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Effect of propionic acid on *Campylobacter jejuni* attached to chicken skin during refrigerated storage

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Summary. The ability of propionic acid to reduce *Campylobacter jejuni* on chicken legs was evaluated. Chicken legs were inoculated with *Campylobacter jejuni*. After dipping legs in either water (control), 1% or 2% propionic acid solution (vol/vol), they were stored at 4ºC for 8 days. Changes in *C. jejuni*, psychrotrophs and *Pseudomonas* counts were evaluated. Washing in 2% propionic acid significantly reduced ($P < 0.05$) *C. jejuni* counts compared to control legs, with a decrease of about 1.62 log units after treatment. Treatment of chicken legs with 1 or 2% propionic acid significantly reduced ($P < 0.05$) numbers of psychrotrophs 1.01 and 1.08 log units and *Pseudomonas* counts 0.75 and 0.96 log units, respectively, compared to control legs. The reduction in psychrotrophs and *Pseudomonas* increased throughout storage. The highest reductions obtained for psychrotrophs and *Pseudomonas* counts in treated legs were reached at the end of storage, day 8, being 3.3 and 2.93 log units, respectively, compared to control legs. Propionic acid treatment was effective in reducing psychrotrophs and *Pseudomonas* counts on chicken legs throughout storage. It is concluded that propionic acid is effective for reducing *C. jejuni* populations in chicken. **[Int Microbiol** 18(3):171-175 (2015)]

Keywords: *Campylobacter jejuni* · *Pseudomonas* spp. · poultry · meat safety · pathogen reduction

Introduction

Human campylobacteriosis is one of the most frequently reported food-borne diseases in the European Union, with 214,779 confirmed cases in 2013. Consumption of contaminated chicken meat is often the source of infection [10]. Various strategies to control *Campylobacter* in chicken have been suggested [9]. The treatment with organic acids is one approach to decontaminate chicken [2,11–13].

Propionic acid has antibacterial activity and could play a role in reducing pathogens in meat and poultry. Mani-López et al. [12] suggested that propionic acid has promising applications in meat and poultry products since it is more effective against *Salmonella* than are other organic acids such as acetic or lactic acids.

Because *Campylobacter jejuni* is a pathogen often associated with chicken, it would be of particular importance to reduce the levels of this bacterium on chicken. The activity of organic acids such as acetic or lactic acid on *C. jejuni* has been investigated by other authors [6]. The efficacy of propionic acid on *C. jejuni* has been investigated in vitro [16]. However, there are few works on the efficacy of propionic acid against *C. jejuni* in chicken [16].

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The purpose of this study was to evaluate the ability of a propionic acid dip to reduce *Campylobacter jejuni* in chicken stored at 4ºC.

Material and methods

Preparation of inocula. *Campylobacter jejuni* ATTCC 33291 was grown in Preston Campylobacter enrichment broth (Oxoid, Hampshire, UK) under microaerobic conditions (85% N_2 10% CO_2 and 5% O_2) at 42ºC for 24h. Anaerobic jars and Gas Generating Kits (BR 56, Oxoid) were used to create microaerobic conditions. After, the culture was centrifuged at 10000 g for 15 min at 4ºC (Sorvall RC-5B, GMI Inc., Minnesota, USA). The supernatant was decanted and the pellet resuspended in sterile peptone water (0.1%) (Merck, Darmstadt, Germany) by vortexing. The suspension of washed cells was diluted in a sterile peptone water (0.1%) to obtain an appropriate cell concentration for inoculation.

Inoculation of chicken legs and treatment. Ninety fresh chicken legs were collected from a commercial chicken processing plant (Logroño, La Rioja, Spain). The legs were transported on crushed ice to the laboratory. Fresh chicken legs with skin were inoculated with *C. jejuni* by dipping them into a suspension of this pathogen for 5 min at room temperature. After the inoculation, the legs were removed and kept for 30 min at room temperature to allow the bacteria to attach to the skin. The inoculated chicken legs were randomly divided into three batches, each containing 30 legs. Samples of each batch were immersed for 5 min into sterile distilled water (control) (batch one), 1% (batch 2) or 2% (batch 3) propionic acid (Scharlab, Barcelona, Spain). After immersion, all legs were removed and drained for 5 min at room temperature. Afterwards, legs were placed individually in sterile bags and stored at 4ºC for 8 days.

Microbiological analyses and pH determination. Analyses were performed on days 0 (after immersion treatment), 1, 3, 6 and 8. On the sampling days, six legs of each batch were taken out from storage to carry out microbiological and pH analysis. Ten grams of skin were aseptically weighed and homogenized in a Stomacher (IUL, Barcelona, Spain) for 2 min with 90 ml of 0.1% sterile peptone water (Oxoid). Serial decimal dilutions were prepared using the same diluent. The number of psychrotrophs was determined on Plate Count Agar (Merck) using the pour plate method. The plates were incubated at 7ºC for 10 days [18]. *Pseudomonas* spp were determined on King´s B medium with an incubation temperature of 25ºC for 48h [29]. Enumeration of *C. jejuni* was conducted on modified charcoal-cefoperazone-deoxycolate agar (mCCDA) (Oxoid, Basingstoke, UK) with an incubation temperature of 42ºC for 48 hours under microaerobic conditions. Suspected *C. jejuni* colonies were confirmed microscopically [2]. Measurements of pH were made using a Crison model 2002 pHmeter (Crison Instruments, Barcelona, Spain).

Statistical analyses. Plate count data were transformed to logarithms prior to their statistical treatment. Analysis of variance was performed using the SYSTAT program for Windows; Statistics version 5.0 (Evanston, Illinois). Tukey's test for comparison of means was performed using the same program. All experiments were performed in duplicate. Significance level was defined at $P < 0.05$.

Results

Microbiological quality. Tables 1 and 2 show the effect of different propionic acid concentrations on psychrotrophs and *Pseudomonas* counts, respectively. Immersion of chicken legs in 1 or 2% propionic acid reduced psychrotrophs counts between 1.01 and 3.3 log units compared to the control legs throughout storage. After treatment (day 0), psychrotrophs counts were 1.08 log units higher in control samples than in legs treated with 2% propionic acid. On day 8, psychrotrophs counts in legs washed with 2% propionic acid were 3.3 log units lower compared to control samples.

After treatment, *Pseudomonas* counts were 0.96 log units lower in legs treated with 2% propionic acid than in control ones (day 0). *Pseudomonas* reductions varied between 0.75 (day 0, control-1% propionic acid) and 2.93 log units (day 8, control-2% propionic acid) for propionic acid treated legs compared to the control ones throughout storage.

Propionic acid was found to reduce significantly ($P < 0.05$) the population of psychrotrophs and *Pseudomonas*. Significant differences ($P < 0.05$) in psychrotroph and *Pseudomonas* counts were also found between the legs treated with 1 and those treated with 2% propionic acid on day 3, 6 and 8 of storage. The psychrotrophs and *Pseudomonas* reductions increased throughout storage. The highest reductions compared to control were reached at the end of storage, day 8. Propionic acid was effective in reducing microbial counts both immediately after treatment and during storage.

Table 1. Psychrotroph counts on chicken legs dipped in propionic acid solutions and stored up to 8 days at 4ºC (log CFU/g)

	Days of storage				
Batch	0	1	3	6	8
Control	3.91 ± 0.06[a]	5.13 ± 0.35[a]	7.18 ± 0.09[a]	8.80 ± 0.24[a]	9.50 ± 011[a]
1% Propionic acid	2.90 ± 0.11[b]	3.62 ± 0.35[b]	5.17 ± 0.30[b]	6.89 ± 0.11[b]	7.16 ± 0.26[b]
2% Propionic acid	2.83 ± 0.03[b]	3.53 ± 0.12[b]	4.31 ± 017[c]	5.53 ± 0.13[c]	6.20 ± 0.28[c]

Mean ± standard deviation, n = 6

Means within columns followed by the same letter were not significantly different ($P > 0.05$).

Table 2. *Pseudomonas* counts on chicken legs dipped in propionic acid solutions and stored up to 8 days at 4ºC (log CFU/g)

	Days of storage				
Batch	0	1	3	6	8
Control	3.22 ± 0.13[a]	4.52 ± 0.13[a]	6.76 ± 0.07[a]	8.34 ± 0.23[a]	9.30 ± 011[a]
1% Propionic acid	2.47 ± 0.18[b]	3.54 ± 0.17[b]	5.19 ± 0.26[b]	6.45 ± 0.28[b]	7.14 ± 0.13[b]
2% Propionic acid	2.26 ± 0.17[b]	3.45 ± 0.15[b]	4.49 ± 015[c]	5.50 ± 0.35[c]	6.37 ± 015[c]

Mean ± standard deviation, n = 6
Means within columns followed by the same letter were not significantly different ($P > 0.05$).

Campylobacter jejuni. Analysis of *C. jejuni* counts (Table 3) show that propionic acid caused significant reductions ($P < 0.05$) in the *C. jejuni* populations. When legs were treated with 2% propionic acid *C. jejuni* counts were reduced 1.62 log units. After 8 days of storage, *C. jejuni* counts were 1.71 log units lower in legs treated with 2% propionic acid than in control ones. Significant differences ($P < 0.05$) were obtained between legs treated with 1 and 2% propionic acid. On day 8, *C. jejuni* counts were 0.7 log units lower in samples treated with 2% propionic acid than in those treated with 1%.

pH changes. Propionic treatment significantly reduced ($P < 0.05$) the pH of chicken legs. The pH was lower when the propionic acid concentration was higher. Initial pH values in legs immersed in 1 or 2% propionic acid (day 0) were 5.75 ± 0.18 and 5.31 ± 0.10, respectively (0.92 and 1.36 units lower than in control legs). The pH differences did not decrease throughout storage.

Discussion

The reductions in psychrotrophs counts obtained in the present work are in agreement with the findings of other authors when using organic acids. Organic acids (1–3%) reduce microbial counts by 1–2 log units [14,28].

In an earlier study, it was found that a washing with 2% propionic acid reduced psychrotrophs counts between 1.27 and 2.19 log units in chicken legs [15]. In the current study, a washing with 2% propionic acid decreased psychrotrophs counts between 1.08 and 3.3 log units. After treatment with 2% propionic acid the reductions of psychrotrophs counts obtained were very similar (1.08 log units in the present study and 1.32 in the previous study).

In the current work, propionic acid at concentrations of 1 or 2% reduced *Pseudomonas* counts in 2.16 and 2.93 logs units in chicken legs after 8 days of storage at 4ºC, compared to control samples. Odgen et al. [25] observed higher *Pseudomonas* count reductions in pork meat treated with 1% propionic acid (3 log units after 13 days of storage at 4ºC). The higher efficacy of propionic acid in pork meat could be explained by the pH, since pork meat has a lower pH than chicken. Propionic acid has optimal inhibitory activity at low pH because it favors the uncharged form, which has stronger antimicrobial activity than the dissociated form [7,8].

Spoilage of poultry meat is mainly attributed to growth and metabolic activity of bacteria. *Pseudomonas* is the major spoilage bacterium in chicken meat [20]. The shelf life of chicken meat depends on the level of its microbial contamination. Therefore, reducing the spoilage bacteria in chicken, mainly *Pseudomonas*, could extend their shelf life. Bacterial counts by 9 log cfu/g are related to the detection of off-odors

Table 3. *Campylobacter jejuni* counts on chicken legs dipped in propionic acid solutions and stored up to 8 days at 4ºC (log CFU/g)

	Days of storage				
Batch	0	1	3	6	8
Control	4.58 ± 0.09[a]	4.25 ± 0.12[a]	4.00 ± 0.21[a]	4.02 ± 0.04[a]	4.01 ± 0.12[a]
1% Propionic acid	3.70 ± 0.70[b]	3.31 ± 0.03[b]	3.39 ± 0.22[b]	3.26 ± 0.20[b]	3.00 ± 0.14[b]
2% Propionic acid	2.96 ± 0.29[c]	2.78 ± 0.19[c]	2.65 ± 0.17[c]	2.50 ± 0.10[c]	2.30 ± 0.10[c]

Mean ± standard deviation, n = 6
Means within columns followed by the same letter were not significantly different ($P > 0.05$).

and spoilage in chicken [15]. In the present work after 6 days of storage, psychrotrophs reached populations by 8.80 log cfu/g in control legs. However, in the legs treated with 1 or 2% propionic acid, psychrotroph and *Pseudomonas* counts were below 8 log cfu/g at the end of storage, day 8. In consequence, propionic acid could extend the shelf life of chicken meat.

On the other hand, *Pseudomonas* could affect the survival of pathogens in chicken. Hilbert et al. [17] reported that *C. jejuni* isolated from chicken meat were able to benefit from cocultivation with *Pseudomonas* spp. This interaction could explain the survival of *C. jejuni* on chicken meat.

In the present work, no growth of *C. jejuni* was detected in chicken legs. *C. jejuni* do not grow on meat at low temperatures, 30ºC being the minimum temperature for growth [5]. This pathogen has a low infective dose, thus the main problem is its survival. Futhermore, *C. jejuni* survives better in refrigerated foods than in food held at room temperature [19]. Another factor that affects its survival is pH. *C jejuni* is very sensitive to low pH. Its survival is optimal in the range 6.5 to 7.5 [1]. In the present study, initial pH of control legs was 6.6, the treatment with 1 or 2% of propionic decreased the pH, reaching values of pH of 5.75 and 5.31, respectively.

The efficacy of propionic acid against *C. jejuni*i has been studied in vitro [3, 16]. Chaveerach et al. [3] found that propionic acid has a very strong bactericidal effect on *Campylobacter jejuni* culturability at low pH. Grilli et al. [16] reported that the minimum inhibitory concentration (%) for propionic acid against *C. jejuni* was 0.46.

Only a few works have investigated the effect of propionic acid on *C. jejuni* in chicken. Propionic acid is effective as feed additive in broilers to reduce caecal *C. jejuni* [16]. Shin et al. [27] studied the effect of propionic acid against *Campylobacter jejuni* in a chicken model system. These authors observed that the addition of propionic acid showed strong antibacterial activity against *C. jejuni* at pH 5.5 or 6.5.

Propionic acid has been investigated for its ability to reduce *Salmonella* [21]. Propionic acid inhibits the growth of *Salmonella* at higher pH values (pH 5.5) than do lactic (pH 4.4) or citric acid (pH 4.05) [4]. Tamblyn and Conner [31] found that 2% propionic reduced *Salmonella* attached to chicken skin in 1.2 log units.

In an earlier study, the ability of propionic acid to reduce the populations of *L. monocytogenes* on poultry meat was evaluated [15]. Legs washed with 2% propionic acid showed a significant ($P < 0.05$) reduction in *L. monocytogenes* counts compared to control legs, with a decrease of about 2.72 log units after 3 days of storage.

The efficacy of propionic acid against *C. jejuni* on chicken observed in the present study is higher than the efficacy reported by other authors in chicken treated with other organic acids such as lactic or acetic acids. Cosansu and Ayhan [6] found that after washing with 1 and 3% lactic acid reduced *C jejuni* counts on chicken 0.36 and 1.06 log units, respectively, while acetic acid at concentrations of 1% and 3% reduced *C jejuni* in 0.78 and 1.27 log units, respectively. Zhao and Doyle [32] found that a treatment with 2% acetic acid caused a reduction of 1.2 log units in *C. jejuni* in chicken wings. In the present study 2% propionic acid reduced *C. jejuni* populations 1.62 log units after treatment. Therefore, propionic acid was more effective against *C. jejuni* than lactic or acetic acid. Grilli et al. [16] also reported that propionic acid was more effective against *C jejuni* than acetic, lactic and citric acids in vitro assays.The higher efficacy of propionic acid compared to other organic acids (lactic, acetic and citric acid) has also been observed against *Salmonella* [23] and *L. monocytogenes* [11,12,13,14].

Propionic acid exerts a greater antimicrobial effect compared to lactic acid, despite the fact that lactic acid is a stronger acid with a pKa value of 3.66, whereas the pKa value of propionic acid is 4.87 [30]. The greater antimicrobial effect of propionic acid could be explained since propionic acid is more lipophilic and hence is transported through the bacterial cell wall quicker [24]. Moreover, antimicrobial activity of propionic acid is attributed to both the undissociated and dissociated acid forms [7].

Contamination of chicken meat with *C. jejuni* can occur at many stages of processing [9]. *C. jejuni* has been found on chicken skin during slaughter process, whereas internal tissues are sterile [1]. Therefore, it is of particular importance to reduce *C. jejuni* on the surface of chicken.

The relevance of reducing *Campylobacter jejuni* counts on chicken for decreasing the incidence of human campylobacteriosis has been shown by quantitative risk assessment. The incidence of campylobacteriosis associated with consumption of chicken could be reduced 30 times by introducing a 2 log reduction of the number of *Campylobacter* on the chicken carcasses [26]. In the current work reduction of 1.62 log units of *C. jejuni* were achieved by decontamination with 2% propionic acid. This fact is of particular interest since reducing the levels of *C. jejuni* may help to decrease the incidence of human campylobacteriosis.

Alterations in sensorial characteristics should be taken into account in the selection and application of organic acids as carcass decontaminants. In a previous study it was observed

that chicken sensory quality was not adversely affected by propionic acid [15].

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Competing interests. Not declared.

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