EFFECTS OF LACTIC ACID, LINALOOL AND CINNAMALDEHYDE AGAINST CAMPYLOBACTER JEJUNI IN VITRO AND ON BROILER BREAST FILLETS

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Abstract. *Campylobacter jejuni* is the leading cause of bacterial human gastroenteritis in the European Union. Poultry products contaminated with *C. jejuni* are considered the main source of human campylobacteriosis. The aim of this study was to evaluate the effect of natural antimicrobials (lactic acid, linalool and cinnamaldehyde) against campylobacters in culture medium and on poultry breast fillets. In addition, total aerobic bacterial count was estimated on broiler breast fillets treated with different concentrations of bioactive compounds to determine whether it could prolong the shelf life of poultry product.

Despite the significant reduction of *C. jejuni* numbers in culture medium by lactic acid, linalool and cinnamaldehyde, these bioactive compounds had considerably lower effect on poultry product. *C. jejuni* numbers were reduced by 1.22 $\log_{10}$ CFU/g when treated with 5% lactic acid ($P \geq 0.05$), by 1.21–2.72 $\log_{10}$ CFU/g depending on the time after exposure to 2 % cinnamaldehyde ($P \leq 0.05$) and by 1.09 $\log_{10}$ CFU/g after treatment with 2 % linalool ($P \geq 0.05$). Total aerobic bacterial count was reduced significantly by 3 % and 5 % lactic acid and 2.5 % and 3 % cinnamaldehyde ($P \leq 0.05$).

This research offers new effective control measures for campylobacters as possible replacement to chemicals now suggested for decontamination purposes.

Keywords: bioactive compounds, foodborne microorganisms, food safety and quality, storage

Introduction

Campylobacter jejuni is the leading cause of bacterial human gastroenteritis in the European Union (EFSA, 2014). Raw poultry meat contaminated with *C. jejuni* and cross-contamination of ready-to-eat food are considered to pose the greatest risk for human health (Humphrey, 2001; Sheppard et al., 2009; Nichols et al., 2012). Poultry and poultry products are the source of 50 %– 70 % of *C. jejuni*-related gastroenteritis (Keener et al., 2004). Therefore, decontamination of contaminated with campylobacters poultry meat could be an effective measure to reduce human campylobacteriosis cases. Chemical substances like trisodium phosphate, chlorine and acidified sodium chlorite (Keener et al., 2004; Riedel et al., 2012) are used in various developed countries yet EU has not authorised chemical decontaminants for poultry meat. Currently, only potable water is permitted for poultry decontamination in the EU (EFSA, 2011).

Chlorine dioxide, acidified sodium chlorite, trisodium phosphate and peroxyacids, considerably reducing *Campylobacter* spp. numbers, are considered as possible poultry decontaminants in the EU, (EFSA, 2011). However, there is a growing need to use natural compounds for effective decontamination purposes (Fisher and Philips, 2006; Nannapaneni et al., 2009). The efficiency of various natural bioactive compounds is discussed in literary sources (Rattanachaikunsopon and Phumkhachorn, 2010; Engels et al., 2011) but there is no solid view as to which compound is the most effective. Besides, researchers often get incomparable results while testing the same bioactive compound because different techniques and bacterial strains are used for the experiments.

Lactic acid is a well-known natural substance, effective against *Campylobacter jejuni*, *Listeria monocytogenes* and *Listeria innocua*, *Escherichia coli O157:H7*, *Salmonella enteritidis* (Lecompte et al., 2009; Rajkovic et al., 2009; Smigic et al., 2010). However, researchers often find working concentrations of lactic acid to be very different. For example, Riedel et al. (2009) determined that 2.5 % lactic acid effectively reduced *C. jejuni* numbers on poultry product, while others have found that 5 % lactic acid shows no significant change in *C. jejuni* numbers on broiler skin and meat (Lecompte et al., 2009). Therefore, further research is needed to estimate which lactic acid concentration is effective against this pathogen.

On the other hand, bioactive compounds of plant origin like cinnamaldehyde and linalool have shown an antimicrobial effect against food pathogens such as *Campylobacter* spp., *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, *Helicobacter pylori*, and *Arcobacter* spp. (Fisher and Philips, 2006; Nannapaneni et al., 2009; Cwikla et al., 2010). Cinnamaldehyde is able to reduce the numbers of antibiotic susceptible and resistant *C. jejuni* strains in culture medium and on broiler breast fillets (Ravishankar et al., 2008; Mild et al., 2011). Likewise, linalool found in many flowers and herbs, is effective against pathogenic bacteria like *Campylobacter* spp., *S. aureus*, *Listeria monocytogenes* and *Bacillus cereus* in culture medium, but less efficient in food models (Fisher and Philips, 2006). However, there is insufficient amount of data about the effect of cinnamaldehyde and linalool against *C. jejuni* on poultry products and it needs further study.

Thus, the aim of this study was to determine the reduction effect of lactic acid, linalool and cinnamaldehyde against *C. jejuni* in culture medium and further on broiler breast fillets in parallel examining the effect of these bioactive compounds on total aerobic bacterial count as possible shelf-life predictor.

Materials and methods

Treatment in culture medium

Three *Campylobacter jejuni* strains representing three different MLST clonal complexes (1034, 464 and 443) were chosen from *C. jejuni* isolates collected from poultry products during previous studies (Kudirkienė et al. 2013). They were tested for the effect of lactic acid in culture medium. Similar growth abilities and vitality were observed with no statistically significant differences between these strains (data not shown). Therefore, one strain with sequence type and clonal complex 464 was chosen for this study.

The experiment was performed according to Rajkovic et al. (2009) technique. Bolton broth (CM 0983, Oxoid, England) was inoculated with 10^7 CFU/ml *C. jejuni.* The control sample was not treated with bioactive compound. The experimental samples were exposed to 0.125 %, 0.25 %, 0.5 %, 2 % concentrations of lactic acid ((L-(+)-Lactic acid, 50 %, 69778, 1L, Sigma-Aldrich), 0.05 %, 0.1 %, 0.2 % linalool (Linalool, 97%, L2602, 500g, Sigma-Aldrich) and 0.01 %, 0.05 %, 0.1 %, 0.2 % cinnamaldehyde (Cinnamaldehyde, ≥93 %, W228613-1KG-K, Sigma-Aldrich) for 10 min with successive centrifugation for 2 min at 10400 x g. After the removal of supernatant, decimal dilutions were prepared and *C. jejuni* numbers were determined by cultivating on Blood agar base No.2 (REF 610188, Liofilchem, Italy) plates for 48h at 37 ºC under microaerofilic conditions.

Treatment on broiler breast fillets

The effect of lactic acid, linalool and cinnamaldehyde against *C. jejuni* on poultry meat was determined using broiler breast fillets with skin. They were taken from the same broiler flock and stored at -80ºC. Sample contamination was checked before the study and they were determined to be free from campylobacter. At the beginning of the experiment, 4x10 g pieces were cut from one broiler breast fillet to form one sample. Different samples represented various decontamination treatments and control. The samples were inoculated with 50 ml bacterial suspension containing 10^8 CFU/ml *C. jejuni* bacteria for 2 min and left for 1 h at 4 ºC temperature for the attachment of bacteria. After 1 h, the samples were decontaminated for 2 min with 50 ml of lactic acid (3 %, 5 %), linalool (0.5 %, 1 %, 1.5 %, and 2 %) and cinnamaldehyde (0.5 %, 1 %, 1.5 %, 2 %, 2.5 %, and 3 %). One sample (control) was not treated to show the initial count of *C. jejuni* on broiler breast fillets after inoculation. One sample was treated with water to show if washing without bioactive compound can reduce *C. jejuni* numbers. Solutions used for every treatment were removed without rinsing. Decimal dilutions of each sample were performed immediately after decontamination, after 4, 24, and 96 hours of storage at 4 ºC temperature to examine if prolonged residual treatment could reduce *C. jejuni* numbers. The counts of *C. jejuni* were determined on Campylobacter blood free medium base (REF 610130, Liofilchem) with Campylobacter Charcoal Cefoperazone Desoxycholate Agar (CCDA) supplement (REF 81037, Liofilchem) incubated at 37 °C for 48 h under microaerophilic conditions. In parallel total bacterial count was determined at 30 ºC incubation for 72 h with pour plate method using Plate count agar (REF 610040, Liofilchem, Italy).

Statistical analysis

Statistical analysis of the quantitative data was performed using the SPSS software 9.0 version. One-way ANOVA was performed to determine whether there were significant differences among *Campylobacter jejuni* numbers at different concentrations of lactic acid, linalool and cinnamaldehyde. Differences among samples based on the time after exposure were analysed using the Bonferroni method for multiple comparisons and Dunnet test when control group was present. For all statistical analyses, $P \leq 0.05$ was considered statistically significant. All experiments were repeated three times.

Results

The reduction effect of bioactive compounds against *C. jejuni* in culture medium and on broiler breast fillets

C. jejuni numbers in culture medium varied significantly between samples treated with different bioactive compounds (Table 1). The count of campylobacters in water treated sample was slightly lower compared to control ($P \geq 0.05$). Linalool showed the lowest effect as *C. jejuni* numbers were reduced by 0.15 $\log_{10}$ CFU/g, 0.47 $\log_{10}$ CFU/g and 1.08 $\log_{10}$ CFU/g compared to control when treated with 0.05 %, 0.1 % and 0.2 % linalool, respectively ($P \geq 0.05$). Lactic acid was significantly more efficient as *C. jejuni* numbers were reduced by 2.05 $\log_{10}$ CFU/g, 4.25 $\log_{10}$ CFU/g ($P \leq 0.05$), 5.67 $\log_{10}$ CFU/g ($P \leq 0.05$) and 5.94 $\log_{10}$ CFU/g ($P \leq 0.05$) compared to control when treated with 0.125 %, 0.25 %, 0.5 % and 2 % lactic acid, respectively. Cinnamaldehyde, on the other hand, showed a similar to water reduction effect at low concentrations (0.01 %, 0.05 %, and 0.1 %), while no *C. jejuni* cells were detected in culture medium after treatment with 0.2 % cinnamaldehyde ($P \leq 0.05$). The reduction effect of higher lactic acid (0.25 %–2 %) and cinnamaldehyde (0.2 %) concentrations was also significant compared to water treated sample.

The reduction effect of bioactive compounds on broiler breast fillets was significantly lower compared to culture medium counting overall pathogen reduction in 96 h period (Table 2). Water treatment reduced campylobacter numbers by 0.35 $\log_{10}$ CFU/g ($P \geq 0.05$), compared to control sample. Five percent lactic acid concentration was more efficient compared to 3 % lactic acid, as it was expected. However, the reduction effect was similar – 1.22 $\log_{10}$ CFU/g and 0.9 $\log_{10}$ CFU/g, respectively ($P \geq 0.05$). Linalool reduced *C. jejuni* numbers by 0.59 $\log_{10}$ CFU/g, 0.67 $\log_{10}$ CFU/g, 0.98 $\log_{10}$ CFU/g and 1.09 $\log_{10}$ CFU/g when treated with 0.5 %, 1 %, 1.5 % and 2 % bioactive compound concentrations, respectively ($P \geq 0.05$). Therefore, lower linalool concentration (2 %) made a slightly (0.19 $\log_{10}$ CFU/g) higher reduction compared to 3 % lactic acid concentration. However, no statistically significant difference was determined between *C. jejuni* numbers treated with different lactic acid, linalool concentrations

in comparison to control sample.

As in culture medium, cinnamaldehyde was also the most effective bioactive compound against *C. jejuni* on broiler breast fillets (Table 2). 2 %–3 % cinnamaldehyde made a significant reduction of campylobacter numbers compared to control sample by 1.96 $\log_{10}$ CFU/g, 2.09 $\log_{10}$ CFU/g, 2.28 $\log_{10}$ CFU/g, respectively ($P \leq 0.05$), while 2.5 %, 3 % bioactive compound concentrations also made a significant difference in comparison to water treated sample reducing campylobacter numbers by 1.74 $\log_{10}$ CFU/g and 1.93 $\log_{10}$ CFU/g, respectively ($P \leq 0.05$).

Table 1. **Effect of different concentration of lactic acid, linalool and cinnamaldehyde on *C. jejuni* in culture medium** ($\log_{10}$ CFU/g)

Sample No.	Treatment	Campylobacter counts log CFU/g, Mean±SD
1	Control	$6.73\pm0.21^{a/defm}$
2	Inoculated+water	$6.57\pm0.14^{b/defm}$
3	Inovulated+0.125% Lactic acid	$4.68\pm0.22^{c/efm}$
4	Inoculated+0.25% Lactic acid	$2.48\pm0.33^{d/abghijklm}$
5	Inoculated+0.5% Lactic acid	$1.06\pm1.5^{e/abcghijkl}$
6	Inoculated+2.0% Lactic acid	$0.79\pm1.37^{f/abcghijkl}$
7	Inoculated+0.05% Linalool	$6.58\pm0.17^{g/defm}$
8	Inoculated+0.1% Linalool	$6.26\pm0.27^{h/defm}$
9	Inoculated+0.2% Linalool	$5.65\pm1.29^{i/defm}$
10	Inoculated+0.01% Cinnamaldehyde	$6.66\pm0.32^{j/defm}$
11	Inoculated+0.05% Cinnamaldehyde	$6.50\pm0.49^{k/defm}$
12	Inoculated+0.1% Cinnamaldehyde	$6.52\pm0.43^{l/defm}$
13	Inoculated+0.2% Cinnamaldehyde	$ND^{m/abcdghijkl}$
SD – standard deviation, ND – not detected. Letters a, b, c, d, e, f, g, h, i, j, k, l, m shows a significant difference between samples 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13		

Table 2. **Effect of different concentration of lactic acid, linalool and cinnamaldehyde on *C. jejuni* on broiler breast fillets** ($\log_{10}$ CFU/g)

Sample No.	Treatment	Campylobacter counts log CFU/g, Mean±SD
1	Control	$7,41\pm0,90^{a/lmn}$
2	Inoculated+water	$7,06\pm0,88^{b/mn}$
3	Inoculated+3% Lactic acid	6,51± 0,63
4	Inoculated+5% Lactic acid	6,19±1,07
5	Inoculated+0,5% Linalool	6,82±1,21
6	Inoculated+1,0% Linalool	6,74±1,34
7	Inoculated+1,5% Linalool	6,43±1,02
8	Inoculated+2,0% Linalool	6,32±0,36
9	Inoculated+0,5% Cinnamaldehyde	$7,04\pm0,91^{i/n}$
10	Inoculated+1,0% Cinnamaldehyde	6,83±1,01
11	Inoculated+1,5% Cinnamaldehyde	6,28±1,10
12	Inoculated+2.0% Cinnamaldehyde	$5,45\pm1,10^{l/a}$
13	Inoculated+2.5% Cinnamaldehyde	$5,32\pm1,30^{m/ab}$
14	Inoculated+3.0% Cinnamaldehyde	$5,13\pm1,76^{n/abi}$
SD – standard deviation. Letters a, b, c, d, e, f, g, h, i, j, k, l, m, n shows a significant difference between samples 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14		

The reduction effect of tested bioactive compounds varied at different times of the experiment (0 h, 4 h, 24 h, 96 h; Table 3). *C. jejuni* numbers were reduced in all tested samples with an exception of 3 % cinnamaldehyde after 4 h compared to 0 h. However, 3 % cinnamaldehyde showed almost the same reduction effect at both 0 h and 4 h. However, the reduction made in 3 % and 5 % lactic acid treated sample by 0.22 $\log_{10}$ CFU/g and 0.29 $\log_{10}$ CFU/g was similar to control and water treated samples – 0.13 $\log_{10}$ CFU/g and 0.09 $\log_{10}$ CFU/g, respectively. The reduction was more significant in 1.5 %, 2 % linalool treated samples (0.64–1.18 $\log_{10}$ CFU/g) and 2 %, 2.5 % cinnamaldehyde treated samples (0.57–0.62 $\log_{10}$ CFU/g) ($P \geq 0.05$). Similar reduction trend remained after 24 h, when *C. jejuni* numbers continued to decline, except for samples treated with 2 % linalool and control sample, where pathogen numbers increased slightly, and 1.5 % linalool, where the increase of *C. jejuni* numbers was more considerable (from 5.64±0.77 $\log_{10}$ CFU/g to 6.39±0.45 $\log_{10}$ CFU/g). This effect can be explained by a

lower linalool concentration (1.5 %) inability to maintain antimicrobial effect for a longer period than 4 h. However, both tested linalool concentrations were less effective than lactic acid and cinnamaldehyde as *C. jejuni* numbers proceeded to increase until the end of the experiment (96 h).

Cinnamaldehyde was the only tested bioactive compound to show statistically significant reduction in certain time periods as 2–2.5 % concentrations reduced *C. jejuni* numbers by 1.19–1.21 $\log_{10}$ CFU/g after initial treatment (0 h) compared to control sample and further 2.72–3.2 $\log_{10}$ CFU/g reduction was made after 96 h ($P \leq 0.05$, Table 3). Statistically significant differences were determined between *C. jejuni* numbers on broiler breast fillets after 0 h, 4 h, 24 h in comparison to pathogen numbers after 96 hours in samples treated with 2 %, 2.5 % cinnamaldehyde ($P \leq 0.05$). However, no significant difference was found between *C. jejuni* numbers at the beginning of experiment (0 h) and after 96 h in samples treated with 3 % cinnamaldehyde. This finding can be explained by a high standard deviation (SD), despite the considerable decline of campylobacter numbers from 5.84±0.77 $\log_{10}$ CFU/g at 0 h to 3.32±3.1 $\log_{10}$ CFU/g at 96 h.

Table 3. **Cinnamaldehyde, linalool and lactic acid effect against *C. jejuni* on broiler breast fillets based on time after exposure** ($\log_{10}$CFU/g)

Sample No.	Treatment/Storage	Campylobacter counts log CFU/g, Mean±SD			
		0 h	4 h	24 h	96 h
1	Control	$7.75\pm0.26^{a/j}$	7.62±0.37	$7.77\pm0.23^{a/dghij}$	7.33±0.49
2	Water	$7.27\pm0.11^{b/j}$	7.18±0.28	7.13±0.26	7.1±0.31
3	3%LA	7.12±0.35	6.9±0.25	5.99±0.66	6.01±0.30
4	5%LA	7.06±0.27	6.77±0.64	$5.62\pm0.95^{d/a}$	5.3±1.51
5	1.5%L	6.82±0.07	5.64±0.77	6.39±0.45	6.85±1.87
6	2.0%L	6.7±0.0	6.06±0.22	6.18±0.16	6.35±0.61
7	1.5%C	$7.05\pm0.10^{g/j}$	6.72±0.08	$6.05\pm0.33^{g/a}$	4.23±0.97
8	2.0%C	$6.54\pm0.08^{A/D}$	$5.97\pm0.47^{B/D}$	$5.47\pm0.06^{C/D,h/a}$	$3.82\pm0.25^{D/ABC}$
9	2.5%C	$6.56\pm0.11^{A/D}$	$5.94\pm0.08^{B/D}$	$5.42\pm0.08^{C/D,i/a}$	$3.36\pm0.52^{D/ABC}$
10	3.0%C	$5.84\pm0.77^{j/abg}$	5.85±0.16	$5.52\pm0.12^{j/a}$	3.32±3.1
SD – standard deviation. LA-Lactic acid, L-Linalool, C-Cinnamaldehyde. Letters a, b, c, d, e, f, g, h, i, j shows a significant difference between samples 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 in certain hour, respectfully, and letters A, B, C, D shows significant differences between hours 0, 4, 24, 96, respectfully, in the same sample					

Variation in total aerobic bacterial count on broiler breast fillets treated with bioactive compounds

All examined bioactive compounds (lactic acid, linalool and cinnamaldehyde; Table 4) lowered the total bacterial count. Statistically insignificant reductions in comparison to control were determined in samples treated with 1.5 %, 2 % linalool and 2 % cinnamaldehyde, when total aerobic bacterial count declined from 6.32±1.60 to 6.20±1.26, 5.20±1.74 and 4.89±1.06, respectively ($P \geq 0.05$). However, 3 %, 5 % lactic acid, 1.5 %, 2.5 %, 3 % cinnamaldehyde proved to be effective in reducing total aerobic bacterial counts on broiler breast fillets by lowering it from 6.32±1.60 to 4.04±0.64, 3.5±0.48, 4.60±1.14, 4.32±1.22, 4.28±1.22 $\log_{10}$CFU/g, respectively ($P \leq 0.05$, Table 4).

Table 4. **Effect of different concentration of lactic acid, linalool and cinnamaldehyde on total aerobic bacterial count on broiler breast fillets** ($\log_{10}$ CFU/g)

Sample No.	Treatment	Total aerobic bacterial count log CFU/g, Mean±SD
1	Control	$6.32\pm1.6^{a/cdgij}$
2	Inoculated+water	$5.94\pm1.82^{b/cd}$
3	Inoculated+3% Lactic acid	$4.04\pm0.64^{c/ab}$
4	Inoculated+5% Lactic acid	$3.48\pm0.48^{d/ab}$
5	Inoculated+1,5% Linalool	6.20±1.26
6	Inoculated+2,0% Linalool	5.20±1.74
7	Inoculated+1,5% Cinnamaldehyde	$4.60\pm1.14^{g/a}$
8	Inoculated+2.0% Cinnamaldehyde	4.89±1.06
9	Inoculated+2.5% Cinnamaldehyde	$4.32\pm1.22^{i/a}$
10	Inoculated+3.0% Cinnamaldehyde	$4.28\pm1.22^{j/a}$
SD – standard deviation. Letters a, b, c, d, e, f, g, h, i, j shows a significant difference between samples 1, 2, 3, 4, 5, 6, 7, 8, 9, 10		

On the other hand, 2 % cinnamaldehyde did not make a statistically significant change in total aerobic bacterial count in comparison to control sample. However, the reduction of total aerobic bacterial count in samples treated with 1.5 % and 2 % cinnamaldehyde counting overall experiment time was very similar – 1.72 $\log_{10}$ CFU/g (from 6.32±1.6 $\log_{10}$ CFU/g to 4.60±1.14 $\log_{10}$ CFU/g) and 1.43 $\log_{10}$ CFU/g (from 6.32±1.6 $\log_{10}$ CFU/g to 4.89±1.06 $\log_{10}$ CFU/g), respectively. Therefore, 0.29 $\log_{10}$ CFU/g difference in bacterial counts might be attributed to sampling, but not the inefficiency of tested bioactive compound solution. Thus, we may assume that total aerobic bacterial count might be effectively reduced by concentrations of cinnamaldehyde starting from 1.5 %.

Our experiment showed that the application of 3 % and 5 % lactic acid was effective in reducing total aerobic bacterial count on broiler breast fillets in comparison to control sample. It was also the only tested compound to make a significant difference between aerobic bacterial count in water treated sample and samples treated with 3 % and 5 % lactic acid (Table 4). Nonetheless, these solutions sustained a stable total aerobic bacterial count throughout the entire storage (Table 5). Bacterial counts varied between 3.82±0.37 $\log_{10}$ CFU/g at 0 h to 4.69±1.00 $\log_{10}$ CFU/g at 96 h ($P \leq 0.05$) when treated with 3 % lactic acid and between 3.40±0.34 $\log_{10}$ CFU/g at 0 h to 3.62±0.56 $\log_{10}$ CFU/g at 96 h ($P \leq 0.05$), when treated with 5 % lactic acid. No significant difference between total aerobic bacterial counts was determined between 0 h and 96 h in these samples.

A significant reduction was also determined in sample treated with 3 % cinnamaldehyde between 0 h, 4 h, 24 h and 96 h ($P \leq 0.05$). However, total aerobic bacterial count was lowered considerably at 0 h from 5.38±1.63 $\log_{10}$ CFU/g to 3.90±0.80 $\log_{10}$ CFU/g ($P \geq 0.05$) and remained relatively stable for 24 h, but as bioactive compound was not able to maintain antimicrobial activity, total aerobic bacterial count increased to 6.16±0.17 $\log_{10}$ CFU/g.

Table 5. **The effect of lactic acid, linalool and cinnamaldehyde against total aerobic bacterial count on broiler breast fillets** ($\log_{10}$ CFU/g) **based on time after exposure**

Sample No.	Treatment/ Storage	Total aerobic bacterial count log CFU/g, Mean±SD			
		0 h	4 h	24 h	96 h
1	Control	5.38±1.63	5.46±0.91	5.83±0.52	$8.59\pm0.25^{A/D,a/cd}$
2	Water	5.19±1.62	4.65±0.71	5.32±0.55	$8.61\pm0.46^{A/D,b/cd}$
3	3%LA	3.82±0.37	4.07±0.04	3.58±0.28	$4.69\pm1.00^{c/abe}$
4	5%LA	3.40±0.34	3.59±0.68	3.31±0.53	$3.62\pm0.56^{d/abef}$
5	1.5%L	6.07±0.22	$5.13\pm0.99^{B/D}$	5.59±0.25	$8.04\pm0.49^{D/B,e/cd}$
6	2.0%L	4.99±1.55	4.08±0.40	4.58±1.73	$7.17\pm2.11^{f/d}$
7	1.5%C	5.06±1.81	3.94±0.32	3.72±0.34	5.67±0.66
8	2.0%C	4.95±1.50	4.47±0.96	4.26±1.27	5.87±0.28
9	2.5%C	4.43±1.41	3.47±0.22	3.45±0.24	5.93±0.39
10	3.0%C	$3.90\pm0.80^{A/D}$	$3.51\pm0.09^{B/D}$	$3.57\pm0.40^{C/D}$	$6.16\pm0.17^{D/ABC}$
SD – standard deviation. LA-Lactic acid, L-Linalool, C-Cinnamaldehyde. Letters a, b, c, d, e, f, g, h, i, j shows a significant difference between samples 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 in certain hour, respectfully, and letters A, B, C, D shows significant differences between hours 0, 4, 24, 96, respectfully, in the same sample					

Discussion

Our study showed that the reduction effect of bioactive compounds was different when tested in culture medium and on broiler breast fillets. The concentrations of bioactive compounds needed to reduce *C. jejuni* numbers in culture medium were considerably lower compared to their application on broiler breast fillets with skin (Table 1, Table 2). For example, *C. jejuni* numbers in culture medium were reduced significantly (5.67 $\log_{10}$ CFU/g) by 0.5 % lactic acid compared to control sample ($P \leq 0.05$, Table 1). These results correspond to Birk et al. (2010) collected data where the application of 0.5 % lactic acid reduced *C. jejuni* numbers by approximately 5 $\log_{10}$ CFU/g after 5 hours of treatment in chicken juice. However, our study showed that only 5 % lactic acid reduced *C. jejuni* numbers on broiler breast fillets by more than 1 $\log_{10}$ CFU/g (from 7.41±0.9 $\log_{10}$ CFU/g to 6.19±1.07 $\log_{10}$ CFU/g; Table 2). This effect was determined by counting overall pathogen reduction with no significant difference between *C. jejuni* numbers in control and treated sample. Additionally, these findings correspond to Lecompte et al. (2009) study results when 5 % lactic acid solution was not effective against *C. jejuni* on chicken breast skin.

Likewise, the difference between effective lactic acid concentrations in culture medium and on chicken meat was investigated in two different Rajkovic et al. studies (2009, 2010). It was determined that 3 % lactic acid reduced *C. jejuni* numbers in culture medium by approximately 3.8 $\log_{10}$ CFU/g after initial treatment (Rajkovic et al., 2009). Meanwhile, other study (Rajkovic et al., 2010) investigated the combined effect of buffered lactic acid and high O_2 modified atmosphere on chicken legs and determined that 10 % lactic acid-sodium lactate buffer solution reduced *C. jejuni* numbers by approximately 1.8 $\log_{10}$ CFU/g with an additional reduction of 1.2 $\log_{10}$ CFU/g induced by modified atmosphere packaging.

These results would support the idea that lower than 5 % lactic acid concentrations could not effectively

reduce *C. jejuni* numbers on poultry meat. However, according to Riedel et al. (2009) experiment results, 2.5 % lactic acid was effective against *C. jejuni* on chicken skin and meat and reduced campylobacter numbers by 1.7 $\log_{10}$ CFU/g after 1 min treatment and the reduction reached 3.87 $\log_{10}$ CFU/g when treatment was prolonged for 24 hours. The reduction was even higher on chicken skin than on meat. Nonetheless, we revealed that the numbers of *C. jejuni* on broiler fillets with skin, were also reduced by 0.63 $\log_{10}$ CFU/g compared to control sample when treated for 2 min and 1.78 $\log_{10}$ CFU/g after 24 h in 3 % lactic acid solution ($P \geq 0.05$, Table 3), yet with no significant difference between treated sample and control. However, 5 % lactic acid reduced campylobacter numbers statistically significantly by 2.15 $\log_{10}$ CFU/g after 24 h in comparison with control sample ($P \leq 0.05$, Table 3). Possible explanation for this difference in *C. jejuni* numbers in Riedel et al. (2009) and our research could be attributed to the different *C. jejuni* strain used for these studies. Nevertheless, as Riedel et al. (2009) study does not provide us with an information about *C. jejuni* strain sensitivity to lactic acid in culture medium, no comparison could be done between two tested pathogen strains.

Linalool was less effective than lactic acid in culture medium as 0.2 % linalool reduced *C. jejuni* numbers only by 1.12 $\log_{10}$ CFU/g in comparison to control sample. These results contradict to Fisher and Phillips (2006) findings, where such low concentration as 0.06 % linalool suppressed the growth of *C. jejuni* when applied on Campylobacter agar base by disc diffusion method. However, extended essential oil treatment (10 min in our experiment and an entire cultivation time in Fisher and Phillips (2006) study) could have enhanced the effect of low linalool concentrations against *C. jejuni* in mentioned study. Moreover, about 3 $\log_{10}$ CFU/g reduction was determined by on-food treatment method when tested by dipping small (2cmx2cm) chicken skin squares to the established minimal inhibition concentration of linalool for 60 s with no additional effect after 10 min. However, as the treatment of skin and meat samples separately usually show different sensitivity to bioactive compound (Riedel et al., 2009), experimental treatment of skin and meat samples together (like it was done in our study) is closer to the real poultry processing conditions.

As distinct from lactic acid effect, we have determined that cinnamaldehyde was more effective on broiler breast fillets than in culture medium as only 0.2 % cinnamaldehyde showed a statistically significant effect against *C. jejuni* numbers in culture medium ($P \leq 0.05$). On the other hand, Friedman et al. (2002) determined that even 0.04 % cinnamaldehyde concentration might reduce *C. jejuni* numbers by 50 % in culture medium. However, in our study 0.2 % cinnamaldehyde reduced *C. jejuni* numbers below detection level. While differences between these two experiments might be assigned to diverse testing techniques, Ravishankar et al. (2008) results confirms our findings as 0.2 % of cinnamaldehyde in their study also completely inactivated *C. jejuni* in culture medium.

The effect of cinnamaldehyde on broiler breast fillets in our experiment is similar to Mild et al. (2011) study results according to which 3 % cinnamaldehyde reduced *C. jejuni* numbers by 1.8–6.0 $\log_{10}$ CFU/g depending on bacterial strain and temperature after 72 hours of treatment. Our findings revealed that 3 % of this bioactive compound lowered *C. jejuni* numbers from 7.41±0.90 $\log_{10}$ CFU/g to 5.13±1.76 $\log_{10}$ CFU/g within 96 h (Table 2). Additionally, 2.5 % and 3 % cinnamaldehyde also reduced the numbers of *C. jejuni* in comparison to water treated control samples (Table 2), therefore showing that it is considerably more beneficial to use this bioactive compound for the decontamination purposes on poultry meat than just potable water which is now permitted for decontamination (EFSA, 2011).

Cinnamaldehyde was also efficient in the reduction of total aerobic bacterial count. These results are supported by Das et al. (2012) and Chan et al. (2013) findings, suggesting that cinnamaldehyde is a highly antimicrobial substance, able to reduce a wide range of bacteria like *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus, Enterobacter* sp., *Klebsiela* sp., *Bacillus* sp. and *Enterococcus* sp.

On the other hand, the efficiency of lactic acid against total bacterial count and various spoilage bacteria like *Pseudomonas* spp., *Enterobacter* sp., *Citrobacter* and lactic acid bacteria is examined in a number of studies (Ruby et al., 2007; Rajkovic et al., 2010; Anang et al., 2010; Burfoot and Mulvey, 2011; EFSA, 2011). However, working concentration of lactic acid on poultry vary from 1 % when subjected with additional bioactive compounds like lauricidin (Anang et al., 2010), to 4–5 % when applied by spraying (Ruby et al., 2007). Nonetheless, it was already approved for the surface decontamination of bovine carcasses (Comission regulation (EU) No 101/2013), therefore encouraging further studies for the effect of lactic acid against other food matrices.

Linalool was less efficient in *C. jejuni* reduction on broiler breast fillets. These results were expected, as other studies like Zengin and Baysal (2014) showed that *Shewanella putrefaciens* and *Carnobacterium divergens*, often found in raw meat stored under aerobic conditions (Doulgeraki et al., 2012), were significantly reduced only by 2 % and higher concentrations of linalool when MIC values were determined in culture medium. In addition, as our study revealed, significantly higher concentrations would be necessary to reduce bacterial numbers on food product in comparison to culture medium.

Total aerobic bacterial count exceeding 7 $\log_{10}$ CFU/g usually shows that poultry product is already spoiled (Anang et al., 2010). Therefore, cinnamaldehyde and lactic acid solutions could effectively prolong the shelf-life of broiler breast fillets as total aerobic bacterial count in samples treated with 3 %, 5 % lactic acid and 1.5 %–3 % cinnamaldehyde did not reach 7 $\log_{10}$ CFU/g after 96 h at 4°C, while in control sample it was 8.59±0.25 $\log_{10}$ CFU/g. Therefore, our results showed that cinnamaldehyde and lactic acid are able to reduce microbial load on poultry products, while linalool was

ineffective in reducing total aerobic bacterial count.

Conclusions

1. The bioactive compound concentrations needed to reduce *C. jejuni* numbers in culture medium are considerably lower compared to their application on broiler breast fillets with skin.
2. Plant origin bioactive compounds like cinnamaldehyde and lactic acid could be an effective alternative to chemical bactericidal treatments now used for poultry decontamination and prolong the shelf life of poultry product.
3. Cinnamaldehyde is the only tested bioactive compound to reveal statistically significant reductions on both *C. jejuni* numbers and total aerobic bacterial count in comparison to control sample at 2.5 %, 3 % bioactive compound concentrations.
4. Lactic acid is effective against total aerobic bacteria, but does not make a considerable effect in *C. jejuni* reduction on poultry breast fillets, while linalool does not show an expected significant reduction of either *C. jejuni* numbers or total aerobic bacterial count.

Acknowledgements

This research was funded by a Grant (No. SVE 05/2011) from the Research Council of Lithuania

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Received 29 June 2015
Accepted 15 October 2015