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Effect of some organic acids on microbial quality of dressed cattle carcasses in Damietta abattoirs, Egypt.

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A B S T R A C T

This experimental study aimed to investigate the anti-microbial effect of some organic acids (OA) represented by Acetic and Lactic acids of (1 and 2%), and assess its reflection on the microbiological quality of dressed cattle carcasses slaughtered in Damietta city abattoirs. Samples were grouped according the concentration of the used acid to five groups, where each group consisted of five carcasses. Acids were applied as nozzle sprays over the external surface of the carcasses and kept for 20 minutes before swab sampling. Swabs were examined for aerobic plate count (APC), Enterobacteriacae count (EC), Coliform count (CC), Staphylococcus count (SC), mould and yeast counts before and after spraying. Results revealed significant reductions of the assessed microbial counts in both lactic and acetic acids of both concentrations, except fungal counts which revealed insignificant reductions for both acids. Moreover, 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.

Keywords: Acetic acid, Lactic acid, Cattle carcass, Microbiological Quality.

1. Introduction

Meat considered as a significant source of valuable nutritious protein, fat, vitamins and minerals, for that, a great diversity of microbes inhabit fresh meat generally, from which, different types may survive and infect consumers depending on pH, textures, storage, temperature, and transportation means of raw meat (Adu-Gyamfi et al., 2012). Soiled hide and hair of the slaughtered animals, knives, hands, arms, workers clothes and accidental piercing of GIT during skinning and evisceration process are considered the main sources of fresh carcass meat contamination (Gracey et al., 1999). Large amounts of foods are condemned yearly due to microbial spoilage by different foodborne bacteria, yeasts and fungi (Lind et al., 2005). So that, antimicrobial preservatives include weak organic acids (OA) such as acetic and lactic acids are commonly applied to inhibit the microbial growth in various foods (Kang et al., 2003). Several efforts of diminishing carcass's surface microbial contamination and avoiding or limiting the microbial growth, and augment shelf life of fresh meat which significantly improves the quality and safety of the consumed meat and meat products.

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carcasses contamination which starts from slaughter house (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 applied due to their ability to decrease the pH which has significant antimicrobial records through disturbance of cell membrane permeability of and on the metabolic enzymes (El-Kadi et al., 2003 and Hauka et al., 2005). Organic acids generally used as safe agents to keep foods wholesome by reducing cytoplasmic pH and stop metabolic activities. Moreover, organic acids caused the death by acting on the plasmic membrane by neutralizing its electrochemical potential and increasing its permeability (Dalie et al., 2010).

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 current work pointed to evaluate the anti-microbial effect of acetic and lactic acid sprays (1 and 2% conc.) in surface decontamination of freshly dressed cattle carcass in slaughterhouse level immediately after evisceration before any further factors effects like transportation or chilling.

2. Materials and Method

2.1. Collection of samples

Twenty random cattle carcasses (5/group) were examined post-dressing and washing at random abattoirs in Damietta governorate, Egypt. Forty swabs (10/group) were taken from hind quarter in area about 10 cm2. Swabs were collected before and after spraying of lactic and acetic acids in concentration of (1.0 and 2.0%). Swabs were collected after 20 min. of organic acids spraying; swabs were identified and transferred to the laboratory in icebox under complete aseptic conditions without undue delay in which APC, Enterobacteriacae, coliform, Staphylococcus, mould and yeast counts were measured.

2.2. 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 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.

2.3. Experiment groups

The swabs groups were divided into four groups. Swabs were taken from the hind quarter of each carcass before and after spraying organic acids in the following groups:

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

2.4. Preparation of swab samples (ISO 18593:2018).

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 as recommended by ISO 6887-1:2017, next microbial parameters were investigated as follow:

A. Aerobic plate count "APC" according to (ISO 4833-2, 2013).
One ml from the previously prepared serial dilutions was mixed with melted plate count agar by pour-plate technique, and incubated at 30±1oC for 72 hours. Colonies were counted as CFU/cm2 and recorded.
B. Enterobacteriaceae count "EC" according to (ISO 21528-2, 2017).
One ml from the previously prepared serial dilutions was mixed with melted Violet Red bile Glucose (VRBG) agar by pour-plate technique, and incubated at 37˚C for 24 hours. All purple suspected colonies surrounded by purple haloes were counted and recorded.
C. Coliform count "CC" according to (ISO 4832, 2006).
One ml from the previously prepared serial dilutions was mixed with melted Violet Red bile (VRBA) agar by pour-plate technique, and incubated at 37˚C for 24 hours. All purple suspected colonies surrounded by purple haloes were counted and recorded.
D. 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.
E. Mould and yeast counts according (ISO 21527:2008)
0.1 ml from the previously prepared serial dilutions was spread over Di-Chloran Rose Bengal-Chloramphenicol (DRBC) agar plates, and incubated at 25±2oC for 5-7 days. Mould and yeast colonies were counted and recorded separately.
Colonies of the previously mentioned tests were counted pre- and post-organic acids application, 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 Tables (1, 2 and 3), showed high anti-microbial effect with significant decreases of the assessed bacteriological and yeast parameters when ($P \leq 0.05$) as recorded in all groups of pre- and post-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 microbial counts than the lower concentrations. Furthermore, Gram-negative bacteria (Enterobacteriacae) were more sensitive to the applied organic acids than Gram-positive bacteria (Staphylococci); furthermore, however high reduction percent, mould showed insignificant declined counts. Moreover, results proved that lactic acid spray recorded higher anti-microbial effect comparing with acetic acid of the same concentrations.

4. Discussion

Contamination of fresh carcasses usually occurs following unhygienic slaughtering, dressing, transportation, storage, and handling procedures required to production of fresh retail meats. Several practices have been applied to control microbial contamination of fresh carcasses, but the total avoidance of foodborne pathogens is nearly impossible. Application of OA sprays for carcass decontamination is one of anti-microbial used techniques which has a significant reducing effect on pathogenic bacteria (Hardin et al., 1995), especially microbial food spoilage including coliforms, Staphylococci, and other aerobic pathogens (Kotula and Kotula, 2000). Therefore, Jay et al. (2005) previously recorded that OAs, especially acetic and lactic acids, were used as warm showers to the whole carcass surfaces.
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 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; furthermore, Carranza et al. (2013) found that carcass spray with acetic acid following water washing reduced microbial load on beef carcasses at a commercial Mexican slaughter house. They reported total reduction of plate count, coliform and staphylococci counts by 0.8-log, 1.54-log and 1.4-log, respectively, when carcasses were sprayed with a 2% acetic acid solution for 60 seconds.
The antimicrobial effects of OA may be attributed to the lipophilic nature of their undissociated form, which make it able to cross the cell membrane leading to lethal modification of inter-cytoplasmic pH concentrations (Dibner and Buttin, 2002); consequently, molecular bases and essential metabolic enzymes are unfavorably affected, so cellular viability declined. In addition, OA were recorded to have strong antiseptic action which may be connected with its ability to defect the surface tension, plus its toxic effect due to its H+ ions. Antimicrobial effect of OA 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).
Although the great recorded anti-microbial effect of the used acid concentrations, no adverse organoleptic changes were noticed. This result was previously reported by Stratakos and Grant (2018) that the organic acids carcass sprays (up to 3% conc.) generally do not alter the characteristic organoleptic properties of fresh meat.
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) and Saad et al. (2020) who cleared that, the lactic acid is more efficient in decontamination of meat carcasses than the acetic acids, which may be attributed to the ordinary production of lactic acid post-mortem.
It is worth mentioning that the used acids were more effective against Enterobacteriacae, coliform, mould and yeast than Staphylococci which may be attributed to their ability to cross the lipo-polysaccheride 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; and Saad et al. (2020) who recorded higher reduction against Enterobacteriacae than staphylococci.
Great variations with other cited results mainly referred to variation in the concentrations and types of the used organic acids by different authors; the method of application; the types of samples tested, and the initial microbial load of samples.

5. Conclusion

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 one. Therefore, recommended to improve quality and safety of freshly dressed cattle carcasses.

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Table (1): Effect of different concentrations of acetic and lactic acids on APC and Staphylococci (SC) Count (CFU/cm2) in the examined swab samples (n=10).

Groups	APC				SC			
	Before	After	R %	p-value	Before	After	R %	p-value
AA (1%)	$1.6x10^5 \pm 0.2x10^5$	$5.8x10^3 \pm 0.5x10^2$*	96.37	0.002	$7.7x10^2 \pm 0.04x10^2$	$9.0x10 \pm 0.05x10$*	88.31	0.001
AA (2%)	$2.2x10^5 \pm 0.19x10^5$	$8.8x10^2 \pm 0.58x10^2$*	99.60	0.000	$7.3x10^2 \pm 0.41x10^2$	$5.0x10 \pm 0.1x10$*	93.15	0.008
LA (1%)	$1.1x10^5 \pm 9.8x10^4$	$7.5x10^3 \pm 5.7x10^2$*	93.18	0.001	$6.1x10^2 \pm 0.91x10^2$	$4.0x10 \pm 0.05x10$*	93.44	0.003
LA (2%)	$2.5x10^5 \pm 3.0x10^4$	$6.3x10^2 \pm 0.16x10^2$*	99.74	0.001	$6.5x10^2 \pm 0.1x10^2$	$2.0x10 \pm 0.06x10$*	96.92	0.007

- 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 Enterobacteriaceae (EC) and Coliforms (CC) Counts (CFU/cm2) of the examined swab samples (n=10)

Groups	EC				CC			
	Before	After	R %	p-value	Before	After	R %	p-value
AA (1%)	$1.6x10^3 \pm 0.3x10^2$	$3.0x10^2 \pm 0.3x10^2$*	81.25	0.009	$6.0x10^2 \pm 0.08x10^2$	$5.0x10 \pm 0.08x10$*	91.66	0.002
AA (2%)	$4.1x10^3 \pm 0.05x10^3$	$6.0x10 \pm 0.61x10$*	98.54	0.002	$4.2x10^2 \pm 0.06x10^2$	$2.0x10 \pm 0.08x10$*	95.24	0.005
LA (1%)	$5.1x10^3 \pm 1.1x10^2$	$4.0x10^2 \pm 0.05x10^2$*	92.15	0.031	$5.6x10^2 \pm 0.07x10^2$	$5.0x10 \pm 0.01x10$*	91.10	0.012
LA (2%)	$3.9x10^3 \pm 3.6x10^2$	$7.0x10 \pm 0.01x10$*	98.21	0.000	$5.2x10^2 \pm 0.15x10^2$	$1.0x10 \pm 0.06x10$*	98.10	0.026

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

Table (3): Effect of different concentrations of acetic and lactic acids on Mold and Yeast Counts (CFU/cm2) of the examined swab samples (n=10)

Groups	Mold				Yeast			
	Before	After	R %	p-value	Before	After	R %	p-value
AA (1%)	$2.9x10^2 \pm 0.03x10^2$	$6.0x10 \pm 0.01x10$	68.96	0.085	$1.04x10^3 \pm 0.01x10^3$	$3.8x10^2 \pm 0.09x10^2$*	63.46	0.001
AA (2%)	$2.8x10^2 \pm 0.09x10^2$	$4.0x10 \pm 0.1x10$	85.71	0.133	$1.5x10^3 \pm 0.21x10^3$	$1.2x10^2 \pm 0.22x10$*	92.00	0.003
LA (1%)	$3.8x10^2 \pm 0.07x10^2$	$8.0x10 \pm 0.1x10$	78.94	0.073	$1.9x10^3 \pm 0.1x10^3$	$3.4x10^2 \pm 0.01x10^2$*	82.11	0.003
LA (2%)	$3.6x10^2 \pm 0.13x10^2$	$1.0x10 \pm 0.1x10$	97.22	0.115	$1.1x10^3 \pm 0.01x10^3$	$5.0x10 \pm 0.01x10$*	95.45	0.001