Organic acids effect on spoilage psyhrotrophic microflora during the shelf life of bovine carcasses

Sorin Daniel DAN*[1], Marian MIHAIU[1], Oana REGET[1], Alexandra TĂBĂRAN[1]
[1]University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Department of Animal Production and Food Safety, 3-5 Mănăştur Street, Cluj-Napoca, Romania, sorindan@usamvcluj.ro

Abstract

The aim of this research was to assess the residual antimicrobial effect of 3% lactic and acetic acid solutions on the load and configuration of psychrotrophs at bovine carcasses. During October 2016 and December 2016, 18 bovine meat samples were collected from a comercial slaughterhouse in Transylvania. Collected samples were sprayed with 3% acetic acid and lactic acid solutions by spraying on the surface of meat samples. Each sample was divided into three sub-samples, from which two were treated with 3% organic acids solutions and one was the control sample. Experimental design were carried out over a 14-day period with microbial analyses at day 0, 1, 5, 9, 14. After spraying with organic acid solutions, the samples were kept at 2-4°C for 24 hours, and the following microbiological determinations were carried out: total load of psychrotrophic germs and isolation of microorganisms from the genera Pseudomonas, Aeromonas, Yersinia and Enterobacteriaceae family. The initial psychrotrophs load of the control sample presented during the experiment an ascendant evolution, from 3.23±0.2 log CFU/cm^2 to 6.21 ± 0.25 log CFU/cm^2, maximum admitted level being exceeded on day 10. After application of 3% acetic acid solution, the total load of psychrotrophic germs decreases to 2.05 ± 0.15 log CFU/cm^2, afterwards showing a constant increase until the last day of the experiment to 4.89 ± 0.21 log CFU/cm^2. The most sensitive psychrotrophic bacteria regarding the decontamination effect of lactic and acetic acid were Aeromonas spp. Lactic and acetic acid solution shown an obvious residual antimicrobial effect during the shelf life of bovine carcasses, when compared with control ($p<0.05$). Although acetic acid has more pronounced residual antimicrobial effect, we recommend using 3% lactic acid because it is a natural metabolite of muscle tissue and does not induce organoleptic changes in meat compared to acetic acid. The use of these methods of decontamination of carcasses should be considered as complementary measures to ensure hygienic quality and meat, and must be integrated within HACCP systems.

Keywords: *residual antimicrobial effect, organic acids, spoilage psychrotrophs, bovine carcasses*

Introduction

The effectiveness of using a wide range of antimicrobial treatments to reduce the prevalence of spoilage and pathogenic bacteria from food producing aimals carcasses has been extensively studied and documented (Alakomi *et al.,* 2000; Castelo *et al.,* 2001; Staruch *et al.*, 2001; Strivarius *et al.,* 2002; Ockerman *et al.*, 2001a, b; Pipek *et al.*, 2004, 2006; Bosilevac *et al.*, 2006). Different procedures have been tested over the past 25 years to reduce microbial contamination from carcasses immediately after the slaughter. The most effective and practical methods for reducing microbial contamnation have been technically proven to apply solutions of diluted organic acids or hot water to the carcass surface, exposure to water vapor under pressure (steam pasteurization) and the use of steam or hot water in combination with the vacuum packaging of the meat. Other types of treatments have been tested, but with less extensibility, as follows: rinsing of carcasses with different chemical solutions (chlorination, trisodium phosphate, pulsating light exposure, pulsating electric fields or ionizing radiation (Pipek *et al.*, 1997; Stradford *et al.*, 1999; Castelo *et al.,* 2000; Staruch *et al.,* 2001; Strivarius *et al.,* 2002 a, b). Of the organic acids, the most used for reducing the microbial load on the carcass surface are: lactic and acetic acids in variable concentrations, between 2-5%. By using the mentioned organic acids solutions on carcasses a reduction of microbial contamination was observed to 1.5 log. Some studies have shown that some meat pathogens are particularly susceptible to organic acids (*Yersinia enterocolitica, Aeromonas hydrophila*) while others are more resistant (*E. coli* O157: H7). One possible advantage of organic acid treatments compared to other types of treatments is that there is residual activity after applying them. Instead, some research has shown that reducing the bacterial load from the carcass surface was not correlated with improved meat hygiene, due to recontamination and microbial growth during post-processing and storage (Gill and Landers, 2003). Therefore, our research aims to assess the residual antimicrobial effect of 3% lactic and acetic acid solutions on the load and configuration of psychrotrophs at bovine carcasses.

Material and methods

During October 2016 and December 2016, 18 bovine meat samples were collected from a comercial slaughterhouse in Transylvania. Collected samples were sprayed with 3% acetic acid and lactic acid solutions by spraying on the surface of meat samples (2.5-3 ml/100 cm^2). Each sample was divided into three sub-samples, from which two were treated with 3% organic acids solutions and one was the control sample (not decontaminated). Experimental design were carried out over a 14-day period (shelf life of the bovine carcasses), with microbial analyses at day 0, 1, 5, 9, 14. After spraying with organic acid solutions, the samples were kept at 2-4°C for 24 hours, and the following microbiological determinations were carried out: total load of psychrotrophic germs and isolation of microorganisms from the genera *Pseudomonas, Aeromonas, Yersinia* and *Enterobacteriaceae* family. Psychrotroph plate count was performed according with the protocol described by Nottingham *at. al* (1982). For isolation of psychrotrophs specific selectiv media were used, as follows: Aeromonas and Pseudomonas - GSP agar (Merck), Yesinia - CIN agar (Merck), Enterobacteriaceae – VRBD agar (Merck). Serial decimal dilutions (10^{-1} : 10^{-6}) were obtained from 10 grams of meat and 90 ml water buffered peptone. Spreading method was used to inoculate 0.1ml of inoculum on to the surface of 2 Petri plates. Incubation was realized at 20°C, for 72 hours. Biochemical confirmation test was realized using API 20 E and API 20 NE (Biomerieux). The statistical calculations were using program Origin 8.5. Data interpretation was made by calculating the monthly averages, based on standard deviation (6 samples/month). The statistical test used was ANOVA cathegorial monofactorial analisys. Result were depicted as log CFU/cm^2.

Results and discussions

The initial psychrotrophs load of the control sample presented during the experiment an ascendant evolution, from 3.23±0.2 log CFU/cm^2 to 6.21 ± 0.25 log CFU/cm^2, maximum admitted level being exceeded on day 10. After application of 3% acetic acid solution, the total load of psychrotrophic germs decreases to 2.05 ± 0.15 log CFU/cm^2, afterwards showing a constant increase until the last day of the experiment to 4.89 ± 0.21 log CFU/cm^2. A similar trend was noticed in the case of lactic acid. Total psychrotrophs counts decreased after 24 hours to 2.12 ± 0.19 log CFU/cm^2, followed by a steady increase up to 4.95 ± 0.16 log CFU/ cm^2 on day 14. Based on these considerations, we can mention that the residual antimicrobial effect of both organic acid solutions was maintained until the 14th day, maximum admitted level vas not exceeded and no statistical differences ($p>0.05$) were recorded between acetic and lactic acid, , the maximum recommended limit being exceeded on day 12 (Figure 1). Similar results were obtained by Strivarius *et al.* (2002 a, b), in the dynamic studies performed to assess the effect of lactic acid and

and acetic acid, regarding the decrease of the total plate count at bovine carcasses. The study revealed that acetic acid lowers the microbial load after application, with 1.0 log CFU/cm^2 when compared with initial load, and the antimicrobial effect being maintained until day 7 of the experiment. Also, Pipek *et al*. (2006), in a research regarding the antimicrobial effect of 3% lactic acid on the psychotropic germs at the surface of the bovine carcass observed a reduction of 2.0 log CFU/cm^2, and the maintenance of this effect during the experiment (5 days).

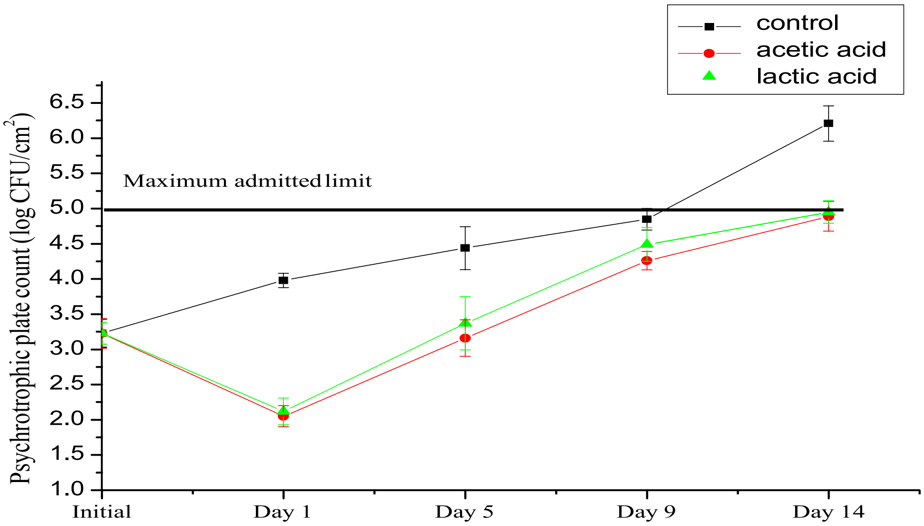

Figure 1. Decontamination effect of 3% organic acid solution regarding psychrotrophic plate count at the surface of bovine meat (n=6)

Regarding the *Enterobacteriaceae* psychrotrophic microflora, an ascending trend from an initial load of 1.89 ± 0.15 log CFU/cm^2 to 5.12 log CFU/cm^2 on the 14th day of the experiment was observed for the control sample, the maximum admissible limit being exceeded in Day 6 (Figure 2). Both organic acid solutions, acetic lactic acid caused after 24 hours a reduction of the number of *Enterobacteriaceae* at 0.87 ± 0.05 log CFU/cm^2 and 1.1 ± 0.41 log CFU/cm^2 respectively, followed by a constant increase up to 3.2 ± 0.25 log CFU/cm^2, respectively 2.89 ± 0.25 log CFU/cm^2 in the last day of the experiment (figure 2). Analyzing these results, we can assume that the antimicrobial effect of 3% acetic and lactic acid solutions was maintained for about 9 days.

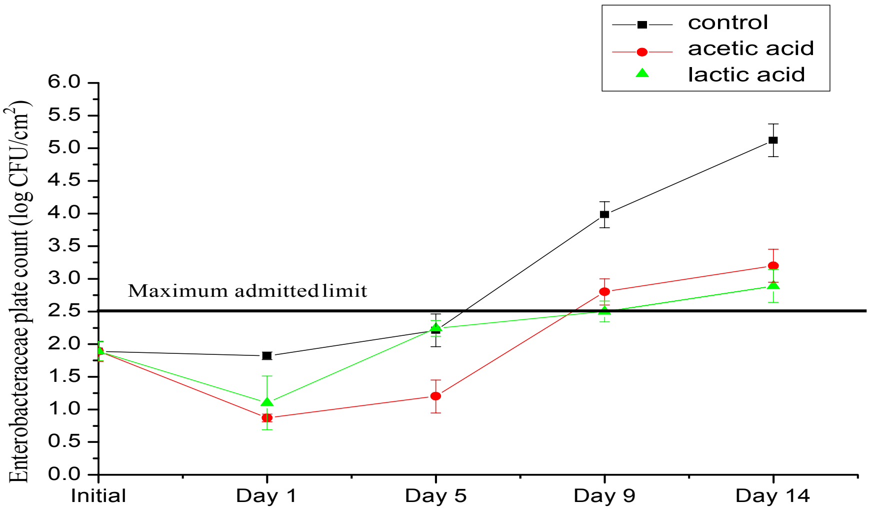

Figure 2. Decontamination effect of 3% organic acid solution regarding *Enterobacteriaceae* load at the surface of bovine meat (n=6)

Following 24 hours since the application of 3% lactic acid solution, initial microbial load of *Aeromonas* spp. decreased from 2.21 ± 0.15 log CFU/cm^2, to 0.89 ± 0.14 log CFU/cm^2. Starting with the 5th day, *Aeromonas* spp. was totally inhibited. Acetic acid solution produced total inhibition of *Aeromonas* spp. after 24 hours since spraying due to their increased susceptibility to acetic acid (Figure 3). Significant differences were recorded when compared acid with lactic acid ($p<0.05$).

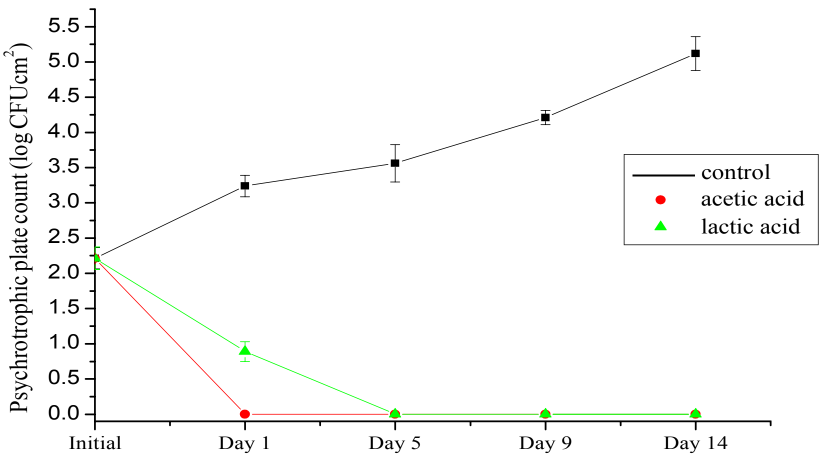

Figure 3. Decontamination effect of 3% organic acid solutions regarding *Aeromonas* spp. at the surface of bovine meat (n=6)

In case of the pseudomonads, an ascending trend regarding microbial load was noticed, ranged from 2.41 ± 0.15 log CFU/cm^2 to 5.6 ± 0.35 log CFU/cm^2 in the final day of the experiment. After the application of 3% acetic acid solution, *Pseudomonas* spp. initial load decreased to 1.96 ± 0.28 log CFU/cm^2, afterwards having an ascending evolution until the last day of the experiment, when they reached 4.02 ± 0.34 log CFU/cm^2. A similar evolution was also noticed for the sample treated with 3% lactic acid (Figure 4). Both solutions of organic acids used caused a decrease of pseudomonads load after 24 hours (1.5 log CFU/cm^2 in case of 3% acetic acid). Afterwards, the results showed a more obvious effect regarding reduction of *Pseudomonas* spp. in case of lactic acid, but no differences were noticed between organic acid solution ($p>0.05$).

With regard to *Yersinia* spp. load, we recorded a constant evolution from an initial load of ± 0.20 log CFU/cm^2 to 5.12 ± 0.15 log CFU/cm^2 on 14th day of the experiment (Figure 120). 24 hours after application of the acetic acid solution, *Yersinia* spp. load decreased to 2.31 ± 0.16 log CFU/cm^2, following then by a moderate ascending trend until day 14, when they reached 3.89 ± 0.19 log CFU/cm^2. A similar evolution was established in case of the lactic acid solution, with a more pronounced decrease of the initial germ load, at the end of the experiment (3.56 ± 0.19 log FCU/cm^2).

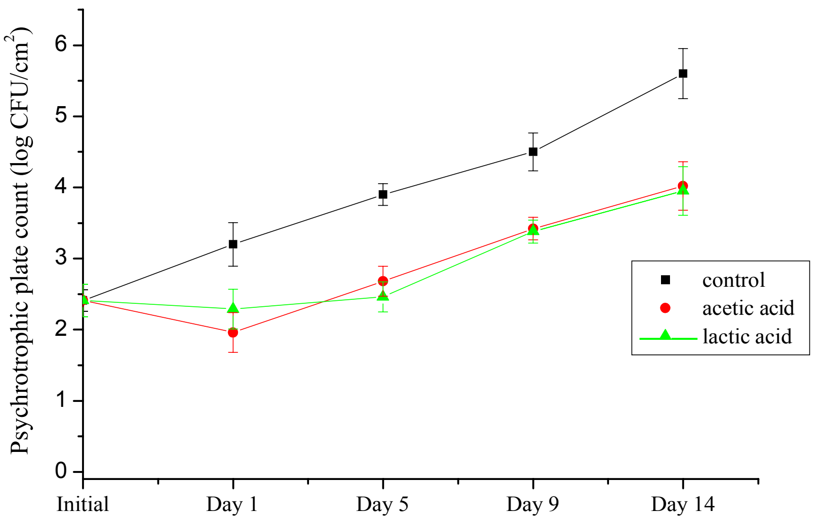

Figure 4. Decontamination effect of 3% organic acid solutions regarding *Pseudomonas* spp. at the surface of bovine meat (n=6)

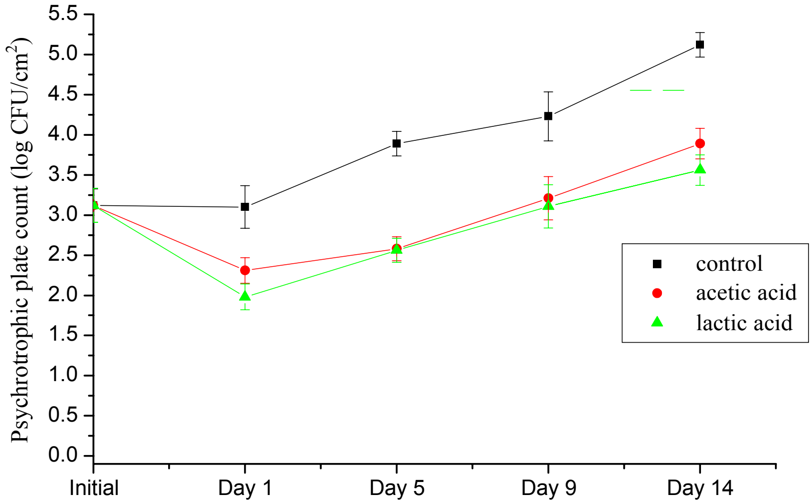

Figure 5. Decontamination effect of 3% organic acid solutions regarding *Yersinia* spp. at the surface of bovine meat (n=6)

Although the antimicrobial effect of the organic acid solutions used to reduce total psychrotrophs, *Enterobacteriaceae*, as well as *Aeromonas* spp., *Pseudomonas* spp. and *Yersinia* spp. load, distinct significant differences were obtained only for aeromonads ($p \leq 0.05$), in which both acetic acid and lactic acid determined total inhibition. These results can be explained by the fact that after the microbial reduction, a variable lag phase (1-5 days), specific for different bacteria followed, to allow some microorganisms to adapt to the new environmental conditions. Similar results were reported by, Alakomi *et al.* (2000), who observed that *Pseudomonas aeruginosa* load from the bovine carcasses surface was reduced from 2.0 ± 0.1 log CFU/cm^2 to 0.23 ± 0.1 log CFU/cm^2 as a result of 2-3% lactic acid spray application. Prasai *et al.* (1991), using lactic acid solutions at different concentrations, from 0.75 to 2.5%, observed that 1.25% lactic acid reduced the number of *Enterobacteriaceae* by 1.0 log CFU/cm^2.

Conclusions

The most sensitive psychrophic bacteria regarding the decontamination effect of lactic and acetic acid were *Aeromonas* spp. Lactic and acetic acid solution shown an obvious residual antimicrobial effect during the shelf life of bovine carcasses, when compared with control ($p<0.05$). Although acetic acid has more pronounced residual antimicrobial effect, we recommend using 3% lactic acid because it is a natural metabolite of muscle tissue and does not induce organoleptic changes in meat compared to acetic acid. The use of these methods of decontamination of carcasses should be considered as complementary measures to ensure hygienic quality and meat, and must be integrated within HACCP systems.

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