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EFFECT OF LACTIC ACID AND ASCORBIC ACID ON SURVIVAL OF *LISTERIA MONOCYTOGENES* IN THE RAW BEEF STORED UNDER REFRIGERATION

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ABSTRACT

The purpose of the experiment was the estimation of *L. monocytogenes* survival in the raw beef samples stored under the refrigeration. We also tried to verify the potential inhibitory effect against the test bacteria of the separately added organic acids (10% lactic acid or 10% ascorbic acid) or their mixture in equal proportions, applied on the meat with the surface spraying technique. The treatment with the organic acids did not affected the pH of the meat, which was similar as in the control samples. The water activity of the chilled beef was not significantly altered and amounted about 0,98 in course of the entire experiment. The acid application significantly decreased the survival of *L. monocytogenes* (by 1.2 and 3.7 log on total for lactic acid and the mixture of the acids, respectively). The reduction was the most efficient after the application of the organic acid mixture, when it was more than twice faster than in case of the addition of ascorbic or lactic acid alone. The rate of *L. monocytogenes* reduction in the control samples was nearly 5 times lower than after the acid mixture application and 2.5 times slower than in case of the separately added lactic or ascorbic acid. The acid mixture application reflected also in the reduction of the *Enterobacteriaceae* as well as in the inhibition of the aerobe growth in the meat samples. Either the separate application of 10% lactic acid or 10% ascorbic acid, as well as the addition of the acid mixture did not affected the number of the lactic acid bacteria in the raw beef.

Key words: *Listeria monocytogenes*, raw beef, lactic acid, ascorbic acid

INTRODUCTION

Listeria monocytogenes is relatively frequently isolated from the stocks and food products of animal origin [4, 5, 7, 12, 14]. From 8.7% to 64.3% of the raw beef is contaminated with the bacteria [1], mainly due to its environmental prevalence. *Listeria monocytogenes* is commonly observed either in the water, sewage and bottom sediments, or in the soil. It is frequently present in the plants, particularly the rotten or stagnant ones. Consequently, the environment, and especially the fodder plants or ensilages, constitute the reservoir of *L. monocytogenes* for farming animals [15]. Healthy animals carry the bacteria on the skin, hoofs or mucosal membranes.

The occurrence of *L. monocytogenes* on the digestive tract mucosal membranes in the slaughter animals results in primary contamination of meat with the bacteria. The technological process, and particularly the accidental gut rupture in course of post-slaughter treatment, and subsequent contamination of the carcass with the digestive tract contents are responsible for meat colonization. The inappropriate procedures of food production as well as the cross-contact of carcasses and meat elements are the main reasons for secondary contamination with *L. monocytogenes* [2].

Following factors promote the growth of *L. monocytogenes* in the food products: 1) elimination of the microflora responsible for food decay but simultaneously antagonistic against the listeria, 2) product storage under the vacuum or refrigeration, especially for the prolonged time [8, 10].

Listeria monocytogenes infections in human occur mainly through the alimentary tract (food-borne listeriosis) [3, 6, 9, 11, 16]. Besides the state of the gut mucosa, the number of listeria in the food is crucial for the infection incidence. The number of 10^3 - 10^4 CFU \times g⁻¹ *L. monocytogenes* in the food is hazardous for the consumer health, whereas the level of 10^5 - 10^7 CFU \times g⁻¹ is connected with very high probability of breaking the intestinal barrier. Consequently, the decrease of *L. monocytogenes* number in the food to the safe level is the crucial objective from the point of view of food hygiene and consumer health.

The purpose of this experiment was the estimation of *L. monocytogenes* survival in the refrigerated raw beef samples, treated with the ascorbic and lactic acid alone or with their mixture, applied on the meat with the surface spraying technique.

MATERIAL AND METHODS

The experiment was performed on the omotransversarius muscle (*m. omotransversarius*) taken from the beef carcasses directly after the slaughter. The pieces of 250 g each were sent to the laboratory within the 6 hours post slaughter and subsequently divided into 4 samples (I, II, III, IV). The samples were inoculated with PCM 2191 strain of *L. monocytogenes* provided by the Institute of Immunology and Therapy, Polish Academy of Sciences, Wrocław. One hour after the inoculation, by means of surface spraying, the samples I, II and III were treated with: I) 10% lactic acid solution, II) 10% ascorbic acid solution, III) the mixture of 10% lactic acid and 10% ascorbic acid in the equal proportion. Sample IV was left as the control.

The experiment was carried out on total number of 140 samples. The samples were stored in at $4 \pm 1^\circ\text{C}$ for 14 days, with the analyses performed on days: 0, 1, 7 and 14. The estimations considered pH and water activity of the meat, the numbers of *L. monocytogenes*, *Enterobacteriaceae* and lactic acid bacteria and the total plate count.

The pH values were measured with the aid of V 628 pH-meter, type N 517, whereas the water activity was controlled with RTD-33 TH-1-NOVASINA avumeter. The number of *L. monocytogenes* cells was determined on the PLS (Palcam Selective Agar, Merck) solid medium after 72 hour incubation at 30°C. The total plate count was measured according to Polish Standard PN-93A-86034/04 [13]. Number of lactic acid bacteria was determined on MRS medium (Oxoid), whereas *Enterobacteriaceae* count - on VRBG agar medium.

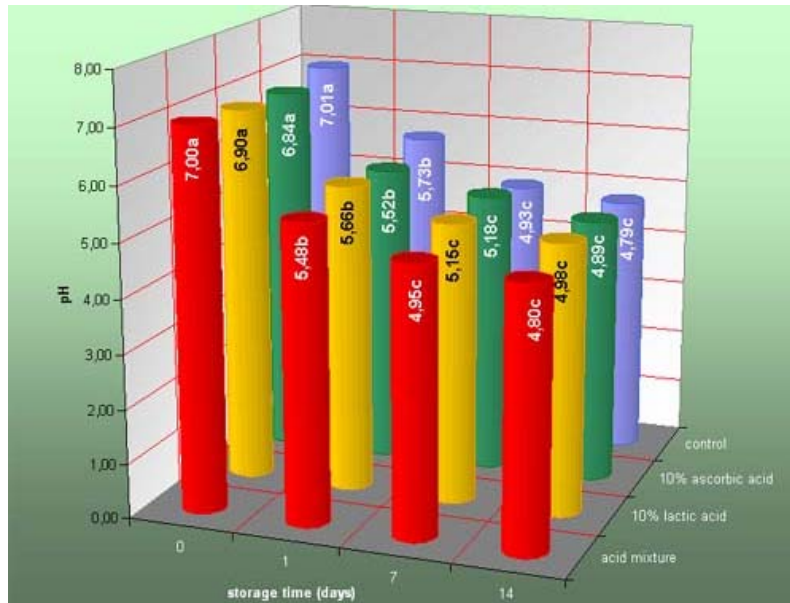
The bacterial counts were transformed to logarithms and statistical calculations were carried out using Microsoft® Excel 2000 and Statistica 5, Version 97 software. Mean values were compared with the aid of Student's test. The T-4D values, time required for reduction of *L. monocytogenes* by 4 log units, were calculated from the regression analysis.

Graphic presentation of the data was done with the aid of Microsoft® Excel 2000 software.

RESULTS AND DISCUSSION

The decrease of the pH values was revealed in all the raw beef samples, but it was statistically significant only between 0 and 24th hour and between 1st and 7th day of storage under the refrigeration. The organic acid addition did not affected the pH of the meat, which was similar as in the control samples (Fig. 1).

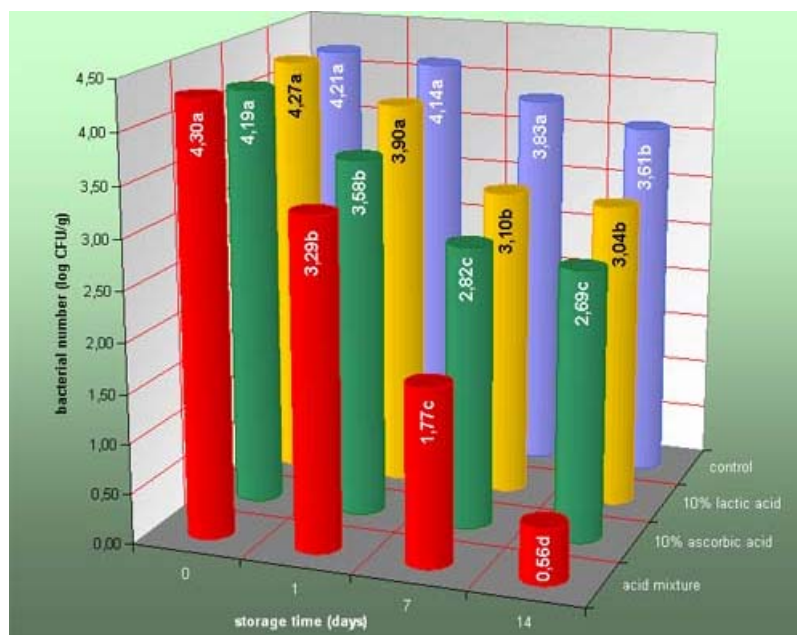
Fig. 1. Effect of the organic acid addition on the pH of the raw beef stored under the refrigeration (a, b, c - statistically significant differences, $P < 0.01$)



The water activity of the samples was not significantly altered during the storage and amounted about 0.98.

The number of *L. monocytogenes* cells in the control samples constantly decreased with time (by 0.6 log on total), but the reduction was statistically significant only between 7th and 14th day of the storage. The organic acid addition significantly reduced the survival of *L. monocytogenes* (by 1.2 and 3.7 log on total for lactic acid and the mixture of the acids, respectively). The inhibition of *L. monocytogenes* was uniform due to the mixture of acids (statistically important decrease in all the periods studied), whereas for the ascorbic acid the significant bacterial reduction was revealed between 0 and 24th hour and between 1st and 7th day, and for lactic acid – only between 1st and 7th day of sample storage (Fig. 2).

Fig. 2. Effect of the organic acid addition on *L. monocytogenes* number in the raw beef stored under the refrigeration (a, b, c, d - statistically significant differences, $P < 0.01$)



The T-4D values, figured out from the regression analysis, indicated the most efficient reduction of *L. monocytogenes* after the application of the organic acid mixture. The subsequent listerial inhibition was more than twice faster than in case of the addition of ascorbic or lactic acid alone. The rate of *L. monocytogenes* reduction in the control samples was nearly 5 times lower than after the acid mixture application and 2.5 times slower than in case of the separately added lactic or ascorbic acid (Table 1).

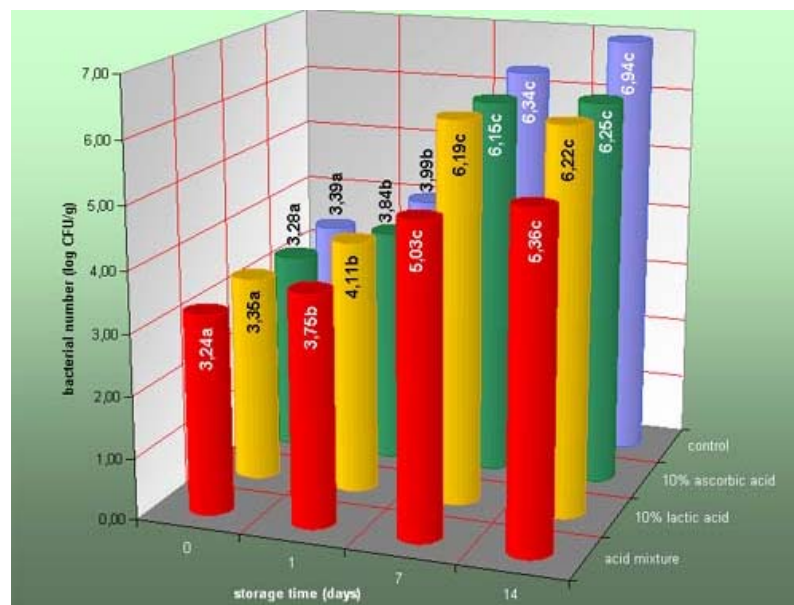
Table 1. T-4D (the time required for the bacterial reduction by 4 log units) for *L. monocytogenes* in the raw beef stored at 4°C and treated with the lactic acid and ascorbic acid alone or with the mixture of both the acids

	mean	confidence range (95%)
10% lactic acid	30.0	26.6-33.4
10% ascorbic acid	31.1	28.7-33.5
10% lactic acid + 10% ascorbic acid	13.1	12.3-13.9

It is very likely that as high efficiency of the organic acids against *L. monocytogenes* is not directly dependent on the pH decrease, since the pH of the acid-treated meat was not significantly different from the one in the control samples. Moreover, according to the available literature, acid adaptation of *L. monocytogenes* is relatively quick [5, 15, 17].

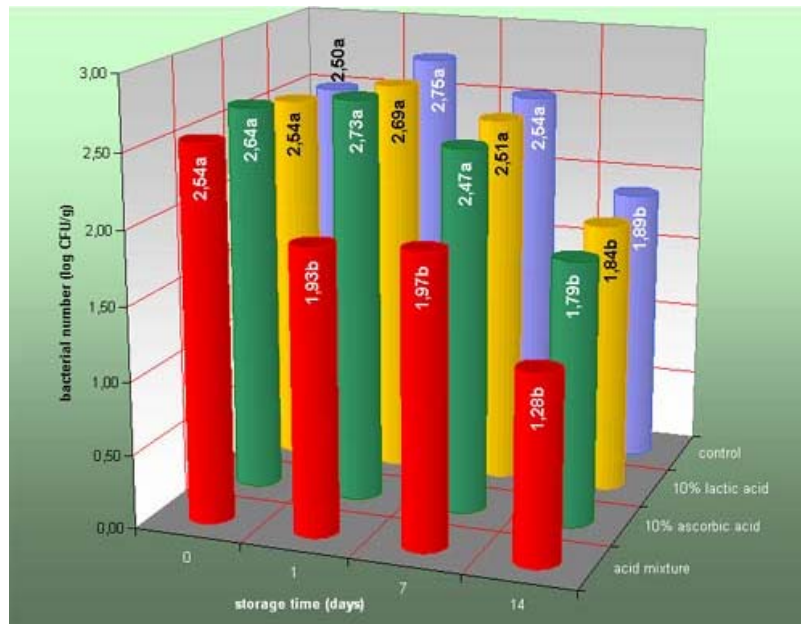
The total plate count in the control samples increased with time, but the differences were statistically important only between 0 and 24th hour and between 1st and 7th day of the storage. The similar rates were observed after the separate lactic or ascorbic acid application and the resulting bacterial counts were only insignificantly lower than in the untreated meat. The aerobic growth was inhibited by the acid mixture. After 7 and 14 days of the storage, the subsequent total plate counts were 1 log and 1.5 log lower than in the control samples, respectively. In course of the experiment, the total number of aerobic bacteria increased by 3.6 log for the control and ascorbic acid samples, by 2.9 log for lactic acid, and only by 2 log for the acid mixture, comparing with the initial levels (Fig. 3).

Fig. 3. Effect of the organic acid addition on the total plate count in the raw beef stored under the refrigeration (a, b, c - statistically significant differences, P < 0.01)



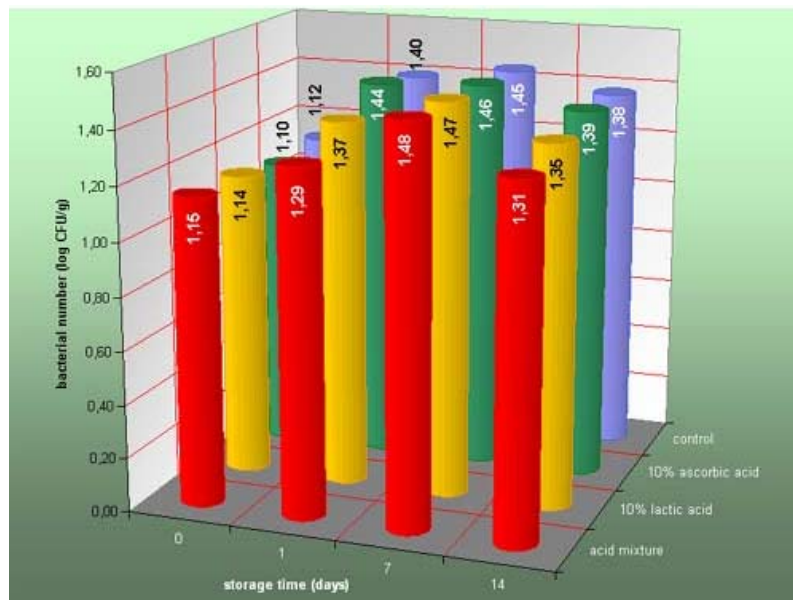
The statistically insignificant increase of *Enterobacteriaceae* count was noted after 24 hours of the control sample storage. Subsequently, the number of the bacteria was on the decrease, with the statistically significant differences revealed only between 7th and 14th day of the experiment. The similar trends were observed due to the lactic or ascorbic acid application and the number of the bacteria was only insignificantly lower than in the untreated meat. The reduction of *Enterobacteriaceae* due to the acid mixture was statistically significant only between 0 and 24th hour of the storage, and by 14th day of the experiment the count was 1.26 log lower than the initial level. Comparatively, the total decrease of *Enterobacteriaceae* in ascorbic acid, lactic acid and control samples amounted 0.85; 0.7 and 0.6 log, respectively (Fig. 4).

Fig. 4. Effect of the organic acid addition on the *Enterobacteriaceae* count in the raw beef stored under the refrigeration (a, b - statistically significant differences, $P < 0.01$)



The number of lactic acid bacteria remained at the similar level during the sample storage, irrespective of the separate organic acid or their mixture addition (Fig. 5). The survival of lactic acid producing bacteria is very likely the reason for the reduction of *L. monocytogenes* in the raw beef, since there is the evidence of their antilisterial activity [8, 10].

Fig. 5. Effect of the organic acid addition on the lactic acid bacteria number in the raw beef stored under the refrigeration (differences statistically insignificant, $P < 0.01$)



CONCLUSIONS

1. The mixture of 10% lactic acid and 10% ascorbic acid in equal proportions is more efficient than the separate acid for the reduction of *Listeria monocytogenes* in the raw beef stored under the refrigeration.
2. The treatment with the acid mixture results in the reduction of *Enterobacteriaceae* count, as well as in the inhibition of the aerobe growth in the meat samples.
3. Either the application of 10% lactic acid and 10% ascorbic acid alone, or the addition of the acid mixture do not affect the number of lactic acid bacteria in the chilled raw beef.
4. The surface application of the organic acids seems to be the efficient technique of raw meat protection, preventing either the bacterial decay, or the transmission of food-borne listeriosis.

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