Original article

Efficacy of citric acid against *Listeria monocytogenes* attached to poultry skin during refrigerated storage

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Summary The aim of this study was to evaluate the effect of citric acid washing on the growth of *Listeria monocytogenes* on poultry legs stored at 4 °C for 8 days. Fresh inoculated chicken legs were dipped into either a 0.052, 0.104 or 0.156 M citric acid solution for 5 min or distilled water (control). Surface pH values, sensorial characteristics and *L. monocytogenes*, mesophiles and psychrotrophs counts were evaluated. Legs washed with 0.156 M citric acid for 5 min showed a significant ($P < 0.05$) inhibitory effect on *L. monocytogenes* compared with control legs, being about 1.55 log units lower in the first ones than in control legs after 1 day of storage. Treatments with 0.156 M citric acid reduced bacterial growth and preserved reasonable sensorial quality after storage at 4 °C for 8 days.

Keywords Citric acid, decontamination, food safety, *Listeria monocytogenes,* poultry.

Introduction

Meat and poultry products are often identified as the source of foodborne disease outbreaks (ICMSF 1998). Raw poultry is a well-recognised source of *Listeria monocytogenes*, and many surveys have confirmed the presence of this pathogen on fresh poultry (Bailey *et al*., 1989; Genigeorgis *et al*., 1989; Uyttendaele *et al*., 1997). Some authors have associated cases of listeriosis with the consumption of undercooked chicken (Schuchat *et al*., 1992).

The contamination of raw chicken with bacterial pathogens has important implications for public health. There is a great interest in reducing surface microbial contamination of carcass meat, with particular regard to reducing the levels of pathogens. One approach has been the application during processing of decontamination treatments such as chlorine, organic acids, phosphates, bacteriocins, hydrogen peroxide, ozone, water, ultrahigh hydrostatic pressure, irradiation, pulsed-field electricity, ultrasonic energy and UV light (Bolder, 1987). On the contrary, the shelf life of raw chicken depends on the level of its microbial contamination, and thus reducing spoilage and foodborne pathogenic microorganisms of chicken carcasses is an important objective of food processors.

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Organic acids and their salts (acetic, citric and lactic) exert antibacterial activity. They have been traditionally used as food preservatives and are generally recognised as safe substances (GRAS) approved as food additives by E.C., FAO/WHO and FDA (Surekha & Reddy, 2000). In November 1992, pre-evisceration organic acid rinses were approved by the Food Safety and Inspection Service of the U.S. Department of Agriculture (FSIS, USDA) for use in commercial slaughterhouses as a means of enhancing product safety and extending the shelf life of beef and pork carcasses (FSIS, 1992). In Europe, Regulation 853/2004 of the European Parliament and Council provides a legal basis for the use of substances other than potable water to remove surface contamination from foods of animal origin.

Organic acids are required at high concentrations to be effective as decontaminating agents, but it is important to consider the effect of high concentrations of acids on product quality (Siragusa, 1995). The application of organic acids can result in discolouration and flavour defects (Smulders & Greer, 1998). Generally, treatments with organic acids at varying concentrations result in population reductions ranging from 1 to 3 log units on meat surfaces (Dickson & Anderson, 1992).

Some organic acids, such us lactic acid, have been extensively investigated as antimicrobial agents for use in meat, including poultry, to extend its shelf-life and inhibit the growth of pathogens (Mulder *et al*., 1987; Zeitoun & Debevere, 1990; El-Khateib *et al*., 1993;

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Conner *et al*., 1997). However, there are few studies on the effect of citric acid in meat or poultry (Cutter & Siragusa, 1994). Moreover, the decontamination effect is studied in combination with other organic acids, being the citric acid at low levels (0.25%) (Acuff *et al*., 1987). Thus, there are few data available on the effect of citric acid in poultry.

The ability of citric acid to inhibit *L. monocytogenes* has been studied in laboratory media (Ahamad & Marth, 1989) and in some foods such as fish (Bal'A & Marshall, 1998). However, there are no studies on the effect of citric acid on *L. monocytogenes* growth on poultry. There are studies of the effect of citric acid against other pathogens such as *Escherichia coli* O157:H7 or *Salmonella* (Cutter & Siragusa, 1994; Tamblyn & Conner, 1997).

The aim of this work was to evaluate the effectiveness of several concentrations of a citric acid dip to control the growth of *L. monocytogenes* on poultry stored at 4 °C (common temperature in the marketing stage). Microbiological and sensorial quality were also evaluated.

Material and methods

Preparation of bacterial inoculum

The *L. monocytogenes* serotype 1/2a strain CECT 932 was grown in Tryptone soya broth (Oxoid, Hampshire, UK) at 30 °C for 18 h to achieve a viable cell population of 9 log CFU mL^{-1}. The culture was then transferred to a sterile centrifuge bottle and centrifuged at 10 000 × ***g*** for 10 min at 4 °C. The supernatant was decanted and the pellet resuspended in sterile 0.1% peptone solution (Merck, Darmstadt, Germany) (pH 6.2) by vortexing. The washing step was repeated twice. The suspension of washed cells was diluted in a sterile 0.1% peptone solution to obtain an appropriate cell concentration for inoculation of sterile distilled water.

Inoculation of poultry and treatment

A total of forty fresh chicken legs were obtained from a poultry processing plant (La Rioja, Spain). The legs were placed on crushed ice and transported to the laboratory.

Fresh chicken legs were inoculated with *L. monocyto genes* by dipping them into a suspension of this pathogen (6 log CFU mL^{-1}) for 5 min at room temperature. After inoculation, the legs were removed and kept for 30 min at room temperature to allow the attachment of inoculated cells to the skin.

The inoculated poultry legs were divided into four groups, each containing ten legs. Samples in one group were dipped for 5 min into sterile distilled water (control). Samples in the other three groups were dipped into 1% w/v (0.052 M), 2% (0.104 M) or 3% (0.156 M) citric acid (Scharlau, Barcelona, Spain) solutions for 5 min, respectively.

After these treatments, the legs were removed and drained for 5 min and stored individually in sterile bags at 4 °C for 8 days.

Samples were taken on days 0 (after the dipping treatment), 1, 3, 6 and 8. On the sampling days, three legs of each group were taken out of storage to perform microbiological, pH and sensorial analysis.

Sensorial analysis

The samples were evaluated for overall acceptability with regard to odour, colour, texture and overall appearance by a panel of nine members. A structured hedonic scale (Anzaldúa-Morales, 1994) with numerical scores ranging from 7 (I like it very much) to 1 (I dislike it very much) was used. A score of 3 was considered the borderline of acceptability.

Microbiological analyses and pH determination

Ten grams of skin were aseptically weighed and homogenised in a Stomacher (IUL, Barcelona, Spain) for 2 min with 90 mL of sterile peptone water (Oxoid). Further decimal dilutions were made with the same diluent. The total number of mesophilic microorganisms was determined on plate count agar (PCA, Merck) using the pour plate method, incubating at 30 °C for 72 h (ICMSF, 1978). Psychrotrophs were determined on PCA (Merck) with an incubation temperature of 7 °C for 10 days, using the pour plate method (ICMSF (International Commission on Microbiological Specifications for Foods), 1978). *Listeria spp*. were determined following the surface plate method on Palcam agar at an incubation temperature of 30 °C for 48 h (Mossel *et al*., 1995). Suspect colonies grown on Palcam agar were subcultured for purity on tryptone soya agar (TSA, Merck) and incubated for 24 h at 30 °C. The following identification tests for *L. monocytogenes* were performed: Gram stain, catalase reaction, oxidase test, tumbling motility at 20–25 °C, umbrella motility in the SIM medium (Oxoid, Unipath, England) and CAMP test (Seeliger & Jones, 1986). Five suspect isolates were also identified by using API *Listeria* strips (BioMérieux, Marey Lètoile, France). All analyses were performed in duplicate.

For pH determination, 5 g of skin were blended with 10 mL of distilled water. The pH of the homogenised sample was measured with a Crison model 2002 pH meter (Crison Instruments, Barcelona, Spain). Determination of pH was performed in duplicate.

Statistical analysis

Analysis of variance was performed using the SYSTAT program for Windows, Statistics version 5.0 (1992,

Evanston, IL, USA). Tukey's test for comparison of means was performed using the same program. Plate count data were converted to logarithms prior to their statistical treatment. All experiments were carried out in duplicate. Significance level was defined at $P < 0.05$.

Results

Microbiological quality

The effect on mesophiles and psychrotrophs of dipping the legs into different citric acid concentrations is shown in Figs 1 and 2, respectively. Significant differences ($P < 0.05$) in mesophile counts were observed between the legs treated with 0.156 M citric acid and the control legs. The data obtained showed that a 5-min dip in 0.156 M citric acid reduced mesophile counts between 0.71 and 1.28 log cycles compared with the control legs throughout storage. Significant differences ($P < 0.05$) were also found between the legs treated with 0.104 M citric acid and the control legs on days 3, 6 and 8. However, no significant differences ($P > 0.05$) were found for these bacterial counts between the samples treated with 0.052 M citric acid and the control samples except on day 3.

No significant differences ($P > 0.05$) in psychrotroph counts were found between samples treated with 0.156 and 0.104 M citric acid except on day 0. Significant differences ($P < 0.05$) were observed between control samples and those treated with citric acid until day 6. After 8 days of storage, the differences between control samples and those treated with citric acid were only significant with samples treated with 0.104 and 0.156 M citric acid.

Listeria monocytogenes

Figure 3 shows the effect of citric acid treatment on the growth of *L. monocytogenes* inoculated onto legs. Significant differences ($P < 0.05$) in the *L. monocytogenes* populations were observed on legs treated with 0.156 M citric acid compared with the control samples. After 1 day of storage, *L. monocytogenes* counts were 1.55 log cycles lower in legs treated with 0.156 M citric acid than in control ones. Significant reductions in the *L. monocytogenes* populations were also observed on legs treated with 0.104 M citric acid on days 0, 1, 3 and 6 of storage compared with the control samples. No significant differences were observed between legs treated with 0.052 M citric acid and control legs on days 6 and 8.

pH evolution

The pH values of legs treated with citric acid are shown in Fig. 4. Significant differences were found in pH values

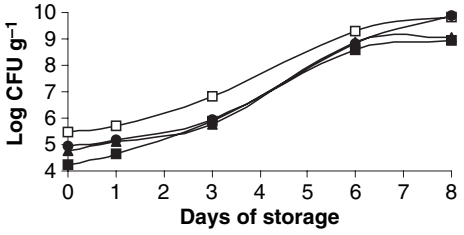

Figure 1 Evolution of mesophile counts in chicken legs treated with citric acid. Control (□), citric acid 0.052 M (●), citric acid 0.104 M (▲), citric acid 0.156 M (■).The data are the mean values of two replicates.

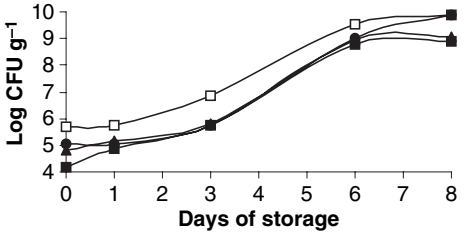

Figure 2 Evolution of psychrotrophs in chicken legs treated with citric acid. Control (□), citric acid 0.052 M (●), citric acid 0.104 M (▲), citric acid 0.156 M (■).The data are the mean values of two replicates.

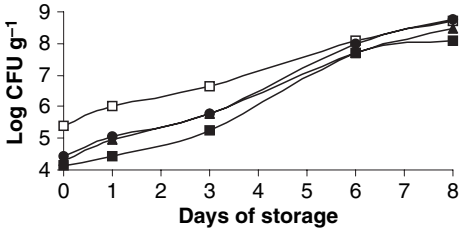

Figure 3 Effect of a 5-min citric acid dip on the growth of *Listeria monocytogenes* on chicken legs. Control (□), citric acid 0.052 M (●), citric acid 0.104 M (▲), citric acid 0.156 M (■).The data are the mean values of two replicates.

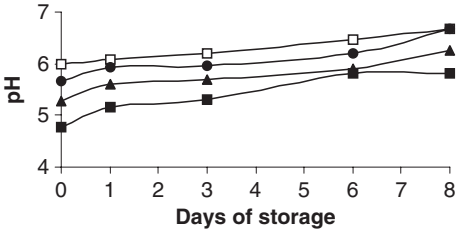

Figure 4 Evolution of pH in chicken legs treated with citric acid. Control (□), citric acid 0.052 M (●), citric acid 0.104 M (▲), citric acid 0.156 M (■).The data are the mean values of two replicates.

between samples treated with 0.156 M citric acid and control samples. No significant differences in pH were observed after 8 days of storage between samples treated with 0.052 M citric acid and control samples. The pH was lower when the citric acid concentration was higher. These pH differences decreased throughout storage. Initial pH values in legs treated with 0.156 M citric acid (day 0) were 1.24 units lower than in control legs. During storage, pH increased 1.06 units after 8 days, being 0.86 units lower than in control samples.

Sensorial quality

The changes in colour, odour and overall appearance of poultry legs are shown in Table 1. A higher score on colour was observed in control samples and those treated with 0.052 M citric acid compared with other citric acid treatments on days 0 and 1. However, no significant differences ($P > 0.05$) in colour were observed between samples treated with citric acid and control samples on day 3. After 6 days of storage, the worst score was obtained by the control legs. After 8 days of storage, the colour of control legs was unacceptable, while samples treated with 0.104 and 0.156 M citric showed scores above 3; this value was the borderline of acceptability.

When treatments were compared at day 6 of storage, treatments with 0.052, 0.104 and 0.156 M citric acid reduced ($P < 0.05$) the presence of off-odours compared with controls. After 8 days of storage, all samples had strong off-odours and were rejected, except those treated with 0.156 M citric acid. The samples treated with 0.156 M citric acid were not severely discoloured, and unacceptable odours were not detected throughout storage. Consequently, legs receiving treatments with 0.156 M citric acid remained acceptable until 8 days of storage, at least 2 days longer than control samples.

Discussion

The mean log reductions observed by us were in agreement with those reported by other authors when using organic acids. In general, the use of organic acids (1–3%) reduces bacterial counts by 1–2 log cycles (Cutter & Siragusa, 1994; Siragusa, 1995). We found that a washing with 0.104 M citric acid (2%) reduced mesophiles counts between 0.45 and 1.08 log cycles compared with the control legs throughout storage. Van der Marel *et al*. (1988) have also reported reductions of 1 log cycle in mesophiles in poultry after washing with 1–2% lactic acid. Cutter & Siragusa (1994) studied the effect of 1%, 3% and 5% acetic, lactic and citric acids against *Escherichia coli* O157:H7 and *Pseudomonas fluorescens* in beef. The authors reported that citric and lactic acids were equally effective in reducing the populations of bacteria studied. According to the authors, the acid type is not a significant treatment factor. However, they observed that acid concentration was a significant factor that influenced the reduction of bacterial populations. In contrast, in a previous work, we observed that a treatment with 2% lactic acid (0.22 M) reduced mesophile counts between 0.67 and 2.32 log cycles compared with the control legs throughout storage (González-Fandos & Dominguez, 2006).

Table 1 Mean scores ± standard deviation of different sensory characteristics (colour, odour and overall appearance) of chicken legs treated with citric acid stored at 4 °C

		Treatment			
			Citric acid		
Sensory characteristic	Storage time (days)	Control	0.052 M	0.104 M	0.156 M
Colour	0	7.00 ± 0.00	7.00 ± 0.00	6.44 ± 0.50	6.11 ± 0.31
	1	7.00 ± 0.00	7.00 ± 0.00	6.44 ± 0.50	6.11 ± 0.31
	3	6.11 ± 0.31	6.11 ± 0.31	6.00 ± 0.00	6.00 ± 0.00
	6	3.00 ± 0.00	4.67 ± 0.47	5.00 ± 0.00	5.11 ± 0.31
	8	1.66 ± 0.47	3.00 ± 0.00	3.33 ± 0.47	4.11 ± 0.31
Odour	0	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	6.33 ± 0.47
	1	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	6.11 ± 0.31
	3	6.11 ± 0.31	6.11 ± 0.31	6.11 ± 0.31	6.00 ± 0.00
	6	2.11 ± 0.31	3.67 ± 0.47	4.33 ± 0.47	5.33 ± 0.47
	8	1.00 ± 0.07	2.11 ± 0.31	2.44 ± 0.50	4.11 ± 0.31
Overall appearance	0	7.00 ± 0.00	7.00 ± 0.00	6.44 ± 0.50	6.11 ± 0.31
	1	7.00 ± 0.00	7.00 ± 0.00	6.44 ± 0.50	6.11 ± 0.31
	3	6.11 ± 0.31	6.11 ± 0.31	6.00 ± 0.00	6.00 ± 0.00
	6	2.11 ± 0.31	3.67 ± 0.47	4.33 ± 0.47	5.11 ± 0.31
	8	1.00 ± 0.00	2.11 ± 0.31	2.44 ± 0.50	4.11 ± 0.31

Key to the scores: 7 = I like it very much, 3 = unacceptable, 1 = I dislike it very much.

The antimicrobial effect of citric acid could be lower than lactic acid if we compare the percentage added.

After 6 days of storage, mesophiles and psychrotrophs reached populations above 9 log CFU g^{-1} in control legs. However, in legs treated with 0.156 M citric acid, mesophile and psychrotroph counts were below 9 log CFU g^{-1} after 8 days of storage at 4 °C, and signs of spoilage were not detected after 8 days of storage. When legs were treated with 0.052 or 0.104 M citric acid, populations around 9 log CFU g^{-1} were detected on day 8 of storage, although sensorial scores were higher than those recorded for control legs, and these legs were rejected on day 8 of storage. To compare our results with those reported by other authors, the data were converted to log CFU cm^{-2}. It was found that 1 g of skin corresponded to an average of 6.88 cm^2 of skin. Thus, 9 log CFU g^{-1} corresponded to 8.16 log CFU cm^{-2}. Other authors have reported spoilage odours in poultry when counts approached 7–8 CFU cm^{-2} (Barnes, 1976; Elliot *et al*., 1985; Studer *et al*., 1988).

The pH data indicated that reductions of bacterial populations may have been due to the effects of acidic pH. Thus, lower counts were observed in legs with lower pH. The antimicrobial effect of organic acids has been attributed to undissociated acid molecules that interfere with cellular metabolism or a decrease in biological activity as a result of pH changes in the cell's environment (Doores, 1983; Cherrington *et al*., 1991). Citric acid has higher dissociation constant than other organic acids such as acetic acid, and it is considered less detrimental to pathogens (Ahamad & Marth, 1989). However, citric acid produces a lower pH in the surface of meat that could reduce microbial growth (Osthold *et al*., 1984). According to Young & Foegeding (1993), the antimicrobial activity of citric acid is dependent on pH concentration and anion effects. A number of studies have suggested that the antimicrobial activity of citric acid is due to the chelation of metal ions that are essential for microbial growth (Beuchat & Golden, 1989; Stratford, 2000). In this study, the application of 0.156 M citric acid reduced the surface pH immediately after treatment, thereby creating an unfavourable environment for bacterial growth. Our results agree with those reported by Bal'A & Marshall (1998), who observed that citric acid treatments caused a decline in the pH of catfish fillets. Cutter & Siragusa (1994) also indicated that reductions of microbial counts in beef when using citric acid may be due to lower pH.

The ability of citric acid to inhibit *L. monocytogenes* may be higher in laboratory media than in foods, according to the results reported by Ahamad & Marth (1989). These authors found that the presence of up 0.1% citric acid (0.0052 M) in tryptone broth inhibited the growth of *L. monocytogenes*, and that the degree of inhibition increased as the temperature of incubation decreased. These authors reported that *L. monocytogenes* was inactivated when citric acid concentration in the medium was 0.3% or greater (0.0156 M). According to these authors, acetic acid was most detrimental to *L. Monocytogenes*, followed in order by lactic and citric acids.

Bal'A & Marshall (1998) studied the effect of a dip treatment in a solution of 2% citric acid on catfish filets. They observed that dip treatment reduced mesophile and *L. monocytogenes* counts. In addition, Palumbo & Williams (1994) observed the efficacy of a 2-min dip in a 1% solution of citric acid for reducing the *L. monocyto genes* counts on the surface of frankfurters inoculated.

Glass & Doyle (1989) reported that *L. monocytogenes* grew well on those meat products with a pH value near or above 6.0, whereas on meats near or below pH 5.0, the organism grew poorly or not at all. Poultry has a higher pH than other types of meat. It should be pointed out that poultry leg muscles have a pH of 6.4–6.7, while its other parts like breast muscles have lower pH values (5.7–5.9) (Barnes, 1976). The higher pH can explain why poultry supports the growth of *L. monocytogenes* better than other meats. Hence, decreasing the pH with citric acid treatment could contribute to control the growth of *L. monocytogenes*.

Although treatments with citric acid did reduce populations of *L. monocytogenes* on poultry meat, the pathogens could not be reduced to zero levels. Depending on the initial populations of the pathogen, reductions ranging from 1 log CFU g^{-1} may not be sufficient as the only means to improve the overall microbiological safety of poultry carcasses. However, citric acid treatments may be beneficial as part of an overall hazard analysis critical control point (HACCP) approach that can be implemented in order to enhance the microbiological safety and extend the shelf life of poultry meat.

Sensorial data (Table 1) indicate that the panel members could not detect negative effects in legs dipped in citric acid, being the scores observed above 6 until day 3. Thus, citric acid treatment did not have adverse effects on poultry legs' quality characteristics. Moreover, the highest acid concentration-treated samples (0.156 M) gave better colour score after day 6 of storage. This fact could be explained because citric acid has a bleaching effect as well as a reducing effect, and thus it maintains the desirable colour in comparison with the non-acid-treated samples (Surekha & Reddy, 2000). This also depends on the type of colour preferable in the particular country or population. Other authors have also reported that solutions of organic acids (1–3%) have no sensorial negative effects in meat when used as a decontaminant (Smulders & Greer, 1998).

Conclusions

The shelf life of samples washed with 0.156 M citric acid was extended by at least 2 days over the control samples

washed with distilled water. Legs washed with 0.156 M citric acid showed a significant ($P < 0.05$) inhibitory effect on *L. monocytogenes* compared with control legs. Sensory quality was not adversely affected by citric acid.

This study demostrates that, while citric acid did reduce the populations of *L. monocytogenes* on meat, it could not completely inactivate the pathogen. Of the concentrations tested, treatments with 0.156 M were the most effective for reducing populations of *L. monocytogenes*.

The application of citric acid cannot replace the rules of strict hygiene and good manufacturing practice, but it may be used as an additional hurdle contributing to extend the shelf life of raw poultry.

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