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Research Note

Microbicidal Activity of Tripotassium Phosphate and Fatty Acids toward Spoilage and Pathogenic Bacteria Associated with Poultry†

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ABSTRACT

The ability of solutions of tripotassium phosphate (TPP) and fatty acids (lauric and myristic acids) to reduce populations of spoilage and pathogenic microorganisms associated with processed poultry was examined. In vitro studies were conducted with cultures of bacteria (*Campylobacter jejuni, Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella* Typhimurium, and *Staphylococcus aureus*) and yeasts (*Candida ernobii* and *Yarrowia lipolytica*). Cultures of the bacteria and yeasts were suspended in solutions of TPP or mixtures of TPP with lauric or myristic acid and mixed for 5 min. Viable numbers (log CFU per milliliter) in the suspensions were enumerated on microbiological agar. Results indicated that TPP solutions are highly bactericidal toward gram-negative bacteria and that mixtures of TPP and fatty acids are highly microbicidal toward gram-negative bacteria, gram-positive bacteria, and yeasts. The microbicidal activity of mixtures of TPP and fatty acids toward the native bacterial flora of skin of processed broiler carcasses was also examined. Skin samples were washed in mixtures of TPP and fatty acid, and the populations of total aerobic bacteria, campylobacters, enterococci, *E. coli,* lactic acid bacteria, pseudomonads, staphylococci, and yeasts in the skin rinsates were enumerated on the appropriate microbiological media. Results indicated that washing the skin in mixtures of TPP and fatty acids produced significant reductions in the number of aerobic bacteria, campylobacters, *E. coli,* pseudomonads, and yeasts recovered from skin rinsates, but there was no significant reduction in the populations of enterococci, lactic acid bacteria, or staphylococci. These findings indicate that mixtures of TPP and fatty acids possess microbicidal activity against several microorganisms associated with processed poultry and that these solutions could be useful as microbicides to reduce the populations of some bacteria and yeasts associated with some poultry processing operations.

The native flora of processed broiler carcasses comprises pathogenic microorganisms that cause human foodborne diseases and spoilage microorganisms that reduce the shelf life of fresh poultry products *(3, 8, 13).* Contaminated poultry products continue to be one of the major sources of human cases of campylobacteriosis and salmonellosis *(4).* Opportunistic pathogens (e.g., staphylococci) can also be recovered from the skin of processed broilers carcasses. The presence of high levels of enteric bacteria such as *Escherichia coli* and enterococci in processing environments might indicate undesirable levels of fecal contamination of processed carcasses *(7).* Although psychrotrophic bacteria *(1, 10)* and yeasts *(9, 10)* are not considered threats to food safety, these microorganisms are of concern in poultry processing because they can cause microbial spoilage of fresh refrigerated poultry.

Although chlorine is the most widely used antimicrobial agent for reducing contamination in poultry processing *(18),* other chemicals have also been used. Trisodium phosphate *(15)* and organic acids *(16)* are among the compounds that have been examined as alternatives to chlorine in poultry processing. Research has indicated that washing poultry skin in solutions of fatty acid, potassium oleate *(11),* or mixtures of potassium oleate and tripotassium phosphate (TPP) *(12)* can significantly reduce the number of bacteria on poultry skin and in rinsates of the skin. Fatty acids other than oleic acid have also been reported to possess antimicrobial activity that can reduce the number of microorganisms associated with food products *(14).* The purpose of this study was to examine the antimicrobial activity of lauric and myristic acids with TPP in vitro and on poultry skin.

MATERIALS AND METHODS

Microbial cultures. The bactericidal activity of TPP solutions and mixtures of TPP and lauric or myristic acid in vitro was examined with microorganisms that had been previously isolated from commercially processed poultry carcasses *(12).* Fresh cultures of *E. coli, Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella* Typhimurium, and *Staphylococcus aureus* were grown in Difco tryptic soy broth (Difco, Becton Dickinson, Sparks, Md.) at 35°C for 18 to 24 h. *Campylobacter jejuni* cultures

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were grown on Difco Campylobacter agar (Difco, Becton Dickinson) Blaser plates, at 42°C under microaerophilic conditions in a BD BBL GasPak Jar (BBL, Becton Dickinson) with an activated CampyPak Plus hydrogen + CO_2 with integral palladium catalyst (Becton Dickinson) for 48 h. *Candida ernobii* and *Yarrowia lipolytica* were grown in Difco Sabouraud dextrose broth (Difco, Becton Dickinson) incubated at 28°C for 48 h. All microbial cultures were harvested and suspended in a solution of 0.1% Difco Bacto peptone (Difco, Becton Dickinson) water, as previously described *(12).*

Test solutions. For in vitro studies, solutions of TPP (K_3PO_4, Sigma Chemical Co., St. Louis, Mo.) at concentrations of 2.0, 3.0, and 4.0% (wt/vol) were prepared by dissolving TPP in distilled water. Mixtures of TPP and fatty acids were prepared by dissolving 0.5% (wt/vol) lauric and myristic acids (Sigma) in separate aliquots of each TPP solution. For poultry skin studies, mixtures of TPP and fatty acids were prepared by dissolving 0.5% fatty acids in 1.0% TPP, 1.0% fatty acids in 2.0% TPP, 1.5% fatty acids in 3.0% TPP, and 2.0% fatty acids in 4% TPP. The pH of the solutions was measured with a Corning 450 pH/ion meter (Corning Inc., Corning, N.Y.). All solutions were sterilized by passage through 0.2-μm-pore-size filters.

In vitro trials. In vitro trials were conducted by suspending each microbial isolate in separate tubes containing 10 ml of peptone water, TPP solution, or TPP–fatty acid mixtures to produce a final concentration of approximately 10^6 CFU/ml. The inoculated test tubes were placed on a laboratory rotator (Glas-Col, Terre Haute, Ind.), and the microbial suspensions were mixed for 5 min. After mixing, the suspensions were plated on agar media with an Autoplate 4000 automated spiral plater (Spiral Biotech, Bethesda, Md.). *E. coli*, *L. monocytogenes*, *P. aeruginosa*, *Salmonella* Typhimurium, and *S. aureus* were plated onto Difco plate count agar (Difco, Becton Dickinson) and incubated aerobically at 35°C for 18 to 24 h; *C. jejuni* suspensions were plated onto Difco Campylobacter agar Blaser plates and incubated microaerophilically at 42°C for 48 h; *C. ernobii* and *Y. lipolytica* were plated onto Difco Sabouraud dextrose agar and incubated aerobically at 28°C for 48 h. After incubation, WinCount (version 1.2, Spiral Biotech) was used to calculate numbers (log CFU per milliliter) of each isolate recovered. Each experiment was repeated six times.

Poultry skin trials. The ability of TPP and mixtures of TPP and fatty acids to reduce the population of microorganisms in the native population of the skin from processed broiler carcasses was also determined. Skin was removed from carcasses obtained from a local commercial poultry processing plant as previously described *(12).* The 5-g skin samples were washed in 45 ml of 0.1% peptone water, TPP, or TPP and fatty acids by blending in a Stomacher 400 (Seward Limited, Thetford, Norfolk, UK) for 2 min on high speed. Liquids were decanted from the bag after washing, and 45 ml of sterile distilled water was added to the skin samples in the bags. The skin was rinsed in water by blending in the stomacher for 1 min on high speed, and aliquots of the water rinsates were removed for immediate microbial analyses. The automated spiral plater was used to spread the skin rinsates onto the appropriate agar media. Total plate counts of rinsates were performed by spreading rinsates on Difco plate count agar and incubating aerobically at 35°C for 24 h; *Campylobacter* spp. were enumerated on the Difco Campylobacter agar Blaser plates incubated at 42°C for 48 h; *Enterococcus* spp. were enumerated on Difco m-Enterococcus agar and incubated at 35°C for 48 h; lactic acid bacteria were enumerated on lactic acid bacteria medium *(2)* and incubated anaerobically at 35°C for 48 h; *Pseudomonas* spp. were enumerated on Pseudomonas agar base with CFC selective agar supplement (Oxoid Ltd., Hampshire, UK) incubated aerobically at 25°C for 18 to 24 h; *Staphylococcus* spp. were enumerated on Difco Bacto Baird-Parker agar base (Difco, Becton Dickinson) supplemented with BBL egg yolk tellurite enrichment (BBL, Becton Dickinson) incubated at 35°C for 24 h; and yeasts were enumerated on Difco acidified potato dextrose agar incubated at 28°C for 5 days. *E. coli* was enumerated by plating on *E. coli*–coliform 3M Petrifilm (St. Paul, Minn.) incubated at 35°C for 48 h. Gram stains of bacterial isolates and wet mounts of yeast isolates were examined microscopically.

Statistical analysis. Group means for the number of viable cells recovered from in vitro tests and from poultry skin trials were analyzed with GraphPad InStat (version 3.05, 32 bit for Windows 95/NT, GraphPad Software, San Diego, Calif.). One-way analyses of variance (ANOVA) were performed, and the Tukey-Kramer multiple comparisons test was used to determine which treatment groups differed significantly when the ANOVA detected significant differences in group means. The minimum level of detection for all of the direct plating techniques, except for *E. coli*, was 2.0×10^1 CFU/ml, and when no microorganisms were recovered by direct plating, a value of 1.9×10^1 CFU/ml was assigned. The minimal level of detection for *E. coli* was 1 CFU/ml. All significant differences were determined at $P < 0.05$.

RESULTS AND DISCUSSION

Results of in vitro trials indicated that TPP solutions and mixtures of TPP with lauric or myristic acid are microbicidal toward several bacteria and yeasts associated with processed poultry (Table 1). No viable gram-negative bacteria (*C. jejuni*, *E. coli*, *P. aeruginosa*, or *Salmonella* Typhimurium) were recovered from cultures suspended in solutions of 2.0, 3.0, or 4.0% TPP. Although populations of gram-positive bacteria (*L. monocytogenes* and *S. aureus*) and yeasts (*C. ernobii* and *Y. lipolytica*) suspended in TPP were reduced significantly, viable cells were recovered from all of the gram-positive bacterial suspensions and yeast suspensions. Other studies have shown that most gram-negative bacteria are more sensitive to the bactericidal activity of TPP than gram-positive bacteria *(6, 12).* Apparently, the structure of the cell wall of gram-positive bacteria and yeasts provides these microorganisms with a greater degree of protection against the lethal effects of TPP. The high pH of solutions of a related compound, trisodium phosphate, has been identified as one of the factors responsible for the antimicrobial activity of the compound *(5).*

In vitro mixtures of TPP and fatty acids exhibited greater microbicidal activity than solutions containing only TPP (Table 1). Although viable yeasts were recovered from cultures suspended in TPP, they were not recovered from yeast cultures suspended in mixtures of TPP and lauric or myristic acid. Additionally, significantly fewer gram-positive bacteria were recovered from suspensions added to mixtures of TPP and fatty acids than from suspensions in TPP solutions. Although significantly fewer gram-positive bacteria and yeasts were recovered from cell suspensions in mixtures of TPP and fatty acids than from suspensions in solutions of TPP, the pHs of TPP solutions were significantly higher than the pH of mixtures of TPP and fatty

TABLE 1. *Average pH of solutions and numbers of microbial isolates recovered in vitro after 5 min of mixing in peptone water, tripotassium phosphate, or tripotassium phosphate and fatty acids*[a]

Solution	pH[b]	Isolates recovered (log CFU/ml)[c]							
		Campylobacter jejuni	*Candida ernobii*	*Escherichia coli*	*Listeria monocytogenes*	*Pseudomonas aeruginosa*	*Salmonella* Typhimurium	*Staphylococcus aureus*	*Yarrowia lipolytica*
PW	6.40 A	5.82 ± 0.08 B	6.44 ± 0.15 E	6.50 ± 0.14 B	6.61 ± 0.31 C	6.63 ± 0.16 B	6.89 ± 0.09 B	5.87 ± 0.06 C	6.38 ± 0.12 D
2.0% TPP	12.2 C	NR A	4.69 ± 0.28 D	NR A	6.31 ± 0.05 B	NR A	NR A	5.41 ± 0.29 C	6.00 ± 0.17 C
2.0% TPP and 0.5% LA	11.6 B	NR A	NR A	NR A	NR A	NR A	NR A	1.52 ± 1.22 B	NR A
2.0% TPP and 0.5% MA	11.6 B	NR A	NR A	NR A	NR A	NR A	NR A	0.81 ± 1.12 B	NR A
3.0% TPP	12.3 C	NR A	4.23 ± 0.30 C	NR A	6.30 ± 0.09 B	NR A	NR A	5.14 ± 0.90 C	5.93 ± 0.28 BC
3.0% TPP and 0.5% LA	11.8 B	NR A	NR A	NR A	NR A	NR A	NR A	1.24 ± 1.28 B	NR A
3.0% TPP and 0.5% MA	11.8 B	NR A	NR A	NR A	NR A	NR A	NR A	0.72 ± 0.96 B	NR A
4.0% TPP	12.4 C	NR A	3.46 ± 0.19 B	NR A	6.17 ± 0.15 B	NR A	NR A	5.37 ± 0.11 C	5.61 ± 0.27 B
4.0% TPP and 0.5% LA	11.8 B	NR A	NR A	NR A	NR A	NR A	NR A	NR A	NR A
4.0% TPP and 0.5% MA	11.8 B	NR A	NR A	NR A	NR A	NR A	NR A	NR A	NR A

[a] Within columns, different letters indicate significant differences in the number of isolates recovered from cell suspensions. PW, peptone water; TPP, tripotassium phosphate; LA, lauric acid; MA, myristic acid; NR, no viable cells recovered from any cell suspension.

[b] $n = 6$.

[c] Values are means ± standard deviations; $n = 6$.

TABLE 2. *Average pH of solutions and numbers of microbial isolates recovered from rinsates of poultry skin on nonselective[a] and selective[b] media after washing skin in peptone water, tripotassium phosphate, or tripotassium phosphate and fatty acids[c]*

		Isolates recovered (log CFU/ml)[e]							
Wash	pH[d]	Total plate count[a]	Campylobacters[b]	Enterococci[b]	*Escherichia coli*[b]	Lactic acid bacteria[b]	Pseudomonads[b]	Staphylococci[b]	Yeasts[b]
0.1% PW	6.40 A	4.45 ± 0.29 B	3.10 ± 0.38 C	2.62 ± 0.73 B	1.20 ± 0.73 B	3.80 ± 0.60 A	2.30 ± 0.28 C	2.81 ± 1.45 A	1.29 ± 0.60 B
1% TPP and 0.5% MA	10.97 B	3.37 ± 0.45 A	2.56 ± 1.24 BC	1.80 ± 1.02 A	0.70 ± 0.48 AB	3.45 ± 0.56 A	1.73 ± 0.84 ABC	2.51 ± 1.44 A	0.52 ± 0.60 AB
1% TPP and 0.5% LA	11.12 B	3.38 ± 0.69 A	1.39 ± 0.70 BC	1.41 ± 1.10 A	0.18 ± 0.32 AB	3.26 ± 0.95 A	1.31 ± 0.94 ABC	2.18 ± 1.07 A	0.52 ± 0.60 AB
2% TPP and 1.0% MA	11.04 B	3.61 ± 0.60 AB	1.87 ± 0.97 ABC	1.58 ± 0.92 A	0.62 ± 0.55 AB	3.14 ± 0.89 A	1.12 ± 0.84 ABC	2.49 ± 1.38 A	0.52 ± 0.60 AB
2% TPP and 1.0% LA	11.19 B	3.58 ± 0.50 AB	1.01 ± 0.69 AB	1.59 ± 1.26 A	0.13 ± 0.21 A	3.25 ± 0.34 A	1.05 ± 0.79 ABC	2.29 ± 1.11 A	0.52 ± 0.60 AB
3% TPP and 1.5% MA	11.10 B	3.60 ± 0.62 AB	2.17 ± 0.62 BC	1.87 ± 0.95 A	0.85 ± 0.49 AB	3.43 ± 0.69 A	1.47 ± 1.05 AB	2.61 ± 1.41 A	NR A
3% TPP and 1.5% LA	11.25 B	3.10 ± 0.50 A	1.46 ± 0.75 AB	1.74 ± 0.94 A	0.20 ± 0.31 AB	3.07 ± 1.06 A	0.41 ± 0.67 ABC	2.41 ± 1.21 A	NR A
4% TPP and 2.0% MA	11.13 B	3.40 ± 0.58 A	1.72 ± 0.91 ABC	1.20 ± 1.19 A	1.00 ± 1.00 AB	3.15 ± 0.60 A	1.26 ± 1.00 A	2.57 ± 1.46 A	NR A
4% TPP and 2.0% LA	11.31 B	2.91 ± 0.49 A	0.44 ± 0.76 A	1.33 ± 1.02 A	0.16 ± 0.38 A	3.32 ± 0.95 A	NR A	2.43 ± 1.15 A	NR A

[a] Plate count agar.

[b] Campylobacter agar Blaser plates; m-Enterococcus agar; *E. coli*–coliform 3M Petrifilm; lactic acid bacteria medium; Pseudomonas agar base with CFC selective agar supplement; Difco Bacto Baird-Parker agar base supplemented with BBL egg yolk tellurite enrichment; Difco acidified potato dextrose agar.

[c] Within columns, different letters indicate significant differences in the number of isolates recovered from cell suspensions. PW, peptone water; TPP, tripotassium phosphate; LA, lauric acid; MA, myristic acid; NR, no viable cells recovered from any skin rinsate.

[d] $n = 6$.

[e] Values are means ± standard deviations; $n = 6$.

acids. Therefore, factors other than pH must also play a role in the antimicrobial activity of TPP and fatty acids.

The antibacterial activity of fatty acids is related to the ability of these compounds to act as surfactants *(14, 17)* that can disrupt the cell walls of microorganisms. The alkali salts of fatty acids are surfactants that are formed when fatty acids combine with alkaline compounds similar to TPP and are known to possess antimicrobial activity *(14)*. No viable *L. monocytogenes* cells were recovered from 2.0, 3.0, or 4.0% TPP supplemented with 0.5% lauric or myristic acid. *S. aureus* cultures were more resistant to the synergistic antibacterial activity of TPP and fatty acids. Viable *S. aureus* cells were recovered from cultures suspended in mixtures of lauric or myristic acid in 2.0 or 3.0% TPP, but the bacteria were not recovered from cultures suspended in mixtures of fatty acids and 4.0% TPP.

Washing poultry skin in mixtures of TPP and fatty acids significantly altered the native microflora of the skin (Table 2). The skin might provide microorganisms some protection against the microbicidal activity of TPP and fatty acids because proteins and lipids in the skin can combine with surface-active agents and reduce their antimicrobial activity *(14)*. Nonetheless, washing skin in mixtures of TPP and lauric or myristic acid produced significant reductions in the number of total aerobic bacteria, gram-negative bacteria (campylobacters, *E. coli*, pseudomonads), and yeasts recovered from rinsates of poultry skin. No yeasts were recovered from rinsates of skin washed in mixtures of 3.0% TPP and 1.5% lauric or myristic acid or 4.0% TPP and 2.0% lauric or myristic acid. Lauric acid appeared to have significantly more antibacterial activity than myristic acid because significantly fewer total bacteria and pseudomonads were recovered from skin washed in 4% TPP and 2.0% lauric acid than from skin washed in 4% TPP and 2.0% myristic acid. Washing the skin in mixtures of TPP and lauric or myristic acid produced no significant change in the number of gram-positive rods and cocci (enterococci, lactic acid bacteria, and staphylococci) recovered from the skin rinsates, however. Other research has shown that washing skin in mixtures of TPP and potassium oleate significantly reduces the number of aerobic bacteria, *Enterobacteriaceae*, *Campylobacter*, and enterococci recovered from skin rinsates and skin samples *(12)*.

Reducing microbial contamination of processed poultry might reduce the number of human foodborne diseases related to processed poultry and extend the shelf life of fresh poultry products. The ability of components of poultry skin to reduce the antimicrobial activity of mixtures of TPP and fatty acids could limit the use of these types of compounds as carcass decontaminants; however, these compounds could potentially be used as processing equipment sanitizers. Additional research will examine potential uses of these types of compounds in poultry processing environments.

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