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## **EFFECT OF LACTIC ACID AND ACETIC ACID ON THE QUALITY OF LOCAL BEEF** (With 5 Tables and 3 Figures)

By

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**تأثير حمض اللاكتيك وحمض الخليك على جودة اللحوم البقرية المحلية**

**عزة على حسين التابعي ، زينب إبراهيم سليمان**

تعرض لحوم الذبائح للتلوث بالميكروبات أثناء الذبح والتجهيز مما يؤثر سلبا على جودة اللحوم وصلاحيتها لذلك تم استبيان اثر استخدام حمض اللاكتيك والخليك كمواد أمنة على جودة اللحوم البقرية المحلية وفترة صلاحيتها. أجريت الدراسة على خمس مجموعات من اللحوم البلدية فور نبحها وتجهيزها. تم معالجة المجموعة الأولى والثانية بالرش بمحلول حمض الخليك بتركيزات ٢ و ٤% على التوالي والمجموعة الثالثة والرابعة بحمض اللاكتيك بتركيزات ٢ و ٤% على التوالي وتركت المجموعة الخامسة كمجموعة ضابطة بدون معالجة. تم حفظ العينات بالتبريد عند درجة ٤ م لمدة ٩ ايام. تم اخذ عينات من كل مجموعة قبل المعالجة وبعد المعالجة على فترات محددة ( ٣٠ دقيقة و ١، ٣، ٥، ٧، ٩ يوم). هذا وقد اعتمد التقويم على إجراء العدد الكلي لكل من البكتريا الهوائية والبكتريا المحبة للبرود والمجموعة القولونية وكذلك الكشف الحسي وقياس الأس الهيدروجيني وقياس المواد النيتروجينية الطيارة. وقد ثبت من النتائج أن لكل من حمض الخليك وحمض اللاكتيك تأثير مباشر على جودة اللحوم من الناحية البكتريولوجية من بداية التجربة مقارنة بالمجموعة الضابطة حيث قل الحمل الميكروبي للمجموعات المعالجة مما كان له الأثر في زيادة مدة صلاحية اللحوم حيث تراوحت مدة صلاحية اللحوم المعالجة من ٧-٩ يوم مقارنة بالمجموعة الضابطة التي كانت فترة صلاحيتها ٣ يوم. هذا وقد كان للمعالجة بحمض اللاكتيك بتركيز ٤% أعلى تأثير في القضاء على الميكروبات إلا انه لوحظ تغير في الصفات الحسية من حيث اللون والرائحة. وتبين من النتائج أن حمض اللاكتيك ذو تأثير يفوق حمض الخليك في القضاء على الميكروبات وكذلك على جودة اللحوم من حيث الكشف الحسي والكيميائي وذلك لكل من التركيزين المستخدمين. وفي نفس الوقت وجد تحسن ملحوظ في الخواص الحسية في المجموعة التي تم معالجتها بحامض اللاكتيك بتركيز ٢% مقارنة بالمجموعة الضابطة مما يوصى باستخدام تركيز ٢% حمض اللاكتيك كمادة أمنة وذلك في المجازر بعد الذبح والتجهيز مباشرة للحصول على لحوم أمنة تتوافق مع متطلبات سلامة الغذاء وكذلك الحفاظ على صلاحية اللحم المحلية حتى تصل للمستهلك.

## SUMMARY

Effect of lactic acid and acetic acid spray on the quality and shelf-life extension of fresh beef stored at 4°C was investigated. Lactic and acetic acid were sprayed over the meat at various concentrations of 0 (control), 2% lactic acid (L.A.), 4% L.A, 2% acetic acid (A.A.) and 4% A.A. then stored at 4°C for 9 days. Samples were examined in the fresh state (0 day), prior to spay. At specified time (30 min,1, 3, 5, 7 and 9 days), samples were analyzed for microbial counts, sensorial properties, pH and total volatile basic nitrogen(TVB-N). Bacterial groups count to indicate effectiveness of treatments included aerobic plate counts (APCs), Psychrophilic counts (PTCs) and total coliform counts (TCCs). Since the beginning of the experiment, the inhibitory effect of lactic acid and acetic acid has been observed. Lactic and acetic acid reduced the microbial counts immediately after the treatment and retarded microbial growth during storage. At first day of display, APCs were reduced by 1.5 to 3 log cycles, log psychrophilic counts were consistently reduced and log coliform counts were reduced to undetectable levels in acid treated groups. The inhibitory effect on bacterial growth was pronounced when the concentration of lactic acetic and lactic acids increased. Acetic and lactic acid treatments improved the microbiological and physicochemical qualities of meat and consequently prolongs their shelf life. Shelf-life of acid treated samples from microbial quality standpoints was 7 -9 days according to treatment used against 3 days in untreated samples. Four percent lactic acid treatments appeared to be more effective in delaying the microbial growth however; meat sample could not keep their color and odor. Comparing the effects of the two acids treatments, lactic acid was found to be more effective in both concentrations used. Lactic acid sprayed samples, particularly with 2%, showed the greater acceptability than did those sprayed with acetic acid throughout the storage days. From microbial and visual quality standpoints, meat sample sprayed with 2% seemed to be more acceptable regarding meat color and bacterial numbers with proper hygiene and handling procedures could provide safer meat with good quality and to ensure safe public health.

*Key words: Meat quality, beef, acetic acid, lactic acid.*

## INTRODUCTION

Fresh meat has unique biological and chemical properties and its nutrients composition represents an optimum medium for microbial growth. The deep tissues of meat carcasses are intrinsically sterile with the majority of microorganisms being found on the skin or any surfaces exposed during slaughter processing (Gill, 1980). Meat carcasses may become contaminated from fecal material, paunch contents and the hide (Lahr, 1996). Additional sources of cross contamination exist in the slaughter process such as processing tools and equipment, a structural component of the facility, human contact and carcass-to-carcass contact (Institute of Food Technologists, 2002).

Fresh meat has a short shelf life due to microbial spoilage of both pathogenic and non-pathogenic (Dickson and Anderson, 1992). Spoilage can be defined as any change in a food product that makes it unacceptable to the consumer from a sensory point of view (Gram *et al.*, 2002). In the case of meat, microbial spoilage leads to the development of off-odors and often slime formation, which make the product undesirable for human consumption (Hilario *et al.*, 2004).

The color of fresh meat is an important quality parameter that determines a consumer's response and decision to buy or not to buy that product at retail. The bright red oxygenated form of oxymyoglobin is most commonly associated with desirability in fresh beef, while oxidation of heme iron to its ferric state (metmyoglobin) is a prime factor in unacceptability as judged by consumers (Strange *et al.*, 1974). According to Seideman *et al.* (1984) the discoloration of meat is predominately due to bacterial growth and reduced oxygen tensions which facilitate the formation of metmyoglobin. The development of organoleptic spoilage is related to microbial consumption of meat nutrients such as sugars and free amino acids and the release of undesired volatile metabolites. Total volatile basic nitrogen (TVB-N) values could potentially be used as indicators in predicting the microbial quality of beef during chilled storage under aerobic conditions (Byun *et al.*, 2003).

Since microbial growth in beef and its products occurs primarily at the surface, attempts have been made to delay spoilage by using an organic acid spray or dips as antimicrobial agents (Siragusa and Dickson, 1992). Lactic acid and acetic acid have been used as food preservatives and are generally recognized as safe (Branen *et al.*, 1990). Solutions of lactic and acetic acid are commonly used by the slaughter

industry as antimicrobial spray wash interventions to reduce the microbial load on freshly slaughtered carcasses (Berry and Cutter, 2000). The effectiveness of organic acids is best achieved shortly after hide removal when the carcass is still warm (Huffman, 2002). In the US, organic acids are applied as part of a carcass wash pre-chill (USDA/FSIS, 2004).

Several studies demonstrated the effectiveness of lactic acid as an antimicrobial intervention in red meat processing (Hardin *et al.*, 1995). The natural content of lactic acid in meat is approximately 10 g /kg it contributes to the flavor of meat and owing to its antimicrobial effects and keeping quality (Bolder, 1997). The advantage of using lactic acid is that it does not require the use of high pressure spraying to eliminate the pathogens from meat carcasses. In addition, it remains on the meat carcasses, and works as antimicrobial agent on carcass surfaces (Ramirez *et al.*, 2001). Moreover, the lactate anion slows down the growth of surviving microbes during storage (Dincer and Baysa, 2004).

Acetic acid is a mono carboxylic acid with a pungent odor and taste. It is commonly known as vinegar which has antimicrobial capabilities due to its ability to lower the pH and cause instability of bacterial cell membranes (Jay, 1992).

The mode of action of organic acid is that the undissociated acid penetrates the cell of microorganisms by means of diffusion and then dissociates and acidify the cell interior, thus interfering with cellular metabolism or decreasing the biological activity as a result of pH changes of the cell's environments (Cherrington *et al.*, 1991). The ultimate pH of meat was significant for its resistance to spoilage because most bacteria grow optimally at about pH 7 and not well below pH 4 or above pH 9 (Walker and Betts, 2000). According to many studies the ultimate pH and meat color are the most important indices of meat quality (Brunso *et al.*, 2005).

Consumers demand high quality, natural, nutritious, fresh appearance and convenient meat with natural flavor and taste and an extended shelf-life. Generally, the quality of beef can be based on color, odor, appearance, taste, culinary properties as well as bacteriological status (Eneji *et al.*, 2007). Therefore, to match all these demands without compromising safety the present study was undertaken to evaluate effect of lactic and acetic acids spray on microbial, sensory and physico-chemical qualities and on shelf-life of fresh meat.

## **MATERIALS and METHODS**

### **1 - Acetic and lactic acid spray solutions:**

Normal household vinegar contains 5% acetic acid and lactic acid (Loba Chemie Pvt, Ltd., 88%) were used. Acid spray solutions were prepared fresh on the day of use. Solution for spray (2% and 4%) were prepared by dilution of acetic acid and lactic acid in tap water. A manual sprayer was used for application of the solution to the meat surface. Meat surface sections were sprayed with 2 and 4 % acid solutions at an amount 2 ml per 100 gm (Sundar and Zhang, 2006).

### **2- Experimental samples:**

Twenty five kilograms of fresh beef were obtained from recent slaughtered animal after arrival of the meat to butcher's shop in Port Said, Egypt. The collected meat was taken from hindquarter after preparation (without visible fat). Meat was rapidly transferred as possible to the laboratory in an ice box with minimum delay. Samples were divided into five groups. Meat samples were thoroughly rinsed with tap water before the acid spray was applied. The first and second groups were sprayed with 2% and 4% lactic acid respectively. Third and fourth groups were sprayed with 2% and 4% acetic acid respectively. The fifth group was kept as controls, untreated sample.

### **3- Sampling:**

A representative parts from each group was examined for background bacterial levels, in the fresh state (0 day) prior to spray. After packaging, samples were stored at 4 °C (9 days). At specified time intervals (30 min, 1, 3, 5, 7 and 9<sup>th</sup> days of display), samples were withdrawn and assessed for aerobic plate count (APCs), psychophilic counts (PTCs), total coliforms count (TCCs), pH, total volatile basic nitrogen (TVB-N) and sensory attributes. Five replications were performed for each group.

### **4- Bacteriological analysis:**

Meat samples of 25 g were aseptically removed from the bags and homogenized in 225 ml of sterile peptone water (0.1%) for 1 min using a Stomacher. From this homogenate, decimal serial dilutions were made in the same sterile peptone water and used for microbiological analyses of the beef samples at each of the appropriate time intervals during refrigerated storage. Aerobic plate counts (APCs) were determined by inoculating 0.1 ml of the sample homogenate, at selected dilutions, onto duplicate sterile plates of plate count agar using the surface spread technique, then the plates were incubated for 72 h at 30°C (ISO: 4833, 2003, Standard). Psychophilic counts (PTC) were

determined in a similar method to that for APC except that plates were incubated at 5°C for 10 days. (ISO: 17410, 2001, Standard). To determine total coliforms counts (TCCs), 1 ml of the appropriate dilution was inoculated by the pour-plated method on violet red bile agar and overlaid with approximately 5 ml of the same growth medium then the plates were incubated at 37°C for 24 h.(ISO: 4832,2006, Standard). Colonies were counted and expressed as log<sub>10</sub> forming units (Log<sub>10</sub> CFU)/g of meat.

#### **5- Sensory analysis:**

Sensory characteristics of samples were investigated by 9 panelists in Food Hygiene Department, Port-Said Lab to color and odor at the same display intervals of bacteriological analysis. Evaluation was performed under white fluorescent light. Sample being judged for color was placed in a glass Petri dish and viewed under daylight fluorescent light against a neutral gray background. For odor evaluation, a portion of the meat sample was presented in glass stoppered 125-ml Erlenmeyer flasks wrapped in tissue to mask appearance. The odors of samples were judged in air-conditioned. Five replicates of each sample were submitted to the sensory analysis.

A 5-point structured hedonic scale was used to score the following: where (5) is the best and (1) is the lowest. Sensory panelists evaluated overall color (5=bright red, 4=dull red, 3=slightly brownish red, 2=moderately brownish red, 1=brown) .Panelists also evaluated beef odor (5=no off odor, 4=slight off odor, 3=small off odor, 2=moderate off odor, and 1=extreme off odor) (Hunt *et al.*, 1991).

Panel members were also asked to note whether the meat samples were acceptable or unacceptable in overall appearance following AMSA (1991) guidelines. 8=Extremely desirable, 7=desirable , 6= moderately desirable, 5= slightly desirable, 4= slightly undesirable , 3= moderately undesirable, 2= undesirable, 1= Extremely undesirable .Each package was evaluated until at least 80% of the samples in that group had been assigned a mean overall appearance score of 3 (moderately undesirable) or lower. The development of unpleasant odor, color, softening, and the formation of a sticky layer on the surface were evaluated as the indicators of spoilage.

#### **6- Determination of pH:**

Meat samples (10 g) from each treatment (n = 5/ treatment) at each sampling day were separately homogenized with 100 ml of distilled water in a stomacher. The pH of the homogenates was measured using a pH electrode attached to a pH meter (AOAC, 1984).

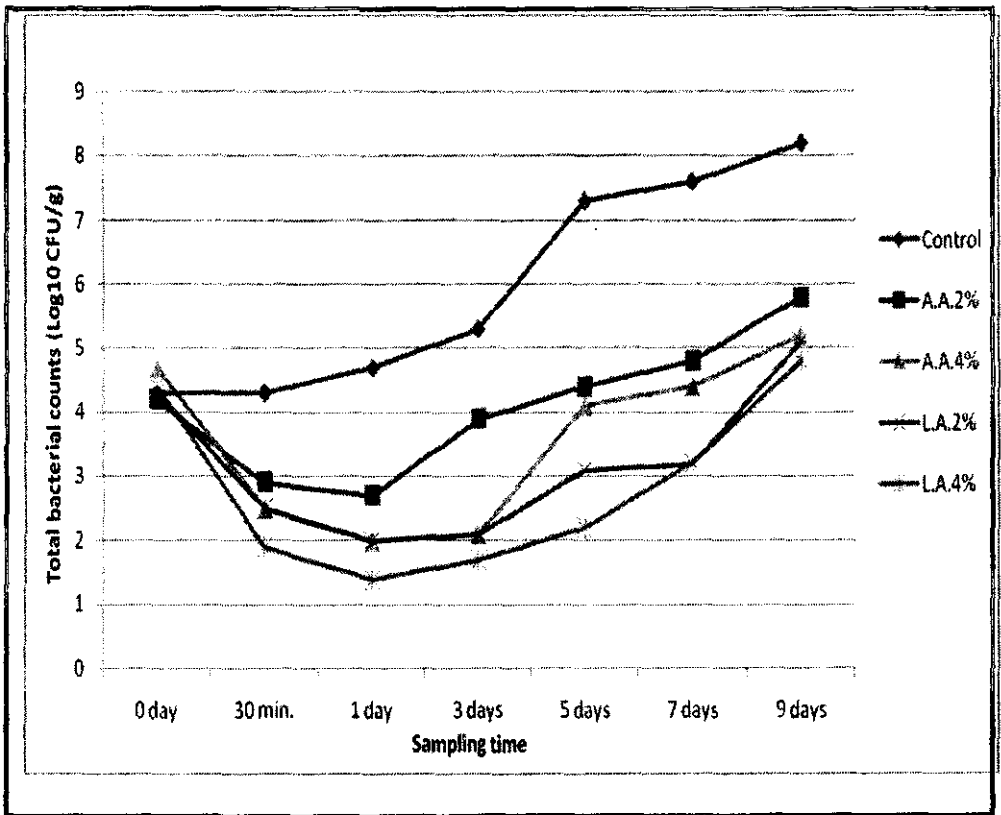
**7- Total volatile bases nitrogen (TVB-N) determination:**

Total volatile bases nitrogen (TVB-N) content was determined according to the method described by Inal (1992).

**8- Statistical Analysis:**

Mean and standard error of mean were used to describe data. One-Way ANOVA test was used to compare the effects of different treatments. P value was considered significant if less than 0.05 and highly significant if less than 0.01. These tests were analyzed using the Statistical Package for Social Scientists (SPSS) for windows 16.0 (SPSS Inc., Chicago, IL, and USA).

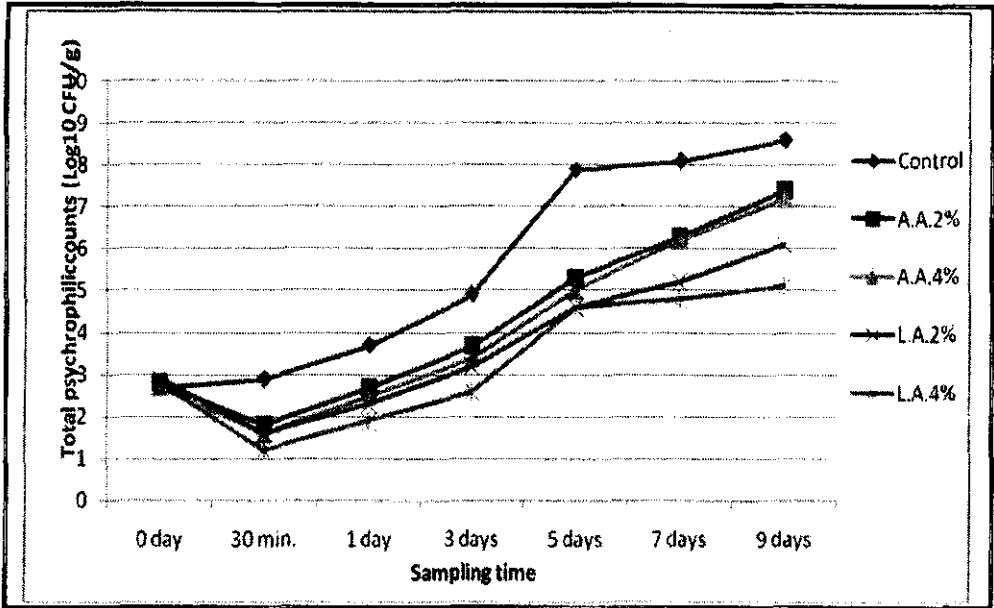
**RESULTS**



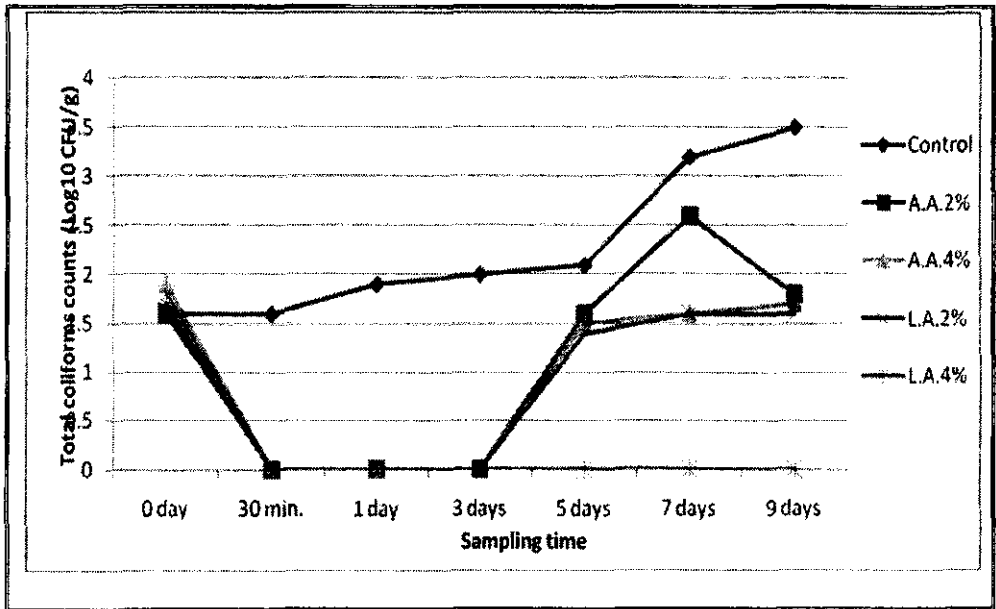
**Fig. 1:** Mean aerobic plate counts ( $\log_{10}$  CFU/g) of control and acid treated beef during storage at 4°C (n=5).

A.A.: Acetic acid

L.A.: Lactic acid



**Fig. 2:** Mean psychrophilic counts ( $\log_{10}$  CFU/g) for control and acid sprayed beef during storage at 4°C (n=5).  
 A.A.: Acetic acid                      L.A. : Lactic acid



**Fig. 3:** Mean total coliforms counts ( $\log_{10}$  CFU/g) for control and acid treated beef during storage at 4°C (n=5).  
 A.A.: Acetic acid                      L.A. : Lactic acid



**Table 1: Mean color evaluation scores for control and acid treated beef during storage at 4°C (n=5).**

Storage period	Control	2%A.A.	4%A.A.	2%L.A.	4%L.A.
0	4.8±0.12	4.7±0.11	4.7±0.12	4.7±0.18	4.6±0.16
30 min.	4.7±0.12	4.0±0.16*	3.5±0.13**	4.7±0.15	4±0.18*
1 day	4.6±0.14	3.5±0.18**	3.2±0.18**	4.8±0.13	4±0.11
3 day	2.3±0.18	3.0±0.15*	2.5±0.18*	4.9±0.1**	4±0.18**
5 day	1±0.18	2.1±0.18**	2.0±0.12**	4.5±0.18**	4±0.15**
7 day	NA	1.2±0.18	1.5±0.18	3.5±0.16	3.6±0.16
9 day	NA	1.0±0.00	1.0±0.00	2.5±0.12	3.0±0.12

NA= Not analyzed (Rejected), A.A.: Acetic acid, L.A.: Lactic acid

Overall color (5=Bright red, 4=Pale red, 3= slightly brownish red, 2=moderately brownish red, 1=Brown).

\*:Significant correlation at P < 0.05, relative to control at the same storage period.

\*\* :Highly Significant correlation at P < 0.01, relative to control at the same storage period.

**Table 2: Mean (± SE) odor evaluation scores for control and acid treated beef during storage at 4°C (n=5).**

Storage period	Control	2%A.A.	4%A.A.	2%L.A.	4%L.A.
0	4.9±0.18	4.8±0.18	4.9±0.18	4.9±0.18	4.9±0.18
30 min.	4.9±0.18	3.2±0.18**	2.5±0.18**	4.5±0.13	3.8±0.20*
1 day	3.6±0.18	3.1±0.18*	2.4±0.18**	4.1±0.18*	3.7±0.18
3 day	2±0.18	3±0.18**	3±0.18**	4.1±0.18**	3.5±0.18**
5 day	1±0.18	3.2±0.18**	3±0.18**	3.6±0.18**	3.2±0.18**
7 day	NA	2±0.18	2.5±0.18	3.2±0.18	3.1±0.18
9 day	NA	1±0.18	1.2±0.18	3.1±0.18	2.6±0.18

NA= Not analyzed (Rejected), A.A.: Acetic acid, L.A.: Lactic acid

NB: Off-odor =Any change in odor

Odor score : 5=no off odor, 4=slight off odor, 3=small off odor, 2=moderate off odor, 1=extreme off odor.

\*:Significant correlation at P < 0.05, relative to control at the same storage period.

\*\* :Significant correlation at P < 0.01, relative to control at the same storage period.

**Table 3: Overall appearance score of meat samples during storage.**

Storage period	Control	2%A.A.	4%A.A.	2%L.A.	4%L.A.
0	7.8±0.12	7.9±0.15	7.7±0.15	7.7±0.15	7.8±0.15
30 min.	7.9±0.15	7±0.15	6.5±0.13	7.9±0.15	6.9±0.15
1 day	6.5±0.13	6±0.15	6.0±0.05	7.9±0.13	6.4±0.15
3 day	4.8±0.15	6±0.05	5.2±0.04	7.9±0.12	6.2±0.15
5 day	3.2±0.13	5.1±0.05	4.9±0.15	6.3±0.15	6.2±0.15
7 day	1±0.00	4.0±0.03	3.7±0.15	5.4±0.15	5.3±0.15
9 day	NA	3.0±0.12	2.7±0.05	3.4±0.15	3.6±0.15

NA= Not analyzed (Rejected), A.A.: Acetic acid , L.A.: Lactic acid

Overall appearance score: 8=Extremely desirable, 7= desirable, 6= moderately desirable, 5= slightly desirable, 4= slightly undesirable , 3= moderately undesirable, 2= undesirable, 1= Extremely undesirable

A mean score of 3 (“moderately undesirable”) or lower was considered the point at which samples would be considered unacceptable.

\*: Significant correlation at  $P < 0.05$ , relative to control at the same storage period.

\*\*: Significant correlation at  $P < 0.01$ , relative to control at the same storage period.

**Table 4: Effects of acid treatments and storage time on the pH of meat samples during refrigerated storage at 4°C (n=5).**

Storage time	Control	A.A.2%	A.A. 4%	L.A.2%	L.A. 4%
0	5.75±0.01	5.73±0.01	5.73±0.01	5.74±0.01	5.74±0.01
30min	5.89±0.10	4.94±0.20**	4.82±0.30**	5.12±0.10**	5.04±0.10**
1 day	5.88±0.20	4.85±0.20*	4.75±0.20*	5.05±0.10*	5.03±0.10*
3 days	5.74±0.10	4.72±0.20*	4.67±0.10*	5.39±0.10	5.12±0.20
5 day	6.58±0.10	4.88±0.20**	4.74±0.20**	5.40±0.20**	5.30±0.20**
7 day	6.68±0.10	5.17±0.02**	5.06±0.02**	5.85±0.01**	5.37±0.02**
9 day	6.72±0.10	5.47±0.02**	5.40±0.02**	5.90±0.01**	5.90±0.01**

A.A. :Acetic acid , L.A. : Lactic acid

\*:Significant correlation at  $P < 0.05$ , relative to control at the same storage period.

\*\*: Significant correlation at  $P < 0.01$ , relative to control at the same storage period.

**Table 5:** Total volatile bases nitrogen (mg/100g) contents of control and acid treated samples during storage (n=5).

Storage period	Control	2%A.A.	4%A.A.	2%L.A.	4%L.A.
0	3.60±0.03	3.49±0.03	3.67±0.03	3.57±0.03	3.55±0.03
30 min.	6.44±0.20	3.57 ±0.23**	3.67±0.31**	3.57±0.33**	3.59±0.34**
1 day	12.55±0.50	7.69±0.31**	6.52±0.33**	6.69±0.36**	5.97±0.30**
3 day	16.55±0.55	12.20±0.35**	10.97±0.27**	11.20±0.33**	9.27±0.30**
5 day	25.89±0.63	17.02±0.38**	15.94±0.35**	16.23±0.38**	11.23±0.34**
7 day	27.43±0.63	21.42±0.23**	20.51±0.33**	20.44±0.03**	15.49±0.30**
9 day	27.48±0.65	26.24±0.10	25.12±0.03	24.51±0.03*	22.23±0.30**

A.A.: Acetic acid , L.A. : Lactic acid

\*:Significant correlation at  $P < 0.05$ , relative to control at the same storage period.

\*\* : Significant correlation at  $P < 0.01$ , relative to control at the same storage period.

## DISCUSSION

### 1- Microbiological evaluation:

Microbiological quality of control and treated meat samples were determined based on aerobic plate count (APCs), psychrophilic count (PTCs) and total coliform counts (TCCs) .

#### 1-1- Aerobic plate counts:

Mean aerobic plate counts ( $\log_{10}$  CFU/g) for control and for treated beef sample groups at storage intervals were graphically represented in Fig. 1. The initial total bacterial count (before spray, 0 time) ranged from 4.3 to 4.7  $\log_{10}$  CFU/g, indicated acceptable quality meat samples. Since the beginning of the experiment the inhibitory effect of lactic acid and acetic acid has been observed. After 30 min of acid spray, all samples treated with acid showed significant reduction of the initial bacterial counts ( $P < 0.05$ ). The magnitude of the reduction in bacterial numbers was highly significant ( $P < 0.01$ ) after 24 hours as the average log reductions in counts were 1.5, 2.7, 2.3 and 3 log cycles in samples treated with 2% acetic acid, 4% acetic acid, 2% lactic acid and 4% lactic acid respectively. Meanwhile, after 24 hours the control meat allowed increase (0.4 log cycles) in the microbial population. According to the literatures, a bacterial reduction up to 3 Log can be achieved, but

the highest reductions are generally observed with high concentration (Prasai *et al.*, 1992; Van-Netten *et al.*, 1994; Jensen *et al.*, 2002; Fabrizio *et al.*, 2004). The antimicrobial properties of acid are attributed to the undissociated molecule and to a reduction of pH below the level at which the growth of many bacteria is inhibited (Nykanen *et al.*, 1998). Undissociated weak acids possess the ability to cross membranes of microorganisms, become dissociated inside the cell and acidify the cell interior. The intracellular dissociation of acid results in denaturation of enzymes and disruption of membrane (Freese *et al.*, 1973).

The increase in storage time produced increase in APCs, the increase was more rapid in control samples. At the day 3 of storage, the mean total APCs of the control ( $5.3 \pm 0.3 \log_{10}$  CFU/g) exceeded the maximal recommended limit of 5 log CFU/g for total APCs in raw and chilled meat (E.O.S.Q.C., 2001 and 2004). This differed significantly ( $P < 0.05$ ) with the APCs in 2% A.A, 4% A.A and 2% L.A. treated meat samples and highly significantly ( $P < 0.01$ ) as compared with the value of APCs in 4% lactic acid treated meat samples on the same day. Spoilage changes with greenish discoloration and putrid odor were noticed in untreated control samples on 5<sup>th</sup> day with corresponding increases in APCs to  $7.3 \pm 0.2 \log_{10}$  CFU/ g. Bacterial counts are generally thought to be an indicator of early spoilage, with "off" odors becoming apparent when bacterial number reach approximately  $10^7$  cells per g of meat (Borch *et al.*, 1996). It is accepted that a number of 7 log CFU/g is the approximate point at which meat reaches spoilage as to become unsalable (Dainty and Mackey, 1992). The APCs values were lower than 5 log CFU/g in day 7 in samples treated with 2% acetic acid, 2% lactic acid and 4% acetic acid and in days 9 in group treated with 4% lactic acid. These results indicate that acetic or lactic acid treatments of beef reduced microbial count immediately after treatment, moreover, a residual influence was observed and inhibited bacterial growth as compared with the non-treated control sample. At day 9<sup>th</sup> of storage, sample containing lactic acid 4% had a highly significant lower APCs ( $4.8 \pm 0.3 \log_{10}$  CFU/g) than the maximal recommended limit and the effect of lactic acid is apparent even at the end of the 9 days storage, while all other samples exhibited a higher APC indicating that 4% L.A. was more effective. The differences in total aerobic counts between treated and control samples at the end of the storage were 2-3 log cycles order.

### **1-2- Psychrophilic counts:**

Although the effect of the acid spray treatments was similar in both psychrophilic and aerobic plate counts, the growth of psychrophilic

during the cold storage was faster than that of mesophilic. The initial PTCs in beef samples ranged from 2.7- 2.9log<sub>10</sub> CFU/g (Fig. 2). The initial delay of PTCs growth in treated samples can be ascribed to the effect of acetic acid and lactic acid on the surface layer. Length of refrigerated storage had significant effect on PTCs, which tended to increase as the storage time increased. During storage significantly higher counts (P < 0.01) of psychrophilic bacteria were observed in control sample compared with those sprayed with acids. Also, psychrophilic counts in samples treated with 4% lactic acid were significantly lower (P < 0.01) than other samples.

The changes in PTC were approximately similar to those of APC, with control being the highest at day 3 (4.9±0.1 log<sub>10</sub> CFU/g) followed by samples treated with A.A. 2% (3.7±0.4 log<sub>10</sub> CFU/g), then 4% A.A. (3.4±0.4 log<sub>10</sub> CFU/g), then 2% L.A. (3.2± 0.3 log<sub>10</sub> CFU/g) while lower counts were detected in samples treated with lactic acid 4% L.A. (2.6± 0.5 log<sub>10</sub> CFU/ g). Spoilage changes with greenish discoloration and putrid odor were noticed in untreated control samples on 5<sup>th</sup> day with corresponding increases in total psychrophilic counts to 7.9 ± 0.4log<sub>10</sub> CFU/g .This reduction in color acceptability has been shown to be related to the growth of psychrophilic bacteria at the meat surface (Jeremiah *et al.*, 1972). In this concern, Kandeepan and Biswas (2007) reported that the increased enzyme activity of psychrophilic at low temperature hugely contributed to deterioration of meat quality. At day 9 of storage, samples containing L.A 4% had a lower PTC (5.1±0.6 log<sub>10</sub> CFU/g) while all other samples exhibited a higher PTC indicating that lactic acid 4% is more effective.

### **1-3- Total coliform counts:**

The initial coliform counts of samples were low (1.6-1.9log<sub>10</sub> CFU/g) .Coliform counts increased from 1.6±0.1 log<sub>10</sub> CFU/g in control samples at day 0 to a count of 3.5± 0.30 log<sub>10</sub> CFU/g by day 9 of storage (Fig.4). The growth of coliform was slower than that of APCs or PTCs. Undetectable level was noticed in all acid treated samples during first 3days of storage but it reached counts of 1.8± 0.2, 1.7±0.2 and 1.6±0.1 log<sub>10</sub> CFU/g in meat samples treated with 2% A.A, 4% A.A and L.A 2%, respectively by day 9 of storage. This was most likely due to the low initial coliform populations present in samples. Also, coliforms do not grow well at 4°C that may also explain why reductions were not evident. However, even any small reduction in microbial counts represents a prolongation of the shelf-life. However, during storage time, sample treated with 4% L.A. showed reduced coliform growth to undetectable level. Castillo *et al.* (2001) found that Log coliform count were

consistently reduced to undetectable levels when 4% L.A sprayed on chilled beef carcasses. They added that the small reductions observed for coliforms are attributable to counts on untreated carcasses already being near the lower detection limit of the counting method. In this concern, Gill and Landers (2003) stated that when relatively high levels of bacterial contamination were present, spray washing reduced bacterial counts, but it had little effect at relatively low levels. However, Gill and Badoni (2004), reported a reduction in coliform population of meat treated with 4% lactic acid by  $\geq 1.5$  log unit during chilling storage.

Comparing the effects of the two acids, the present results in Tables 1, 2 and 3 revealed that lactic acid inhibited the growth of bacteria effectively, the microbial counts in samples treated with lactic acid were lower than that recorded in acetic acid treated samples in both concentrations. Our results were in agreement with Lin and Chuang (2001) who reported that lactic acid was more inhibitory toward microorganisms than acetic acid in pork loin chop. Also Samelis *et al.* (2002) reported that lactic acid is higher in effectiveness than acetic acid against inhibiting the growth of microorganisms.

From the present results, it has been found that treatment with 4% of both acid appears to be more effective when compared to 2% solutions. This may be due to the reason that acid exhibits effective antimicrobial action, only when appropriate amounts of undissociated molecules of that acid penetrate bacterial cell by means of diffusion and interfere with intra cellular enzymes (Smulders *et al.*, 1986). Increasing the amount of acid applied or lowering the pH will increase the amount of undissociated acid molecules, thereby, antimicrobial action. The results of present study are in confirmation with the work done by Saoji *et al.* (1990) who observed similar results in buffalo meat streaks treated with progressively higher concentration of lactic acid solutions. It is very certain that the efficacy of organic acid depends on the load of initial microbial contamination as well as the pH and the concentration of the organic acid solution (Koutsoumanis *et al.*, 2006).

It is evident from the data of bacteriological evaluation that the treatment with 4% lactic acid was more effective in delaying the microbial growth and extended the shelf life of the meat. This finding is consistent with that reported by Castillo *et al.* (2001) who reported that increased lactic acid concentration up to 4% resulted in increased reduction rates in the numbers of microorganifsms on carcasses surface.

## **2- Sensory evaluation:**

Sensory characteristics, particularly the odor and color of fresh meats are extremely important from the standpoint of consumer acceptability (Ingram and Simonsen, 1980).

### **2-1-Sensory Evaluated Color:**

Effect of lactic acid and acetic acid treatments (2 and 4 %) on color of meat samples were summarized in Table 1. Initially, treatment with 2 % L.A. had minimal effect on the color of meat, however this often disappears or becomes less evident after chilling. Meanwhile, samples treated with 2, 4% A.A. and 4% L.A. showed discoloration (bleaching) as compared to the control on 30 min. of display. At days 1 of display all treatments except 2 % L.A. showed discoloration compared with the control .Meat treated with 2 % L.A. was redder in color when compared with the control on days 1 to 3 of display and there was an improved in the sensory scores until 3 days ( $P < 0.01$ ) with no further improvement when storage was extended to 9 days. Saoji *et al.* (1990) also observed similar discoloration in buffalo meat streaks treated with 4% lactic acid. No effect of spraying beef carcasses with lactic acid (2%) was observed by Prasai *et al.* (1991). Meanwhile, Stivarius *et al.* (2002) reported that acetic acid caused changes in ground beef color and odor characteristics. Also Pipek *et al.* (2005) observed that the surface spraying of beef muscle by 2% lactic acid had a negligible effect on the color. At 3 days of display, reduction of color score of control group was observed. This reduction in color acceptability has been shown to be related to the growth of mesophilic and psychrophilic bacteria at the meat surface. The color scores decreased with increasing concentrations of acids and the days of storage with better color shown in samples treated with 2% lactic acid during first 3 days followed by samples treated with 4% lactic acid and to a lesser extent to samples treated with acetic acid in both concentrations. At 3 days, sensory evaluation indicated that the samples treated with 2% and 4% acetic acid were exhibited reduction in color score as compared to samples treated with lactic acid. Color deterioration of whole muscle cut surfaces have been reported with acetic acid treatment (Mikel *et al.*, 1996). In this concern, 2 percent acetic acid and 5 percent acetic acid had been reported to cause premature browning of lean meat color (Miller, 2005).

As bacteria grow on the meat surface they accelerate the oxidation of meat pigments which results in a progressive deterioration of the acceptable red color and the appearance of varying degrees of brown surface discoloration .After beef has been refrigerated for 5 days, control meat lost red color, turn brown, had an off-odor, and be sticky to

the touch and become visually unacceptable to panelist indicating shelf life of about 3-5 days. Similar results were reported by Abd El-Rhman *et al.* (2007) who found an unacceptable color and off-odor after 5 days of storage at 4°C of air-packaged beef. Microbial results appeared to be consistent with results of color. In this respect, Koutsoumanis *et al.* (2006) reported that the micro-organisms can cause discoloration, off-odors, off-flavors, gas formation and/or changes in texture and slime. The surface of the samples treated with 2% L.A. was not severely discolored and remained acceptable even after 7 days storage. Four percent lactic acid treatments controlled microbial load more significantly among the treatments. However, these sample could not keep their color stable. In this concern, Sundar and Zhan (2006) reported that 4 or 6% lactic acid spray treatment could be better if color and lipid oxidation could be stabilized by appropriate stabilizers.

## **2-2- Sensory Evaluated Odor:**

The changes in sensory evaluated odor for all studied samples showed similar trends to those found for sensory evaluated color. After 30 min. of acid spray, highly significant ( $P < 0.01$ ) change in odor was detected in samples treated with acetic acid either 2 or 4% concentrations (Table 2). The panelists characterized the odor as mild acidic (vinegar odor). However, no change in odor was noticed in sample sprayed with 2% lactic acid and a significant ( $P < 0.05$ ); mild acidic odor was detected in samples treated with 4% lactic acid solution. Saoji *et al.* (1990) also observed a mild acidic odor in buffalo meat streaks treated with 4% lactic acid solution. At 3<sup>rd</sup> day, the samples treated with acetic acid continued to have the strongest acid odor as compared to other samples. After 5<sup>th</sup> days of storage, control sample exhibited significant ( $P < 0.01$ ) unpleasant odors. Data from sensory analysis confirmed those from microbiological tests. Panelists rejected control samples after 5 days, where samples reached or exceeded the spoilage onset ( $10^7$ - $10^8$  CFU/g). The offensive odor and change in color were appeared when the count reached  $10^7$ CFU/g (Byun *et al.*, 2003). Sensory evaluation indicated that the samples treated with 2% and 4% acetic acid were unacceptable on the 9<sup>th</sup> day of storage. The increased enzyme activity of psychrophilic at low temperature hugely contributed to deterioration of meat quality (Kandeepan and Biswas, 2007). At 9<sup>th</sup> day storage samples treated with L.A. had small to moderate off-odor. Data from sensory analysis confirmed those from microbiological tests.



### **2-3- Sensory overall appearance:**

Appearance of beef in the retail case affects acceptability of the product to consumers bright-red lean color suggests freshness to retail customers (Liu *et al.*, 1996).

Time at which samples were scored moderately undesirable in overall appearance by members of a panel was considered unacceptable. Acid treated samples had mean overall appearance scores of 3 (moderately undesirable) at 7 to 9 days whereas control group had mean overall appearance scores of 3 at 5<sup>th</sup> day (Table 3). Sensory data and microbial populations were in general agreement and showed correlations across the observed time periods. Off-odors, declining color scores and increased surface discoloration became detectable which corresponded with peak bacterial growth occurring at 5<sup>th</sup> days in control group. From the sensory and overall appearance results, it could be concluded that the shelf life of meat treated with acid could be extended from 2-4 days under refrigerator storage. Data from sensory analysis confirmed those from microbiological tests. Kofitsyo (1988) recorded that spraying the meat surface of skinned cow heads with lactic acid resulted in reduction in total viable counts of bacteria and the shelf lives were observed to have been extended for about three days at 4°C. The predominant reason for meat shelf life is microbial spoilage activity (Koch *et al.*, 2009). There have been several studies investigating the use of organic acids as sanitizing solutions to improve shelf-life and safety of fresh meat (Hardin *et al.*, 1995; Kochevar, 1997).

From microbial quality standpoints, shelf-life of acid treated samples was 7-9 days according to treatment used against 3 days in untreated samples.

### **3- Physicochemical-evaluation:**

#### **3-1- pH Changes:**

Changes in pH of meat as affected by lactic and acetic acid treatments during storage are presented in Table 4. The initial mean pH values of all meat samples ranged from  $5.63 \pm 0.01$  to  $5.75 \pm 0.01$  which reflected the acceptable quality state of meat samples. After acid treatment, the pH values of acid sprayed samples dropped significantly ( $P < 0.01$ ). Mean pH values recorded after 30 min. in control, 2% A.A., 4% A.A, 2% L.A. and 4% L.A. treated meat samples were  $5.89 \pm 0.02$ ,  $4.94 \pm 0.01$ ,  $4.82 \pm 0.03$ ,  $5.12 \pm 0.01$  and  $5.04 \pm 0.01$ . During the initial storage period, the pH of treated meat samples was consistently low. The low pH values of samples treated with acetic and lactic acid treated samples may have altered the growth of spoilage microorganisms, hence, extending the shelf life of the meat. One effective means of

limiting microbial growth is to increase the acidity of beef, thereby creating an unfavorable environment for the growth. The lower pH disturbs the homeostasis of the bacterial cells, including pathogens and spoilage bacteria (Leistner, 2000).

A gradual increase in pH was observed in control meat sample. On 5<sup>th</sup> day, the pH of control sample ( $6.58 \pm 0.01$ ) was significantly higher ( $P < 0.01$ ) than the acid treated meat samples. The increase in pH values reflected the production of alkaline bacterial metabolites in spoiling meat (Kyraa *et al.*, 1997) and coincided with the increase in microbial population, sensory changes and total volatile basic nitrogen (TVB-N). In general, bacteria prefer a pH near neutrality (Walker and Betts, 2000). Upon storage, the pH values of all treated samples increased gradually which was also observed by Jayesh and Venkataramanujam (2000). Comparing acid treatment used, lactic acid treated samples had a pH decline during the initial 3 days then increased gradually, giving a final pH 0.24 unit above the initial reading above the initial reading. Meanwhile, samples treated with acetic acid had a lower pH during storage giving final readings about 0.16- 0.33 pH unit below the initial readings. The lower capacity of acetic acid to enter bacteria cell is compensated by their greater capacity to dissociate inside the cell and thus acidify the cell cytoplasm (Young and Foegeding, 1993). Watts (1954) points out that lower pH values associated with extended periods of storage may accelerate metmyoglobin formation in fresh meats. In this concern, Abril *et al.* (2002) concluded that the low pH affects beef color by promoting oxidation of the haem pigments from the purple or red myoglobin and oxymyoglobin to the brown met myoglobin. Data from pH confirmed those from sensory analysis after 3 days, sensory evaluation indicated that the samples treated with 2% and 4% A.A. were exhibited reduction in color score as compared to samples treated with lactic acid and turn brown on the 5<sup>th</sup> day of storage.

### **3-2- Total volatile basic nitrogen (TVB-N):**

Total volatile basic nitrogen (TVB-N) contents of control and acid treated samples were tabulated in Table 5. Generally, the significantly higher ( $P < 0.01$ ) TVB-N content was found in control beef sample when compared with acid treated samples for the same storage period. For the control group, TVB-N content increased rapidly and reached  $25.89 \pm 0.23$  mg /100 g after 5 days of storage. Data from sensory analysis and microbiological tests confirmed those where control sample reached or exceeded the spoilage onset ( $10^7$ - $10^8$  CFU/g). However, all samples sprayed with acids had TVB-N contents less than 20 mg/100g within 5 days of storage which meet the safety and quality standards

.Since the total bacteria were low in number, the corresponding TVB-N value was equally low. TVB-N are products of bacterial spoilage and it is often used as an index to assess the keeping quality and shelf-life (Goulas *et al.*, 2005). TVB-N values of fresh and good quality meat are less than 20 mg/100g. TVB-N values in the range 20-25 mg/100g and above 25 mg/g indicate meat that are slightly decomposed/ edible and decomposed/ inedible, respectively (Lannelongue *et al.*, 1982). The increase in storage time produced increase in TVB-N values of all groups which were related to bacterial growth. The TVB-N values were higher in acetic acid sprayed samples than that of lactic acid sprayed samples at the same concentrations used for the same storage period which coincides with the bacterial counts and sensory evaluation. At 7<sup>th</sup> day, all samples had TVB-N contents more than 20 mg/100g except samples treated with lactic acid 4% which showed the lowest TVB-N content and reached  $22.23 \pm 0.03$  at 9<sup>th</sup> day of storage .The present results were in accordance with the results of bacterial counts. Byun *et al.* (2003) reported that TVB-N contents showed the best correlation with bacterial counts for beef. They added that TVB-N could potentially be used as indicators in predicting the microbial quality of beef during chilled storage.

### **Conclusion**

Microbial population that comes in contact with fresh meat during slaughtering, dressing and processing presents a challenging problem to the meat industry. Results from the present study suggest that lactic acid and acetic acid treatment improved the microbial quality of raw meat. In the light of data obtained from the analyses of the bacteriological, sensory and chemical qualities it was concluded that 2%lactic acid spray treatment had less effect on odor and did not negatively influence the surface color, it can be used in slaughterhouses to comply with food safety regulations, prolong the shelf-life of carcasses and to increase the safety of meat. Reduction of pathogen prevalence on meat post-slaughter leads to a reduced probability that errors occurring in subsequent parts of the food chain will lead to foodborne illness and yield products that should be safe for consumption following proper cooking and serving.

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