**Meat Research**
Vol 5, Issue 6: 1-8, Article 130, December 2025
ISSN: 2790-1971
https://doi.org/10.55002/mr.5.6.130 http://www.bmsa.info/meatresearch

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**Keywords:**
Natural antioxidants
Butylated hydroxy toluene
Lipid oxidation
Meat quality
Microbial load

**Article Info:**
Received: October 24, 2025
Accepted: December 09, 2025
Published online: December 31, 2025

**Research Article**

# Effect of natural and synthetic antioxidants on the quality of broiler meat in short term preservation

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## Abstract

This study evaluated the comparative efficacy of natural and synthetic antioxidants on the quality, oxidative stability, and microbial safety of fresh broiler chicken meat during refrigerated storage (4° C) for 9 days. Breast meat samples were allocated to four treatments: $T_0$ (Control; no additive), $T_1$ (1% holy basil leaf extract), $T_2$ (1% mandarin orange peel extract), and $T_3$ (0.01% butylated hydroxytoluene, BHT). Samples were analyzed at 0, 3, 6, and 9 days for sensory attributes, color coordinates (L*, a*, b*), proximate composition, physicochemical properties (pH, drip loss, cooking loss), lipid oxidation (TBARS), and microbial loads (total viable count). Antioxidant treatments significantly suppressed lipid oxidation and microbial proliferation compared to the control ($P < 0.001$). The synthetic group ($T_3$) exhibited the highest stability in meat color, dry matter, and ether extracts retention ($P < 0.001$), while $T_1$ demonstrated superior crude protein preservation. Physicochemical analysis revealed that $T_3$ optimized water-holding capacity and minimized drip loss, whereas the highest pH and cooking loss values were recorded in $T_1$ and $T_2$ respectively. Throughout storage, $T_3$ maintained the lowest TBARS values and TVC ($P < 0.001$). However, both holy basil ($T_1$) and mandarin peel ($T_2$) extracts significantly retarded deterioration compared to $T_0$. While 0.01% BHT ($T_3$) demonstrated the highest overall preservative efficacy, plant-derived extracts ($T_1$ and $T_2$) present viable, bioactive alternatives for clean-label shelf-life extension of refrigerated poultry.

## Introduction

Meat and meat products are highly nutritious food matrices rich in high-quality proteins, essential amino acids, bioavailable vitamins, and minerals required for human health (Azad et al., 2021; Prabakaran, 2012). However, fresh poultry meat is inherently prone to rapid quality degradation due to its chemical composition, high water activity, and abundance of polyunsaturated fatty acids (PUFAs). These characteristics facilitate both microbial proliferation and lipid oxidation, severely compromising product safety, sensory attributes, and consumer acceptability during storage (Azad et al., 2022; Kumudavally et al., 2005). Lipid oxidation represents a primary mechanism of non-microbial quality deterioration in muscle foods. This oxidative cascade begins with the interaction between molecular oxygen and PUFAs, inducing rancidity, discoloration, texture degradation, and the formation of potentially toxic secondary metabolites, such as malondialdehyde (Islam et al., 2025; Mostafa et al., 2025; Nunez de Gonzalez et al., 2008).

To mitigate oxidative decay and extend commercial shelf-life, the meat industry has historically relied on synthetic antioxidants, including butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl gallate (Decker and Mei, 1996; Sadakuzzaman et al., 2021 and 2024; Biplob et al., 2024; Shohiduzjaman et al., 2024). Although these synthetic formulations are highly effective and economically viable, growing consumer anxiety and toxicological evidence suggest that their long-term systemic accumulation may induce hepatic toxicity and elevate carcinogenic risks (Kahl and Kappus, 1993). Consequently, a profound paradigm shift is underway toward identifying plant-derived, bioactive natural antioxidants capable of replacing synthetic counterparts without sacrificing food safety or quality standards (Bithi et al., 2020; Dish et al., 2020; Kumar et al., 2013).

Plant extracts rich in phenolic acids, flavonoids, and tannins offer a robust dual-action framework by exhibiting both radical-scavenging and antimicrobial properties in muscle foods (Jung et al., 2010). Among these, holy basil (Ocimum sanctum) leaf extract and mandarin (Citrus reticulata) orange peel extract are promising candidates. Holy basil leaves possess elevated concentrations of eugenol, rosmarinic acid, and apigenin, which effectively retard lipid peroxidation and suppress bacterial growth in meat systems (Gupta and Sharma, 2016; Hasan et al., 2024). Concurrently, mandarin orange peel serves as a rich reservoir of vitamin C and polymethoxyflavones (e.g., hesperidin and naringin) that deliver substantial antioxidant protection (Anagnostopoulou et al., 2006; Dhanavade et al., 2011). Furthermore, utilizing citrus peel—a major byproduct of the juice processing sector promotes environmental sustainability through agro-industrial waste valorization (Manthey and Grohmann, 2001; Masum et al., 2025).

While the separate functionalities of these botanicals are recognized, comprehensive comparative studies evaluating their performance against industry-standard synthetic agents like BHT across a

complete matrix of sensory, proximate, physicochemical, and microbial parameters in broiler meat remain limited. Therefore, this study aimed to investigate and compare the efficacy of holy basil leaf extract, mandarin orange peel extract, and BHT on the quality profiles and shelf-life extension of broiler meat under refrigerated storage (4° C) for up to 9 days.

## Materials and Methods

### Sample Collection and Experimental Design

Fresh broiler chicken breast meat (Pectoralis major) was obtained from the Kamal-Ronjit market at Bangladesh Agricultural University (BAU), Mymensingh. Birds of similar weight and age were humanely slaughtered in accordance with official Halal standards. Skeletal muscle tissue was carefully deboned, trimmed of external fat, and transported under chilled conditions (4°C) to the Animal Science Laboratory, Department of Animal Science, BAU, for immediate processing. The meat samples were randomly allocated into four distinct treatment groups such as $T_0$: Control or untreated fresh chicken meat, $T_1$: Treated with 1.0% (w/w) holy basil (Ocimum sanctum) leaf extract, $T_2$: Treated with 1.0% (w/w) mandarin orange (Citrus reticulata) peel extract, $T_3$: Treated with 0.01% (w/w) butylated hydroxytoluene (BHT). All samples were packaged and stored under refrigeration at 4°C. Quality parameters were systematically evaluated at specific storage intervals: days 0, 3, 6, and 9.

### Preparation of Plant Extracts

Fresh holy basil leaves were collected from the BAU residential quarters, and fresh mandarin oranges were purchased from a local market. Plant materials were washed thoroughly with distilled water. The edible portions of the mandarin peels were separated, and both the peels and basil leaves were homogenized using a laboratory grinder. The respective extracts were recovered by filtering the homogenates through sterile sieving cloth, stored at 4°C, and used within 24 hours of extraction.

### Sensory and Instrumental Color Evaluation

Sensory characteristics, focusing primarily on surface color stability, were evaluated throughout the storage period by a trained panel. Instrumental color parameters (lightness, redness, and yellowness) were verified to monitor surface discoloration, fading, and oxidative browning during refrigerated storage.

### Proximate Composition Analysis

Proximate composition parameters such as dry matter (DM), crude protein (CP), ether extract (EE), and ash was determined in triplicate following standard AOAC (1995) methodologies.

### Determination of dry matter

Samples were dried in a laboratory forced-air oven at 105°C for 24 hours until a constant weight was achieved. DM percentage was calculated as:

$$DM\% = (\text{weight after drying} \div \text{weight of fresh sample}) \times 100$$

### Determination of crude protein

Total nitrogen content was quantified via the micro-Kjeldahl method. Samples were digested with concentrated sulfuric acid ($H_2SO_4$) in the presence of a catalyst mixture ($K_2SO_4$, $CuSO_4$ and selenium powder). The released ammonia was distilled into a 2% boric acid solution and titrated against standard hydrochloric acid (HCl). Total nitrogen (TKN) was calculated using the equation:

$$\% \text{ N} = \frac{\text{Titrate required (ml)} \times .014 \text{ (milliequivalent of N2)} \times \text{Strength of HCl}}{\text{weight of the sample}} \times 100$$

The crude protein content was obtained by multiplying the nitrogen percentage by a conversion factor of 6.25.

% CP = % of nitrogen × conversion factor (6.25)

### Determination of ether extract

Fat content was determined via Soxhlet extraction using a continuous solvent extraction apparatus with diethyl ether as the solvent at 40–45°C for 7–8 hours.

$$\% \text{ ether extract} = \frac{(\text{Weight of ether})}{(\text{Weight of sample})} \times 100$$

### Determination of Ash

Samples were pre-ashed in porcelain crucibles and subsequently incinerated in a muffle furnace at 550°C for 6 hours to burn off organic matter.

$$\text{The formula is mentioned as \% of ash content} = \frac{E}{C} \times 100$$

Where, E = Weight of ash, C = Weight of sample

### Physicochemical Measurements

### Determination of pH

The pH value of the raw meat was measured using a digital pH meter. Samples (5 g) were homogenized with 10 mL of distilled water prior to immersion of the glass electrode.

### Determination of drip loss

Drip loss was determined gravimetrically. Uniformly sliced meat samples were weighed, suspended inside inflation bags without contacting the inner surface, and held at 4°C for up to 48 hours. Samples were gently blotted with filter paper to remove surface moisture and reweighed.

### Drip loss calculating formula is mentioned as

$$\% \text{ drip loss} = \frac{W1-W2}{W1} \times 100$$

Where, $W_1$=Initial Weight $W_2$=Final Weight

### Determination of cooking loss

Pre-weighed meat samples (20 g) were wrapped in heat-resistant foil and cooked in a water bath at 70°C for 30 minutes. Cooked samples were cooled to room temperature, blotted dry, and reweighed.

$$CL\ (\%) = \frac{(\text{Weight before cooking of sample} - \text{weight after cooking})}{\text{Weight before cooking of sample}} \times 100$$

### Biochemical Analysis (TBARS)

Lipid oxidation was monitored in triplicate via the 2-thiobarbituric acid reactive substances (TBARS) assay, following the method of Schmedes and Holmer (1989). Meat samples (5 g) were homogenized with 25 mL of a 20% trichloroacetic acid (TCA) solution prepared in a 135 mL/L phosphoric acid matrix. The homogenate was filtered through Whatman No. 4 filter paper. A 2 mL aliquot of the filtrate was reacted with 2 mL of 0.02 M aqueous TBA solution (3 g/L) in a sealed test tube and heated in a boiling water bath (100°C) for 30 minutes. After cooling under running water, the absorbance was measured at 532 nm using a UV-VIS spectrophotometer (UV-1200, Shimadzu, Japan). TBARS values were expressed as milligrams of malondialdehyde per kilogram of meat sample (mg MDA/kg).

### Microbial Assessment

Microbiological profiles for Total Viable Count (TVC) were evaluated according to standard ISO (1995) protocols. A 10 g meat sample was aseptically transferred to a sterile container containing 90 mL of 0.1% peptone water and homogenized using a mechanical blender to obtain a base dilution. Serial ten-fold dilutions were prepared in 0.1% peptone water using a vortex mixer. A 0.1 mL aliquot of each dilution was spread onto duplicate Plate Count Agar (PCA) plates. Plates were incubated at 35°C for 24–48 hours. Plates containing 30–300 colonies were counted using a colony counter, and values were expressed as colony-forming units per gram of meat.

### Statistical Analysis

All experimental data were analyzed using SAS software (SAS Institute, Cary, NC, USA). Data were subjected to analysis of variance (ANOVA), and mean separations were verified using Duncan's Multiple Range Test (DMRT). Statistical significance was defined at $p<0.05$, with highly significant differences noted at $p<0.01$.

## Results and Discussion

### Instrumental color profile of chicken meat

The effects of natural and synthetic antioxidant treatments (T), storage day intervals (DI), and their interaction (T × DI) on the instrumental color attributes such as lightness (l*), redness (a*), and yellowness (b*) of broiler chicken meat are presented in Table 1.

The lightness (l*) values of chicken meat were highly significantly affected ($p < 0.001$) by treatment groups, storage intervals, and their mutual interaction $p < 0.001$. Throughout the storage period, the untreated control group ($T_0$) consistently exhibited the lowest mean value (39.2), which was significantly lower ($p < 0.001$) than all antioxidant-treated groups (47.19 in $T_1$, 48.79 in $T_2$ and 49.44 in $T_3$). Among the treatments, the synthetic antioxidant group ($T_3$) retained the highest cumulative mean lightness (49.44), followed closely by the mandarin orange peel extract group ($T_2$: 48.79) and the holy basil leaf extract group ($T_1$: 47.19). Over time, the pooled storage means experienced a slight but significant increase, rising from Day 0 to Day 9. The lower lightness values observed in indicate that the absence of antioxidants permitted rapid surface moisture exudation and accelerated protein oxidation, which characteristically causes untreated muscle tissues to become dark and dull during post-mortem storage (Janisch et al., 2011; Joo et al., 1995).

Statistical analysis revealed that both treatment application and storage duration exerted a highly significant influence ($p < 0.001$) on meat redness (a*), whereas the interaction effect (T × DI) was non-significant (NS). Meat samples treated with 0.01% BHT ($T_3$) maintained the highest cumulative redness mean (5.85), which differed significantly from all other groups. Among the natural formulations, the incorporation of 1% holy basil leaf extract ($T_1$) yielded a significantly higher redness value (4.84) compared to both the control (4.27) and mandarin orange peel extract (3.84) groups. Over the 9-day storage cycle, a gradual, highly significant upward trend in values was documented across all groups, with the pooled mean advancing from Day 0 to Day 9. BHT and holy basil extracts proved highly effective at delaying the structural oxidation of oxymyoglobin into metmyoglobin. The marginal rise in values across all samples over time may be attributed to a progressive concentration of pigments resulting from moisture loss or the presence of specific microbial metabolites during storage (Azad et al., 2022; Tushar et al., 2023).

For the yellowness (b*) parameter, the main effect of treatment (T), individual storage interval (DI), and their interaction (T × DI) were statistically significant. Cumulative treatment means ranged from a minimum of in the control (5.31) to a maximum of in the mandarin peel extract group (10.89). In terms of storage duration, the pooled yellowness mean showed a descending trend over time, significantly dropping from on Day 0 to by Day 9. Notably, on Days 6 and 9, samples treated with 1% holy basil extract ($T_1$) and 1% mandarin peel extract ($T_2$) displayed superior color stability compared to the BHT-treated group ($T_3$), which dropped noticeably to on the final day of storage. The high initial yellowness in is biologically expected, as mandarin peel is a rich reservoir of yellow-orange carotenoids and flavonoids (such as hesperidin), which naturally impart a higher value to the raw meat matrix upon application (Mostafa et al., 2025). The progressive decline in color metrics over extended storage reflects pigment and lipid co-oxidation, which triggers non-enzymatic browning reactions between oxidized lipids and amino acid residue networks (Mancini and Hunt, 2005; Nerín et al., 2006).

**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 |
| L* | 0 | 41.47±3.06 | 47.43±3.04 | 44.62±6.13 | 52.02±4.04 | $47.19^{a}$ | ** | ** | ** |
| | 3 | 39.92±1.08 | 51.56±1.08 | 48.86±1.14 | 49.33±6.04 | $46.67^{a}$ | | | |
| | 6 | 41.35±1.03 | 48.82±2.95 | 52.33±5.08 | 48.29±3.03 | $48.2^{b}$ | | | |
| | 9 | 38.85±2.90 | 50.93±4.05 | 51.35±1.92 | 46.12±3.98 | $49.56^{b}$ | | | |
| | Mean | $39.2^{d}$ | $47.19^{c}$ | $48.79^{b}$ | $49.44^{a}$ | | | | |
| a* | 0 | 4.59±0.47 | 3.32±0.21 | 2.22±0.39 | 4.12±0.60 | $3.96^{d}$ | ** | ** | NS |
| | 3 | 3.69±0.58 | 4.83±0.54 | 3.50±0.50 | 5.63±0.35 | $4.31^{b}$ | | | |
| | 6 | 4.53±0.15 | 5.16±0.13 | 3.09±0.17 | 4.81±0.95 | $4.62^{c}$ | | | |
| | 9 | 4.88±0.23 | 5.76±1.04 | 5.63±0.86 | 6.55±0.19 | $5.53^{a}$ | | | |
| | Mean | $4.27^{c}$ | $4.84^{b}$ | $3.86^{d}$ | $5.85^{a}$ | | | | |
| b* | 0 | 5.80±1.06 | 11.22±3.95 | 11.77±1.59 | 11.61±0.26 | $10.21^{a}$ | ** | * | * |
| | 3 | 4.92±0.47 | 10.68±0.97 | 10.60±1.93 | 10.87±0.58 | $9.26^{b}$ | | | |
| | 6 | 4.89±0.4 | 9.96±0.23 | 12.16±1.96 | 10.12±0.14 | $9.28^{ab}$ | | | |
| | 9 | 5.68±1.12 | 11.49±1.73 | 9.83±1.28 | 9.84±0.59 | $8.21^{b}$ | | | |
| | Mean | $5.31^{b}$ | $10.54^{ab}$ | $10.89^{a}$ | $10.61^{a}$ | | | | |

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% holy basil leaf extract), $T_2$ = (1 % mandarin 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 not significant.

## Proximate composition of chicken meat

The changes in dry matter (DM), total ash, and crude protein (CP), and ether extract (EE) contents of broiler chicken meat are presented in Table 2.

Statistical analysis indicated that the main effect of antioxidant treatments and the interaction were non-significant (NS) on the dry matter content of broiler chicken meat. However, the individual effect of storage duration (DI) exerted a highly significant influence ($p<0.05$) on percentages. The pooled mean values experienced a progressive upward trend over time, escalating from on Day 0 to on Day 3, on Day 6, and reaching a maximum value of by Day 9. This highly significant increase in dry matter percentage across all groups is primarily driven by moisture loss via evaporation from the muscle tissues during refrigerated storage. As water content decreases, the proportional percentage of total solids (dry matter) within the matrix naturally raises, which aligns with trends documented by Islam et al. (2025) and Masum et al. (2025).

The total ash percentage of the chicken meat was highly significantly affected ($P < 0.01$) by both individual treatment applications and storage intervals, whereas their mutual interaction was non-significant (NS). Among the treatment groups, the cumulative mean ash content was highest in ($T_2$: 1.43%), followed sequentially by $T_1$ (holy basil leaf extract; 1.37%), and control group ($T_0$: 1.32%). Regarding the storage duration, a highly significant linear increase in ash percentages was detected as storage advanced, rising from a pooled mean of on Day 0 to on Day 9. The significantly higher ash contents in the plant extract groups are structurally logical because natural plant materials, particularly mandarin peel and holy basil leaves are rich in essential mineral elements (calcium, potassium, magnesium, and phosphorus). Incorporating these extracts into the meat matrix directly increases its baseline inorganic mass (Das et al., 2022). The rise in ash over time also correlates with the concentration effect caused by ongoing moisture loss.

Statistical analysis demonstrated that the main effect of antioxidant treatments, storage duration, and their mutual interaction were all statistically non-significant (NS) on the of the broiler meat. The overall mean values for crude protein remained uniform across all experimental groups, fluctuating within a narrow baseline range. Crude protein is a structural macro-nutritional component bound within the myofibrillar and sarcoplasmic protein networks of muscle tissue. While antioxidants are effective at inhibiting chemical pathways like lipid oxidation and microbial spoilage, they do not synthesize new protein molecules or alter the baseline amino acid mass of the meat. Therefore, a non-significant treatment effect is biologically expected (Boby et al., 2021).

The ether extract (EE) content ranged between and across the treatments, showing significant variations ($p<0.01$) between the control and antioxidant-treated groups. Among the four treatments, the cumulative mean was lowest in the group ($T_3$: 2.42), while the control group ($T_0$) displayed the highest value (2.69). When considering storage intervals (DI), the pooled content rose significantly ($p < 0.01$) from on Day 0 to on Day 9 across all treatments. This progressive increase in percentage over time does not represent actual lipid synthesis but rather a concentration effect driven by the simultaneous evaporation of moisture from the meat tissue during refrigerated storage.

**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 | 26.46±0.44 | 26.75±0.24 | 26.43±0.14 | 26.24±0.34 | $26.38^{d}$ | NS | ** | NS |
| | 3 | 26.95±0.71 | 26.88±0.35 | 27.19±0.46 | 27.03±0.11 | $27.01^{c}$ | | | |
| | 6 | 27.76±0.43 | 27.83±0.56 | 28.22±0.39 | 27.41±0.63 | $27.83^{b}$ | | | |
| | 9 | 28.81±0.51 | 28.50±0.48 | 28.71±0.55 | 28.14±0.78 | $28.42^{a}$ | | | |
| | Mean | $27.95^{a}$ | $27.62^{b}$ | $27.41^{c}$ | $27.24^{d}$ | | | | |
| Ash (%) | 0 | 1.12±0.30 | 1.19±0.004 | 1.33±0.003 | 1.12±0.15 | $1.29^{d}$ | ** | ** | NS |
| | 3 | 1.27±0.08 | 1.37±0.02 | 1.34±0.02 | 1.18±0.11 | $1.32^{c}$ | | | |
| | 6 | 1.39±0.05 | 1.46±0.01 | 1.43±0.02 | 1.29±0.06 | $1.44^{b}$ | | | |
| | 9 | 1.48±0.04 | 1.52±0.01 | 1.55±0.12 | 1.31±0.01 | $1.46^{a}$ | | | |
| | Mean | $1.32^{d}$ | $1.37^{c}$ | $1.43^{b}$ | $1.21^{a}$ | | | | |
| CP (%) | 0 | 23.75±0.60 | 23.59±0.45 | 23.52±0.31 | 23.68±0.17 | $23.54^{a}$ | **NS** | ** | **NS** |
| | 3 | 22.89±0.15 | 22.75±0.03 | 22.85±0.14 | 22.61±0.41 | $21.53^{b}$ | | | |
| | 6 | 21.04±0.96 | 21.96±0.49 | 21.69±0.22 | 21.56±0.10 | $20.89^{c}$ | | | |
| | 9 | 20.33±0.35 | 20.48±0.70 | 20.98±0.06 | 20.31±0.02 | $20.58^{d}$ | | | |
| | Mean | 22.00 | 21.95 | 21.46 | 21.25 | | | | |
| EE% | 0 | 2.49±0.16 | 2.41±0.19 | 2.33±0.17 | 2.25±0.08 | $2.36^{c}$ | ** | ** | ** |
| | 3 | 2.58±0.11 | 2.48±0.10 | 2.54±0.07 | 2.36±0.08 | $2.45^{a}$ | | | |
| | 6 | 2.66±0.12 | 2.59±0.03 | 2.65±0.10 | 2.48±0.07 | $2.57^{b}$ | | | |
| | 9 | 2.85±0.12 | 2.77±0.11 | 2.79±0.14 | 2.61±0.09 | $2.73^{a}$ | | | |
| | Mean | $2.64^{a}$ | $2.58^{b}$ | $2.69^{a}$ | $2.42^{c}$ | | | | |

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% holy basil leaf 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 parameters of broiler meat

Physicochemical properties including raw pH, drip loss (DL), and cooking loss (CL) are summarized in Table 3.

Table 3 displays the pH changes in chicken meat stored at 4°C. The main effects of treatment and storage interval were highly significant ($p<0.01$), whereas the interaction effect was non-significant (NS). The overall treatment means pH values ranged from 5.85 to 6.12, with the group exhibiting the lowest cumulative pH in $T_3$ (from 5.58% to 6.08%). Throughout storage, the pooled pH levels across all samples gradually increased from Day 0 to Day 9. This progressive rise in pH is a classic indicator of late-stage storage deterioration, driven by the microbial accumulation of basic volatile nitrogen compounds, such as ammonia and primary amines, derived from amino acid deamination (Nychas et al., 1998).

Statistical analysis revealed that the main effect of antioxidant treatments was statistically non-significant (NS) on the drip loss percentages of the meat, with cumulative treatment means remaining tightly aligned ($T_1$ to $T_3$). The interaction between treatment groups and storage duration (T × DI) was also non-significant (NS). In contrast, the duration of refrigerated storage (DI) exerted a highly significant influence ($P < 0.01$) on drip loss, showing a linear upward trend from a pooled mean of on Day 0 to on Day 9. This continuous increase in drip loss over the 9-day storage cycle is a standard physiological process in post-mortem muscle tissue. As meat ages under refrigeration, structural myofibrillar proteins undergo gradual denaturation and proteolytic degradation, reducing their net charge and capacity to chemically bind water. Consequently, cellular moisture escapes into the extracellular spaces as exudate (Yu et al., 2005).

Similarly, statistical analysis revealed that the main effect of antioxidant treatments and the interaction were non-significant (NS) on cooking loss percentages, which ranged uniformly between and across groups. However, the individual effect of storage interval (DI) exerted a highly significant influence ($P < 0.01$) on the cooking loss of the meat. A clear, linear descending trend was observed across all treatment groups as storage advanced, with the highest pooled mean cooking loss recorded on Day 0 (26.80%), which significantly decreased to by Day 9 (26.96%). This progressive drop in cooking loss over time is directly linked to the cumulative drip loss and moisture evaporation that occurred during raw storage. Because the raw meat had already lost a substantial amount of its free water content prior to processing, less moisture remained within the tissue to be expelled during the subsequent thermal cooking process (Chakrabartty et al., 2024; Liza et al., 2024). The non-significant difference among the treatment groups indicates that while natural and synthetic antioxidants are highly effective at intercepting chemical pathways (such as lipid oxidation), they do not alter the structural capillary forces or protein net charges responsible for trapping moisture within the muscle beds (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 | 5.77±0.05 | 5.82±0.04 | 5.86±0.03 | 5.90±0.03 | $5.67^{c}$ | ** | ** | NS |
| | 3 | 5.93±0.09 | 5.99±0.08 | 5.96±0.10 | 5.87±0.08 | $5.91^{d}$ | | | |
| | 6 | 6.07±0.10 | 5.84±0.08 | 5.99±0.11 | 6.09±0.19 | $6.03^{b}$ | | | |
| | 9 | 6.28±0.08 | 5.97±0.11 | 6.04±0.10 | 6.17±0.15 | $6.12^{a}$ | | | |
| | Mean | $5.92^{a}$ | $5.85^{c}$ | $5.91^{bc}$ | $5.96^{ab}$ | | | | |
| Drip loss (%) | 0 | 2.34±0.18 | 2.45±0.17 | 2.36±0.11 | 2.31±0.16 | $2.34^{c}$ | 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 | $3.25^{a}$ | | | |
| | Mean | $2.87^{a}$ | $2.76^{a}$ | $2.79^{a}$ | $2.64^{a}$ | | | | |
| Cooking loss (%) | 0 | 29.64±0.21 | 28.92±0.10 | 28.84±0.02 | 28.96±0.10 | $29.10^{a}$ | NS | ** | ** |
| | 3 | 28.81±0.15 | 28.06±0.1 | 28.17±0.72 | 27.91±0.18 | $28.29^{b}$ | | | |
| | 6 | 26.76±0.58 | 26.32±0.03 | 26.88±0.07 | 26.87±0.44 | $26.43^{c}$ | | | |
| | 9 | 24.92±0.20 | 24.72±0.39 | 25.15±0.23 | 24.75±0.61 | $23.41^{d}$ | | | |
| | Mean | 26.93 | 26.85 | 26.96 | 26.80 | | | | |

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% holy basil leaf extract), $T_2$ = (1 % mandarin 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

The individual and interactive effects of different antioxidant treatments (T), storage day intervals (DI), and their mutual interaction (T × DI) on TBARS values and Total Viable Counts (TVC) are presented in Table 4.

Statistical analysis revealed that the application of both natural and synthetic antioxidants had a highly significant effect ($P < 0.01$) on suppressing lipid oxidation in the meat matrix. The untreated control group ($T_0$) exhibited the highest cumulative mean TBARS value (0.276 mg-MA/kg), indicating advanced lipid degradation. In contrast, the synthetic antioxidant group ($T_3$: 0.01% BHT) proved most effective at inhibiting oxidation, maintaining the lowest overall mean TBARS value (0.193 mg-MA/kg). Among the natural extract treatments, mandarin orange peel extract ($T_2$) demonstrated a significantly stronger antioxidant capacity with a lower cumulative mean value (0.216 mg-MA/kg) compared to the holy basil leaf extract group ($T_1$: 0.228 mg-MA/kg).

The duration of refrigerated storage (DI) exerted a highly significant influence ($P < 0.01$) on the development of rancid compounds. A sharp, progressive linear increase in TBARS values was observed across all experimental groups as time advanced, rising from a pooled baseline mean of on Day 0 to a peak of on Day 9. While all groups started with low, closely aligned values on Day 0, the protective divergence of the treatments became highly apparent by Day 9, where the untreated control ($T_0$) experienced a massive spike to (0.124 mg-MA/kg), which rose steadily to 0.139 mg-MA/kg on day 3, advanced to 0.173 mg-MA/kg on day 6, and underwent a substantial, abrupt escalation to peak at 0.391 mg-MA/kg by the final day of storage (day 9). Conversely, 0.01% BHT ($T_3$) restricted this rise most effectively (0.193 mg-MA/kg), closely followed by holy basil extract ($T_1$: 0.228 mg-MA/kg) and mandarin orange peel extract ($T_2$: 0.216 mg-MA/kg). The phenolic compounds, flavonoids, and essential oils in holy basil and mandarin peel act as potent free-radical scavengers and hydrogen donors, successfully terminating the radical chain reaction of lipid peroxidation before it degrades muscle lipids into secondary oxidation products like malondialdehyde (Kim et al., 2013; Shohiduzjaman et al., 2024).

Regarding microbial quality, the main effects of treatments and storage intervals exerted a highly significant influence ($P < 0.01$) on the Total Viable Count (TVC) of the broiler meat. Across the entire storage period, the untreated control group ($T_0$) consistently exhibited a significantly higher (5.90 log CFU/g) cumulative mean microbial load compared to all antioxidant-treated groups. Conversely, the inclusion of antioxidants successfully restricted bacterial proliferation, with the synthetic group ($T_3$: 0.01% BHT) maintaining the lowest overall TVC load (5.63 log CFU/g), closely followed by mandarin peel extract ($T_2$: 5.77 log CFU/g) and holy basil extract ($T_1$: 5.82 log CFU/g).

Regarding the storage intervals, the pooled mean TVC values experienced a progressive, highly significant linear increase over time, advancing from on Day 0 to on Day 9. The significant suppression of TVC in and compared to the control is driven by the rich profile of bioactive compounds in the botanicals. Holy basil leaves contain high amounts of eugenol and rosmarinic acid, while mandarin orange peels are rich in polymethoxyflavones and hesperidin. These molecules disrupt microbial cell membranes, inhibit key cellular enzymes, and interfere with bacterial metabolic pathways, thereby delaying lag phases and slowing down the log phase of growth during refrigerated storage (Mostafa et al., 2025; Torun et al., 2023; Fernandez-Lopez et al., 2004).

**Table 4:** Effect of different types of anti-oxidants on TBARS (mg MDA/kg) Total viable count (logCFU/g) 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 | 0.154±0.009 | 0.143±0.013 | 0.128±0.014 | 0.124±0.01 | 0.124$^{d}$ | ** | ** | NS |
| | 3 | 0.162±0.007 | 0.158±0.01 | 0.143±0.011 | 0.138±0.013 | 0.139c | | | |
| | 6 | 0.207±0.017 | 0.195±0.01 | 0.179±0.014 | 0.186±0.01 | 0.173b | | | |
| | 9 | 0.445±0.008 | 0.407±0.014 | 0.418±0.014 | 0.394±0.01 | 0.391a | | | |
| | Mean | 0.276a | 0.228b | 0.216c | 0.193c | | | | |
| TVC (logCFU/g) | 0 | 5.85±0.07 | 5.64±0.09 | 5.76±0.09 | 5.57±0.10 | 5.61$^{c}$ | ** | ** | NS |
| | 3 | 5.98±0.12 | 5.84±0.10 | 5.82±0.17 | 5.68±0.13 | 5.73bc | | | |
| | 6 | 6.12±0.08 | 5.93±0.12 | 5.96±0.11 | 5.73±0.11 | 5.81b | | | |
| | 9 | 6.25±0.13 | 5.96±0.13 | 6.05±0.09 | 5.92±0.10 | 6.07a | | | |
| | Mean | 5.90a | 5.82ab | 5.77b | 5.63b | | | | |

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% Holy basil leaf extract), $T_2$ = (1 % Mandarin 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.

## Conclusion

This study systematically evaluated the comparative efficacy of natural plant extracts (1% holy basil leaf extract and 1% mandarin orange peel extract) against a conventional synthetic antioxidant (0.01% BHT) in preserving the quality and extending the shelf-life of broiler chicken meat during a 9-day refrigerated storage period. The experimental findings demonstrate that while 0.01% BHT ($T_3$) maintained the highest overall oxidative stability (lowest TBARS values), surface lightness (l*), and microbial suppression, the plant-derived formulations offered highly competitive preservative advantages. Although synthetic BHT remains highly efficient, holy basil leaf and mandarin orange peel extracts represent potent, biologically active, and eco-friendly alternatives. Utilizing mandarin peel also advances agro-industrial waste valorization. Consequently, these botanical extracts can be effectively utilized by the poultry processing sector as safe, clean-label functional ingredients to extend the commercial shelf-life of refrigerated broiler meat without sacrificing quality or safety.

### Future Perspectives

To facilitate commercial adoption, future investigations should focus on:

1. Optimizing the extraction yields and identifying the specific synergistic combinations of these natural phenols.
2. Conducting comprehensive sensory panel evaluations to monitor the flavor and aroma thresholds of these extracts on cooked poultry products.
3. Evaluating cost-effective encapsulation techniques to enhance the thermal stability of these bio-compounds during industrial processing.

### Conflict of interest

The authors do not have any conflicts of interest.

## Acknowledgements

The authors are highly appreciated to the Bangladesh Agricultural University Research System (BAURES) for funding this research.

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