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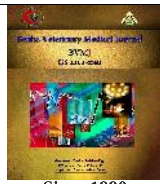

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Efficiency of some organic acids as decontaminants in sheep carcasses

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ABSTRACT

Potent and food-safe antibacterial chemicals capable of decontaminating carcass surfaces have been researched for a long time due to their great value for the quality and shelf life of the meat. The aim of current study is to test the antibacterial effect of lactic acid (LA) and acetic acid (AA) spray treatments using three concentrations (1, 1.5 and 2 %) on the aerobic plate count, Enterobacteriacae count, coliform count, and Staphylococcus count of fresh sheep carcasses surface after 20 minutes of spraying. Results of the investigated bacteriological parameters showed significant reductions after being exposed to organic acids, especially Gram-negative bacteria (Enterobacteriacae) which showed greater sensitivity to the used organic acids than Gram positive bacteria (Staphylococcus), where greater concentration gave greater reduction in the bacterial counts. Moreover, spray wash of lactic acid resulted in higher reduction of bacterial counts on meat surface than acetic acid. From the obtained results, organic acids showed safe, simple, efficient, cheap, and highly effective modality of meat decontamination, on addition, application of lactic acid 2.0% spray showed higher anti-bacterial effect, therefore, it is recommended to improve safety of sheep carcasses for industrial scales.

1. INTRODUCTION

Sheep, as food animal, is one of the most numerous domestic small ruminant that is important in the tropic climates of Africa; sheep is reared worldwide for their ability to turn of low cost feed into high value food products such as red meat, milk and milk products, and wool fibers (Wilsmore, 2006). The accelerated increase in the human populations basically increased their demands to higher good quality red meat; meanwhile, the foodborne poisoning outbreaks increase worldwide which many of them associated with meat consumption (Goksoy et al., 2000).

Bacterial contamination of meat starts up with the arrival of microorganisms to the carcass surface penetrating deeper in layers of the meat; meat can be contaminated through many sources such as contact with the hide, gastrointestinal tract contents, water sources, the dressing instrument (knives, saws, cleavers or hooks), and even air quality of slaughtering halls (Ashok and Kashyap, 2007).

Foodborne diseases remain responsible for high morbidity and mortality rates among human population but particularly dangerous in infants, pregnant women, and elderly or immunocompromised people which were estimated that about 76 million cases of food related illness, resulting in 5,000 deaths and 325,000 hospitalizations, occur in the United States each year (Mead et al., 1999).

Several attempts of reducing carcass's surface contamination and avoiding or limiting the microbial growth and extend the shelf life of carcasses which significantly improves the quality and safety of the consumed meat and meat products. carcass wash with organic acids up to 2.5% concentration diluted with hot water recorded as the most used technique used to decontaminate fresh carcasses in the industry to reduce the microbial loads before cold-storage (USDA/FSIS, 2004 and Harris et al., 2006), where lactic and acetic acids were especially approved by USDA for use on beef carcasses, offal and variety of meats (i.e. pre- and post-chill) (FDA, 2003).

Organic acids were generally recorded due to their ability to decrease the environmental pH which has antimicrobial capabilities through disturbance in the bacterial cell membranes (Jay, 1992 and Bromberg et al., 2004).

Organic acids are generally recognized as safe (GRAS) antimicrobial agents, where acetic and lactic acid dilute solutions are the most frequently used chemical interventions in commercial plants for both beef and lamb dressing due to having no adverse effect on the desirable sensory properties of meat with significantly antimicrobial effects (Jay et al., 2005).

So, the main target of this study was to evaluate the anti-bacterial effect of acetic and lactic acid spray of different concentrations in surface decontamination of freshly dressed sheep carcass in slaughterhouse level immediately after evisceration before any further factors' effects like transportation or chilling.

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2. MATERIAL AND METHODS

2.1 .Collection of samples

Thirty random sheep carcasses (5/group) were examined after dehiding, evisceration, and washing at random abattoirs in Shark El-Owainat, New Valley province, Egypt. Swabs were taken from hind quarter in area about 10 cm2, before and after spraying of lactic and acetic acids in concentration of (1.0, 1.5, and 2.0%). Swabs were collected after twenty minutes of application of organic acids; swabs were identified, packed and transferred to the laboratory in icebox under complete aseptic conditions without undue delay in which APC, Enterobacteriacae, coliform, and Staphylococcus counts were measured.

Organic acids used:

- Acetic acid glacial 99-100% a.r. (Chem-Lab NV) and Lactic acid 88% (Guangzhou Zio Co., LTD) were purchased and prepared with sterile distilled water (DW) to reach (1.0, 1.5, and 2.0% concentration). Maximum 2.0% concentration was prepared by blank DW (without heating) to avoid adverse effect of acidity and hotness on the sensory properties of the carcass surface.

Experiment groups

The swabs groups divided into 6 groups. Swabs were taken from each carcasses before and after spraying organic acids in the following groups:

Group 1: treated with acetic acid.(%1.0)
Group 2: treated with acetic acid.(%1.5)
Group 3: treated with acetic acid.(%2.0)
Group 4: treated with lactic acid.(%1.0)
Group 5: treated with lactic acid.(%1.5)
Group 6: treated with lactic acid.(%2.0)

Preparation of swab samples (ISO 18593:2004).

Swabs were taken from the confined area with a template loop of 5cm x 2cm dimensions (10 cm2); after swabbing, cotton buds ware immediately placed in 1ml of 0.1% solution of peptone broth and held at 4OC until plating was accomplished. After appropriate dilutions, followed bacteria were investigated as follow:

2.2 .Aerobic plate count "APC" according to (ISO 4833-2, 2013).

0.1 ml from the previously prepared serial dilutions was spread over plate count agar plates and incubated at 30±1oC for 72 hours. Colonies were counted as CFU/cm2 and recorded.

2.3. Enterobacteriaceae count "EC" according to (ISO 21528-2, 2017).

0.1 ml from the previously prepared serial dilutions was spread over Violet Red bile Glucose (VRBG) agar plates and incubated at 37˚C for 24 hours. All purple suspected colonies surrounded by purple haloes were counted and recorded.

2.4. Coliform count "CC" according to (ISO 4832, 2006).

0.1 ml from the previously prepared serial dilutions was spread over Violet Red bile (VRBA) agar plates and incubated at 37˚C for 24 hours. All purple suspected colonies surrounded by purple haloes were counted and recorded.

2.5. Staphylococci count "SC" according to (ISO 6888-1:1999, A1:2003).

0.1 ml from the previously prepared serial dilutions was spread over Baird-Parker agar plates and incubated at 35±2oC for 24-48 hours. Black, shiny, circular, smooth, convex colonies were counted .

APC, EC, CC, and SC were performed like mentioned before by surface plating technique. After which, colonies were counted and recorded as CFU/cm2 of sample .

2.6 .Statistical analysis :

A logarithmic transformation of the obtained results was then analyzed using paired samples T-test on SPSS application according to Feldman et al. (2003).

3. RESULTS

Results of lactic and acetic acid spray application, as mentioned in Table (1 and 2), showed high anti-bacterial effect with significant decreases of the assessed bacteriological parameter when ($P \leq 0.5$) as recorded in all groups of pre- and post-organic acids treatment within the same group. Greater reductions were recorded with increasing the organic acid concentration, where 2% lactic and acetic acid concentration revealed more reduction in bacterial counts than the lower concentrations. Furthermore, Gram-negative bacteria (Enterobacteriacae) were more sensitive to the applied organic acids than Gram-positive bacteria (Staphylococci). Moreover, results proved that lactic acid spray recorded higher anti-bacterial effect comparing with acetic acid of the same concentrations.

4. DISCUSSION

Microbial contamination of animal carcasses usually occurs as a consequence of the following slaughtering, transportation, storage, and handling procedures required to production of fresh retail meats. The contamination can be controlled by GMP practices, but the total elimination of foodborne pathogenic microorganisms is extremely difficult. Application of organic acids as sanitizing sprays for carcass decontamination is one of microbial reducing techniques which has received considerable attention and has shown to be effective in reducing the presence of pathogenic bacteria (Hardin et al., 1995), especially meat spoilage microorganisms including coliforms, Staphylococci, and other aerobic bacteria (Kotula and Kotula, 2000). Organic acids were typically used as warm showers to the whole carcass surfaces; of the organic acids evaluated, acetic and lactic acids have been most widely accepted as carcass decontamination rinses (Jay et al., 2005).

From the obtained results, it appeared that the used lactic and acetic acids had high potential antibacterial effect especially with increasing the concentration of the used organic acid. This result is in agree with the conclusion of Acuff (2005) and Laury et al. (2009) who reported that, the lactic acid and acetic acid are the best organic acids that of a high effect for decontamination of sheep carcass from total bacteria and the higher concentration of these acids gave better decontamination than the lower concentration of these organic acids.

The antimicrobial effects of organic acids may be attributed to the lipophilic nature of their undissociated form, which enables it to cross the cell membrane, due to, modifying the proton and associated anion concentrations in the cytoplasm (Dibner and Buttin, 2002); consequently, purine bases and essential enzymes are negatively affected and bacterial viability decreases. Moreover, certain types of bacteria that presented as pH- sensitive cannot survive a wide internal and external pH gradient. In addition, the antiseptic action of organic acid has been connected with its disturbance effect on the surface tension or contributed to its toxic effect due to its molecule as whole rather than to H+ ions alone. Antibacterial activity of organic acids is mainly attributed to the direct reduction of pH, decrease the intracellular pH by ionization of the undissociated acid molecule or disruption of substrate transport by alteration of cell membrane permeability, and therefore pH dependent (Warnecke and Gill, 2005). Carranza et al. (2013) found that an acetic acid spray treatment following water washing was effective at reducing microbial load on beef carcasses at a commercial Mexican slaughterhouse. They reported 0.8-log, 1.54-log and 1.4-log reductions in total plate count, total coliform and staphylococci counts, respectively, when carcasses were sprayed with a 2% acetic acid solution for 60 seconds.

Table 1 Effect of different concentrations of acetic and lactic acids on APC and Staphylococci Count (log_{10} CFU/g) in the examined swab samples (n=5).

Groups	APC				SC			
	Before	After	R%	p-value	Before	After	R%	p-value
AA (1%)	4.31 ± 0.04	3.00 ± 0.02*	30.39	0.000	3.16 ± 0.05	2.64 ± 0.03*	16.45	0.003
AA (1.5%)	4.34 ± 0.02	2.88 ± 0.02*	33.64	0.000	3.06 ± 0.03	1.92 ± 0.03*	37.25	0.000
AA (2%)	4.85 ± 0.06	2.46 ± 0.05*	49.27	0.000	3.16 ± 0.05	1.55 ± 0.05*	50.94	0.000
LA (1%)	4.66 ± 0.09	3.06 ± 0.07*	34.33	0.001	3.07 ± 0.04	2.66 ± 0.04*	13.35	0.000
LA (1.5%)	4.70 ± 0.05	2.55 ± 0.07*	45.86	0.000	3.20 ± 0.07	1.94 ± 0.09*	39.25	0.001
LA (2%)	4.70 ± 0.05	1.90 ± 0.04*	59.66	0.000	3.66 ± 0.04	1.43 ± 0.04*	60.65	0.000

- AA: Acetic Acid. LA: Lactic Acid. R%: Reduction percent. *: means significant difference between before and after bacteriological counts when ($P \leq 0.05$).

Table 2 Effect of different concentrations of acetic and lactic acids on Enterobacteriacae and Coliform Counts (log_{10} CFU/g) of the examined swab samples (n=5).

Groups	EC				CC			
	Before	After	R%	p-value	Before	After	R%	p-value
AA (1%)	3.09 ± 0.04	2.01 ± 0.02*	34.95	0.000	2.68 ± 0.03	2.28 ± 0.08*	14.92	0.014
AA (1.5%)	3.08 ± 0.03	1.55 ± 0.12*	49.67	0.001	2.78 ± 0.03	1.77 ± 0.06*	36.33	0.000
AA (2%)	3.59 ± 0.24	0.88 ± 0.08*	75.49	0.000	2.57 ± 0.11	1.22 ± 0.05*	52.52	0.001
LA (1%)	3.21 ± 0.05	2.19 ± 0.08*	31.77	0.000	2.52 ± 0.09	2.05 ± 0.08*	18.65	0.015
LA (1.5%)	3.24 ± 0.04	1.32 ± 0.07*	59.26	0.000	2.50 ± 0.11	1.59 ± 0.05*	36.40	0.003
LA (2%)	4.14 ± 0.02	0.91 ± 0.03*	78.02	0.000	2.70 ± 0.04	1.08 ± 0.03*	60.0	0.000

AA: Acetic Acid. LA: Lactic Acid. R%: Reduction percent. *: means significant difference between before and after bacteriological counts when ($P \leq 0.05$).

Although the used concentrations of lactic and acetic acids showed a great antibacterial effect, they have no adverse effect on the sensory and organoleptic examinations of the carcass's meat. This character was previously reported by (Stratakos and Grant, 2018) where they reported that, the diluted solutions of organic acids (1 to 3%) are generally don't affect the wholesome organoleptic properties of fresh meat when used as a carcass decontaminant.

In addition, this study recorded that lactic acid showed greater inhibitory effect than acetic acid in the same concentrations. This result agreed with that reported by Arthur et al. (2008) who cleared that, the lactic acid is more efficient in decontamination of meat carcasses than the acetic acids.

It is worth mentioning that the used organic acids revealed higher reduction ability against Gram negative bacteria (Enterobacteriacae) than Gram positive bacteria (Staphylococci) which may be referred to their ability to cross the lipo-polysaccharide cell membrane of Gram negative bacteria, due to the lipophilic nature of their undissociated form decreasing bacterial cell availability (Dibner and Buttin, 2002). This result is in line with the results of Abdul Qadir and Ahmed (2013) who recorded a greater inhibitory effect against E. coli than S. aureus in their study.

There is a great variation in the literature in terms of the cited reductions which will be achieved. This is mainly due to differences in the concentrations and types of acids used by different researchers, the method of application, the types of samples tested, and the initial microbial load of samples. Warm organic acids rinse (50-55°C) appeared to be the most effective carcass decontamination technique (Acuff, 2005).

5. CONCULSION

Finally, the present study allowed concluding that the use of acetic and lactic acids potential decontaminants and lactic acid (2%) proved to be more efficient antibacterial one. Therefore, recommended to improve quality and safety of sheep carcasses.

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