THE EFFECTS OF LACTIC AND ACETIC ACID TREATMENT ON THE PSYCHROTROPHIC GERM GROWTH FROM THE SURFACE OF BEEF AND PORK

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Abstract. The research material was represented by 11 meat samples: 4 pork and 7 beef, collected in November 2005 – May 2006 period, from a slaughterhouse in Cluj County.

The aim of the study was to test the treatment effect of lactic and acetic acids on the reduction of psychrotrophic germ load from the surface of beef and pork.

From the obtained data, it had been established that on the surface of the carcasses the psychrotrophe count differed, with values situated between $9.0 \times 10^{-5}$ and $1.0 \times 10^{-9}$ ufc/cm$^2$ in the case of beef, and $1.48 \times 10^{-5}$ and $1.68 \times 10^{-6}$ ufc/cm$^2$ in the case of pork. After applying the acetic and lactic acids solutions, of 3% and 5% concentration, on the surface of the bovine carcasses, the total germ number was situated between $1.1 \times 10^{-5}$ and $2.48 \times 10^{-8}$ ufc/cm$^2$ and for the pig carcasses between $5.18 \times 10^{-3}$ and $1.62 \times 10^{-7}$ ufc/cm$^2$.

Following the treatment with organic acids, a reduction of total germ number on the meat surface is observed, of up to 109 times compared to control sample, in the case of beef and up to 147 times for the pork carcasses.

INTRODUCTION

The efficiency of using a wide range of antimicrobial treatments to reduce the prevalence of alteration and the pathogenic once, from the cattle and pig carcasses was intensely studied and documented. In the last 25 years several techniques were tested in the way of microbial contamination reduction in live animals as well as on carcasses, immediately after the slaughter and final washing. The most efficient and practical methods of limited surface hygienisation in the case of evident contamination were proved to be – from the technical point of view, the application of diluted organic acids or hot water on the carcass surfaces, the exposure to steam pressure (steam pasteurization) and the use of steam and hot water in combination with vacuum or modified atmosphere packaging.

The use of acidulation agents of the carcasses before processing was proved to reduce, but entirely eliminate the germs from the carcass surface. Kotula and Rough, cited by M.R. Strivarius et al. (2002), suggested the fact that every time the carcasses are processed in smaller pieces, the germs on the surface are inoculated on the newly created surfaces as following the cutting.

M.R. Strivarius et al. confirm the efficiency of acetic acid on *E. coli* 0157:H7 and *S. typhymurium*, who uses a 2 % acetic acid solution treatment that reduces with over 2,5 log CFU/g the number of microorganisms on a period of 7 days. These results were confirmed also by the results obtained by Bell et al. (1984) and Kotula Thelapurate (1994) who observed a similar reduction of aerobic bacteria from the muscle surface after acetic acid application.

Van der Marrel et al (1998) studied the effect of broiler carcasses imersation in different solutions of 1 – 2 % acetic acid (pH=2.2 at 19°C) for 15 seconds at different stages of the
technological process with the purpose of determining the inhibition of bacterial development from the psychrophilic members *Enterobacteriaceae* family and *Staphylococcus aureus*. They immediately after the treatment observed that colonization per skin gram, in generally, was reduced by 1 log and pH with values between 3.2 and 4. The treatment with 2 % lactic acid stopped the post-decontamination bacterial development, more efficiently than the 1 % concentration solution, the effect being more obvious if it was followed by immediate carcass freezing.

As following of the presented aspects, in our research we tried to appreciate the microbial psychrotrophe load and configuration after the application of 3 and 5 % lactic and acetic acid solutions for the beef carcasses, processed in a slaughterhouse from Cluj County.

**MATERIAL AND METHOD**

The studied material was represented by 8 beef samples and 5 pork samples, collected between November 2005 – May 2006, from a slaughterhouse from Cluj County. The samples were collected from the surface of refrigerated carcasses at 24 – 48 hours, from the chilling spaces of the abattoir in accordance with the methodical norms recommended by Veterinary National Agency and Food Safety. From the surface there were collected slices of superficial muscle tissue of a thickness of 2 – 3mm, square shaped with the side of 10 cm (100 cm$^2$ each), collected from different anatomic regions: the internal part of the thy, the chest and the flank.

The samples obtained from bovine and pork carcasses were treated with solutions of acetic and lactic acid, through surface aspersion of the meat pieces (2,5 – 3 ml/100 cm$^2$). Each collected sample was portioned in other 3 samples, from which 2 were treated with organic acids and one was the control sample, to compare the results regarding the germ number of the organic acid treated samples. The samples were cut small (200 cm$^2$) with scissors and homogenized with 200 ml sterile 0,9% NaCl solution, for 5 minutes, with the mechanic homogenizer, obtaining the base solution (10$^{-1}$) in which 1 ml liquid represents 1 cm$^2$ from the controlled surface, than successive dilutions were obtained: 10$^{-2}$, 10$^{-3}$, 10$^{-4}$, 10$^{-5}$, 10$^{-6}$, 10$^{-7}$ (when it was considered necessary).

Identifying the psychrotrophic bacteria was made on a basis of morphological confirmation tests (colony aspect, Gram stained smears, the 3% KOH test to differentiate the Gram negative from Gram positive bacteria) and biochemical confirmation tests using API 20NE and 20E commercial kits. The obtained data was systematized and graphically expressed, average values being established, which were compared with the literature. The microbial load was estimated as log mean value.

**RESULTS AND DISSCUSSION**

Regarding the psychrotrope counts, we observed in beef an average decrease with 1,41 log$_{10}$ CFU/cm$^2$ in the case of 3 % acetic acid, and 1,59 log$_{10}$ CFU/cm$^2$ in the case of 3 % lactic acid. In the case of 5% concentration solutions we revealed a decrease in the germ number of average 0,87 log$_{10}$/cm$^2$ CFU, in the case of acetic acid and 1,04 log$_{10}$ CFU/cm$^2$ in that of lactic acid, as it can be observed in graphic 1. In all 8 samples taken into study, the microbial load of the control sample (24 hours after acid application), was higher than 10$^6$ CFU/cm$^2$, but lactic acid decreased the level of bacterial contamination to acceptable limits in 4 of the samples, (50 % of the sample number), while lactic acid determined a decrease of germ number to acceptable limits in only 2 of the samples (25 % of the total sample number).
Analyzing this graphic it can be observed that 3 and 5% lactic acid solutions has a more obvious effect on the number of psychrotrophic bacteria, but also – surprisingly, the 3% concentration acids being more efficient. Similar studies conducted by Woolthuis and Smulders (1985), to test the efficacy of some lactic acid solutions of different concentrations (from 0.75 to 2.5%) to reduce the microbial load of beef carcasses, revealed that using a 1.25% concentration lactic acid solution was more efficient in decreasing the psychrotrophic count, that of $1.0 \log_{10} \text{CFU/cm}^2$.

A possible explanation for these results could be that the germ number of these samples tested with 5% concentration organic acids may be much higher. Also there could be discussed some biochemical reactions at bacterial membrane level, with some precipitation reactions in the superficial areas, fact that couldn’t permit the penetration of concentrated acid solutions inside the microbial cell, keeping its viability.

Strivarius M.R. (2001, 2002), in the studies made to test the efficacy of some methods of reducing the microbial load, revealed that, for the 5% lactic acid solution, a decrease of $0.64 \log_{10} \text{CFU/cm}^2$, and in the case of 5% acetic acid solution a decrease of $1.25 \log_{10} \text{CFU/cm}^2$.

For the germs from *Aeromonas* genus, we obtained an average decrease in their number of $0.8 \log_{10} \text{CFU/cm}^2$ in the case of 3% acetic acid solution and no effect in that of 3% lactic acid solution.
% lactic acid, compared to the control sample. In the case of 5% acetic acid, we observed an average decrease of $2,45 \log_{10} \text{CFU/cm}^2$ and $1,02 \log_{10} \text{CFU/cm}^2$ in that of 5% lactic acid. For samples 2, 3 and 7 there weren’t any Aeromonas genus bacteria isolated (fig. 2).

From this data, it can be deduced that germs from Aeromonas genus are more sensitive to acetic acid that to lactic acid.

For bacteria from Pseudomonas genus we revealed an average decrease of their number of $1,52 \log_{10} /\text{cm}^2$ CFU in the case of 3% acetic acid and $0,82 \log_{10} \text{CFU/cm}^2$ in that of 3% lactic acid. As in the case of Aeromonas genus bacteria, an increased sensitivity of bacteria from Pseudomonas genus to 3% acetic acid can be observed. After applying 5% concentration acid solutions, a decrease of the bacterial average number was observed, of $0,89 \log_{10} \text{CFU/cm}^2$ in the case of acetic acid and $1,03 \log_{10} \text{CFU/cm}^2$ in that of lactic acid, aspects that can be noticed from fig. 3, but as we can see, in the case of 5% concentration solutions, lactic acid is more efficient.

For bacteria from Yersinia genus we revealed an average decrease of their number of $5.16 \log_{10} /\text{cm}^2$ CFU in the case of 3% acetic acid and $3.18 \log_{10} \text{CFU/cm}^2$ in that of 3% lactic acid. As in the case of Aeromonas genus bacteria, an increased sensitivity of bacteria from Yersinia genus to 3% acetic acid can be observed. After applying 5% concentration acid solutions, a decrease of the bacterial average number was observed, of $4.53 \log_{10} \text{CFU/cm}^2$ in the case of acetic acid and $4.14 \log_{10} \text{CFU/cm}^2$ in that of lactic acid, aspects that can be noticed from fig. 4, but as we can see, in the case of 5% concentration solutions, lactic acid is more efficient.
In the case of *Yersinia spp.*, we observed a decrease with an average of $1.42 \log_{10} \text{CFU/cm}^2$ for 3% acetic acid, and $1.35 \log_{10} \text{CFU/cm}^2$ for 3% lactic acid. From the 5% acetic acid, a decrease of $1.44 \log_{10} \text{CFU/cm}^2$ was observed, and for the 5% lactic acid, a decrease of $1.07 \log_{10} \text{CFU/cm}^2$. From this data it can be deduced that *Yersinia spp* is more sensitive to acetic acid compared to the lactic one. From samples 3, 4, 5, 6, 7 there weren’t any *Yersinia spp* bacteria isolated. The efficiency of organic acid solutions on the germ development and growth from *Enterobacteriaceae* family, in the case of collected samples was studied for the 5% concentration, only (fig. 5). In this case, a decrease of average $1.53 \log_{10} \text{CFU/cm}^2$ was observed for the acetic acid and $1.28 \log_{10} \text{CFU/cm}^2$ for the lactic acid. It can be noticed that acetic acid is more efficient than lactic one.

**Graphic 5** The effect of acid solutions treatments to development of germs from *Enterobacteriaceae* family at the surface of beef

From the presented data by Bell M.F. et al. (1986) who studied the antimicrobial effect of acetic and formic acids, observed that 1.2% concentration acetic acid reduced with 65% the *Yersinia enterocolitica* number and *Pseudomonas aeruginosa*, the number of *Enterobacteriaceae* family germs decreased with 46%. Woolthuis and Smulders (1985), using different concentration solutions of lactic acid (from 0.75% to 2.5%) observed that 1.25% lactic acid reduced the number of *Enterobacteriaceae* with $1.0 \log_{10} \text{CFU/cm}^2$.

**CONCLUSIONS**

Our results, obtained in the study regarding the antimicrobial effect of acetic acid and lactic acid solutions, made in November 2005 – May 2006 period, permit us to conclude:

1. In the case of beef, a decrease of psychrotrophe count with $0.8 \log_{10}/\text{cm}^2 \text{ CFU} – 2.45 \log_{10} \text{CFU/cm}^2$ was revealed, after the application of acetic and lactic acids solutions (3 – 5%);
2. Lactic acid is more efficient in reducing the aerobic plate count, compared to acetic acid, which is more efficient in decreasing the germs from *Aeromonas, Yersinia* genus and *Enterobacteriaceae* family; for the germs belonging to *Pseudomonas* genus, acetic and lactic acids had a similar effect;
3. From our study, we can conclude that 3% lactic and acetic acid solutions are more efficient than the 5% ones, probably because of the action that higher concentration acid solutions have on the bacterial cell wall, precipitating the surface areas but keeping the viability of the bacterial cell.

Based on our results, we recommend the spraying of 3 – 5% organic acid solutions to the surface of beef carcasses after their final washing, in the purpose of germ load reduction.
The findings suggest that objective assessment of the microbiological effects on carcasses of beef carcass dressing processes will be required to ensure that Hazard Analysis: Critical Control Point and Quality Management Systems are operated to control the microbiological condition of carcasses.

BIBLIOGRAPHY