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In vitro EFFECTS OF SODIUM HYPOCHLORITE, TRISODIUM PHOSPHATE AND ORGANIC ACIDS ON THE DECONTAMINATION OF *Salmonella enterica* Serovar Enteritidis ON CHICKEN SKIN

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Abstract

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In vitro EFFECTS OF SODIUM HYPOCHLORITE, TRISODIUM PHOSPHATE AND ORGANIC ACIDS ON THE DECONTAMINATION OF *Salmonella enterica* Serovar Enteritidis ON CHICKEN SKIN

This study was conducted to evaluate the effectiveness of five chemical disinfectants, including sodium hypochlorite (CL), trisodium phosphate (TSP) and organic acids; acetic acid (AA), citric acid (CA) and lactic acid (LA), in reducing of the contamination level of *Salmonella* enterica Serovar Enteritidis (*S.* Enteritidis) on chicken skin. Chicken drumsticks were artificially contaminated with the kanamycin resistant *S.* Enteritidis (SEnKmr) to obtain 10^5-10^6 CFU/cm^2. The five disinfectants used were: CL; 25, 50 and 100 ppm, TSP; 4, 8 and 12%, AA, LA and CA; 0.6, 1.2 and 2.4%. The disinfectants were applied by either dipping for 1, 3 and 5 min or spraying for 10 s. The number of viable cells were enumerated after 30 min incubation at room temperature (RT;ca 25^{o}C) and compared to that from untreated controls. The results showed good potential for 4% and 8% TSP and 0.6% and 1.2% acetic acid, lactic acid and citric acid, as disinfectants that caused no changes in the physical appearance of the skin. Serovar Enteritidis cells were inhibited most when dipped for 5 min or sprayed with 2.4% acetic acid (1.70 and 1.78 log CFU/cm^2 reduction, respectively). Chicken carcasses sprayed with 2.4% AA were further investigated by storage at RT for 1, 6 and 24 h and at 4^{o}C for 1, 24, 72 and 120 h. The surviving cells were enumerated at the specified times. Storage at 4^{o}C produced a rapid loss of viable cells, with no detectable viability after 120 h. A combination of spraying with disinfectants and 4^{o}C storage prolonged the activity of the disinfectants. The results support the benefit of decontamination and warrant further research.

Keywords : disinfectants, decontamination, *S.* Enteritidis

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***ผู้รับผิดชอบบทความ**

บทคัดย่อ

รุ่งทิพย์ ชวนชื่น* ไฉไล คูวัฒนานุกูล วลาศินี รักขาว ฐานิส ดำรงวัฒนโภคิน

ประสิทธิภาพของโซเดียมไฮโปคลอไรท์ ไตรโซเดียมฟอสเฟต และกรดอินทรีย์ต่อการลดการปนเปื้อนของ ซาลโมเนลลา เอ็นเตอริก้า ซีโรวาร์ เอ็นเตอรัยติดิส บนหนังไก่

ทำการศึกษาประสิทธิภาพของสารเคมีที่ใช้เป็นยาฆ่าเชื้อ 5 ชนิด ได้แก่ โซเดียมไฮโปคลอไรท์ ไตรโซเดียมฟอสเฟต กรดอะซิติก กรดแลคติค และกรดซิติคต่อการลดระดับการปนเปื้อนซาลโมเนลลา เอ็นเตอรัยติดิสบนหนังไก่ โดยทำการเพาะ เชื้อซาลโมเนลลา เอ็นเตอรัยติดิสที่ดื้อต่อยากานามัยซินบนหนังไกให้ได้จำนวน 10^5-10^6 CFU/cm^2 และให้สัมผัสยาฆ่าเชื้อด้วยการจุ่มนาน 1, 3 และ 5 นาทีหรือฉีดพ่นนาน 10 วินาทีที่ความเข้มข้นต่างๆ ดังนี้ โซเดียมไฮโปคลอไรท์ 25, 50 และ 100 ppm ไตรโซเดียมฟอสเฟต 4, 8 และ 12% และกรดอินทรีย์ ได้แก่ กรดอะซิติก กรดแลคติคและกรดซิตริค 0.6, 1.2 และ 2.4% เก็บตัวอย่างที่อุณหภูมิห้องเฉลี่ย 25 องศาเซลเซียส (°C) นาน 30 นาทีแล้วนับจำนวนโคโลนีเปรียบเทียบกับกลุ่มควบคุม พบว่าสารที่สามารถลดการปนเปื้อนได้ดีโดยที่ไม่ส่งผลให้เกิดการเปลี่ยนแปลงทางกายภาพคือ ไตรโซเดียมฟอสเฟต 4 และ 8% กรดอะซิติก กรดแลคติคและกรดซิตริค 0.6 และ 1.2% การจุ่มและฉีดพ่นด้วยกรดอะซิติก 2.4% สามารถลดการปนเปื้อนได้มากที่สุดคือ 1.70 และ 1.78 log CFU/cm2 จากนั้นทำการศึกษาต่อด้วยการฉีดพ่นกรดอะซิติค 2.4% โดยเก็บตัวอย่างที่อุณหภูมิห้องแล้วนับจำนวนโคโลนีที่ 4, 6 และ 24 ชั่วโมง และเก็บที่ 4°C แล้วนับจำนวนโคโลนีที่ 1, 24, 72 และ 120 ชั่วโมง พบว่าการเก็บที่ 4°C สามารถลดจำนวนเชื้อได้อย่างรวดเร็วและลดถึงระดับที่ไม่สามารถตรวจพบได้เมื่อเก็บไว้นาน 120 ชั่วโมง โดยการใช้ยาฆ่าเชื้อด้วยการฉีดพ่นร่วมกับการเก็บที่ 4°C ช่วยยืดระยะเวลาในการออกฤทธิ์ของสาร ผลการศึกษาในครั้งนี้ชี้ให้เห็นถึงคุณประโยชน์ของการใช้ยาฆ่าเชื้อเพื่อลดการปนเปื้อนเชื้อซาลโมเนลล่าและควรที่จะมีการศึกษาวิจัยเพิ่มเติมต่อไป

คำสำคัญ : ยาฆ่าเชื้อ การลดการปนเปื้อน ซาลโมเนลลา เอ็นเทอรัยติดิส

Introduction

All species and strains of *Salmonella* may be presumed to be pathogenic to man (Tietjen and Fung, 1995) and poultry and poultry products have been known as the major sources of *Salmonella* infections (Grijspeerdt and Herman, 2003). *Salmonella enterica* Serovar Enteritidis (*S.* Enteritidis) is one of the most common causes of food-borne disease (Poppe, 1995). Recently, a prosthetic valve endocarditis, caused by *S.* Enteritidis, has been reported (Gonen et al., 2003). The incidence of *S.* Enteritidis infections has increased in many countries and its virulence characteristics have been analyzed (Lu et al., 1999). In the United States, infections by the pathogen have increased and exceed those of *S.* Typhimurium (Poppe, 1995). In Thailand, the incidence rapidly increased from 1.33% to 16.98% during 1991-1992 and the bacterium is the most common *Salmonella* isolated from both chicken and from patients with Salmonellosis (Jerngklinchan et al., 1994). Poultry products are the major source of human Salmonellosis in the country.

Salmonella spp. usually contaminate chicken carcasses during slaughter and primary processing. They invade, attach and multiply on the surface of the chicken's skin (Sampathkumar et al., 2003). These pathogenic bacteria contaminate food products and promote food-borne diseases. It follows that decontamination of the pathogens from chicken carcasses would be useful. Several decontamination methods have been developed to either reduce or eliminate *Salmonella* from chicken carcasses, including dipping or spraying with disinfectants. None of them, however, can guarantee the complete removal of *Salmonella*. An alternative process, ionizing radiation, has been developed (Sherry et al., 2004). It has been shown to be highly effective, safe and not significantly affecting nutritional value in raw chicken or causing any apparent changes (Lewis et al., 2002; Nassar et al., 1997). However, it does not remove physical contaminants and as the cost of the process is rather high,

it may not be applicable in all abattoirs, especially those in developing countries. Decontamination is one of the methods in a holistic approach. Dipping and spraying with effective disinfectants are still the practical methods of choice and should not be overlooked.

In the past 40 years, decontamination of *Salmonella* in raw chicken carcasses has been widely studied. However, the published literature reporting the efficacy of several disinfectants contains inconsistencies. This could be attributed to the complexity of the interaction of a number of variables, for example, type, concentration and temperature of the acids and the experimental design. To date, there is only sparse information on the decontamination of *S.* Enteritidis as most of the studies were conducted with *S.* Typhimurium. In this study, we evaluated the effectiveness of the potential disinfectants in reducing of *S.* Enteritidis contamination under defined and controlled conditions in the laboratory. The candidate compounds were sodium hypochlorite (CL), trisodium phosphate (TSP), acetic acid (AA), lactic acid (LA) and citric acid (CA). Although chlorine has been the predominant disinfectant used in poultry processing, chlorine residue on chicken carcasses has become a public concern and a hindrance to raw chicken exports. TSP, AA, LA and CA are commonly used and generally recognized as safe. This research served as a pilot study for decontamination in processing plant conditions.

Materials and Methods

Bacterial strains, media and chemicals used

A *S.* Enteritidis, kanamycin resistant strain (SEnKm^r), for which the minimal inhibitory concentra tion (MIC) was at least 200 parts per millions (ppm), was obtained from the National Institute of Health, Department of Medical Sciences (Nontatburi, Thailand). Xylose lysine desoxycholate (XLD) medium, Brain heart infusion (BHI) broth and bacto peptone were purchased from Difco Laboratories (Detroit, MI) and used as the enriching medium. For the selection medium, XLD was supplemented with 200 ppm of kanamycin (XLD.KM200) (Sigma, St. louis, MO). TSP, AA and CA were obtained from E.Merck (Darmstadt, Germany). CL and LA were purchased from Ajax Chemicals (N.S.W., Australia) and BDH laboratories (TD, England), respectively.

Preparation of cell suspensions for artificial contamination

SEnKm^r was grown on XLD.KM200 at 37^{o}C for 24 ± 2 h. A single colony was inoculated into 100 ml of BHI and incubated for 18 h at 37^{o}C and 130 rpm on a shaking incubator (Lab-Therm®, Birsfelden, Switzerland). The inoculum was serially diluted 10-fold to 10^7-10^8 colony forming units per milliliter (CFU ml^{-1}) with 0.1% peptone saline diluent (PSD, 0.85% sodium chloride and 0.1% bacto peptone). Cell suspensions were used immediately.

Artificial contamination of chicken skin

Fresh chicken drumsticks were obtained from a retail outlet in Bangkok, Thailand. All pieces weighed between 150-200 mg, to yield at least 50 cm^2 of skin. The carcasses were tested for the presence of kanamycin resistant *Salmonella* and found to be negative. Chicken skin was inoculated with SEnKn^r as previously described with some modifications (Lillard, 1986). Briefly, chicken drumsticks were immersed in the cell suspensions at room temperature (RT; *ca* 25^{o}C) for 30 min, dipped in 500 ml of 0.1% PSD 5 times for 2 s each and allowed to drain on a stainless tray at RT for 30 min. The diluent was changed every 2 drumsticks. The procedure was first tested in the laboratory and yielded the attached cells on chicken skin of 10^5-10^6 CFU cm^{-2}. For each experiment, ten inoculated samples were removed for a viable count of SEnKm^r and served as untreated controls.

Decontamination of chicken carcasses artificially-contaminated with S. Enteritidis

The experiments were divided into 2 parts as follows:

Test 1 was carried out to determine the most efficient disinfectant(s) and treatment condition(s). The in-

oculated chicken drumsticks were treated with CL, TSP, AA, CA or LA by dipping for 1, 3 or 5 min or spraying for 10 s at specific concentrations: CL; 25, 50 and 100 ppm, TSP; 4, 8 and 12 %, organic acids; 0.6, 1.2 and 2.4 %. For the spraying treatment, a spray gun model SG112ABC (ABC®, PA, USA) with a 2-mm diameter nozzle was connected to a dynamic pump at 20-45 pound per square inch (PSI) to generate a spray volume of 100-110 ml min^{-1}. The spraying distance was 15-20 cm from the samples and covered an area of 50-100 cm^2. After treatment, 50-cm^2 of skin from each treated sample was removed, individually placed in stomach bags and incubated at RT for 60 min. Then, 100 ml of 0.1% PSD was added and each sample was blended using Stomacher Model 400 BA7021 (Seward Medical, London, England) for 3 min. One-ml aliquots of each cell suspension were removed and serially diluted with 0.1% PSD. Appropriate dilutions were plated on XLD.KM200. The viable cells were counted after incubation at 37°C for 18 h. The untreated controls were the inoculated samples stored under the same conditions without treatment and yielded the initial SEnKm^r cells.

For each disinfectant, ten drumsticks were used for each treatment (dipping or spraying) at specific concentration per time point. A 50-cm^2 sample was taken from each drumstick. No samples were used for retesting. Three inoculated samples were treated with distilled water following the same procedure (DwControl). The presence of SEnKm^r was confirmed as described in the Bacteriological Analytical Manual (BAM) (Andrews et al., 1992).

Test 2 further determined the effectiveness of 2.4% acetic acid spraying that yielded the greatest reduction of SEnKm^r in Test 1. A total of 210 drumsticks were artificially contaminated with SEnKm^r as described above. After treatment, 50-cm^2 of skin from each treated sample was individually placed in stomach bags. Ninety and 120 skin samples were stored at RT and 4°C, respectively. Thirty samples were withdrawn for viable counts as follows: RT; 1, 6 and 24 h, 4°C; 1, 24, 72 and 120 h. The viability of SEnKm^r was identified as described above. The initial cells from 30 drumsticks served as the untreated controls, being the inoculated samples without treatment.

pH measurement

The pH of each disinfectant at the desired concentrations was measured at RT using Microprocessor pH/mV/°C meter model 8417N (Hanna Instruments, Singapore) (Table 1). The measurements were performed in duplicate on 4 separate occasions.

Table 1 pH of sodium hypochlorite, trisodium phosphate, acetic acid, lactic acid and citric acids at various concentrations at 25°C (data shown for four applications)

Disinfectant	**pH (± SD)***
Chlorine	
25 ppm	7.38 ± 0.02
50 ppm	7.95 ± 0.21
100 ppm	8.72 ± 0.05
Trisodium phosphate	
4%	12.13 ± 0.01
8%	12.30 ± 0.10
12%	12.39 ± 0.04
Acetic acid	
0.6 %	2.95 ± 0.02
1.2 %	2.84 ± 0.03
2.4 %	2.68 ± 0.02
Citric acid	
0.6%	2.56 ± 0.01
1.2%	2.41 ± 0.11
2.4%	2.26 ± 0.01
Lactic acid	
0.6%	2.51 ± 0.10
1.2%	2.34 ± 0.04
2.4%	2.19 ± 0.03

*SD = Standard deviation

Statistical analysis

The number of cells was analyzed by both means and logarithm. The effectiveness of the selected disinfectants under specific conditions was determined by calculating the differences in number of SEnKm^r at each time point and the corresponding untreated controls. Data were statistically analyzed using a t-test, described elsewhere. A value of $P<0.05$ was considered statistically significant.

Results

Table 2-7 summarize the reductions of SEnKm^r on chicken skin by dipping and spraying with selected disinfectants under specific conditions. Dipping with 25 and 50 ppm CL did not eliminate SEnKm^r cells whereas 100 ppm resulted in a 0.32 to 0.41 log reduction. Concentrations of 25, 50 and 100 ppm CL by spraying caused 0.21, 0.27 and 0.46 log reductions, respectively. There was, however, no significant difference in the viable counts among the three concentrations ($P>0.05$). The chlorine residue was not determined in this study.

Inoculated chicken skin dipped with 4% TSP for 5 min, 8 and 12% for 1, 3 and 5 min or sprayed with TSP at any concentration resulted in a significant reduction of SEnKm^r, in comparison to the untreated control ($P<0.05$). Reductions by 4 and 8% TSP dipping over any length of time were not significantly different ($P>0.05$). For 12% TSP dipping, the 5-min exposure was significantly different from 1-min treatment ($P<0.05$) but not from the 3-min treatment ($P>0.05$). The 12 % concentration of TSP spraying resulted in a 1.47-log reduction, which is the greatest reduction by TSP treatment in this study.

All treatments with the selected organic acid disinfectants significantly reduced the attached SEnKm^r cells compared to the untreated samples ($P<0.05$). CA dipping at the same concentration for 1, 3 and 5 min did not yield significantly different reductions ($P>0.05$). For LA, 2.4 % LA dipping for 5 min resulted in a 1.29-log reduction in CFU cm^{-2}. Dipping with 2.4% LA for 5 min duration was significantly different from a 1-min duration ($P<0.05$) but not from a 3-min duration ($P>0.05$). When applied by spraying, 2.4% LA had a maximum reduction of 1.41 log. AA produced results comparative to LA and CA, with 2.4% AA spraying yielding the highest level of reduction. The 2.4% AA dipping for 5 min and spraying resulted in reductions of 1.70 log and 1.78 log, respectively, which were not significantly different ($P>0.05$). Due to the higher effectiveness of AA and the spraying treatment, 2.4% AA spraying was chosen for further investigation. Dipping with distilled water (Dwcontrol) did not reduce the population of SEnKm^r cells on chicken skin but water spraying yielded 0.08 - 0.09 log reduction (data not shown).

In test 2, inoculated samples were sprayed with 2.4% AA and stored at either RT or 4^{o}C (Table 7). For RT storage, the number of SEnKm^r cells was reduced by 1.76, 2.53 and 3.55 log reductions after 1, 6 and 24 hr, respectively. The SEnKm^r cells on the untreated and DW-sprayed samples showed growth with a healthy appearance. At 4^{o}C storage, 2.4% AA spraying decreased the SEnKm^r cells by 2.07, 3.12 and 5.4 log reductions after 1, 24 and 72 h, respectively, with no detectable survivors after 120 hr. The number of SEnKm^r cells on the untreated controls was reduced by 0.48, 0.16 and 0.98 log reductions after 24, 72 and 120 h, respectively. SEnKm^r cells on Dwcontrol decreased by up to 1.09 log reductions after 120 h (data not shown).

Table 2 Effect of sodium hypochlorite (CL) on the reduction of *S.* Enteritidis on chicken skin

Treatment	Concentration (ppm)	Exposure time	*S.* Enteritidis (log CFU ml^{-2})		
			Untreated* Control	Treated+ samples	Reduction‡
Dipping	25	1 min	5.58 ± 0.30	5.85 ± 0.31	-
		3 min	5.58 ± 0.30	5.88 ± 0.19	-
		5 min	5.58 ± 0.30	6.08 ± 0.15	-
	50	1 min	5.58 ± 0.30	5.69 ± 0.29	-
		3 min	5.58 ± 0.30	6.02 ± 0.17	-
		5 min	5.58 ± 0.30	5.92 ± 0.08	-
	100	1 min	5.58 ± 0.30^{a}	5.26 ± 0.23^{b}	0.32
		3 min	5.58 ± 0.30^{a}	5.25 ± 0.15^{b}	0.33
		5 min	5.58 ± 0.30^{a}	5.17 ± 0.31^{b}	0.41
Spraying	25	10 s	5.47 ± 0.15^{a}	5.26 ± 0.34^{b}	0.21
	50	10 s	5.47 ± 0.15^{a}	5.20 ± 0.37^{b}	0.27
	100	10 s	5.47 ± 0.15^{a}	5.01 ± 0.26^{b}	0.46

For the dipping treatment, values in the same concentration not bearing a common superscript are significantly different (P<0.5). For the spraying treatment at any concentration, values not bearing a common superscript are significantly different (P<0.5).

*,+Values are mean log ± SD; number of samples (n) = 10

‡Mean log of untreated control - Mean log of treated samples

Table 3 Effect of trisodium phosphate (TSP) on the reduction of *S.* Enteritidis on chicken skin

Treatment	Concentration (%)	Exposure time	*S.* Enteritidis (log CFU ml^{-2})		
			Untreated* Control	Treated+ samples	Reduction‡
Dipping	4	1 min	5.09 ± 0.14^{a}	4.80 ± 0.19^{ab}	0.29
		3 min	5.09 ± 0.14^{a}	4.78 ± 0.30^{ab}	0.31
		5 min	5.09 ± 0.14^{a}	4.73 ± 0.34^{b}	0.36
	8	1 min	5.09 ± 0.14^{a}	4.42 ± 0.23^{b}	0.67
		3 min	5.09 ± 0.14^{a}	4.38 ± 0.62^{b}	0.71
		5 min	5.09 ± 0.14^{a}	4.23 ± 0.27^{b}	0.86
	12	1 min	5.09 ± 0.14^{a}	4.08 ± 0.48^{b}	1.01
		3 min	5.09 ± 0.14^{a}	3.76 ± 0.35^{bc}	1.33
		5 min	5.09 ± 0.14^{a}	3.72 ± 0.39^{c}	1.37
Spraying	4	10 s	5.35 ± 0.16^{a}	4.80 ± 0.31^{b}	0.42
	8	10 s	5.72 ± 0.37^{a}	4.82 ± 0.30^{b}	0.90
	12	10 s	5.72 ± 0.37^{a}	4.25 ± 0.20^{c}	1.47

For the dipping treatment, values in the same concentration not bearing a common superscript are significantly different (P<0.5). For the spraying treatment at any concentration, values not bearing a common superscript are significantly different (P<0.5).

*,+Values are mean log ± SD; (n) = 10

‡Mean log of untreated control - Mean log of treated samples

Table 4 Effect of acetic acid (AA) on the reduction of *S.* Enteritidis on chicken skin

Treatment	Concentration (%)	Exposure time	*S.* Enteritidis (log CFU ml^{-2})		
			Untreated* Control	Treated+ samples	Reduction‡
Dipping	0.6	1 min	5.32 ± 0.21^{a}	4.69 ± 0.39^{b}	0.63
		3 min	5.32 ± 0.21^{a}	4.68 ± 0.39^{b}	0.64
		5 min	5.32 ± 0.21^{a}	4.64 ± 0.40^{b}	0.68
	1.2	1 min	5.23 ± 0.15^{a}	4.57 ± 0.15^{b}	0.66
		3 min	5.23 ± 0.15^{a}	4.50 ± 0.23^{b}	0.73
		5 min	5.23 ± 0.15^{a}	4.41 ± 0.29^{b}	0.82
	2.4	1 min	4.94 ± 0.24^{a}	3.71 ± 0.41^{b}	1.23
		3 min	4.94 ± 0.24^{a}	3.56 ± 0.33^{bc}	1.38
		5 min	4.94 ± 0.24^{a}	3.24 ± 0.27^{c}	1.70
Spraying	0.6	10 s	5.61 ± 0.24^{a}	4.92 ± 0.25^{b}	0.69
	1.2	10 s	5.61 ± 0.24^{a}	4.79 ± 0.26^{b}	0.82
	2.4	10 s	5.61 ± 0.24^{a}	3.83 ± 0.11^{c}	1.78

For the dipping treatment, values in the same concentration not bearing a common superscript are significantly different (P<0.5). For the spraying treatment at any concentration, values not bearing a common superscript are significantly different (P<0.5).

*,+Values are mean log ± SD; n = 10

‡Mean log of untreated control - Mean log of treated samples

Table 5 Effect of lactic acid (LA) on the reduction of *S.* Enteritidis on chicken skin

Treatment	Concentration (%)	Exposure time	*S.* Enteritidis (log CFU ml^{-2})		
			Untreated* Control	Treated+ samples	Reduction‡
Dipping	0.6	1 min	5.23 ± 0.15^{a}	4.84 ± 0.31^{b}	0.39
		3 min	5.23 ± 0.15^{a}	4.82 ± 0.35^{b}	0.41
		5 min	5.23 ± 0.15^{a}	4.72 ± 0.41^{b}	0.51
	1.2	1 min	5.36 ± 0.22^{a}	4.85 ± 0.11^{b}	0.47
		3 min	5.36 ± 0.22^{a}	4.80 ± 0.11^{b}	0.52
		5 min	5.36 ± 0.22^{a}	4.74 ± 0.05^{b}	0.58
	2.4	1 min	5.36 ± 0.22^{a}	4.29 ± 0.33^{b}	1.07
		3 min	5.36 ± 0.22^{a}	4.16 ± 0.27^{bc}	1.20
		5 min	5.36 ± 0.22^{a}	4.03 ± 0.37^{c}	1.29
Spraying	0.6	10 s	5.35 ± 0.16^{a}	4.80 ± 0.31^{b}	0.55
	1.2	10 s	5.72 ± 0.37^{a}	4.89 ± 0.21^{b}	0.83
	2.4	10 s	5.72 ± 0.37^{a}	4.51 ± 0.38^{c}	1.41

For the dipping treatment, values in the same concentration not bearing a common superscript are significantly different (P<0.5). For the spraying treatment at any concentration, values not bearing a common superscript are significantly different (P<0.5).

*,+Values are mean log ± SD; (n) = 10

‡Mean log of untreated control - Mean log of treated samples

Table 6 Effect of citric acid (CA) on the reduction of *S.* Enteritidis on chicken skin

Treatment	Concentration (%)	Exposure time	*S.* Enteritidis (log CFU ml^{-2})		
			Untreated* Control	Treated* samples	Reduction‡
Dipping	0.6	1 min	5.50 ± 0.26^{a}	5.44 ± 0.27^{b}	0.06
		3 min	5.50 ± 0.26^{a}	5.42 ± 0.18^{b}	0.08
		5 min	5.50 ± 0.26^{a}	5.39 ± 0.26^{b}	0.11
	1.2	1 min	5.50 ± 0.26^{a}	5.19 ± 0.17^{b}	0.31
		3 min	5.50 ± 0.26^{a}	4.98 ± 0.38^{b}	0.52
		5 min	5.50 ± 0.26^{a}	4.97 ± 0.25^{b}	0.53
	2.4	1 min	5.36 ± 0.22^{a}	4.69 ± 0.39^{b}	0.67
		3 min	5.36 ± 0.22^{a}	4.68 ± 0.39^{b}	0.68
		5 min	5.36 ± 0.22^{a}	4.64 ± 0.40^{b}	0.72
Spraying	0.6	10 s	5.35 ± 0.16^{a}	5.00 ± 0.16^{b}	0.35
	1.2	10 s	5.72 ± 0.37^{a}	5.20 ± 0.16^{b}	0.52
	2.4	10 s	5.72 ± 0.37^{a}	4.99 ± 0.23^{b}	0.73

For the dipping treatment, values in the same concentration not bearing a common superscript are significantly different (P<0.5). For the spraying treatment at any concentration, values not bearing a common superscript are significantly different (P<0.5).

*,*Values are mean log ± SD; n = 10

‡Mean log of untreated control - Mean log of treated samples

Table 7 Effect of 2.4%-acetic acid spraying on the reduction of *S.* Enteritidis on chicken skin stored at RT or 4°C

Storage temperature	Duration of storage (h)	*S.* Enteritidis (log CFU ml^{-2})		
		Untreated* Control	Treated* samples	Reduction‡
RT	0	5.75 ± 0.25^{a}	NT	NT
	1	5.81 ± 0.26^{a}	3.99 ± 0.25^{c}	1.76
	6	5.84 ± 0.21^{a}	3.22 ± 0.32^{d}	2.53
	24	6.60 ± 0.14^{b}	2.20 ± 0.34^{e}	3.55
4°C	0	5.64 ± 0.28^{a}	NT	NT
	1	5.69 ± 0.20^{a}	3.57 ± 0.62^{b}	2.07
	24	5.16 ± 0.32^{b}	2.52 ± 1.17^{c}	3.12
	72	4.48 ± 0.27^{c}	0.24 ± 0.62^{b}	5.40
	120	4.66 ± 0.25^{c}	ND	5.64

For each storage temperature, values not bearing a common superscript are significantly different (P<0.5).

*,*Values are mean log ± SD; n = 30

‡Mean log of untreated control at O h - Mean log of treated samples at desired duration

RT = room temperature *ca* 25°C

NT = No test

ND = Not detected

Discussion

It is well documented that contamination of enteric bacteria in chicken carcasses occurs mostly during primary processing, including scalding, defeathering, rinsing, cutting and chilling (Lillard, 1989, 1986). As the attachment of bacterial cells to the outer surface of the skin is irreversible, the firmly attached cells are a major cause of food-borne diseases (Dickson and Anderson, 1992). To be fully effective, the potential disinfectants must attack the attached cells. The experimental contamination in the present study was carried out using liquid medium inoculum followed by washing off the loose cells, allowing the effectiveness of selected disinfectants against the attached cells to be calculated per cm^2 (log of CFU cm^{-2}). The initial number of cells used was 10^5-10^6 CFU cm^{-2}, as previously suggested (Geer and Dilts, 1992). The argument can be made that the number was unrealistically high and would never be reached naturally. However, the number was in the susceptible level for a comparison study (Geer and Dilts, 1992). Bender and Brosktly (1992) reported that spraying at 25-120 PSI produced sufficient physical force to reduce the number of *Salmonella* cells without causing damage to the chicken carcasses (Bender and Brostky, 1992), 25-45 PSI spraying was used in this experiment.

Our results showed that the 100-ppm CL treatment had a significant effect on SEnKm^r but the 20- and 50-ppm CL did not. This was in agreement with Wabeck et al. (1968) who reported that 20 and 40 ppm did not eliminate *Salmonella* cells on chicken carcasses (Wabeck et al., 1968). However, it was suggested that an increased water temperature and chlorine concentration had an additive effect (Kotula et al., 1974). In this study, the 100-ppm CL spraying produced a maximum magnitude of reduction. This result is in agreement with Marshall et al. (1977) who showed that spraying with sodium hypochlorite reduced the number of aerobic bacteria on beef (Marshall et al., 1977).

TSP treatment either by dipping or spraying significantly reduced SEnKm^r cells compared to untreated controls. TSP was shown to have bacteriocidal activity when used at ≥ 8% or at a pH of 12.0 to 13.5. The high pH of TSP causes membrane damage and destruction of *Salmonella* cells (Sampathkumar et al., 2003). In this study, the pH of 4, 8 and 12% TSP was 12.13, 12.3 and 12.39, respectively, which was in the effective range (Table 1).

The inhibitory action of organic acids is primarily attributable to low pH that dissociates protonmotive force. Recently, lactic acid was shown as a membrane permeabilizer and AA was suggested to have a similar action (Alakomi et al., 2000). Even though the susceptibility of *Salmonella* to organic acids was noted, *S.* Typhimurium has been shown to be the least sensitive, in comparison to other spoilage bacteria (Geer and Dilts, 1992). LA and AA were slightly synergistic in their inhibitory effects on this pathogen (Rubin, 1978). Acuff et al. (1987) reported that neither LA nor AA reduced spoilage flora on beef subprimals (Acuff et al., 1987). Later, Cutter and Siragura (1994) showed that AA, CA and LA had equal effects on *E. coli* O157:H7 reduction on beef tissue (Cutter and Siragura, 1994). The authors concluded that the acid type was not a significant factor. Contrary to these findings our study showed that CA had the least effect on *S.* Enteritidis in comparison to AA and LA, as 2.4% AA dipping for 5 min and spraying yielded the greatest reduction of the SEnKm^r population. Distilled water spraying also reduced SEnKm^r cells purely by physical removal.

A combination of 2.4% AA spraying and low-temperature storage (4^{o}C) dramatically decreased SEnKm^r cells on chicken carcasses. The efficacy was prolonged when used for up to 120 h. Refrigeration temperatures extend the lag phase of microorganisms, thus minimizing growth and preserving the products for a longer time (Jay, 1996). Spraying treatment prevents the recontamination of the carcasses that usually happens during the dipping process, facilitates the concentration control of disinfectants used and reduces water holding in chicken

carcasses.

In this experiment, physical changes in the carcasses due to the treatments were not systemically recorded. However, the highest concentration of CL, TSP and organic acids caused changes of color, texture and odors in the carcasses. Such changes were not noticed in samples treated with 25 and 50 ppm CL, 4 and 8% TSP, and 0.6 and 1.2% organic acids when used by either dipping or spraying. The 2.4 % AA spraying with RT storage gave similar changes. No color and texture changes were apparent under 4°C storage but an acid odor was detected. One of the reasons for the smell could be the packing of treated samples in closed bags during the experiment.

The results from this study showed that a reduction of SEnKm^r was dependent on the type of disinfectant, duration of exposure, type of application and storage temperature. For the dipping method, the longer the exposure time, the less the number of the viable cells. At the same concentration for each disinfectant, the spraying treatment was more effective than the dipping treatment. The following disinfectants: 8 and 12% of TSP, and 0.6 and 1.2% of AA, LA and CA are effective in reducing *S.* Enteritidis contamination.

There are many variables that affect the susceptibility of meat borne pathogens. However, a definite solution cannot be achieved in a single experiment. Further research is required before concrete recommendations of the applications can be made. Future work should include the recovery of sub-lethally injured cells and the representation of *Salmonella* spp. The damaged cells may become more susceptible to selective agents in the media used and resulted in a lower enumeration (Jay, 2000). Resuscitation in liquid medium may have to be considered. The strain used in this experiment was a clinical isolate and facilitated the protocol. However, physiological differences among strains are unavoidable. Challenge studies with spiked cocktails are suggested.

In conclusion, disinfectant treatment can result in an economically beneficial extension of storage time and is therefore feasible for use in developing countries. The susceptibility of *S.* Enteritidis to disinfectants is worthy of further investigation. On a precautionary note, one particular concern is the disposal of the alkaline waste, which also needs evaluation.

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