# Effect of steam, lactic acid and combined treatments for inactivating *Campylobacter jejuni* inoculated on chicken skin

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## Introduction

Zoonoses are infections and diseases that are naturally transmissible directly or indirectly between animals and humans. Food-borne zoonoses are caused by pathogenic microbes such as bacteria, viruses, and parasites, or their toxins present in contaminated foods. The most common food-borne infections in the world are caused by the bacteria such as *Campylobacter, Salmonella* or *Listeria*. In 2009, campylobacteriosis is the most commonly reported zoonosis in the European Union with 198 252 confirmed human cases and its cost to public health systems and to lost productivity is estimated to be around 2.4 billion euros each year across the EU (EFSA, 2011). *Campylobacter jejuni* is the most frequently reported cause of acute inflammatory gastroenteritis in industrialized countries (Friedman, 2000; Mishu Allos *et al.*, 1998). Typically, *C. jejuni* cause a self-limited gastrointestinal illness characterized by diarrhea, abdominal pain, and fever.

Numerous studies have shown that the consumption of raw or undercooked poultry products are the major risks for human campylobacteriosis (Altekruse *et al.*, 1999; Stern, 1992).

Control of chicken carcass contamination becomes then a major interest for the food industry. Moreover, poultry meat consumption has increased in the last two decades; therefore, it is necessary to find suitable intervention strategies to decrease the contamination levels by *Campylobacter jejuni* on chicken carcasses and its prevalence.

Further to best practices in husbandry and slaughterhouse, decontaminating treatments might be required to control microbial hazards. Among the available treatments, heat and acid treatments are good candidates as their efficiency has been shown on other pathogens and because they benefit a more natural image than the use of chlorine or irradiation for example.

The aim of our study was to determine the efficiency of lactic acid and/or heat treatments for the decontamination of chicken skins inoculated with *Campylobacter jejuni*. This study focused on the chicken skin because it has been shown that contamination at the slaughterhouse is principally located on the skin and in the digestive system.

**Material and Methods**

Strain of *Campylobacter jejuni* subsp. *jejuni* ATCC 33291 was stored on beads (VWR, France) at -80°C. One bead of *Campylobacter jejuni* was placed and spread onto a Karmali plate (Oxoid, France) and the plate was incubated at 41.5 °C for 24 h in a micro-aerophilic atmosphere (Campygen, Oxoid). After 24 h of incubation, three loopfuls of the culture onto Karmali were placed into a flask containing 100 ml of Preston broth with Preston supplements (Oxoid, France) and was incubated at 41.5 °C for 24 h in a micro-aerophilic atmosphere and under stirring 150 rpm. The suspension was centrifuged at 5000 g for 20 min and the pellet was resuspended in sterile saline to obtain a concentration of 6.5 ± 0.5 log cfu.ml$^{-1}$ for the decontamination trials.

The skins used were taken from chicken upper legs purchased from a supermarket. The chickens were slaughtered 1 to 3 days before the experiments were performed.

The chicken skins were placed on a sterile baking paper and on a stainless steel support. The whole material was placed between a Teflon support and a stainless steel dish. Two holes drilled in the top of the dish allowed filling and draining of the dish. This laboratory-developed system was used to place 15.61 cm$^2$ of the external surface of the skin in contact with 10 ml of inoculation solution for 1 min. The skins were then drained for 10 min at room temperature (25 °C) under a laminar flow hood, allowing adhesion of bacteria (Lecompte *et al.*, 2008). No rinsing step was performed. Untreated controls were inoculated the same way.

Inoculated skins were then untreated (control) or treated by steam (100 °C - 8 s), lactic acid (5% - 1 min - 25 ml) or by a combined treatment (steam followed by acid lactic treatment). Combined and acid lactic treated skins were also rinsed off (2 x 50 ml of distilled sterile water - 30 s) or unrised off after contact with lactic acid. The acid treatments were performed using the same device than for the skins inoculation. A steam treatment unit, small enough to fit on a laminar flow hood, was developed for this study. Steam was produced by a generator (Phillips, France) at 100 °C and normal pressure, and introduced in a closed chamber. The skin disk, fixed on a stainless sample holder, was placed in a drawer. When the drawer was closed, the skin was in contact with saturated steam at 100 °C. At the end of the treatment, the

drawer was opened and the skin cooled with a 20 °C air flow until its surface temperature dropped to 35 °C.

The surviving bacteria on skins were enumerated either immediately after treatment (T0), or after 7 days (T7) of storage at 4 °C in Petri dishes closed with self-sealing plastic film.

To enumerate the surviving bacteria, a disk of 7.35 $cm^2$ was cut out of the skin and homogenized in stomacher for 5 min in 25 ml of Preston broth with Preston supplements (Oxoid, France). For the decontamination trials, the Most Probable Number method (MPN) was used in order to low as much as possible the detection threshold. This method permitted here detection threshold at 0.1 log $cfu.cm^{-2}$. Pre-enrichment of decimal dilutions (D0-D7) was done in Preston broth with Preston supplements and laked horse blood (Oxoid, France) and incubated 24 h ± 1 h at 41.5 °C in a micro-aerophilic atmosphere. After 24 h, 20 µl of decimal dilutions (D0-D7) were dropped on Karmali agar contained in 12 round wells plates (Fisher Scientific, France) and incubated for 48 h at 41.5 °C in a micro-aerophilic atmosphere. 3 series of dilutions are dropped onto Karmali agar to follow the MPN procedure (Scherer *et al.*, 2006). Every colony present on the selective agar matched a well considered as positive. Knowing the number of positive wells for each dilution level, it is therefore possible to calculate the bacterial concentration at the surface of the skin. The bacterial concentration was calculated with a MPN Calculator software (MPN Calculator build 23, Myke Curiale). For each sample, the bacterial concentration was expressed in $cfu.cm^{-2}$, and the log of this value was used for calculations. ANOVA analysis were performed using Statistica software with (Statsoft, USA). Fisher least significant difference test was used to study difference between means with a confidence threshold of 95%.

**Results**

Immediately after inoculation, the concentration of *C. jejuni* on the surface of the control skins was 5.54 ± 0.60 log $cfu.cm^{-2}$. Bacterial populations of controls significantly decreased to 4.18 ± 1.35 log $cfu.cm^{-2}$ after 7 days of storage at 4 °C.

After lactic acid treatments, the T0 bacterial counts were significantly lower than for the control. The reduction in bacterial counts reached 1.54 ± 0.78 log $cfu.cm^{-2}$ for unrised off skins and 1.67 ± 0.60 log $cfu.cm^{-2}$ for rinsed off skins. There was no significant difference between counts for rinsed off and unrised off skins directly after lactic acid treatment.

At T0, steam and combined treated skins showed significantly lower concentrations than the control and the acid lactic treated ones. Mean concentrations were inferior to 1 log $cfu.cm^{-2}$

with respectively 0.25 ± 0.20, 0.35± 0.39, 0.69 ± 1.15 log cfu.cm$^{-2}$ for steam, unrised off and rinsed off combined treatment.

After 7 days of storage, all treated skins showed significantly lower bacterial concentration than the control.

The decimal reductions obtained using a rinse off or not after lactic acid treatment were significantly different at T7. When a rinse off was applied, reduction (1.41 ± 1.79 log cfu.cm$^{-2}$) was significantly lower than without the rinse off (3.84 ± 1.35 log cfu.cm$^{-2}$).

After steam treatment and 7 days of storage at 4 °C, the number of *C. jejuni* was below the detection threshold in 4 cases out of 5 and the reduction reached 3.95 ± 1.35 log cfu.cm$^{-2}$.

At T7, the reduction obtained with the combined treatments (rinse off or not) reached 4.08 ± 1.35 log cfu.cm$^{-2}$ and all samples (5/5) were below the detection threshold.

**Discussion**

The decrease of the *C. jejuni* counts between T0 and T7 is in agreement with previous studies (Davis and Conner, 2007). El-Shibiny *et al.* (2009) also reported a decline in viability of 3.3 log cfu.cm$^{-2}$ after 9 days of storage at 4 °C. However, the mechanisms promoting survival of *C. jejuni* in the cold remain poorly understood despite several investigations (Haddad *et al.*, 2009). Moreover, *C. jejuni* is well known for using a viable non culturable (VNC) form to survive in hostile environment (Stern *et al.*, 1991; Beumer *et al.*, 1992). It would then be very interesting to quantify the passage to VNC during the 7 days of storage by molecular technics.

Reduction of *C. jejuni* counts of 1.5 log cfu.cm$^{-2}$ directly after lactic acid treatment had been showed in previous studies. Loretz *et al.* (2010) reported on their literature survey reductions by 0.2 to 1.7 log orders of magnitude.

For acid lactic treatment without rinse off used alone, we observed a marked bactericidal effect during storage in agreement with previous studies (Smigic *et al.*, 2010; Rajkovic *et al.*, 2010). This remaining bactericidal effect has been showed by Lecompte *et al.* (2008) on decontamination of chicken skins inoculated with *Listeria innocua* and *Salmonella enteritidis*. This result is interesting because showing a similar behavior of differents food major pathogens to the lactic acid decontamination. Moreover, Lecompte *et al.* (2009) reported that a 5% lactic acid treatment 1 min did not significantly increase the lactic acid in the skin (and so even less in the whole product) and therefore did not cause any organoleptic modifications to the product. Lactic acid seems then being a good candidate for carcass decontamination.

After 7 days of storage, the lactic acid effects on the skins were not as important when skins were rinsed off directly after the acid treatment but reduction were significantly different compared to the control skins with a reduction of 1.41 ± 1.79 log cfu.cm$^{-2}$ compared to a reduction of 3.84 ± 1.35 log cfu.cm$^{-2}$ for unrised off skins. According to the EFSA regulation, the use of any substance other than potable water to remove/reduce surface contamination from products of animal origin is not authorized in the EU, unless the use of the substances has been approved in accordance with the regulation. Moreover, to follow the EFSA regulation, the use of lactic acid for carcass decontamination would impose a rinse off of the carcass. However, lactic acid is a natural component of poultry skin which is not detrimental to human health at antimicrobial concentrations.

The most effective treatments are heat treatment and combined treatments with reductions of approximately 5 log cfu.cm$^{-2}$ at T0 and 4 log cfu.cm$^{-2}$ at T7. The heat treatment would be sufficient to decontaminate carcass because we showed no significant differences at T0 and T7 between reductions obtained with heat treatments and combined treatments. The immediate steam treatment on *Campylobacter* was so efficient in our study that an eventual synergistic effect of steam and lactic acid couldn't have been showed. However on *Listeria* (Lecompte et *al.*, 2008) and *Salmonella* (unpublished data), combined treatment was more effective than each single treatment. Moreover, the bactericidal or bacteriostatic effect of lactic acid used in the combined treatment could protect skins from recontamination during storage. It will be studied in further studies.

**Abstract**

Raw poultry carcasses are often contaminated with pathogens, including *Salmonella spp.*, *Campylobacter jejuni* and *Listeria monocytogenes*. Campylobacteriosis is an emerging and one of the most frequently reported zoonotic infectious disease. Further to the best management practices in husbandry, slaughtering and processing, decontaminating treatments might be required to control microbial hazards.

A previous study has shown that combining heat and lactic acid treatments for decontaminating chicken skin inoculated with *Listeria innocua* or *Salmonella Enteritidis* seems particularly promising as it cumulates the advantages of each treatment: an immediate bacterial reduction due to the heat treatment while acid treatments leads to a bacteriostatic or bactericidal effect during storage. After 7 days of storage at 4°C, the combined treatment was even more effective than each single treatment.

The present work evaluated the effectiveness of lactic acid, heat and combined heat and lactic acid treatments for inactivating *Campylobacter jejuni*.

Chicken skins were inoculated with *Campylobacter jejuni* (6.5 $\log_{10}$ cfu.cm$^{-2}$) and treated with steam (100 °C during 8 s) and/or lactic acid (5% v/v for 1 min at 25 °C). Residual bacteria on the skin were enumerated immediately after treatment and after 7 days of storage at 4°C. Bacterial concentration was expressed in $\log_{10}$ cfu.cm$^{-2}$. Each treatment was repeated 5 times.

Results showed a reduction of 1.5 log cfu.cm$^{-2}$ thanks to the lactic acid treatment compare to the control directly after treatment. After 7 days of storage, results showed a remaining bactericidal effect of the unrised off acid treatment with a reduction of 3.9 log cfu.cm$^{-2}$. With a rinse off, the reduction of 1.5 log ufc.cm$^{-2}$ is significantly lower. Heat treatment and combined treatments were very efficient at T0 and T7 with reductions of, respectively, 5 and 4 log cfu.cm$^{-2}$.

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Table 1. Effect of decontamination treatments on *C. jejuni* inoculated chicken skins

| Treatments | Mean Bacterial Concentration (MBC) (log cfu.cm$^{-2}$) | | | | Mean Bacterial Reduction (MBR) (log cfu.cm$^{-2}$) | |
|---|---|---|---|---|---|---|
| | T0 | Number of samples <DT | T7 | Number of samples <DT | T0 | T7 |
| Control | 5.54 ± 0.60$^{a}$ | 0/5 | 4.18 ± 1.35$^{b}$ | 0/5 | - | - |
| Lactic Acid 5% - 1 min | 4.00 ± 0.78$^{b}$ | 0/5 | 0.34 ± 0.48$^{c}$ | 3/5 | 1.54 | 3.84 |
| Lactic Acid 5% - 1 min + Rinse Off | 3.87 ± 0.54$^{b}$ | 0/5 | 2.77 ± 1.79$^{d}$ | 0/5 | 1.67 | 1.41 |
| Steam 100 °C - 8 s | 0.25 ± 0.20$^{c}$ | 3/5 | 0.23 ± 0.29$^{c}$ | 4/5 | 5.29 | 3.95 |
| Combined Steam + Lactic Acid | 0.35 ± 0.39$^{c}$ | 2/5 | < 0.1$^{c}$ | 5/5 | 5.19 | ≥ 4.08 |
| Combined Steam + Lactic Acid + Rinse Off | 0.69 ± 1.15$^{c}$ | 3/5 | < 0.1$^{c}$ | 5/5 | 4.85 | ≥ 4.08 |

n=5, ± standard deviation. Different letters indicate significant ($p<0.05$) differences between reported values.

DT : Detection Threshold.

MBR = (Control MBC - Sample MBC)