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Influence of Catfish Skin Mucus on Trisodium Phosphate Inactivation of Attached *Salmonella* Typhimurium, *Edwardsiella tarda,* and *Listeria monocytogenes*

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ABSTRACT

This study examined the antimicrobial effectiveness of trisodium phosphate (TSP) on *Edwardsiella tarda, Listeria monocytogenes,* and *Salmonella* Typhimurium attached to catfish skin with and without mucus. *Salmonella* Typhimurium and *E. tarda* attached more readily to catfish skin than did *L. monocytogenes*. At high inoculum levels (10^7 CFU/ml), TSP treatments (at 2 to 6%) for 10 min reduced bacterial counts of *E. tarda* by >2.5 to >3.3 $\log_{10}$ CFU per skin sample for firmly attached cells and by 3.5 to 3.6 $\log_{10}$ CFU per skin sample for loosely attached cells. Counts of *L. monocytogenes* declined by 0.6 to >1.8 $\log_{10}$ CFU per skin sample for firmly attached cells and by 1.2 to 2.2 $\log_{10}$ CFU per skin sample for loosely attached cells. Counts of *Salmonella* Typhimurium were reduced by 3.6 to >3.8 $\log_{10}$ CFU per skin sample for firmly attached cells and by 3.5 to >3.8 $\log_{10}$ CFU per skin sample for loosely attached cells. Overall, counts of firmly attached bacteria on TSP-treated skins with mucus were higher than counts on skin without mucus. Firmly attached *L. monocytogenes* was more resistant to TSP than was firmly attached *Salmonella* Typhimurium or *E. tarda.* The presence of mucus on skins slightly decreased the antimicrobial effect of TSP. Significant ($P < 0.05$) reduction in the numbers of all three bacteria can be achieved by treatment with 6% TSP for 10 min.

Trisodium phosphate (TSP) is a food additive that is generally recognized as safe and is approved by the U.S. Department of Agriculture for use in poultry processing *(16).* Several researchers have reported that TSP is effective in reducing the numbers of bacteria on animal carcasses *(9–11, 21, 24, 25),* including catfish *(29),* and in biofilms *(34).*

To our knowledge, aquacultured catfish have never been identified as a vehicle for the transmission of human foodborne diseases, possibly because of the low incidence of enteric pathogens and because such products are cooked before consumption. However, some investigators have reported the presence of a variety of microorganisms, including potential human pathogens, in catfish *(1, 7, 14).* Thus, even if foodborne outbreaks related to catfish have not been reported, it is possible for catfish to serve as a vehicle for foodborne pathogens.

Salmonella Typhimurium and *Listeria monocytogenes* are well-recognized human and animal pathogens *(8, 12).* Because these pathogens can be spread through the aquaculture farm environment by water, feed, birds, reptiles, and other sylvatic animals, their presence on catfish is unavoidable. Since Meyer and Bullock *(31)* first reported the occurrence of *Edwardsiella tarda* in catfish, the results of several subsequent studies have indicated that this bacterium causes human gastroenteritis and bacteremic infections, including wound abscesses and meningitis *(17, 18, 26, 35, 37). E. tarda* is often recovered from catfish and may possibly infect people through the ingestion of contaminated products or as a cross-contaminant of other foods.

Previous microbial decontamination research on catfish has concentrated on catfish fillets *(2, 13, 19, 20, 30, 38)* and frames *(29).* Since catfish skin is an important source of bacterial contamination during catfish processing and the microbial load of catfish skin can be transferred to retail products such as skinless and boneless fillets, bacterial decontamination research should also focus on catfish skin. Catfish skin contains mucus, which is a part of the catfish immune system. We previously demonstrated that this mucus reduced the antimicrobial effectiveness of lactic acid against *Salmonella* Typhimurium, *E. tarda,* and *L. monocytogenes (22, 23).* Using a previously developed catfish skin attachment model *(22),* the present research was conducted to test the antimicrobial efficacy of TSP on these three pathogens attached to catfish skin with and without mucus.

MATERIALS AND METHODS

Catfish skin sample preparation. Fresh aquacultured channel catfish (*Ictalurus punctatus*) were obtained from a local catfish processing plant and transported on ice to the laboratory. On the same day, catfish skin was cut into 2.54-cm^2 samples by excision with a surgical blade. Skin without mucus was obtained by scraping the skin with a surgical blade. Skin samples were mounted into sterile skin holders *(22),* which served as the attachment sur-

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face (1.3 cm^2), and were stored at −20°C and used within 3 weeks. Preliminary experiments demonstrated no difference between numbers of pathogens attached on unfrozen and frozen skin samples that were stored for up to 3 weeks (results not shown). Samples stored frozen for >3 weeks had moisture (mucus) drying on the skin but still showed no difference in bacterial attachment rates. Thus, skins were used within 3 weeks of frozen storage.

Inoculation and TSP treatment. Three stock strains each of *E. tarda* (ATCC 15947 and two environmental isolates), *L. monocytogenes* (F5027, Scott A, and ATCC 43256), and *Salmonella* Typhimurium (ATCC 13311, 14028, and 19585) were individually subcultured at 37°C for 24 h in tryptic soy broth (Difco Laboratories, Detroit, Mich.). The two environmental *E. tarda* strains were obtained from Dr. F. W. Austin (College of Veterinary Medicine, Mississippi State University). For each species, 1-ml aliquots of each strain (10^9 CFU/ml) were combined and then diluted in 0.1% (wt/vol) peptone buffer (Difco) to 10^5 CFU/ml (low inoculum) or 10^7 CFU/ml (high inoculum). Mounted skins with or without mucus were inoculated by depositing 1 ml of diluted multistrain culture on the exposed skin surface for 30 min at 21°C to achieve inoculum levels of 10^4 or 10^5 CFU per skin sample for firmly attached *E. tarda,* 10^3 or 10^4 CFU per skin sample for firmly attached *L. monocytogenes,* and 10^4 or 10^6 CFU per skin sample for firmly attached *Salmonella* Typhimurium. The remaining inoculum liquid was then decanted aseptically. Immediately following inoculation, samples were treated with 1-ml volumes of membrane-filter-sterilized TSP (Fisher Scientific Co., Pittsburgh, Pa.) solutions at 0, 2, 4, and 6% (wt/vol) for 1, 5, and 10 min at 21°C. The remaining treatment solution was then decanted aseptically.

Microbiological analysis. Treated samples were vigorously rinsed with two 1-ml volumes of peptone buffer. Vigorous rinsing was carried out by sucking and releasing buffer with a sterile plastic transfer pipette (Fisher Scientific) three times. The two volumes of rinse buffer were pooled and analyzed to obtain counts of loosely attached bacterium cells *(36).* Rinsed skin samples were swabbed twice with two cotton-tipped applicators (Fisher Scientific) to detach firmly attached bacterial cells *(36).* Both applicators were aseptically placed into 9-ml peptone buffer in a test tube and vortexed for 3 min. After appropriate serial dilutions, the *E. tarda, L. monocytogenes,* and *Salmonella* Typhimurium obtained were enumerated by spread plating on Hektoen enteric agar, modified Oxford agar, and bismuth sulfide agar plates (all from Difco Laboratories), respectively, which were incubated for 24 h at 37°C. Cell numbers were expressed as mean $\log_{10}$ CFU per skin sample.

Statistical analyses. Means of the data from three replicate experiments conducted on separate days were obtained. Each treatment involved one skin sample per day. Microbial count data were analyzed by analysis of variance and the general linear models procedure of the Statistical Analysis System *(33).* An a × b × c factorial design was used for the statistical analysis, and the three factors were lactic acid concentration, treatment time, and skin type (with or without mucus). Fisher's least significant difference test was used to separate treatment combination means at a significance level of $P < 0.05$.

RESULTS

Sensitivity of *E. tarda*. For firmly attached *E. tarda* cells, bacterial counts decreased in proportion to the concentration of TSP and the treatment time (Tables 1 and 2). At a high inoculum level (10^7 CFU/ml), the bacterium was

TABLE 1. *Change in numbers (mean $\log_{10}$ CFU per skin sample ± standard deviation) of low inocula (10^5 CFU/ml of bacterial suspension) of* Edwardsiella tarda, Listeria monocytogenes, *and* Salmonella *Typhimurium firmly attached to catfish skin with mucus (WM) and without mucus (WOM) after treatment with various concentrations of TSP for various times*

Treatment	Change for bacterium and treatment time (min)[a]														
	E. tarda					*L. monocytogenes*					*Salmonella* Typhimurium				
	0	1	5	10		0	1	5	10		0	1	5	10	
2.0% TSP WM	3.4 ± 0.12 A	2.0 ± 0.00 B	<2.0	<2.0		2.6 ± 0.06 A	2.3 ± 0.00 B	<2.0	<2.0		3.7 ± 0.06 A	2.8 ± 0.15 B	2.0 ± 0.00 D	<2.0	
2.0% TSP WOM	3.3 ± 0.06 A	<2.0	<2.0	<2.0		2.6 ± 0.00 A	2.3 ± 0.00 B	<2.0	<2.0		3.7 ± 0.12 A	2.3 ± 0.00 C	<2.0	<2.0	
4.0% TSP WM	3.3 ± 0.06 A	<2.0	<2.0	<2.0		2.6 ± 0.06 A	<2.0	<2.0	<2.0		3.7 ± 0.06 A	2.0 ± 0.00 D	<2.0	<2.0	
4.0% TSP WOM	3.3 ± 0.10 A	<2.0	<2.0	<2.0		2.6 ± 0.00 A	<2.0	<2.0	<2.0		3.7 ± 0.10 A	<2.0	<2.0	<2.0	
6.0% TSP WM	3.4 ± 0.06 A	<2.0	<2.0	<2.0		2.6 ± 0.00 A	<2.0	<2.0	<2.0		3.6 ± 0.06 A	<2.0	<2.0	<2.0	
6.0% TSP WOM	3.4 ± 0.06 A	<2.0	<2.0	<2.0		2.6 ± 0.06 A	<2.0	<2.0	<2.0		3.7 ± 0.15 A	<2.0	<2.0	<2.0	

[a] For each bacterium, means followed by the same letter are not significantly different ($P > 0.05$).

TABLE 2. *Change in numbers (mean $\log_{10}$ CFU per skin sample ± standard deviation) of high inocula (10^7 CFU/ml of bacterial suspension) of* Edwardsiella tarda, Listeria monocytogenes, *and* Salmonella *Typhimurium firmly attached to catfish skin with mucus (WM) and without mucus (WOM) after treatment with various concentrations of TSP for various treatment times*

Treatment	Change for bacterium and treatment time (min)[a]															
	E. tarda				*L. monocytogenes*				*Salmonella* Typhimurium							
	0	1	5	10	0	1	5	10	0	1	5	10	0	1	5	10
2.0% TSP WM	5.3 ± 0.10 A	2.8 ± 0.06 C	2.4 ± 0.12 D	<2.0	3.7 ± 0.12 AB	3.6 ± 0.17 BC	3.2 ± 0.12 D	3.1 ± 0.12 DE	5.6 ± 0.10 B	3.7 ± 0.10 C	2.8 ± 0.10 EF	2.0 ± 0.00 H				
2.0% TSP WOM	4.6 ± 0.15 B	2.5 ± 0.12 D	<2.0	<2.0	3.8 ± 0.06 A	3.5 ± 0.15 C	3.1 ± 0.15 DE	2.9 ± 0.06 EF	5.6 ± 0.00 B	3.6 ± 0.06 CD	2.3 ± 0.25 G	<2.0				
4.0% TSP WM	5.3 ± 0.15 A	2.8 ± 0.12 C	<2.0	<2.0	3.7 ± 0.10 AB	2.9 ± 0.10 F	2.5 ± 0.10 G	2.2 ± 0.17 H	5.8 ± 0.06 A	3.5 ± 0.25 D	2.7 ± 0.17 F	<2.0				
4.0% TSP WOM	4.6 ± 0.17 B	2.1 ± 0.17 E	<2.0	<2.0	3.7 ± 0.17 AB	2.9 ± 0.10 F	2.3 ± 0.00 H	2.0 ± 0.00 I	5.8 ± 0.06 A	2.9 ± 0.06 E	<2.0	<2.0				
6.0% TSP WM	5.3 ± 0.15 A	<2.0	<2.0	<2.0	3.8 ± 0.06 A	2.6 ± 0.12 G	2.2 ± 0.17 H	<2.0	5.6 ± 0.06 B	<2.0	<2.0	<2.0				
6.0% TSP WOM	4.5 ± 0.06 B	<2.0	<2.0	<2.0	3.7 ± 0.06 AB	2.6 ± 0.06 G	2.0 ± 0.00 I	<2.0	5.7 ± 0.10 AB	<2.0	<2.0	<2.0				

[a] For each bacterium, means followed by the same letter are not significantly different ($P > 0.05$).

better able to attach to skin with mucus (counts were higher by 0.7 $\log_{10}$ CFU per skin sample) than to skin without mucus (Table 2). Also at a high inoculum level, counts were reduced to below the limit of detection (10^2 CFU per skin sample) by TSP exposure for 10 min at all treatment concentrations (Table 2). Treatment of firmly attached *E. tarda* cells with 6% TSP reduced counts to below the limit of detection with 1 min of exposure (Tables 1 and 2).

For loosely attached *E. tarda* cells, counts decreased proportionally as the concentration of TSP and the treatment time increased (Tables 3 and 4). Like firmly attached *E. tarda* cells, treatment with 6% TSP was effective in inactivating loosely attached *E. tarda* cells to below the limit of detection with 1 min of exposure time. Unlike the case for firmly attached cells, there was no significant difference in counts of *E. tarda* cells loosely attached to skin with mucus and skin without mucus (Tables 3 and 4). For each cell type, skin mucus appeared to have little influence on TSP efficacy.

Sensitivity of *L. monocytogenes*. For firmly attached *L. monocytogenes* cells, counts decreased proportionally as the concentration of TSP and the treatment time increased (Tables 1 and 2). At each inoculum level, there was no difference in the attachment of *L. monocytogenes* on skin with mucus and its attachment on skin without mucus. However, the results presented in Table 2 show that counts of TSP-treated *L. monocytogenes* firmly attached to skin without mucus were slightly lower than counts on skin with mucus. To achieve high inoculum count reductions to below the limit of detection, treatment with 6% TSP for 10 min was required (Table 2).

For loosely attached *L. monocytogenes* cells, counts decreased proportionally as the concentration of TSP and the treatment time increased (Table 3), with the exception of a slight increase in counts for the high inoculum level after 10 min of TSP exposure (Table 4). This anomaly may have been an artifact of the experiment or may demonstrate that TSP poorly inactivates *L. monocytogenes* on catfish skin and merely acts to increase the detachment of viable cells from the skin surface. Thus, at longer exposure times, the reattachment of cells may occur, resulting in the count increase observed after 10 min of exposure. Like the case for *E. tarda,* there was no significant difference in counts of *L. monocytogenes* cells loosely attached to skin with mucus and skin without mucus (Tables 3 and 4).

Sensitivity of *Salmonella* Typhimurium. For firmly attached *Salmonella* Typhimurium cells, counts decreased (i.e., the effectiveness of TSP treatment increased) as the concentration of TSP and the exposure time increased (Tables 1 and 2). At the high inoculum level, counts of firmly attached cells were higher on TSP-treated skins with mucus than on skins without mucus (Table 2). Like the case for *E. tarda,* treatment of firmly attached *Salmonella* Typhimurium cells with 6% TSP reduced counts to below the limit of detection, even at the highest inoculum level, within 1 min (Table 2).

For loosely attached *Salmonella* Typhimurium cells, counts decreased as the concentration of TSP and the treat-

TABLE 3. *Change in numbers (mean $\log_{10}$ CFU per skin sample ± standard deviation) of low inocula (10^5 CFU/ml of bacterial suspension) of* Edwardsiella tarda, Listeria monocytogenes, *and* Salmonella *Typhimurium loosely attached to catfish skin with mucus (WM) and without mucus (WOM) after treatment with various concentrations of TSP for various treatment times*

Treatment	Change for bacterium and treatment time (min)[a]											
	E. tarda				*L. monocytogenes*				*Salmonella* Typhimurium			
	0	1	5	10	0	1	5	10	0	1	5	10
2.0% TSP WM	3.5 ± 0.06 B	2.3 ± 0.10 C	<2.0	<2.0	4.0 ± 0.12 A	3.0 ± 0.00 FG	2.9 ± 0.06 G	2.7 ± 0.10 HI	3.7 ± 0.06 A	2.1 ± 0.15 B	<2.0	<2.0
2.0% TSP WOM	3.6 ± 0.17 AB	2.3 ± 0.00 C	<2.0	<2.0	4.0 ± 0.00 A	3.3 ± 0.06 E	3.2 ± 0.06 EF	2.8 ± 0.06 H	3.8 ± 0.06 A	2.1 ± 0.17 B	<2.0	<2.0
4.0% TSP WM	3.7 ± 0.12 A	2.1 ± 0.10 D	<2.0	<2.0	3.6 ± 0.15 D	2.8 ± 0.25 H	2.2 ± 0.35 I	<2.0	3.8 ± 0.10 A	<2.0	<2.0	<2.0
4.0% TSP WOM	3.7 ± 0.06 A	<2.00	<2.0	<2.0	3.7 ± 0.06 CD	2.8 ± 0.06 H	2.2 ± 0.06 I	<2.0	3.8 ± 0.00 A	<2.0	<2.0	<2.0
6.0% TSP WM	3.7 ± 0.12 A	<2.00	<2.0	<2.0	3.8 ± 0.06 BC	2.6 ± 0.06 I	2.0 ± 0.00 K	<2.0	3.7 ± 0.06 A	<2.0	<2.0	<2.0
6.0% TSP WOM	3.7 ± 0.06 A	<2.00	<2.0	<2.0	3.8 ± 0.06 BC	2.6 ± 0.12 I	2.0 ± 0.00 K	<2.0	3.7 ± 0.06 A	<2.0	<2.0	<2.0

[a] For each bacterium, means followed by the same letter are not significantly different ($P > 0.05$).

ment time increased (Tables 3 and 4). Like the case for firmly attached cells, 6% TSP treatment inactivated loosely attached *Salmonella* Typhimurium within 1 min. Also like the case for the other two test microorganisms, there was no significant difference in counts of cells loosely attached to skin with mucus and those loosely attached to skin without mucus (Table 4).

Comparison of the three test microorganisms. For firmly attached cells, attachment levels of the three test bacteria (10^4 or 10^5 CFU per skin sample for *E. tarda,* 10^3 or 10^4 CFU per skin sample for *L. monocytogenes,* and 10^4 or 10^6 CFU per skin sample for *Salmonella* Typhimurium) were different even though inocula preparations were the same (10^5 or 10^7 CFU/ml). In contrast, none of the three bacteria showed differences in attachment levels of loosely attached cells (10^4 or 10^6 CFU per skin sample). When counts of firmly attached cells at the inoculum level of 10^4 CFU per skin sample were compared, counts of TSP-treated *L. monocytogenes* were higher than those of *E. tarda* or *Salmonella* Typhimurium (Tables 1 and 2). Counts of TSP-treated, firmly attached *E. tarda* cells were lower than those of *Salmonella* Typhimurium cells. Thus, firmly attached *L. monocytogenes* was the most resistant to TSP, while *E. tarda* was the most sensitive. Likewise, counts of TSP-treated *L. monocytogenes* cells loosely attached to catfish skin were significantly higher than those of loosely attached *E. tarda* or *Salmonella* Typhimurium cells (Tables 3 and 4). There was no significant difference in counts between loosely attached *E. tarda* and *Salmonella* Typhimurium cells.

DISCUSSION

Phosphates have been widely used for texture modification and surface decontamination of muscle foods. Phosphates have broad-spectrum antimicrobial activity and improve the microbiological quality and shelf life of muscle foods through changes in pH and the ability to chelate metal ions essential in bacterial metabolism *(32).* Since TSP has been approved for use as a carcass-decontaminating agent during poultry processing, several researchers have reported on the effectiveness of TSP for the inactivation of pathogens. Dickson et al. *(9)* reported that TSP reduced the numbers of *Salmonella* Typhimurium, *Escherichia coli* ATCC 25922, *E. coli* O157:H7, and *L. monocytogenes* on beef tissue. Dorsa et al. *(10, 11)* stated that a TSP wash inactivated *E. coli* O157:H7, *Listeria innocua,* and *Clostridium sporogenes* inoculated on beef carcasses and that a TSP wash before inoculation inhibited the growth of *E. coli* O157:H7, *L. innocua, C. sporogenes,* and *Salmonella* Typhimurium. Kim and Slavik *(24)* reported that TSP treatment of the surface of beef reduced the level of attached *E. coli* O157:H7 and *Salmonella* Typhimurium by 0.5 to 1.4 logs and was more effective on fat surfaces than on fascia surfaces. Likewise, Kim et al. *(25)* showed that TSP treatment reduced the number of *Salmonella* cells on chicken carcasses by 1.6 to 1.8 logs. Marshall and Jindal *(29)* reported that 10% TSP reduced the levels of coliforms on catfish frames to nearly undetectable levels and that TSP was more effective than sodium metaphosphate and sodium

TABLE 4. *Change in numbers (mean $\log_{10}$ CFU per skin sample ± standard deviation) of high inocula (10^7 CFU/ml of bacterial suspension) of* Edwardsiella tarda, Listeria monocytogenes, *and* Salmonella *Typhimurium loosely attached to catfish skin with mucus (WM) and without mucus (WOM) after treatment with various concentrations of TSP for various treatment times*

Treatment	Change for bacterium and treatment time (min)[a]																			
	E. tarda					*L. monocytogenes*					*Salmonella* Typhimurium									
	0	1	5	10		0	1	5	10		0	1	5	10	
2.0% TSP WM	5.6 ± 0.23 A	3.4 ± 0.15 B	2.8 ± 0.12 C	2.1 ± 0.15 D		6.0 ± 0.21 A	5.0 ± 0.19 C	4.5 ± 0.04 D	4.8 ± 0.12 C		5.6 ± 0.00 B	3.6 ± 0.30 C	2.8 ± 0.15 D	2.1 ± 0.17 E	
2.0% TSP WOM	5.6 ± 0.15 A	3.4 ± 0.17 B	2.8 ± 0.28 C	2.1 ± 0.17 D		6.0 ± 0.04 A	5.0 ± 0.12 C	4.5 ± 0.07 D	4.8 ± 0.08 C		5.8 ± 0.15 A	3.7 ± 0.15 C	2.9 ± 0.31 D	2.1 ± 0.17 E	
4.0% TSP WM	5.6 ± 0.15 A	2.6 ± 0.10 C	2.0 ± 0.00 D	<2.0		5.8 ± 0.30 AB	4.2 ± 0.31 FG	3.2 ± 0.32 K	3.6 ± 0.25 I		5.8 ± 0.15 A	2.8 ± 0.15 D	<2.0	<2.0	
4.0% TSP WOM	5.6 ± 0.15 A	2.6 ± 0.11 C	2.0 ± 0.00 D	<2.0		5.8 ± 0.21 AB	4.3 ± 0.32 EF	3.4 ± 0.12 J	3.7 ± 0.12 I		5.8 ± 0.10 A	2.8 ± 0.20 D	<2.0	<2.0	
6.0% TSP WM	5.6 ± 0.15 A	<2.0	<2.0	<2.0		5.7 ± 0.06 B	3.8 ± 0.10 HI	3.0 ± 0.10 K	4.0 ± 0.03 GH		5.7 ± 0.06 AB	<2.0	<2.0	<2.0	
6.0% TSP WOM	5.5 ± 0.06 A	<2.0	<2.0	<2.0		5.8 ± 0.12 AB	4.2 ± 0.10 FG	3.5 ± 0.06 J	4.1 ± 0.06 FG		5.7 ± 0.10 AB	<2.0	<2.0	<2.0	

[a] For each bacterium, means followed by the same letter are not significantly different ($P > 0.05$).

tripolyphosphate. In the present study, TSP treatment effectively inactivated *E. tarda, L. monocytogenes,* and *Salmonella* Typhimurium attached to catfish skin, even if the sensitivities of these bacteria to TSP were different.

Several previous studies have demonstrated different sensitivities of microorganisms to TSP. Kim and Slavik *(24)* indicated that TSP was more effective in removing *E. coli* O157:H7 than it was in removing *Salmonella* Typhimurium. Dickson et al. *(9)* reported that *L. monocytogenes* was less sensitive to TSP than were *E. coli* O157:H7 and *Salmonella* Typhimurium. Likewise, Somers et al. *(34)* indicated that *L. monocytogenes* was less sensitive to TSP than were *Campylobacter jejuni, E. coli* O157:H7, and *Salmonella* Typhimurium. Dorsa et al. *(10, 11)* reported that TSP treatments were similar in effectiveness to organic acids in controlling the growth of *E. coli* O157:H7, *Salmonella* Typhimurium, and *C. sporogenes* inoculated on refrigerated beef carcasses but were less effective in controlling mesophilic aerobic bacteria, lactic acid bacteria, and *L. innocua.* The present study showed similar results in that gram-positive *L. monocytogenes* was less sensitive to TSP than were gram-negative *Salmonella* Typhimurium and *E. tarda.*

The attachment of bacteria to surfaces is a common phenomenon in nature, and it is known to be an initial step in product contamination *(15).* Blankenship *(3)* stated that knowledge of microbial attachment characteristics might be useful in devising decontamination processing techniques. For this reason, many food microbiologists have studied bacterial attachment on animal carcasses. Firstenberg-Eden *(15)* reported that bacterial attachment was dependent on various factors, such as the bacterial strain and the type of meat surface. Butler et al. *(5)* stated that gram-negative, motile bacteria showed more extensive attachment than did gram-positive, nonmotile species. However, Lillard *(28)* concluded that nonflagellated bacteria attached as readily as flagellated bacteria under the same controlled conditions and that bacterial adhesion to poultry skin could not be attributed to the presence or absence of flagella. Kim and Marshall *(23)* reported that *E. tarda* attached to catfish skin more readily than did *L. monocytogenes.* Likewise, the results of the present study indicated that gram-negative *Salmonella* Typhimurium and *E. tarda* more readily attached to catfish skin than did gram-positive *L. monocytogenes.*

Kim and Marshall *(22, 23)* used a catfish skin attachment model based on the method of Conner and Bilgili *(6)* to test the effectiveness of lactic acid against *E. tarda, L. monocytogenes,* and *Salmonella* Typhimurium attached to catfish skin. These authors reported that counts of all three pathogens declined as the concentration of lactic acid increased from 0.5 to 2% *(22, 23).* Counts of lactic acid–treated bacteria firmly attached to skin with mucus were higher than counts for skin without mucus. These results for lactic acid are similar to the results reported here for TSP.

Catfish skin is covered by mucus secreted by specific globet cells in the epidermal layer. Krovacek et al. *(27)* reported that fish mucus might enhance the accumulation of *Vibrio anguillaricum* and *Aeromonas hydrophila.* Bordas et al. *(4)* reported that mucus has several important func-

tions, including locomotion, mechanical protection, and antimicrobial activities. In the present study, catfish skin mucus slightly decreased the antimicrobial activity of TSP.

CONCLUSIONS

Salmonella Typhimurium and *E. tarda* attached more readily to catfish skin than did *L. monocytogenes*. Attached *L. monocytogenes* was more resistant to TSP than were *E. tarda* and *Salmonella* Typhimurium. Significant reduction in the counts of all three bacteria could be achieved with 6% TSP treatment for 10 min. Catfish skin mucus slightly decreased the antimicrobial effect of TSP, suggesting that the removal of catfish skin mucus prior to TSP application may improve decontamination effectiveness.

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REFERENCES

1. Andrews, W. H., C. R. Wilson, P. L. Poelma, and A. Romero. 1977. Bacterial survey of the channel catfish (*Ictalurus punctatus*) at the retail level. J. Food Sci. 42:359–363.
2. Bal'a, M. F. A., and D. L. Marshall. 1998. Organic acid dipping of catfish fillets: effect on color, microbial load, and *Listeria monocytogenes*. J. Food Prot. 61:1470–1474.
3. Blankenship, L. C. 1977. Attachment characteristics of *Clostridium perfringens* spores to broiler carcass skin. Abstr. Annu. Meet. Am. Soc. Microbiol. 77:245.
4. Bordas, M. A., M. C. Balebona, I. Zorrilla, J. J. Borregoand, and M. A. Morinigo. 1996. Kinetics of adhesion of selected fish-pathogenic *Vibrio* strains to skin mucus of gilt-head sea bream (*Spraus aurata* L.). Appl. Environ. Microbiol. 62:3650–3654.
5. Butler, J. L., J. C. Stewart, C. Vanderzant, Z. L. Carpenter, and G. C. Smith. 1979. Attachment of microorganisms to pork skin and surface of beef and lamb carcasses. J. Food Prot. 42:401–406.
6. Conner, D. E., and S. F. Bilgili. 1994. Skin attachment model for improved laboratory evaluation of potential carcass disinfectants for their efficacy against *Salmonella* attached to broiler skin. J. Food Prot. 57:684–688.
7. Cotton, L. N., and D. L. Marshall. 1998. Predominant microflora on catfish processing equipment. Dairy Food Environ. Sanit. 18:650–654.
8. D'Aoust, J. Y. 1994. *Salmonella* and the international food trade. Int. J. Food Microbiol. 24:11–31.
9. Dickson, J. S., C. N. Cutter, and G. R. Siragusa. 1994. Antimicrobial effects of trisodium phosphate against bacteria attached to beef tissue. J. Food Prot. 57:952–955.
10. 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.
11. Dorsa, W. J., C. N. Cutter, and G. R. Siragusa. 1998. Long-term effect of alkaline, organic acid, or hot water washes on the microbial profile of refrigerated beef contaminated with bacterial pathogens after washing. J. Food Prot. 61:300–306.
12. Farber, J. M., and P. I. Peterkin. 1991. *Listeria monocytogenes,* a food-borne pathogen. Microbiol. Rev. 55:476–511.
13. Fernandes, C. F., G. J. Flick, Jr., J. Cohen, and T. B. Thomas. 1998. Role of organic acids during processing to improve quality of channel catfish fillets. J. Food Prot. 61:495–498.
14. Fernandes, C. F., G. J. Flick, Jr., J. L. Silva, and T. A. McCaskey. 1997. Influence of processing schemes on indicative bacteria and quality of fresh aquacultured catfish fillets. J. Food Prot. 60:54–58.
15. Firstenberg-Eden, R. 1981. Attachment of bacteria to meat surface: a review. J. Food Prot. 44:602–607.
16. Giese, J. 1993. *Salmonella* reduction process receives approval. Food Technol. 47(1):110.
17. Janda, J. M., and S. L. Abbott. 1993. Infections associated with the genus *Edwardsiella tarda*: the role of *Edwardsiella tarda* in human disease. Clin. Infect. Dis. 17:742–748.
18. Jordan, G. W., and W. K. Hadley. 1969. Human infection with *Edwardsiella tarda.* Ann. Intern. Med. 70:283–288.
19. Kim, C. R., and J. O. Hearnsberger. 1994. Gram negative bacteria inhibition by lactic acid culture and food preservatives on catfish fillets during refrigerated storage. J. Food Sci. 59:513–516.
20. Kim, C. R., J. O. Hearnsberger, and J. B. Eun. 1994. Gram-negative bacteria in refrigerated catfish fillets treated with lactic culture and lactic acid. J. Food Prot. 58:639–643.
21. 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.
22. Kim, J., and D. L. Marshall. 2000. Lactic acid inactivation of *Salmonella typhimurium* attached to catfish skin. J. Food Saf. 20:53–64.
23. Kim, J., and D. L. Marshall. 2001. Catfish skin mucus decreases lactic acid inactivation rate of attached *Listeria monocytogenes* and *Edwardsiella tarda.* Food Microbiol. 18:589–596.
24. Kim, J.-W., 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.
25. Kim, J.-W., M. F. Slavik, M. D. Pharr, D. P. Raben, C. M. Lobsinger, and S. Tsai. 1994. Reduction of *Salmonella* on post-chill chicken carcasses by trisodium phosphate (Na_3PO_4) treatment. J. Food Saf. 14:9–17.
26. King, B. M., and D. L. Adler. 1964. A previously undescribed group of Enterobacteriaceae. Am. J. Clin. Pathol. 41:230–232.
27. Krovacek, K., A. Faris, W. Ahne, and I. Masson. 1987. Adhesion of *Aeromonas hydrophila* and *Vibrio anguillarum* to mucus-coated glass slides. FEMS Microbiol. Lett. 42:85–89.
28. Lillard, H. S. 1985. Bacterial cell characteristics and conditions influencing their adhesion to poultry skin. J. Food Prot. 48:803–807.
29. Marshall, D. L., and V. Jindal. 1997. Microbial quality of catfish frames treated with selected phosphates. J. Food Prot. 60:1081–1083.
30. Marshall, D. L., and C. R. Kim. 1995. Microbiological and sensory analyses of refrigerated catfish fillets treated with acetic and lactic acids. J. Food Qual. 19:317–329.
31. Meyer, F. P., and G. L. Bullock. 1973. *Edwardsiella tarda,* a new pathogen of channel catfish (*Ictalurus punctatus*). Appl. Microbiol. 25:155–156.
32. Molins, R. A. 1991. Phosphates in food. CRC Press, Boca Raton, Fla.
33. SAS Institute, Inc. 1989. SAS STAT User's Guide, version 6, 4th ed., vol. 2. SAS Institute, Cary, N.C.
34. Somers, E. B., J. L. Schoeni, and A. 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.
35. Sonnenwirth, A. C., and B. A. Kallus. 1968. Meningitis due to *Edwardsiella tarda.* Am. J. Clin. Pathol. 49:92–95.
36. 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 treatment. Poult. Sci. 76:1318–1323.
37. Van Damme, L. R., and J. Vendepitte. 1980. Frequent isolation of *Edwardsiella tarda* and *Plesiomonas shigelloides* from healthy Zairese freshwater fish: a possible source of sporadic diarrhea in the tropics. Appl. Environ. Microbiol. 39:475–479.
38. Verhaegh, E. G. A., D. L. Marshall, and D. H. Oh. 1996. Effect of monolaurin and lactic acid on *Listeria monocytogenes* attached to catfish fillets. Int. J. Food Microbiol. 29:403–410.