

EFFECT OF ORGANIC ACIDS ON THE MICROBIAL PROFILE AND SHELF LIFE OF BUFFALO MEAT SAUSAGES

Original Article

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ABSTRACT

Background: The meat industry faces significant challenges in meeting consumers' nutritional requirements, quality expectations, and food safety standards. These demands have heightened interest in biopreservation methods to enhance the shelf life and safety of meat products. Organic acids, such as lactic acid and acetic acid, have been widely recognized for their preservative properties, effectively controlling spoilage microorganisms while maintaining product quality and sensory attributes.

Objective: This study aimed to evaluate the microbial profile, shelf-life stability, and sensory attributes of buffalo meat sausages treated with organic acids during chilled storage.

Methods: Buffalo meat sausages were treated with an organic acid mixture of lactic acid and acetic acid at concentrations of 0.75%, 1%, and 2% (v/v), with untreated sausages serving as the control. A total of 96 samples were prepared and stored at chilling temperatures (0–4°C) for 12 days. Microbial analysis included total viable count (TVC), *E. coli* count, and *Pseudomonas* count, while physicochemical parameters such as pH and water activity were assessed. Sensory evaluations were conducted to assess odor, flavor, texture, juiciness, and overall acceptability. Data were analyzed using factorial ANOVA in SAS software, with Duncan's Multiple Range Test identifying significant differences at $P \leq 0.05$.

Results: Sausages treated with 2% organic acids showed the lowest TVC (4.49 log CFU/g), *E. coli* (2.01 log CFU/g), and *Pseudomonas* (3.97 log CFU/g) compared to the control group with TVC (5.99 log CFU/g), *E. coli* (2.73 log CFU/g), and *Pseudomonas* (5.20 log CFU/g). Treated sausages demonstrated stable pH values (5.30 for 2% acid vs. 5.73 for control) and improved sensory scores, with overall acceptability highest in 1% acid-treated sausages.

Conclusion: Organic acids effectively reduced microbial load, stabilized physicochemical properties, and improved sensory attributes of buffalo meat sausages, thereby enhancing shelf life and quality during chilled storage.

Keywords: *E. coli*, Lactic Acid, Meat Preservation, Meat pH, Organic Acids, Sensory Evaluation, Total Viable Count.

INTRODUCTION

Meat and its derived products are integral sources of essential nutrients such as proteins, fatty acids, vitamins, and minerals, which are vital for human growth and health (1). However, substantial quantities of these products are wasted due to microbial spoilage at various stages of the supply chain, representing a significant global challenge in food preservation and safety (2). Ensuring the extended shelf life of perishable meat products requires rigorous refrigeration practices, which not only demand substantial energy resources but also underscore the need for alternative preservation methods (3). Meat serves as an ideal substrate for bacterial growth, and improper storage conditions accelerate the proliferation of pathogenic bacteria, leading to food spoilage and safety concerns. This issue has garnered global attention, with foodborne illnesses and quality deterioration becoming pressing matters for public health and industry stakeholders (4). The availability of free water in meat products plays a pivotal role in bacterial survival and activity. Therefore, strategies aimed at reducing water availability can effectively mitigate microbial spoilage and enhance product stability (5).

Incorporating non-meat ingredients into meat products offers a dual advantage by not only enhancing their functional and antioxidant properties but also reducing production costs. While poultry meat processing dominates Pakistan's further processing industry, the rising demand for red meat products, including beef sausages, is noteworthy. Sausages, a versatile meat product with varied classifications such as fresh, cured, and fermented types, are still gaining traction in Pakistan. Their manufacturing also serves as an economical means to utilize low-cost meat cuts while addressing protein requirements (6). Preservation techniques, including physical, chemical, and biological interventions, have been extensively explored, with the addition of organic acids emerging as a safe and effective method to extend shelf life (7). Organic acids such as acetic acid, citric acid, lactic acid, malic acid, propionic acid, and tartaric acid are commonly employed for meat preservation due to their antimicrobial properties and ability to enhance flavor profiles (8). These acids function by disrupting bacterial membranes and cytoplasmic processes through acidification, leading to toxicity in microbial cells. Among these, citric acid demonstrates the highest antibacterial efficacy, followed by lactic acid, propionic acid, and acetic acid. Lactic acid, in particular, is highly effective against gram-negative bacteria, disrupting their outer membranes without compromising the sensory attributes of the meat (9, 10, 11, 12).

Despite advancements in meat preservation, there is a need to explore the combined impact of specific organic acids on the microbial profile and shelf life of buffalo meat sausages under chilling conditions. This study aims to address this gap by evaluating the effectiveness of lactic acid and acetic acid in enhancing the microbial stability and shelf-life of buffalo beef sausages stored at 0–4°C over a 12-day period, ensuring their sensory attributes remain unaltered.

METHODS

This study was conducted at the Department of Meat Science and Technology, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan, utilizing facilities at the Meat Processing Lab, City Campus, Lahore, and the Food Safety and Hygiene Lab, Central Laboratory Complex (CLC), Ravi Campus, Pattoki, Pakistan. The research was designed as a factorial experiment under a Completely Randomized Design (CRD) with four treatment groups and three replicates. The treatments included three concentrations of an organic acid mixture (lactic acid and acetic acid) at 0.75%, 1%, and 2% (v/v), along with a control group without organic acid. Observations were recorded on the 0th, 4th, 8th, and 12th days of storage at chilling temperatures (0–4°C). A total of 96 samples were evaluated across the storage period, with statistical analysis performed to assess treatment effects.

Table 1: Experimental Layout

Treatment (Organic Acids With Equal Ratio)	Storage Period (Days)	Sample Size
Control: without organic acid addition		Concentration = 4 Storage = 4
0.75 % + 0.75 % concentration of both (lactic and acetic acid)	0, 4, 8, 12	Sample/replicate = 2 Replicate = 3 n = 32 x 3 = 96
1 % + 1 % concentration of (lactic and acetic acid)		
2 % + 2 % concentration of (lactic and acetic acid)		

Approximately 4.8 kg of cube roll meat and 1.2 kg of edible animal fat were obtained from buffalo calves (*Bubalus bubalis*) slaughtered at the UVAS Meat Processing Facility, Lahore. The calves were procured from commercial fattening farms and rested for 24 hours prior to slaughter, during which they were provided water and feed as per standard pre-slaughter protocols. Ante-mortem inspections were conducted, and the animals were slaughtered using the Halal method, following PS-3733 (2016) guidelines. After slaughter, the carcasses were chilled for 24 hours before deboning to extract cube roll meat. All ingredients used in sausage preparation, including spices, collagen-based casings, and organic acids, were Halal-certified and sourced from verified local suppliers or reputable international manufacturers.

Table 2: Composition of the Batter (2kg)

Ingredients	Quantity(grams)
Meat	1800
Fat	200
Spices	18
Nitrite-NaCl (0.5%)	70
Ascorbic acid	1.5
Phosphate	10
Ice	100

The organic acid solutions were prepared by diluting 80% pure lactic acid (Appli Chem GmbH, Germany) and 100% pure acetic acid with distilled water to achieve concentrations of 0.75%, 1%, and 2% (v/v). These solutions were mixed in equal proportions and applied to sausages using the sprinkling method at a rate of 1 ml per 10 g of meat. The sausage batter, composed of minced beef, edible fat, spices, and additives (as outlined in Table 2), was prepared using standardized meat processing equipment. The batter was homogenized with ice to maintain a low temperature during preparation. Organic acid solutions were sprayed onto the batter, which was then allowed to cure for 24 hours in a chiller before stuffing into Halal-certified, collagen-based casings. Sausages were cooked in a hot air oven at 85°C until the internal temperature reached 75°C, ensuring food safety standards, and then cooled at room temperature for 15 minutes before being stored in a display chiller.

Microbial analysis was conducted to assess the total viable count (TVC), *E. coli* count, and *Pseudomonas* count. TVC was determined using nutrient agar following ISO6222:1999 protocols, with colony counts recorded after 48 hours of incubation at 37°C. *E. coli* was identified using EMB agar based on ISO16654:2001, while *Pseudomonas* was assessed using cetrimide agar in accordance with ISO13720:2010. All microbial analyses employed serial dilution techniques, with results calculated as colony-forming units (CFU) per gram of sample. Water activity (*A_w*) was measured using a LabSwift-AW meter (Novasina, Switzerland), with readings taken on specified days of storage. pH was measured using a calibrated WTW pH meter, with values recorded thrice for each sample. Sensory evaluation was conducted by a trained panel using a nine-point hedonic scale to assess sensory attributes, including odor, flavor, tenderness, juiciness, and overall acceptability.

Ethical considerations were meticulously addressed. The study protocol was reviewed and approved by the Institutional Review Board (IRB) of UVAS (approval number: UVAS-2025/IRB/02). All animals used in the study were handled in compliance with institutional animal welfare guidelines, and humane slaughter practices were ensured. Informed consent was obtained from the sensory panel members before their participation. Data were analyzed using factorial ANOVA with PROC GLM in SAS software (Version 9.1.3), treating organic acid concentrations and storage periods as fixed effects. Mean differences were assessed using Duncan's Multiple Range Test, with significance set at $P \leq 0.05$. The study design and statistical methods ensured robust analysis, yielding reliable results to address the research objectives.

RESULTS

The results demonstrated significant differences across all parameters, reflecting the effect of organic acids on the shelf-life stability, microbial profile, and sensory attributes of buffalo meat sausages. The total viable count (TVC) showed a significant reduction with increasing concentrations of organic acids. The highest TVC was observed in the control group (T0) at 5.99 log CFU/g, while the lowest count was recorded in T3 (2% organic acid concentration) at 4.49 log CFU/g. The effect of storage time was also significant, with TVC progressively increasing over the 12-day storage period, from 3.88 log CFU/g on day 0 to 6.56 log CFU/g on day 12 ($P \leq 0.0001$). The *E. coli* count decreased significantly with higher concentrations of organic acids. The highest count was observed in the control group (T0) at 2.73 log CFU/g, while the lowest was recorded in T3 at 2.01 log CFU/g. As storage time increased, a gradual rise in the *E. coli* count was noted, with significant differences observed on the 12th day of storage ($P \leq 0.0001$).

The *Pseudomonas* count exhibited a similar trend, with the control group (T0) showing the highest count of 5.20 log CFU/g and T3 demonstrating the lowest at 3.97 log CFU/g. The storage period also had a significant impact, with counts increasing over time, from 3.67 log CFU/g on day 0 to 5.62 log CFU/g on day 12 ($P \leq 0.0001$). Water activity showed a significant reduction with the application of organic acids, particularly at the highest concentration (T3), which recorded the lowest value of 0.85. Over the storage period, water activity decreased marginally but remained within acceptable ranges, with no significant interaction between treatment and storage time. The pH of the sausages decreased significantly with increasing concentrations of organic acids. The control group (T0) exhibited the highest pH at 5.73, while T3 showed the lowest pH at 5.30. Changes in pH over the storage period followed a fluctuating trend, with initial reductions followed by slight increases on the 12th day. Sensory analysis revealed that organic acid treatments significantly improved the sensory attributes of the sausages, including odor, flavor, texture, juiciness, and overall acceptability. T2 (1% concentration) scored the highest in overall acceptability, while T3 (2% concentration) displayed slightly lower scores, likely due to the stronger acidity. The control group consistently scored the lowest across all sensory parameters. Over the storage period, sensory scores declined slightly, with day 0 samples receiving the highest ratings.

Table 3: Effect of Organic Acids on the Microbial Profile of Buffalo Meat Sausages

Treatment	TVC	E.Coli	Pseudomonas	
T0	5.99 ± 0.54 ^a	2.73 ± 0.13 ^a	5.20 ± 0.37 ^a	
T1	4.96 ± 0.26 ^b	2.40 ± 0.02 ^b	4.76 ± 0.23 ^b	
T2	4.52 ± 0.22 ^c	2.13 ± 0.02 ^c	4.37 ± 0.15 ^c	
T3	4.49 ± 0.23 ^c	2.01 ± 0.01 ^d	3.97 ± 0.13 ^d	
P-value	≤0.0001	≤0.0001	≤0.0001	
Storage(Day)				
0	3.88 ± 0.01 ^d	2.26 ± 0.07 ^b	3.67 ± 0.02 ^d	
4	4.35 ± 0.11 ^c	2.26 ± 0.05 ^b	4.40 ± 0.13 ^c	
8	5.17 ± 0.28 ^b	2.25 ± 0.06 ^b	4.62 ± 0.14 ^b	
12	6.56 ± 0.35 ^a	2.50 ± 0.18 ^a	5.62 ± 0.29 ^a	
P-value	≤0.0001	≤0.0001	≤0.0001	
T0	0	3.92 ± 0.01 ^h	2.50 ± 0.06 ^b	3.73 ± 0.01 ^h
	4	4.82 ± 0.02 ^f	2.45 ± 0.01 ^{bc}	4.81 ± 0.01
	8	6.70 ± 0.12 ^b	2.46 ± 0.02 ^{bc}	5.14 ± 0.02 ^c
	12	8.53 ± 0.20 ^a	3.49 ± 0.01 ^a	7.12 ± 0.02 ^a

Treatment		TVC	E.Coli	Pseudomonas
T1	0	3.90 ± 0.01 ^h	2.45 ± 0.01 ^{bc}	3.70 ± 0.02 ^h
	4	4.61 ± 0.01 ^g	2.38 ± 0.01 ^c	4.77 ± 0.02 ^{de}
	8	5.09 ± 0.02 ^e	2.40 ± 0.06 ^c	4.79 ± 0.02 ^{de}
	12	6.23 ± 0.09 ^c	2.37 ± 0.01 ^c	5.78 ± 0.19 ^{de}
T2	0	3.89 ± 0.01 ^h	2.10 ± 0.06 ^e	3.62 ± 0.01 ^h
	4	3.99 ± 0.01 ^h	2.20 ± 0.06 ^d	4.32 ± 0.01 ^f
	8	4.45 ± 0.01 ^g	2.11 ± 0.01 ^{de}	4.64 ± 0.02 ^e
	12	5.75 ± 0.03 ^d	2.11 ± 0.01 ^{de}	4.91 ± 0.05 ^d
T3	0	3.81 ± 0.01 ^h	1.98 ± 0.01 ^f	3.62 ± 0.02 ^h
	4	3.97 ± 0.01 ^h	2.02 ± 0.01 ^{ef}	3.71 ± 0.01 ^g
	8	4.44 ± 0.02 ^g	2.02 ± 0.01 ^{ef}	3.89 ± 0.06 ^g
	12	5.72 ± 0.04 ^d	2.03 ± 0.01 ^{ef}	4.67 ± 0.02 ^e
P-value		≤0.0001	≤0.0001	≤0.0001

Superscripts on various means within column showing significant results at P ≤ 0.05

Table 4: Effect of Organic Acids on the pH and Water Activity of Buffalo Meat Sausages

Treatment		pH	Water Activity
T0		5.73 ± 0.06 ^a	0.91 ± 0.01
T1		5.42 ± 0.05 ^b	0.91 ± 0.001
T2		5.39 ± 0.03 ^b	0.91 ± 0.001
T3		5.30 ± 0.03 ^c	0.85 ± 0.06
P-value		≤0.001	0.4351
Storage(Day)			
	0	5.52 ± 0.06 ^a	0.92 ± 0.001
	4	5.37 ± 0.04 ^b	0.85 ± 0.06
	8	5.36 ± 0.05 ^b	0.91 ± 0.001
	12	5.58 ± 0.08 ^a	0.89 ± 0.001
P-value		≤0.001	0.4092
T0	0	5.80 ± 0.06 ^b	0.93 ± 0.001
	4	5.50 ± 0.06 ^{cd}	0.92 ± 0.01
	8	5.60 ± 0.06 ^c	0.92 ± 0.001
	12	6.02 ± 0.01 ^a	0.87 ± 0.01
T1	0	5.58 ± 0.01 ^c	0.92 ± 0.001
	4	5.40 ± 0.12 ^{cde}	0.92 ± 0.001
	8	5.34 ± 0.01 ^{de}	0.91 ± 0.01
	12	5.36 ± 0.13 ^{de}	0.89 ± 0.01
T2	0	5.40 ± 0.12 ^{cde}	0.92 ± 0.001
	4	5.37 ± 0.01 ^{de}	0.91 ± 0.001
	8	5.30 ± 0.06 ^{de}	0.91 ± 0.001
	12	5.49 ± 0.01 ^{cd}	0.89 ± 0.01
T3	0	5.30 ± 0.06 ^{de}	0.92 ± 0.001

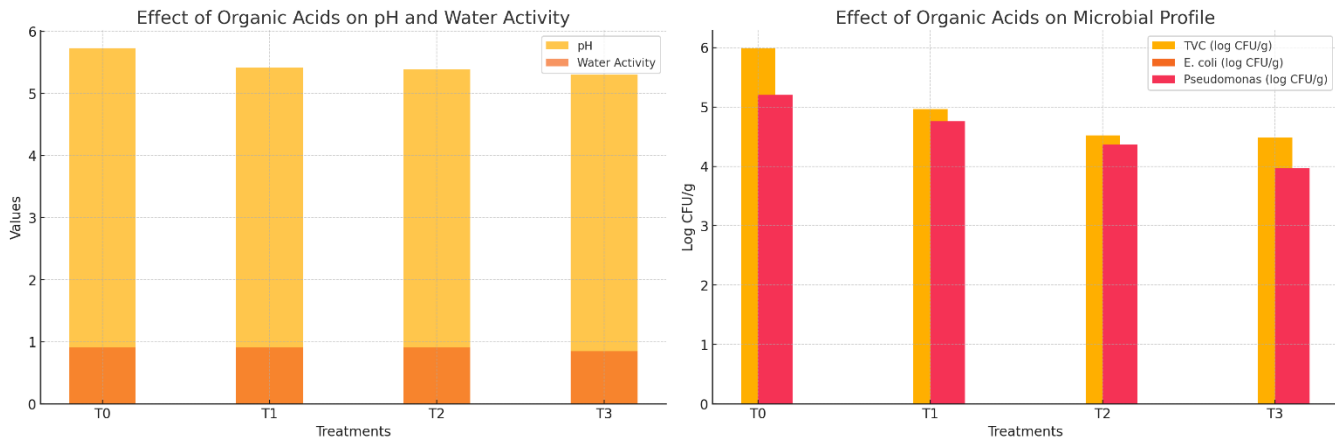
Treatment	pH	Water Activity
4	5.23 ± 0.01 ^c	0.66 ± 0.25
8	5.20 ± 0.06 ^c	0.92 ± 0.001
12	5.45 ± 0.001 ^{cd}	0.89 ± 0.01
P-value	0.0102	0.4294

Superscripts on various means within column showing significant results at P ≤ 0.05

Table 5: Sensory Evaluation of Organic Acid-Treated Beef Sausages and Control without Treated with Acid

Treatment	Odor	Flavor	Texture	Juiciness	Overall acceptability	
T0	4.79 ± 0.04 ^d	4.63 ± 0.01 ^c	5.32 ± 0.02 ^d	5.38 ± 0.01 ^c	4.60 ± 0.01 ^d	
T1	6.51 ± 0.01 ^c	6.90 ± 0.07 ^b	6.61 ± 0.08 ^c	6.60 ± 0.08 ^b	7.19 ± 0.01 ^b	
T2	7.13 ± 0.01 ^b	7.33 ± 0.03 ^a	7.22 ± 0.01 ^b	7.21 ± 0.03 ^a	7.33 ± 0.01 ^a	
T3	7.17 ± 0.01 ^a	7.29 ± 0.01 ^a	7.27 ± 0.02 ^a	7.18 ± 0.01 ^a	7.00 ± 0.06 ^c	
P-value	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	
Storage(Day)						
0	6.46 ± 0.28 ^a	6.62 ± 0.34 ^a	6.68 ± 0.23 ^a	6.69 ± 0.22 ^a	6.63 ± 0.34 ^a	
4	6.44 ± 0.28 ^a	6.58 ± 0.34 ^{ab}	6.66 ± 0.23 ^{ab}	6.66 ± 0.22 ^a	6.54 ± 0.34 ^b	
8	6.38 ± 0.30 ^b	6.55 ± 0.34 ^b	6.62 ± 0.24 ^b	6.56 ± 0.22 ^b	6.51 ± 0.34 ^b	
12	6.33 ± 0.31 ^c	6.40 ± 0.33 ^c	6.46 ± 0.25 ^c	6.47 ± 0.23 ^c	6.45 ± 0.34 ^c	
P-value	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	
T0	0	4.92 ± 0.01 ^c	4.67 ± 0.01 ^c	5.38 ± 0.01 ^c	5.43 ± 0.01 ^c	4.65 ± 0.01 ^g
	4	4.90 ± 0.01 ^c	4.65 ± 0.01 ^c	5.38 ± 0.01 ^c	5.41 ± 0.01 ^c	4.61 ± 0.01 ^{gh}
	8	4.70 ± 0.06 ^f	4.62 ± 0.01 ^c	5.27 ± 0.01 ^f	5.37 ± 0.01 ^c	4.59 ± 0.01 ^{gh}
	12	4.63 ± 0.01 ^g	4.59 ± 0.01 ^c	5.23 ± 0.02 ^f	5.32 ± 0.01 ^c	4.54 ± 0.01 ^h
T1	0	6.54 ± 0.01 ^c	7.07 ± 0.02 ^c	6.76 ± 0.01 ^c	6.90 ± 0.06 ^b	7.21 ± 0.01 ^{bc}
	4	6.52 ± 0.01 ^{cd}	7.04 ± 0.01 ^c	6.75 ± 0.01 ^c	6.80 ± 0.06 ^b	7.21 ± 0.01 ^{bc}
	8	6.51 ± 0.01 ^{cd}	7.00 ± 0.06 ^c	6.73 ± 0.02 ^c	6.50 ± 0.06 ^c	7.19 ± 0.01 ^c
	12	6.47 ± 0.01 ^d	6.50 ± 0.06 ^d	6.20 ± 0.12 ^d	6.21 ± 0.01 ^d	7.16 ± 0.01 ^c
T2	0	7.17 ± 0.01 ^a	7.40 ± 0.12 ^a	7.26 ± 0.02 ^{ab}	7.22 ± 0.01 ^a	7.35 ± 0.01 ^a
	4	7.15 ± 0.01 ^{ab}	7.32 ± 0.02 ^{ab}	7.23 ± 0.01 ^{ab}	7.22 ± 0.01 ^a	7.34 ± 0.01 ^a
	8	7.12 ± 0.01 ^{ab}	7.29 ± 0.01 ^{ab}	7.19 ± 0.02 ^{ab}	7.20 ± 0.06 ^a	7.34 ± 0.02 ^a
	12	7.09 ± 0.01 ^b	7.29 ± 0.01 ^{ab}	7.18 ± 0.01 ^b	7.20 ± 0.12 ^a	7.29 ± 0.01 ^{ab}
T3	0	7.19 ± 0.01 ^a	7.34 ± 0.01 ^{ab}	7.30 ± 0.06 ^a	7.22 ± 0.01 ^a	7.30 ± 0.06 ^{ab}
	4	7.19 ± 0.01 ^a	7.32 ± 0.01 ^{ab}	7.29 ± 0.01 ^{ab}	7.19 ± 0.01 ^a	7.00 ± 0.06 ^d
	8	7.17 ± 0.01 ^a	7.29 ± 0.02 ^{ab}	7.28 ± 0.01 ^{ab}	7.17 ± 0.01 ^a	6.90 ± 0.06 ^c
	12	7.12 ± 0.01 ^{ab}	7.22 ± 0.01 ^b	7.22 ± 0.01 ^{ab}	7.13 ± 0.02 ^a	6.80 ± 0.06 ^f
P-value	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	

Superscripts on various means within column showing significant results at P ≤ 0.05



DISCUSSION

The findings of this study provide valuable insights into the use of organic acids, such as lactic acid and acetic acid, as effective preservatives for enhancing the shelf life and sensory attributes of buffalo meat sausages. The significant reduction in microbial counts, including total viable counts, *E. coli*, and *Pseudomonas*, with increasing concentrations of organic acids highlights their efficacy in controlling spoilage and pathogenic bacteria. Sausages treated with a 2% concentration of lactic and acetic acids exhibited the lowest microbial counts, while untreated sausages (control) displayed the highest levels. These results are consistent with prior studies that reported the antibacterial properties of organic acids, which act by disrupting bacterial membranes and reducing microbial survival during storage. The gradual increase in microbial counts over time, even in treated samples, aligns with previous findings that storage duration significantly impacts bacterial proliferation under chilled conditions (3, 17). The pH of the sausages showed a significant decrease with increasing organic acid concentrations, reflecting the acidic nature of the treatments. The lowest pH values were observed in sausages treated with 2% organic acids, indicating effective acid penetration into the meat matrix. However, a gradual increase in pH was noted during the storage period, likely due to protein degradation and the production of basic compounds such as ammonia and amines. These findings are consistent with studies suggesting that initial pH reduction is followed by an increase during prolonged storage due to biochemical changes within the meat (24). While the reduction in pH aids in inhibiting bacterial growth, the later increase could promote spoilage if storage is extended beyond optimal limits.

Water activity, a critical parameter for microbial growth and meat preservation, showed minimal changes across treatments. While sausages treated with 2% organic acids exhibited slightly lower water activity values, the differences were not statistically significant. The results are consistent with observations that chilled storage reduces water activity due to gradual moisture evaporation but does not cause drastic changes in meat products over short storage periods (28). The limited impact of organic acids on water activity suggests that their preservative effect is primarily due to their antibacterial properties rather than changes in meat moisture content. Sensory evaluation revealed that the incorporation of organic acids improved the sensory attributes of buffalo meat sausages. Panelists consistently rated acid-treated sausages higher in terms of odor, flavor, texture, juiciness, and overall acceptability compared to untreated sausages. Sausages treated with a 1% concentration of organic acids were preferred over those treated with 2%, likely due to the stronger acidic taste at higher concentrations. These findings align with previous studies reporting that organic acids enhance sensory qualities by reducing spoilage bacteria while maintaining the natural flavor profile of the product. However, the slight decline in sensory scores over the storage period reflects the gradual deterioration of meat quality, even under chilled conditions.

The study's strengths lie in its comprehensive evaluation of microbial, physicochemical, and sensory parameters, providing a holistic understanding of the effects of organic acids on meat preservation. The factorial experimental design and the use of validated methodologies ensure the reliability of the findings. However, limitations include the relatively short storage duration, which restricts the ability to assess the long-term effects of organic acids. The study also focused exclusively on buffalo meat sausages, limiting the generalizability of the results to other meat types or products. Future research could explore the use of organic acids in combination with other preservation methods, such as vacuum packaging or modified atmosphere storage, to further enhance shelf life. Additionally, studies investigating the impact of organic acids on the nutritional profile of meat products would provide a more comprehensive assessment of their suitability as preservatives. The findings underscore the potential of organic acids as natural and economical alternatives to synthetic preservatives. Their availability, affordability, and effectiveness in controlling spoilage and pathogenic bacteria

make them suitable for widespread use in the meat industry. While the 2% concentration demonstrated the greatest microbial control, the 1% concentration emerged as a more balanced option, offering both preservation and sensory benefits without compromising taste. These findings pave the way for optimizing organic acid treatments to meet consumer preferences and industry requirements for safe, high-quality meat products.

CONCLUSION

The study concluded that the combined use of lactic acid and acetic acid as natural preservatives effectively enhanced the quality and shelf life of buffalo meat sausages stored at chilling temperatures. The treatment demonstrated significant improvements in microbial stability, pH regulation, and sensory attributes, offering a practical solution for maintaining meat safety and quality. While higher concentrations of organic acids provided greater microbial control, they also slightly impacted sensory acceptability due to altered taste profiles. These findings underscore the potential of organic acids as economical and efficient alternatives to synthetic preservatives, contributing to safer and higher-quality meat products for consumers.

Author Contribution

Author	Contribution
Haseeb Ahmad	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Fazal Ur Rehman*	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Zia Ullah	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Muhammad Awais	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Haider Ali	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Naveed Sabir	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Zabeeh Ullah	Contributed to study concept and Data collection Has given Final Approval of the version to be published
Obaid Muhammad Abdullah	Writing - Review & Editing, Assistance with Data Curation
Faisal Rasool	Writing - Review & Editing, Assistance with Data Curation
Abdul Aziz	Writing - Review & Editing, Assistance with Data Curation

REFERENCES

1. Li Y, Xue C, Quan W, Qin F, Wang Z, He Z, et al. Assessment the influence of salt and polyphosphate on protein oxidation and N ϵ -(carboxymethyl)lysine and N ϵ -(carboxyethyl)lysine formation in roasted beef patties. *Meat Sci.* 2021;177:108489.
2. Linghu Z, Karim F, Taghvaei M, Albashabsheh Z, Houser T, Smith J. Amino acids effects on heterocyclic amines formation and physicochemical properties in pan-fried beef patties. *J Food Sci.* 2020;85(8):2337–46.
3. Ahmad, S., et al. Quality and shelf life evaluation of fermented sausages of buffalo meat with different levels of heart and fat. *Meat Science.* 2007. 75(4): p. 603-609.
4. Brooks, J.D., et al. Biofilms in the food industry: problems and potential solutions. *International Journal of Food Sciences and Technology.* 2008. 43(12): p. 2163-2176.
5. Lawrie, R. The structure, composition and preservation of meat. In P. G. Campbell & P. E. Cook (Eds.), *Fermented Meats.* 1995. 1(1): p. 1-38.
6. Li Y, He J, Quan W, He Z, Qin F, Tao G, et al. Effects of polyphosphates and sodium chloride on heterocyclic amines in roasted beef patties as revealed by UPLC-MS/MS. *Food Chem.* 2020;326:127016.

7. Sousa AMB, Alves R, Madeira D, Santos RM, Pereira ALF, Lemos T, et al. Storage of beef burgers containing fructooligosaccharides as fat replacer and potassium chloride as replacing sodium chloride. *J Food Sci Technol*. 2020;57(11):4354–60.
8. Cirstea NL, Nour V, Boruzi AI. Effects of pork backfat replacement with emulsion gels formulated with a mixture of olive, chia and algae oils on the quality attributes of pork patties. *Foods*. 2023;12(3):519.
9. Mani-Lopez, E., et al. Organic acids as antimicrobials to control Salmonella in meat and poultry products. *Food Research International*. 45(2): 2012. p. 713-721.
10. Bolder N. Decontamination of meat and poultry carcasses. *Trends in Food Science and Technology*. 1987. 8(7): p. 221-227.
11. Ouattara, B., et al. Inhibitory effect of organic acids upon meat spoilage bacteria. *Journal of Food Protection*. 1997. 60(3): p. 246-253.
12. Alakomi, H.L., et al. Lactic acid Permeabilizes gram-negative bacteria by disrupting the outer membrane. *Applied Environmental Microbiology*. 2000. 66 (5): p. 2001-2005
13. Seo, J.K., et al. Properties of Frankfurter-type sausages with pork back-fat replaced with bovine heart surimi-like materials. *Korean Journal of Food Science and Animal Resources*. 2016. 36(4): p. 523-531.
14. Maturin, L., et al. Aerobic Plate Count. In *Bacteriological Analytical Manual* (8th ed., Revision A). AOAC International. 2017. P. 1-78.
15. Saha, A., et al. Consumer acceptance of broiler breast fillets marinated with varying levels of salt. *Poultry Science*. 2009. 88(2): p. 415-423.
16. Carvalho C, Madrona G, Mitcha JG, Valero MV, Guerrero A, Scapim M, et al. Effect of active packaging with oregano oil on beef burgers with low sodium content. *Acta Sci Technol*. 2019;42(1):1–7.
17. Sachindra, N., et al. Microbial profile of buffalo sausage during processing and storage. *Food Control*. 2005. 16(1): p. 31-35.
18. Verma, S.P., et al. Improvement in the quality of ground chevon during refrigerated storage by tocopherol acetate preblending. *Meat Science*. 2000. 56(4): p. 403-413.
19. Acuff, G.V., et al. Effect of acid decontamination of beef subprimal cuts on the microbiological and sensory characteristics of steaks. *Meat Sci*. 1987. 19(3): p. 217-226.
20. Bosilevac, J.M., et al. Treatments using hot water instead of lactic acid reduce levels of aerobic bacteria and Enterobacteriaceae and reduce the prevalence of Escherichia coli O157: H7 on pre evisceration beef carcasses. *J Food Protection*. 2006. 69(8): p. 1808-1813.
21. Stivarius, M., et al. Effects of hot water and lactic acid treatment of beef trimmings prior to grinding on microbial, instrumental color, and sensory properties of ground beef during display. *Meat Science*. 2002. 60(4): p. 327-334.
22. Hardin, H., et al. Comparison of methods for contamination removal from beef carcass surfaces. *International Association for Milk, Food, and Environmental Sanitarians*. 1995. 58: p. 368-370.
23. Ozdemir, H., et al. Effects of lactic acid and hot water treatments on Salmonella Typhimurium and Listeria monocytogenes on beef. *Food Control*. 2006. 17(4): p. 299-303.
24. Karabagias, I.K., et al. Shelf-life extension of lamb meat using thyme or oregano essential oils and modified atmosphere packaging. *Meat Science*. 2011. 88(1): p. 109-116.
25. Stanisic, N., et al. Changes of physical-chemical properties of beef during 14 days of chilling. *Biotechnology in Animal Husbandry*. 28(1): p. 77-85.
26. Perez, M.L., et al. Effect of calcium chloride marination on calpain and quality characteristics of meat from chicken, horse, cattle, and rabbit. *Meat Science*. 1998. 48(1-2): p. 125-134.
27. Dikeman, M., et al. Effects of post exsanguination vascular infusion of cattle with a solution of saccharides, sodium chloride, and phosphates or with calcium chloride on quality and sensory traits of steaks and ground beef. *Journal of Animal Science*. 2003. 1(1): p. 221-229.
28. Arief, M.A., et al. Influence of packaging (wrapping) materials and storage periods on certain chemical and organoleptic characteristics of broiler cut up parts. *Kerala Journal of Veterinary Science*. 1989. 20: p. 107-114.
29. Andres, S., et al. The effect of whey protein concentrates and hydrocolloids on the texture and color characteristics of chicken sausages. *International Journal of Food Science and Technology*. 2006. 41(8): p. 954-961.