			After 7 d of storage		
	Control	TSP	<i>P</i>	Control	TSP	<i>P</i>
3	8/10	3/10	0.0001	1/10	1/10	1.0000
4	1/10	3/10	0.0350	3/10	0/10	0.0384
5	10/10	5/10	0.0001	5/10	1/10	0.0114
6	1/10	0/10	0.2919	0/10	0/10	1.0000
7	3/10	2/10	0.4901	1/10	0/10	0.2919
Total	23/50	13/50	0.0045	10/50	2/50	0.0047
Percentage	46	26		20	4	

TABLE 3.3. Carcass bag chill water pH values for trials 4 through 7

Treatment	Trial	1 st Chiller ¹	2 nd Chiller ²
Control	4	7.94	7.43
	5	7.80	7.38
	6	8.00	7.59
	7	7.98	7.68
Average		7.93 ^{A,Z}	7.52 ^{B,Z}
TSP	4	9.07	8.16
	5	8.96	8.24
	6	9.05	8.32
	7	8.83	8.28
Average		8.98 ^{A,Y}	8.25 ^{B,Y}

^{A-B} Values within a row with no common superscripts differ significantly ($P < 0.0001$).

^{Y-Z} Values within a column with no common superscripts differ significantly ($P < 0.0001$).

¹ After first 20 min chill for individually bagged carcasses.

² After second 20 min chill for individually bagged carcasses.

CHAPTER 4
EFFECT OF ENRICHMENT pH ON SALMONELLAE RECOVERY FROM TSP-
TREATED BROILER CARCASSES¹

¹D. V. Bourassa, D. L. Fletcher, R. J. Buhr, M. E. Berrang, and J. A. Cason, To be Submitted to Poultry Science

ABSTRACT Trisodium phosphate (TSP) has been reported to reduce the recovery of salmonellae from processed poultry carcasses. It has been suggested that the high pH of TSP solutions as well as the detergent-like properties are responsible for the reduction in salmonellae recovery. This project was conducted to determine the efficacy of TSP and modified enrichment pH on the recovery of salmonellae. Carcasses were obtained from a commercial processing plant immediately after the final inside-outside carcass washer, prior to chilling. Carcasses were subjected to one of four treatment groups; 1) TSP and alkaline enrichment, 2) TSP and neutral enrichment, 3) non-TSP and alkaline enrichment, 4) non-TSP and neutral enrichment. Carcasses were individually placed into plastic bags, 500 mL of the pH adjusted peptone was added, the carcasses shaken for 1 min, and pre-incubation pH measured. Carcasses with rinse solution were incubated at 37 C for 24 h and presence/absence of salmonellae determined. The pH of the pre-incubation enrichment peptone was 8.4 for the TSP alkaline enrichment, 7.2 for the TSP neutral enrichment, 8.6 for the non-TSP alkaline enrichment, and 7.1 for the non-TSP neutral enrichment. Salmonellae were detected from 40% of the TSP alkaline enrichment carcasses, 44% of the TSP neutral enrichment carcasses, 54% of the non-TSP alkaline enrichment carcasses, and 38% of the non-TSP neutral enrichment carcasses. Neither TSP treatment nor alkaline whole carcass enrichment influenced carcass salmonellae detection.

Key Words: trisodium phosphate, salmonellae, pH, whole carcass enrichment

INTRODUCTION

Salmonellae are known to be a common cause of food-borne gastroenteritis. The Center for Disease Control and Prevention (CDC) estimates that 1.4 million cases of salmonellosis including over 500 fatalities occur annually in the United States (CDC, 2004). Therefore, the reduction of salmonellae levels on food is an essential area in food safety research. Processing aids such as chlorine, cetylpyridinium chloride, and trisodium phosphate are used to assist in the reduction of food-borne pathogens on processed poultry.

It has been reported that the use of trisodium phosphate as an antimicrobial wash can significantly reduce carcass salmonellae contamination levels (Bender and Brotsky, 1991; Kim et al., 1994a, 1994b; Li et al., 1994; Lillard, 1994; Somers et al., 1994). However, the mechanisms of salmonellae reduction by TSP are not fully understood. The high pH (12) (Teo et al. 1996; Sampathkumar et al., 2003), detachment of bacteria from the carcass surface (Lee, et al., 1994), and lipid removal from skin surfaces (Bender and Brotsky, 1992; Giese, 1992) have been reported to be factors that lead to the reduction of carcass salmonellae numbers.

In commercial processing plants, carcasses are flushed with 10% TSP at 24 C for 2-3 s and then TSP is allowed to drip off the carcass for about 1 min before immersion chilling. Chiller water pH is neutralized with carbon dioxide gas to increase the antimicrobial efficiency of chlorine. However, in most studies reporting salmonellae reduction by TSP, carcasses are treated for a longer period of time (10 to 30 min) or pH is not neutralized before sampling (Bender and Brotsky, 1991; Li et al., 1994; Lillard, 1994; Somers et al., 1994).

The objective of the current study was to compare the effect of TSP treatment with and without pH neutralization after treatment on the detection of salmonellae using whole carcass enrichment.

MATERIALS AND METHODS

Carcass Treatment

In each of five trials, 40 pre-chill broiler carcasses were collected after an inside-outside carcass washer of a commercial processing plant, individually bagged, and transported to University of Georgia pilot processing facility. Carcasses were divided into four treatment groups. Treatment 1 (TSP plus alkaline enrichment) carcasses were dipped two at a time in 10% TSP for 5 s at 24 C, allowed to drip for 1 min, placed into a clean plastic bag, and 500 mL buffered peptone water¹ (BPW) was added. Treatment 2 (TSP plus neutral enrichment), was identical to Treatment 1 except the 500 mL of BPW was pre-loaded with 7.5 mL of 2 N HCl to adjust the enrichment pH to neutral. Treatment 3 (non-TSP plus alkaline enrichment) carcasses were not treated with TSP and the 500 mL BPW had 4.5 mL of 2 N NaOH added to adjust the pH to a level similar to that of Treatment 1 (pH 8.5). Treatment 4 (non-TSP plus neutral enrichment) carcasses were not dipped in TSP and 500 mL BPW was added to each bag. All carcasses were shaken for 1 min and a 5 mL aliquot of rinse was removed from each for an initial enrichment pH determination.

¹Becton Dickinson, Sparks, Maryland

Salmonellae Sampling

Carcasses were sampled using a modified whole carcass enrichment method (Cox and Blankenship, 1975; Simmons, et al., 2003). Each bag containing a carcass and BPW was incubated 24 h at 37 C. After incubation, a 0.1 mL of rinsate was transferred to 10 mL Rappaport-Vassiliadis broth² and 0.5 mL rinsate was transferred to 10 mL tetrathionate (HANJA) broth² and incubated 24 h at 42 C. Each broth was then streaked onto brilliant green sulfa² and modified lysine iron³ agar and incubated 24 h at 35 C. Triple sugar iron² and lysine iron² agar slants were inoculated with suspect salmonellae colonies and incubated for 24 h at 35 C. Poly O² and Poly H⁴ agglutination tests were done to confirm presumptive positives as salmonellae.

Statistical Analysis

Salmonellae recovery data were analyzed using the Chi-square test procedure. Initial enrichment pH data were analyzed using the General Linear Models procedure of SAS (SAS Institute, 1998). Sources of variation were treatment (4) and trial (5). For all analyses significance was determined at $P < 0.05$ level, and the mean square error was the error test statistic.

RESULTS AND DISCUSSION

²Difco Laboratories, Detroit, MI

³Oxoid, Basinstoke, Hampshire RG24 8PW, UK

⁴Microgen, Camberly, Surrey GU15 3DT, UK

Enrichment pH

The pH of the pre-incubation enrichment peptone was 8.4 for the TSP plus alkaline enrichment, 7.2 for the TSP plus neutral enrichment, 8.6 for the non-TSP plus alkaline enrichment, and 7.1 for the non-TSP plus neutral enrichment (Table 4.1). Neutralization of residual TSP high pH in the enrichment by preloading the BPW with HCl was successful in bringing the enrichment pH close to that of the non-TSP plus neutral enrichment group. Alkalinization of the BPW with NaOH was also successful in increasing the enrichment pH of the non-TSP plus alkaline enrichment group to a pH similar to the TSP-treated plus neutral enrichment group. Although significant differences were found between the initial enrichment pHs, the magnitude of the difference being only 0.2 pH units is unlikely to have affected salmonellae recovery.

Salmonellae Recovery

Salmonellae recovery was not significantly affected by either TSP treatment or enrichment pH. Salmonellae were detected from 40% of the TSP alkaline enrichment carcasses, 44% of the TSP neutral enrichment carcasses, 54% of the non-TSP alkaline enrichment carcasses, and 38% of the non-TSP neutral enrichment carcasses (Table 4.2).

The lack of salmonellae reduction between the TSP-treated and non-TSP treated carcasses may have been due to the shortness of the pH shock (< 2 min), a lack of an immersion chilling step, and the sensitivity of the method of salmonella recovery.

The high pH shock (12) from the initial 5 s TSP treatment and 1 min of resident time did not have a significant impact on salmonellae. Similar short term pH shock results were found in

a study reported by Teo et al. (1996) where only minimal reductions of *Salmonella enteritidis* cells were detected after 1 min of exposure to a pH 11. In another study, a 6 second dip in 10% TSP also did not decrease salmonellae incidence on broiler carcasses (Ellerbroek et al., 1996).

The lack of an immersion chilling step that would have had a washing or scrubbing action on the carcass may have prevented loose or detached salmonellae from being physically removed from the carcass. In a previous study TSP treatment significantly reduced salmonellae recovery in comparison to non-treated controls when the methods of TSP treatment and salmonellae detection were the same as in the present study, except carcasses were rinsed off and chilled between TSP-treatment and salmonellae recovery (D. V. Bourassa et al., unpublished data).

The use of the whole carcasses enrichment method may have also contributed to the lack of TSP effectiveness in the present study compared to other studies where TSP significantly reduced salmonellae (Coppen et al., 1998; Yang et al., 1998; Whyte et al., 2001). Whole carcass enrichment has been previously reported as a more sensitive salmonellae detection method than the rinse aliquot type method (Cox and Blankenship, 1975; Simmons et al., 2003). Although TSP may have decreased salmonellae numbers, the numbers were not low enough to prevent detection by the whole carcass enrichment method.

There was also no significant difference in salmonellae recovery between the neutral and alkaline pH initial enrichments. This may have occurred because the alkaline pH of 8.5 was not high enough to reduce salmonellae numbers low enough that they would not be detected by whole carcass enrichment. According to Bell and Kyriakides (2002), the minimum and maximal pH levels for salmonellae survival are 3.8 and 9.5. The pH of 8.5 was within that range

therefore, the initial enrichment pH appears not to have significantly affected salmonellae recovery in comparison to pH 7.1.

The results presented in this study indicate that salmonellae prevalence was not reduced due to the high pH TSP treatment. Other factors such as rinsing TSP off or immersion chilling may be necessary to achieve the full antimicrobial effects from TSP.

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REFERENCES

- Bell, C. and A. Kyriakides, 2002. *Salmonella*: A practical approach to the organism and its control in foods. Blackwell Science Company, IA, pp: 1-317
- Bender, F. G., and E. Brotsky, 1991. Process for treating poultry carcasses to control salmonellae growth. U.S. Patent 5,069,922.
- Bender, F. G., and E. Brotsky, 1992. Process for treating poultry carcasses to control salmonellae growth. U.S. Patent 5,143,739.
- CDC, Center for Disease Control and Prevention, Division of Bacterial and Mycotic Diseases: Disease Information: Salmonellosis. http://www.cdc.gov/ncidod/dbmd/diseaseinfo/salmonellosis_t.htm. Accessed March 2004.
- Coppen, P., S. Fenner, and G. Salvat, 1998. Antimicrobial efficacy of AvGard® carcass wash under industrial processing conditions. Br. Poult. Sci. 39:229-234.
- Cox, N. A. and L. C. Blankenship, 1975. Comparison of rinse sampling methods for detection of salmonellae on eviscerated broiler carcasses. J. Food Sci. 40:1333-1334.
- Giese, J., 1992. Experimental process reduces *Salmonella* on poultry. Food Technol. 46:112.
- Giese, J., 1993. *Salmonella* reduction process receives approval. Food Technol. 46:110.
- Kim, J. W., M. F. Slavik, and F. G. Bender. 1994a. Removal of *Salmonella typhimurium* attached to chicken skin by rinsing with trisodium phosphate solution: scanning electron microscopic examination. J. Food Safety 14:77-84.
- Kim, J. W., M. F. Slavik, M. D. Pharr, D. P. Raben, C. M. Lobsinger, and S. Tsai. 1994b. Reduction of *Salmonella* on post-chill chicken carcasses by trisodium phosphate

- (Na_3PO_4) treatment. J. Food Safety 14:9-17.
- Lee, R. M., P. A. Hartman, D. G. Olsen, and F. D. Williams, 1994. Metal ions reverse the inhibitory effects of selected food-grade phosphates in *Staphylococcus aureus*. J. Food Prot. 57:284-288.
- Li, Y., J. W. Kim, M.F. Slavik, C. L. Griffis, J. T. Walker, and H. Wang. 1994. *Salmonella typhimurium* attached to chicken skin reduced using electrical stimulation and inorganic salts. J. Food Science 59:23-25.
- SAS Institute. 1998. SAS User's Guide. Version 8. SAS Institute Inc., Cary, NC.
- Simmons, M., D. L. Fletcher, J. A. Cason, and M. E. Berrang. 2003. Recovery of *Salmonella* from retail broilers using a whole carcass enrichment procedure. J. Food Prot. 66:446-450.
- Somers, E. B., J. L. Schoeni, and C. L. Wong. 1994. Effect of trisodium phosphate on biofilm and planktonic cells of *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella typhimurium*. Int. J. Food Microbiol. 22:269-276.
- Teo, Y. L., T. J. Raynor, K. R. Ellajosyula, and S. J. Knabel, 1996. Synergistic effect of high temperature and high pH on the destruction of *Salmonella enteritidis* and *Escherichia coli* O157:H7. J. Food Prot. 59:1023-1030.
- Whyte, P., J. D. Collins, K. McGill, C. Monahan, and H. O'Mahony, 2001. Quantitative investigation of the effects of chemical decontamination procedures on the microbiological status of broiler carcasses during processing. J. Food Prot. 64:179-183.

Yang, Z., Y. Li, and M. Slavik, 1998. Use of antimicrobial spray applied with an inside-outside birdwasher to reduce bacterial contamination on prechilled chicken carcasses. J. Food Prot. 61:829-832.

TABLE 4.1. Enrichment media pH post-treatment and prior to carcass incubation

Trial	Treatments			
	TSP		Non-TSP	
	Enrichments			
	Alkaline	Neutral	Alkaline	Neutral
1	8.62	7.48	8.64	7.11
2	8.53	7.30	8.50	7.03
3	8.35	7.06	8.72	7.18
4	8.15	7.02	8.81	7.18
5	8.55	7.33	8.60	7.11
Average	8.44 ^B	7.24 ^C	8.65 ^A	7.12 ^C

^{A-B} Values within a row with no common superscripts differ significantly ($P < 0.05$).

TABLE 4.2. Recovery of salmonellae from TSP-treated and control carcasses with and without initial enrichment pH adjustments.

Treatments				
TSP		Non-TSP		
Enrichments				
	Alkaline	Neutral	Alkaline	Neutral
Trial				
1	3/10	1/10	2/10	0/10
2	6/10	5/10	9/10	7/10
3	10/10	10/10	10/10	10/10
4	1/10	4/10	4/10	0/10
5	0/10	2/10	2/10	2/10
Total	20/50	22/50	27/50	19/50
Probability	<i>P</i> - value			
Treatment	0.4222			
Enrichment	0.2225			

CHAPTER 5

SUMMARY AND CONCLUSIONS

The effectiveness of trisodium phosphate on reduction of salmonellae on poultry carcasses was evaluated. According to the previous literature it is generally accepted that the use of TSP is beneficial to the microbiological status of poultry carcasses.

The treatment of broiler carcasses with TSP reduced the recovery of salmonellae both on the day the carcasses were processed and after being held for 7 days in refrigerated conditions.

However, in the second experiment when carcasses were sampled immediately following treatment, without chilling or rinsing. TSP did not have any effect on the recovery of salmonellae in comparison to control carcasses.