



## Research Article

**Effect of organic acid and natural antioxidant on the quality and shelf life of raw chicken meat at refrigerated storage**MR Islam<sup>1</sup>, MM Hasan<sup>1</sup>, MMR Masum<sup>1</sup>, M Khan<sup>1</sup>, MM Rahman<sup>1\*</sup>

## Abstract

The present study was conducted to evaluate the shelf life and microbiological quality of raw chicken meat incorporated with organic acid and natural antioxidants at refrigerated storage (4±1°C). The meat was stratified into four experimental groups: T<sub>0</sub> (Control), T<sub>1</sub> (1% Vinegar), T<sub>2</sub> (1.5% Lemon Pulp), T<sub>3</sub> (0.3% Tulsi leaf Extract). Assessments were systematically performed on days 0, 3 and 7 to determine a wide range of parameters including physicochemical characteristics oxidative stability, color value, Odor, microbial safety, and proximate composition. Throughout the storage period, organic acid and natural antioxidants-treated samples exhibited significantly reduced pH levels and higher water retention capacities (P<0.01) relative to the control. Throughout the storage period, comparatively lower viable count was detected in T<sub>1</sub> treatment. Among them, 1% vinegar (T<sub>1</sub>) demonstrated superior oxidative stability, as evidenced by its significantly lower TBARS (P<0.01) values. The most preferable good odor was observed from T<sub>1</sub> treatment and the lowest odor from T<sub>0</sub> group. In different treatment groups color content significantly (P<0.05) decreased but in the control group color content decreased rapidly. From this comparative study it can be concluded that in case of sensory evaluation, 1% of vinegar is more appreciated and nutrient quality is more satisfactory.

## Introduction

Chicken meat is a highly nutritious food, rich in protein, vitamins, and minerals essential for human health. It provides key nutrients like vitamin B12, niacin (B3), vitamin B6, iron, zinc, phosphorus, and selenium, which support various bodily functions, including immune health, energy metabolism, and bone health (Kaur et al., 2023; Simopoulos, 2016). Additionally, it contains bioactive compounds such as carnosine, creatine, and taurine, known for their antioxidant, anti-inflammatory, and neuroprotective effects. Despite these benefits, chicken meat is highly perishable and prone to microbial contamination, which presents significant challenges in preservation. Its affordability, versatility, and health advantages over red meat have contributed to its increasing global consumption (Jayathilakan et al., 2012; FAO, 2020; Rahman et al., 2023). Meat is highly perishable and prone to spoilage due to microbial growth, enzymatic activity, and physicochemical changes. Microbial contamination, including bacteria like *Pseudomonas*, *Enterobacteriaceae*, and lactic acid bacteria, is a primary cause of spoilage, leading to off-flavors, odors, and visual changes (Liza et al., 2024; Sajib et al., 2023; Torun et al., 2023; Jay et al., 2005). Improper storage, temperature abuse, and contamination during processing further exacerbate microbial growth and increase the risk of foodborne illness. Enzymatic processes also contribute to spoilage: proteolytic enzymes degrade proteins, altering texture and flavor (Disha et al., 2020; Toldrá and Flores, 1998), while lipolytic enzymes induce rancidity and off-odors through lipid oxidation (Frankel et al., 2003). Physicochemical changes, such as oxidative reactions from exposure to oxygen, lead to off-flavors and nutrient loss, particularly in fatty acids and fat-soluble vitamins. Changes in pH, water activity, and ion concentrations also play a role in accelerating spoilage (Biplob et al., 2024; Hasan et al., 2024; Hossan et al., 2024; Sarker et al., 2024). Proper storage is essential to maintain the quality, safety, and nutritional value of meat. Refrigeration below 5°C (41°F) inhibits microbial growth, reducing the risk of foodborne illness by controlling bacteria like *Salmonella*, *Escherichia coli*, and *Listeria monocytogenes* (Jay et al., 2005). Freezing at temperatures below -18°C (0°F) further extends shelf life by preventing microbial spoilage and enzymatic activity (Boby et al., 2021). Natural antioxidants, such as those found in *Ocimum sanctum* (Tulsi), lemon extract, and vinegar, are increasingly used to extend meat shelf life and prevent oxidative rancidity. Tulsi leaves, rich in polyphenols and flavonoids, have antioxidant properties that help preserve meat without affecting its quality. Vinegar, containing acetic acid, lowers meat pH, creating an environment unfavorable for microbial growth and directly disrupting bacterial membranes, thereby extending shelf life (Sarker et al., 2021). Similarly, lemon extract, rich in citric acid, flavonoids, and vitamin C, lowers pH and neutralizes free radicals to prevent oxidative spoilage and inhibit the growth of spoilage microorganisms like *E. coli* and *Staphylococcus aureus* (Jayaprakasha and Patil, 2007; Tajkarimi et al., 2010). Studies have demonstrated the effectiveness of lemon extract in maintaining meat quality, reducing microbial load, and extending shelf life during cold storage (Disha et al., 2020; Azad et al., 2022).

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Tulsi's effectiveness as a natural preservative in meat products is primarily due to its bioactive compounds, including phenolic compounds, flavonoids, and essential oils, which exhibit strong antioxidant and antimicrobial properties. These compounds, such as cirsilineol, cirsimaritin, isothymusin, apigenin, rosmarinic acid, and eugenol, scavenge free radicals and inhibit microbial growth, thus preventing oxidative rancidity and spoilage in meat (Pandey and Madhuri, 2010). The present study aims to achieve the following objectives: i. to evaluate the effect of organic acids, ii. To assess the impact of natural antioxidants, and iii. To compare the combined effectiveness of organic acids and natural antioxidants in extending the shelf life of raw chicken meat.

## **Materials and Methods**

### **Place of experiment**

The experiment was carried out in the laboratory of the Department of Animal Science in Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh

### **Preparation of Sample**

Chicken meat samples, sourced from Bangladesh Agricultural University Sheshmor market, were slaughtered using the Halal method and transported to the Animal Science Laboratory for analysis. The muscle part of the chicken was used, with bones and fat removed. Vinegar, lemons, and tulsi leaves were collected from the university market and gardens. The lemons were washed, peeled, and the peel was pulverized to extract juice, while fresh tulsi leaves were boiled to make an infusion. All materials and equipment were properly cleaned before use. The chicken meat samples were treated with the lemon, tulsi, and vinegar extracts, and then subjected to sensory, physicochemical, and microbial analyses to study the effects of these treatments.

### **Experimental layout**

The chicken meat samples were divided into four parts: the control group ( $T_0$ ), treated with no additives, and three experimental groups:  $T_1$  (1% vinegar),  $T_2$  (1.5% lemon pulp extract), and  $T_3$  (tulsi leaf extract). Each treatment was mixed with its respective portion of the meat, and the samples were then placed in polythene bags. The meat was stored at 4°C for 7 days. At intervals of 0 days, 3 days, and 7 days, sensory, physicochemical, and microbial analyses were conducted on the samples in the laboratory to observe any changes due to the treatments.

### **Instrumental color Analysis**

Instrumental color measurement was conducted on meat from the longissimus muscle. Color was assessed using a Konica Minolta Chroma Meter (CR 410, Konica Minolta Sensing, Inc., Osaka, Japan), a Miniscan Spectro colorimeter set to the CIE Lab system, which includes  $L^*$ ,  $a^*$ , and  $b^*$  values (International Commission on Illumination). Here,  $L^*$  indicates lightness,  $a^*$  indicates redness, and  $b^*$  indicates yellowness. The analysis focused on the medial surface (bone side) of the meat 24 hours post-mortem. Prior to measurement, the colorimeter was calibrated using a specific whiteboard. Each color value was the average of three measurements taken from a meat area of 4–5 cm<sup>2</sup> to ensure a representative evaluation. The  $L^*$  value ranges from 0 (black) to 100 (white), while both  $a^*$  and  $b^*$  values range from -60 to +60;  $a^*$  indicates green when negative and red when positive, while  $b^*$  indicates blue when negative and yellow when positive.

### **Proximate Analysis**

Proximate composition, including Dry Matter, Crude Protein, Ether Extract, and Ash, was determined according to AOAC (1995) methods. Crude protein was determined using the micro Kjeldahl method. Ether extract content was determined using a Soxhlet apparatus with diethyl ether. Ash content was determined by pre-ashing the samples and then heating them in a muffle furnace.

### **Physicochemical Analysis**

The pH meter is calibrated with standard buffer solutions at pH 4 and 7, ensuring proper stabilization and adjustments. A fresh piece of meat is prepared by cutting it into sections, exposing fresh muscle tissue while avoiding fat and connective tissue. The electrode is inserted into the muscle, and the pH reading is allowed to stabilize before being recorded. After measuring, the electrode is rinsed with distilled water and stored according to the manufacturer's instructions. To measure the water holding capacity (WHC) of meat, a fresh sample is prepared, weighed, and placed into centrifuge tubes. The samples are centrifuged at around 10,000 RPM for 10 minutes to expel excess water. To assess the drip loss of meat, a fresh sample is prepared, weighed to determine its initial weight, and placed in a container to refrigerate for 24 hours. After this period, the meat is removed, allowed to reach room temperature, and then weighed again to find its final weight.

### **Biochemical Analysis**

Lipid oxidation was evaluated by using the 2-thiobarbituric acid (TBA) method. Chicken breast meat samples (5 g) were mixed with 25 ml of a 20% trichloroacetic acid solution and vortexed for 60 seconds, then filtered through Whatman filter paper number 4. The filtrate (2 mL) was combined with 2 mL of a 0.02 M TBA solution and incubated at 100°C for 30 minutes, then cooled with tap water. Absorbance was measured at 532 nm using a UV-VIS spectrophotometer, and the TBA value was reported as mg of malonaldehyde per kg of meat sample.

### **Microbiological Analysis**

Microbial assessment of meat ensures food safety and quality by identifying and quantifying microorganisms, including pathogens and spoilage organisms. Samples are prepared by blending 10 g of chicken meat with sterile diluent, creating a homogenized suspension, and performing serial dilutions. For bacteriological analysis, media like Plate Count Agar (PCA), MacConkey Agar (MA), and Potato Dextrose Agar (PDA) are prepared, sterilized, and used to culture microorganisms. The Total Viable Count (TVC), Total Coliform Count (TCC), and Yeast-Mould count are then determined by spreading diluted samples on the respective agar plates, incubating, and counting colonies, with results expressed as CFU/g of chicken meat.

## Statistical Model and Analysis

The statistical model used for the experiment was a factorial design with two factors, A (Treatments) and B (Days of Intervals). Data analysis was performed using SAS Statistical Discovery software, and the significance of differences among treatment means was determined using the DMRT test.

## Results and Discussion

### Physicochemical analysis

#### pH value

The pH changes of chicken treated with vinegar during refrigerated storage (4°C) are shown in Table 1. The control samples exhibited an increase in pH over the storage period, while chicken samples treated with 1% vinegar, 1.5% lemon pulp, and 0.3% tulsi leaf extract showed significantly lower pH (5.62) values ( $P < 0.05$ ) compared to the control. The pH range for the treated samples was between 5.60 and 5.85, while the control samples ranged from 5.52 to 5.74 by the end of the 7-day storage period. The pH of all samples increased gradually over the 7 days, with significant differences observed at the 0, 3, and 7-day intervals. The rise in pH was attributed to the increase in volatile base compounds produced by microbial or enzymatic activity, as well as the decomposition of nitrogenous components. These findings align with studies of Sarker et al. (2021) and Serdaroglu et al. (2005), who noted similar pH increases in meat samples treated with additives.

#### Cooking loss

Cooking loss refers to the weight reduction of meat during cooking, caused by thawing, dripping, and evaporation. Thawing loss is related to fluid loss due to freezing and thawing, while dripping refers to fluid loss from the meat during cooking. The results of cooking loss (CL) in chicken treated with vinegar are presented in Table 1. Significant differences ( $P < 0.05$ ) were observed in all treatment groups, with the CL content decreasing compared to the control. Among the treatments, the 1% vinegar treatment showed the most favorable cooking loss (25.15%), indicating it was preferred by consumers. Significant differences were noted across the 0, 3, and 7-day intervals, with Duncan grouping letters (a, b, c, d) showing significant variations. Cooking loss decreased as the storage period increased, with the least cooking loss observed on the 7th day and the most on the 0th day.

**Table 1.** Effect of organic acid and natural antioxidant on physicochemical parameters in chicken meat at different day intervals

Parameters	DI	Treatments					Mean	Level of significance		
		T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	Treat.		DI	T*DI	
pH	0	5.52±0.029	5.79±0.029	5.54±0.031	5.69±0.036	5.70 <sup>a</sup> ±0.031	P<.001	P<.001	P<.001	
	3	5.63±0.012	5.83±0.032	5.58±0.034	5.83±0.033	5.71 <sup>a</sup> ±0.27				
	7	5.74±0.006	5.98±0.028	5.74±0.029	5.96±0.041	5.85 <sup>b</sup> ±0.26				
	Mean	5.632 <sup>a</sup> ±0.016	5.866 <sup>a</sup> ±0.030	5.621 <sup>c</sup> ±0.031	5.793 <sup>b</sup> ±0.036					
Cooking Loss	0	30.81±0.033	28.46±0.032	30.45±0.037	28.30±0.034	29.50 <sup>a</sup> ±0.034	P<.001	P<.001	P<.001	
	3	28.76±0.041	24.62±0.030	27.73±0.027	24.13±0.043	26.31 <sup>b</sup> ±0.035				
	7	22.45±0.034	22.45±0.033	20.36±0.038	20.06±0.037	21.33 <sup>c</sup> ±0.033				
	Mean	27.341 <sup>a</sup> ±0.036	26.180 <sup>b</sup> ±0.033	26.180 <sup>b</sup> ±0.033	24.163 <sup>d</sup> ±0.037					

The mean in each row having different superscripts varies significantly at values  $P < 0.05$ . Again, mean values with the same superscript in each row did not differ significantly at  $P > 0.05$ . T<sub>0</sub> = (Control group), T<sub>1</sub> = (1% vinegar), T<sub>2</sub> = (1.5% lemon pulp), T<sub>3</sub> = (0.3% tulsi leaf extract) and DI=Day Intervals, Treat= Treatment, T×DI=Interaction of Treatment and Day Intervals. \*Means significant at 5% level of probability, \*\* means significant at 1% level of probability, NS means non-significant

### Biochemical properties

The TBA values (Table 2) were significantly lower ( $P < 0.05$ ) in the 1% vinegar (T<sub>1</sub>)(0.12) and 1.5% lemon pulp (T<sub>2</sub>) treatments compared to the control and tulsi leaf extract (T<sub>3</sub>) at the start of storage. Vinegar maintained the lowest TBA values throughout the storage period, consistent with the findings of Sarker et al. (2021), which showed vinegar's superior antioxidant activity. TBA values increased over time, with T<sub>3</sub> showing the highest values and T<sub>1</sub> the lowest by the end of storage. All three preservatives acted as effective antioxidants, with vinegar performing the best, as supported by Okon et al. (2024).

**Table 2.** Effect of organic Acid and natural Anti-oxidants on physicochemical parameters (Mean ± SE) in chicken meat at different day intervals

Parameters	DI	Treatments					Mean	Level of significance		
		T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	Treat		DI	T*DI	
TBARS Value	0	0.11±0.0049	0.12±0.0055	0.13±0.0045	0.13±0.0039	0.1225 <sup>b</sup> ±0.0047	P<.001	P<.001	P<.001	
	3	0.12±0.0039	0.12±0.0050	0.13±0.0031	0.13±0.0034	0.1250 <sup>b</sup> ±0.0038				
	7	0.14±0.0043	0.13±0.0048	0.14±0.0021	0.14±0.0032	0.1375 <sup>a</sup> ±0.0036				
	Mean	0.12 <sup>a</sup> ±0.0044	0.12 <sup>a</sup> ±0.0051	0.13 <sup>b</sup> ±0.0032	0.13 <sup>b</sup> ±0.0035					

### Sensory Evaluation

#### Color

The results of the color analysis of chicken samples treated with T<sub>1</sub> (1% vinegar), T<sub>2</sub> (1.5% lemon pulp extract), and T<sub>3</sub> (0.3% tulsi leaf extract) during refrigerated storage are shown in Table 3. Color was significantly affected ( $P < 0.05$ ) by the treatments, with the T<sub>1</sub>, T<sub>2</sub>, and T<sub>3</sub> groups showing significant differences compared to the control. The control group exhibited a rapid decrease in color content, whereas the treatment groups showed a slower decline, with T<sub>1</sub> being the most preferable. The addition of natural antioxidants and organic acids influenced color ( $P < 0.05$ ), with effects depending on the concentration similar with the findings of (Tushar et al., 2023). Abdel Hamied et al. (2009) also found that organoleptic properties of rosemary-treated beef were more acceptable than untreated samples.

## Odor

The odor scores for different treatments at various intervals are shown in Table 3. The odor scores ranged from 4.33 to 4.43 across the four treatments, with significant ( $P < 0.05$ ) differences observed. The most preferable odor was found in T<sub>1</sub> (1% vinegar), while the lowest odor was observed in the control group (T<sub>0</sub>). The odor score decreased with increased storage time, ranging from 4.74 to 4.03. Significant differences were noted at the 0 to 7-day intervals, indicating a decline in odor quality as storage progressed. Odor is a major factor in quality deterioration, as it affects other sensory attributes like color, texture, flavor, and nutritional value. The meat industry seeks effective natural antioxidants that can replace synthetic ones without compromising product quality or consumer perception. Sagoo et al. (2002) found that adding chitosan to sausages did not result in off-odors or affect the appearance, preventing consumer rejection. The decrease in flavor scores is likely due to oxidative rancidity and microbial deterioration during storage.

**Table 3.** Effect of organic Acid and natural Anti-oxidants on sensory parameters (Mean  $\pm$  SE) in chicken at different day intervals

Parameters	DI	Treatments					Level of significance		
		T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	Mean	Treat.	DI	T*DI
Color	0	4.83 $\pm$ 0.0040	4.33 <sup>c</sup> $\pm$ 0.0052	4.68 $\pm$ 0.0049	4.76 $\pm$ 0.0033	4.75 <sup>a</sup> $\pm$ 0.0041			
	3	4.69 $\pm$ 0.0038	4.69 $\pm$ 0.0045	4.59 $\pm$ 0.0057	4.63 $\pm$ 0.0039	4.65 <sup>b</sup> $\pm$ 0.0044	P<.001	P<.001	P<.001
	7	4.03 $\pm$ 0.0043	4.24 $\pm$ 0.0033	4.42 $\pm$ 0.0051	4.23 $\pm$ 0.0037	4.23 <sup>c</sup> $\pm$ 0.0041			
	Mean	4.51 <sup>c</sup> $\pm$ 0.0043	4.55 <sup>ab</sup> $\pm$ 0.0041	4.56 <sup>a</sup> $\pm$ 0.0049	4.54 <sup>b</sup> $\pm$ 0.0038				
Odor	0	4.81 $\pm$ 0.0050	4.71 $\pm$ 0.0052	4.73 $\pm$ 0.0033	4.71 $\pm$ 0.0045	4.74 <sup>a</sup> $\pm$ 0.0045			
	3	4.39 $\pm$ 0.0047	4.43 $\pm$ 0.0043	4.42 $\pm$ 0.0057	4.44 $\pm$ 0.0043	4.42 <sup>b</sup> $\pm$ 0.0047	P<.001	P<.001	P<.001
	7	3.81 $\pm$ 0.0060	4.10 $\pm$ 0.0039	4.06 $\pm$ 0.0047	4.14 $\pm$ 0.0036	4.03 <sup>c</sup> $\pm$ 0.0045			
	Mean	4.33 <sup>c</sup> $\pm$ 0.0052	4.41 <sup>b</sup> $\pm$ 0.0042	4.40 <sup>b</sup> $\pm$ 0.0044	4.43 <sup>a</sup> $\pm$ 0.0041				

The mean in each row having different superscripts varies significantly at values  $P < 0.05$ . Again, mean values with the same superscript in each row did not differ significantly at  $P > 0.05$ . T<sub>0</sub> = (Control group), T<sub>1</sub> = (1% vinegar), T<sub>2</sub> = (1.5% lemon pulp), T<sub>3</sub> = (0.3% tulsi leaf extract) and DI=Day Intervals, Treat= Treatment, T\*DI=Interaction of Treatment and Day Intervals. \*Means significant at 5% level of probability, \*\* means significant at 1% level of probability, NS means non-significant

## Proximate analysis

### Dry Matter

The Dry Matter (DM) content showed in (Table 4) no significant differences across treatments, days of interval, or the interaction between treatment and days of interval, with mean values ranging from 25.13 to 25.46 across all groups. The most preferable DM content was observed in the T<sub>1</sub> (26.21%) group, while the lowest was found in the T<sub>3</sub> (25.32%) group, indicating it was less preferable. DM content increased over the storage period due to decreased moisture loss, with the most preferable content observed on day 0 and the least preferable on day 9, although it was still accepted by consumers. Similar findings were reported by Naveena et al. (2008) for extracts of pomegranate peel and rind, while a decrease in DM content was noted in low-fat chicken nuggets by Santhi et al. (2017).

### Crude Protein

The crude protein (CP) content of chicken samples with different treatments at various intervals is shown in Table 4. The CP content ranged from 21.50% to 21.88% across the treatments, with no significant ( $P < 0.05$ ) differences observed between the natural antioxidant treatments. The highest CP content, which is preferable for consumer health, was found in the control group (21.88%), while the lowest was observed in the T<sub>2</sub> (21.50%) group. The CP content ranged from 21.34% to 21.95% across the different storage intervals. Significant ( $P < 0.05$ ) differences were noted at the 0, 3, and 7-day intervals, with CP content decreasing over time. The highest CP content was observed on day 0, and the lowest on day 7. This trend is consistent with the findings of Konieczny et al. (2007), who reported a decrease in CP content during frozen storage. Higher CP content is beneficial for consumers, especially for growing children, pregnant women, and lactating women, as protein is essential for growth and productive functions. Therefore, higher CP levels in products can meet nutritional needs while potentially reducing expenditure on meat and meat products.

### Ether Extract

The study assessed the ether extract (EE) content in chicken meat treated with various natural antioxidants. The EE content across treatments ranged from 2.63% to 2.71%, with significant differences ( $p < 0.05$ ) between the control (T<sub>0</sub>) and antioxidant treatments. The T<sub>2</sub> (2.63%) group showed the most preferable EE content (Table 4.), indicating it was the healthiest option for consumers, while the control group (T<sub>0</sub>) had the highest EE content, making it less preferable. Additionally, EE content ranged from 2.59% to 2.73% across different storage days, with significant differences ( $p < 0.05$ ) observed between day 0, day 3, and day 7. The lowest EE content was observed on day 0 (2.5%), while the highest was on day (72.7%), indicating that the EE content increased as the storage period lengthened.

### Ash

Table presents the ash content results for chicken meat treated with various antioxidants, showing significant differences ( $p < 0.05$ ) between treatments, days of storage, and the interaction between the two (Table 4). The mean ash content ranged from 1.47% to 1.58%. Among the treatments, the T<sub>1</sub> (1.51%) group showed the most preferable ash content, indicating a lower and more favorable amount for consumer health. The T<sub>3</sub> (1.58%) group had the highest ash content, making it less preferable. Ash content increased over the storage period, with the lowest ash content observed on day 0 and the highest on day 7. Despite this increase, the higher ash content after 7 days (1.47%) was still considered acceptable for consumers. Similar findings were reported by Serdaroglu et al. (2005), and Bhosale et al. (2011), who noted changes in ash content with different treatments and ingredients.

**Table 4.** Effect of organic Acid and natural Anti-oxidants on proximate components (Mean ± SE) in chicken at different day intervals.

Parameters	DI	Treatments					Mean	Level of significance		
		T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	Treat.		DI	T×DI	
DM (%)	0	25.36±0.041	25.84±0.038	26.59±0.044	26.56±0.057	26.08c±0.045	P<.001	P<.001	P<.001	
	3	25.67±0.058	26.43±0.034	26.73±0.057	26.93±0.049	26.44b±0.049				
	7	25.98±0.063	26.24±0.021	26.71±0.047	26.86±0.055	26.45a±0.046				
	Mean	25.67d±0.054	26.17c±0.031	26.67b±0.049	26.78a±0.053					
CP (%)	0	22.48±0.011	21.79±0.034	21.61±0.011	21.90±0.011	21.95a±0.016	P<.001	P<.001	P<.001	
	3	21.85±0.017	21.62±0.010	21.56±0.012	21.47±0.012	21.63b±0.013				
	7	21.31±0.017	21.40±0.012	21.34±0.011	21.30±0.015	21.34c±0.014				
	Mean	21.88a±0.015	21.60b±0.018	21.50d±0.011	21.56c±0.012					
EE (%)	0	2.57±0.003	2.65±0.005	2.54±0.005	2.61±0.005	2.59c±0.004	P<.001	P<.001	P<.001	
	3	2.69±0.003	2.71±0.005	2.63±0.004	2.65±0.004	2.67b±0.004				
	7	2.74±0.006	2.76±0.003	2.72±0.005	2.69±0.005	2.73a±0.004				
	Mean	2.67b±0.007	2.71a±0.003	2.63d±0.003	2.65c±0.001					
Ash (%)	0	1.25±0.017	1.37±0.014	1.38±0.011	1.49±0.026	1.37 c±0.017	*	*	*	
	3	1.46±0.015	1.53±0.010	1.59±0.017	1.57±0.022	1.54b±0.016				
	7	1.69±0.015	1.64±0.008	1.57±0.015	1.68±0.016	1.65a±0.013				
	Mean	1.47c±0.015	1.51b±0.011	1.51b±0.015	1.58a±0.021					

The mean in each row having different superscripts varies significantly at values  $P < 0.05$ . Again, mean values with the same superscript in each row did not differ significantly at  $P > 0.05$ . T<sub>0</sub> = (Control group), T<sub>1</sub> = (1% vinegar), T<sub>2</sub> = (1.5% lemon pulp), T<sub>3</sub> = (0.3% tulsi leaf extract) and DI=Day Intervals, Treat= Treatment, T×DI=Interaction of Treatment and Day Intervals. \*Means significant at 5% level of probability, \*\* means significant at 1% level of probability, NS means non-significant

### Microbiological assessments

The study assessed the presence of micro-flora (total viable count, TVC) and foodborne pathogens in chicken treated with different natural preservatives. The results indicated in (Table 5) that the TVC of the treated chicken was significantly lower ( $P < 0.05$ ) than the control group (T<sub>0</sub>). Fresh chicken had an initial TVC of 5.68 log<sub>10</sub> CFU/g, indicating good quality. Over the 7-day storage period, the TVC increased in all treatments, with the control sample showing the highest count (5.73 log<sub>10</sub> CFU/g), while the treated samples (T<sub>1</sub>, T<sub>2</sub>, and T<sub>3</sub>) had lower counts, with T<sub>1</sub> (vinegar treatment) showing the most significant reduction. The TVC values ranged from 5.61 to 5.47 log<sub>10</sub> CFU/g over the storage period, and significant differences ( $P < 0.05$ ) were observed between treatment groups and across days. The study supports the antimicrobial effects of vinegar, which showed the most pronounced inhibition of microbial growth, similar to findings from Sadakuzzaman et al. (2023), Sarker et al. (2021) and Akhter et al., (2022), who also observed lower microbial counts with vinegar. Overall, the natural preservatives, particularly vinegar, were effective in controlling microbial growth during storage.

**Table 5.** Effect of organic Acid and natural Anti-oxidants on different microbial population (Mean ± SE) in chicken at 4°C temperature

Parameters	DI	Treatments					Mean	Level of significance		
		T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	Treat		DI	T*DI	
TVC (log CFU/g)	0	5.68±0.0040	5.34±0.0034	5.49±0.0043	5.52±0.0052	5.47c±0.0042	P<.001	P<.001	P<.001	
	3	5.72±0.0039	5.41±0.0023	5.52±0.0030	5.59±0.0050	5.56b±0.0035				
	7	5.79±0.0042	5.45±0.0039	5.59±0.0035	5.63±0.0045	5.61a±0.0040				
	Mean	5.73a±0.0040	5.40d±0.0032	5.53c±0.0036	5.58b±0.0049					

The mean in each row having different superscripts varies significantly at values  $P < 0.05$ . Again, mean values with the same superscript in each row did not differ significantly at  $P > 0.05$ . T<sub>0</sub> = (Control group), T<sub>1</sub> = (1% vinegar), T<sub>2</sub> = (1.5% lemon pulp), T<sub>3</sub> = (0.3% tulsi leaf extract) and DI=Day Intervals, Treat= Treatment, T×DI=Interaction of Treatment and Day Intervals. \*Means significant at 5% level of probability, \*\* means significant at 1% level of probability, NS means non-significant.

### Conclusion

The study found that chicken can be preserved for 7 days using various treatments, with 1% vinegar and 1.5% lemon pulp receiving the best sensory scores. These treatments improved nutrient quality, while 0.3% tulsi leaf extract performed poorly. Over storage, dry matter increased, but crude protein decreased, likely due to absorption of organic acids and antioxidants. Vinegar treatment also enhanced antioxidant and antimicrobial properties, reducing lipid oxidation and extending shelf life. Thus, 1% vinegar is recommended for preserving chicken, offering a natural alternative to synthetic antioxidants.

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