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

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ABSTRACT This work evaluated the effect of malic acid washing on the growth of *Listeria monocytogenes* on poultry legs stored at 4°C for 8 d. Fresh inoculated chicken legs were dipped into a 1 or 2% malic acid solution (vol/vol) for 5 min or distilled water (control). Surface pH values, sensorial characteristics (odor, color, texture, and overall appearance) and *L. monocytogenes*, mesophile, psychrotroph, and *Enterobacteriaceae* counts were evaluated after treatment (d 0) and after 1, 3, 6, and 8 d of storage at 4°C. Legs washed with 2% malic acid showed a significant ($P < 0.05$) inhibitory effect on *L. monocytogenes* compared with control legs, with a decrease of about 1.66 log units after treatment. Sensory quality was not adversely affected by malic acid. Treatments with malic acid reduced bacterial growth and preserved reasonable sensorial quality after storage at 4°C for 6 d. This study demonstrates that, although malic acid did reduce populations of *L. monocytogenes* on poultry, it did not completely inactivate the pathogen. The application of malic acid may be used as an additional hurdle contributing to extend the shelf life of raw poultry.

Key words: poultry, decontamination, pathogen reduction, organic acid, *Listeria monocytogenes*

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INTRODUCTION

Meat and poultry products are often identified as the source of foodborne pathogens (ICMSF, 1998). Raw poultry is a well-recognized 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. Reducing poultry contamination with foodborne pathogens during slaughter is particularly important. Because hygienic practices during slaughter cannot completely prevent contamination of poultry carcasses, decontamination treatments are gaining increasing interest in the slaughter process (González-Fandos and Dominguez, 2007; Loretz et al., 2010).

Organic acids (acetic, lactic, citric, malic) have a long history of being used as food additives and preservatives for preventing food deterioration and extending shelf life of perishable foods (Ricke, 2003). Organic acids are required at high concentrations to be effective as decontaminating agents (Siragusa, 1995). Generally, treatments with organic acids at varying concentrations result in population reductions ranging from 1 to 3 log units on meat surfaces (Dickson and Anderson, 1992).

The ability of malic acid to inhibit *L. monocytogenes* has been studied in laboratory media (Friedly et al., 2009; Over et al., 2009) and in vegetables and fruits (Raybaudi-Massilia et al., 2009; Sagong et al., 2011). The effect of malic acid against other pathogens such as *Arcobacter butzleri* has been studied in poultry by Skřivanová et al. (2011). However, there is no information on the effect of malic acid on *L. monocytogenes* growth on poultry. The aim of this work was to evaluate the effectiveness of a malic acid dip to control the growth of *L. monocytogenes* on poultry stored at 4°C. Microbiological and sensorial quality was also evaluated.

MATERIALS 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. 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

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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 90 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, where they were inoculated with *L. monocytogenes* by dipping them into a suspension of this pathogen (7 log cfu/mL) for 5 min at room temperature. After the 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 3 groups, each containing 15 legs. Samples of each group were dipped for 5 min into sterile distilled water (control; group 1), 1% (vol/vol; group 2), or 2% (group 3) malic acid (Scharlau, Barcelona, Spain). After these treatments, the legs were removed and drained for 5 min and stored individually in sterile bags at 4°C for 8 d. All experiments were carried out in duplicate.

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

Sensorial Analysis

The samples were evaluated for overall acceptability with regard to odor, color, texture, and overall appearance by a panel of 9 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 was aseptically weighed and homogenized 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 (Merck) following the pour plate method, incubating at 30°C for 72 h (ICMSF, 1978). Psychrotrophs were determined on Plate Count Agar (Merck) with an incubation temperature of 7°C for 10 d, using the pour plate method (ICMSF, 1978). Enumeration of *Enterobacteriaceae* was carried out on violet red bile glucose (Merck) following the pour plate method with an incubation temperature of 37°C for 48 h (ICMSF, 1978). *Listeria spp.* were determined following the surface plate method on Palcam agar with an incubation temperature of 30°C for 48 h (Mossel et al., 1995). Suspected 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 to 25°C, umbrella motility in the SIM medium (Oxoid, Unipath, UK), and CAMP test (Seeliger and Jones, 1986). Five suspected 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 was blended with 10 mL of distilled water. The pH of the homogenized sample was measured with a Crison model 2002 pH meter (Crison Instruments, Barcelona, Spain). Determinations of pH were performed in duplicate.

Statistical Analysis

Each experiment was replicated 2 times (2 different batches) with 3 samples analyzed each time (6 replicates). An ANOVA was performed using the SYSTAT program for Windows; Statistics version 5.0 (Evanston, IL). Tukey's test for comparison of means was performed using the same program. Plate count data were converted to logarithms before their statistical treatment. All experiments were carried out in duplicate. Significance level was defined at $P < 0.05$.

RESULTS

Microbiological Quality

The effect on viability of mesophiles and psychrotrophs on chicken legs dipped into different malic acid concentrations is shown in Tables 1 and 2, respectively. Microbiological quality was not evaluated on d 8 because all the samples were rejected by their sensorial quality. Significant differences ($P < 0.05$) in mesophile counts were found between the legs treated with 2% malic acid and the control legs. The data obtained showed that a 5 min dip in 2% vol/vol malic acid reduced mesophile counts between 1.14 and 1.83 log cycles compared with the control legs throughout storage. After treatment, mesophile counts were about 1.14 log units ($P < 0.05$) lower than in control samples. Significant differences ($P < 0.05$) were found for these bacterial counts between the samples treated with 1% malic acid and control samples except on d 0. No significant differences ($P > 0.05$) were found in mesophile counts between the samples treated with 1% malic acid and those treated with 2% except on d 0 and 3. After 6 d, mesophile counts on samples treated with 1 or 2% malic acid were 1.12 and 1.22 log units lower compared with control samples, respectively.

Significant differences ($P < 0.05$) in psychrotroph counts were found between the legs treated with 2%

Table 1. Effect of malic acid on mesophile counts on poultry legs (log cfu/g)[1]

	Days of storage				
Batch	0	1	3	6	8
Control	5.20 ± 0.18[a]	6.11 ± 0.09[a]	7.96 ± 0.11[a]	9.21 ± 0.06[a]	NI
1% Malic acid	5.05 ± 0.02[a]	5.00 ± 0.42[b]	7.98 ± 0.02[b]	8.09 ± 0.07[b]	NI
2% Malic acid	4.06 ± 0.02[b]	4.69 ± 0.04[b]	6.13 ± 0.09[c]	7.99 ± 0.01[b]	NI

[a–c]Means within columns followed by the same superscript were not significantly different ($P > 0.05$).

[1]Mean ± SD. NI = not investigated.

malic acid and the control legs. We found that washing with 2% malic acid reduced psychrotroph counts between 0.94 and 1.69 log cycles compared with the control legs throughout storage.

Table 3 shows the effect of malic acid treatment on the growth of *Enterobacteriaceae*. Significant differences ($P < 0.05$) in the *Enterobacteriaceae* counts were observed on legs treated with 2% malic acid compared with the control samples. After treatment, *Enterobacteriaceae* counts were 0.29 log cycles lower in legs treated with 2% malic acid than in control ones. After 1 d, *Enterobacteriaceae* counts on samples treated with 1 or 2% malic acid were 0.79 and 1.33 log units lower compared with control samples, respectively. No significant differences ($P > 0.05$) were found between the legs treated with 1% malic acid and those treated with 2% malic acid except on d 1.

L. monocytogenes

Table 4 shows the effect of malic 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 1 or 2% malic acid compared with the control samples. After treatment (d 0), *L. monocytogenes* counts in samples treated with 1 or 2% malic acid were 1.56 and 1.66 log cycles lower than in control ones, respectively. No significant differences ($P > 0.05$) were observed between legs treated with 1 or 2% malic acid.

pH Evolution

The pH values of the legs treated with malic acid are shown in Figure 1. Significant differences ($P < 0.05$) were found in pH values between samples treated with 1 or 2% malic acid and control samples, except on d 6. No significant differences ($P > 0.05$) were found in pH values between samples treated with 1% malic acid and those treated with 2%, except on d 0. Initial pH values in legs treated with 2% malic acid (d 0) were 4.34 ± 0.02, 1.76 units lower than in control legs. Initial pH values in legs treated with 1% malic acid (d 0) were 4.62 ± 0.06, whereas the values in control legs were 6.1 ± 0.03. The differences in pH between treated legs and control legs decreased during storage.

Sensorial Quality

The changes in color, odor, and overall appearance of the poultry legs are shown in Table 5. Sensory quality was not adversely affected by malic acid treatment, being the scores for treated samples above 6 until d 3. No significant differences ($P > 0.05$) in color were observed between samples treated with malic acid and control samples until d 3. After 6 d of storage, the worst score was obtained by control legs. Control legs were rejected on d 6. When treatments were compared at d 6 of storage, treatment with malic acid reduced ($P < 0.05$) the presence of off-odors compared with control. The samples treated with 1 or 2% malic acid were not severely discolored and unacceptable odors were not detected until d 8.

DISCUSSION

The mean log reductions found by us are 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 to 2 log cycles (Smulders and Greer, 1998). Raybaudi-Massilia et al. (2007) also reported malic acid at concentrations of 2% reduced the mesophile and psychrotroph counts in sliced apple.

Table 2. Effect of malic acid on the psychrotroph counts on poultry legs (log cfu/g)[1]

	Days of storage				
Batch	0	1	3	6	8
Control	5.00 ± 0.09[a]	5.94 ± 0.01[a]	7.35 ± 0.16[a]	9.22 ± 0.05[a]	NI
1% Malic acid	4.78 ± 0.01[a]	5.58 ± 0.06[a]	6.58 ± 0.15[b]	8.39 ± 0.13[b]	NI
2% Malic acid	4.06 ± 0.03[b]	4.72 ± 0.16[b]	5.66 ± 0.16[c]	7.81 ± 0.01[c]	NI

[a–c]Means within columns followed by the same superscript were not significantly different ($P > 0.05$).

[1]Mean ± SD. NI = not investigated.

Table 3. Effect of malic acid on the enterobacteria counts on poultry legs (log cfu/g)[1]

	Days of storage				
Batch	0	1	3	6	8
Control	3.97 ± 0.10^{a}	6.00 ± 0.06^{a}	6.40 ± 0.16^{a}	6.92 ± 0.01^{a}	NI
1% Malic acid	3.77 ± 0.00^{ab}	5.21 ± 0.05^{b}	5.54 ± 0.06^{b}	5.76 ± 0.06^{b}	NI
2% Malic acid	3.68 ± 0.02^{b}	4.67 ± 0.10^{c}	5.37 ± 0.09^{b}	5.63 ± 0.10^{b}	NI

[a–c]Means within columns followed by the same superscript were not significantly different ($P > 0.05$).

[1]Mean ± SD. NI = not investigated.

According to Gill and Landers (2003), decontaminating treatments must be regarded as trivial when the numbers of bacteria recovered before and after a treatment do not differ by a minimum of 0.5 log unit. In consequence, in our study malic acid treatments could be considered as effective.

In a previous work, we observed that a treatment with 2% lactic acid reduced mesophile counts between 0.67 and 2.32 log cycles compared with the control legs throughout storage (González-Fandos and Dominguez, 2006). In the present work, a treatment with 2% malic acid reduced mesophile counts between 1.14 and 1.83 log cycles. The antimicrobial effect of malic acid was higher than lactic acid on d 0, if we compare the percentage added being mesophile count reductions reached 1.14 and 0.67 log cycles, respectively. However, the antimicrobial effect of malic acid was lower than lactic acid after d 1 of storage. The efficacy of malic acid was higher than citric acid because a treatment with 2% citric acid reduced mesophile counts between 0.45 and 1.08 log cycles (González-Fandos et al., 2009).

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). In this study, the application of malic acid reduced the surface pH immediately after treatment, thereby creating an unfavorable environment for bacterial growth. Mean skin pH value of untreated samples was 6.1. Washing with 1 or 2% malic acid solution resulted in a decrease in pH of about 1.48 and 1.76 units, respectively.

The higher efficacy against *L. monocytogenes* in apple, pear, and melon juice reported by Raybaudi-Massilia et al. (2009) could be explained by these products having a lower pH (3.94, 4.60, and 5.45, respectively) than poultry legs.

Sagong et al. (2011) have also observed that malic acid was effective against *L. monocytogenes* in lettuce. These authors reported that 1% malic acid reduced *L. monocytogenes* counts in 1.38 log units. We observed reduction of 1.56 log cycles in poultry. Our results are in agreement with those reported by Sagong et al. (2011), who observed that concentrations of 2% of malic acid only resulted in an additional 0.15 log reductions of *L. monocytogenes* compared with 1% malic acid. Reduction levels of *L. monocytogenes* increased with increasing malic acid concentration up to 1%, whereas reduction did not increase much between 1 to 2% organic acid concentration.

Organic acids have optimal inhibitory activity at low pH because it favors the uncharged undissociated state of the molecule, which is responsible for the bactericidal activity. In our study, we did not find significant differences ($P > 0.05$) in pH values between samples treated with 1% malic acid and those treated with concentrations of 2% except on d 0, which may explain why a higher concentration of washing solution did not result in a significant higher reduction of *L. monocytogenes*.

According to our results, malic acid was more effective against *L. monocytogenes* than citric acid in poultry in terms of total acid added, because in a previous work we observed that a treatment with 2% citric acid resulted in 1.12 log cycle reductions of the pathogen on d 0 compared with the control legs, whereas reductions obtained in the present work with 2% malic acid were 1.66 log cycles compared with the control legs (González-Fandos et al., 2009). Our results are consistent with those reported by Eswaranandam et al. (2004) who indicated that malic acid was more effective than citric acid to reduce pathogens as a consequence

Table 4. Effect of malic acid on *Listeria monocytogenes* counts on poultry legs (log cfu/g)[1]

	Days of storage				
Batch	0	1	3	6	8
Control	4.68 ± 0.02^{a}	5.51 ± 0.09^{a}	7.19 ± 0.08^{a}	9.05 ± 0.04^{a}	NI
1% Malic acid	3.12 ± 0.11^{b}	5.01 ± 0.04^{b}	6.71 ± 0.15^{b}	8.36 ± 0.27^{b}	NI
2% Malic acid	3.02 ± 0.01^{b}	4.71 ± 0.24^{b}	6.31 ± 0.02^{b}	7.99 ± 0.01^{b}	NI

[a,b]Means within columns followed by the same superscript were not significantly different ($P > 0.05$).

[1]Mean ± SD. NI = not investigated.

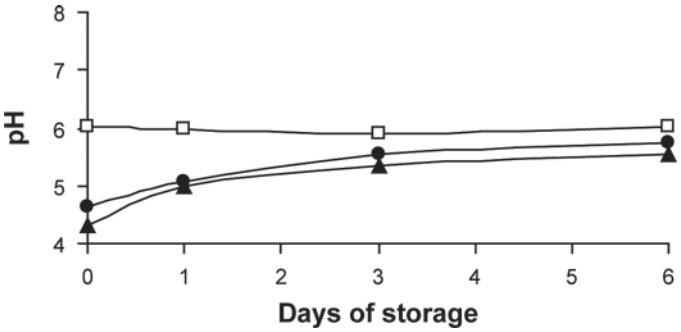

Figure 1. Evaluation of pH in chickens legs treated with malic acid. Control (□), malic acid 1% (vol/vol; ●), and malic acid 2% (vol/vol; ▲). The data are the mean values of 2 replicates.

of its molecular weight, which may facilitate the entrance of malic acid into microbial cells.

It must be highlighted that Uyttendale et al. (1997) reported that among chicken parts, *L. monocytogenes* was predominantly isolated from chicken legs and chicken wings, the parts that are still partially covered with skin. This pathogen is mainly located on the skin surface of poultry carcasses and to a lesser extent in the meat. On the other hand, the higher pH of leg meat may provide more favorable conditions for multiplication of *L. monocytogenes* (ICMSF, 1998).

Although treatments with malic acid did reduce populations of *L. monocytogenes* on poultry meat, they were not able to reduce the pathogen to zero levels. Depending on the initial populations of the pathogen, reductions ranging from 1 log cfu/g may not be sufficient as the only means to improve the overall microbiological safety of poultry carcasses. However, malic acid treatments may be beneficial as part of an overall hazard analysis critical control point (HACCP) approach that can be implemented to enhance the microbiological safety and extended the shelf life of poultry meat.

Malic acid treatment did not have adverse effects on poultry leg quality characteristics. 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 and Greer, 1998). Skřivanová et al. (2011) did not observe any change in color, odor, or appearance in poultry carcasses after washing with malic acid. Other authors have not observed a negative effect of malic acid on sensorial quality in other foods (Bal'a and Marshall, 1998; Singla et al., 2011).

In the present work, off-odors were noticed by the panel members when the counts approached 9 log cfu/g. To compare our results with those reported by other authors the data were transformed 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 corresponded to 8.16 log cfu/cm^2. Other authors have reported spoilage odors in poultry when counts approached 7 to 8 cfu/cm^2 (Barnes, 1976; Elliot et al., 1985; Studer et al., 1988).

After 6 d of storage, mesophiles and psychrotrophs reached populations above 9 log cfu/g in control legs, and off-odors were detected. However, in the legs treated with 1 or 2% malic acid, mesophile and psychrotroph counts were below 9 log cfu/g on d 6 and signs of spoilage were not detected. Control legs were rejected on d 6. On d 8, all the samples were rejected by their sensorial scores.

In conclusion, this study demonstrates that although malic acid reduced populations of *L. monocytogenes* on meat, it did not completely inactivate the pathogen. Of the concentrations tested, treatments with 2% malic acid were the most effective for reducing populations of *L. monocytogenes*. The application of malic 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.

Table 5. Mean scores ± SD of different sensory characteristics (color, odor, and overall appearance) of chicken legs treated with malic acid stored at 4°C[1]

		Treatment		
			Malic acid	
Sensory characteristic	Storage time (d)	Control	1%	2%
Color	0	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
	1	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
	3	4.11 ± 0.31	6.11 ± 0.31	6.22 ± 0.41
	6	1.33 ± 0.47	4.11 ± 0.31	4.22 ± 0.41
	8	1.11 ± 0.31	3.11 ± 0.31	3.22 ± 0.41
Odor	0	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
	1	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
	3	4.22 ± 0.41	6.11 ± 0.31	6.22 ± 0.41
	6	1.22 ± 0.41	4.22 ± 0.41	4.22 ± 0.41
	8	1.11 ± 0.31	3.11 ± 0.31	3.11 ± 0.31
Overall appearance	0	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
	1	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
	3	4.11 ± 0.31	6.11 ± 0.31	6.22 ± 0.41
	6	1.22 ± 0.41	4.11 ± 0.31	4.22 ± 0.41
	8	1.11 ± 0.41	3.11 ± 0.31	3.11 ± 0.31

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

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