

Inhibitory Effects of Different Decontamination Agents on the Levels of *Listeria monocytogenes* in the Experimentally Inoculated Raw Beef Samples in the Laboratory Conditions

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Summary

This study aimed at comparing the inhibitory effects of various decontamination agents (1% lactic acid, 2% lactic acid, 2% acetic acid, 0.1% acidified sodium chloride, 0.1% sodium acetate, and 0.1% cetylpridinium chlorine) on experimentally contaminated raw beef samples with *Listeria monocytogenes* being a significant pathogen microorganism for public health. The highest level of bacterial inhibition was determined in the meat samples treated with 2% lactic acid while the lowest level was in the samples treated with 0.1% acidified sodium chloride. It is very important to specify that initial bacterial load of carcasses, the decontamination technique applied, and the characteristics of acids used (dissociated or undissociated, pH, amount, percentage, application temperature, and different combinations) are essential parameters to be taken into account in the process of reducing microorganism on raw meat samples.

Keywords: *Listeria monocytogenes*, Decontamination, Raw meat, Cetylpridinium chlorine, Acetic acid, Acidified sodium chloride, Sodium acetate, lactic acid

Laboratuvar Koşullarında Çiğ Sığır Etlerine Deneysel Olarak Inoküle Edilen Farklı Dekontaminasyon Ajanlarının *Listeria monocytogenes* seviyesi Üzerine Baskılayıcı Etkisi

Özet

Bu çalışmada, laboratuvar koşullarında, halk sağlığı bakımından önemli bir patojen olan *L. monocytogenes* ile deneysel olarak inoküle edilmiş çiğ sığır etlerinde farklı dekontaminasyon ajanlarının (%1 laktik asit, %2 laktik asit, %2 asetik asit ve %0.1 asitlendirilmiş sodyum klorür, %0.1 sodyum asetat ve %0.1 cetylpridinium chlorine) baskılayıcı etkisinin karşılaştırılması amaçlanmıştır. En yüksek bakteriyel inhibisyon oranı, %2 laktik asit uygulanmış et örneklerinde belirlenmiştir, en düşük oran ise %0.1 asitlendirilmiş sodyum klorür uygulanmış örneklerde gözlemlenmiştir. Çiğ etlerdeki bakteriyel yükü azaltma sürecinde karkasların ilk bakteriyel yükü, uygulanan dekontaminasyon tekniği ve kullanılan asitlerin karakteristik özellikleri (dissosiyasyon yada dissosiyasyon olmaması, pH, miktarı, oranı, uygulama sıcaklığı ve kullanılan farklı kombinasyonlar) gibi gözönüne alınması gereken temel parametrelere vurgu yapmak önem arz etmektedir.

Anahtar sözcükler: *Listeria monocytogenes*, Dekontaminasyon, Çiğ et, Cetylpridinium klorit, Asetik Asit, Asitlendirilmiş Sodyum Klorit, Sodyum Asetat, Laktik Asit



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INTRODUCTION

Listeria monocytogenes is one of the most important pathogens in terms of public health all over the world. It poses an important risk for human with several food including fresh meat and meat products. It is the fact that, it can grow at +4°C and keep vital activation in aerobic conditions as a microaerophilic bacterium ¹.

Contamination of a the cattle carcass mainly starts with the removal of skin after slaughtering. During the slaughtering process; removal of the skin and internal organs, and cooling process of the carcass are the major important critical control points that extremely need to be taken care of. On the other hand, equipments used in the slaughtering process (particularly knives), physical condition of the slaughter house, and personal hygiene are significant parameters that affect the microbial profile of the meat.

In order to reduce microbial contaminations caused by the personal hygiene and improper handling, some chemicals (organic acid, cetylpridinium chlorine, trisodium phosphate, acidified sodium chloride, chlorine, ozone), physical (animal washing, trimming, dehairing, steam pasteurization, hot water), and microbial (bacteriocin) applications are need to be applied to the carcasses, meat and meat products ².

Combined 'multi-hurdle' techniques including several applications such as water, wash + hot water ³, steam vacuuming + hot water + lactic acid or steam vacuuming + lactic acid + hot water ⁴, hot water + lactic acid ⁵⁻⁷, hot water + acidified sodium chloride, hot water + cetylpridinium chlorine, acidified sodium chloride + hot water, cetylpridinium chlorine + hot water ⁸, and lactic acid + cetylpridinium chlorine along with physical and chemical techniques are used in decontamination purpose of various animal carcasses ⁹.

Cetylpridinium chlorine (CPC) is generally recognized as safe (GRAS), and certain chemical components including lactic acid, acidified sodium chloride, sodium acetate, and acetic acid, are suggested by the Food Safety and Inspection Services ¹⁰, United States Department of Agriculture, and frequently applied in the decontamination processes in the food marked.

The antimicrobial activity of acidified sodium chloride is due to the oxidative effects of chlorous acid originated from the conversion of chlorite ion into acid form under acidic conditions. It is very essential that the antibacterial solution should be prepared shortly before spraying as the reactions occur immediately after mixing the sodium chloride with an acid ².

Interaction of basic cetylpyridinium ions with acidic molecules results in antimicrobial activity on bacteria. This reaction then inhibits bacterial metabolism through constructing weak ionic compounds interfering with

bacterial respiration ². Cetylpridinium chlorine also prevents bacterial attachment, and potentially reducing the cross-contamination risk. Treatment of the poultry products with cetylpridinium chlorine has no effect on the physical appearance ¹¹. Pohlman et al.¹² have indicated that use of cetylpridinium chlorine should not only enhance the microbial safety of ground beef, but also prolong the colour stability of shelf-life.

The antibacterial action of organic acids including acetic, tartaric, lactic, and citric acids is due to the effect of low pH and the degree of dissociation of the acid. Since undissociated organic acids are more readily soluble in the bacterial cell membrane, they have more bactericidal effects in the dissociated form. It is also known that the concentration of the acid, temperature of the solution, the method used and time of application influence antimicrobial activity of the acids ¹³⁻¹⁵.

There is no routine usage of organic acid or chemical solution for the carcass decontamination in slaughterhouses in Turkey yet, a few experimental researches have been conducted so far. Several countries in Europe and the United States of America (USA) allowed to the usage of certain chemical solutions for decontamination purpose in red meat supply chains. Carcasses are applied by either chemicals or organic acids at chilling processing stage in the USA while it is forbidden in Europe. Likewise, application of acidified salted water to the carcass after the removal of the skin is permitted in the USA, it is not allowed in the European Union. Similarly, different applications such as hot water and steam pasteurization are applied to the carcass, On the other hand, oil extracts and carbon dioxide applications are preferred at packaging and retail processing stages.

This study aimed to compare the effects of individual decontamination agents including 1% lactic acid, 2% lactic acid, 2% acetic acid, 0.1% acidified sodium chloride, 0.1% sodium acetate, and 0.1% cetylpridinium chlorine on *L. monocytogenes* in the raw beef samples were contaminated experimentally.

MATERIAL and METHODS

This study was carried out at the University of Kafkas, Veterinary Medicine Faculty, Food Hygiene and Technology Department in 2009.

Preparation of the Culture of Bacteria

L. monocytogenes was provided from the Culture Collection Unit of Refik Saydam Laboratory (RSKK 472-1/2b) as a lyophilized stock culture and aerobically incubated in 10 ml *Listeria* Enrichment Broth (Oxoid CM862+*Listeria* selective enrichment suppl. Oxoid SR141) at 30°C for 24 h. The level of *L. monocytogenes* after the incubation period was adjusted to approximately 10⁸cfu/ml by using McFarland 0.5 standard method ¹⁶.

Preparation of the Meat Samples

Fresh beef samples (*Musculus longissimus dorsi*) taken after slaughtering were brought to the laboratory under cold chain conditions (4°C) within 60 min and then cut into eight pieces in the size of 6×4×1 cm (about 22-27 g each) to form different groups.

Attachment of Bacteria

As the volume of each meat sample was small, immersion technique was used for the inoculation of each sample with *L. monocytogenes* stock culture. Firstly, 100 ml *L. monocytogenes* stock culture (10⁸cfu/ml) was put into sterilized aluminum foil cup and each beef sample was then immersed into the stock culture for 5 min. After that, each meat sample was placed into another aluminum foil cup and stored at +4°C for 35 min for bacterial attachment (Stage I).

Decontamination Process

Several decontamination solutions including antimicrobial agents were prepared in the individual sterilized aluminum containers at room temperature (18±1°C) and sterilized using 0.20 micro liter filters (Sartorius). In order to determine the changes of *L. monocytogenes* levels during the processing stage and storage period at 4°C, different groups were conducted as; 1% lactic acid, pH 1.95±0.01, (Merck 100366.2500) in container I (Group I); 2% lactic acid, pH 1.82, (Merck 100366.2500) in container II (Group II); 2% acetic acid, pH 2.42±0.01, (Merck 818755.2500) in container III (Group III); 0.1% acidified sodium chloride, pH 2.72±0.01, (Merck 106404.1000) in container IV [Citric acid (Merck 100244.1000) was used in preparing acidified sodium chloride] (Group IV); 0.1% sodium acetate, pH 6.75, (Merck 106268.1000) in container V (Group V); 0.1% cetylpridinium chlorine, pH 6.35, (Merck 1.02340) in container VI (Group VI); distilled water (Control group), pH 6.9, in container VII (Group VII) and finally, a contaminated meat sample (pH 6.8) placed into the container VIII (Group VIII) without any chemical treatment.

After bacterial attachment (Stage I), each meat sample was dipped into the individually different treatment carrying one of the decontamination agent solutions mentioned above for 15 second at room temperature (Stage II). Then, each treated meat sample was placed on sterilized aluminum foil and all left ones at room temperature for 10 min (Stage III). Subsequently, each sample was wrapped up separately with a piece of aluminum foil and stored at +4°C for five days (Stage IV).

Microbiological Analysis

The presence and counts of *L. monocytogenes* were determined in the fresh beef samples at the beginning of decontamination treatments, and after the decontamination procedures on 1st, 3rd, and 5th days of storage at +4°C. Each meat sample was individually put into a sterile polyethylene

bag and then 198-243 ml Listeria Enrichment Broth (Oxoid CM862+Listeria selective enrichment supplement Oxoid SR141) was added. After the samples were rinsed in the bags for 2 min, an amount of 0.05 ml rinse broth was taken from each bag and inoculated onto Palcam Agar media (Oxoid CM877+Palcam selective supplement Oxoid SR150) on plates by using drop plaque culture technique and incubated at 30°C for 48 h. Finally, *Listeria* spp. colonies with typical morphology were counted^{17,18}.

Measurement of Meat pH

In order to measure pH of the fresh raw meat samples, 5 g of each meat sample was homogenized in a stomacher bag with 15 ml of sterile deionized water for 2 min. The pH value of the meat samples was measured with a digital pH meter.

Statistical Analysis

Statistical analyses of the results were performed using a SPSS 11.5 program (one way anova), and considered as statistically significant at the P<0.05 level¹⁹.

RESULTS

No *L. monocytogenes* was isolated in the fresh raw beef sample after initial microbiological analysis, and the mean value of pH for the raw beef sample was measured as 6.8 before the application of any treatments. Even though the stock culture of *L. monocytogenes* was adjusted to around the counts of 10⁸cfu/ml, after dipping the meat samples into stock culture, the attachment level of *L. monocytogenes* was in the range of between 6.37±0.04 and 7,82±0,03 cfu/g. After treatment of raw beef samples with 1% lactic acid, 2% lactic acid, 2% acetic acid, and 1% cetylpridinium chlorine, *L. monocytogenes* was counted approximately at the level of 6.0 log cfu/g. However, it was around 7.0 log in the 0.1% acidified sodium chloride, 0.1% sodium acetate and distilled water groups. The counts of *L. monocytogenes* in the 8th beef sample with no treatment resulted in 7.76 log cfu/ml. As a result, the bacterial reduction level was the highest in the 2% lactic acid group, and lowest in the group treated with 0.1% acidified sodium chloride in this study (Table 1).

DISCUSSION

The counts of *L. monocytogenes* in the 8th meat sample without treatment did not change considerably. At the end of 1st, 3rd and 5th days of storage at +4°C were 7.34 log cfu/ml, 7.34 log cfu/ml, and 7.30 log cfu/ml, respectively. Likewise, in the distilled water and 2% acetic acid groups, no logarithmic changes were detected in the *L. monocytogenes* levels during 5 days of storage at +4°C. In the study of Ikeda et al.²⁰, beef slices (2.5×5×1 cm) from top rounds inoculated with acid-adapted or nonadapted *L. monocytogenes* (4.6 to 5.0 log CFU/cm²) were grouped as either untreated (control) or dipped into 2% lactic acid for 30 s. As a results

Table 1. The reduction effect of different chemical agents on the levels of *L. monocytogenes* in the experimentally inoculated raw beef samples stored at +4°C for five days*

Tablo 1. Farklı kimyasal ajanların deneysel olarak inoküle edilerek +4°C'de beş gün muhafaza edilen çiğ et örneklerinde *L. monocytogenes* seviyeleri üzerine yıkımlayıcı etkisi*

Time Day	Group I 1% Lactic acid	Group II 2% Lactic acid	Group III 2% Acetic acid	Group IV 0.1% Acidified Sodium chloride	Group V 0.1% Sodium acetate	Group VI 0.1% Cetylpridinium chlorine	Group VII Distilled water (control group)	Group VIII (Without any treatment)	F Value
0**	6.79±0.04 ^{Ad}	6.45±0.04 ^{Ae}	6.37±0.04 ^{Be}	7.32±0.09 ^{Bc}	7.82±0.03 ^{Aa}	6.66±0.06 ^{Ad}	7.50±0.08 ^{Ab}	7.76	99.953
1	6.78±0.07 ^{Ac}	5.65±0.08 ^{Be}	6.83±0.02 ^{Ac}	6.81±0.04 ^{Cc}	7.87±0.02 ^{Aa}	5.83±0.02 ^{Cd}	7.46±0.09 ^{Bb}	7.34	193.429
3	5.55±0.03 ^{Ce}	4.85±0.04 ^{Df}	6.38±0.02 ^{Bd}	6.65±0.04 ^{Cc}	6.92±0.02 ^{Bb}	5.53±0.06 ^{De}	7.30±0.00 ^{Ba}	7.34	600.069
5	6.52±0.04 ^{Bc}	5.29±0.04 ^{Ce}	6.52±0.02 ^{Bc}	7.64±0.02 ^{Aa}	6.42±0.08 ^{Cc}	6.30±0.00 ^{Bd}	7.30±0.00 ^{Bb}	7.30	170.516
F Value	172.762	148.007	11.515	71.598	248.836	146.534	3.150		

*Average bacterial log of five repetitions, **Time after bacterial attachment procedure; 1. A,B,C,D: The differences were statistically significant among the groups displayed with different letters at the same column, (P<0.05); 2. a,b,c,d,e,f: The differences were statistically significant among the groups displayed with different letters at the same line, (P<0.05)

of the study, the one treated with lactic acid resulted in immediate pathogen reductions of 1.8 to 2.6 log CFU/cm². After storage at +10°C for 28 days, the count of *L. monocytogenes* on meat remained at low levels (1.6 to 2.8 log CFU/cm²). In the study of Ikeda et al.²⁰, there was no growth of the pathogen on 14 and 21 days in the untreated and acid-treated samples during storage at +4°C, respectively. This difference between our and above study might be the different strains used.

In the 1% lactic acid group, there was no significant changes in the counts of *L. monocytogenes* between the attachment level and 1st day of sample (6.0 log cfu/ml). However, one log decrease was observed on the 3rd day but increased back to 6.0 log cfu/ml on the 5th day. In the 2% lactic acid group, 1.60 and 1.16 log cfu/ml reductions were determined on the 3rd and 5th days of storage at +4°C, respectively. In the study of Ozdemir et al.⁶, muscle tissue samples inoculated with *L. monocytogenes* were immersed in 1% and 2% lactic acid solutions for 15 s and stored at +4°C. In the 1% lactic acid group, 1.12, 1.14, and 2.16 log cfu/ml and in the 2% lactic acid group 1.70, 1.59, and 2.54 log cfu/ml reductions were reported on the 1st, 3rd, and 5th days, respectively. Comparing our results with Ozdemir et al.⁶, the treatments of 1% and 2% lactic acid revealed similar reduction log units of 1.24 and 1.16 cfu/ml, respectively in 3 days storage but no similarity was observed for the 1st and 5th days storage. In the study of Ozdemir et al.⁶, 1.02 and 0.95 log cfu/ml recovery of *L. monocytogenes* was counted on the storage day 5 after 1% and 2% lactic acid treatments, respectively but in our study the recovery of *L. monocytogenes* was recorded as 0.97 log cfu/ml in the 1% lactic acid treated group and 0.44 log cfu/ml in the 2% lactic acid group. These differences may have arisen due to the fact that our 1% and 2% lactic acid solutions had lower pH and temperature (1.95 and 1.82, 18±1°C) than the values of pH and temperature (2.53 and 2.40, 24–25°C) in the study of Ozdemir et al.⁶. It is known that the concentration of the acid, temperature, and pH of

the solution, the method used and time of application may influence antimicrobial activity of the acids^{13-15,21,22}.

In our study, in the 0.1% acidified sodium chloride group, the bacterial level on the 1st and 3rd days decreased 1 log (6 log cfu/ml) but increased 1 log on the 5th day (7 log cfu/ml) comparing to day 0. The lowest bacterial decline was observed in the group of acidified sodium chloride. In the study of Su and Morrissey²³ the antimicrobial activity of acidified sodium chlorite against *L. monocytogenes* in salmon was similar to our study. Del Rio et al.²⁴ reported that dipping treatments of acidified sodium chlorite, and 2% citric acid on inoculated pathogenic bacteria (*L. monocytogenes*) throughout storage of chicken legs (days 0, 1, 3, and 5) at 3±1°C reduced microbial populations (P<0.001) as compared with the control (untreated). Likewise, in our study, the results showed similarity with Del Rio et al.²⁴

At present study, in the 1% cetylpridinium chlorine group, the levels of *L. monocytogenes* showed 0.83 and 1.13 log cfu/ml reductions on days 1st and 3rd, respectively. However, a slight recovery (0.57 log cfu/ml) was observed on day 5th resulting the similar counts of *L. monocytogenes* as it was on day 0. Dupard et al.²⁵ reported that a soaking treatment of cetylpridinium chlorine had a strong potential effect to eliminate or reduce *L. monocytogenes* on the surfaces of shrimps. In their study, the shrimp samples were treated by soaking in different concentrations of cetylpridinium chlorine (0.05, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0%) solutions for 1 min and then packed, stored at 4°C for 24 h. The counts of *L. monocytogenes* on cooked shrimps were reduced by 3 log cfu/g with 0.1% cetylpridinium chlorine treatment. However, 1 log cfu/g change was observed with 0.1% cetylpridinium chlorine treatment in our study. This might be caused by different pH levels and application periods in the studies.

In the study of Lim and Mustapha²⁶, 0.5% cetylpridinium chlorine was sprayed on the beef surfaces and tray absorbent pads, and then samples were stored at +4°C for 10 days. *L. monocytogenes* was reduced to undetectable levels in 2 h

after spraying with 0.5% cetylpridinium chlorine. Likewise, in the study of Singh et al.²⁷, sliced (cut) and exterior (intact) surfaces of restructured cooked roast beef were inoculated with *L. monocytogenes*, and treated with 1% cetylpridinium chlorine by immersion for 1 min. Then vacuum packed, and stored for 42 days at 0 or +4°C. Immediately after cetylpridinium chlorine treatment, and regardless of inoculation levels, *L. monocytogenes* populations were reduced ($P=0.05$) about 2 log cfu/cm² on sliced surfaces and 4 log cfu/cm² on exterior surfaces and remained lower ($P=0.05$) than those of nontreated samples for both surface types throughout 42 days of refrigerated storage (at both 0 and +4°C).

These studies indicate that cetylpridinium chlorine is effective to reduce the numbers of *L. monocytogenes* but the effectiveness rate seems to be depend on concentration and time. In our study, treatment of 0.1% cetylpridinium chlorine solution caused only 1.13 log cfu/g reduction being lower than results above studies. This may be due to the lower concentration of cetylpridinium chlorine and a shorter time period treatment comparing to the cetylpridinium chlorine treatments in other related studies above.

Similar studies compared the results of bacterial reductions by single or double combinations of different chemical agents. Dubal et al.²⁸ reported that 2% lactic acid treatment is more effective than using 1.5% acetic acid + 1.5% propionic acid combination; on the contrary, suggesting that combined treatments are more effective in the bacterial reduction as far as the shelf life is concerned.

Pohlman et al.²⁹ used a combination of multiple antimicrobial intervention treatment through applying ozone, chlorine dioxide, cetylpridinium chlorine, trisodium phosphate and acetic acid on beef trimmings before grinding and found out that it could be effectively employed to decrease bacterial load. As a result of sensorial evaluation, this treatment had an insignificant effect on fresh ground beef colour and odor peculiarities. Multiple antimicrobial intervention technologies applied to the ground beef processing systems may increase the safety levels in public health without any negative effects on the sensory characteristics of the fresh product.

At the present study, in the 0.1% sodium acetate group, the bacteria levels were 7 log cfu/g on the 1st day, and 6 log cfu/g on the 3rd and 5th days, being different than day 0. Serdengecti et al.³⁰ reported that 2.5% sodium lactate alone, 2.5% sodium lactate and 0.2% sodium diacetate in combination were the most effective alternatives in their beef samples against total aerobic bacteria, lactic acid bacteria, coliforms and psychrotrophs among the different sodium compound solutions. Even though the increase is lower than that of the control group, these results have indicated that the *L. monocytogenes* levels have increased during the storage period in the sodium acetate, sodium lactate, and sodium diacetate applied samples. Upon performing

the decontamination process using sodium acetate at the same concentration as in the study of Serdengecti et al.³⁰, the bacterial reduction levels were similar with the findings of control group, indicating no bacterial inhibition at the significant level.

Serdengecti et al.³¹ reported that sodium salts and their combinations such as sodium lactate, sodium acetate, and sodium diacetate had no significant inhibitory effects on the levels of *L. monocytogenes* in the minced meat samples comparing with initial inoculation levels (4.70 log cfu/g). Likewise, Hwang and Beuchat²² showed that the combination of 0.5% lactic acid, and 0.05% sodium benzoate limited the increasing of *C. jejuni*, *Salmonella* spp., and *E. coli* O157:H7 numbers and inhibited them in the chicken wings samples, However, it did not show a significant effect on the levels of *L. monocytogenes*. The difference between the results of this study and our results is probably because of the solution concentrations and treatment techniques.

In conclusion, it can be drawn from the findings of this study that the highest level of bacterial inhibition was observed in the beef samples treated with 2% lactic acid while the lowest level was acquired in the samples treated with 0.1% acidified sodium chloride. Comparing our study with others, there are some similarities and differences. It is important to specify that the initial bacterial load of carcasses and the decontamination technique applied, and the characteristics of acids being used (dissociated or undissociated, pH, amount, percentage, application temperature, and different combinations) are fundamental parameters to be taken into account in the process of reducing microorganisms *L. monocytogenes* on raw beef meat. In further studies, using different decontaminant agent solutions and combinations against other pathogens beside *L. monocytogenes* might be considered.

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