

Effect of Organic Acids on Microbial Populations and *Salmonella typhimurium* in Pork Loins

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ABSTRACT : The objective of this study was to evaluate the effects of various organic acids on microbial characteristics and *Salmonella typhimurium* in pork loins. Fresh pork loins were sprayed with various organic acids such as lactic acid, citric acid and acetic acid at various concentrations (0.5, 1, 1.5 and 2%). After spraying, the samples were packaged by HDPE film under air and stored at 4°C for 14 days, and analyzed. Microbial deterioration of pork loins during the aerobic cold storage was delayed by organic acid spray. The bactericidal effect of acids increased with the increasing concentration. However, the inhibitory activity of organic acids during the storage varied with the kinds and concentrations of the acids. As for total plate counts, acetic acid was found to have the highest bactericidal activity, whereas citric acid was found to be the most inhibitory for coliform and *S. typhimurium*. (*Asian-Aust. J. Anim. Sci.* 2003. Vol 16, No. 1 : 96-99)

Key Words : Lactic Acid, Citric Acid, Acetic Acid, *Salmonella Typhimurium*, Total Plate Counts, Coliforms

INTRODUCTION

Spoilage microorganisms and pathogenic bacteria such as *Salmonella*, *Campylobacter*, and *Listeria*, are contaminated during slaughter and subsequent processing to produce retail cuts from live animals. Application of organic acids to carcass surfaces has been reported to reduce populations of spoilage bacteria and pathogens, and thus has potential to extend shelf-life and reduce food borne illness in pork (Fu et al., 1994), beef and lamb (Osthold et al., 1984) and poultry (Tamblyn and Conner, 1997). However, acids treatment of beef carcasses had little or no effect on the microbiological quality of meat cuts after fabrication (Prasai et al., 1991). In pork, Fu et al. (1994) reported that organic acids treatments were of relatively limited effectiveness for long term storage. On the other hand, an acid spray immediately prior to packaging can reduce some species of bacteria without adversely affecting the physical properties of beef (Goddard et al., 1996). Therefore, this study was carried out to evaluate the effects of various organic acids including lactic acid, citric acid and acetic acid on microbial characteristics and *Salmonella typhimurium* in pork loins.

MATERIALS AND METHODS

Various organic acids such as lactic acid (85%, fisher

science, Fairlawn, NJ, USA), citric acid (99.8%, anhydrous, Fisher science, St. Louis, Mo, USA), and acetic acid (glacial, food grade, J.T. Baker chemical Co., Phillipsburg, NJ, USA) were used. They were diluted to 0.5% (v/v), 1% (v/v), 1.5% (v/v), 2% (v/v) with distilled water and then sprayed on fresh pork loins for 15sec at 30°C by hand sprayer. The control samples were sprayed with sterilized distilled water. After spraying, the samples were packaged by HDPE film under air and stored at 4°C for 14 days, and analyzed for total plate counts and coliforms (n=9). In another experiment, fresh pork loins were inoculated with *Salmonella typhimurium* ATCC 14028 (from Veterinary Microbiology Lab, College of Veterinary Medicine, Seoul National University) and then sprayed with organic acids in the same way as in the first experiment. After spraying, the samples were packaged and stored at 1°C. For *Salmonella typhimurium* measurements, 10 g of each sample was stomached in 10 ml of ambient distilled water for 1 min. The stomached sample was diluted serially. However, for other microbial measurements, sample's surface was swabbed by using sterile cotton swabs and sterile metal templates (3×4 cm). The swabs were aseptically broken into a sterile 10 ml 0.1% peptone water blank. After then serial dilutions were made. Aerobic and coliform bacteria were enumerated on Petrifilm (Microbiology Products 3M Health Care, USA) for 2 days at 37°C, while SS agar (Difco Lab, ML, USA), salmonella selective medium, was used for enumeration of *Samonella typhimurium* on plates. It was evenly spread on the surface of the plates with a sterile bent glass rod. (Frederick, 1994)

The pH values of samples were measured in the homogenized meat slurry by a pH meter with a glass

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electrode (Model 5985 Digi-Sense pH meter, Cole-Parmer Instrument Co., USA). The pH values of 2% (v/v) lactic acid, citric acid, acetic acid were 3.09, 3.11, 3.80, respectively. Duncan's multiple range test was used to compare among treatments using SAS (1995) program.

RESULTS AND DISCUSSION

Results showed that organic acids treatments reduced the total plate counts of pork in aerobic package after a day of storage at 4°C. The counts of treatment samples at 0.5% were 0.7-1.1 log unit lower than that of the control. (Figure 1). With each organic acid, the antimicrobial effect increased as the concentration increased. The effect during the storage period varied with the kind of organic acid. Lactic and citric acids showed decreasing trends while acetic acid showed an increasing trend except at 0.5% level. (Figure 1). The results of Figure 2 on coliform bacteria revealed a similar pattern to those on total plate counts. The bactericidal effect increased with the concentration. Citric acid above 1% was most effective in keeping the count low during the storage. Fu et al. (1994) reported that at 1.5%, both acetic acid and citric acid showed some initial effects on decreasing aerobic plate counts and coliform count of pork loins, but the effects did not continue beyond 14 days storage under vacuum packaging, while lactic acid had no effect on inhibiting them at any point of storage. Ouattara et al. (1997) suggested that on a weight basis, acetic acid was found to be the most inhibitory, followed by lactic and citric acid. Because acetic acid (pKa values 4.8), under comparable conditions of the acid (same medium and same acid concentration), was considerably less dissociated than lactic acid (pKa 3.8) while citric acid (pK₁ 3.1, pK₂ 4.8, pK₃ 6.3) was the most dissociated of all.

The number of *Salmonella typhimurium* on the fresh pork loins which has been sprayed with organic acids was lower than that of the control sprayed with distilled water (Figure 3). The lactic acid spray reduced *S. typhimurium* by 0.4 to 1.4 log unit, citric acid 0.6 to 1.5 unit and acetic acid 0.5 to 1.5 unit. The bactericidal activity was the highest at 2% for all acids. Among acids, citric acid was found to have most inhibitory effect at all concentrations and acetic acid was the second.

In this experiment, the bactericidal effect of organic acids appeared to be not solely due to the pH lowering effect. The pH changes of pork loins varied with the concentrations and types of organic acids (Figure 4). During storage, pH kept decreasing at varying degrees with different concentrations of organic acids until 7 days. After that storage time, there appeared different pattern of pH changes according to organic acids: pH increased at all

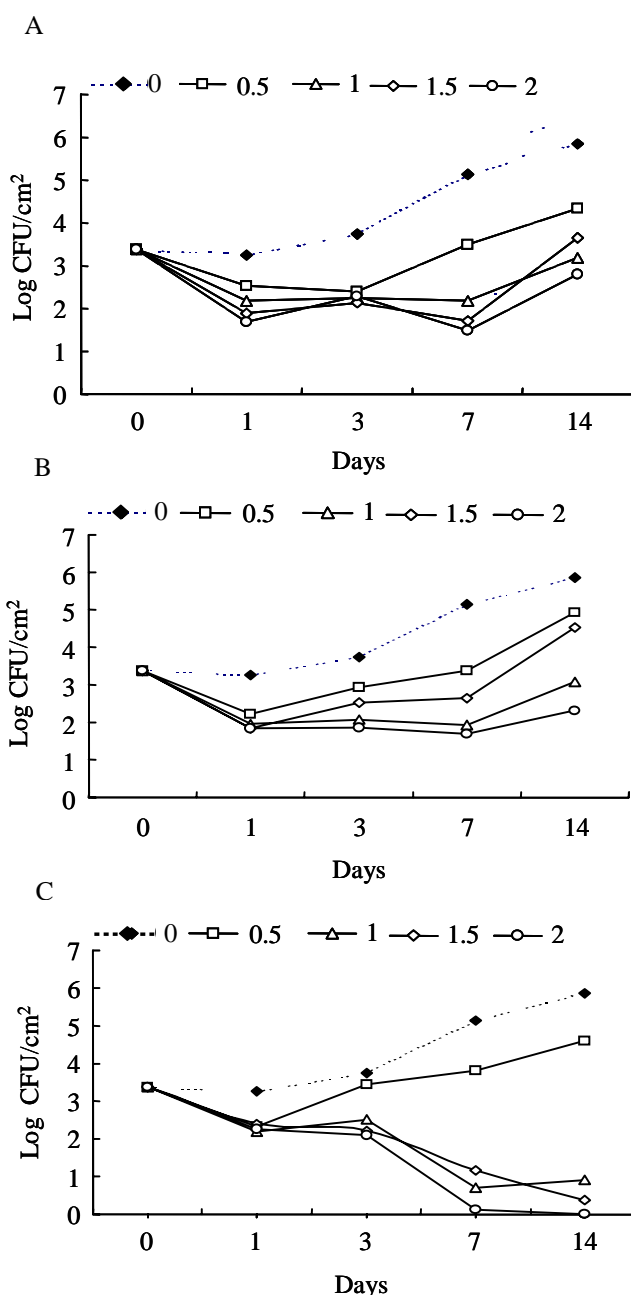


Figure 1. Total plate counts on aerobic-packaged pork loins sprayed with lactic acid, acetic acid and citric acid (from left to right) during storage at 4°C. Zero percentage samples were controls. Means with the different letter in the same day are significantly different. ($p < 0.05$)

A; Lactic acid, B; citric acid, C; acetic acid

levels of lactic acid, increased only at 0.5% level of citric acid and decreased at all levels of acetic acid. Tamblyn and Conner (1997) reported that bactericidal activity of organic acids increased linearly with increasing concentration and the activity of organic acids depended on the concentration and the method of application. Acetic acid was found to

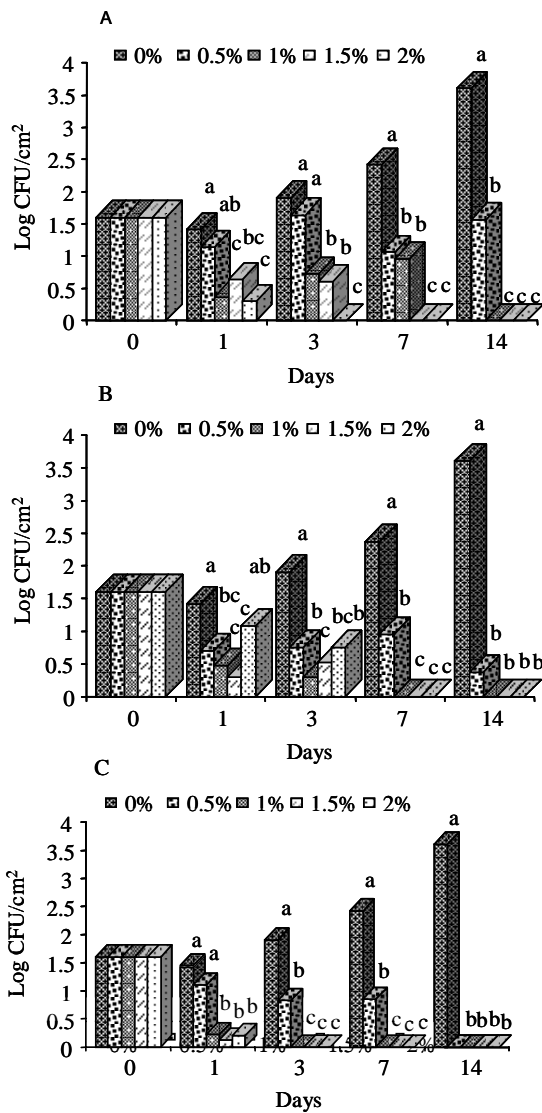


Figure 2. Coliform counts of aerobic-packaged pork loins sprayed with lactic acid, acetic acid and citric acid (from left to right) during storage at 4°C. Zero percentage samples were controls. Means with the different letter in the same day are significantly different. (p<0.05)

A; Lactic acid, B; citric acid, C; acetic acid

have most inhibitory effect against salmonellae, whereas lactic acid exhibited intermediate activity, and citric acid was least inhibitory (Chung and Goepfert, 1970). Effective use of an acid depends upon the dissociation constant (pKa) or the pH at which 50% of the total acid is dissociated (Doores, 1993). In our results, the pH values of lactic acid, citric acid, acetic acid were slightly higher than each pKa values on 7th storage days. It can be suggested that all these acids must have been dissociated, even though the citric acid above 1% showed bacteriostatic effect. The lower capacity of citric acids to enter bacterial cells is

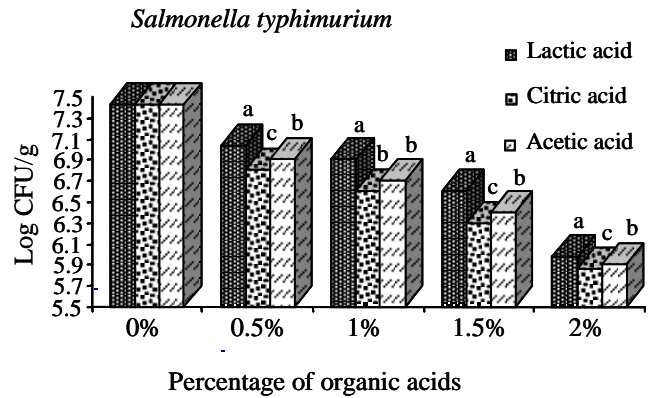


Figure 3. *Salmonella typhimurium* of aerobic-packed pork loins sprayed with organic acids after 24 h at 4°C. Zero percentage samples were controls. Means with the different letter in the same percentage of organic acids are significantly different (p<0.05).

compensated by their greater capacity to dissociate inside the cell and thus acidify the cell cytoplasm. Indeed, partially ionized citrate can enter the cells of many bacteria (e.g., *Bacillus subtilis*, numerous members of the family *Enterobacteriaceae*, *Lactococcus lactis*, and *Leuconostoc spp.*) through the action of a proton-or cation dependent citrate permease. (Quattara et al., 1997) We assume that the reason why the citric acid showed high bacteriostatic effects in spite of higher pH values than pKa values was that undissociated acid broke into *S. typhimurium* so that it was dissociated inside.

CONCLUSIONS

Microbial deterioration of pork loins during the aerobic cold storage was delayed by organic acid spray. The bactericidal effect of acids increased with the increasing concentration. However, the inhibitory activity of organic acids during the storage varied with the kind and the concentration of the acid. For total plate counts, acetic acid was found to have the highest bactericidal activity, whereas citric acid was the most inhibitory for coliform and *S. typhimurium*. Because human infectious dose of *S. typhimurium* is known to be around 10⁰ to 10², the decreased number of *S. typhimurium*, by ca. 2 log cycle may help consumer be a bit safe from the possible link from it. Therefore, it can be concluded that organic acid spray could be utilized to extend the shelf-life of pork loins and ensure safety.

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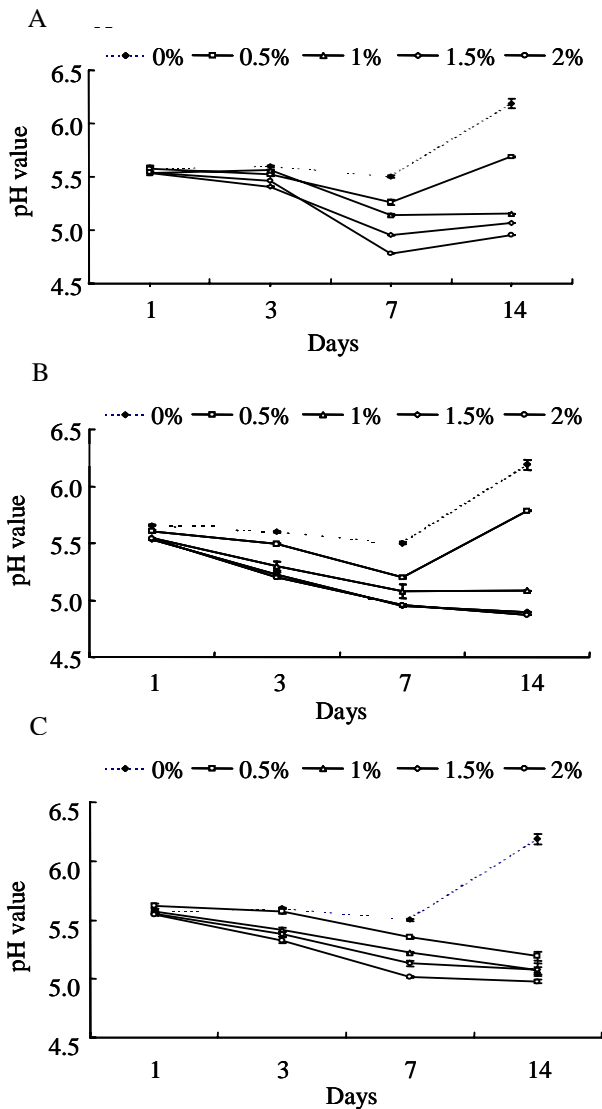


Figure 4. The pH changes of pork loins during the storage at 4°C after being sprayed with different levels of lactic acid, citric acid and acetic acid (from left to right). Bars at values show standard deviations of measurements.

A; Lactic acid, B; Citric acid, C; Acetic acid

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