

# Survival of *Salmonella typhimurium* on Sterile Ground Chicken Breast Patties After Washing with Salt and Phosphates and During Refrigerated and Frozen Storage

K.S. YOON AND T.P. OSCAR

**ABSTRACT:** This study investigated the effects of 10% (w/v) salt, trisodium phosphate (TSP), sodium tripolyphosphate (STPP), and tetrapotassium pyrophosphate (TKPP) washes on removal of attached *Salmonella typhimurium* from sterile chicken breast patties, as well as on their injury and survival in a refrigerator for 16 d, in a  $-20^{\circ}\text{C}$  freezer for 10 mo, and after 3 freeze-thaw cycles. *S. typhimurium* were grown on chicken patties at  $20^{\circ}\text{C}$  for 20 h, washed, and enumerated by plating on selective and nonselective media. Salt and phosphates washing significantly lowered the survival populations of attached *S. typhimurium* on patties, but did not cause any significant sublethal injury of attached *S. typhimurium*, irrespective of storage treatments. The TSP washes showed superior effects of removing and inactivating *S. typhimurium* compared to other washing treatments.

**Keywords:** *Salmonella typhimurium*, survival, injury, frozen chicken, phosphates

## Introduction

THE ABILITY OF *SALMONELLA* TO SURVIVE in poultry products during frozen storage is a major safety concern to both the poultry industry and consumers because freezing poultry products is a common practice in today's poultry market. Besides, freezing also has been increasingly used in the household for storage of raw poultry between purchasing and cooking, as well as for pre-cooked and ready-to-eat frozen chicken dinner packages. Sublethally injured microorganisms as a result of freezing and frozen storage have been observed (Ray and Speck 1973; Speck and Ray 1977) and are still very important because they are potentially as dangerous as their uninjured counterparts (Ray 1986). Besides, sublethally injured microorganisms, which have lost the ability to form colonies on a selective media, could produce misleading test results and other potential problems after recovering from injury.

In 1992, the USDA approved the use of trisodium phosphate ( $\text{Na}_3\text{PO}_4$ , TSP) as a post-chill antimicrobial treatment of raw poultry to control *Salmonella* during processing (Giese 1992, 1993). Since then, studies have focused on the antimicrobial effects of TSP against *Salmonella* on beef (Dickson and others 1994; Kim and Slavik 1994; Dorsa and others 1997), pork (Morris and others 1997), poultry (Lillard 1994; Kim and

others 1994a, 1994b; Li and others 1994, 1997; Salvat and others 1997; Wang and others 1997; Xiong and others 1998), and tomatoes (Zhuang and Beuchat 1996). Various applications of TSP against *Salmonella typhimurium* have been reported, such as immersing chicken carcasses for 15 min in a 10% TSP solution (Kim and others 1994a, 1994b; Lillard 1994; Li and others 1994; Salvat and others 1997) and spraying pre-chill chicken carcasses with 10% TSP (Wang and others 1997; Li and others, 1997; Xiong and others 1998). All of these studies showed a 1.5 to 2.3  $\log_{10}$  reduction of *Salmonella*, but their application was limited to loosely attached *Salmonella* on chicken carcasses, where the attachment period was only 20 to 30 min. Some studies also showed that polyphosphate could control *Salmonella* on poultry carcasses (Mead and Adams 1979; Thomson and others 1979) and reduce the survival of *Salmonella typhimurium* in chicken meat during frozen storage (Foster and Mead 1976; Obafemi and Davies 1985). However, injury and survival of *S. typhimurium* on poultry products washed with salt and various phosphate solutions, including trisodium phosphate (TSP), sodium tripolyphosphate (STPP), and tetra potassium pyrophosphate (TKPP), during refrigerated and extended frozen storage have not been adequately addressed. In addition, the effect of

such chemicals on more tightly attached and grown *S. typhimurium* on poultry has not been studied.

Therefore, the objective of this study was to investigate washing effects of 10% salt, TSP, STPP, and TKPP on attached *Salmonella typhimurium* on sterile ground chicken breast patties, as well as on their subsequent injury and survival during refrigerated storage, frozen storage, and after 3 freeze-thaw cycles.

## Materials and Methods

### Materials

Food grade trisodium phosphate (TSP), sodium tripolyphosphate (STPP), and tetra potassium pyrophosphate (TKPP) were obtained from the FMC Corp. (Philadelphia, Pa., U.S.A.). Salt (NaCl) and fresh, boneless chicken breasts were purchased from a local supermarket (Salisbury, Md., U.S.A.). Trypticase soy agar (TSA) and brilliant green agar with sulfadiazine (BGS) were purchased from Becton Dickinson Microbiology System (Cockeysville, Md., U.S.A.) and used as nonselective and selective media, respectively. *Salmonella typhimurium* (ATCC 14028, Antigenic formula; 4,5,12:i:1,2) was purchased from the American Type Culture Collection (Rockville, Md., U.S.A.) and was maintained at  $-70^{\circ}\text{C}$  at a concentration of 9.0 to 9.4  $\log_{10}$  colony forming units (CFU) per ml in brain heart infusion broth (BHIB; Dif-

co Laboratories, Detroit, Mich., U.S.A.) that contained 15% glycerol.

### Starter culture

Stock cultures of *S. typhimurium* were thawed at room temperature and diluted in 0.1% sterilized peptone water by  $10^{-3}$ . Starter cultures were initiated by adding 5  $\mu$ l of the diluted stock culture to 5 ml of BHIB (pH 6.4), resulting in an initial concentration of 3.0 to 3.4  $\log_{10}$  CFU/ml. Starter cultures were incubated at 37 °C for 23 h in 25 ml Erlenmeyer flask sealed with a foam plug and shaken at 150 orbits per min (opm). One ml of the starter culture was transferred into 9 ml of 0.1% sterilized peptone water, which was serially diluted to  $10^{-5}$ . Fifty  $\mu$ l of the diluted starter culture was spiral plated (Whitley Automatic Spiral Plater; Don Whitley Scientific Limited, West Yorkshire, U.K.) onto trypticase soy agar (TSA). Spiral plates were inverted and incubated aerobically at 37 °C for 24 h. Colonies on TSA plates were counted using an automated colony counter (Protos Colony Counter; Synoptics, Cambridge, England). Viable counts of the starter cultures at the end of the incubation were between 10.0 and 10.4  $\log_{10}$  CFU/ml.

### Preparation and inoculation of ground chicken patties

Fresh chicken breast was ground through a three-sixteenth inch plate of an electric meat grinder (Oster, Bay Springs, Miss., U.S.A.). Ten grams of ground chicken were formed into a circular patty by finger kneading followed by flattening with a 100-ml beaker. An indentation (1.2 cm<sup>2</sup>) was made in the center of the chicken patty with a dilution tube cap to serve as an inoculation well. The prepared chicken patties were autoclaved at 121 °C for 15 min to remove background microflora, placed in sterile petri dishes, and kept in sealed plastic bags at 4 °C until being used. Cooked chicken breast patties were incubated overnight at 20 °C to equilibrate the temperature of the patties before inoculation. Cooked chicken breast patties were surface-inoculated with a 100  $\mu$ l of the diluted starter culture of *S. typhimurium* using a sterile repeater pipette for a target population of approximately 6.0  $\log_{10}$  CFU/patty, aseptically transferred into sterile petri dishes, and placed in sealed plastic bags to prevent drying. They were held at 20 °C for 20 h for growth and attachment of *S. typhimurium* on chicken patties. Growth ensured high numbers for following survival, whereas attachment provided a model

system for assessing the ability of the salt and phosphates washes to remove the *Salmonella* physically. The mean population ( $n = 9$ ) of *S. typhimurium* was 9.06  $\log_{10}$  CFU/patty, which was determined immediately after inoculation and before washing treatment.

### Washing with salt and phosphate solutions

At levels of 10% (w/v), NaCl, TSP, STPP, and TKPP were prepared by adding 10 g of each chemical to 100 ml of sterile distilled water and stored in 118 ml sterile, specimen container with lid (VWR, Bridgeport, N.J., U.S.A.) at 4 °C until being used. The pH values of the salt, TSP, STPP, and TKPP solutions were 7.32, 13.11, 10.36, and 10.53, respectively. Cooked chicken breast patties (6 g after autoclaving) with approximately 9.0  $\log_{10}$  CFU of *S. typhimurium* were washed at 4 °C for 10 min with agitation at 180 opm using an orbital shaking incubator (Model 4230; New Brunswick Scientific, Edison, N.J., U.S.A.) before refrigeration and frozen storage. Control samples were treated the same as above except that distilled water was used instead of phosphate or salt solutions. After washing, the washed chicken breast patties were immediately transferred into the sterile petri dishes using a sterile applicator to inactivate action of all phosphate solutions, covered, placed in sealed plastic bags, and then divided into 3 groups. Each group was stored in a refrigerator for 16 d, in a -20 °C freezer for 10 mo, or subjected to 3 freeze-thaw cycles. The numbers of *S. typhimurium* on cooked chicken breast patty in each group were quantified at predetermined intervals; 0, 4, 8, 12, and 16 d for refrigerated samples and monthly over 10 mo for frozen samples and after 3 freeze-thaw cycles for freeze-thaw cycles samples, respectively. Three freeze-thaw cycles involved storage at -20 °C for 1 mo, thawing at 4 °C overnight, and repeated 3 times.

### Enumeration of uninjured and injured *S. typhimurium*

The numbers of uninjured and injured *S. typhimurium* due to salt and phosphate washing, the extended refrigerated and frozen storage, and freeze-thaw cycles were determined by plating on the nonselective and selective media. Both uninjured and injured *S. typhimurium* form colonies on the nonselective media, while only uninjured cells form colonies on the selective media (Dickson and Siragusa 1994). The difference between the numbers of

*S. typhimurium* on the nonselective and selective media was considered to represent the numbers of sublethally injured *S. typhimurium*.

After washing and on each sampling day, a cooked chicken breast patty was homogenized (Model 400 Stomacher; Seward, London, English) for 2 min in 94 ml of 0.1% sterilized peptone water. One ml of the homogenized sample was transferred into 9 ml of 0.1% sterilized peptone water, which was serially diluted to  $10^{-5}$ . Fifty  $\mu$ l of the appropriate diluted homogenate was spiral plated (Whitley Automatic Spiral Plater; Don Whitley Scientific Limited, West Yorkshire, U.K.) onto TSA as the nonselective media and onto BGS as the selective media. Fifty  $\mu$ l of each chemical solution (salt, TSP, STPP, and TKPP) and control (water) after washing was also spiral plated on TSA and BGS. Spiral plates were inverted and incubated aerobically at 37 °C for 24 h. Colonies on quadruplicate plates of each sample were counted using an automated colony counter (Protos Colony Counter; Synoptics, Cambridge, England). Results were converted to  $\log_{10}$  CFU/ml of homogenate.

### Statistical analysis

Each experiment was independently replicated 3 times in a completely randomized block design. Data were analyzed by one-way analysis of variance (ANOVA) using SAS statistical analysis software program, version 8 (SAS Institute Inc., Cary, N.C. U.S.A.). When the ANOVA indicated a significant effect of treatment ( $P < 0.05$ ), means were compared using Duncan's multiple range test. A paired t-test was used to determine the significant difference in the numbers between TSA and BGS plates.

### Results and Discussion

TABLE 1 SUMMARIZES THE EFFECTS OF washing and storage on survival populations of attached *S. typhimurium* on sterile ground chicken breast patties. Chicken breast patties inoculated with *S. typhimurium* were washed with 10% solutions of salt and various phosphates, stored in a refrigerator for 16 d, in a -20 °C freezer for 10 mo, or subjected to 3 freeze-thaw cycles. Washing the inoculated chicken breast patties with salt, TSP, STPP, and TKPP solutions before storage reduced *S. typhimurium* by 0.62 to 1.18  $\log_{10}$ . However, no significant differences were detected among the wash solutions tested ( $P > 0.05$ ). In this study, *S. typhimurium* was grown for 20 h on the sterile chick-

en breast patty to allow attachment and growth of *Salmonella* to early stationary phase before washing. Even though sterile chicken patties were rigorously shaken for 10 min in salt, TSP, STPP, and TKPP solutions, the most effective reduction by TSP was only 1.18 log<sub>10</sub>. A 10% TSP solution has been investigated for its effectiveness in reducing attached *S. typhimurium* on chicken skin. Immersing inoculated chicken skin in a 10% TSP solution for 15 min (Lillard 1994) or spraying them with 10% TSP for 30 s at 206 kPa (Xiong and others 1998) resulted in a 2.0 or 2.2 log<sub>10</sub> reduction, respectively. Attachment periods in these previous studies were only 20 min, while the attachment period in our study was 20 h at 20 °C, which resulted in a 3-log<sub>10</sub> increase of *S. typhimurium* before washing treatment. This long period of attachment in our study may explain why most effective TSP washing did not cause significant reduction of *Salmonella* on chicken patties in comparison with the previous studies with this chemical.

Further reduction in *S. typhimurium* number with salt and phosphates washing was noticed after 16 d of refrigerated storage, 3 freeze-thaw cycles, and 10 mo of frozen storage at -20 °C (Table 1). After refrigerated storage, 10% solutions of salt and TSP reduced further the survival populations of *S. typhimurium* by 2.56 and 2.35 log<sub>10</sub>, respectively, which was a significant reduction compared to the control (1.44 log<sub>10</sub> reduction) ( $P < 0.0001$ ). However, STPP and TKPP had little or no inhibitory effects on survival of *S. typhimurium* in comparison with the control (water). After 3 freeze-thaw cycles and 10 mo of storage at -20 °C, greater reductions of *S. typhimurium* were noticed for all samples washed with salt, TSP, STPP, and TKPP compared to the control. The greatest reduction of *S. typhimurium* was found in chicken patties washed with TSP. The higher pH of the 10% TSP solution (13.11) compared to STPP (10.36) and TKPP (10.53) could explain its superior detrimental effect on *S. typhimurium* in this study. The highest pH (9.90) was also observed at the patty surface washed with TSP, followed by TKPP (7.87), STPP (6.82), control (6.30), and salt (6.15). Additionally, it was noteworthy that 10 mo of frozen storage at -20 °C caused a greater reduction of survival population of *S. typhimurium* than 3 freeze-thaw cycles did.

To detect injury of *S. typhimurium* after washing and storage, the detached *S. typhimurium* in wash solutions after

**Table 1—Effects of washing and storage on survival populations of attached *Salmonella typhimurium*<sup>1</sup> on sterile ground chicken breast patties**

Wash solutions	After washing & before storage	After 16 d	After 3 F-T <sup>2</sup>	After 10 mo
		at 4 °C	at -20 °C	
		(Log <sub>10</sub> CFU/ml of Homogenate)		
Control (water)	8.07 (0.99 <sup>a</sup> ) <sup>3</sup>	7.62 (1.44 <sup>d</sup> )	5.38 (3.68 <sup>d</sup> )	3.27 (5.79 <sup>e</sup> )
10% Salt	7.99 (1.07 <sup>a</sup> )	6.50 (2.56 <sup>a</sup> )	4.55 (4.51 <sup>b</sup> )	3.03 (6.03 <sup>d</sup> )
10% TSP	7.88 (1.18 <sup>a</sup> )	6.56 (2.35 <sup>b</sup> )	4.14 (4.92 <sup>a</sup> )	1.91 (7.15 <sup>a</sup> )
10% STPP	8.24 (0.82 <sup>a</sup> )	7.91 (1.15 <sup>e</sup> )	4.76 (4.30 <sup>c</sup> )	2.85 (6.21 <sup>c</sup> )
10% TKPP	8.44 (0.62 <sup>a</sup> )	7.37 (1.69 <sup>c</sup> )	4.76 (4.30 <sup>c</sup> )	2.70 (6.36 <sup>b</sup> )

<sup>1</sup>Mean of initial concentration (n = 9): 9.06 log<sub>10</sub> CFU/patty.

<sup>2</sup>3 F-T: Samples were subjected to 3 freeze-thaw cycles (frozen at -20 °C for 1 mo and thawed at 4 °C overnight, repeated 3 times).

<sup>3</sup>The numbers in parentheses indicate the log<sub>10</sub> reduction of *Salmonella typhimurium* numbers on sterile ground chicken breast patties from the mean population of unwashed control.

Values in a column with different superscripts are significantly different at  $P < 0.001$ .

**Table 2—Survival and sublethal injury of *Salmonella typhimurium* in wash solutions and on sterile ground chicken breast patties**

Wash solutions	In wash solution (Log <sub>10</sub> CFU/ml)	On chicken patties (Log <sub>10</sub> CFU/ml homogenate)	
		After 3 F-T	After 10 mo at -20 °C
Control (water)	5.96 <sup>1</sup> (5.98 <sup>2</sup> ) <sup>NS*</sup>	5.38 (5.82) <sup>NS</sup>	3.27 (3.44) <sup>NS</sup>
10% Salt	5.95 (6.03) <sup>NS</sup>	4.55 (4.51) <sup>NS</sup>	3.03 (3.00) <sup>NS</sup>
10% TSP	—	4.14 (4.22) <sup>NS</sup>	1.96 (2.00) <sup>NS</sup>
10% STPP	5.48 (4.3) <sup>S</sup>	4.76 (4.74) <sup>NS</sup>	2.85 (2.44) <sup>NS</sup>
10% TKPP	4.33 (1.9) <sup>S</sup>	4.76 (4.71) <sup>NS</sup>	2.7 (2.32) <sup>NS</sup>

<sup>1</sup>The numbers indicate total injured and uninjured *S. typhimurium* on TSA (trypticase soy agar, nonselective media).

<sup>2</sup>The numbers in parentheses indicate uninjured *S. typhimurium* on BGS (brilliant green agar with sulfadiazine, selective media).

\*Level of significance in the numbers between TSA and BGS plates was determined by a paired t-test. NS: means not different at  $P < 0.05$ , S: significantly different at  $P < 0.0001$ .

washing and undetached *S. typhimurium* on chicken patties were enumerated by plating on selective and nonselective media after 3 freeze-thaw cycles or 10 mo of frozen storage (Table 2). In control (water) and 10% salt treatments, no significant differences in numbers of detached *S. typhimurium* between TSA and BGS were noticed ( $P > 0.05$ ), which indicates no injured cells were detected. These data indicate that both the control and 10% salt solution (pH 7.32) removed *S. typhimurium* from patties during washing, but did not kill or injure *S. typhimurium*. But we observed some injuries in 10% STPP (21.5%) and TKPP (55.9%), indicating that TKPP was more effective at injuring *S. typhimurium* than STPP during washing (Table 2). On the other hand, no survivors among the detached *S. typhimurium* were detected in wash solution with 10% TSP. This result was consistent with the results of Li and others (1994) and Hwang and Beuchat (1995). Alexandra and others (1998) also reported that no survivors were detected when cells of *Salmonella* were suspended for 10 min in 10mM TSP at 4 or 37 °C. The mechanism(s) of bacterial reduction on chicken carcasses during TSP treatment are still not fully understood. Kim and Slavik (1994) from scanning electron microscopic examination sug-

gested that detachment of contaminants from the skin surface is one of the major mechanisms of TSP on *Salmonella* reduction in addition to the killing effect.

No significant injuries of attached *S. typhimurium* on chicken patties were found after 3 freeze-thaw cycles and 10 mo of frozen storage, irrespective of treatments ( $P > 0.05$ ) (Table 2). It has been shown that when bacteria are subjected to freezing, freeze-thaw cycles, and sanitizers, sublethal injury can occur. Olson and others (1981) used repeated freeze-thaw treatments to investigate its effectiveness in reducing numbers of *S. typhimurium* on experimentally inoculated chicken wings. They reported that 5 freeze-thaw cycles resulted in 99% reduction in the numbers of *S. typhimurium* cells and 75% of the surviving cells were sublethally injured. However, our results did not show any significant sublethal injury due to frozen storage and freeze-thaw cycles in addition to chemical washing treatments, including TSP, STPP, and TKPP. In this study, we observed some injuries of the detached *S. typhimurium* in 10% STPP (21.5%) and TKPP (55.9%) wash solutions, while no significant injuries of nondetached *S. typhimurium* on patties were noticed (Table 2). Since there was no inactivation step in wash

solutions, the detached *S. typhimurium* may be continuously affected and injured by STPP and TKPP in wash solution, while the stationary phase of attached *S. typhimurium* may be more resistant to chemical washing or frozen storage. Therefore, the *Salmonella* reduction after 3 freeze-thaw cycles and 10 mo of frozen storage can be considered as a death of *S. typhimurium* attached to sterile chicken breast patties (Table 1). In our study, the extended frozen storage alone reduced the viable count of *Salmonella* by more than 5 log<sub>10</sub> cycles, but death of *S. typhimurium* was further enhanced by prewashing with salt, TSP, STPP, and TKPP.

Figure 1 also shows the effect of washing on the total survivor of uninjured and injured *S. typhimurium* during frozen storage at -20 °C. *S. typhimurium* survived on sterile ground chicken breast patties for 10 mo, irrespective of treatments. Within the first mo, total populations of *S. typhimurium* were reduced from 1.75 to 2.90 log<sub>10</sub> for chicken patties washed with salt, TSP, STPP, and TKPP, while only 1.17 log<sub>10</sub> reduction was observed in the control. Overall, the numbers of *S. typhimurium* gradually decreased over the 10 mo of storage with a similar pattern on both TSA and BGS plates, indicating no significant effects of washing treatments and frozen storage on injury of stationary phase of *S. typhimurium* attached on chicken patties ( $P > 0.05$ ), which have not been washed away by washing treatment before storage. Superior killing effect of TSP on *S. typhimurium* to other treatments was noticed at -20 °C after 9 mo of storage.

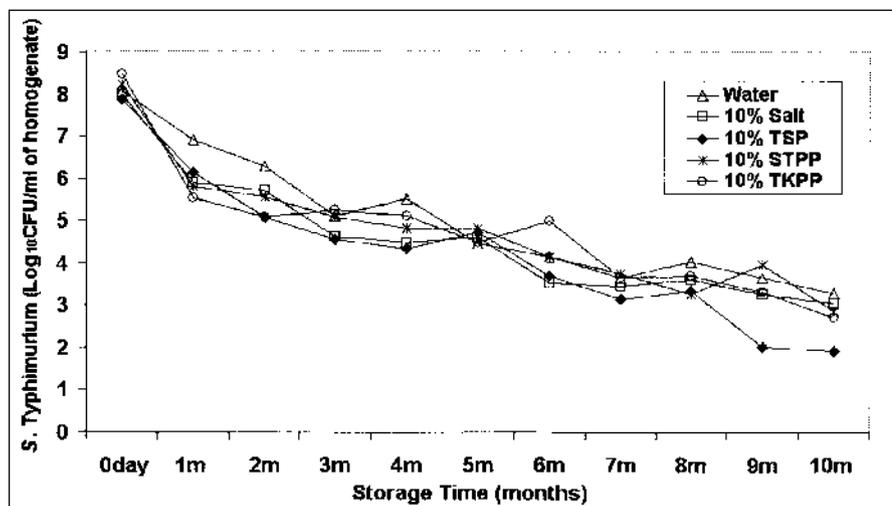
## Conclusions

**I**N CONCLUSION, WASHING CHICKEN breast patties with 10% salt, TSP, STPP, and TKPP lowered the surviving populations of attached *S. typhimurium* on chicken patties after 16 d of refrigeration, 10 mo of frozen storage, or 3 freeze-thaw in comparison to treatments using plain water. TSP washing was more effective at removing and inactivating attached *S. typhimurium* on chicken patties than water, salt, STPP, or TKPP, irrespective storage treatments. However, salt and all phosphates washing treatments in conjunction with 16 d of refrigeration, 10 mo of frozen storage, or 3 freeze-thaw cycles did not cause complete destruction and injury of attached *S. typhimurium* on chicken breast patties. Therefore, careful handling and cooking methods are still required in retail markets and in the consumer's kitchen to reduce the health risk involved with refrigerated and frozen chicken consumption.

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**Figure 1—Survival of *Salmonella typhimurium* on sterile ground chicken breast patties after washing with salt and various phosphates solutions during 10 mo of frozen storage at -20 °C and enumerated on trypticase soy agar (TSA) plate.**

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Author Yoon is with the Dept. of Human Ecology, Univ. of Maryland Eastern Shore, 2103 Trigg Hall, Backbone Road, Princess Anne, MD 21853. Author Oscar is with the U. S. Dept. of Agriculture, Agricultural Research Service, Princess Anne, Md. Direct inquiries to author Yoon at (E-mail: ksyoon@mail.umes.edu).