Pathogens Contamination Level Reduction on Beef Using Organic Acids Decontamination Methods

Sorin Daniel DAN^{*1}, Marian MIHAIU¹, Oana REGET¹, Delia OLTEAN² and Alexandra TĂBĂRAN¹

¹Department of Animal Production and Food Safety, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

²Sanitary Veterinary and Food safety laboratory Alba County, Department of Food Microbiology, Alba Iulia, 7A Lalelelor Street, Alba County

*corresponding author: sorindan@usamvcluj.ro

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ABSTRACT

In this study we aimed to assess the efficiency of organic acids in different concentrations regarding pathogens as *Salmonella*, *Listeria* and *Escherichia* on beef, which can cause food borne illness in humans. The samples were sterilized using UV radiation for 30 minutes, afterwards being contaminated with 1 ml of microbial suspension (0.5 MacFarland). We used reference bacterial strains for *Salmonella Enteritidis*, *Escherichia coli* and *Listeria monocytogenes*. The samples were subjected to decontamination procedure by introducing 25mL of solution of lactic, acetic or citric acid in concentration of 1%, 2% and 3%. The results showed a reduction of initial pathogen load, ranging from 0.32 to 7.78 log CFU/g, depending on the type of acid, concentration and pathogen sensitivity. After decontamination, standardized methods have been used for the isolation of pathogenic germs. Based on statistical analysis we conclude that pathogens have a different sensitivity to the action of acid solutions, their sensitivity in ascending order being: *Listeria monocytogenes*, *Salmonella Enteritidis* and *Escherichia coli*. Among the organic acids, the most efficient was lactic acid, followed by acetic acid and less efficient citric acid. The greatest reduction of germs was determined by the concentration of 3%.

Keywords: pathogens, decontamination methods, bovine carcasses

INTRODUCTION

Bovine carcasses can become contaminated with pathogens bacteria during slaughtering process, mainly due to lack of Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP) in the hiding and evisceration steps (Smulders and Greer, 1998; Sofos, 2008; Loretz *et al.*, 2011). Pathogenic microorganisms, once in the body through food consumption, can cause food poisoning especially in developing countries, affecting a large number of consumers (Boslaugh, 2008). For these reasons, the competent authorities of each country has an interest in adopting appropriate prevention measures to reduce the prevalence of food-borne illnesses. Numerous studies have demonstrated the effectiveness

of using decontamination methods in order to reduce the level of pathogens, among which the most commonly used are: organic acids (lactic acid, citric acid, acetic acid), alkaline solutions (trisodium phosphate), steam, chlorinated water or ozone (Dorsa *et al.*, 1998; Smulders and Greer, 1998; Castillo *et al.*, 1999; Gonzales and Domongoz, 2006; Carpenter *et al.*, 2011, Loretz *et al.*, 2011). Depending on the concentration of the chemical solutions used, the reduction of pathogen load was variable, ranging from 0.4 log CFU/cm²-4.5 log CFU/cm² (Ramson *et al.*, 2003; Sohaib *et al.*, 2016). Based on EFSA's scientific opinion, in accordance with current legislation, only lactic acid solutions in bovine carcasses can be used in the EU, with the indication that it is introduced in the HACCP plan

(EFSA, 2011; Reg CE 101/2013). Therefore, the aim of this study was to evaluate the effectiveness of different organic acid solutions regarding pathogenic microorganisms from *Salmonella*, *Escherichia* and *Listeria* genera from bovine meat.

MATERIALS AND METHODS

Sixteen samples of chilled meat from a bovine slaughterhouse located in Alba County were collected between November 2016 and April 2017. The samples were packed in sterile polyethylene foil, placed in isothermal bags at 4 – 6°C and transported to the Sanitary Veterinary and Food Safety Laboratory Alba County, within the department of Food microbiology, where they were processed. In order to achieve the aforementioned objectives, the following reference strains for pathogenic germs were used: *Salmonella* Enteritidis ATCC 13076 (Microbiologics, USA), *Escherichia coli* ATCC 25922 (Microbiologics, USA), *Listeria monocytogenes* ATCC 19114 (Microbiologics, USA). For the contamination of samples with the above-mentioned pathogenic germs, the lyophilized cultures were suspended in a nutrient broth maintained at 37°C for 24 hours in *Salmonella* spp. and *E. coli* and TSYEB maintained for 48 hours in case of *Listeria monocytogenes*. After incubation, inoculation was performed on specific selective specific media (XLD-*Salmonella* spp., TBX - *E. coli*, ALOA - *Listeria monocytogenes*), which were thermostated at 37°C for 24-48 hours. For each type of pathogen germ, 5 specific colonies were subjected to cultural and morphological confirmation tests. From the selective media, the colonies were cultured on nutrient agar, which was incubated at 37°C for 24 hours. Afterwards, biochemical and serological tests were performed to confirm that the isolated strains were pure. In order to obtain microbial suspensions with a specific number of bacteria, colonies developed on the nutrient agar were homogenized in 5 ml sterile saline solutions in test tubes until a turbidity of 0.5 on the MacFarland scale was obtained, verified with the Densimat apparatus (Biomerieux). According to the manufacturer's stipulations, the value of 0.5 corresponds to a load of microorganisms of 150×10^6 CFU/ml. The collected samples were sterilized inside the microbiological cabinet using UV radiation for 30 minutes in order to inactivate any pathogenic germs. Three solutions of organic acids: lactic,

acetic and citric in different concentrations (1%, 2% and 3% respectively) were used to assess the antimicrobial potential.

In order to evaluate the decontamination effect of some organic acid solutions, the samples were processed as follows: each beef sample was divided into 11 sub-samples of 25 g, from which one was the negative control (un-contaminated). 9 samples have undergone the decontamination process with the aforementioned organic acid solutions, and the latter has been a positive control (non-decontaminated). Samples 2-10 (25 g of meat each) were contaminated with 1 ml of microbial suspension (0.5 MacFarland), then homogenized for 30 seconds in the Stomacher device (230 rpm), leaving 30 minutes at 20°C to ensure the adhesion of bacteria to muscle tissue. Afterwards, samples 2-10 were subjected to the decontamination procedure by adding 25 ml of 1%, 2% and 3% citric acid solution, acetic acid and citric acid respectively, followed by homogenization in Stomacher (230 rpm, for 30 seconds), being maintained for 1 minute at 20°C for the decontamination effect. For isolation of *Salmonella*, *Escherichia* and *Listeria* strains, 225 ml of buffered peptone water (APT), semi-Fraser broth and Bolton broth were added to samples 1-11, performing serial dilutions: 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} . For the isolation of pathogenic germs standardized methods have been used, according to the current legislation (SR EN ISO 6579 AC/2009, SR EN ISO 11290-1/A1/2005, and SR EN ISO 16649-2/2007). Statistical analysis of the results was carried out using Origin 8.5 software program by comparison of means by analysis of variance through ANOVA test.

RESULTS AND DISCUSSIONS

The decontamination effect of organic acid solutions on Salmonella Enteritidis

Based on the results, it was found that 1% acetic acid solution showed a reduction of *Salmonella* Enteritidis from 6.78 to 5.64 ± 0.16 log CFU/g (1.14 log reduction), of 5.38 ± 0.11 log CFU/g in case of the lactic acid solution 1% (1.4 log reduction), and of 5.96 ± 0.07 log CFU/g (0.90 log reduction) in case of citric acid 1% (Figure 1). After application of 2% organic acids solutions, the *Salmonella* load decreased at 4.56 ± 0.04 log CFU/g for acetic acid (2.22 log reduction), to 4.54 ± 0.07 log logCFU/g for lactic acid, respectively (of

Fig.1. Decontamination effect of 1%, 2% and 3% of acetic, lactic and citric acid regarding *Salmonella* Enteritidis on beef samples (n=3)

2.24 log reduction), and 5.56 ± 0.09 log CFU/g for citric acid (1.22 log reduction) (Fig. 1).

A significant reduction was noticed in case of using organic acids in concentrations of 3%. Thus, the *Salmonella* load was reduced to 4.44 log CFU/g after using acetic and lactic acid (2.34 log reduction) and in the case of citric acid the load decreased to 4.43 log CFU/g (2.35 log reduction) (Figure 1). Statistical analyzes revealed significant differences regarding *Salmonella* Enteritidis load between positive samples (contaminated) and decontaminated samples with organic acid solutions ($p < 0.05$), and no differences were recorded between the same concentration of organic acid solutions.

Results similar to those obtained by us were published by Castillo *et al.* (2001), in a study on the efficacy of *Salmonella* Enteritidis reduction methods from the surface of chilled cattle carcasses, which showed a reduction between 1.6-1.9 log when using 2% lactic acid. Similarly, Harris *et al.* (2006) showed a reduction of *E. coli* and *Salmonella* spp. between 1.5 and 2.0 log, in the case of lactic acid concentration of 2% and 4%, respectively.

The decontamination effect of organic acid solutions on *E. coli*

Following application of the 1% acetic acid solution, the initial load of *Escherichia coli* decreased to 6.72 ± 0.08 log CFU/g (0.06 log reduction), to $6.56 \log \pm 0.10$ CFU/g after lactic acid use (0.12 log reduction) and 6.56 ± 0.16 log CFU/g after utilization of citric acid (0.12 log reduction).

Following the use of 2% organic acid solutions the initial load of *E. coli* decreased to 6.46 ± 0.06 log CFU/g (0.32 log reduction) in case of acetic acid, 6.37 ± 0.04 log CFU/g for lactic acid and 6.38 ± 0.04 log CFU/g for citric acid (Figure 2), with a reduction of 0.40 log. A more significant decrease was recorded in case of the use of 3% organic acids solutions. Thus, after the application of acetic acid, a decrease to 5.56 ± 0.13 log CFU/g (1.22 log reduction), to 5.45 ± 0.08 log CFU/g for lactic acid (1.33 log reduction) and to 5.85 ± 0.08 log CFU/g for citric acid (Figure 2). Significant differences were recorded only in case of 2% and 3% acetic and lactic acid solutions ($p < 0.05$) in comparison with the control sample regarding the effect of on the total load of *Escherichia coli*. Regarding the efficiency of lactic acid solutions (1%, 2%, 3%) in comparison with acetic acid solutions (1%, 2%,

Fig.2. Decontamination effect of 1%, 2% and 3% of acetic, lactic and citric acid regarding *E. coli* on beef samples (n=3)

Fig.3. Decontamination effect of 1%, 2% and 3% of acetic, lactic and citric acid regarding *Listeria monocytogenes* on beef samples (n=3)

3%), significant differences were found only in case of 1% concentration ($p < 0.05$).

A study by Castilo *et al.* (2001) on the efficiency of *E. coli* reduction methods on chilled bovine carcass surfaces revealed a more pronounced reduction compared to those obtained in our study, ranging from 2.0-2.4 log when using 2% lactic acid. Similar results have been mentioned by Delazari *et al.* (1998), who found lower values than 0.3 log CFU/cm² when used 1.5% acetic acid to the contaminated carcasses. Different results regarding acetic acid solutions were reported by Gorman *et al.* (1995), who reported an *E. coli* reduction of 2.0 log cm² when using acetic acid 2%. Similarly, Algino *et al.* (2007) reported a reduction in *E. coli* of 0.71 log CFU/cm² and 0.81 CFU/cm² after application of 2.5% acetic acid.

The decontamination effect of organic acid solutions on *Listeria monocytogenes*

The results regarding *L. monocytogenes* showed a decrease of initial microbial load after application of 1% organic acid solutions, as follows: in case of acetic acid, the level of microorganisms decreased to 5.56 ± 0.11 log CFU/g (1.22 log reduction), to 5.38 ± 0.06 log CFU/g (1.40 log reduction) for lactic acid and 5.66 ± 0.08 log CFU/g for citric acid, with a decrease of 1.12 log (Figure 3). A more pronounced decrease in microbial load was recorded in case of using of 2% organic acids solutions, since from the initial load of 6.78 log CFU/g, it reached 5.31 ± 0.18 log CFU/g using acetic acid (reduction of 1.47 log), 4.32 ± 0.14 log CFU/g for lactic acid (reduction of 2.46) and 5.06 ± 0.16 log CFU/g in case of citric acid (reduction of 1.72) (Figure 3). The greatest reduction of microbial load was recorded in the use after application of 3% lactic acid solution, which determined a complete inactivation of *Listeria monocytogenes*. In the case of acetic acid the load of *Listeria monocytogenes* was reduced to 4.58 ± 0.06 log CFU/g (3.20 log reduction) and 4.33 ± 0.30 log CFU/g for citric acid (3.45 log reduction) (Figure 3).

Statistical analysis found significant differences regarding the effect of acetic acid in comparison with lactic acid solutions in regard with the total load of *Listeria monocytogenes* compared to the control sample for the three concentrations used ($p < 0.05$). From the comparative analysis of the efficacy of lactic acid solutions (1%, 2%, 3%) compared to those treated with acetic acid

(1%, 2%, 3%), significant differences were found only in case of 2% , respectively 3% organic acid solution ($p < 0.05$).

CONCLUSION

Based on our results we conclude that the pathogens have a different sensitivity to the action of the decontaminating acid solutions, their sensitivity in ascending order being the following: *Escherichia*, *Salmonella* and *Listeria*, 3% lactic acid solution caused total inhibition of *Listeria monocytogenes*; the effectiveness of solutions of organic acids in descending order was: lactic acid, acetic acid and citric acid. These methods of decontamination should be considered as complementary measures to increase the safety of meat, in addition to GMP and GHP.

REFERENCES

1. Algino RJ, Ingham SC, Zhu J (2007). Survey of antimicrobial effects of beef carcass intervention treatments in very small state-inspected slaughter plants. *Journal of Food Science*, 72: 173-179.
2. Bacon RT (2000). Comparison of three commercial beef carcass decontamination systems. The Department of Animal Sciences Colorado State University, 2000. Research Report.
3. Carpenter CE, Smith JV, Broadbent JR (2011). Efficacy of washing meat surfaces with 2% levulinic, acetic, or lactic acid for pathogen decontamination and residual growth inhibition. *Meat Science*, 88: 256-260.
4. Castillo A, Lucia LM, Roberson DB, Stevenson TH, Mercado I, Acuff JR (2001). Lactic acid sprays reduce bacterial pathogens on cold beef carcass surfaces and in subsequently produced ground beef, *J Food Prot.*, 64(1): 58-62.
5. Castillo A, Lucia LM, Goodson KJ, Savell JW, GR (1999). Decontamination of beef carcass surface tissue by steam vacuuming alone and combined with hot water and lactic acid sprays. *Journal of Food Protection*, 61: 823-828.
6. Delazari I, Iaria ST, Riemannm HT, Cliver DO, Mori T (1998). Decontaminating beef for *Escherichia coli* O157:H7. *Journal of Food Protection*, 61: 547-550.
7. Dorsa WJ, Cutter CN, Siragusa GR (1998). Long-term effect of alkaline, organic acid or hot water washes on the microbial profile of refrigerated beef contaminated with bacterial pathogens after washing. *Journal of Food Protection*, 61: 300-306.
8. Gorman BM, Sofos JS, Morgan JB, Schmidt GR, Smith GC (1995). Evaluation of hand-trimming, various sanitizing agents, and hot water spray washing as decontamination interventions for beef brisket adipose tissue. *Journal of Food Protection*, 58: 899-907.
9. Harris K, Miller MF, Loneragan GH, Brashears MM (2006). Validation of the use of organic acids and acidified sodium chlorite to reduce *Escherichia coli* O157 and *Salmonella*

- typhimurium in beef trim and ground beef in a simulated processing environment. J. Food Prot., 69:1802-1807.
10. Loretz M, Stephan R, Zweifel C (2008). Antibacterial activity of decontamination treatments for cattle hides and beef carcasses. Meat Science, 88: 256-260.
 11. Ransom JR, Belk KE, Sofos JN, Stopforth JD, Scanga JA, Smith GC (2003). Comparison of intervention technologies for reducing *Escherichia coli* O157:H7 on beef cuts and trimmings. Food Protection Trends, 23: 24-34.
 12. Smulders FJ, Greer GC (1998). Integrating microbial decontamination with organic acids in HACCP programs for muscle foods: prospects and controversies. International Journal of Food Microbiology, 44: 149-169.
 13. Sofos JN (2008). Challenges to meat safety in the 21st century. Meat Science, 78: 3-13.
 14. *** Reg. (EU) 101/2013 of 4 February 2013 concerning the use of lactic acid to reduce microbiological surface contamination on bovine carcasses.
 15. *** SR EN ISO 6579/2003, SR EN ISO 6579 AC/2009, Metoda orizontală pentru detectarea bacteriilor din genul *Salmonella*.
 16. *** SR EN ISO 11290-1/2000, SR EN ISO 11290-1/A1 2005, Metoda orizontală pentru detectarea și numărarea *Listeria monocytogenes*.
 17. *** SR EN ISO 16649-2/2007, Metoda orizontală pentru numărarea *Escherichia coli* pozitivă la β -glucuronidază.