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Lactic and acetic acids as bacterial decontaminators on chicken carcasses

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ABSTRACT

This study was done to evaluate the efficiency of two decontaminators (Lactic and acetic acids) for decreasing the contamination level in chicken carcasses (cut up), which had been artificially inoculated with food poisoning microorganisms (*Salmonella Enteritidis* with infective dose $10^5$and *Staphylococcus aureus* with infective dose 10^7). A total of 42 random samples chicken breast samples were collected from different markets in Gharbia governorate, Egypt. The collected chicken samples were then inoculated with the tested microorganisms *Salmonella Enteritidis* and *Staphylococcus aureus* (21 Samples for each microorganism). Thus, the samples were dipped into containers containing distilled water (Control), lactic acid 1%, 2%, 3% and acetic acid 0.5 %, 1 %, 1.5%for 20 seconds and 1 minute, respectively. The results indicated that the reduction % of acetic acid for *Salmonella Enteritidis* were 90.69%, 93.80%, 95.17% for 20 seconds and 91.39%, 96.56%, 96.90% for 1 minute, respectively. Regarding lactic acid, the reduction % was 61.43%, 94.49%, 97.41% for 20 seconds and 92.07%, 99.28%, 99.24% for 1 minute, respectively. While the reduction of *Staphylococcus aureus* by acetic acid were 95.64%, 99.63%, 99.78% for 20 seconds and 97.84%, 99.94%, 99.98% for 1 minute but it was 99.88%, 99.98%, 99.99% for 20 seconds and 99.98%, 99.99%, 100% for 1 minute for lactic acid, respectively. Lactic acid with a concentration of 3% showed the highest reduction on *Salmonella Enteritidis* and *Staphylococcus aureus.* The effect of such organic acids on Gram positive bacteria as *Staphylococcus aureus* was higher than Gram negative ones as *Salmonella Enteritidis.*

1. INTRODUCTION

Chicken meat is characterized by excellent nutritional profile as it contains protein of high biological value, vitamin, and mineral content as well as low-fat content (a large portion of which unsaturated fatty acids) empowers these meats to be optimally essential at the eating regimen in all ages (Marangoni, 2015). However, various food-borne pathogens *(Salmonella* and *Staphylococcus aureus)* can contaminate chicken meat (Bohaychuk et al., 2006).

Foodborne outbreaks of Salmonellosis are most considerably related with Salmonella in chicken meat (Ejo *et al*., 2016) and especially with nontyphoidal *Salmonella Enteritidis* and *Salmonella Typhimurium* (Saravanan *et al*., 2015).

Staphylococcus aureus is an important food-borne disease throughout the world. In several countries, the foods that most frequently incriminated in this type of food poisoning are red meat and poultry (Genigeorgis, 1989)

Organic acids (acetic and lactic) are used widely in food products as preservatives, because of their antibacterial activity. Organic acids are generally known as safe substances (GRAS) by FDA and approved as food additives by European Commission, (Surekha, 2000). Organic acids decrease in intracellular pH, the alteration of the membrane functions could be also liable for the microbial inactivation. The high concentration of anions inside the cells might increase osmolarity and consequently to the metabolic disturbance (Hirshfield et al., 2003). Lactic acid can be used to remove pathogens and reduce the microbial loads in meat carcasses. The advantage of using lactic acid is that it does not need the utilization of high spraying to eliminate the pathogens from meat. Additionally, it remains on the meat carcasses, and act as anti-microbial agent on carcass surface (Ramirez et al., 2001). Acetic acid has been investigated as an antimicrobial agent for use in poultry, to prolong its shelf-life and inhibit the growth of pathogens, such as Salmonellae (Jiménez, 2007).

Therefore, this work was planned to check the antimicrobial effect of lactic acid (1%, 2%, 3%) and acetic acid (0.5 %, 1 %, 1.5%) on chicken carcasses (cut up) experimentally inoculated with *Salmonella Enteritidis* and *Staphylococcus aureus* after 20 seconds and 1 minute.

2. MATERIAL AND METHODS

2.1. Chicken carcasses:

Accurately, 42 different samples of chicken breast (200 g each) were collected from retail markets in Gharbia Governorates. The collected samples were kept in plastic bags, transferred to laboratory in an insulated Ice box under possible aseptic conditions without delay and divided into four equal groups (21 samples per group).

2.2. Preparation of microbial suspension:

Reference strains of *S. Enteritidis* and *S. aureus* were obtained from Food Hygiene Department, Animal Health

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Research Institute, Dokki, Giza. Accordingly,4 to 5 isolated colonies of the tested strains were picked up by sterile inoculating loop and inoculated in sterile peptone water 0.1% (Merck, Germany) and were then incubated at 37°C for 24 hrs and (*Salmonella Enteritidis* with infective dose of 10^5, *Staphylococcus aureus* with infective dose of 10^7).

2.3. Preparation of decontaminators:

2.3.1. Lactic acid: lactic acid was used to prepare 1%, 2%, 3% solution.

2.3.2. Acetic acid: Acetic acid was used to prepare 0.5 % and 1% and 1.5 % (Sigma).

2.4. Inoculation of chicken breasts with the tested microorganisms:

Forty-two chicken samples were dipped in 500 ml sterile peptone water 0.1% (Merck, Germany) which contain 24 hrs-old cultures of the tested bacteria as *Salmonella Enteritidis* with infective dose of 10^5 and *Staphylococcus aureus* with infective dose of 10^7 (21 samples for each bacterium). Then the inoculated chicken samples were left for 30 minutes at temperature (25 °C) to permit attachment and absorption of the inoculated bacteria. Then, the tested microorganisms (*Salmonella Enteritidis* and *Staphylococcus aureus*) were enumerated in the control samples.

2.5. Application of the tested decontaminators:

Fourteen contaminated chicken sample with of the tested microorganisms (7 samples for *Salmonella Enteritidis* and *Staphylococcus aureus*) were dipped into containers containing 500 ml of distilled water (control) and lactic acid 1%, 2%, 3% plus acetic acid 0.5 %, 1 %, 1.5% at room temperature (25 ± 1oC) for 20 Sec, and 1 Min, respectively. Then dip the control samples in 500 ml sterile distilled water. All the containers were properly labelled. all surface of the chicken samples were covered with Solutions. The experiment was repeated three times. *Salmonella Enteritidis* count on xylose lysine deoxycholate (XLD) agar medium and *Staphylococcus aureus* counts on Baird-Parker agar plates agar medium were conducted after 20 seconds and 1 minute using the serial dilutions and spread plate technique after decontamination to work out the reduction percentages.

2.6. Statistical analysis:

The data was statistically treated by one-way ANOVA using SPSS program for windows (Version 16) (SPSS Inc. Chicago, IL, and USA) and Duncan's post hoc test with $p < 0.05$ considered to be statistically significant.

3. RESULTS

3.1. Reduction by lactic acid

Salmonella *Enteritidis* count was $9.67x10^5 \pm 4.06x10^{5a}$ (control count), it was reduced to $3.73x10^5 \pm 1.19x10^{5b}$, $5.33x10^4 \pm 6.67x10^{3b}$ and $2.50x10^4 \pm 2.89x10^{3b}$ CFU/g, when inoculated chicken parts dipped in lactic acid solution in concentrations of 1 %, 2% and 3%, for 20 seconds, respectively. (Table 1), when dipped in acetic acid with the same concentrations for 1 minutes it was reduced to $7.67x10^4 \pm 8.82x10^{3b}$, $7.00x10^3 \pm 2.65x10^{3b}$ and $37.33x10^3 \pm 4.49x10^{3b}$ CFU/g respectively (table 1). On the other hand, the reduction percentages of *Staphylococcus aureus* treated with the same concentrations were 99.88%, 99.98% and 99.99%, respectively (Table 4). *S. aureus* count was decreased from $7.73x10^8 \pm 4.27x10^{8a}$ CFU/g (control count), to $9.33x10^5 \pm 5.36x10^{5b}$, $1.93x10^5 \pm 1.55x10^{5b}$ and $6.67x10^4 \pm 3.33x10^{4b}$ CFU/g, for 20 seconds respectively (table 3) and for 1 minute it was reduced to $1.20x105 \pm 4.16x10^{4b}$, $1.03x10^5 \pm 9.84x10^{4b}$ and $3.33x10^4 \pm 3.33x10^{4b}$ CFU/g respectively (Table 3).

3.2. Reduction by acetic acid

Salmonella *Enteritidis* count was $9.67x10^5 \pm 4.06x10^{5a}$ (control count), it was reduced to $9.00x10^4 \pm 1.73x10^{4b}$, $6.00x10^4 \pm 2.08x10^{4b}$ and $4.67x10^4 \pm 1.33x10^{4b}$ CFU/g, when inoculated chicken parts dipped in acetic acid solution in concentrations of 0.5 %, 1% and 1.5%, for 20 seconds, respectively (Table 1), when dipped in acetic acid with the same concentrations for 1 minutes it was reduced to $8.33x10^4 \pm 4.48x10^{4b}$, $3.33x10^4 \pm 8.82x10^{3b}$ and $3.00x10^4 \pm 5.77x10^{3b}$ respectively (Table 1). However, the reduction percentages of *Staphylococcus aureus* treated with the same concentrations were 95.64%, 99.63% and 99.94%, respectively (Table 4). *Staphylococcus aureus* count was decreased from $7.73x10^8 \pm 4.27x10^{8a}$ CFU/g (control count), to $3.37x10^7 \pm 3.32x10^{7b}$, $2.87x10^6 \pm 1.71x10^{6b}$ and $1.67x10^6 \pm 1.17x10^{6b}$ CFU/g, for 20 seconds, respectively (table 3) and for 1 minute the reduction was $1.67x10^7 \pm 1.67x10^{7b}$, $4.63x10^5 \pm 2.68x10^{5b}$ and $1.73x10^5 \pm 7.33x10^{4b}$, respectively (Table 3).

Table 1 The effects of different concentrations of acetic and lactic acids on *Salmonella Enteritidis* (cfu/g) inoculated into the fresh chicken samples.

Groups	20 seconds	1 minute
Control	$9.67x10^5 \pm 4.06x10^{5a}$	$9.67x10^5 \pm 4.06x10^{5a}$
Acetic acid 0.5%	$9.00x10^4 \pm 1.73x10^{4b}$	$8.33x10^4 \pm 4.48x10^{4b}$
Acetic acid 1%	$6.00x10^4 \pm 2.08x10^{4b}$	$3.33x10^4 \pm 8.82x10^{3b}$
Acetic acid 1.5%	$4.67x10^4 \pm 1.33x10^{4b}$	$3.00x10^4 \pm 5.77x10^{3b}$
Lactic acid 1%	$3.73x10^5 \pm 1.19x10^{5b}$	$7.67x10^4 \pm 8.82x10^{3b}$
Lactic acid 2%	$5.33x10^4 \pm 6.67x10^{3b}$	$7.00x10^3 \pm 2.65x10^{3b}$
Lactic acid 3%	$2.50x10^4 \pm 2.89x10^{3b}$	$7.33x10^3 \pm 4.49x10^{3b}$

The mean inside the column followed by different letters is significantly different ($P < 0.05$).

Table 2 Reduction % of *Salmonella Enteritidis* (cfu/g) in the fresh chicken samples treated with different concentrations of lactic and acetic acids.

Groups	20 seconds	1 minute
Acetic acid 0.5%	90.69	91.39
Acetic acid 1%	93.80	96.56
Acetic acid 1.5%	95.17	96.90
Lactic acid 1%	61.43	92.07
Lactic acid 2%	94.49	99.28
Lactic acid 3%	97.41	99.24

Table 3 The effects of different concentrations of lactic and acetic acids on *Staphylococcus aureus* (cfu/g) in the examined fresh chicken samples.

Groups	20 seconds	1 minute
Control	$7.73x10^8 \pm 4.27x10^{8a}$	$7.73x10^8 \pm 4.27x10^{8a}$
Acetic acid 0.5%	$3.37x10^7 \pm 3.32x10^{7b}$	$1.67x10^7 \pm 1.67x10^{7b}$
Acetic acid 1%	$2.87x10^6 \pm 1.71x10^{6b}$	$4.63x10^5 \pm 2.68x10^{5b}$
Acetic acid 1.5%	$1.67x10^6 \pm 1.17x10^{6b}$	$1.73x10^5 \pm 7.33x10^{4b}$
Lactic acid 1%	$9.33x10^5 \pm 5.36x10^{5b}$	$1.20x10^5 \pm 4.16x10^{4b}$
Lactic acid 2%	$1.93x10^5 \pm 1.55x10^{5b}$	$1.03x10^5 \pm 9.84x10^{4b}$
Lactic acid 3%	$6.67x10^4 \pm 3.33x10^{4b}$	$3.33x10^4 \pm 3.33x10^{4b}$

There were significant differences between the examined samples ($P < 0.05$).

Table 4 Reduction % of *Staphylococcus aureus* (cfu/g) in the examined chicken samples treated with different concentrations of lactic and acetic acids.

Groups	20 seconds	1 minute
Acetic acid 0.5%	95.64	97.84
Acetic acid 1%	99.63	99.94
Acetic acid 1.5%	99.78	99.98
Lactic acid 1%	99.88	99.98
Lactic acid 2%	99.98	99.99
Lactic acid 3%	99.99	100

4. DISCUSSION

Contamination of food with *Staphylococcus aureus* occurs through workers who have skin lesions contain *Staphylococcus aureus*, or through sneezing or coughing. Other sources of *Staphylococcus aureus* are soil, water, and air. The surface of raw poultry meat usually contaminated with Salmonella pathogens at slaughtering, primary processing (scalding, defeathering, rinsing, cutting, mincing, and mixing) and should attach and reside within the regular and irregular surfaces of the skin, multiply and after that contaminate food preparation surfaces, hands, and utensils. The antibacterial effect of the organic acids is mainly because of the undissociated type of organic acids (Dibner and Buttin, 2002). Non-dissociated organic acids can passively diffuse through bacterial cell wall and once internalized into the neutral pH of the cell cytoplasm, they dissociate into anions and protons, which show an inhibitory effect on bacteria (Ricke, 2003).

The effect of different concentration of lactic acid (0.5%, 1%, 2% and 3%) on *S. aureus* contaminating chicken meat revealed that the counts reduced from $8x10^3$ cfu/g to 79 x 10 cfu/g (Sudershan et al., 2011).

Higher reduction percentage was achieved by (Aksoy, 2003) who decreased S. aureus count on chicken meat from $74x10^5$ to $93x10^2$ cfu/g by dipping inoculated samples in 2 % lactic acid solution.

Staphylococcus aureus count was decreased from $67x10^5$CFU/g to $69x10^4$±0.26,$46x10^4$±0.34 and $09x10^4$± 0.29 CFU/g, respectively, Where the reduction percentages achieved in the experiment were 17.28 %, 21.34 % and 27.87 % for the 3 concentrations of lactic acid used (0.75%, 1.25% and 2% solution), respectively (Saad et al., 2015).

The reduction of *Salmonella Enteritidis* prevalence using acetic acid solution was relevant only for the 2% solution, from $7x10^7$ cfu/g (positive control) at $50x10^5$±0.09 cfu/g, respectively 3%, at $81x10^4$±0.18 cfu/g. Thus, the lactic acid solutions 1%, 2%, 3% are more efficient than the acetic acid 1%, 2%, 3% (Dan et al. 2012). The 1.0% concentration of lactic acid, with a pH value of 2.47 in the processing water, achieved complete elimination of Salmonella from the 10 carcasses (Nassar, et al. 1997).

5. CONCULOSIONS

Acetic acid 1.5 % and lactic acid 3% showed the highest reduction % of *Salmonella enteritidis* after 20 seconds and 1 minute, respectively. Also, lactic acid 3% showed the highest reduction effect of *Salmonella Enteritidis* and *Staphylococcus aureus* after 20 seconds and 1 minute. Thus, good personal hygiene will help in decreasing the contamination load of chicken meat.

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