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Improving the Microbial Quality and Shelf Life of Chicken Carcasses by Trisodium Phosphate and Lactic Acid Dipping

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Abstract: This study determined the influence of Trisodium Phosphate (TSP) and Lactic Acid (LA) dipping on the microbial load and shelf life of broiler chicken carcasses during refrigerated storage for 8 days at $2\pm 1^{\circ}\text{C}$. The results indicated that both TSP (12%) and LA (2%) dipping significantly reduced the initial microbial load of Aerobic Plate Counts (APC), Psychrotrophic Counts (PTC), Total Proteolytic Counts (PLC) and *Enterobacteriaceae* Counts (EBC) just after dipping and throughout the storage period in comparison with the control. At the beginning of storage (day 0), no significant differences in the microbial reductions were detected between TSP and LA treatments. By the day 8 of the storage, however, LA-dipping indicated a higher ($p < 0.01$) mean reductions in APC, PTC and PLC than the corresponding reductions obtained by TSP-dipping. The untreated carcasses would have a refrigerated shelf life between 4 and 5 days while after chemical dipping, the shelf life extended to about 7 days in TSP-treated carcasses and 8 days in LA-treated carcasses. Therefore, both TSP and LA can be applied on poultry carcasses to reduce their microbial load and extend their shelf life during refrigerated storage.

Key words: Chicken, decontamination, lactic acid, trisodium phosphate, shelf life

INTRODUCTION

At present, poultry constitute about 30% of the world's total meat consumption (FAO, 2006). Chicken and other types of poultry, however, have higher pathogenic and spoilage bacterial counts than most other foods. Carcass contamination can occur at several points throughout the processing operation of poultry. The microbial load can greatly increase on poultry carcasses due to cross contamination during immersion scalding, de-feathering and evisceration as well as from other birds and from the processing equipment. The shelf life of chicken meat thus depends on the level of its microbial contamination. Therefore enhancing the keeping quality, reducing or killing spoilage causing and food borne pathogenic microorganisms of chicken carcasses are very important objectives of food technologists and microbiologists.

In addition to general hygienic practice, the use of organic chemicals as decontaminating agents for reduction or elimination of spoilage and pathogenic organisms from poultry meat and for extension of shelf life had been reported. Organic acids, such as lactic, citric, acetic and propionic acids and/or their salts were evaluated, both at the laboratory level and in processing plants (Dorsa *et al.*, 1997; Smulders and Greer, 1998; Sallam and Samejima 2004a; Theron and Lues, 2007). Of all the organic acids evaluated, Lactic Acid (LA) is one of the most widely accepted meat decontaminant (Huffman, 2002). Extensive research on the application of lactic acid to control both spoilage and pathogenic

organisms in foods of animal origin has been reported (Smulders, 1987; van der Marel *et al.*, 1988; Zeitoun and Debevere, 1992; Deumier, 2006; Anang *et al.*, 2007).

Trisodium Phosphate (TSP) is generally recognized as safe by the US Food and Drug Administration and has been approved by the US Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) at levels of 8-12% as an antimicrobial agent on raw chilled poultry carcasses that have been passed for wholesomeness. Treatment of poultry carcasses with TSP is effective in reducing populations of food spoilage bacteria as well as food-borne pathogens including *Salmonella*, *Campylobacter*, *Escherichia coli* O157:H7, *Listeria* and *Staphylococcus aureus* (Giese, 1993; deLedesma, *et al.*, 1996; Colin and Salvat, 1996; Capita *et al.*, 2002; Sallam and Samejima, 2004b).

This work is aimed to study the effects of TSP and LA dipping on the microbial growth and shelf life of whole chicken carcasses during refrigerated storage at 2°C .

MATERIALS AND METHODS

Sample preparation: A total of 60 native breed chicken carcasses were purchased immediately after slaughtering from a local commercial source in Sharkia Governorate, Egypt and transported in coolers containing crushed ice to the Meat Hygiene Laboratory, Department of Food Control, Zagazig University. Chickens were divided into three batches (twenty chickens per batch). Each chicken batch was dipped for 10 min in aqueous solution of either 12% TSP (w/v; pH

12.73) (El Nasr Pharm. Chem. Co., Egypt), 2% LA (w/v; pH 2.40) (El Nasr Pharm Chem Co., Egypt), or sterile distilled water (control) and gently swirled with a sterile glass rod. The temperature of each solution tested was $15\pm 1^\circ\text{C}$. Chickens were then removed from the solution and allowed to drain on a stainless wire mesh screen for 3 min. Subsequently, the carcasses were individually placed in sterile polyethylene bags, labeled and stored at $2\pm 1^\circ\text{C}$. Chicken meats from the 3 groups were sampled at storage days 0, 3, 5, 7 and 8 for microbiological analyses.

Microbiological analysis: Twenty five grams of composite chicken samples (meat with skin) were aseptically excised from the whole carcass and homogenized in 225 ml of sterile buffered 0.1% peptone water (Difco, Detroit, MI) for 1 min using a Stomacher 400 Lab Blender (Seward Medical, London, UK). From this homogenate, decimal serial dilutions were made in the same sterile peptone water and used for microbiological analyses of the chicken meat samples at appropriate time intervals during refrigerated storage. Samples were examined bacteriologically for Aerobic Plate Counts (APC), Psychrotrophic Count (PTC), *Enterobacteriaceae* Count (EBC) and Proteolytic Bacterial Count (PLC) on each of the predetermined sampling days during the refrigerated storage.

Aerobic Plate Counts (APC): Aerobic Plate Counts (APC) were determined by surface spreading of 0.1 ml of the sample homogenate, at selected dilutions, onto duplicate sterile plates of pre-poured and dried standard plate count agar (Oxoid, CM463), then the plates were incubated for 48 h at 35°C (APHA, 1992).

Psychrotrophic Counts (PTC): Psychrotrophic counts were determined in a similar method to that for APC except that plates were incubated at 7°C for 10 days. (Cousin *et al.*, 1992).

Proteolytic Bacterial Count (PLC): Total proteolytic counts were enumerated on Skim Milk Agar medium containing 10% Skim Milk (Difco Laboratories, Detroit, MI, USA) and 20% Agar (Oxoid, Basingstoke, Hampshire, England). The plates were inoculated with the diluted sample homogenate and incubated at 30°C for 3 days and examined for clear zone around growth to indicate proteolytic activity.

***Enterobacteriaceae* Count (EBC):** *Enterobacteriaceae* Counts (EBC) were enumerated by the pour-plating method on violet red bile glucose agar (VRBA; Difco Laboratories Inc., Detroit, Michigan, USA). The plates were overlaid with a virgin layer of the same growth medium before incubation at 37°C for 24 h (ICMSF, 1978).

Statistical analysis: All values are presented as means \pm SE and all measurements were carried out in triplicates. All microbial counts were converted into base-10 logarithms of colony forming units per g of chicken samples (\log_{10} CFU g^{-1}). Data were subjected to one-way analysis of variance (ANOVA) to determine the differences in the bacterial counts among the different treatments. Significant differences among the means were determined by Tukey Honestly Significant Difference (HSD) test. All data analysis was performed using the VassarStats web site for statistical computation (<http://faculty.vassar.edu/lowry/VassarStats.html>).

RESULTS AND DISCUSSION

Reduction of the initial microbial populations in chicken carcasses by TSP and LA dipping: The Effect of TSP and LA dipping on the initial microbial load of broiler chicken carcasses is shown in (Fig. 1). Tukey HSD test indicted significant reductions ($p < 0.01$) in the initial microbial counts of APC, PTC, PLC and EBC in the TSP- and LA-dipped chicken carcasses in comparison with the controls. No differences were observed in the initial microbial reductions between TSP- and LA-treated samples. The *Enterobacteriaceae* were the most affected organisms, where TSP- and LA-dipping reduced their initial counts in chicken meat by 1.41 and 1.22 \log_{10} CFU g^{-1} , respectively. On the contrary, psychrotrophs were the least affected bacteria as the TSP- and LA-dipping achieved mean log reductions of 0.62 and 0.75 \log_{10} CFU g^{-1} in their initial count, respectively. On the other hand, dipping of chicken carcasses in TSP and LA resulted in reductions of APC by 0.9 and 1.02 logs, respectively as well as reductions of PLC by 0.78 and 0.88 logs, respectively in comparison with the controls. Significant reduction in the initial aerobic plate count, psychrotrophic count and *Enterobacteriaceae* count had been also verified in chicken after dipping in lactic acid and trisodium phosphate (Van der Marel *et al.*, 1988; Sakhare *et al.*, 1999; Capita *et al.*, 2000; Whyte *et al.*, 2001; Okolocha and Ellerbroek, 2005; Deumier, 2006; del Río *et al.*, 2007).

Influence of TSP and LA dipping on the microbial load and shelf life of chicken carcasses during refrigerated storage: Under normal aerobic packaging conditions, the shelf life of refrigerated meat is limited by the growth and biochemical activities of aerobic, psychrotropic strains of bacteria (Lambert *et al.*, 1991). Chemical decontamination prior to packaging can be used to extend the shelf life of fresh meat. In this study TSP and LA dipping were applied to control the microbial growth and to extend the shelf life of fresh chicken carcasses during refrigerated storage.

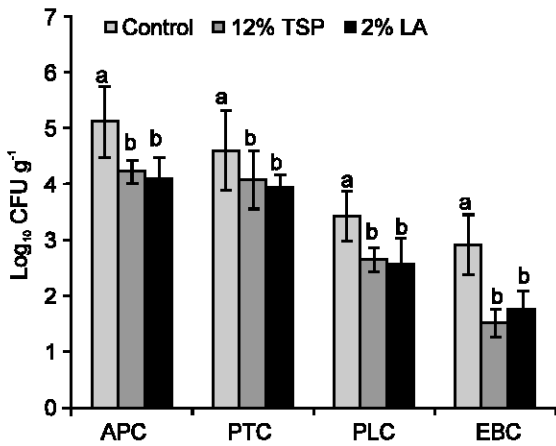


Fig. 1: Effect of TSP and LA dipping on the initial microbial load of broiler chicken carcasses. Columns with different letters differ significantly ($p < 0.01$)

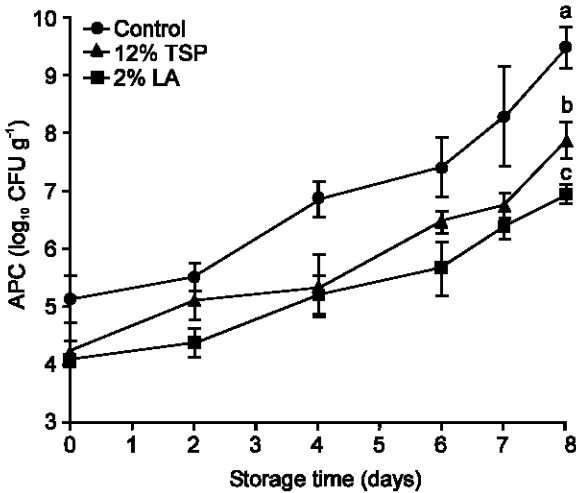


Fig. 2: Effects of Trisodium Phosphate (TSP) and Lactic Acid (LA) dipping on Aerobic Plate Counts (APC) of chicken carcasses during storage at $2 \pm 1^\circ\text{C}$. Values represent means \pm SE of three samples and each sample was measured in triplicates. Points on the curves with different letters differ significantly ($p < 0.01$)

Aerobic Plate Count (APC): Amongst the studied microbial categories, the population counts in control as well as in treated chicken carcasses followed the order: APC > PTC > PLC > EBC throughout the storage period. The increase in storage time produced significant proliferations in APC, regardless of the treatment conditions (Fig. 2). The initial (day 0) mean of APC (\log_{10} CFU g⁻¹) in control chicken meat samples was 5.13. On the fourth day of storage, APC of control meat reached a log mean count of 6.87, which is closed to the maximal recommended limit ($7 \log_{10}$ CFU g⁻¹) set by ICMSF

(1986) for APC in processed chickens. On the sixth day of storage, APC in control increased to $7.41 \log_{10}$ CFU g⁻¹, while signs of spoilage started to appear as a slight foul smell, which indicating a shelf life of 4-5 days for control chicken. On the other hand, samples dipped in TSP or LA showed delayed growth for APC in comparison with controls. TSP (12%) and LA (2%) dipping significantly ($p < 0.01$) reduced the microbial load of APC throughout the storage period in comparison with the control. While at the end of the storage APC in LA-dipped sample were almost 1 log lower ($p < 0.01$) than the corresponding count in TSP-dipped chickens. On the seventh day of storage TSP- and LA-dipped samples demonstrated mean APC of 6.75 and 6.40, respectively vs. 8.28 for control, while by the end of storage (day 8), TSP- and LA-dipped samples exhibited log mean APC of 7.88 and 6.94, respectively vs. 9.48 in controls (Fig. 2). This indicating a refrigerated shelf life of about 7 and 8 days for TSP- and LA-dipped samples, respectively. The estimated shelf life for TSP-treated samples in the present study (7 days) is lower than our previously determined shelf life for TSP-dipped chicken breast samples (12 days) (Sallam and Samejima, 2004b). This could be resulted from the difference of the initial microbial load of the mean log APC (5.13 vs. 3.48) determined at the beginning of the storage.

The extension of the refrigerated shelf life of chickens under the influence of TSP dipping has been reported in previous studies. Kim and Marshall (1999) reported that dipping of chicken legs in 5-10% TSP could extend shelf-life up to 8 days during refrigerated storage without adversely affecting sensory. Likewise, Sallam and Samejima (2004b) estimated a shelf life of 12 days for chicken breasts dipped in 10% TSP during refrigerated storage at 2°C . Lactic acid treatment has been also shown to result in increased shelf life of fresh poultry meat during refrigerated storage (Van der Marel *et al.*, 1988; Zeitoun and Debevere, 1992).

Psychrotrophic Count (PTC): The psychrotrophic counts in broiler chicken carcasses were slightly lower than the APC. This was true for all of the three groups analyzed. As the storage time increased, the PTC steadily increased in the control group from a mean count of 4.62 \log_{10} CFU g⁻¹ at the beginning of the storage (day 0) to higher counts of 7.08 and 8.63 \log_{10} CFU g⁻¹ on the sixth and eighth day of storage, respectively (Fig. 3). On the other hand, both TSP- and LA-dipped samples exhibited significantly ($p < 0.01$) lower PTC in comparison with the control throughout the storage time. By the end of the storage time (day 8), the mean PTC in TSP-treated samples was 1.45 logs lower than that of the control (7.18 vs. 8.63), while the mean PTC in LA-treated samples was 2.21 logs lower than that of the control (6.42 vs. 8.63). The results also indicated a significant

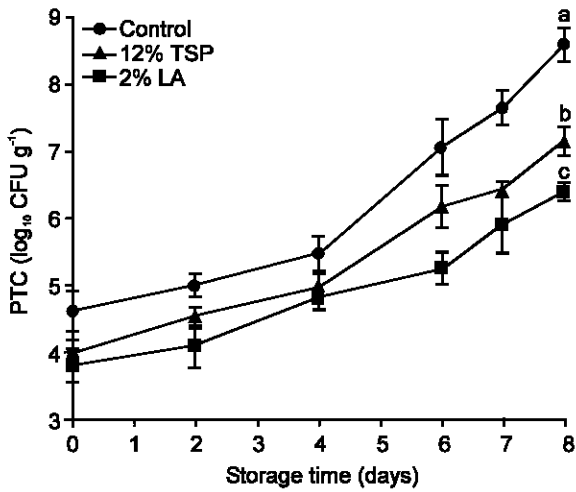


Fig. 3: Effects of Trisodium Phosphate (TSP) and Lactic Acid (LA) dipping on Psychrotrophic Counts (PTC) of chicken carcasses during storage at 2±1°C. Values represent means±SE of three samples and each sample was measured in triplicates. Points on the curves with different letters differ significantly (p<0.01)

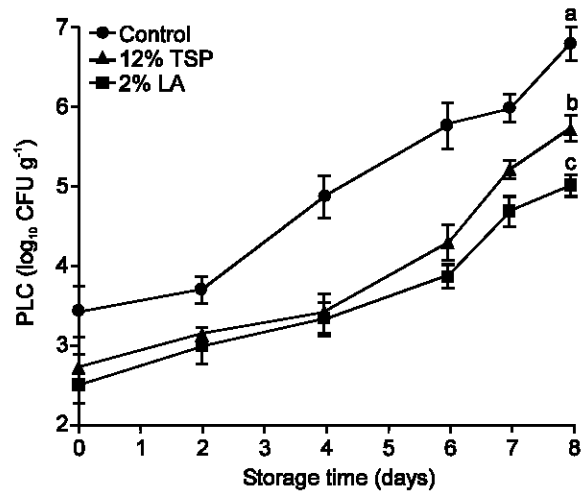


Fig. 4: Effects of Trisodium Phosphate (TSP) and Lactic Acid (LA) dipping on Proteolytic Counts (PLC) of chicken carcasses during storage at 2±1°C. Values represent means±SE of three samples and each sample was measured in triplicates. Points on the curves with different letters differ significantly (p<0.01)

difference in the PTC between TSP and LA treated samples at the end of storage period.

Our result is in accordance with that of Capita *et al.* (2000) who noted a significant decrease in psychrotrophic population on the skin of chicken carcasses immersed in 8-12% solution of TSP for 15 min during refrigerated storage at 2°C and also with the results of Sallam and Samejima (2004b), who demonstrated a significant reduction in psychrotrophic count in chicken breasts treated by dipping in 10% TSP for 10 min and stored at 2°C.

Similar to our findings in LA-dipped chickens, various researches reported significant reductions in the populations of psychrotrophic bacteria in chickens treated with lactic acid during refrigerated storage (Dorn *et al.*, 1989; Sawaya *et al.*, 1994; Hwang and Beuchat, 1995).

Total Proteolytic Count (PLC): Proteolytic bacteria are protease-producing microorganisms that break down protein in meat and results in foul odours. Bacteria are confined to the surface of meat during the logarithmic phase of growth. When proteolytic bacteria approach their maximum cell density, extracellular proteases secreted by the bacteria apparently break down the connective tissue between muscle fibers, allowing the bacteria to penetrate the meat. Non-proteolytic bacteria do not penetrate meat, even when grown in association with proteolytic species (Gill and Penney, 1977). The penetration of poultry muscle strips by proteolytic species; *Pseudomonas putida*, *P. fragi* and

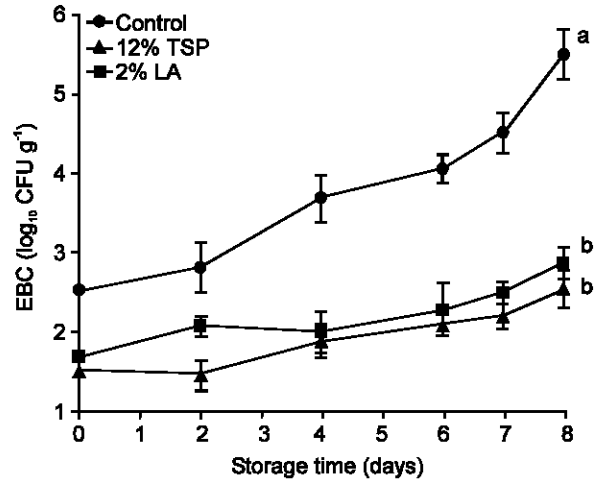


Fig. 5: Effects of Trisodium Phosphate (TSP) and Lactic Acid (LA) dipping on *Enterobacteriaceae* counts (EBC) of chicken carcasses during storage at 2±1°C. Values represent means±SE of three samples and each sample was measured in triplicates. Points on the curves with different letters differ significantly (p<0.01)

Lactobacillus plantarum had been reported by Gupta and Nagamohini (1992).

The obtained results in (Fig. 4) revealed that the mean log counts (log₁₀ CFU g⁻¹) of total proteolytic bacterial (PLC) were 3.44, 2.66 and 2.56 in control, TSP- and LA-treated chicken samples, respectively at the initial time

of storage (day 0). Dipping of chicken carcasses in TSP or LA resulted in significant reduction ($p < 0.01$) in the PLC throughout the storage period in comparison with the control. By the end of the storage (day 8), the PLC increased to a high level of 6.81 in control chicken samples, while TSP- and LA-dipped samples exhibited lower counts of 5.74 and 5.04, respectively. Although there was no significant difference between LA- and TSP-dipped samples during the first 6 days of the storage, LA-dipped samples revealed a significant lower PLC than TSP-dipped samples by the end of the storage period (Fig. 4).

Enterobacteriaceae Counts (EBC): The initial EBC of control chicken carcass samples increased from 2.53 on the first day of storage, to a high count of 5.53 on the eighth day of storage (Fig. 5). Chicken samples dipped in TSP or LA solutions, however, indicated significantly lower counts ($p < 0.01$) in comparison with control throughout the storage time and they remained below the count of $3 \log_{10} \text{CFU g}^{-1}$ even on the end of storage period (day 8). Similarly, Okolocha and Ellerbroek (2005) reported significant reduction in EBC on whole chicken carcasses during 6-days storage at 4°C ; while Ellerbroek *et al.* (1997) declared that spraying or dipping of chicken meat with TSP or lactic acid significantly reduced the *Enterobacteriaceae* count during refrigerated storage.

Length of refrigerated storage ($2 \pm 1^{\circ}\text{C}$) had a significant ($p < 0.01$) effect on EBC which tended to increase as the storage time increase. EBC in TSP- and LA-dipped samples in the present study, increased slowly from an initial count of 1.52 and 1.69 on the first day of storage to 2.54 and 2.88, respectively at the end of storage. Nonetheless, Van der Marel *et al.* (1988) claimed that chicken carcasses treated after chilling with lactic acid (2%) tended to decrease from $3.3 \log_{10} \text{CFU g}^{-1}$ just after treatment to a low count of $2.8 \log_{10} \text{CFU g}^{-1}$ by the end of refrigerated storage (day 25) at $0 \pm 1^{\circ}\text{C}$.

Generally, LA was more potent than TSP in reducing the population of APC, PTC and PLC in the present study. This may be due to the destructive effect of lactic acid on proteolytic bacteria particularly gram negative through induction of low pH and liberation of undissociated acid molecules that change the permeability of microbial cell membrane.

This study concluded that both TSP and LA are efficient in the reduction of the microbial contamination on chicken carcasses and therefore they can be utilized to improve the microbial safety and extend the shelf life of chicken carcasses during refrigerated storage.

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