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SHORT COMMUNICATION

Effect of lactic acid and steam treatments on *Campylobacter jejuni* on chicken skin

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ABSTRACT

Minimization of *Campylobacter jejuni* contaminations in poultry meats is important for public health. Certain chemical agents and physical processes to be used on carcasses to destroy pathogenic microorganisms. One of the most common used chemical is lactic acid. The bactericidal activity of different concentrations of lactic acid and hot steam on the *C. jejuni* populations on chicken skin samples were determined. Chicken breast skin samples were inoculated with *C. jejuni* and dipped into different lactic acid solutions and hot steam. reduction of *C. jejuni* and pH values were determined after 0., 1., 3. and 5 days of the storage at 4 ± 1°C. according to microbiological analysis. Compared with the control group, reductions in *C. jejuni* populations were determined as 1.72 and 2.02 log at 22°C, as 1.91 and 2.34 log at 54°C on day 0, in 2%, 3% concentrations respectively. On the other hand, beginning from day 0, bacterial counts reached undetectable ($<1.0x10^2$ cfu/g) levels after the decontamination treatments with 4% LA for 60 s at 22°C and 54°C, after the treatments with 2% LA followed by HS concentrations of 97 ± 1°C for 15 s and 133 ± 1°C for 3 s. It was determined that decontamination with lactic acid and hot steam application had a significant reduction effect on *C. jejuni* in chicken skin samples and the effect of lactic acid was increased depending on the concentration.

Keywords: *Campylobacter jejuni*; Chicken meat; Decontamination; Hot steam; lactic acid

INTRODUCTION

Campylobacter jejuni is one of the most common causes of bacterial originated gastroenteritis in humans. Besides, raw or undercooked contaminated poultry meat consumption is primarily responsible for the transmission to humans of the bacteria. Therefore minimization of *C. jejuni* contaminations in poultry meats is important for public health. *C. jejuni* is primarily responsible for foodborne gastroenteritis in developed countries such as the USA, Canada, Australia and Japan, particularly in European countries. It is stated that about 9 million cases of campylobacteriosis occur annually in the European Union countries, which causes serious problems in terms of public health. *Campylobacter* contamination in poultry varies according to the country. Bacteria can easily be transmitted to the chicken carcasses as a result of cross-contamination during the slaughtering process, due to the colonization of bacteria in the intestines from the 3rd week (Hashem and Parveen, 2016; Skarp et al., 2016).

Studies have shown that chicken carcasses are contaminated with *C. jejuni* at different levels (Hue et al., 2011; Ma et al., 2014; Wieczorek and Osek, 2015). Hue et al. (2011) reported that *C. jejuni* contamination incidence in chicken carcasses was 87.5%. Similarly, Ma et al. (2014) reported that contamination rate in cecum of broiler chickens and carcass samples was 72.5% and 34.1% respectively. In another study, *C. jejuni* contamination percentage in chicken carcasses determined by Wieczorek and Osek (2015) was found between 36.3% and 70.5%. In studies conducted in Turkey, *C. jejuni* contamination in neck skin and chicken carcass were 74.8% and 86.25, respectively (Koluman, 2010; Savaçı and Ozdemir, 2006).

Although the European Union's (EU) food legislation does not permit use of chemical agents or physical processes for decontamination in poultry carcasses, the use of lactic acid in cattle carcasses is permitted by directive EU Directive 101/2013 published in 2013. In Turkey, similar directives are also adapted in legislation (Anon, 2013).

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The US Department of Agriculture Food Safety and Inspection Service (USDA/FSIS) has allowed certain chemical agents and physical processes to be used on carcasses to destroy pathogenic microorganisms at certain stages of slaughtering of cattle, swine and poultry. The most common chemicals used in these applications are organic acids, acidified sodium chloride and trisodium phosphate, whereas hot water and pressurized hot steam applications are used as physical processes (Bolder, 1997; Sofos and Smith, 1998; Oezdemir et al., 2006; Anon, 2015).

Studies conducted by different researchers (Anang et al., 2007; Chaine et al., 2013; Liu et al., 2016) have reported that there are significant declines in the number of pathogens such as *C. jejuni, Salmonella* spp.*, Listeria monocytogenes, E. coli* 0157:H7 in poultry meat as a result of decontamination with lactic acid at different concentrations and temperatures. Anderson and Marshall (1990) reported that the reduction effect of lactic acid to bacteria counts varies with the concentration and temperature of the lactic acid solution and duration of the treatment. Liu et al. (2016) reported that 1.5% lactic acid at 50°C caused a drop of about 1.88-2.03 log on *Clostridium* spp. and *Pseudomonas fluorescens*.

This study was conducted to determine the reduction effect of lactic acid and hot steam applications on *C. jejuni* population and the difference between lactic acid at different concentrations and temperatures and hot steam applications at a temperature of about 97±1°C and 133±1°C.

MATERIALS AND METHODS

Bacterial inoculum preparation

Campylobacter jejuni ATCC 33291 (Oxoid, Hampshire, U.K.) was used as a stock strain for the preparation of the inoculum. Stock strain was plated onto Blood Free Campylobacter Selective Agar Base (CCDA-Oxoid CM0739, Supplement Oxoid SR0155) and was incubated for 48–72 h at 42ºC in microaerobic conditions (Campygen, Oxoid CN025). After the incubation, colonies were transferred into Brain Heart Infusion Broth (Oxoid CM1135) by sterile swab with the volume of 10 mL. The bacterial density of the inoculum was controlled by plating eightfold serial dilutions onto CCD agar base in duplicate, before the inoculation of the chicken breast skin samples. Broth cultures of *C. jejuni* ATCC 33291 was determined to reach about 9.0 x10^5 cfu/mL.

Sample inoculations

Chicken breast skin samples were obtained from 50 samples from the whole chicken carcasses purchased from local supermarkets and were transported to the laboratory within 30 min. under refrigerated conditions. From the whole breast skins, samples were excised aseptically with the approximate size of 8x8 cm, and weight about 13-15 g, in the laboratory. Afterwards, the samples were immersed into the suspension that contain freshly prepared *C. jejuni* at 10^5 cfu/mL level, for 5 min in sterile bottles at room temperature. After inoculation, samples were held in sterile bags for 30 min at room temperature to allow the attachment of bacteria.

Antimicrobial treatment and sampling

All antimicrobial solutions were prepared freshly before the experiments. The inoculated skin samples were immersed respectively into the antimicrobial solutions of 2% (pH 1.93), 3% (pH 1.86) and 4% (pH 1.81) lactic acid (LA) (Merck 1.00366.2500) for 60 s at 22–54°C± 1 and hot steam (HS) 97-133°C± 1 respectively, 15 and 3 s. These concentrations are the most common and economic ones for the treatments. The inoculated samples were divided into seven groups, each containing 10 chicken skin samples and were treated with 7 different applications alone or in combination. Treatment groups were; sterile distilled water; (22–54°C); 2% LA; 3% LA; 4% LA; HS for 15 s at 97ºC; 2% LA followed by HS for 15 s at 97ºC; HS for 3 s at 133°C. Following the treatment, the samples were held at room temperature for 5 min, and they were stored at 4°C in the sealed sterile bottles. pH values of all samples were evaluated on 0,1,3 and 5 days of storage and microbiological analysis were done at the same days. Each experiment was repeated twice on different days, and a total of 112 skin samples were used.

Microbiological analysis and pH determination

For each treatment, half of each skin sample was used for microbiological analysis while the other half was used for pH determination. Each sample was prepared by cutting 10 g of skin with sterile scissors to use them in microbiological analysis. The samples were placed into a sterile bag containing 90 mL of sterile peptone (0.1%) water and homogenized in a stomacher (Laboratory-Blender 400 Seward, London, U.K.) for 2–3 min. Decimal dilutions were prepared from the skin homogenates by using sterile peptone water after the homogenization. For *C. jejuni* enumeration, 0.1 mL volume of each homogenate was plated onto both Blood Free Campylobacter Selective Agar and Palcam Agar (Oxoid CM0877; Supplement SR0155). All plates were incubated for 48–72 h at 42°C in microaerobic conditions and enumerated (Baumgart, 1997). For pH evaluation, 5 g of skin sample was weighed in the sterile bag containing 15 mL of sterile deionized water and homogenized in the stomacher for 2 min (Capita et al., 2002). The pH values of the samples was measured by electronic pH meter (Mettler Toledo-Inlab 427, Urdorf, Switzerland).

Statistical analysis

Variance analysis in this study were done by the SPSS 10.0 statistical package program (Reference No. 651544). All bacterial counts and each contamination level were converted to $\log_{10}$ cfu/g-values and the variance analysis was carried out to detect the statistical significance between the effects of antimicrobial treatments on the reduction of *C. jejuni* and the time of storage period.

RESULTS

The effects of antimicrobial treatments on the reduction of *C. jejuni* populations are summarized in Table 1. The microbiological analysis on day 0 of storage showed that compared to the control group, *C. jejuni* reductions were 1.72 log for 2% lactic acid and 2.02 log for 3% lactic acid at the temperature 22°C. In the group where 4% lactic acid used, the bacterial counts decreased to undetectable levels ($<1.0 \times 10^2$ cfu/g) on day 0. No reduction was observed in *C. jejuni* levels in the group treated with distilled water at 22°C from day 0 to the end of the storage period (day 5). Similar results obtained after using lactic acid solutions at a temperature of 54°C. Compared to the control group, *C. jejuni* reductions were 1.91 and 2.34 log for 2% and 3% lactic acid, respectively. Bacterial counts were found under the detection limits at 4% lactic acid treatment group. Also, no reduction was observed in the control group. In other treatments, where hot steam, hot steam and lactic acid combinations were used, the bacterial counts decreased to undetectable levels (<1.0 × 102 cfu/g) on day 0.

In addition to the microbiological analysis, the pH values of the samples were also measured, while the pH values of the control samples were found to be 6.43-6.62, whereas the pH values of the samples treated with lactic acid at concentrations of 2%, 3% and 4% at 22°C and 54°C were observed to be low depending on the concentration. The average pH-values of the lactic acid-immersed samples at concentrations of 2%, 3% and 4%, 22°C and 54°C were measured at the levels of 3.02-3.10, 2.97-3.00 and 2.85-2.88, respectively, on the 0th day of storage. Also, pH levels of the samples, monitored to the end of the storage period (data not shown).

Statistical analysis was performed to determine the difference between groups according to the lactic acid concentration in the study. It was determined that the decrease was dependent on the lactic acid concentration and this was statistically significant (P <0.005).

DISCUSSION

In our study about 1.72-2.34 log *C. jejuni* reduction was detected on day 0, after the immersion of the samples into the lactic acid solutions at 2 % and 3 % concentrations, at different temperatures (22°C, 54°C). Also, *C. jejuni* levels were determined under the detection limits ($<1.0x10^2$ cfu/g) when lactic acid and steam used together. The obtained data is similar to the results reported by Chaine et al. (2013). In their study Chaine et al. (2013) determined 3.8 log reduction in *Salmonella* Enteritidis and *C. jejuni* levels on chicken skin samples which are treated with 5% lactic acid solution. Anang et al. (2007) reported that *L. monocytogenes, S.* Enteritidis and *E. coli* 0157:H7 levels in poultry meats which are treated with lactic acid, were reduced to 1.97, 1.71 and 2.59 log, respectively.

Izat et al. (1990) reported that broilers treated with 1% and 2% lactic acid solutions at different stages during processing at different temperatures and different contact times showed reductions on *Salmonella* levels. Okolocha and Ellerbroek (2005) conducted a study to determine the influence of acid and alkaline treatments on pathogens and the shelf life of poultry meat. Also they compared the spraying and dipping method. Their results showed that

Table 1: The effects of antimicrobial treatments on the reduction of C. *jejuni* ($\log_{10}$ CFU/g) on chicken breast skin samples stored at 4°C for up to 5 days

Treatment groups		Days of storage (at 4°C)			
		0	1	3	5
22°C	Control	4.62±0.7^c	4.55±0.2^c	4.60±0.4^c	4.50±0.3^c
	2% LA	2.90±0.4^b	<2.0±0.0^a	<2.0±0.0^a	<2.0±0.0^a
	3% LA	2.60±0.1^b	<2.0±0.0^a	<2.0±0.0^a	<2.0±0.0^a
	4% LA	<2.0±0.0^a	<2.0±0.0^a	<2.0±0.0^a	<2.0±0.0^a
54°C	Control	4.75±0.9^c	4.75±0.9^c	4.74±0.8^c	4.73±0.7^c
	2% LA	2.84±0.3^b	<2.0±0.0^a	<2.0±0.0^a	<2.0±0.0^a
	3% LA	2.41±0.2^b	<2.0±0.0^a	<2.0±0.0^a	<2.0±0.0^a
	4% LA	<2.0±0.0^a	<2.0±0.0^a	<2.0±0.0^a	<2.0±0.0^a
	HS (97±1°C),15s	<2.0±0.0^a	<2.0±0.0^a	<2.0±0.0^a	<2.0±0.0^a
	2% LA+HS (97±1°C),15s	<2.0±0.0^a	<2.0±0.0^a	<2.0±0.0^a	<2.0±0.0^a
	HS (133±1°C), 3s	<2.0±0.0^a	<2.0±0.0^a	<2.0±0.0^a	<2.0±0.0^a

Log reduction = ($\log_{10}$ cfu/g before treatment) − ($\log_{10}$ cfu/g after treatment). a-c: Different letters within same column are significant (P<0.005). Results are reported as means (n=23). LA, lactic acid; HS, hot steam

the mean log reductions on the *Enterobacteriaceae* counts with 1% lactic acid showed the highest reduction effect on day 0 of storage with a decrease and then a slight increase on days 3 and 6 respectively. Additionally, they indicated that the dipping treatment gave the best overall reduction effect. Anderson and Marshall (1990) reported that, the highest reduction of *S*. Typhimurium was determined when beef samples were dipped in 3% lactic acid for 15 s. This difference between all these results thought to be due to difference in the application method. Lj et al. (1997) signified the effectiveness of LA treatment was affected by the method of application, and the concentration-time combination.

In our study, it was indicated that in microbiological analyzes performed during different days of storage, the reduction in samples increased during the storage period. In this context, bacterial counts at day 0 were found at countable levels in lactic acid treated groups at concentrations of 2% and 3%, but below the detection limit at day 1. Researchers (Anderson and Marshall 1990; Mani-Lopez et al., 2012) reported that this was due to the residual effect of lactic acid.

Apart from lactic acid treatments, the effects of hot steam applications on *C. jejuni* reduction in skin samples were also investigated in this study. For this purpose, 97 ± 1°C and 133 ± 1°C steam was applied to skin samples contaminated with *C. jejuni* for 15 and 3 seconds. *C. jejuni* was found below the detection limits (<1.0x10 2 cfu/g) in both treatments. The results of this study are consistent with the results of Chaine et al. (2013). Indeed, Chaine et al. (2013) reported that *C. jejuni* reduction was approximately 5 log in the wing skin samples after the treatment with steam at 100°C for approximately 8 seconds. Similarly, Morgan et al. (1996) and Phebus et al. (1997) reported that hot steam applications significantly reduced the pathogenic microorganisms in cattle carcass and poultry meats. Morgan et al. (1996) reported that steam application in cattle, swine and poultry meat contaminated with *Listeria innocua* resulted in a reduction of 4 log levels. Phebus et al. (1997) determined a reduction at a level of 4.2-5.3 log after 15 seconds of steaming in cattle meat contaminated with *L. monocytogenes*, *E. coli* O157: H7 and *S*. Typhimurium at approximately 5 log cfu/cm^2. Likewise, Özdemir et al. (2006) reported a reduction in *S*. Typhimurium and *Listeria monocytogenes* counts at 0.54-0.09 log immediately after treatment in cattle meat immersed in hot water at 82°C for 15 seconds, respectively.

CONCLUSIONS

It was determined that decontamination with lactic acid and hot steam application had a significant reduction effect on *C. jejuni* in chicken skin samples and the effect of lactic acid was increased depending on the concentration. For this reason, both lactic acid and hot steam treatments are considered to be important for the reduction of *C. jejuni*, which is primarily responsible for foodborne infections, and for the production of safe poultry meat in terms of public health.

Authors' contributions

Haydar Ozdemir conceived and planned the experiments and he supervised the work. Gorkem Cengiz and Erdi Sen verified the methods and carried out the experiments. Guzin Iplikcioglu Cil, and Bahar Onaran contributed to the interpretation of the results. Haydar Ozdemir, took the lead in writing the manuscript. Guzin Iplikcioglu Cil, and Bahar Onaran wrote the paper with input from all authors and prepared the manuscript according to the journal.

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