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Bacteria Recovered from Whole-Carcass Rinsates of Broiler Carcasses Washed in a Spray Cabinet with Lauric Acid-Potassium Hydroxide

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Abstract: Effects of spray washing carcasses with Lauric Acid (LA)-Potassium Hydroxide (KOH) on bacteria recovered from Whole-Carcass Rinsates (WCR) were examined. Carcass skin was inoculated with antibiotic resistant strains of *Escherichia coli*, *Salmonella* Typhimirum and *Campylobacter coli*. The first trial examined effects of washing carcasses with water, 0.25% LA-0.125% KOH, 0.50% LA-0.25% KOH, 1.00% LA-0.50% KOH, or 2.00% LA-1.00% KOH at 80 psi for 15 sec. Findings indicated that significantly fewer Total Plate Count (TPC) bacteria, *E. coli* and *Salmonella* Typhimirum were recovered from carcasses washed with 2.00% LA-1.00% KOH than from carcasses washed with water and that no *C. coli* were recovered from carcasses washed with 2.00% LA-1.00% KOH. Another trial examined effects of washing carcasses at 60, 100, or 150 psi of pressure with 2.00% LA-1.00% KOH for 15 sec. Findings indicated that significantly fewer TPC bacteria were recovered from rinsates of carcasses washed with 100 psi than from carcasses washed with 60 or 150 psi. Finally, a trial was conducted to examine effects of washing carcasses for 0, 5, 15, or 30 sec with 2.00% LA-1.00% KOH at 100 psi. Results indicated that significantly fewer bacteria were recovered from carcasses washed for 5 sec than from unwashed carcasses. Furthermore, significantly fewer TPC bacteria and *Salmonella* Typhimirum were recovered from carcasses washed for 15 sec than for 5 sec and no *C. coli* were recovered from carcasses washed for 15 or 30 sec. Findings indicate that spray washing carcasses with LA-KOH can affect the number of bacteria recovered from WCR. These studies also provide data that may be useful in designing applications for using of microbicidal surfactants in processing operations.

Key words: Lauric acid, potassium hydroxide, spray washing, broilers, carcasses

INTRODUCTION

The use of on-line carcass washers to remove fecal contamination and other debris from carcasses during processing was approved in the Unites States after it was shown that spray washing was as effective as carcass trimming in removing carcass contamination (Blankenship *et al*., 1975). Carcass washers include such devices as brush washers with bristles that scrub carcasses during washing, inside-outside carcass washers that spray water inside of the body cavity and outside of the carcass and spray cabinet washers composed of a cabinet that encloses a battery of spray nozzles that spray-wash passing carcasses (Keener *et al*., 2004). Spray cabinets and other carcass washers may be employed at various sites in the line to wash carcasses during processing; however, spray washing varies in its ability to reduce microbial carcass contamination (Bashor *et al*., 2004; Berrang and Bailey, 2009; Northcutt *et al*., 2003; Northcutt *et al*., 2005; Li *et al*., 2002; Sakhare *et al*., 1999). The utilization of surfactants (Keener *et al*., 2004) and other food grade chemicals (Li *et al*., 1997) during spray washing might improve the ability of washing to reduce carcass contamination.

Alkaline salts of fatty acids, such as oleic, myristic, or lauric acids, are microbicidal surfactants that can kill bacteria *in vitro* and on poultry skin (Hinton and Ingram, 2003; Hinton *et al*., 2007). Alkaline salts of fatty acids are Generally Recognized as Safe (GRAS) substances that possess little or no human toxicity (Kabara *et al*., 1977; Kabara, 1979). Many plant oils serve as sources of fatty acids and lauric acid is the major fatty acid associated with coconut oil which is widely used in soap manufacturing. Adding these microbicidal surfactants to water used in spray cabinets may improve the efficacy of carcass washing to reduce microbial contamination of processed broiler carcasses by reducing the surface tension of water and by killing microorganisms. The purpose of the present study was to examine changes in the bacterial flora of rinsates of whole broiler carcasses washed in solutions of lauric acid-potassium hydroxide.

MATERIALS AND METHODS

Collection and inoculation of broiler carcasses: For each trial eviscerated carcasses were taken from the processing line of a local, commercial poultry

processing facility and placed in separate plastic bags. Also, broiler intestinal tracts with ceca were obtained from the carcass evisceration line and pooled in one plastic bag. Bagged carcasses and intestinal tracts were placed on crushed ice in coolers and transported to the pilot plant poultry processing facility.

An inoculated cecal paste was prepared by mixing fresh bacterial cultures with pooled cecal contents from broiler intestinal tracts using modifications of a previously described procedure (Northcutt *et al*., 2005). Cultures of nalidixic acid resistant (nal^r) *Escherichia coli* and *Salmonella* Typhimirum were grown in Tryptic Soy Broth (Becton, Dickson and Co., Sparks, MD 21152) and harvested as previously described (Hinton and Ingram, 2003). Gentamicin resistant (gent^r) cultures of *Campylobacter coli* was grown under microaerophilic conditions in a BBL GasPak Jar System with an activated BBL CampyPak Plus gas generator envelope for 24 h at 42^{o}C on Remel Blood Agar Base #2 (Remel Products, Lenexa, KS 66215) supplemented with 7% lysed horse blood (Lampire Biological Laboratories, Pipersville, PA 18947). After incubation, *C. coli* cultures were harvested as previously described (Hinton and Ingram, 2003). A bacterial suspension containing approximately 10^8 cfu/ml each of *E. coli* nal^r, *Salmonella* Typhimirum nal^r and *C. coli* gent^r was prepared by combining equal volumes of suspensions of these isolates. Ceca were cut from the intestines and cecal contents were collected. Five grams of pooled cecal contents and 0.3 ml of the bacterial suspension were mixed with a sterile spatula. A bacterial spreader was then used to spread 0.1 g of the inoculated cecal paste over the outer surface of the carcasses and the inoculated carcasses were allowed to hang for 15 min to simulate the longest possible time of carcass contamination in processing facilities (Northcutt *et al*., 2005).

Spray washing carcasses: The effect of LA-KOH concentration used during spray washing on recovery of bacteria from carcass rinsates was determined by washing carcasses in solutions containing various concentrations (% w/v) of Lauric Acid (LA) (Sigma Chemical Co., St. Louis, MO 63178) and Potassium Hydroxide (KOH) (Spectrum Chemical Manufacturing Corp., Gardena, CA 90248). LA-KOH solutions were prepared by dissolving KOH in deionized water then dissolving LA in KOH solutions. Concentrations of 0.25% LA-0.125% KOH, 0.5% LA-0.25% KOH, 1.00% LA-0.50% KOH, 2.00% LA-1.00% KOH were prepared, and sterile deionized water served as the control wash solution.

Fifty carcasses and 5 intestinal tracts with ceca were obtained from the processing facility.

Carcasses were hung on shackles, inoculated with 0.1 g of cecal paste containing antibiotic resistant bacterial isolates and divided into 5 groups of 10 carcasses each. After hanging for 15 min, each group of carcasses was washed for 15 sec at 80 psi with water or one concentration of LA-KOH. During washing, carcasses were manually oscillated back and forth through a 20^o arc within the spray cabinet to simulate movement along the processing line. After washing, carcasses were rinsed with sterile deionized water from a garden sprayer for 15 sec to remove excess LA-KOH. Washed carcasses were then allowed to hang for 1 min to allow liquid to drip from the carcasses before performing a Whole Carcass Rinse (WCR) to recover bacteria remaining on the carcass after washing.

The effect of spray wash pressure and the effect of washing time on the recovery of bacteria from carcass rinsates were also determined. The spray wash pressure trials were conducted by collecting 30 carcasses and 5 intestinal tracts with ceca from the processing facility. The cecal paste with antibiotic resistant bacterial isolates was applied and carcasses were divided into 3 groups of 10 carcasses each. After 15 min, each group of carcasses was washed for 15 sec at 60, 100, or 150 psi with a solution of 2.00% LA-1.00% KOH during manual oscillation within the spray cabinet. Finally, the spray wash time trials were conducted by collecting 40 carcasses and 5 intestinal tracts with ceca from the processing facility. After carcasses were inoculated with the cecal paste, carcasses were divided into 4 groups of 10 carcasses each. Each group of carcasses was washed for 0, 5, 15, or 30 sec at 100 psi with a solution of 2.00% LA-1.00% KOH. After spray washing for each trial, a garden sprayer was used to rinse carcasses with deionized water, as described above.

Whole carcass rinses: Each washed carcasses was transferred to a plastic bag and 200 ml of sterile Phosphate Buffered Saline (PBS, pH 7.4) was added. The bags were closed, placed in containers on an automated mechanical shaker (Dickens *et al*., 1985), and carcasses were shaken in PBS for 2 min. After shaking, aliquots of the carcass rinsates were decanted for microbial analysis and pH measurement.

Selected bacterial populations in the rinsates were enumerated by culturing serial dilutions of WCR samples on nonselective and selective bacteriological media. Total aerobic and facultative bacteria (TPC) were enumerated on Difco Plate Count Agar (Becton, Dickson and Co.) incubated aerobically at 35^{o}C for 24 h. *E. coli* nal^r were enumerated on Levine Eosin Methylene Blue (EMB) Agar supplemented with 200 mg/liter nalidixic acid (Sigma). After incubation at 35^{o}C for 24 h, typical *E. coli*-like colonies with a blue-black metallic sheen on EMB Agar were counted. *Salmonella* Typhimirum nal^r were enumerated on BGS Sulfa Agar supplemented with 25 mg/liter nalidixic acid and 100 mg/liter novobiocin

(Sigma). Inoculated plates were incubated aerobically at 35°C for 24 h. Typical colonies were selected from BGS agar for confirmation as *Salmonella* by examining biochemical reactions on Difco Triple Sugar Iron Agar (Becton, Dickson and Co.) and Difco Lysine Iron Agar (Becton, Dickson and Co.) and serological reactions with Difco Salmonella O antiserum, Group B factors 1, 4, 5 and 12 (Becton, Dickson and Co.). *C. coli* gent^r were enumerated on Oxoid Blood Agar Base (Oxoid Limited, Basingstoke, Hampshire RG24 8PW, England) supplemented with 7.0% lysed horse blood (Lampire Biological Laboratories, Pipersville, PA 18947), Oxoid *Campylobacter* Selective Supplement (Blaser-Wang, Oxoid Limited) and 200 mg/liter of gentamicin (Sigma). Inoculated *Campylobacter* plates were incubated at 42°C for 48 h in a BBL GasPak Jar System (Becton, Dickson and Co.) with an activated BBL CampyPak Plus gas generator envelope (Becton, Dickson and Co.). Typical colonies were confirmed as *Campylobacter* by latex agglutination using the Latex-CAMPY(jcl)^{TM} *Campylobacter* Culture Confirmation Test Latex-CAMPY (jcl)^{TM} (Integrated Diagnostics, Inc. Baltimore, MD 21227). The pH of rinsates was measured electronically with a Corning pH/Ion meter (Corning Inc., Corning, NY 14831).

Statistical analysis of data: The number of Colony-Forming-Units (CFU)/ml of rinsate was transformed to $\log_{10}$ cfu/ml before conducting statistical analysis using GraphPad InStat® version 4.00 for Windows 95 (GraphPad Software. San Diego, CA. 92130). One-way Analysis of Variance (ANOVA) with Tukey-Kramer Multiple Comparison test was performed to determine significant differences in group means. The P value for all statistical tests was $\leq$0.05.

RESULTS AND DISCUSSION

Effect of LA-KOH concentration on bacteria recovered from WCR solutions after washing carcasses in a spray cabinet: The concentration of LA-KOH used to wash carcasses in a spray cabinet affected the number of bacteria recovered from carcass rinsates (Table 1). Significantly fewer TPC bacteria were recovered from rinsates of carcasses washed in 2.00% LA-1.00% LA than from rinsates of carcasses washed in water or in more dilute concentrations of LA-KOH. Additionally, significantly fewer *E. coli* nal^r and *Salmonella* Typhimirum nal^r were recovered from rinsates of carcasses washed in 2.00% LA-1.00% KOH than from carcasses washed in water, although there was no significant difference in the number of these bacteria recovered from rinsates of carcasses washed in 0.25% LA-0.125% KOH, 0.5% LA-0.25% KOH, 1.00% LA-0.5% KOH or water. No *C. coli* gent^r were recovered from rinsates of carcasses washed in 2.00% LA-1.00% KOH, and significantly fewer of these bacteria were recovered from rinsates of carcasses washed in 1.00% LA-0.5% KOH than from carcasses washed in water or in 0.5% LA-0.25% KOH. The concentration of sanitizer used in carcass washing operations is an important consideration and increasing the concentration of sanitizers during spray washing has been shown to decrease carcass contamination (Huang and Chang, 1997; Li *et al*., 1997).

Although a buffer, PBS, was used to rinse carcasses after washing with LA-KOH, the pH of rinsates from carcasses washed in higher concentrations of LA-KOH was significantly higher than the pHs of carcasses washed in lower concentrations of LA-KOH (Table 1). The increase in pH of WCR rinsates associated with washing carcasses in LA-KOH probably indicated that there was a carry-over of the alkaline salt of the fatty acid from the carcass to the rinsates. The high pH of LA-KOH solutions is produced by KOH which is bactericidal towards Gram negative bacteria; when fatty acids are added to KOH solutions there is a significant increase in the bactericidal activity of these solutions although no significant change in pH occurs (Hinton and Ingram, 2006). High rinsate pH levels might have produced some portion of bacterial death in the rinsates after WCR samples were collected.

In order for LA-KOH solutions to be effective carcass sanitizers, the microbicidal surfactants must increase

Table 1: Log colony-forming-units/ml[1] recovered from carcasses inoculated with cecal paste containing *Escherichia coli* nal^r, *Salmonella* Typhimurium nal^r and *Campylobacter coli* gent^r and washed in spray cabinet with various concentrations of lauric acid-potassium hydroxide

	CFU/ml rinsate recovered				
Spray wash[3] (% w/v)	Rinsate pH	Total plate count	*E. coli* nal^r	*Salmonella* Typhimirum nal^r	*C. coli* gent^r
0.0 LA-0.0KOH	7.27^c±0.04	4.06^a±0.18	2.11^a±0.35	2.23^a±0.17	2.84^a±0.22
0.250 LA-0.125KOH	7.41^c±0.03	4.01^a±0.43	1.74^{ab}±0.44	1.98^{ab}±0.32	2.51^{ab}±0.25
0.50 LA-0.25KOH	7.57^c±0.10	3.94^a±0.34	1.86^{ab}±0.37	2.03^{ab}±0.35	2.55^a±0.17
1.00 LA-0.50KOH	8.00^b±0.10	4.02^a±0.32	2.07^a±0.29	2.26^a±0.34	1.99^b±0.83
2.00 LA-1.00KOH	9.94^a±0.23	3.46^b±0.34	1.35^b±0.54	1.64^b±0.63	NR[2]

[1]Values are averages ± standard deviation. n = 10. [2]NR = None Recovered. [3]Carcasses spray washed for 15 sec at 80 psi.

[abcd]Within columns, different letters indicate significant ($p\leq0.05$) differences in the number of bacteria from recovered from carcasses rinsates

the cleansing activity of water by decreasing water tension (Keener *et al*., 2004) remove fats and proteins that provide protection to microorganisms on the surface of the skin and then wash away or kill bacteria found on the skin of the carcass. When the effect of LA-KOH concentration on the native bacterial flora of carcasses was examined by shaking carcasses on a mechanical shaker in solutions of LA-KOH for 1 min, a solution of 0.50% LA-0.25% KOH produced a significant reduction in the number of TPC bacteria recovered, while no native *Campylobacter* sp. were recovered from carcass rinsates when the concentration was increased to 1.00% LA-0.50% KOH and no native *E. coli* were recovered when the concentration was increased to 2.00% LA-1.00% KOH (Hinton *et al*., 2007). However, this concentration of LA-KOH should not cause undesirable organoleptic changes in the washed carcasses that would be detected by the consumer.

Effect of spray pressure on bacteria recovered from WCR solutions after washing with LA-KOH in a spray cabinet: The pressure used during spray washing may affect the number of bacteria recovered from carcass rinses under some circumstances (Huang and Chang, 1997), but washing carcasses with 2.00% LA-1.00% KOH within the range of pressures examined in the present study only played a limited role in influencing the number of bacteria recovered from carcass rinses (Table 2). There was no significant difference in the number of *E. coli* nal^r or *Salmonella* Typhimurium nal^r recovered from rinsates of carcasses washed at 60, 100, or 150 psi with LA-KOH. No *C. coli* gent^r were recovered from any carcasses washed with this solution, but this was probably due to the bactericidal properties of the concentration of LA-KOH used. TPC from rinsates of carcasses washed with 100 psi were significantly lower that counts from carcasses rinsed with 60 or 150 psi, however. Research that examined using different pressures to spray wash carcasses with chlorine indicated that there was no significant difference in the number of bacteria recovered from the carcasses washed with 60 or 100 psi, but significantly more bacteria were recovered from carcasses when pressure was reduced to 30 psi or lower (Huang and Chang, 1997).

Effect of time of spray washing with LA-KOH on bacteria recovered from WCR solutions: The amount of time that carcasses were washed with LA-KOH had a significant effect on the number of bacteria recovered from carcass rinses (Table 3). Significantly fewer TPC bacteria, *E. coli* nal^r, *Salmonella* Typhimirum nal^r and *C. coli* gent^r were recovered from rinsates of carcasses spray washed for 5 sec than from rinsates of carcasses that were not washed.

Additionally, when wash time was increased from 5-15 sec, the number of TPC bacteria and *E. coli* nal^r recovered were significantly reduced, but increasing

Table 2: Log colony-forming-units/ml[1] recovered from carcasses inoculated with cecal paste containing *Escherichia coli* nal^r, *Salmonella* Typhimurium nal^r and *Campylobacter coli* gent^r and washed at various pressures in a spray cabinet with a solution of lauric acid-potassium hydroxide

	CFU/ml rinsate recovered				
Spray wash, PSI[3]	Rinsate pH	TPC	*E. coli* nal^r	*Salmonella* Typhimirum nal^r	*C. coli* gent^r
60	9.12^a±0.32	2.88^a±0.38	0.37^a±0.63	0.76^a±0.71	NR[2]
100	9.18^a±0.20	2.31^b±0.29	0.33^a±0.54	0.70^a±0.95	NR
150	9.24^a±0.24	2.91^a±0.49	0.33^a±0.54	1.23^a±0.26	NR

[1]Values are averages ± standard deviation. n = 10. [2]NR = None Recovered.

[3]Carcasses spray washed for 15 sec with a solution of 2.00% LA-1.00%KOH.

[ab]Within columns, different letters indicate significant ($p \leq 0.05$) differences in the number of bacteria from recovered from carcasses rinsates

Table 3: Log colony-forming-units/ml[1] recovered from carcasses inoculated with cecal paste containing *Escherichia coli* nal^r, *Salmonella* Typhimurium nal^r and *Campylobacter coli* gent^r and washed at various times in spray cabinet with lauric acid-potassium hydroxide

	CFU/ml rinsate recovered				
Spray wash time, sec[3]	Rinsate pH	TPC	*E. coli* nal^r	*Salmonella* Typhimirum nal^r	*C. coli* gent^r
0	7.16^d±0.10	4.37^a±0.16	3.12^a±0.07	3.21^a±0.10	3.44^a±0.14
5	8.12^c±0.27	3.44^b±0.47	1.49^b±0.62	1.76^b±0.42	0.55^b±0.89
15	9.05^b±0.28	2.42^c±0.29	0.13^c±0.41	1.33^{bc}±0.58	NR
30	9.44^a±0.20	2.77^c±0.36	0.16^c±0.51	1.04^c±0.75	NR

[1]Values are averages ± standard deviation. n = 10. [2]NR = None Recovered.

[3]Carcasses spray washed at 100 psi with a solution of 2.00% LA-1.00%KOH.

[abcd]Within columns, different letters indicate significant ($p \leq 0.05$) differences in the number of bacteria from recovered from carcasses rinsates

wash time from 15-30 sec did not produce further significant decrease in the number of these bacteria recovered from the rinsates. No *C. coli* gent^r were recovered from rinsates of carcasses washed for 15 or 30 sec and significantly fewer *Salmonella* Typhimirum nal^r were recovered from rinsates of carcasses rinsed for 30 sec than from carcasses rinsed for only 5 sec. Increasing the length of time that carcasses are washed with sanitizers generally produces a greater reduction in carcass bacterial contamination (Li *et al*., 1997). Additionally, increasing the amount of time that carcasses were washed with LA-KOH also significantly increased the pH of carcass rinsates. The higher pH of rinsates indicated that by increasing the length of time of exposure to LA-KOH more of the solution was absorbed by the skin of the carcass; therefore, there was probably a greater amount of carry-over of LA-KOH wash solution into carcass rinsates.

Alkaline salts of lauric acid are soaps that can reduce microbial contamination by acting as surfactants that aid in the physical removal of bacteria from surfaces during washing or by acting as microbicides that can kill microorganisms by rupturing cellular membranes and causing leakage of the cellular contents (Galbraith and Miller, 1973). Although popular sanitizers such as chlorine (Lillard, 1979) and trisodium phosphate (Lillard, 1994) are currently used by commercial poultry processors to reduce contamination of poultry meat by pathogenic and spoilage microorganisms, microbial contamination of processed poultry continues to be a major food safety and storage issue (Zhao *et al*., 2001). Furthermore, the European Union has banned the import of poultry processed with chlorinated water (Russell, 2007) because of health concerns related to the possible formation of human carcinogens in the meat. Since fatty acids are GRAS substances that are normally found in food, carcasses washed in these surfactants should not present similar concerns. Studies which examine the utilization of natural microbicides, such as alkaline salts of fatty acids, in poultry processing operations may lead to the practical application of these microbicidal surfactants as sanitizers to decrease microbial contamination of processed poultry products.

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