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IMPACT OF SOME ORGANIC ACIDS AND THEIR SALTS ON MICROBIAL QUALITY AND SHELF LIFE OF BEEF

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ABSTRACT

The main purpose of this investigation was to study the effect of processing fresh meat with some organic acids and their salts on the shelf-life and microbial quality of these meat during chill storage. Fresh beef samples (144) were collected from an abattoir in Benha city then prepared and treated with dipping in 1 and 2% lactic acid (LA), 1 and 2% acetic acid (AA), 2.5 and 5% sodium lactate (SL) and 2.5 and 5% sodium acetate (SA) solutions then stored at 4°C at interval period 3, 6, 9, 12, 15, 18 and 21 days and examined sensory, physicochemically and microbiologically. The results of the physical analysis revealed a significant decrease in the pH value. The pH value of control group was 5.82±0.13 at the beginning of the experiment and 4.6±0.14, 4.4±0.13, 4.9±0.13, 4.40±0.19, 5.73±0.08, 5.55±0.08, 5.84±0.08 and 5.69±0.12 for both lactic acid (1% and 2%), acetic acid (1% and 2%), sodium lactate (2.5% and 5%) and lead acetate (2.5% and 5%), respectively. The mean of total volatile basic nitrogen value (TVBN) at the beginning of the experiment was 12.50±1.0 for the control group. On day 21 of the experiment the best mean of TVBN value was 20.70±2.07 for acetic acid (2%) and the highest was 25.70±2.84 for the control group. The results also showed that the mean of thiobarbituric acid reactive substances (TBARs) value at the beginning of the experiment was 0.43±0.02 for the control group. On day 21 of the experiment the lowest value of TBARs was 0.51±0.04 for acetic acid (2%) while the highest was 1.17±0.04 for the control group. Also, treatments were efficient against the proliferation of various spoilage microorganisms including aerobic, psychrotrophic, pseudomonas, enterobacteriaceae, *Staphylococcus aureus*, yeast and mould counts. The analysis of sensory characteristics revealed that the highest general acceptance rate was 8.1±0.2 for the acetic acid-treated beef samples (1%), while the lowest level was 6.2±0.1 for the samples treated with lactic acid (2%). Samples treated with acetic acid 1% and lactic acid 1% showed the best sensory properties in terms of color, texture and flavor. Overall, the use of lactic, acetic acid, and their salts for treating fresh beef improved its microbial safety and extended its shelf-life. This can open new opportunities for beef preservation using efficient, safe, and cost-effective preservatives.

Key words: Organic acids, Organic salts, Microbial quality, Shelf life, Beef meat.

INTRODUCTION

Meat has long been known for its nutritive composition which could explain why it is being the first choice source of animal protein for many people all over the world. Meat consumption is continuously increasing worldwide. The annual per capita consumption of beef increased from 10 kg in the 1960 to 26 kg in 2000 and will reach 37 kg by the year 2030 (Heinz and Hautzinger, 2007). On the other hand, significant portion of meat and meat products are spoiled every year. If 5% of this meat loss is preserved it could satisfy the daily needs of approximately 320,000 people for meat (Cerveny *et al.*, 2009).

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Beef has a short shelf-life of one day or less at ambient temperature and few days at refrigerated temperature due to microbial spoilage (Dickson and Anderson, 1992) and/or lipid oxidation (Houben *et al.*, 2000), which are strongly influenced by initial beef quality, package parameters and storage conditions (Zhao *et al.*, 1994). Spoilage of meat is caused by contamination and subsequent decomposition of meat by microbes which are borne by the animal itself, by the people handling the meat, and by their implements (Singh *et al.*, 2014). Meat spoilage and foodborne infections in human, resulting in economic and health losses (Komba *et al.*, 2012). Meat borne infections could spread and acquire epidemic status, which could pose serious health hazards (Antwi-Agyei and Maalekuu 2014). Among pathogenic bacteria that associated with fresh beef were *E. coli O157:H7, Salmonella, Campylobacter Jejuni, Listeria monocytogenes, Pseudomonas* and *Staphylococcus aureus* (Schylter *et al.*, 1993). Minimizing meat contamination and delaying or inhibiting growth of

spoilage and pathogenic organisms are major keys for improving fresh meat shelf-life and increasing consumer safety (Sallam and Samejima 2004).

Lactic and acetic acids have being utilized as preservatives for preventing food deterioration and extending the shelf-life of perishable food (Ricke, 2003), as these acids and their salts can minimize meat contamination by inhibiting growth of spoilage and pathogenic microorganisms. Lactic acid, acetic acid and their salts have been generally recognized as safe (GRAS), which provides for unregulated use (Anonymous, 1987).

Considering the above, the aim of the work was to evaluate effect of different concentrations of lactic acid, acetic acid and their sodium salts on shelf-life extension of fresh beef during the refrigerated storage at 4°C for 21 days.

MATERIALS AND METHODS

Collection of samples:
A total of 144 random samples of fresh beef was purchased immediately after slaughter from abattoir in Qalubia Governorate, packed in clean polyethylene bags then transported in insulated iced containers to laboratory under hygienic condition for treatment and analysis. To minimize the number of resident surface microflora, 2 to 3 mm of the beef surface were trimmed off.

Preparation of treatment solutions:

1- Lactic acid 1% (v/v): lactic acid 85% (El- Naser Phar. Co.) was diluted with sterile distilled water to obtain a solution containing 1 % lactic acid (1.18 ml of 85% lactic acid + 98.82 ml sterile distilled water).

2- Lactic acid 2% (v/v): lactic acid 85% (El- Naser Phar. Co.) was diluted with sterile distilled water to obtain a solution containing 2 % lactic acid (2.36 ml of 85% lactic acid + 97.64 ml sterile distilled water).

3- Acetic acid 1% (v/v): acetic acid 96% (El- Naser Phar. Co.) was diluted with sterile distilled water to obtain a solution containing 1 % acetic acid (1.04 ml of 96% acetic acid + 98.96 ml sterile distilled water).

4- Acetic acid 2% (v/v): acetic acid 96% (El- Naser Phar. Co.) was diluted with sterile distilled water to obtain a solution containing 2 % acetic acid (2.08 ml of 96% acetic acid + 97.92 ml sterile distilled water).

5- Sodium lactate 2.5% (v/v): sodium lactate 85% (El- Naser Phar. Co.) was diluted with sterile distilled water to obtain a solution containing 2.5% sodium lactate (2.95 ml of 85% sodium lactate + 97.05 ml sterile distilled water).

6- Sodium lactate 5% (v/v): sodium lactate 85% (El- Naser Phar. Co.) was diluted with sterile distilled water to obtain a solution containing 5% sodium lactate (5.89 ml of 85% sodium lactate + 94.11 ml sterile distilled water).

7- Sodium acetate 2.5% (w/v): sodium acetate 99% (El- Naser Phar. Co.) was diluted with sterile distilled water to obtain a solution containing 2.5% sodium acetate (2.525 gm of 99% sodium acetate + 97.475 ml sterile distilled water).

8- Sodium acetate 5% (w/v): sodium acetate 99% (El- Naser Phar. Co.) was diluted with sterile distilled water to obtain a solution containing 5% sodium acetate (5.05 gm of 99% sodium acetate + 94.95 ml sterile distilled water).

Treatment of samples:
The lean beef was cut to cubes (150 gram each) then divided into 9 groups (16 cubes for each group). One group (Group 1) left as untreated (control) group. The remaining 8 groups were treated with solutions as following:

Group 2: immersed in 1 % of lactic acid solution (1% LA).
Group 3: immersed in 2 % of lactic acid solution (2% LA).
Group 4: immersed into 1 % of acetic acid solution (1% AA).
Group 5: immersed into 2 % of acetic acid solution (2% AA).
Group 6: immersed in 2.5 % of sodium lactate solution (2.5% SL).
Group 7: immersed in 5 % of sodium lactate solution (5% SL).
Group 8: immersed in 2.5 % of sodium acetate solution (2.5% SA).
Group 9: immersed in 5 % of sodium acetate solution (5% SA).

Beef cubes from each treated group were individually immersed in 250 ml of each treatment solution at 23 °C for 10 minutes and drip-dried for 15 minutes. Then, beef samples were individually sealed in clean polyethylene bags and stored at 4 °C for up to 21 days. Beef samples were taken for physicochemical, microbiological and sensory analyses every 3days during the storage period.

Experimental Design:
The experiment was set up as 9 groups and 8 storage periods (0, 3, 6, 9, 12, 15, 18, and 21 days). Three replications of the experiment were conducted.

Physicochemical analyses:

1. Measurement of hydrogen ion concentration (pH):
According to ISO (1999), 10 g of the sample were homogenized with 50 ml distilled water and pH value was measured by a digital pH-meter (HM-5S; TOA Electric Industrial Co. Ltd., Tokyo, Japan).

2. Measurement of total volatile basic nitrogen (TVBN):
A sample (10g) was mixed with 100 ml distilled water and washed into a distillation flask with 100 ml distilled water; then 2g of magnesium oxide and an antifoaming agent were added. The mixture was distilled using the micro Kjeldahl distillation apparatus. Distillate was collected for 25min into 25 ml 4% boric acid and five drops of Tashero indicator. The solution was titrated using (0.1 M) HCl to calculate the total volatile basic nitrogen in the sample in terms of mg VBN/100g meat as described by Harold *et al.* (1981).

3. Measurement of thiobarbituric acid reactive substances (TBARs):
The thiobarbituric acid (TBA) assay was carried out according to the procedure of Vyncke (1970). Sample (20g) was mixed with 100 ml of 7.5% trichloroacetic acid solution and homogenized in a blender for 2 minutes. The homogenate was filtrated. After filtration, 5ml of the filtrate were added to 5ml TBA reagent (0.02M TBA) in a test tube with screw cap. The test tubes were placed in a water bath for 40 minutes then the absorbance was measured at 538 nm by using spectrophotometer. TBARs value was expressed as mg malonaldehyde (MA) per kg of meat (mg MA/kg meat).

Calculation: concentration of malonaldehyde = 0.016 + 2.872x mg/kg
where, x = the absorbance.

Microbiological analyses:
From each group, 25 g of meat were taken randomly and aseptically using sterile forceps and scissors. The removed muscles were placed in a sterile homogenizer flask contained 225 ml of (0.1%) peptone water. The content of each flask were homogenized at 14000 rpm for 2.5 minutes to obtain a dilution of 10^{-1}, from which 1 ml was transferred with a sterile pipette to a sterile test tube containing 9 ml of (0.1%) peptone water, from which a decimal serial dilution were prepared in a sequential manner up to 10^{-10}, to cover all expected range of samples contamination. For microbial counts, colonies were counted and recorded in colony forming units per gram (cfu/g) of meat sample using the formula:

cfu/g = level of dilution plated x number of colonies counted/volume plated.

These were further expressed in mean colony forming units per gram (mean cfu/g) and converted to $\log_{10}$ base values ($\log_{10}$cfu/g).

1- Determination of total aerobic plate count (APC):
According to ICMSF (2006) one ml from previously prepared dilution was aseptically transferred into a sterile petri dish, and then about 15 ml of sterile standard plate count agar previously melted and cool at 45 °C were added and thoroughly mixed in a horizontal position. After solidification, the inoculated plates as well as control one were inverted and incubated at 37 °C for 24 hours. The plates were counted and recorded as a total colony count/g.

2- Determination of total psychrotrophic counts (PTC):
According to ISO (2004a) a rapid method called modified psychrotrophic bacterial count has been formulated to enumerate psychrotrophic bacteria. Psychrotrophic counts were determined in a similar method to that of APC except that the plates were incubated at 7°C for 10 days.

3- Determination of total pseudomonas count:
According to Mead and Adams (1977), pseudomonas were enumerated on pseudomonas agar base supplemented with cetrimide, fucidin, and cephaloridine. Incubate spread plates, at 25°C for 48 hours. Pseudomonas appear as round, cream colored colonies. The colonies were counted and recorded as pseudomonas cfu/g.

4- Determination of total enterobacteriaceae count (EBC):
According to ISO (2004b), from previously prepared serial dilution, a one ml was transferred into a sterile petri dish and carefully mixed with about 15 ml of melted and adjusted (45±1°C) Violet Red Bile Glucose Agar. After solidification, the plates were overlay by pouring another 5 ml of the same medium. The plates were incubated at an inverted position at 35°C for 24-48 hours. Typical colonies characterized by red purple color, > 0.5 mm diameter and surrounded by a purple halo of precipitated bile. The colonies were counted and recorded as enterobacteriaceae cfu/g.

5- Determination of staphylococcus count:
According to ISO (2003), 1 ml of the dilution was transferred into two petri dishes by means of a sterile pipette. 15 ml of *Staphylococcus aureus* Baired-parker agar at 44°C to 47°C was added into each petri dish. The inoculum was carefully mixed with the medium by rotating the petri dishes and the mixture was left in a cool horizontal surface to allow it to solidify. An overlaying layer medium of 4 ml at 44°C to 47°C was added into the surface of the inoculated medium. The layer was allowed to solidify by putting it in a cool

horizontal surface. The prepared dishes were inverted and placed in an incubator at 37°C ±1°C for 24 h ± 3 h. After the complete incubation period, plates were colony counted according to the ISO methods ISO (2003).

6- Determination of total yeasts and molds count:

According to Bailey and Scott (1978), plates of Sabouraud's medium (containing 0.05 mg of chloramphenicol per ml) were inoculated each with one ml from the prepared serial dilutions. Inoculated plates were incubated at 25°C for 5 days in upright position. The first examination of the plates was after 3 days of incubation to determine the degree of yeast growth, and if large numbers are visible, a count was made and repeated on the fifth day. The yeast and molds colonies were counted. The total yeast and mould counts per gram of the sample was then calculated and recorded.

Sensory Evaluation:

It was carried out according to (Meilgaard *et al.*, 1999). The sensory attributes were evaluated by staff members in the Animal Health Research Institute (Benha branch). Each person had to assess levels of color, texture (toughness or juiciness), and flavor (sourness or sweetness). Representative samples of the different treatments were cooked in hot water at 75°C for 25 min. and presented in covered small porcelain dishes to each member in a separate area where distracters, noises, and odors were minimized. The judges were not informed about the experimental approach and the samples were blind coded with 3 digit random numbers. A 9 point hedonic scale (9 = Excellent, 8 = Very very good, 7 = Very good, 6 = Good, 5 = Medium, 4 = Fair, 3 = Poor, 2 = Very poor, 1 = Very very poor) was used for the evaluation of the overall acceptability.

Statistical Analysis:

All measurements were carried out in triplicate. Results were expressed as means ± standard deviations (SD). Statistical analysis of data was done by one way analysis of variance (ANOVA) using the statistical package for social sciences (SPSS-version 22.0 statistical software package 2013). Differences among the mean values of the various treatments and storage periods were determined by the least significant difference (LSD) test and significance was defined at $p<0.05$. The differences that were equal or more than the identified least significant difference values were considered statistically significant.

RESULTS

Table 1: Changes in the means of pH value of beef samples during refrigerated storage at 4°C.

Treatment	Days							
	0	3	6	9	12	15	18	21
Control	5.82±0.13^{a}	5.77±0.09^{a}	5.68±0.15^{a}	5.59±0.23^{a}	5.95±0.12^{a}	6.10±0.15^{a}	6.33±0.11^{a}	6.65±0.14^{a}
Lactic A 1%	4.60±0.14^{c}	5.10±0.20^{c}	5.30±0.20^{c}	5.40±0.18^{c}	5.45±0.12^{c}	5.51±0.12^{c}	5.55±0.13^{c}	5.61±0.10^{c}
Lactic A 2%	4.40±0.13^{c}	4.90±0.07^{c}	5.08±0.13^{c}	5.14±0.15^{c}	5.25±0.14^{c}	5.35±0.14^{c}	5.41±0.19^{c}	5.46±0.21^{c}
Acetic A 1%	4.90±0.13^{b}	5.20±0.15^{b}	5.40±0.16^{b}	5.48±0.18^{b}	5.50±0.22^{b}	5.59±0.20^{b}	5.65±0.19^{b}	5.68±0.11^{b}
Acetic A 2%	4.40±0.19^{c}	4.80±0.16^{c}	5.25±0.12^{c}	5.30±0.09^{c}	5.40±0.15^{c}	5.50±0.10^{c}	5.57±0.12^{c}	5.63±0.09^{b}
Sodium lactate 2.5%	5.73±0.08^{b}	5.70±0.07^{b}	5.71±0.07^{b}	5.62±0.07^{a}	5.73±0.07^{a}	5.74±0.05^{a}	5.72±0.08^{b}	5.71±0.04^{b}
Sodium lactate 5%	5.55±0.08^{b}	5.51±0.12^{c}	5.52±0.09^{b}	5.53±0.14^{b}	5.53±0.07^{b}	5.54±0.08^{b}	5.52±0.09^{c}	5.51±0.14^{c}
Sodium acetate 2.5%	5.84±0.08^{a}	5.78±0.08^{a}	5.68±0.09^{a}	5.63±0.07^{a}	5.84±0.07^{a}	5.85±0.08^{a}	5.83±0.04^{b}	5.51±0.14^{c}
Sodium acetate 5%	5.69±0.12^{b}	5.65±0.12^{b}	5.66±0.07^{b}	5.67±0.11^{b}	5.59±0.12^{a}	5.69±0.08^{a}	5.67±0.09^{b}	5.65±0.10^{b}

Data given as mean ± SD of 3 replicates. Means with different alphabetical superscripts in the same column are significantly different at $P<0.05$.

Table 2: Changes in the means of total volatile basic nitrogen value (mg N/100g meat) of beef samples during refrigerated storage at 4°C.

Treatment	Days							
	0	3	6	9	12	15	18	21
Control	12.5±1.0^{a}	15.1±0.82^{a}	17.0±1.59^{a}	18.3±0.95^{a}	20.2±1.13^{a}	22.3±1.11^{a}	23.90±1.8^{a}	25.70±2.84^{a}
Lactic A 1%	11.5±1.57^{b}	11.7±0.7^{b}	13.5±0.85^{b}	14.8±1.04^{b}	16.7±1.2^{b}	18.6±1.7^{b}	20.90±1.95^{b}	22.40±2.13^{b}
Lactic A 2%	10.5±0.95^{b}	10.8±0.36^{c}	12.5±0.96^{c}	13.4±1.04^{c}	15.7±1.45^{c}	17.6±1.4^{c}	19.80±1.40^{c}	21.00±2.43^{c}
Acetic A 1%	11.0±1.04^{b}	11.4±0.78^{b}	13.2±0.79^{b}	14.4±1.08^{b}	16.5±1.3^{b}	18.4±1.4^{b}	20.60±1.73^{b}	22.10±3.22^{b}
Acetic A 2%	10.0±1.06^{b}	10.3±0.46^{c}	12.1±0.61^{c}	13.2±1.13^{c}	15.3±1.2^{c}	17.2±1.78^{c}	19.50±1.83^{b}	20.70±2.07^{b}
Sodium lactate 2.5%	12.3±0.44^{a}	13.7±0.50^{a}	15.2±0.79^{b}	16.9±1.47^{b}	18.7±1.45^{b}	20.4±1.39^{a}	22.60±2.20^{a}	24.30±2.35^{a}
Sodium lactate 5%	12.0±0.82^{a}	12.5±0.52^{b}	14.2±0.72^{b}	15.9±1.13^{b}	17.7±2.01^{b}	19.5±1.65^{b}	21.50±2.20^{b}	23.20±2.43^{b}
Sodium acetate 2.5%	12.1±0.66^{a}	13.4±0.53^{a}	15.1±0.70^{b}	16.7±1.41^{b}	18.5±1.78^{b}	20.2±1.61^{a}	22.40±2.93^{a}	24.10±2.78^{a}
Sodium acetate 5%	11.9±1.15^{a}	12.3±0.5^{b}	14.0±0.82^{b}	15.6±1.08^{b}	17.4±1.93^{b}	19.1±1.54^{b}	21.30±2.08^{b}	23.00±2.42^{b}

Data given as Mean ± SD of 3 replicates. Means with different alphabetical superscripts in the same column are significantly different at *P<0.05.*

Table 3: Changes in the means of thiobarbituric acid value (mg MA/kg meat) of beef samples during refrigerated storage at 4°C.

Treatment	Days							
	0	3	6	9	12	15	18	21
Control	0.43±0.02^{a}	0.53±0.03^{a}	0.64±0.03^{a}	0.78±0.03^{a}	0.92±0.04^{a}	1.06±0.04^{a}	1.12±0.04^{a}	1.17±0.04^{a}
Lactic A 1%	0.41±0.03^{b}	0.47±0.02^{b}	0.53±0.02^{b}	0.54±0.03^{b}	0.55±0.03^{b}	0.58±0.04^{b}	0.59±0.04^{b}	0.61±0.03^{b}
Lactic A 2%	0.39±0.02^{c}	0.42±0.02^{c}	0.43±0.03^{c}	0.44±0.02^{c}	0.47±0.02^{c}	0.49±0.04^{c}	0.51±0.03^{c}	0.53±0.04^{c}
Acetic A 1%	0.40±0.01^{b}	0.44±0.02^{b}	0.50±0.03^{b}	0.52±0.03^{b}	0.54±0.03^{b}	0.56±0.03^{b}	0.56±0.04^{b}	0.57±0.04^{c}
Acetic A 2%	0.37±0.02^{c}	0.39±0.02^{c}	0.41±0.02^{c}	0.43±0.03^{c}	0.44±0.03^{c}	0.46±0.03^{c}	0.48±0.03^{c}	0.51±0.04^{c}
Sodium lactate 2.5%	0.43±0.02^{a}	0.54±0.03^{a}	0.62±0.03^{a}	0.65±0.04^{b}	0.69±0.04^{b}	0.73±0.03^{b}	0.76±0.04^{b}	0.80±0.03^{b}
Sodium lactate 5%	0.42±0.02^{b}	0.50±0.02^{b}	0.57±0.03^{b}	0.61±0.03^{b}	0.64±0.03^{b}	0.66±0.04^{b}	0.68±0.04^{b}	0.72±0.04^{b}
Sodium acetate 2.5%	0.42±0.02^{b}	0.51±0.02^{a}	0.60±0.02^{a}	0.63±0.03^{b}	0.67±0.03^{b}	0.69±0.0^{b}	0.72±0.05^{b}	0.77±0.04^{b}
Sodium acetate 5%	0.41±0.03^{b}	0.47±0.02^{b}	0.55±0.03^{b}	0.58±0.0^{b}	0.60±0.03^{b}	0.61±0.04^{b}	0.65±0.04^{b}	0.68±0.03^{b}

Data given as mean ± SD of 3 replicates. Means with different alphabetical superscripts in the same column are significantly different at *P <0.05*.

Table 4: Statistical analytical results of total aerobic plate count (log_{10}cfu/g) of beef samples depending on treatment solutions and refrigerated storage period at 4°C.

Treatment	Days							
	0	3	6	9	12	15	18	21
Control	3.86±0.45^{a}	4.83±0.16^{a}	5.92±0.24^{a}	6.73±0.19^{a}	7.75±0.19^{a}	8.25±0.35^{a}	8.66±0.18^{a}	8.98±0.23^{a}
Lactic A 1%	3.73±0.19^{b}	3.91±0.11^{b}	4.35±0.22^{b}	4.77±0.25^{b}	5.23±0.28^{b}	5.68±0.19^{b}	6.17±0.33^{b}	6.85±0.12^{b}
Lactic A 2%	3.65±0.21^{c}	3.73±0.18^{c}	4.22±0.29^{c}	4.48±0.22^{c}	4.96±0.22^{c}	5.48±0.24^{c}	5.92±0.24^{c}	6.48±0.18^{c}
Acetic A 1%	3.70±0.26^{b}	3.86±0.32^{b}	4.31±0.35^{b}	4.74±0.14^{b}	5.16±0.27^{b}	5.52±0.27^{b}	6.04±0.36^{b}	6.67±0.21^{b}
Acetic A 2%	3.62±0.35^{c}	3.65±0.14^{c}	4.07±0.15^{c}	4.42±0.20^{c}	4.79±0.25^{c}	5.31±0.12^{c}	5.85±0.20^{c}	6.39±0.23^{c}
Sodium lactate 2.5%	3.84±0.16^{a}	4.43±0.20^{a}	5.15±0.16^{b}	5.64±0.19^{b}	6.12±0.10^{b}	6.59±0.25^{b}	7.05±0.21^{b}	7.15±0.15^{b}
Sodium lactate 5%	3.75±0.21^{b}	4.05±0.23^{b}	4.45±0.09^{b}	4.86±0.28^{b}	5.44±0.20^{c}	5.86±0.18^{b}	6.43±0.19^{b}	6.87±0.19^{b}
Sodium acetate 2.5%	3.82±0.21^{a}	4.49±0.23^{b}	5.33±0.21^{b}	5.72±0.20^{b}	6.15±0.17^{b}	6.73±0.21^{b}	7.18±0.19^{b}	7.25±0.15^{b}
Sodium acetate 5%	3.81±0.13^{a}	4.22±0.14^{b}	4.48±0.20^{b}	5.31±0.15^{b}	5.49±0.20^{c}	6.04±0.17^{b}	6.47±0.24^{b}	6.89±0.12^{b}

Data given as mean ± SD of 3 replicates. Means with different alphabetical superscripts in the same column are significantly different at *P <0.05.*

Table 5: Statistical analytical results of total psychrotrophic count (log_{10}cfu/g) of beef samples depending on treatment solutions and refrigerated storage period at 4°C.

Treatment	Days							
	0	3	6	9	12	15	18	21
Control	3.30±0.16^{a}	5.15±0.18^{a}	6.25±0.27^{a}	7.15±0.26^{a}	7.95±0.21^{a}	8.43±0.34^{a}	8.65±0.27^{a}	9.15±0.19^{a}
Lactic A 1%	2.92±0.15^{b}	3.66±0.18^{b}	4.67±0.29^{b}	5.08±0.23^{b}	5.58±0.32^{b}	5.82±0.24^{b}	6.14±0.27^{b}	6.47±0.19^{b}
Lactic A 2%	2.67±0.28^{c}	3.26±0.25^{c}	4.43±0.28^{c}	4.92±0.07^{c}	5.31±0.33^{c}	5.67±0.26^{c}	5.70±0.22^{c}	6.24±0.25^{c}
Acetic A 1%	2.90±0.16^{b}	3.63±0.17^{b}	4.64±0.16^{b}	5.03±0.22^{b}	5.57±0.29^{b}	5.76±0.28^{b}	6.13±0.19^{b}	6.33±0.19^{b}
Acetic A 2%	2.56±0.21^{c}	3.23±0.10^{c}	4.31±0.16^{c}	4.88±0.12^{c}	5.26±0.19^{c}	5.63±0.16^{c}	5.69±0.24^{c}	5.86±0.28^{c}
Sodium lactate 2.5%	3.26±0.23^{a}	4.06±0.14^{b}	4.95±0.14^{b}	5.47±0.20^{b}	6.03±0.16^{b}	6.25±0.20^{b}	6.90±0.13^{b}	7.28±0.22^{b}
Sodium lactate 5%	3.06±0.24^{a}	3.77±0.21^{b}	4.83±0.09^{b}	5.18±0.14^{b}	5.69±0.24^{b}	5.96±0.07^{b}	6.26±0.15^{b}	6.76±0.19^{b}
Sodium acetate 2.5%	3.20±0.14^{ab}	3.97±0.11^{b}	4.92±0.04^{b}	5.34±0.10^{b}	6.00±0.08^{b}	6.19±0.13^{b}	6.76±0.20^{b}	7.23±0.16^{b}
Sodium acetate 5%	3.00±0.10^{ab}	3.72±0.23^{b}	4.79±0.12^{b}	5.16±0.11^{b}	5.63±0.15^{b}	5.93±0.05^{b}	6.18±0.09^{b}	6.73±0.13^{b}

Data given as mean ± SD of 3 replicates. Means with different alphabetical superscripts in the same column are significantly different at *P <0.05.*

Table 6: Statistical analytical results of total pseudomonas count (log_{10}cfu/g) of beef samples depending on treatment solutions and refrigerated storage period at 4°C.

Treatment	Days							
	0	3	6	9	12	15	18	21
Control	2.81±0.10^{a}	3.24±0.13^{a}	3.63±0.17^{a}	4.36±0.22^{a}	5.84±0.14^{a}	6.22±0.08^{a}	6.83±0.08^{a}	7.25±0.22^{a}
Lactic A 1%	1.97±0.11^{b}	2.49±0.13^{b}	2.86±0.10^{b}	3.37±0.10^{b}	4.79±0.14^{b}	5.19±0.13^{b}	5.72±0.14^{b}	6.13±0.10^{b}
Lactic A 2%	1.57±0.11^{c}	2.10±0.10^{c}	2.39±0.10^{c}	2.88±0.17^{c}	3.82±0.12^{c}	4.34±0.11^{c}	4.76±0.12^{c}	5.48±0.11^{c}
Acetic A 1%	1.92±0.07^{b}	2.40±0.09^{b}	2.78±0.13^{b}	3.34±0.17^{b}	4.71±0.11^{b}	5.13±0.11^{b}	5.68±0.14^{b}	6.09±0.09^{b}
Acetic A 2%	1.49±0.12^{c}	1.94±0.08^{c}	2.32±0.15^{c}	2.79±0.11^{c}	3.54±0.18^{c}	4.15±0.11^{c}	4.53±0.13^{c}	5.21±0.08^{c}
Sodium lactate 2.5%	2.45±0.12^{a}	2.91±0.08^{b}	3.11±0.13^{b}	3.85±0.12^{b}	5.11±0.07^{b}	5.62±0.07^{b}	6.24±0.07^{b}	6.73±0.07^{b}
Sodium lactate 5%	2.15±0.07^{b}	2.63±0.13^{b}	2.98±0.08^{b}	3.62±0.07^{b}	4.89±0.09^{b}	5.47±0.08^{b}	5.98±0.08^{b}	6.25±0.08^{b}
Sodium acetate 2.5%	2.31±0.09^{a}	2.86±0.08^{b}	3.14±0.08^{b}	3.81±0.08^{b}	5.14±0.08^{b}	5.59±0.09^{b}	6.16±0.10^{b}	6.68±0.09^{b}
Sodium acetate 5%	2.03±0.04^{b}	2.54±0.11^{b}	2.88±0.08^{b}	3.53±0.11^{b}	4.81±0.07^{b}	5.26±0.09^{b}	5.78±0.08^{b}	6.22±0.08^{b}

Data given as mean ± SD of 3 replicates. Means with different alphabetical superscripts in the same column are significantly different at *P <0.05.*

Table 7: Statistical analytical results of total enterobacteriaceae count (log_{10}cfu/g) of beef samples depending on treatment solutions and refrigerated storage period at 4°C.

Treatment	Days							
	0	3	6	9	12	15	18	21
Control	1.83±0.16^{a}	3.21±0.40^{a}	3.57±0.17^{a}	4.23±0.20^{a}	4.44±0.18^{a}	4.95±0.04^{a}	5.71±0.18^{a}	6.23±0.26^{a}
Lactic A 1%	<1^{d}	2.15±0.15^{b}	2.66±0.29^{b}	2.75±0.19^{c}	2.88±0.19^{c}	2.95±0.10^{c}	3.05±0.18^{b}	3.2±0.22^{c}
Lactic A 2%	<1^{d}	< 1^{c}	2.47±0.19^{c}	2.48±0.20^{c}	2.69±0.14^{c}	2.82±0.14^{c}	2.91±0.12^{c}	3.02±0.13^{c}
Acetic A 1%	<1^{d}	2.12±0.19^{b}	2.61±0.21^{b}	2.75±0.21^{b}	2.83±0.17^{c}	2.92±0.08^{c}	3.03±0.08^{c}	3.15±0.08^{c}
Acetic A 2%	<1^{d}	< 1^{c}	2.45±0.21^{c}	2.49±0.23^{c}	2.65±0.15^{c}	2.74±0.13^{c}	2.85±0.14^{c}	2.94±0.05^{c}
Sodium lactate 2.5%	1.49±0.19^{b}	2.86±0.16^{b}	3.05±0.09^{b}	3.14±0.18^{b}	3.25±0.26^{b}	3.5±0.22^{b}	3.87±0.13^{b}	4.01±0.10^{b}
Sodium lactate 5%	1.18±0.14^{c}	2.55±0.27^{b}	2.73±0.2^{c}	2.85±0.10^{c}	2.99±0.13^{c}	3.25±0.20^{b}	3.45±0.17^{b}	3.91±0.07^{b}
Sodium acetate 2.5%	1.42±0.18^{b}	2.78±0.10^{b}	2.98±0.08^{b}	3.14±0.09^{b}	3.23±0.18^{b}	3.48±0.11^{b}	3.75±0.15^{b}	3.97±0.07^{b}
Sodium acetate 5%	1.16±0.08^{c}	2.55±0.14^{b}	2.72±0.16^{c}	2.83±0.11^{c}	2.95±0.12^{c}	3.22±0.17^{b}	3.45±0.16^{b}	3.88±0.08^{b}

Data given as mean ± SD of 3 replicates. Means with different alphabetical superscripts in the same column are significantly different at *P <0.05.*

Table 8: Statistical analytical results of staphylococcus count (log_{10}cfu/g) of beef samples depending on treatment solutions and refrigerated storage period at 4°C.

Treatment	Days							
	0	3	6	9	12	15	18	21
Control	1.45±0.10^{a}	2.14±0.11^{a}	2.63±0.07^{a}	3.36±0.07^{a}	3.52±0.08^{a}	3.66±0.11^{a}	3.81±0.08^{a}	4.05±0.08^{a}
Lactic A 1%	1.06±0.07^{b}	1.26±0.08^{c}	1.63±0.13^{c}	1.82±0.06^{b}	1.94±0.07^{b}	2.15±0.10^{b}	2.31±0.07^{b}	2.56±0.12^{c}
Lactic A 2%	< 1^{c}	< 1^{c}	1.02±0.09^{c}	1.35±0.08^{c}	1.67±0.11^{c}	1.94±0.06^{c}	2.18±0.10^{c}	2.25±0.09^{c}
Acetic A 1%	< 1^{c}	1.19±0.10^{c}	1.38±0.14^{c}	1.69±0.13^{c}	1.79±0.11^{c}	1.99±0.10^{c}	2.25±0.16^{b}	2.52±0.13^{c}
Acetic A 2%	< 1^{c}	< 1^{c}	< 1^{c}	1.03±0.10^{c}	1.31±0.08^{d}	1.63±0.13^{d}	1.96±0.09^{d}	2.19±0.12^{d}
Sodium lactate 2.5%	1.15±0.08^{b}	1.72±0.13^{b}	1.94±0.06^{b}	2.17±0.11^{b}	2.38±0.09^{b}	2.55±0.19^{b}	2.79±0.12^{b}	3.03±0.08^{b}
Sodium lactate 5%	< 1^{c}	1.33±0.07^{c}	1.55±0.15^{c}	1.72±0.08^{c}	1.84±0.08^{c}	2.06±0.07^{c}	2.31±0.10^{c}	2.57±0.10^{c}
Sodium acetate 2.5%	1.12±0.10^{b}	1.69±0.19^{b}	1.88±0.08^{b}	2.13±0.10^{b}	2.14±0.05^{b}	2.35±0.12^{b}	2.67±0.10^{b}	2.96±0.07^{b}
Sodium acetate 5%	< 1^{c}	1.24±0.12^{c}	1.41±0.11^{c}	1.70±0.16^{c}	1.82±0.08^{c}	1.99±0.06^{c}	2.28±0.10^{c}	2.53±0.13^{c}

Data given as mean ± SD of 3 replicates. Means with different alphabetical superscripts in the same column are significantly different at *P <0.05.*

Table 9: Statistical analytical results of total yeast and mould count (log_{10}cfu/g) of beef samples depending on treatment solutions and refrigerated storage period at 4°C.

Treatment	Days							
	0	3	6	9	12	15	18	21
Control	2.52±0.11^{a}	2.85±0.11^{a}	3.36±0.14^{a}	3.71±0.12^{a}	4.50±0.10^{a}	5.60±0.21^{a}	5.97±0.11^{a}	6.20±0.15^{a}
Lactic A 1%	1.60±0.18^{b}	1.66±0.13^{c}	1.79±0.14^{c}	1.86±0.09^{c}	1.95±0.05^{c}	2.29±0.08^{c}	2.57±0.10^{b}	2.98±0.12^{b}
Lactic A 2%	1.18±0.12^{c}	1.49±0.21^{c}	1.67±0.16^{c}	1.79±0.10^{c}	1.88±0.08^{c}	2.21±0.20^{c}	2.43±0.15^{b}	2.84±0.08^{b}
Acetic A 1%	1.51±0.16^{b}	1.64±0.11^{b}	1.78±0.16^{c}	1.85±0.15^{b}	1.95±0.07^{c}	2.27±0.13^{c}	2.55±0.19^{b}	2.92±0.07^{b}
Acetic A 2%	1.14±0.14^{c}	1.47±0.11^{c}	1.64±0.17^{c}	1.73±0.15^{d}	1.88±0.10^{c}	2.15±0.12^{c}	2.36±0.16^{b}	2.51±0.20c
Sodium lactate 2.5%	2.31±0.07^{a}	2.48±0.12^{b}	2.48±0.14^{b}	2.74±0.15^{b}	2.92±0.06^{b}	3.52±0.17^{c}	3.97±0.08c	4.89±0.08^{d}
Sodium lactate 5%	1.70±0.13^{b}	1.88±0.11^{c}	1.92±0.11^{c}	2.15±0.14^{b}	2.25±0.13^{b}	2.38±0.11^{c}	2.89±0.10d	3.26±0.21e
Sodium acetate 2.5%	2.22±0.10^{a}	2.44±0.18^{b}	2.47±0.14^{b}	2.69±0.12^{b}	2.92±0.13^{b}	3.50±0.16^{b}	3.95±0.06c	4.87±0.08d
Sodium acetate 5%	1.68±0.24^{b}	1.85±0.11^{c}	1.89±0.21^{c}	2.02±0.11^{b}	2.22±0.21^{b}	2.36±0.13^{c}	2.89±0.18d	3.23±0.15e

Data given as mean ± SD of 3 replicates. Means with different alphabetical superscripts in the same column are significantly different at *P <0.05.*

Table 10: Statistical analytical results of the sensory attributes for the examined beef samples.

Sensory attribute	Control	LA 1 %	LA 2%	AA1 %	AA 2%	SL 2.5%	SL 5 %	SA 2.5%	SA 5 %
Color	5.4±0.2^{a}	6.8±0.2^{b}	5.0±0.8^{a}	7.1±0.8^{b}	5.2±0.8^{a}	6.3±0.4^{b}	5.3±0.3^{a}	5.6±0.2^{c}	5.5±0.4^{c}
Texture	6.7±0.1^{a}	7.8±0.1^{b}	5.4±0.1^{c}	7.9±0.1^{b}	5.6±0.2^{c}	6.9±0.1^{a}	6.6±0.2^{e}	7.0±0.2^{d}	6.6±0.3^{a}
Flavor	7.0±0.3^{a}	7.8±0.1^{b}	6.9±0.1^{a}	8.2±0.1^{c}	7.0±0.1^{a}	7.1±0.1^{a}	6.9±0.1^{a}	7.3±0.2^{d}	7.1±0.2^{a}
Overall acceptability	6.6±0.1^{a}	7.9±0.1^{b}	6.2±0.1^{c}	8.1±0.2^{b}	6.3±0.1^{c}	7.2±0.2^{d}	6.5±0.1^{a}	7.3±0.1^{d}	6.5±0.1^{e}

Data given as mean ± SD of 3 replicates. Means with different alphabetical superscripts in the same raw are significantly different at *P <0.05.*

DISCUSSION

Physicochemical analyses:

1. Hydrogen ion concentration (pH) values:
Table (1) revealed that the pH values at zero day ranged from 5.82±0.13 in control samples to 4.6±0.14, 4.4±0.13, 4.9±0.13, 4.4±0.19, 5.73±0.08, 5.55±0.08, 5.84±0.08 and 5.69±0.12 for LA 1%, LA 2%, AA 1%, AA 2%, SL 2.5%, SL 5%, SA 2.5% and SA 5% treated samples, respectively. The previous data demonstrated that the pH value of LA (1&2%), AA (1&2%) treatments were significantly *(P<0.05)* lower than of control samples at zero day.

Nearly similar results were obtained by Mustapha *et al.* (2002) who found that the pH on zero day was 5.67 for untreated control beef sample, while the pH for samples dipped in LA 2% and SL 2% were 4.90 and 4.95, respectively, which significantly ($P<0.05$) different from the control one. Elabbasy *et al.* (2014) found that at zero day the pH value of minced beef stored at 4°C was 5.82±0.34, and 5.35±0.125 for control and acetic acid treated samples, respectively. Then, the pH value of control samples reduced till reaching 5.57±0.14 at day 12. This reduction can be attributed to breakdown of the glycogen of the slaughtered animal into glucose. Glucose undergoes glycolysis but, in the absence of oxygen, lactic acid is formed, which causes the pH in the muscles to drop (Muchenje *et al.,* 2009). After day 12 the pH value of the control samples begin to increase till reaching 6.65±0.14 at day 21. This increase in pH reflects the degree of meat spoilage through protein breakdown for the production of free amino acids, leading to the formation of NH_3 and amines, compounds of alkaline reactions Kesavan *et al.* (2014). These findings agree with Kenawi *et al.* (2009) who found that the pH value for untreated control minced beef samples (5.83) at zero day then reduced till the day twelve of storage (5.58) and then started to increase again reaching (5.76) on day 24.

During 21days of storage, the pH values for all treated samples underwent incremental increases reaching a maximum at day 21 as 5.61±0.10, 5.46±0.21, 5.68±0.11, 5.63±0.09, 5.71±0.04, 5.51±0.14, 5.51±0.14 and 5.65±0.10 for LA 1%, LA 2%, AA 1%, AA 2%, SL 2.5%, SL 5%, SA 2.5% and SA 5%, treated samples, respectively. This agreed with the results obtained by Sallam and Samejima (2004) who found that pH value was (5.71±0.02) for beef samples treated with SL 3% at day 21.

2. Total volatile basic nitrogen (TVBN) values: (mg N/100g)
Accurately, TVBN can be used as an indicator of freshness and quality deterioration in meat. EOS (2008) stated that the maximum recommended limit of total volatile basic nitrogen is 20 mg N/100g and above this limit meat begin spoilage. From table (2) the TVBN value of control beef samples was 12.50±1.00 at zero day then underwent incremental increases till reaching 20.20±1.13 at day 12 which revealed the onset of spoilage of control samples at this stage. Nearly similar results were obtained by El-Aal (2005) who found that the TVN value at zero day for control buffalo meat samples was 12.5mg N/100g, Agunbiade *et al.* (2010) who stated that fresh beef TVBN at zero day was 12.6±0.1mg N/100g at 1-2°C.

The TVBN value of all samples underwent significant *(P<0.05)* increases during 21days of storage. These increases in TVBN values as storage time increase may be attributed to protein breakdown caused by microbial activity as well as tissue proteolytic enzymes during storage periods (Moawed, 1995; and Eleiwa-Nessrien, 2003).

By the day 15, SL 2.5% and SA 2.5% samples revealed TVBN value of 20.40 ± 1.39 and 20.20 ± 1.61 mg N/100g, respectively, which indicate a shelf-life of about 12-15 days. On day 21 the lowest TVBN value was 21.00±2.43 and 20.70±2.07 mg N/100g for LA 2% and AA 2% treated samples, respectively, indicating a shelf-life of about 18-21 days of these treatments. This revealed that these treatments have antimicrobial activities and reduce the accumulation of TVBN substances results from microbial activity.

The obtained results agree with EL-Desouky *et al.* (2006) who reported that the TVN of sodium lactate (0.5%) treated minced meat samples at day 15 was 17.97 mg N/100g. The TVN increased significantly for all samples during 15 days of storage at 4°C. Also with, Smaoui *et al.* (2012) who found that the maximal allowed levels of TVN for examined samples were attained at the ninth, thirteenth, and sixteenth days for the combinations 0.3% SL+0.03% LA, 0.6% SL+0.06% LA, and 0.9% SL+0.09% LA, respectively.

3. Thiobarbituric acid reactive substances (TBARs) values: as (mg MA/kg meat)
The TBARs test widely used to estimate the extent of lipid oxidation in meat and meat products (Wu *et al.,* 2000). EOS (2008) stated that the maximum recommended limit of thiobarbituric acid is 0.9 mg MA/kg meat.

From table (3) the mean TBARs value for control samples on zero day was 0.43±0.02, on the day 12 was 0.92±0.04 which exceeded the maximum recommended limit, and then increased till reaching 1.17±0.04 on day 21.

The lowest TBARs values on zero day were 0.39±0.02 and 0.37±0.02, for LA 2% and AA 2% treated samples, respectively. The TBARs values on

day 21 were 0.61±0.03, 0.53±0.04, 0.57±0.04, 0.51±0.04, 0.80±0.03, 0.72±0.04, 0.77±0.04 and 0.68±0.03 for LA 1%, LA 2%, AA 1%, AA 2%, SL 2.5%, SL 5%, SA 2.5% and SA 5% treated samples, respectively, which indicated that all treatments were successful in maintaining TBARs values of the treated samples below the limit recommended by (EOS, 2008) and also were significantly ($p<0.05$) lower than control samples. So, addition of LA 1%, LA 2%, AA 1%, AA 2%, SL 2.5%, SL 5%, SA 2.5% and SA 5% was effective in reducing the oxidative reactions and accumulation of MA resulted from lipid oxidation of beef during 21 days of the refrigerated storage at 4°C.

Such results agree with the results of Sallam and Samejima (2004), they found that the TBA values of ground beef stored at 2°C for control and SL3%, on day zero were 0.19, and 0.17, on day 12 were 0.27, and 0.22 and on day 21 were 0.33, and 0.30, respectively. Also Kenawi *et al.* (2009) found that the TBA values of ground buffalo meat during refrigeration for control and 3% SL, on zero day were 0.22 and 0.18, on day 12 were 0.30 and 0.23 and on day 20 were 0.37 and 0.29, respectively.

Microbiological analyses:

1. Total aerobic plate count (APC):

From table (4) the APC values on the day zero were 3.86±0.45, 3.73±0.19, 3.65±0.21, 3.70±0.26, 3.62±0.35, 3.84±0.16, 3.75±0.21, 3.82±0.21and 3.81±0.13 for control, LA 1%, LA 2%, AA 1%, AA 2%, SL 2.5%, SL 5%, SA 2.5% and SA 5% treated samples, respectively. On the day 12, the APC values for the samples treated with SA 2.5% and SL 2.5% increased to 6.15±0.17 and 6.12±0.10, respectively, indicating a shelf-life of about 12 days. On the day 15, the APC values for the samples treated with AA 1%, LA 1%, SA 5% and SL 5% increased to 6.04±0.36, 6.17±0.33, 6.04±0.17 and 5.86±0.18, respectively, indicating a shelf-life of about 15 days. While, when the concentration of acetic and lactic acid was increased to 2% on the day 18 the treated samples exhibited a delayed growth for APC of 5.85±0.20 and 5.92±0.24 for AA 2% and LA 2%, respectively, which increasing the shelf-life for these samples to 18 days during refrigerated storage at 4°C. From the obtained results, samples treated by different concentrations of lactic acid, acetic acid and their sodium salts showed decreasing count of aerobic plate microorganisms than permissible limit which is 10^6 cfu/g according to (EOS, 2005) when compared to control samples. Nearly similar results were obtained by Sallam and Samejima (2004), who found that the APC of ground beef treated with SL3% was 3.78, 6.73 and 7.57 on days zero, 15 and 21, respectively, extending the self-life up to 14 days. Ibrahim-Ghada (2006) found that the APC on zero day was $2.9x10^3$, $2.6x10^3$, $1.54x10^3$ and $1.30x10^3$ for LA 1%, AA1%, LA 2% and AA 2%, respectively. Kenawi *et al.* (2009) found that the APC of ground buffalo meat treated with 3% SL was 3.8, 6.0 and 6.9 at days zero, 12 and 20, respectively.

2. Total psychrotrophic count (PTC):

From Table (5) the psychrotrophic count for control samples was 3.30±0.16, 7.95±0.21, 8.43±0.34 and 9.15±0.19 at zero, 12, 15 and 21 days, respectively. The psychrotrophic count at day 12 was 5.58±0.32, 5.31±0.33, 5.57±0.29, 5.26±0.19, 6.03±0.16, 5.69±0.24, 6.00±0.08 and 5.63±0.15 for LA 1%, LA 2%, AA 1%, AA 2%, SL 2.5%, SL 5%, SA 2.5% and SA 5% treated samples, respectively. At day 21, the psychrotrophic count underwent incremental increases till reaching 6.47±0.19, 6.24±0.25, 6.33±0.19, 5.86±0.28, 7.28±0.22, 6.76±0.19, 7.23±0.16 and 6.73±0.13 for LA 1%, LA 2%, AA 1%, AA 2%, SL 2.5%, SL 5%, SA 2.5% and SA 5% treated samples, respectively. These results indicated that AA 2% or LA 2% significantly ($P< 0.05$) reduced total psychrotrophic count in refrigerated beef, followed by AA 1%, LA 1%, SA 5%, SL 5%, SA 2.5% and SL 2.5%.

Nearly similar results were obtained by Ibrahim-Ghada (2006), who found that AA 2% significantly (*P<0.05*) reduced psychrotrophic count on fresh lamb carcasses more than treated with AA 1%, LA 1% and LA 2% within 14 days of refrigerated storage.

3. Total pseudomonas count:

From table (6) on day zero the pseudomonas count ranged from 2.81±0.10 in control samples to 1.97±0.11, 1.57±0.11, 1.92±0.07, 1.49±0.12, 2.45±0.12, 2.15±0.07, 2.31±0.09 and 2.03±0.04 for LA 1%, LA 2%, AA 1%, AA 2%, SL 2.5%, SL 5%, SA 2.5% and SA 5% treated samples, respectively. The pseudomonas count underwent incremental increases during 21 days of storage for all examined samples.

On day 21, the pseudomonas count was 7.25±0.22, 6.13±0.10, 5.48±0.11, 6.09±0.09, 5.21±0.08, 6.73±0.07, 6.25±0.08, 6.68±0.09 and 6.22±0.08 for control, LA 1%, LA 2%, AA 1%, AA 2%, SL 2.5%, SL 5%, SA 2.5% and SA 5% treated samples, respectively. The previous data showed that the pseudomonas count for the control samples was significantly *(P<0.05)* higher than those of all treated samples during 21 days of storage and this indicate that the used treatments have antimicrobial effects. From the previously mentioned data we found that AA 2% and LA 2% were the most significantly *(P<0.05)* effective treatments in reducing the pseudomonas count.

Nearly similar results were obtained by Aksu and Alp (2012) they found that the highest pseudomonas count on zero day was 2.41 $\log_{10}$cfu/g for control beef samples that reached 7.81 $\log_{10}$cfu/g by day 14 of storage. Our results agree with the results

obtained by Gammariello *et al.* (2014), they found that the SL 3.60% was the most effective treatment against Pseudomonas spp. A decrease in pseudomonas populations for treated samples was observed from day 1 until the last day of storage, in comparison to the control sample.

4. Total enterobacteriaceae count:

Table (7) showed that on zero day the enterobacteriaceae count for control samples was 1.83±0.16, 4.44±0.18, 4.95±0.04 and6.23±0.26 at zero, 12, 15 and 21 days. On day zero the Enterobacteriaceae count was <1, <1, <1, <1, 1.49±0.19, 1.18±0.14, 1.42±0.18 and 1.16±0.08 for LA 1%, LA 2%, AA 1%, AA 2%, SL 2.5%, SL 5%, SA 2.5% and SA 5% treated samples, respectively, and this indicated that application of LA 1%, LA 2%, AA 1% and AA 2% on beef samples significantly *(P<0.05)* lowered the enterobacteriaceae count to undetectable levels in comparison to control and other treatments. Also, the enterobacteriaceae count remained undetectable on day 3 for LA 2% and AA 2% treated samples.

Nearly similar results recorded by Smaoui *et al.* (2012) who found that treating chicken thighs with 0.6% SL plus 0.6% LA and 0.75% SL plus 0.075% LA lowered the enterobacteriaceae count to undetectable levels until the day 3, while treating them with 0.9% SL+0.09% LA lowered the enterobacteriaceae count to undetectable levels until the day 15 of storage. By the day 21 the enterobacteriaceae count was 3.2±0.22, 3.02±0.13, 3.15±0.08, 2.94±0.05, 4.01±0.10, 3.91±0.07, 3.97±0.07 and 3.88±0.08 for LA 1%, LA 2%, AA 1%, AA 2%, SL 2.5%, SL 5%, SA 2.5% and SA 5% treated samples, respectively. The current results agree with those obtained by Sallam and Samejima (2004) who found that the initial enterobacteriaceae count increased from 1.8 in control ground beef samples at day zero to a higher count of 7.39 by day 21 of storage, while it reached significant *(P<0.05)* lower counts of 5.19 in beef samples treated with SL3% when compared with control. From the previously mentioned data we found that AA 2% and LA 2% were the most significantly (*P<0.05*) effective treatment in reducing the enterobacteriaceae count followed by AA 1%, LA1%, SA 5%, SL 5%, SA 2.5% and SL 2.5%. These results agree with the results of Ibrahim-Ghada (2006) who found that the enterobacteriaceae count for lamb carcasses treated with LA 2% and AA 2% was significantly lower than LA 1% and AA 1%.

5. Staphylococcus count:

From results given in table (8) the staphylococcus count for control samples was 1.45±0.10, 2.63±0.07, 3.52±0.08 and3.81± 0.08 at zero, 6, 12 and 18 days, respectively. On day zero the staphylococcus count was 1.06±0.07, <1, <1, <1, 1.15±0.08, <1,1.12±0.10 and <1 for LA 1%, LA 2%, AA 1%, AA 2%, SL 2.5%, SL 5%, SA 2.5% and SA 5% treated samples, respectively and this indicated that application of LA 2%, AA 1%, AA 2%, SL 5% and SA 5% on beef samples significantly *(P<0.05)* lowered the staphylococcus count to undetectable levels in comparison to control and other treatments. Also, the staphylococcus count remained undetectable until day 3 for LA 2% and until day 6 for AA 2% treated samples.

The staphylococcus count underwent incremental increases during 21 days of storage for all examined samples. By the day 21 the staphylococcus count was 2.56±0.12, 2.25±0.09, 2.52±0.13, 2.19±0.12, 3.03±0.08, 2.57± 0.10, 2.96±0.07 and 2.53±0.13 for LA 1%, LA 2%, AA 1%, AA 2%, SL 2.5%, SL 5%, SA 2.5% and SA 5% treated samples, respectively.

The current results are in agreement with that of Khurana (2009) who found that acetic acid marinade reduced staphylococcus count in ground beef from 8 to 5 $\log_{10}$cfu/g within 28 days and after that a further reduction to 4 $\log_{10}$cfu/g. Eniolorunda *et al.* (2014) found that the application of acetic acid (5%) on fresh beef cuts significantly *(P<0.05)* reduced staphylococcus count from 6.81 $\log_{10}$cfu/g in control samples to 6.54 $\log_{10}$cfu/g in the acetic acid treated samples during 14 days of storage.

6. Total yeasts and molds count:

From table (9) the total yeast and mould count for control samples was 2.52±0.11, 2.85±0.11, 3.36±0.14, 3.71±0.12, 4.50±0.10 and 6.20±0.15 at zero, 3, 6, 9, 12 and 21 days, respectively. On zero day the yeast and mould count was 1.60±0.18, 1.18±0.12, 1.51±0.16, 1.14± 0.14, 2.31±0.07, 1.70±0.13, 2.22±0.10 and 1.68±0.24 for LA 1%, LA 2%, AA 1%, AA 2%, SL 2.5%, SL 5%, SA 2.5% and SA 5% treated samples, respectively. The yeast and mould count underwent incremental increases during 21 days of storage for all examined beef samples. By the day 21 the yeast and mould count was 2.98±0.12, 2.84±0.08, 2.92±0.07, 2.51±0.20, 4.89±0.08, 3.26±0.21, 4.87±0.08 and 3.23±0.15 for LA 1%, LA 2%, AA 1%, AA 2%, SL 2.5%, SL 5%, SA 2.5% and SA 5% treated samples, respectively.

The obtained results agree with the results obtained by Ibrahim-Ghada (2006) who found that the mean yeast and mould count for control lamb carcasses stored for 14 days was 2.47±1.48, while the mean yeast and mould count was 2.25±1.30, 2.08±1.30, 2.18±1.23 and 1.91±1.08 for LA 1%, LA 2%, AA 1% and AA 2% treated samples, respectively. In addition Bingol & Bostan (2007) found that the yeast and mould count for control sausage samples was 3.73 and 3.50 at 10 and 20 days, respectively, while for samples treated with SL

1.8% was 2.91 and 3.26 at 10 and 20 days, respectively.

Sensory Evaluation:

The most common method in evaluating the freshness of meat is the sensory evaluation. It offers a fast, easy, and immediate information on the product quality. Table (10) revealed the means of color, texture, flavor and overall acceptability for the examined beef samples. The overall acceptability score for examined beef ranged from 6.6±0.1 for control samples to 7.9±0.1, 6.2±0.1, 8.1±0.2, 6.3±0.1, 7.2±0.2, 6.5±0.1,7.3±0.1 and 6.5±0.1 for LA 1%, LA 2%, AA 1%, AA 2%, SL 2.5%, SL 5%, SA 2.5% and SA 5% treated samples, respectively.

From the previously mentioned data we found that 1% LA and 1% AA significantly *(P<0.05)* brought the best overall acceptability score, while 2% LA and 2% AA treatments brought the lowest overall acceptability score among the examined samples and all the samples analyzed were considered acceptable during the sensory analysis. Such results agree with that obtained by Quilo *et al.* (2009) reported that the use of potassium lactate (PL) on beef trimmings before grinding could improve or maintain the same sensory properties (odor and taste) of ground beef. In addition, Smaoui *et al.* (2012) found that samples treated with 0.9% SL and 0.09% LA showed the highest overall acceptability score of 8.1±0.17 while that treated with 0.75% SL/0.075% LA that showed an overall acceptability of 7.4±0.12 which is in contrary with the present findings.

CONCLUSION

Treatment beef samples with dipping in 1 and 2% lactic acid, 1 and 2% acetic acid, 2.5 and 5% sodium lactate and 2.5 and 5% sodium acetate solutions then chilling at 4°C for 3, 6, 9, 12, 15, 18 and 21 days, can reduce the physicochemical changes, delay the microbial growth of treated beef samples. Among the of different concentrations of lactic acid, acetic acid and their sodium salts, AA 2% and LA 2% demonstrated the most potent effect than other concentrations, followed by AA 1%, LA 1%, SA 5%, SL 5%, SA 2.5% and SL 2.5%. However, addition of AA 2% or LA 2%, did not result in a strong flavor and, at the same time, they produced significant antioxidant and antimicrobial effects and extended the shelf-life of the product up to 21 days.

Overall, the use of lactic acid, acetic acid, sodium lactate and sodium acetate for decontaminating fresh beef shortly before chilling resulted in improving microbial safety and extending the shelf-life. So, the previously mentioned organic acids and their salts can be used for beef preservation as efficient, safe, and cost-effective preservatives.

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تأثير بعض الاحماض العضوية وأملاحها على الجودة الميكروبية وفترة صلاحية اللحوم البقرية

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يهدف هذا العمل الي دراسة تأثير معالجة اللحوم الطازجة ببعض الأحماض العضوية واملاحها على فترة الصلاحية والجودة الميكروبية لهذه اللحوم خلال الحفظ بالتبريد حيث انه تم تجميع ١٤٤ عينة من اللحوم البقرية الطازجة من أحد المسالخ بمحافظة القليوبية وتم تجهيزها وتوزيعها الى مجموعات ومعالجتها عن طريق الغمس في محاليل من حمض اللاكتيك (١% و ٢%) وحمض الخليك (١% و ٢%) ولاكتيات الصوديوم (٢،٥ % و ٥ %) وخلات الرصاص (٢،٥ % و ٥ %) تم حفظها مبردة عند درجة حرارة ٤ درجة مئوية لمدة ٢١ يوم. خلال فترة الحفظ تم اجراء الاختبارات الفيز وكيميائية والمكروبيولوجية على العينات المعالجة كل ٣و ٦و ٩و ١٢و ١٥و ١٨و ٢١ يوم. وقد تم أيضا فحص العينات حسيا اثناء فترة الدراسة. كشفت نتائج التحليل الفيز وكيميائية عن وجود انخفاض معنوي في قيمة الأس الهيدروجيني حيث تراوحت متوسطات الأس الهيدروجيني في بداية التجربة من٥,٨٢ ± ٠,١٣ للمجموعة الضابطة الى ٤,٦ ± ٠,١٤ و ٤,٤± ٠,١٣ و ٤,٩ ± ٠,١٣ و ٤,٤٠ ± ٠,١٩ و ٥,٧٣± ٠,٠٨ و ٥,٥٥ ± ٠,٠٨ و ٥,٨٤ ± ٠,٠٨ و ٥,٦٩ ± ٠,١٢ لكلا من حمض اللاكتيك (١%و ٢%) وحمض الخليك (١%و ٢%) ولاكتيات الصوديوم (٢،٥ % و ٥ %) وخلات الرصاص (٢،٥ % و ٥ %) على التوالي وكانت أعلى قيمة للأس الهيدروجيني ٥,٧٣± ٠,٠٨ للمجموعة الضابطة في حين كان أدنى مستوى ٥,٤٦± ٠,٢١ لحمض اللاكتيك (٢%) في اليوم ٢١. كانت متوسطات المركبات النيتروجينية الطيارة في بداية التجربة ١٢,٥± ١,٠ للمجموعة الضابطة وكانت أفضل نسبة انخفاض هي ٢٠,٧٠ ±٢,٠٧ لحمض الخليك (٢%) في اليوم ٢١ من التجربة وكانت أعلى قيمة لمتوسط المركبات النيتروجينية الطيارة ٢٥,٧٠± ٢,٨٤ للمجموعة الضابطة. أثبتت النتائج أيضا ان متوسط قيمة حمض الثيوبابيتيوريك في بداية التجربة ٠,٤٣ ± ٠,٠٢ للمجموعة الضابطة. كانت أعلى قيمة لمتوسط حمض الثيوبابيتيوريك ١,١٧ ± ٠,٠٤ للمجموعة الضابطة بينما أدنى قيمة ٢,٠٧± ٠,٥١ لحمض الخليك (٢%) في اليوم ٢١ من التجربة. وأوضحت النتائج أيضا أن محاليل المعالجة المختلفة سابقة الذكر كانت فعالة على البكتيريا الهوائية والبكتريا المحبة للبرودة وبكتريا السيدوموناس والبكتيريا المعوية وبكتريا المكور العنقودي الذهبي وأيضا الخمائر والفطريات. وكشف تحليل الصفات الحسية أن اعلى نسبة للقبول العام كانت ٨,١ ± ٠,٢ لعينات لحوم البقر المعالجة بحمض الخليك ١% في حين كان أدنى مستوى ٦,٢ ± ٠,١ للعينات المعالجة بحمض اللاكتيك ٢% وكانت نتيجة القبول العام ٦,٦ ± ٠,١ لعينات المجموعة الضابطة. وقد سجلت العينات المعالجة بحمض الخليك ١% وحمض اللاكتيك ١% أفضل سمات حسية من حيث اللون والملمس والنكهة. ووفقا للمواصفة القياسية المصرية (٢٠٠٨) فان فترة الصلاحية الموصي بها للحوم المبردة المحفوظة عند درجة حرارة (٠ – ٢ درجة مئوية) هي ١٤ يوم. وقد وجدنا في دراستنا أن فترة صلاحية اللحوم المبردة والمحفوظة عند درجة حرارة (٤ درجة مئوية) كانت ٩ أيام للمجموعة الضابطة وقد زادت الى ١٢ يوم عند استخدام لاكتيات الصوديوم ٢،٥% وخلات الصوديوم ٢،٥% و ١٥ يوم باستخدام حمض اللاكتيك ٢% وحمض الخليك ٢% وعليه فان استخدام هذه المعالجات قد ساهم بشكل فعال في تحسين السلامة الميكروبية وتمديد فترة صلاحية اللحوم الطازجة المبردة باستخدام مواد حافظة فعالة وامنة وغير مكلفة.