Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

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

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

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2005/1047: received 14 September 2005, revised 16 March 2006 and accepted 17 March 2006

doi:10.1111/j.1365-2672.2006.03022.x

Abstract

Aims: The aim of this study was to evaluate the effect of lactic acid washing on the growth of *Listeria monocytogenes* on poultry legs stored at 4°C for 7 days.
Methods and Results: Fresh inoculated chicken legs were dipped into either a 0·11, 0·22 mol l^{-1} or 0·55 mol l^{-1} lactic acid solution for 5 min or distilled water (control). Surface pH values, sensorial characteristics and *L. monocytogenes*, mesophiles and pychrotrophs counts were evaluated after treatment (day 0) and after 1, 3, 5 and 7 days of storage at 4°C. Legs washed with 0·55 mol l^{-1} lactic acid for 5 min showed a significant ($P < 0·05$) inhibitory effect on *L. monocytogenes* compared with control legs, being about 1·74 log units lower in the first ones than in control legs after 7 days of storage. Sensory quality was not adversely affected by lactic acid, with the exception of colour.
Conclusions: Treatments with 0·55 mol l^{-1} lactic acid reduced bacterial growth and preserved reasonable sensorial quality after storage at 4°C for 7 days. However, it was observed a reduction in the colour score within 1 day post-treatment with 0·55 mol l^{-1} lactic.
Significance and Impact of the Study: This study demonstrates that, while lactic acid did reduce populations of *L. monocytogenes* on poultry, it did not completely inactivate the pathogen. The application of lactic acid may be used as an additional hurdle contributing to extend the shelf-life of raw poultry.

Introduction

Meat and poultry products are often identified as the source of foodborne disease outbreaks' (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; Uyttendale *et al.* 1997; Gudbjörnsdóttir *et al.* 2004). Some authors have associated cases of listeriosis with the consumption of undercooked chicken (Schuchat *et al.* 1992).

The microbial contamination of raw chicken can be minimized by good manufacturing practices, but the total elimination of foodborne pathogenic micro-organisms is difficult if not impossible. A variety of methods have been developed to reduce the levels of contaminating bacteria on carcasses. 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, irradition, pulsed-field electricity, ultrasonic energy and UV light (Bolder 1987). On the other hand, the shelf-life of raw chicken is very short and it would be useful to note what the usual shelf-life is so that it can be seen if the treatments used in this study extended the shelf-life compared not only to the control but also to commercial products.

Organic acids (acetic, lactic and sorbic) have been traditionally used as food preservatives and are generally recognized as safe substances (GRAS) and approved as food additives by E.C., FAO/WHO and FDA (Surekha and Reddy 2000). In November 1992, pre-evisceration organic acid rinses were approved by the Food Safety and Inspection Service of the US 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, Smulders *et al.* (1986) have recommended that public health authorities should allow the use of lactic acid as a decontaminating agent.

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).

Generally, treatments with lactic acid at varying concentrations result in population reductions ranging from 1 to 3 log units on meat surfaces (Dickson and Anderson 1992). The effectiveness of lactic acid for controlling meat-borne pathogens varied between studies and may be attributable to differences in acid concentration as well as methods for acid delivery, temperature of acids, contact time, sampling techniques, tissue type, or organisms (Greer and Dilts 1992). Several research reports have addressed the use of lactic acid for reducing *Salmonella* (Mulder *et al.* 1987; Izat *et al.* 1989; Li *et al.* 1997). The ability of lactic acid to inhibit *L. monocytogenes* has been studied in laboratory media (Ahamad and Marth 1989; Van Netten *et al.* 1994) and in beef (El-Khateib *et al.* 1993; Dickson and Siragusa 1994; Conner *et al.* 1997; Ariyapitipun *et al.* 2000; Stopforth *et al.* 2003). However, there are few studies on the effect of lactic acid on *L. monocytogenes* growth on poultry (Zeitoun and Debevere 1991).

The aim of this work was to evaluate the effectiveness of several concentrations of a lactic acid dip to control the growth of *L. monocytogenes* on poultry stored at 4°C. 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 40 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. monocytogenes* by dipping them into a suspension of this pathogen (7 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 10 legs and dipped for 5 min into sterile distilled water (control) (Batch C), 0·11 mol l^{-1} (Batch L1), 0·22 mol l^{-1} (Batch L2) or 0·55 mol l^{-1} (Batch L5) lactic 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 7 days.

Samples were taken on days 0 (after the dipping treatment), 1, 3, 5 and 7. On the sampling days, two 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 seven (I like it very much) to one (I dislike it very much) was used. A score of three was considered the borderline of acceptability.

Microbiological analyses and pH determination

Ten grams of skin were 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 micro-organisms 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 Plate Count Agar (Merck) with an incubation temperature of 7°C for 10 days, using the pour plate method (ICMSF 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). 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 20–25°C, umbrella motility in the SIM medium (Oxoid, Unipath, England) 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 were blended with 10 ml of distilled water. The pH of the homogenized sample was measured with a Crison model 2002 pHmeter (Crison Instruments, Barcelona, Spain). Determination of pH were performed in duplicate.

Statistical analysis

Analysis of variance was performed using the SYSTAT program for Windows; Statistics Version 5·0 (Evanston, IL, USA, 1992). 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 dupicate. Significance level was defined at $P < 0{\cdot}05$.

Results

Microbiological quality

The effect on mesophiles and psychrotrophs of dipping the legs into different lactic acid concentrations is shown in Tables 1 and 2, respectively. Significant differences ($P < 0{\cdot}05$) in mesophile counts were observed between the legs treated with 0·55 mol l^{-1} lactic acid and the control legs. The data obtained showed that a 5 min dip in 0·55 mol l^{-1} lactic acid reduced mesophile counts between 0·68 and 3·13 log cycles compared with the control legs throughout storage. Significant differences ($P < 0{\cdot}05$) were also found between the legs treated with 0·22 mol l^{-1} lactic acid and the control legs, except after 7 days of storage. However, significant differences ($P < 0{\cdot}05$) were found for these bacterial counts between the samples treated with 0·11 mol l^{-1} lactic acid and the control samples only on days 1 and 5, although lower counts were observed on analyses performed on the other days.

No significant differences in psychrotrophs counts were found between samples treated with lactic acid and the control samples on day 0 ($P > 0{\cdot}05$). After day 1, significant differences were observed between control samples and those treated with lactic acid, as well as differences between the batches treated with lactic acid. After 7 days of storage, the differences between control samples and those treated with lactic acid were smaller and only significant with samples treated with 0·55 mol l^{-1} lactic acid.

Listeria monocytogenes

Table 3 shows the effect of lactic acid treatment on the growth of *L. monocytogenes* inoculated onto legs. Significant differences ($P < 0{\cdot}05$) in the *L. monocytogenes* populations were observed on legs treated with 0·55 mol l^{-1} lactic acid compared with the control samples. After 7 days of storage, *L. monocytogenes* counts were 1·74 log cycles lower in legs treated with 0·55 mol l^{-1} lactic acid than in control ones. Significant reductions in the *L. monocytogenes* populations were also observed on legs treated with 0·22 mol l^{-1} lactic acid on days 1, 3 and 5 of storage compared with the control samples. No significant differences were observed between legs treated with 0·11 mol l^{-1} lactic acid and control legs, although the populations reached were lower. Throughout the 7 days of storage, *L. monocytogenes* counts on legs treated with 0·55 mol l^{-1} lactic acid were 0·9–1·74 log cycles lower than on untreated legs. Samples treated with 0·22 or 0·55 mol l^{-1} lactic acid displayed an extended lag phase of *L. monocytogenes* and lower counts throughout storage compared with control legs. While *L. monocytogenes* grew readily on control legs, growth was slower on acid dipped legs, particularly those dipped in 0·22 and 0·55 mol l^{-1} lactic acid.

pH evolution

The pH values of the legs treated with lactic acid are shown in Fig. 1. Significant differences were found in pH values between samples treated with 0·55 mol l^{-1} lactic acid and control samples. No significant differences in pH were observed after 7 days of storage between samples treated with 0·11 or 0·22 mol l^{-1} lactic acid and control samples. The pH was lower when the lactic acid

Table 1 Effect of lactic acid on mesophiles counts on poultry legs (log CFU g^{-1})

	Days of storage				
Batch	0	1	3	5	7
Control	5·92 ± 0·19^{a}	6·89 ± 0·15^{a}	8·65 ± 0·06^{a}	9·30 ± 0·11^{a}	9·54 ± 0·07^{a}
0·11 mol l^{-1} lactic acid	5·59 ± 0·16^{ab}	5·29 ± 0·08^{b}	7·84 ± 0·26^{a}	8·49 ± 0·11^{b}	9·06 ± 0·20^{ab}
0·22 mol l^{-1} lactic acid	5·25 ± 0·08^{b}	5·26 ± 0·04^{b}	6·32 ± 0·17^{b}	7·68 ± 0·15^{c}	8·77 ± 0·33^{ab}
0·55 mol l^{-1} lactic acid	4·60 ± 0·16^{c}	4·77 ± 0·09^{c}	5·52 ± 0·11^{c}	7·35 ± 0·14^{cd}	8·86 ± 0·06^{b}

Mean ± standard deviation. Means within columns followed by the same letter were not significantly different ($P > 0{\cdot}05$).

Table 2 Effect of lactic acid on the psycrotrophs counts on poultry legs (log CFU g^{-1})

	Days of storage				
Batch	0	1	3	5	7
Control	4·87 ± 0·30^{a}	6·94 ± 0·21^{a}	8·60 ± 0·26^{a}	9·38 ± 0·14^{a}	9·13 ± 0·04^{a}
0·11 mol l^{-1} lactic acid	4·39 ± 0·13^{a}	5·02 ± 0·11^{bc}	7·00 ± 0·16^{b}	8·55 ± 0·08^{b}	8·94 ± 0·08^{ab}
0·22 mol l^{-1} lactic acid	4·09 ± 0·23^{a}	4·98 ± 0·13^{b}	5·95 ± 0·36^{b}	7·83 ± 0·09^{c}	8·80 ± 0·43^{ab}
0·55 mol l^{-1} lactic acid	4·00 ± 0·18^{a}	4·20 ± 0·04^{c}	5·95 ± 0·35^{b}	7·47 ± 0·04^{d}	8·43 ± 0·07^{b}

Mean ± standard deviation. Means within columns followed by the same letter were not significantly different ($P > 0·05$).

Table 3 Effect of lactic acid on *Listeria monocytogenes* counts on poultry legs (log CFU g^{-1})

	Days of storage				
Batch	0	1	3	5	7
Control	4·75 ± 0·22^{a}	5·09 ± 0·01^{a}	6·25 ± 0·16^{a}	6·95 ± 0·08^{a}	7·69 ± 0·27^{a}
0·11 mol l^{-1} lactic acid	4·27 ± 0·13^{ab}	5·52 ± 0·40^{a}	5·68 ± 0·28^{ab}	6·31 ± 0·208^{ab}	7·25 ± 0·20^{a}
0·22 mol l^{-1} lactic acid	4·15 ± 0·21^{ab}	3·75 ± 0·07^{b}	5·21 ± 0·13^{b}	5·91 ± 0·13^{b}	6·79 ± 0·16^{a}
0·55 mol l^{-1} lactic acid	3·70 ± 0·14^{b}	3·80 ± 0·14^{b}	4·34 ± 0·23^{c}	5·07 ± 0·47^{b}	5·95 ± 0·07^{b}

Mean ± standard deviation. Means within columns followed by the same letter were not significantly different ($P > 0·05$).

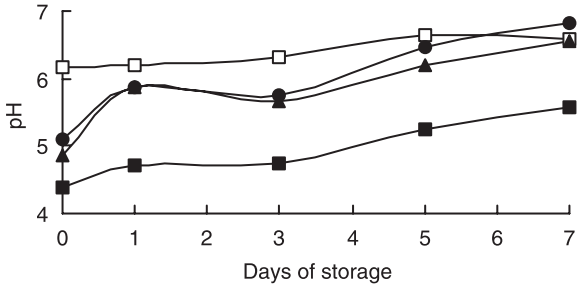

Figure 1 Evolution of pH in chicken's legs treated with lactic acid. Control (□), lactic acid 0·11 mol l^{-1} (w/v) (●), lactic acid 0·22 mol l^{-1} (▲) and lactic acid 0·55 mol l^{-1} (■). The data are the mean values of two replicates.

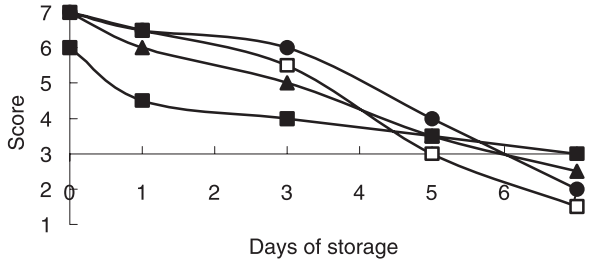

Figure 2 Evolution of colour in chicken's legs treated with lactic acid. Control (□), lactic acid 0·11 mol l^{-1} (w/v) (●), lactic acid 0·22 mol l^{-1} (▲) and lactic acid 0·55 mol l^{-1} (■). The data are the mean values of two replicates.

concentration was higher. These pH differences decreased throughout storage. Initial pH values in legs treated with 0·55 mol l^{-1} lactic acid (day 0) were 4·38 ± 0·04, 1·78 units lower than in control legs. During storage, pH increased 0·86 units after 3 days, being 5·56 after 7 days of storage, 1·02 units lower than in control samples.

Sensorial quality

The changes in colour, odour and overall appearance of the poultry legs are shown in Figs 2–4 respectively. Significant ($P < 0·05$) and severe discolouration was observed at day 1 for legs treated with 0·55 mol l^{-1} lactic acid compared with the other treatments. A pale appearance was observed in the 0·55 mol l^{-1} lactic acid treated samples. However, after 5 days of storage, the worst score was obtained by the control legs. When treatments were

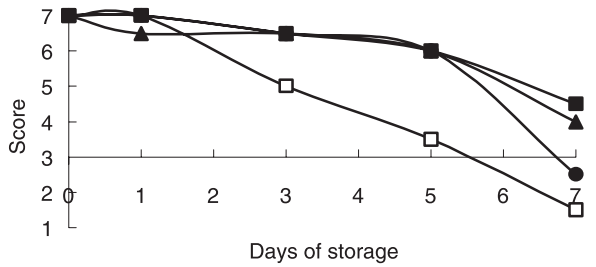

Figure 3 Evolution of odour in chicken's legs treated with lactic acid. Control (□), lactic acid 0·11 mol l^{-1} (w/v) (●), lactic acid 0·22 mol l^{-1} (▲) and lactic acid 0·55 mol l^{-1} (■). The data are the mean values of two replicates.

compared at day 5 of storage, treatments with 0·11, 0·22 and 0·55 mol l^{-1} lactic acid reduced ($P < 0·05$) the presence of off-odours compared with controls. After 7 days of storage, all samples had strong off odours and were

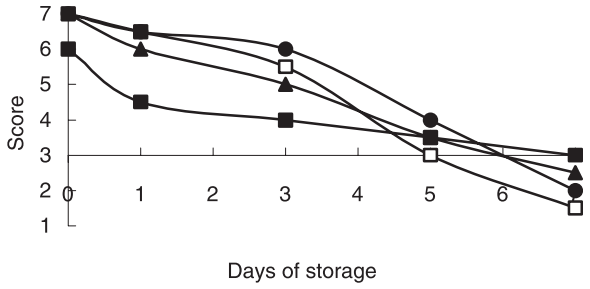

Figure 4 Overall appearance of chicken's legs treated with lactic acid. Control (□), lactic acid 0·11 mol l^{-1} (w/v) (●), lactic acid 0·22 mol l^{-1} (▲) and lactic acid 0·55 mol l^{-1} (■). The data are the mean values of two replicates.

rejected, except those treated with 0·22 or 0·55 mol l^{-1} lactic acid. The samples treated with 0·22 or 0·55 mol l^{-1} lactic acid were not severely discoloured until day 7, unacceptable odours were not detected in these treatments throughout storage. Consequently, legs receiving treatments with 0·22 or 0·55 mol l^{-1} lactic acid remained acceptable until 7 days of storage, 2 days longer than control samples (Fig. 4).

Discussion

Our results agree with those reported by Ismail *et al.* (2001) who found that treatment of chicken wings with 0·22 and 0·55 mol l^{-1} lactic acid reduced mesophiles by 0·75 and 1·07 logs units on day 0, compared with controls, respectively. After 3 days of storage treatment of chicken with 2% (0·22 mol l^{-1}) lactic acid, these authors observed slightly lower reductions (1·92 logs units compared with 2·33 logs units in controls as reported here). Van der Marel *et al.* (1988) also observed mesophiles reductions after treatment with 0·11 or 0·22 mol l^{-1} of lactic acid. After lactic acid treatment, these authors observed similar reductions in mesophiles and psycrotrophs to those reported here. As in the present study, the aforementioned authors did not find significant differences in mesophiles between carcasses washed with 0·11 or 0·22 mol l^{-1} lactic acid immediately after treatment, although differences were observed on days 3 and 7 of storage. After storage for 7 days at 0°C, they reported lower mesophiles and psycrotrophs counts, probably because of the lower storage temperature (0°C compared with 4°C in the present study). Zeitoun and Debevere (1990) applied 1·11 mol l^{-1} lactic acid, buffered to pH 3·0 on chicken legs. They found that the use of 1·11 mol l^{-1} lactic acid buffer resulted in a reduction of psychrotrophs counts in poultry meat of 1·3 log units on day 0 compared with untreated poultry.

After 5 days of storage, mesophiles and psychrotrophs reached populations above 9 log CFU g^{-1} in control legs. However, in the legs treated with 2 or 5% of lactic acid, mesophiles and psychrotrophs counts were below 9 log CFU g^{-1} after 7 days of storage at 4°C and signs of spoilage were not detected after 7 days of storage. When the legs were treated with 0·11 mol l^{-1} lactic acid, populations around 9 log CFU g^{-1} were detected on day 7 of storage, although sensorial scores were higher than those recorded for the control legs, and these legs were rejected on day 7 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 might have been because of the effects of acidic pH. Thus, lower counts were observed in legs with lower pH. The anti-microbial 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). In this study, the application of 0·55 mol l^{-1} lactic acid reduced the surface pH immediately after treatment, thereby creating an unfavourable environment for bacterial growth. Our results agree with those reported by Van der Marel *et al.* (1989) who observed that lactic acid treatments caused a decline in the pH of the poultry skin depending on the lactic acid concentration used.

Despite being considered the most important factor by some authors (Gill and Newton 1982) a decrease in pH is not likely to be the only mechanism by which bacterial growth is inhibited. The specific effect of lactic acid (Ingran 1972), as well as the concentration of the undissociated acid portion are other relevant factors (Grau 1980; Osthold *et al.* 1983). The inhibition of the growth of micro-organisms depends not only on the pH of the growth medium, but also on the nature, the dissociation constant (p K_a) and the concentration of the acidulant used (Debevere 1987). The use of buffered lactic acid systems compared with unbuffered solutions also enhanced the decontaminating effect and increased the shelf-life of chicken legs (Van der Marel *et al.* 1988).

The ability of lactic acid to inhibit *L. monocytogenes* can be higher in laboratory media than in foods according to the results reported by Ahamad and Marth (1989). These authors found that lactic acid concentrations as low as 0·011 mol l^{-1} when incorporated into tryptose broth, inhibited the growth of *L. monocytogenes* and that the degree of inhibition increased as the temperature of incubation decreased. This fact may explain the results

obtained by Van Netten *et al.* (1994) when they studied the effect of 0·22 mol l^{-1} lactic acid in an *in vitro* model. These authors observed that 2% lactic acid at 37°C for 30–60 s was suitable for eliminating salmonellas on meat but not for *L. monocytogenes*.

The differences in efficacy of lactic acid on *L. monocytogenes* found in the bibliography can be explained by variations in the type of meat (beef, pork and poultry), concentration of acid used, application method (spray and immersion), exposure times, temperature, inoculum size, strains of *L. monocytogenes* studied and modified atmosphere packaging.

Zeitoun and Debevere (1991) reported that *L. monocytogenes* inoculated on poultry legs at levels of approximately 2·92 log CFU cm^{-2} and treated with 0·55 mol l^{-1} buffered lactic was reduced after 2 and 6 days of storage at 6°C, 0·9 and 1·39 log CFU cm^{-2}, respectively, compared with untreated samples. The storage temperature used by Zeitoun and Debevere (1991) was 6°C instead of 4°C in the present study. We observed higher reductions in *L. monocytogenes* than those reported by the mentioned authors (1·91 and 1·88 log cycles lower in legs treated with 0·55 mol l^{-1} lactic acid than in control ones after 3 and 5 days of storage, respectively).

To compare our results with those reported by Zeitoun and Debevere (1991) our data were transformed to log CFU cm^{-2} . In the present study the initial levels of *Listeria* were 3·91 log CFU cm^{-2}, 0·99 log units higher than the one mentioned by these authors. Also there are differences in the application method, the method used by Zeitoun and Debevere was spraying instead of immersion. On the other hand these authors reported data on the combined effect of buffered lactic acid treatment and modified atmosphere packaging (90% CO_2 and 10% O_2). According to these authors there is a synergistic effect of CO_2 and lactic acid treatment. However, it is important to note that the growth of *L. monocytogenes* on meat and poultry is highly dependent on the temperature, pH, and the type and amount of background microflora present (Glass and Doyle 1989).

Spoilage aerobic microflora are greatly inhibited by modified atmosphere packaging. However, *L. monocytogenes* is potentially capable of growing under these conditions depending on the CO_2 concentration. There have been conflicting reports on the ability of *Listeria* to grow at high CO_2 levels, mainly because of the influence of factors such as pH, temperature and microbial competition. *Listeria monocytogenes* is unable to grow at 6°C on chicken packaged under 30% CO_2 or at 4°C in an atmosphere of 75% CO_2. However, adding 5% oxygen to the latter atmosphere results in growth of the organism (Wimpfheimer *et al.* 1990; Harth *et al.* 1991). It can be stated that *L. monocytogenes* is able to grow in CO_2 levels up to 50%. Growth of this organism in concentrations higher than this will depend mainly on the interplay between the gas atmosphere, pH, temperature and microbial competition.

Glass and Doyle (1989) reported that the *L. monocytogenes* grew well on those meat products with a pH value near or above 6·0, while 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 legs muscles have a pH of 6·4–6·7, while other parts like breast muscles have lower pH values (5·7–5·9) (Barnes 1976). This higher pH can explain why poultry supports the growth of *L. monocytogenes* better than other meats, for that reason decreasing the pH with lactic acid treatment could contribute to control the growth of *L. monocytogenes*.

The efficacy of lactic acid against *Listeria* could be higher in other types of meat. Dorsa *et al.* (1997) reported that treatments with 0·33 mol l^{-1} lactic acid initially reduced the level of *L. innocua* on inoculated beef by 2·8 log CFU cm^{-2} . These authors reported the ability of lactic acid to inhibit the growth of this pathogen for at least 21 days on beef carcass surface held at 5°C. The same authors reported that *L. innocua* inoculated in beef at levels of approximately 2 log was suppressed after treatment with 0·22 mol l^{-1} lactic acid and also through storage at 4°C for 21 days (Dorsa *et al.* 1998). El-Khateib *et al.* (1993) found that 0·22 mol l^{-1} lactic acid on beef surfaces had an immediate and also a delayed listericidal action during 48 h of refrigeration storage. Ariyapitipun *et al.* (2000) reported that when beef were dipped in 0·22 mol l^{-1} lactic acid the reductions in *L. monocytogenes* were 1·56 log at day 0. Effect of lactic acid on *L. monocytogenes* growth in pork has been also studied. Greer and Dilts (1995) reported that a 0·33 mol l^{-1} solution of lactic acid produced a 2 log cycle reduction in *L. monocytogenes* inoculated on lean pork.

Although treatments with lactic 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 to 2 log CFU g^{-1} may not be sufficient as the only means to improve the overall microbiological safety of poultry carcasses. However, lactic 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 extended the shelf-life of poultry meat.

There was a larger reduction of bacteria but the colour score of the poultry legs could be affected by increasing concentrations of lactic acid. These data support the findings reported by other authors, who indicate that lactic acid may affect the colour of treated meat to a certain

extent, depending on its concentration (Snijders *et al.* 1979; Smulders and Woolthuis 1985; Van Netten *et al.* 1995) According to other authors (Smulders 1987; Izat *et al.* 1989), decontamination at 0·22 mol l^{-1} lactic acid is the highest concentration which gives just acceptable discolouration. However, Singh *et al.* (1988) observed no adverse effect of 0·22 mol l^{-1} lactic acid treatment on colour, flavour and overall acceptability attributes of poultry. In contrast, Mulder *et al.* (1987) reported that immersion of poultry carcass in 0·11 mol l^{-1} lactic acid solution for 15 min resulted in a slightly changed colour.

Discrete decolouration was observed after treatment with 0·55 mol l^{-1} of lactic acid, probably because of protein denaturation caused by the quick decrease of pH (Snijders *et al.* 1979). This fact may account for the results reported by Zeitoun and Debevere (1990), who did not find adverse effect on sensory quality when applying 1·11 mol l^{-1} lactic acid buffer in poultry meat.

Conclusions

Treatments with 0·55 mol l^{-1} lactic acid reduced the bacterial growth and preserved a reasonable sensorial quality after storage at 4°C for 7 days, with the exception of colour which was adversely affected. The increased concentration of lactic acid extended the lag phase of the microbial growth. At the same time, the shelf-life of chilled poultry was prolonged.

This study demonstrates that while lactic acid did reduce populations of *L. monocytogenes* on meat, it did not completely inactivate the pathogen. Of the concentrations tested, treatments with 0·55 mol l^{-1} were the most effective for reducing populations of *L. monocytogenes*.

The application of lactic 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.

Acknowledgements

The authors thank the Regional Government of La Rioja (Spain) for its financial support (Project Reference ANGI 2002/08, Leader researcher Dr E. González-Fandos, Univeristy of La Rioja).

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