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Research Article

Effect of natural and synthetic antioxidant on the quality of broiler meat during refrigeration

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Abstract

This study evaluated the effects of natural and synthetic antioxidants and antimicrobial agents on the quality and shelf life of fresh and refrigerated chicken meat. Fresh chicken meat samples were divided into four treatment groups: T_0 (control, no antioxidant), T_1 (1% lemon peel extract), T_2 (1% orange peel extract), and T_3 (0.01% Butylated Hydroxytoluene, BHT). Samples were stored at 4°C for 9 days, and quality parameters were assessed at intervals of 0, 3, 6, and 9 days. Sensory attributes (color: L*, a*, b* values), proximate composition (dry matter, crude protein, ether extract, and ash), physicochemical properties (pH, water-holding capacity, drip loss, and cooking loss), biochemical stability (thiobarbituric acid reactive substances, TBARS), and microbial quality (total viable count, coliform count, yeast, and mold count) were analyzed. Instrumental color analysis showed that T_3 had the highest L* (47.44) and a* (4.95) values, with peak redness (6.35) on day 9. Significant differences ($P < 0.01$) were observed in color attributes across treatments and storage periods. Proximate composition analysis revealed significant differences ($P < 0.01$) among treatments. T_3 exhibited the highest dry matter (DM) and ash content, while T_1 had the highest crude protein (CP) content. Ether extract (EE) values were most favorable in T_3. Physico-chemical properties indicated significant differences ($P < 0.01$) in pH, water-holding capacity (WHC), drip loss, and cooking loss. T_1 had the most favorable pH (5.72–5.88), while WHC was highest in T_3 (92.05%–93.22%). Biochemical analysis showed TBARS values significantly increased ($P < 0.01$) over time, indicating lipid oxidation. TBARS values ranged from 0.193 to 0.226 across treatments, with T_3 exhibiting the lowest values, signifying better oxidative stability. Microbial analysis confirmed that T_3 had the lowest total viable count (5.43 log CFU/g), coliform count (2.85 log CFU/g), and yeast-mold count (2.60 log CFU/g), demonstrating its superior antimicrobial effectiveness. Overall, 0.01% BHT (T_3) was the most effective antioxidant and antimicrobial agent, preserving meat quality and extending shelf life under refrigerated conditions (4 ± 1°C).

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Introduction

Meat is widely considered a nutritious food and an excellent source of high-quality protein. Poultry meat, in particular, provides all nine essential amino acids, making it a valuable part of the human diet. It is also rich in selenium, vitamins B3 and B6, and choline. Poultry is globally favored for its affordability, availability, and lack of religious restrictions (Biplob et al., 2024; Hasan et al., 2024; Hossan et al., 2024; Prabakaran et al., 2012; Sarker et al., 2024). Chicken is popular due to its low fat, high protein content, and essential fatty acids, offering superior taste and nutritional value compared to beef, pork, and mutton (Duan et al., 2021; Sagar et al., 2024; Sajib et al., 2023). Additionally, during the religious festival of Eid-ul-Adha, an increased beef supply arises due to cattle sacrifices, contributing to an oversupply and potential wastage (Begum et al., 2007). However, meat, particularly beef, is highly perishable, and improper preservation can lead to spoilage and foodborne pathogens (Yasmin et al., 2022; Kumudavally et al., 2005). Meat spoilage is mainly caused by microbial growth and chemical degradation, with lipid oxidation playing a key role in the processed meat industry. This oxidation affects meat quality by altering sensory characteristics (color, texture, odor and flavor) and reducing its nutritional value (Nunez de Gonzalez et al., 2008; Rahman et al., 2023). Antioxidants are commonly used to prevent lipid oxidation, which causes rancid flavors, odors, and reduces shelf life and safety (Lahucky et al., 2010). To extend their freshness, meat is often processed, with cooking being a common method. However, cooking can have both positive and negative effects on meat quality (Torun et al., 2023). The antioxidant capacity of meat is low but can be enhanced by adding flavonoid-rich plant parts (seeds, fruit skin, or extracts) without affecting sensory attributes. Meat contains natural antioxidants, called endogenous antioxidants, such as tocopherols, carnosine, lipoic acid, and various enzymes (Decker and Mei, 1996). Antioxidants, such as plant polyphenols, essential oils (EOs), and synthetic compounds like BHA, BHT, and nitrites, have been used in the meat industry to slow oxidation and extend shelf life (Sadakuzzaman et al., 2021 and 2024; Haque et al., 2020). To prevent oxidation in meat, synthetic antioxidants like BHA, BHT, TBHQ, and PG have been used, but concerns about their safety have arisen due to potential toxic effects, such as liver damage and cancer risks. Natural antioxidants from plants are valued for their ability to improve the taste, stability, and shelf-life of meat products (Jung et al., 2010). Citrus fruits are widely consumed worldwide for their energy content, nutrients, and health benefits.

They are a rich source of bioactive compounds, including flavonoids and vitamin C, which exhibit antioxidant properties by scavenging free radicals (Anagnostopoulou et al., 2006). Citrus extracts contain flavonoid glycosides, coumarins, sterols, and volatile oils, while their fiber is rich in polyphenols, particularly vitamin C. Additionally, citrus fruits provide various macronutrients such as sugars, fiber, potassium, vitamins, and minerals. They are known for their bioactive properties, including antioxidant, anti-inflammatory, anti-cancer, antimicrobial, and anti-allergy effects, and offer benefits for cardiovascular health, neuroprotection, liver protection, and obesity management. Citrus fruit products, such as orange-peel extract, act as effective antimicrobial agents against bacteria and fungi. These products have important physiological roles and substantial commercial value in the food and pharmaceutical industries (Mathur et al., 2011). While citrus fruits are mainly used for juice production, the peels are often discarded, generating a significant amount of waste (Manthey and Grohmann, 2001). In this context, this study aims to evaluate the sensory, proximate, biochemical, physico-chemical, and microbial qualities of chicken meat following the addition of lemon pulp extract and orange peel extract, while also assessing their impact on oxidative changes during storage and their effectiveness in inhibiting microbial growth to extend the shelf life of chicken meat.

Materials and Methods

Place of experiment

The experiment was conducted at the Animal Science Laboratory of Bangladesh Agricultural University (BAU) in Mymensingh, Bangladesh.

Experimental samples

Chicken meat samples were collected from the Kamal-Ronjit market at Bangladesh Agricultural University, Mymensingh. Chickens of similar weight were humanely slaughtered according to Halal standards, and only muscle tissue was used for sampling, excluding bones. The samples were then transported to the Animal Science Laboratory at BAU for sensory, physicochemical, and microbial analyses.

Source of lemon and orange

Lemons and oranges were sourced from the Kamal-Ranajit market at Bangladesh Agricultural University.

Preparation of jar and other instruments

All required instruments and jars were thoroughly washed with hot water and detergent, then properly dried before beginning the experiment.

Preparation of meat sample

The chicken meat was thoroughly rinsed with fresh water, and all visible body fat, tendons, skin, and easily removable connective tissues were carefully trimmed from the boneless meat using a sharp knife.

Preparation of lemon peel and orange peel extract

The edible portions of their peels were carefully removed. The remaining peels were then ground using a grinder. After grinding, the lemon peel and orange peel extracts were obtained by filtering through a sieving cloth. The chicken meat sample was combined with lemon peel and orange peel extracts in the following proportions: T_0 = Control, T_1 = 1.0% lemon pulp extract , T_2 = 1.0% Orange peel extract,T_3 = 0.01% BHT (butylated hydroxyl toluene)

Different analytical characteristics of chicken meat samples

Sensory properties of chicken

Sensory evaluation

Color is a key attribute in the sensory evaluation of chicken meat, as it greatly influences consumer perception and acceptance. Bright, vibrant colors are often associated with freshness and high quality, while discoloration suggests spoilage. Factors like feed, antioxidants (natural and synthetic), and storage conditions can impact meat color. Antioxidants help preserve the visual appeal by delaying oxidation that causes browning. A well-preserved, attractive color is crucial for consumer satisfaction, making it an important factor alongside texture, flavor, and overall appearance in sensory evaluation.

Proximate composition

Proximate composition such as Dry Matter (DM), Ether Extract (EE), Crude Protein (CP) and Ash were carried out according to the methods (AOAC, 1995). All determination was done in triplicate and the mean value was reported.

Physicochemical properties measurement

Raw pH measurement

pH value of raw meat was measured using pH meter from raw meat homogenate. The homogenate was prepared by blending 5g of meat with 10 ml distilled water.

Drip loss

To measure drip loss using the gravimetric method, broiler meat samples are cut into uniform pieces for consistency. The initial weight (W_1) is recorded using a precision balance. The samples are then suspended in a sealed plastic bag or box to prevent contact with surfaces, allowing exudate to drip naturally. After refrigeration for 24 to 48 hours at 4°C, the samples are removed, excess moisture is blotted off, and the final weight (W_2) is recorded. The difference in weight (W_1 - W_2) indicates the drip loss.

Cooking loss

To assess cooking loss, 20 g samples were weighed, wrapped in heat-resistant foil, and placed in a water bath at 70°C for 30 minutes. After cooking, the surfaces of the samples were dried and re-weighed. This procedure was carried out on day 0, day 3, day 6, day and 9 days.

Water holding capacity

To measure water holding capacity, a portion of meat is cut or minced and weighed to record its initial weight. The sample is then placed in a centrifuge tube, with a filter-equipped tube or absorbent paper to collect the expelled water. The centrifuge is set to a speed (typically 1,500-3,000 rpm) and duration (5-10 minutes), depending on the protocol. The centrifugal force expels water trapped in the meat. After centrifugation, the meat and absorbent paper are removed, and the final weight is recorded. The difference between the initial and final weights represents the water lost.

Biochemical analysis

Thiobarbituric acid values (TBARS) (mg-MDA/kg)

Lipid oxidation was measured in triplicate using the 2-thiobarbituric acid (TBA) method as outlined by Schmedes and Holmer (1989). The absorbance was recorded at 532 nm using a UV-VIS spectrophotometer (UV 1200, Shimadzu, Japan). The TBA value was reported as milligrams of malonaldehyde per kilogram of the meatball sample.

Microbial assessment

The microbial analysis of chicken meat involved assessing the total viable count (TVC), total coliform count (TCC), and total yeast and mold count (YMC). Samples were prepared by homogenizing 10 g of chicken meat in 0.1% peptone water and performing serial dilutions. The media used for the analysis included Plate Count Agar (PCA), MacConkey Agar (MA), and Potato Dextrose Agar (PDA). For TVC, 0.1 ml of each dilution was spread on PCA plates and incubated at 35°C for 24-48 hours. Colonies were counted, and results were expressed as CFU/g. For TCC, similar to TVC, 0.1 ml of each dilution was spread on MA plates and incubated at 35°C for 24-48 hours. Colonies were counted and reported as CFU/g. For TYMC, 0.1 ml of each dilution was spread on PDA plates and incubated at 25°C for 48-72 hours. Colonies were counted, and the results were expressed as CFU/g. The results for all counts were recorded based on ISO (1995) guidelines.

Statistical model and analysis

The proposed model for the planned experiment was factorial experiment with two factors A (Treatments) and B (Days of Intervals). Data were statistically analyzed using SAS Statistical Discovery software, NC, USA. DMRT test was used to determine the significance of differences among treatments means.

Results and Discussion

Instrumental color value

The color attributes of fresh chicken meat, including lightness (L*), redness (a*), and yellowness (b*), were evaluated across four treatment groups. The T_3 group had the highest lightness (L*) value (47.44), while T_0 had the lowest (39.2). By day 3, the T_1 group reached a peak L* value of 54.56, which declined to 30.93 by day 9. Significant variations were observed in L* values across treatments, storage days, and their interaction ($P < 0.01$). For redness (a*), T_3 had the highest value (4.95), with T_0 having the lowest (2.67). The highest a* value for T_3 was seen on day 9 (6.35), and it decreased by day 0. Significant differences in redness were found across treatments and storage days ($P < 0.01$), but the interaction was not significant. Regarding yellowness (b*), T_3 exhibited the most desirable value (9.63), while T_2 had the lowest (10.69). On day 9, T_1 had the highest b* value (12.49), which decreased to 8.68 by day 3. Yellowness values were significantly influenced by storage duration and their interaction ($P < 0.01$), but not by treatment groups. Metmyoglobin is the compound that causes the distinct brown color in meat as it degrades during storage in refrigeration (Mancini et al., 2005).

Table 1. Effect of different types of anti-oxidants on instrumental color value (Mean ± SE) in chicken meat at different days of intervals

Parameters	DI	Treatments				Mean	Level of significance		
		T_0	T_1	T_2	T_3		Treat.	DI	T*DI
	0	41.67±3.06	44.43±3.04	42.62±6.13	52.02±4.04	45.19^{a}			
	3	36.92±1.08	54.56±1.08	38.86±1.14	48.33±6.04	44.67^{a}			
L*	6	39.35±1.03	34.82±2.95	42.33±5.08	48.29±3.03	41.2^{b}	**	**	**
	9	38.85±2.90	30.93±4.05	51.35±1.92	41.12±3.98	40.56^{b}			
	Mean	39.2^{c}	41.19^{bc}	43.79^{b}	47.44^{a}				
	0	1.59±0.47	1.72±0.21	2.82±0.39	4.10±0.60	2.56^{d}			
	3	2.69±0.58	2.83±0.54	4.50±0.50	5.23±0.35	3.81^{b}			
a*	6	2.53±0.15	2.66±0.13	3.59±0.17	4.11±0.95	3.22^{c}	**	**	NS
	9	3.88±0.23	5.76±1.04	5.73±0.86	6.35±0.19	5.43^{a}			
	Mean	2.67^{d}	3.24^{c}	4.16^{b}	4.95^{a}				
	0	11.15±2.06	11.22±3.95	11.07±1.59	11.41±0.26	11.21^{a}			
	3	8.92±0.47	8.68±0.97	10.70±1.93	9.54±0.59	9.46^{b}			
b*	6	8.89±0.4	8.96±0.23	13.16±1.96	9.69±0.14	10.18^{ab}	NS	*	**
	9	10.68±1.12	12.49±1.73	7.83±1.28	7.86±0.58	9.72^{b}			
	Mean	9.91^{a}	10.34^{a}	10.69^{a}	9.63^{a}				

Same superscripts in different treatments groups and days of interval did not differ significantly, whereas different superscripts in different treatments groups and days of interval differ significantly. T_0 = (control group), T_1 = (1% lemon peel extract), T_2 = (1 % orange peel extract), T_3 = (0.01% BHT), DI=Day Intervals, Treat= Treatment, T×DI=Interaction of Treatment and Day Interval. ** means significant at 1% level of probability. * Means significant at 5% but more than 1% level of probability. NS means no significance.

Proximate Analysis

Dry Matter

Among the four treatments, the T_3 group showed the most favorable DM (dry matter) content. A lower DM content suggests higher preference, while a higher DM content indicates lower preference. The control group exhibited the least desirable DM content. As the storage period extended, DM content increased, which can be attributed to reduced moisture loss over time. The

most preferred DM content was found on day 0, whereas the least preferred was observed on the 9th day. The main cause is likely the evaporative loss from the hot carcass when it is moved into refrigeration. Similar findings have been reported by Al-Bachir and Zeinou (2014). Naveena et al. (2008) reported that extending the storage period was linked to an increase in the dry matter content of both pomegranate peel extract and pomegranate rind powder extract.

Ash

Among the four treatments, the T_3 group showed the most favorable ash content. A lower ash content is considered better for consumer health, making this treatment the most preferred. Conversely, the control group had the least desirable ash content. Additionally, ash content increased significantly with longer storage periods. The lowest ash content was recorded on day 0, while the highest was noted on the 9th day, though it was still deemed acceptable from a consumer perspective. Bhosale et al. (2011) observed a reduction in ash content in chicken nuggets that included ground carrot and mashed sweet potato, which aligns with the current findings.

Crude Protein

The crude protein (CP) content of chicken meat varied between treatment groups, ranging from 20.68% to 21.25%. Significant differences ($p < 0.01$) were found between treatments with natural and synthetic antioxidants, with the T_1 group showing the highest CP content, beneficial for consumer health, and the control group (T_0) having the lowest. The CP content also fluctuated over the storage period, ranging from 19.88% to 22.04%. Significant differences ($p < 0.01$) were noted across storage days (0, 3, 6, and 9). CP content generally decreased over time, with the highest levels on day 0 and the lowest on day 9, when all treatment groups showed a decline to below 20%. This reduction in CP content with extended storage time is consistent with the findings of Konieczny et al. (2007), who reported a decrease in protein content during frozen storage. The higher CP levels found in products treated with Dawadawa and Curcuma are advantageous for consumers since proteins are essential, especially for children's growth and during periods of increased physiological demand, such as pregnancy and lactation, due to the increased need for protein for conception and milk production (Heinz and Hautzinger, 2007)

Ether Extract

The ether extract (EE) content in chicken meat treated with natural and synthetic antioxidants ranged from 2.32% to 2.62%, with significant differences ($p < 0.01$) between the control and antioxidant-treated groups. T_3 had the most favorable EE content, which is preferable for consumer health, while the control group (T_0) had a higher EE value. Over the storage period (day 0, 3, 6, and 9), EE content ranged from 2.3% to 2.67%, with significant variations ($p < 0.01$). The lowest EE content was observed on day 0, and the highest on day 9, suggesting that storage duration increases fat concentration. Similar trends have been reported in previous studies. Verma et al. (2013) found a reduction in the fat content of sheep meat nuggets when guava powder was added.

Table 2. Effect of different types of anti-oxidants on proximate parameters (Mean ± SE) in chicken meat at different days of intervals

Parameters	DI	Treatments				Mean	Level of significance		
		T_0	T_1	T_2	T_3		Treat.	DI	T*DI
DM (%)	0	26.80±0.52	26.45±0.04	26.43±0.14	26.64±0.44	26.58^{d}	NS	**	NS
	3	27.23±0.71	26.98±0.65	27.19±0.46	26.93±0.11	27.08^{c}			
	6	28.16±0.73	27.83±0.56	28.02±0.46	27.31±0.63	27.83^{b}			
	9	29.21±0.51	28.50±0.48	28.82±0.55	28.73±0.78	28.82^{a}			
	Mean	27.85^{a}	27.62^{a}	27.44^{a}	27.40^{a}				
Ash (%)	0	1.18±0.11	1.22±0.004	1.33±0.003	1.58±0.40	1.33^{c}	**	**	NS
	3	1.31±0.10	1.35±0.02	1.34±0.02	1.36±0.11	1.34^{c}			
	6	1.32±0.06	1.41±0.01	1.43±0.02	1.59±0.06	1.44^{b}			
	9	1.47±0.05	1.52±0.01	1.59±0.02	1.60±0.01	1.54^{a}			
	Mean	1.32^{c}	1.37^{bc}	1.42^{b}	1.53^{a}				
CP (%)	0	22.15±0.60	22.09±0.05	21.92±0.31	22.00±0.17	22.04^{a}	**	**	*
	3	21.19±0.15	21.75±0.03	21.55±0.04	21.61±0.41	21.53^{b}			
	6	20.04±0.06	21.06±0.49	21.19±0.02	21.26±0.10	20.89^{c}			
	9	19.33±0.05	20.08±0.70	19.98±0.06	20.11±0.02	19.88^{d}			
	Mean	20.68^{b}	21.25^{a}	21.16^{a}	21.25^{a}				
EE%	0	2.46±0.16	2.38±0.19	2.21±0.07	2.15±0.08	2.30^{c}	**	**	**
	3	2.54±0.11	2.46±0.10	3.34±0.07	2.26±0.08	2.65^{a}			
	6	2.67±0.12	2.55±0.03	2.45±0.10	2.36±0.07	2.51^{b}			
	9	2.82±0.12	2.74±0.11	2.59±0.14	2.51±0.09	2.67^{a}			
	Mean	2.62^{a}	2.53^{b}	2.65^{a}	2.32^{c}				

Same superscripts in different treatments groups and days of interval did not differ significantly, whereas different superscripts in different treatments groups and days of interval differ significantly. T_0 = (control group), T_1 = (1% lemon peel extract), T_2 = (1 % orange peel extract), T_3 = (0.01% BHT), DI=Day Intervals, Treat= Treatment, T×DI=Interaction of Treatment and Day Interval. ** means significant at 1% level of probability. *Means significant at 5% but more than 1% level of probability. NS means no significant.

Physicochemical properties

pH of raw chicken meat

Table 3 shows pH changes in chicken meat stored at 4°C. After three days, pH decreased but gradually increased with longer storage. The pH ranged from 5.72 to 5.88, with significant differences ($p < 0.01$) between treatments. T_1 had the most favorable raw pH, which is better for consumer health. Over the storage period, pH values ranged from 5.58% to 6.08%, with significant differences across days ($p < 0.01$). The pH levels in all samples gradually increased throughout storage, likely as a result of the buildup of basic substances like ammonia, which are produced by microbial activity (Nychas et al., 1998). Mold spoilage in different dried meat products can be prevented or slowed down by lowering the pH level (Leistner, 1987).

Water holding capacity

Table 3 presents the water-holding capacity (WHC) of chicken meat across different treatments and storage days. WHC ranged from 92.05% to 93.22%, with significant differences ($p < 0.01$) observed among treatments. T_3 exhibited the highest WHC, making it the most preferable treatment. Over the storage period, WHC ranged from 90.45% to 95.00%, with significant differences ($p < 0.01$) across days. WHC decreased with storage time, with the highest values on day 0 and the lowest on day 9. Overall, T_3 showed the best WHC, suggesting its potential to preserve meat quality and offer health benefits. A lower pH in poultry meat has been associated with diminished WHC, which in turn results in higher drip and cooking losses (Allen et al., 1997)

Drip loss

Table 3 shows that while drip loss was significantly affected by storage days ($p < 0.01$), there was no significant effect of different treatments or their interaction with storage time. Drip loss values ranged from 2.73 to 2.85 across treatments, and from 2.37 to 3.21 across storage intervals. The T_3 group had the most favorable drip loss, indicating better juiciness and quality. In conclusion, drip loss varied with storage time but was not influenced by treatment or the interaction between treatment and storage duration. Drip loss refers to the loss of moisture from meatballs due to the evaporation of water, which occurs as muscle proteins like actin and myosin shrink (Yu et al., 2005).

Cooking Loss

Table 3 presents cooking loss data for chicken meat treated with antioxidants. Cooking loss ranged from 26.75% to 26.95%, with no significant differences among treatment groups. The T_2 group had the most favorable cooking loss, indicating better appeal to consumers. Significant differences ($p < 0.01$) were observed across storage periods (days 0, 3, 6, and 9), with cooking loss decreasing over time. Cooking loss refers to the decrease in the weight of meatballs during the cooking process, as reported by Jama et al. (2008). Thawing loss specifically refers to the fluid loss in meatballs due to the formation of exudates after the freezing and thawing process, a finding also noted by Jama et al. (2008).

Table 3. Effect of different types of anti-oxidants on Physicochemical parameters (Mean ± SE) in chicken meat at different days of intervals

Parameters	DI	Treatments				Mean	Level of significance		
		T_0	T_1	T_2	T_3		Treat.	DI	T*DI
pH	0	5.71±0.05	5.63±0.04	5.66±0.03	5.68±0.03	5.67^{c}	**	**	NS
	3	5.63±0.09	5.53±0.08	5.56±0.10	5.60±0.08	5.58^{d}			
	6	5.97±0.10	5.74±0.08	5.77±0.11	5.85±0.19	5.83^{b}			
	9	6.21±0.08	5.97±0.11	6.04±0.10	6.10±0.15	6.08^{a}			
	Mean	5.88^{a}	5.72^{c}	5.76^{bc}	5.81^{ab}				
WHC (%)	0	95.04±0.14	94.96±0.20	94.91±0.52	95.09±0.21	95.00^{a}	**	**	**
	3	93.51±0.13	93.75±0.07	93.94±0.20	94.22±0.17	93.86^{b}			
	6	90.61±0.55	91.56±0.13	91.98±0.32	92.44±0.58	91.65^{c}			
	9	89.04±0.21	90.80±0.62	90.84±0.30	91.12±0.09	90.45^{d}			
	Mean	92.05^{c}	92.77^{b}	92.92^{b}	93.22^{a}				
Drip loss (%)	0	2.34±0.18	2.45±0.17	2.36±0.11	2.31±0.16	2.37^{c}	NS	**	NS
	3	2.75±0.09	2.77±0.12	2.72±0.14	2.65±0.1	2.72^{b}			
	6	2.98±0.03	2.71±0.3	2.77±0.14	2.85±0.18	2.83^{b}			
	9	3.33±0.09	3.12±0.1	3.29±0.22	3.11±0.04	3.21^{a}			
	Mean	2.85^{a}	2.76^{a}	2.79^{a}	2.73^{a}				
Cooking loss (%)	0	29.44±0.21	28.92±0.10	29.28±0.02	28.76±0.10	29.10^{a}	NS	**	**
	3	28.81±0.15	28.16±0.1	28.27±0.72	27.91±0.18	28.29^{b}			
	6	26.56±0.58	26.12±0.03	26.28±0.07	26.77±0.44	26.43^{c}			
	9	22.92±0.20	23.82±0.39	23.15±0.23	23.75±0.61	23.41^{d}			
	Mean	26.93^{a}	26.76^{a}	26.75^{a}	26.80^{a}				

Same superscripts in different treatments groups and days of interval did not differ significantly, whereas different superscripts in different treatments groups and days of interval differ significantly. T_0 = (control group), T_1 = (1% lemon peel extract), T_2 = (1 % orange peel extract), T_3 = (0.01% BHT), DI=Day Intervals, Treat= Treatment, T×DI=Interaction of Treatment and Day Interval. ** means significant at 1% level of probability. * Means significant at 5% but more than 1% level of probability. NS means no significance.

Biochemical properties

Thiobarbituric acid value

The TBARS (thiobarbituric acid reactive substances) values, as detailed in Table 4, significantly increased ($p<0.01$) with extended storage durations, indicating a reduction in shelf life. Across treatment groups, TBARS values ranged from 0.193 to 0.226, with significant differences ($p<0.01$) observed among the groups. The T_3 treatment had the lowest TBARS value, making it the most beneficial for consumers, as lower TBARS levels are associated with better health. During storage (0, 3rd, 6th, and 9th days), TBARS values varied from 0.124 to 0.391, showing significant differences ($p<0.01$) across time points. However, the interaction between treatments and storage durations was not significant. According to Biswas et al. (2012), the TBA value significantly increased in all batches throughout the entire storage period. Several researchers have found that incorporating edible plant extracts notably reduced TBARS values in fresh ground beef compared to untreated samples. Yadav et al. (2018) observed a substantial rise in TBARS values in both control and fiber-enriched sausages as the storage duration increased. Similar findings were reported by Nassu et al. (2003) in goat meat sausage during refrigerated storage.

Table 4. Effect of different types of anti-oxidants on TBARS (mg MDA/kg) value (Mean ± SE) in chicken at different days of intervals

Parameters	DI	Treatments				Mean	Level of significance		
		T_0	T_1	T_2	T_3		Treat.	DI	T*DI
TBARS (mg-MA/kg)	0	0.143±0.009	0.129±0.013	0.108±0.014	0.114±0.01	0.124^{d}			
	3	0.155±0.007	0.138±0.01	0.133±0.011	0.128±0.013	0.139^{c}			
	6	0.193±0.017	0.175±0.01	0.159±0.014	0.166±0.01	0.173^{b}	**	**	NS
	9	0.413±0.008	0.397±0.014	0.388±0.014	0.364±0.01	0.391^{a}			
	Mean	0.226^{a}	0.210^{b}	0.197^{c}	0.193^{c}				

Same superscripts in different treatments groups and days of interval did not differ significantly, whereas different superscripts in different treatments groups and days of interval differ significantly. T_0 = (control group), T_1 = (1% lemon peel extract), T_2 = (1 % orange peel extract), T_3 = (0.01% BHT), DI=Day Intervals, Treat= Treatment, T×DI=Interaction of Treatment and Day Interval. ** means significant at 1%, * means significant 5% level of probability and NS means no significance.

Microbial assessment

Total viable count

Table 5 shows the total viable count (TVC) values across treatments and time intervals. Fresh chicken meat initially had a TVC of 5.41 log CFU/g, reflecting high quality. Despite the presence of some bacteria, proper storage conditions can effectively control their growth (Fernandez-Lopez et al., 2005). The aerobic plate counts (TVC) for chicken meat samples ranged from 5.43 to 5.60 log CFU/g across treatments, with significant differences indicated by distinct superscripts. The control sample had the highest bacterial load (5.60 log CFU/g), while the T_3 treatment recorded the lowest (5.43 log CFU/g), demonstrating its effectiveness in reducing bacterial contamination. TVC values increased over time (5.41–5.67 log CFU/g) across storage intervals (0, 3rd, 6th, and 9th days). Antioxidants minimized fat oxidation and inhibited bacterial growth, supporting findings by Hanan et al. (2013), who reported that fruit by-products significantly ($p<0.05$) reduced bacterial counts and extended shelf life in meat.

Total coliform count

Table 5 presents Total Coliform Count (TCC) values for chicken meat across treatments and storage intervals. The control sample had the highest TCC (3.02 log CFU/g), while antioxidant-treated samples showed lower values (2.85–3.02 log CFU/g), with T_3 being the most favorable ($p<0.01$). No significant difference was observed between T_2 and T_3. Initial TCC for fresh meat was 2.26 log CFU/g, indicating low contamination. Over storage intervals (0, 3, 6, and 9 days), TCC values ranged from 2.66 to 3.25 log CFU/g, decreasing over time due to the antioxidant effects, which inhibited bacterial growth by blocking fat metabolism. Similar findings were reported by Zivanovic et al. (2005), who showed that chitosan films reduced pathogen counts by 1–3 log units. Camo et al. (2008) demonstrated that antioxidant active packaging, including rosemary and oregano-based films, reduced coliform counts in lamb meat stored at 11°C for 13 days under high oxygen and continuous lighting.

Total Yeast-Mold count

Table 5 summarizes the total yeast and mold counts (TYMC) in chicken samples treated with various preservatives (T_0, T_1, T_2, T_3) over 9 days of refrigerated storage. Initial TYMC for fresh chicken was 2.55 $\log_{10}$ CFU/g, indicating good quality. Treatments incorporating 1% lemon/orange pulp extract or 0.01% BHT significantly ($p<0.01$) reduced yeast and mold growth compared to the control group, where counts were significantly higher (2.95 $\log_{10}$ CFU/g). Across treatments, TYMC ranged from 2.60 to 2.95 $\log_{10}$ CFU/g, and across storage intervals, from 2.55 to 2.91 $\log_{10}$ CFU/g. Distinct superscripts indicated significant differences ($p<0.01$) among treatments and storage days (0, 3, 6, 9), with TYMC gradually increasing over time. Despite reduced growth in treated samples, the natural preservatives were not entirely effective in inhibiting yeast and mold. This aligns with Naveena et al. (2001), who observed no significant differences in microbial counts between control and green tea-treated hen meat. Similarly, Bali et al. (2011) reported lower total plate counts in garlic-treated chicken sausages stored at 4°C for 21 days, although yeast and mold growth increased after 7 days in all groups. This aligns with previous studies that have highlighted the antimicrobial properties of natural antioxidants in meat preservation (Azad et al., 2022).

Table 5. Effect of anti-oxidants on different microbial population (Mean ± SE) of chicken meat stored at different days of intervals

Parameters	DI	Treatments				Mean	Level of significance		
		T_0	T_1	T_2	T_3		Treat.	DI	T*DI
TVC (log CFU/g)	0	5.55±0.07	5.44±0.09	5.36±0.09	5.27±0.10	5.41^{c}			
	3	5.48±0.12	5.44±0.10	5.42±0.17	5.38±0.13	5.43^{bc}			
	6	5.62±0.08	5.53±0.12	5.46±0.11	5.43±0.11	5.51^{b}	**	**	NS
	9	5.75±0.13	5.66±0.13	5.65±0.09	5.62±0.10	5.67^{a}			
	Mean	5.60^{a}	5.52^{ab}	5.47^{b}	5.43^{b}				
TCC (log CFU/g)	0	2.76±0.10	2.67±0.09	2.63±0.11	2.59±0.08	2.66^{d}			
	3	2.85±0.08	2.78±0.10	2.74±0.11	2.68±0.13	2.76^{c}			
	6	3.14±0.07	3.06±0.11	3.01±0.14	2.96±0.11	3.04^{b}	**	**	NS
	9	3.34±0.09	3.25±0.11	3.23±0.05	3.18±0.12	3.25^{a}			
	Mean	3.02^{a}	2.94^{ab}	2.90^{b}	2.85^{b}				
TYMC (log CFU/g)	0	2.79±0.08	2.52±0.10	2.48±0.06	2.42±0.07	2.55^{c}			
	3	2.95±0.10	2.64±0.08	2.55±0.10	2.60±0.13	2.69^{b}			
	6	2.97±0.14	2.73±0.17	2.65±0.08	2.62±0.11	2.74^{b}	**	**	NS
	9	3.08±0.10	2.95±0.07	2.85±0.13	2.74±0.12	2.91^{a}			
	Mean	2.95^{a}	2.71^{b}	2.63^{bc}	2.60^{c}				

Same superscripts in different treatments groups and days of interval did not differ significantly, whereas different superscripts in different treatments groups and days of interval differ significantly. T_0 = (control group), T_1 = (1% lemon peel extract), T_2 = (1 % orange peel extract), T_3 = (0.01% BHT), DI=Day Intervals, Treat= Treatment, T×DI=Interaction of Treatment and Day Interval. ** means significant at 1%, * means significant 5% level of probability and NS means no significance. TVC = Total viable count, TCC = Total coliform count, TYMC = Total yeast-mould count.

Conclusions

The research found that chicken meat can be effectively preserved for up to 9 days using both natural and synthetic antioxidants. The group treated with 0.01% BHT (T_3) demonstrated significantly better results compared to the control group in terms of sensory, physicochemical, biochemical, and microbial evaluations. The addition of 0.01% BHT not only improved consumer acceptability but also maintained the nutritional quality, making it an effective preservative for up to 9 days of storage at 4°C.

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