

Impact of organic acids and their salts on microbial quality and shelf life of beef meat.

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ABSTRACT

This study examined the effect of different concentrations of lactic acid (LA) (1 and 2%), acetic acid (AA) (1 and 2%), sodium lactate (SL) (2.5%) and sodium acetate (SA) (2.5%) on the chemical, microbiological and sensory quality of raw beef meat stored at 4°C. The results showed that these additives were efficient ($P < 0.05$) against the proliferation of various spoilage microorganisms; including aerobic, and psychrotrophic bacteria, and Enterobacteriaceae. The general order of antibacterial activity of the different additives used was; AA, LA, SA, SL. The chemical analysis revealed a significant reduction ($P < 0.05$) in the pH value of treated beef meat samples. Significant differences ($P < 0.05$) were detected with the sensory quality, with 1% (AA), the treated samples yielding the highest scores for the color, texture, and flavor attributes. Overall, the findings demonstrate that the addition of 1% AA or 1% LA to beef cuts can delay the proliferation of spoilage microorganisms, improve the sensory attributes and extend the shelf life of the beef during refrigerated storage. These additives have promising properties that can open new pathways and opportunities for beef meat preservation for using efficient, safe, and cost-effective preservatives.

Keywords: beef, organic acids, sodium lactate, sodium acetate, shelf life.

1) INTRODUCTION

Meat consumption is continuously increased worldwide as it is being the first choice source of animal protein and very good source of various micronutrients. 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**). A 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 (**Cervený *et al.*, 2009**). Beef has a short shelf life of one day or less at ambient temperature and a few days at refrigerated temperature due to microbial

spoilage (Dickson, 1992) and/or lipid oxidation (Houben, 2000), which are strongly influenced by initial beef quality, package parameters and storage conditions (Zhao, 1994). Spoilage of meat is caused by infection 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 food borne 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 (Maalekuu *et al.*, 2014). Among pathogenic bacteria that associated with fresh beef were *E. coli* O157:H7, *Salmonella*, *Campylobacter Jejuni*, *Listeria monocytogenes*, *Pseudomonas* and *Staphylococcus aureus* (Schlyter *et al.*, 1993).

Minimizing 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 as well as their salts have been utilized as preservatives for preventing food deterioration and extending the shelf life of perishable food (Ricke, 2003). They have been generally recognized as safe (GRAS), which provides for unregulated use in food products, until sensory characteristics are negatively influenced (Anon, 1987).

Considering the above, the aim of the present study was to investigate the effect of lactic acid, acetic acid, and their salts on the shelf-life extension of fresh beef stored at 4°C.

2) MATERIAL AND METHODS

2.1. Sampling:

Fresh beef was transported immediately after slaughter to the laboratory. The lean beef was cut to cubes (100 gram each) and was divided into seven groups (10 cubes for each group). One was left as the control group. The remaining 6 groups were treated with LA 1%, LA 2%, AA 1%, AA 2%, SL 2.5%, and SA 2.5%, respectively, by immersion for 10 min and drip-dried for 15 min. Examined beef samples were packaged and stored at 4°C. Analyses were carried out on examined beef samples every 3 days during the 21 day storage period.

2.2. pH measurement:

The pH value determined according to (ISO, 1999) using digital pH-meter.

2.3. Microbiological analyses:

From each treatment sample, 25 g of meat were taken aseptically and 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 for obtaining a dilution of 10^{-1} , from which 1 ml was transferred 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.

The microbiological analysis included determination of Total aerobic plate counts (APC) according to the procedure of (ICMSF, 1982), Total psychrotrophic count (PTC) according to (APHA 1992) and Enterobacteriaceae count according to the procedure of (Mercuri and Cox 1979).

2.4. Sensory Evaluation:

It was carried out according to (Pearson and Tauber, 1984). The sensory attributes were evaluated by panelists. Each person had to assess levels of color, texture (toughness or juiciness), and flavor (sourness or sweetness). Samples from the different treatments were individually presented to each panelist. The judges were not informed about the experimental approach. 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.

2.5. Statistical Analysis:

Microbial counts were converted into base-10 logarithms of colony forming units per gram of beef ($\log_{10}\text{cfu/g}$). Results were expressed as means \pm standard deviations(SD) of 3 replicates. Statistical analysis of data was done by one way analysis of variance (ANOVA) using the statistical package for social sciences (SPSS-22 statistical software package). The differences were considered statistically significant when $P<0.05$.

3) RESULTS AND DISCUSSION

3.1. Changes in pH Value:

The pH was determined to assess the storage stability of meat. From the data presented in **Table (1)** the initial pH value ranged from 5.82 ± 0.13 in control samples to 4.6 ± 0.14 , 4.4 ± 0.13 , 4.9 ± 0.19 , 4.68 ± 0.14 , 5.73 ± 0.08 and 5.84 ± 0.08 for LA 1%, LA 2%, AA 1%, AA 2%, SL 2.5% and SA 2.5%, respectively. The previous data demonstrated that the initial pH value of the control sample was significantly ($P<0.05$) higher than those of all treated samples. The SL 2.5% treatment resulted in higher pH values than all other treatments except for SA 2.5%, while the LA 2% added treatments having the lowest values. For all treatments, storage had a significant ($P<0.05$) effect on the pH values, which tended to increase as storage time increased, but the control samples showed reduction in the pH values till the 12th day of the experiment. The reduction in pH values 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 12th of the experiment the pH values of the control samples begin to increase till the end of the experiment. Whereas, the pH values of all treated samples showed slight increase from zero day till the end of the experiment. Nearly similar results were reported by (Ghada 2006) and (Bassma 2011). Smaoui *et al.*, (2012) reported that the determination of pH values for all treated chicken samples during 15 days, showed an increase in pH value especially for the control samples which become 6.87

at the end of the storage period, while samples treated with LA at 1% concentration showed the lower pH value(6.06) and these observations are in accordance with our results.

According to **Gonzalez-Fandos *et al.* (2009)**, the buffering capacity of the acid system seems to be sufficient to maintain a low pH of the meat; these observations are in accordance with our results.

3.2. Microbiological evaluation ($\log_{10}\text{cfu/g}$):

Under normal aerobic packaging conditions, the shelf life of refrigerated meat is limited by the growth and biochemical activities of aerobic, and psychrotrophic strains of bacteria (**Lambert *et al.*, 1992**). In this study AA 1 and 2%, LA 1 and 2%, SA 2.5% and SL 2.5% were applied to control the microbial growth and to extend the shelf-life of fresh beef during refrigerated storage.

3.2.1. Aerobic plate count (APC):

APC is of importance in judging the hygienic condition under which meat has been produced and handled. The data presented in **Table (2)** revealed that the initial APC in examined beef ranged from 4.62 ± 0.35 in AA 2% treated samples to 4.86 ± 0.45 in control samples. By the day 6, the mean APC of control samples were 6.92 ± 0.24 which was close to the maximal limit of APC $7 \log_{10}\text{cfu/g}$ for raw meat recommended by (**ICMSF, 1986**), indicating a shelf life of about 7 - 8 days. The maintenance of APC in control samples below spoilage level until day 6 was attributed to longer acidic phase which started after bleeding of the animal. There was a significant reduction of APC of all treated samples compared to the control and this was attributed to destructive effect of acids(AA1% and 2%, LA1% and 2%) and salts(SA 2.5%, SL 2.5%) on different microbes may be contaminate the meat. On day 9, the mean APC of the control samples increased to 7.73 ± 0.19 and signs of spoilage started to appear as a slight foul smell. On day 12, the mean APC of the samples treated with (SA 2.5% or SL 2.5%) increased to 7.15 ± 0.17 and 7.12 ± 0.10 , respectively and signs of spoilage started to appear. On day 18, the samples treated with (AA 1% or LA 1%) were showed mean APC of 7.04 ± 0.36 and 7.17 ± 0.33 , respectively and signs of spoilage started to appear. While, the samples treated with (AA 2% and LA 2%) exhibited a delayed growth of APC of 6.85 ± 0.20 and 6.92 ± 0.24 , respectively by the day 18. Thus extending shelf life of samples treated with (AA 2% or LA 2%) up to 18 d during storage at 4°C. Nearly similar results were reported by (**Ghada 2006**) and (**Smaoui *et al.*, 2012**). **Sallam and Samejima 2004** found that SL 3% significantly delayed the microbial growth and extended the shelf life of ground beef up to 15 days at which the APC was 6.73 versus 8.69 for control.

3.2.2. Psychrotrophic count (PTC):

The initial PTC **Table (3)** for the treated samples ranged between 2.56 ± 0.21 and 3.26 ± 0.23 . These counts were lower than those recorded for the control and were noted to decrease with the increase in the percentage of the preservative in the treated beef samples. The PTC were noted to increase during the storage period, all treatments were observed to result in significant reductions ($P < 0.05$) in those PTC

compared to the control. The PTC recorded at day 21 for the samples treated with 2.5% SL or 2.5% SA was about 7.28 ± 0.22 and 7.23 ± 0.16 , respectively. While, the samples treated with (LA2% or AA2%) exhibited a delayed growth of PTC of 6.24 ± 0.25 and 5.86 ± 0.28 , respectively. These results indicated that 2% LA or 2% AA significantly ($P < 0.05$) reduced PTC in refrigerated beef. These results attributed to destructive effect of acids (AA 1% and 2%, LA 1% and 2%) and salts (SA 2.5%, SL 2.5%) on different microbes may be contaminate the meat. These results agreed with (Smaoui *et al.*, 2012) and (Sallam and Samejima 2004). Also, Ghada (2006) found that AA 2% significantly ($P < 0.05$) reduced PTC on fresh lamb carcasses more than treated with AA 1%, LA 1% and LA 2% within 14 days of storage.

3.2.3. Enterobacteriaceae count (EBC):

The EBC given in Table (4) showed that the growth of Enterobacteriaceae was slower than that of APC or PTC. The initial EBC ranged from 1.83 ± 0.16 for control samples to <1 , <1 , <1 , <1 , 1.49 ± 0.19 and 1.42 ± 0.18 for LA 1%, LA 2%, AA 1%, AA 2%, SL 2.5% and SA 2.5%, respectively. LA2% and AA 2% treatments brought a significant ($P < 0.05$) reduction of the EBC in treated beef samples which remained under the detection limits until day 3 of storage. On day 21, the lowest EBC was 2.94 ± 0.05 for AA 2% treated samples, so AA 2% treated samples showed a significantly ($P < 0.05$) lower EBC compared to other acid treatments. LA and AA at concentrations 2% led to significantly ($P < 0.05$) lower EBC than LA and AA at 1%, SL and SA at 2.5% during the storage period. These results agree with (Ghada 2006) and (Smaoui *et al.*, 2012). Also, Sallam and Samejima (2004) reported that a combination of SL and NaCl restricted the growth of the Enterobacteriaceae to a lower level of 1.66 and appeared to be the most effective ($P < 0.05$) among the other treatments against the growth of Enterobacteriaceae.

3.3. Sensory Evaluation:

The color, texture and flavor attributes of the treated beef samples are shown in Table (5). The results indicated that samples treated with 1% LA and 1% AA showed the highest overall acceptability score (7.9 ± 0.1 and 8.1 ± 0.2), respectively, followed by the samples treated with 2.5% SL and 2.5% SA that showed an overall acceptability of 7.2 ± 0.2 and 7.3 ± 0.1 , respectively. However, samples treated with 2% LA and 2% AA showed the lowest overall acceptability score among all treated groups. All the samples analyzed were considered as acceptable during sensory analysis. Bacterial populations as well as chemical indicators (pH) coincided with the sensory scores. These results agreed with (Smaoui *et al.*, 2012). Quilo *et al.* (2009) stated that the use of potassium lactate on beef trimmings before grinding could improve or maintain the same sensory characteristics (odor and taste) of ground beef.

4) CONCLUSION

Overall, the present study revealed that treatment with LA1% and 2%, AA1% and 2%, SL2.5% and SA2.5% could reduce the chemical changes, delay the microbial growth and improve or maintain the sensory attributes of treated beef.

Impact of organic acids and their salts on microbial quality and shelf life of beef meat.

Better results were attained with concentrations of 1% AA and 1% LA that extend the shelf life and show the highest overall acceptability score for the sensory attributes during refrigerated storage. The use of LA1% and AA1% can be considered as strong and promising properties that can open new pathways and opportunities for the beef preservation by using efficient, safe, and cost-effective preservatives.

Table: (1) Mean values reveal the changes in the pH values of raw beef samples during storage at 4 °C.

Treatments	Days							
	0	3	6	9	12	15	18	21
Control	5.82±0.13 ^d	5.77±0.09 ^a	5.68±0.15 ^d	5.59±0.23 ^a	5.57±0.14 ^{acd}	5.63±0.13 ^{ace}	5.71±0.11 ^a	5.73±0.08 ^{ac}
LA 1 %	4.6±0.14 ^{bd}	5.1±0.20 ^b	5.3±0.20 ^b	5.4±0.18 ^{ac}	5.45±0.12 ^a	5.51±0.12 ^a	5.55±0.13 ^{ab}	5.61±0.10 ^a
LA 2 %	4.4±0.13 ^b	4.9±0.07 ^{bd}	5.08±0.13 ^c	5.14±0.15 ^{bc}	5.25±0.14 ^{bc}	5.35±0.14 ^{bc}	5.41±0.19 ^b	5.46±0.21 ^b
AA 1 %	4.9±0.19 ^c	5.2±0.15 ^{bc}	5.4±0.16 ^b	5.48±0.18 ^a	5.5±0.22 ^a	5.59±0.20 ^{ae}	5.65±0.19 ^a	5.68±0.11 ^a
AA 2 %	4.68±0.14 ^d	5.1±0.16 ^b	5.25±0.12 ^{bc}	5.3±0.09 ^c	5.4±0.15 ^c	5.5±0.10 ^c	5.57±0.12 ^{ab}	5.63±0.09 ^{ab}
SL 2.5 %	5.73±0.08 ^d	5.7±0.07 ^c	5.71±0.07 ^a	5.72±0.07 ^{ab}	5.73±0.07 ^d	5.74±0.05 ^e	5.72±0.08 ^a	5.71±0.04 ^a
SA 2.5 %	5.84±0.08 ^d	5.8±0.08 ^c	5.81±0.09 ^d	5.83±0.07 ^{ab}	5.84±0.07 ^{de}	5.85±0.08 ^{de}	5.83±0.04 ^{ac}	5.51±0.14 ^{dc}

Data given as mean ± SD of 3 replicates. Means are different P < 0.05 when superscripts differ within column.

Table: (2) Mean values reveal the changes in the APC (log10cfu/g) of raw beef samples during storage at 4 °C.

Treatments	Days							
	0	3	6	9	12	15	18	21
Control	4.86±0.45ab	5.83±0.16a	6.92±0.24 a	7.73±0.19a	8.75±0.19a	9.25±0.35a	9.66±0.18a	9.98±0.23a
LA 1 %	4.73±0.19a	4.91±0.11b	5.35±0.22 b	5.77±0.25b	6.23±0.28bc	6.68±0.19b	7.17±0.33b	7.85±0.12b
LA 2 %	4.65±0.21a	4.73±0.18bd	5.22±0.29 b	5.48±0.22bf	5.96±0.22 c	6.48±0.24bd	6.92±0.24bc	7.48±0.18c
AA 1 %	4.70±0.26a	4.86±0.32bd	5.31±0.35 b	5.74±0.14 b	6.16±0.27bc	6.52±0.27bd	7.04±0.36b	7.67±0.21bc
AA 2 %	4.62±0.35a	4.65±0.14bdf	5.07±0.15be	5.42±0.20bf	5.79±0.25cd	6.31±0.12bd	6.85±0.20b	7.39±0.23c
SL 2.5 %	5.04±0.16ab	5.43±0.21g	6.15±0.16cd	6.64±0.19c	7.12±0.10e	7.59±0.25c	8.05±0.21e	8.15±0.15bde
SA 2.5 %	5.23±0.21b	5.49±0.23ag	6.33±0.21cd	6.72±0.20cd	7.15±0.17e	7.73±0.21c	8.18±0.19e	8.25±0.15e

Data given as mean ± SD of 3 replicates. Means are different P < 0.05 when superscripts differ within column.

Table: (3) Means changes in PTC (log10cfu/g) of raw beef samples during storage at 4 °C.

Treatments	Days							
	0	3	6	9	12	15	18	21
Control	3.3±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
LA 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
LA 2 %	2.67±0.28 ^{bc}	3.26±0.25 ^c	4.43±0.28 ^{bc}	4.92±0.07 ^{bd}	5.31±0.33 ^b	5.67±0.26 ^b	5.7±0.22 ^c	6.24±0.25 ^b
AA 1 %	2.9±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
AA 2 %	2.56±0.21 ^c	3.23±0.10 ^c	4.31±0.16 ^c	4.88±0.12 ^b	5.26±0.19 ^b	5.63±0.16 ^b	5.69±0.24 ^c	5.86±0.28 ^c
SL 2.5 %	3.26±0.23 ^b	4.06±0.14 ^d	4.95±0.14 ^b	5.47±0.20 ^c	6.03±0.16 ^c	6.25±0.20 ^c	6.9±0.13 ^d	7.28±0.22 ^d
SA 2.5 %	3.2±0.14 ^a	3.97±0.11 ^{bd}	4.92±0.04 ^b	5.34±0.10 ^{bc}	6±0.08 ^c	6.19±0.13 ^c	6.76±0.20 ^d	7.23±0.16 ^d

Data given as mean ± SD of 3 replicates. Means are different P < 0.05 when superscripts differ within column.

Table: (4) Means changes in EBC (log10cfu/g) of raw beef samples during storage at 4 °C.

Treatments	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
LA 1 %	<1 ^b	2.15±0.15 ^b	2.66±0.29 ^b	2.75±0.19 ^b	2.88±0.19 ^b	2.95±0.10 ^b	3.05±0.18 ^b	3.2±0.22 ^b
LA 2 %	<1 ^b	<1 ^b	2.47±0.19 ^{bd}	2.48±0.20 ^b	2.69±0.14 ^b	2.82±0.14 ^b	2.91±0.12 ^b	3.02±0.13 ^{bc}
AA 1 %	<1 ^b	2.12±0.19 ^b	2.61±0.21 ^{bd}	2.75±0.21 ^b	2.83±0.17 ^b	2.92±0.08 ^b	3.03±0.08 ^b	3.15±0.08 ^{bc}
AA 2 %	<1 ^b	<1 ^b	2.45±0.21 ^{bd}	2.49±0.23 ^b	2.65±0.15 ^b	2.74±0.13 ^b	2.85±0.14 ^b	2.94±0.05 ^c
SL 2.5 %	1.49±0.19 ^c	2.86±0.16 ^c	3.05±0.09 ^c	3.14±0.18 ^c	3.25±0.26 ^c	3.5±0.22 ^c	3.87±0.13 ^c	4.01±0.10 ^d
SA 2.5 %	1.42±0.18 ^c	2.78±0.10 ^c	2.98±0.08 ^{bc}	3.14±0.09 ^c	3.23±0.18 ^c	3.48±0.11 ^c	3.75±0.15 ^c	3.97±0.07 ^d

Data given as mean ± SD of 3 replicates. Means are different P < 0.05 when superscripts differ within column.

Table: (5) Means changes of the sensory attributes of examined raw beef samples:

Sensory attribute	Control	LA 1 %	LA 2%	AA1 %	AA 2%	SL 2.5%	SA 2.5 %
Color	5.4±0.2 ^{ac}	6.8±0.2 ^b	5.0±0.8 ^{ac}	7.1±0.8 ^b	5.2±0.8 ^{ac}	6.3±0.4 ^{bc}	5.6±0.2 ^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 ^{ad}	7.0±0.2 ^d
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 ^{ad}	7.3±0.2 ^d
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	7.3±0.1 ^d

Data given as mean ± SD of 3 replicates. Means are different P < 0.05 when superscripts differ within row.

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تأثير الاحماض العضويه وأملاحها على الجوده الميكروبيه وفترة صلاحية اللحم البقرية

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فى هذه الدراسه تم فحص تأثير تركيزات مختلفه من حامض اللاكتيك (1 و 2 %), وحامض الخليك (1 و 2 %), وكتات الصوديوم (2.5 %) و خلات الصوديوم (2.5 %) على الجوده الكيماييه و الميكروبيولوجيه و الحسيه للحوم البقرية النيئه و المحفوظه عند درجه حراره 4 درجه مؤويه. وقد اوضحت النتائج ان هذه الاضافات كانت فعاله ($P < 0.05$) فى تثبيط تكاثر العديد من الكائنات الحيه الدقيقه المسببه لفساد اللحم والتي تشمل على: البكتيريا الهوائيه و البكتيريا المحبه للبروده و البكتيريا المعويه. وكان الترتيب العام للنشاط المضاد للبكتيريا للإضافات المختلفه التي استخدمت كالاتى حامض الخليك < حامض اللاكتيك < خلات الصوديوم < لكتات الصوديوم. وكشف التحليل الكيمايى انخفاض معنوي ($P < 0.05$) فى قيمة الرقم الهيدروجيني فى عينات لحم البقر المعالجه. وكشف التحليل الحسي عن وجود اختلافات معنويه ($P < 0.05$) ، وقد وجد ان حامض الخليك 1% أعطى افضل النتائج لكل من سمات اللون والملمس والنكهة. وبشكل عام، تظهر النتائج أن إضافة حامض الخليك (1 %) أو حامض اللاكتيك (1 %) إلى لحم البقر يمكن أن يؤخر انتشار الكائنات الحية الدقيقة المسببه لفساد اللحم ويحسن الخواص الحسية ويزيد فترة صلاحية اللحم البقرية المبرده. وعليه فان هذه الإضافات لها خصائص واعدة كمادة حافظة فعالة، وأمنة، واقتصاديه من حيث التكلفة والتي يمكن أن تفتح فرصا جديدة لحفظ لحوم الابقار.