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Research Article

Effects of natural and synthetic antioxidant on the quality of beef in short-term preservation

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Abstract

The experiment aimed to assess the effects of different natural and synthetic antioxidants and antimicrobial agents on the quality and shelf-life of fresh and preserved beef. Four treatment groups were used: 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). Beef samples were stored at 4°C for 9 days, and various tests were conducted on color, proximate components, physicochemical properties, biochemical markers, and microbial growth. Results showed that antioxidants significantly impacted physicochemical properties, oxidative defense, microbial growth, and sensory attributes, compared to the control. However, no significant changes were found in proximate components (CP, Ash, DM, EE). Antioxidant-treated samples showed lower pH levels but had no significant effect on water holding capacity. BHT (T_3) exhibited the best results for oxidative stability, with lower TBARS values and reduced microbial counts. The sensory qualities, especially color, were improved in the lemon peel extract group (T_1). Overall, BHT (T_3) proved to be the most effective treatment for preserving beef quality, and it can be used up to 9 days for refrigeration-based preservation.

Introduction

Bangladesh's livestock sector is vital to its economy, with a significant portion of the population engaged in livestock rearing. The country's livestock population includes 25.013 million cattle, 27.117 million goats, 3.903 million sheep, and 327.77 million chickens (Livestock Economy, DLS, 2023-2024). Beef, which is a highly nutritious source of protein, comes mainly from bulls, steers, and cows, with some imports from neighboring countries like India and Myanmar. 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 (Baset et al., 2003; Begum et al., 2007). Meat, particularly beef, is highly perishable, and improper preservation can lead to spoilage and foodborne pathogens (Yasmin et al., 2022; Kumudavally et al., 2005). Despite this, there is a lack of knowledge regarding effective preservation methods in Bangladesh, making it essential to develop strategies to ensure both the quality and safety of beef while minimizing wastage (The Business Standard Report, 18 June, 2024). Meat and meat products are highly nutritious but are prone to spoilage shortly after slaughter, which limits their shelf life. 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 (Chakrabartty et al., 2024; Torun et al., 2023; Mojola, 2008). Key attributes such as color, texture, juiciness, and flavor, which influence consumer perception, can vary significantly, even from the same source. The spoilage of meat is mainly caused by microbial growth or chemical deterioration. A significant concern in the processed meat industry is lipid oxidation, which degrades the quality of the meat by affecting its sensory attributes (color, texture, odor, and flavor) and nutritional value (Liza et al., 2024; Nunez de Gonzalez et al., 2008). Oxidation during storage and processing results in changes to muscle lipids and proteins, leading to economic losses and potential health risks (Insani et al., 2008; Karpinska et al., 2001). To combat lipid oxidation, technologies like vacuum packaging, modified atmosphere packaging, and the use of antioxidants are commonly employed (Amaral et al., 2018). 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). Citrus fruit juices, peels, and essential oils, known for their antimicrobial properties, are also utilized in various meat preservation methods (Pandey et al., 2011 ;). Citrus fruits are a valuable source of bioactive compounds, such as flavonoids and vitamin C. The flavonoids found in citrus have antioxidant properties due to their ability to neutralize free radicals. Citrus extracts are rich in flavonoid glycosides, coumarins, β- and γ-sitosterol, glycosides, and essential oils. Additionally, the fiber in citrus fruits contains bioactive compounds like polyphenols, with vitamin C being one of the most significant. Freezing is the primary method available for preserving beef, and the quality of beef can be influenced by the freezing temperature used. There was not more research so far conducted before my experiment on beef preservation with Lemon extract, Orange- peel extract and BHT in Bangladesh. So, in our country aspects there are very few information regarding on beef

marination with Lemon peel extract, Orange-peel extract and butylated hydroxytoluene (BHT) in different storage condition in Bangladesh. The aim of storage is not only to retard the food spoilage but also to control undesirable changes of wholesomeness, nutritive value and growth of microorganisms. In this situation, the present study has under taken with the objectives of, to examine sensory, proximate, biochemical, physicochemical and microbial quality of beef after addition of lemon peel extract, Orange- peel extract and BHT, to investigate the effect of lemon peel extract, Orange- peel extract and BHT on meat spoilage and to control undesirable changes of wholesomeness, nutritive value and growth of microorganisms.

Methodology

Place of Experiment

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

Experimental Samples

The beef was sourced from the "Local Market" (Shesh Mor Bazar) at Bangladesh Agricultural University, Mymensingh, at 8:00 a.m. The bull, approximately two years old with a live weight of 250±5 kg, was slaughtered using the halal method. Following slaughter, the meat sample was promptly transferred to the "Animal Science Laboratory" for sensory, physicochemical, and microbial analyses. The orange was collected from the “Bangladesh Agricultural University Shesh Mor bazar” of Mymensingh Sadar and BHT was bought from a laboratory.

Preparation of sample and Other Instruments

All visible fat and connective tissue were trimmed off as far as possible with the help of knife and the sample was cut into small pieces. All necessary instruments and jars were cleaned with hot water and detergent powder, then, dried properly before starting the experimental activities. Fresh oranges and lemons were collected from local market. Then the oranges and the lemons were washed with clean water. The peels were separated from edible portion and grinded with the grinder machine. After grinding orange peel extract was collected from it by filtrating with sieving cloth.

Experimental Layout

The meat sample was divided into 4 parts. 1% (T_1 lemon peel extract), 1% (T_2 orange peel extract), 0.01% (T_3 BHT) were mixed with the three portions of sample respectively and T_0 is control sample without any antioxidant and placed in polythene bags. Then Stored under 4°C for 9 days. The sample were taken from each treatment at 0, 3rd, 6th and 9th days respectively for different analysis.

Analysis of Different Characteristics of beef Samples in the Laboratory

Instrumental color measurement

Instrumental color measurement was performed on the longissimus muscle of meat using a Konica Minolta Chroma Meter (CR 410, Konica Minolta Sensing, Inc., Osaka, Japan), programmed with the CIE Lab (L*, a*, b*) color system, where L* represents lightness, a* denotes redness, and b* indicates yellowness (CIELAB, 2014). The color was assessed at 24 hours post-slaughter, 3rd, 6th and 9th day on the medial surface of the meat. Calibration was conducted using a specific whiteboard prior to each measurement. Each color value was determined by averaging three readings taken from a 4–5 cm² area of the sample to ensure accurate representation. The L* value reflects the lightness, ranging from 0 (black) to 100 (white), while a* ranges from -60 (green) to +60 (red), and b* ranges from -60 (blue) to +60 (yellow). Color measurements were taken on day 0 and repeated on days 3, 6, and 9, during frozen storage at 4ºC.

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 of Beef

The study involved several procedures to analyze the physicochemical properties of beef samples. A 5 g sample was homogenized in 45 ml distilled water, then centrifuged, and the pH was measured using a pH meter. Water holding capacity (WHC) was assessed by wrapping 1 g samples in absorbent cotton, centrifuging them, and calculating the WHC as the ratio of post-centrifugation weight to initial weight. Drip loss, which impacts the quality, tenderness, and juiciness of the meat, was determined to evaluate water loss from the muscle tissue. Cooking loss was measured by wrapping 10 g samples in foil, heating them in a water bath at 60°C for 30 minutes, and weighing the samples before and after cooking to assess moisture loss. These measurements were taken at 0, 3, 6, and 9 days to monitor changes over time.

Biochemical Analysis

Lipid oxidation in beef samples was evaluated using the 2-thiobarbituric acid (TBA) method. In this process, 5 g of beef was mixed with 25 mL of a 20% trichloroacetic acid solution, then homogenized and filtered. The filtrate was combined with TBA solution and incubated at 100°C for 30 minutes. After cooling, the absorbance at 532 nm was measured using a UV-VIS spectrophotometer. The resulting TBA value, which reflects lipid oxidation levels, was expressed in terms of milligrams of malondialdehyde per kilogram of beef. This procedure was carried out in triplicate to ensure reliability of the results.

Microbial assessment

The study involved microbiological analysis of beef samples to determine total viable count (TVC), total coliform count (TCC), and yeast and mold counts. A 5 g sample of raw beef was homogenized in a sterile 0.1% peptone water solution, then serially diluted (10^{-2} to 10^{-6}). The samples were cultured on three types of media: Plate Count Agar (PCA) for TVC, MacConkey Agar (MA) for TCC, and Potato Dextrose Agar (PDA) for yeast and mold counts. After incubation at the appropriate temperatures, colonies between 30 and 300 were counted using a colony counter. The results were expressed as colony-forming units (CFU) per gram of beef. The preparation of the media involved dissolving the appropriate amounts of PCA, MA, and PDA ingredients in distilled water, boiling to dissolve, and sterilizing at 121°C for 15 minutes. The final pH of the media was adjusted to 7.0 ± 0.1. Samples were plated in triplicate, incubated, and the colonies were counted to calculate the respective counts (TVC, TCC, and yeast and mold count) according to ISO guidelines (1995). The results were expressed as CFU/g of beef.

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

Instrumental color value

The study assessed the color characteristics of fresh beef across four treatment groups, focusing on lightness (L*), redness (a*), and yellowness (b*). In the (table 1) T_1 group showed the most desirable lightness (L*) value of 38.57, while the T_0 group had the lowest at 32.55. T_2 had the highest L* value on day 6 (42.85), which decreased slightly by day 9 (41.92). Significant differences in L* values were found between treatments ($P < 0.01$), across storage days ($P < 0.01$), and in the interaction between treatments and storage intervals ($P < 0.01$). For redness (a*), T_1 again performed best with a mean value of 13.63, whereas T_3 had the lowest at 12.22. The peak redness for T_1 was on day 0 (16.94), which dropped to 9.82 by day 9. Redness values significantly differed across treatments ($P < 0.01$), storage days ($P < 0.01$), and their interaction ($P < 0.01$). For yellowness (b*), T_1 had the most desirable value of 9.39, while T_0 had the least favorable at 9.17. On day 3, T_1 recorded the highest b* value (10.30), which declined to 8.24 by day 6. Again, significant differences in b* values were observed by treatment ($P < 0.01$), storage days ($P < 0.01$), and their interaction ($P<0.01$). Overall, the T_1 treatment showed consistently better color characteristics, with lower L* and higher a* and b* values compared to T_2 and T_3, all of which were significantly different ($P < 0.01$). The study concluded that T_1 was the most preferred treatment, and over time, the color scores decreased as storage duration increased. This reduction was attributed to pigment and lipid oxidation, likely leading to non-enzymatic browning reactions between lipids and amino acids.

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

Parameters	DI	Treatments					Level of significance		
		T_0	T_1	T_2	T_3	Mean	Treat.	DI	T×DI
L*	0	32.93±0.56	35.63±0.45	38.58±0.22	39.18±0.37	36.58^{c}	**	**	**
	3	33.08±0.39	33.89±0.46	37.22±0.55	42.24±0.39	36.61^{c}			
	6	32.05±0.23	42.85±0.29	41.86±0.31	46.16±0.08	40.73^{b}			
	9	32.12±0.49	41.92±0.38	40.72±0.40	52.24±1.38	41.75^{a}			
	Mean	32.55^{d}	38.57^{c}	39.59^{b}	44.95^{a}				
a*	0	16.34±0.46	16.94±0.58	11.41±0.37	11.47±0.61	14.04^{b}	**	**	**
	3	11.33±0.47	12.77±0.42	12.62±0.31	11.49±0.33	12.05^{c}			
	6	12.49±0.34	14.99±0.40	17.23±0.35	15.63±0.34	15.08^{a}			
	9	12.36±0.16	9.82±0.45	10.61±0.66	10.31±0.38	10.77^{d}			
	Mean	13.13^{b}	13.63^{a}	12.97^{b}	12.22^{c}				
b*	0	7.12±0.25	9.53±0.35	10.38±0.38	7.47±0.61	8.63^{c}	**	**	**
	3	9.80±0.56	10.30±0.38	9.17±0.44	9.53±0.54	9.70^{b}			
	6	9.62±0.27	8.24±0.41	12.78±0.56	11.10±0.58	10.44^{a}			
	9	10.13±0.43	9.48±0.35	10.51±0.59	12.63±0.46	10.69^{a}			
	Mean	9.17^{c}	9.39^{c}	10.18^{b}	10.71^{a}				

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_0 = (Control group), T_1 = (1% olive oil), T_2 = (1 % mustard oil), T_3 = (1% sesame seed oil) and T_4 = (1% soyabean oil), 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

This study presents the dry matter (DM) content of beef across different treatments and storage intervals in (Table 2). The DM content ranged from 25.53% to 26.28%, with no significant differences between the treatment groups. The T1 group had the most desirable DM content, as lower DM values are preferred for better product quality. The DM content increased from 25.52% to 26.54% across the storage days, with no significant differences observed between days 0, 3, 6, and 9. This increase was due to moisture loss during storage, making the product less preferable over time. The lowest DM content was recorded on day 0, while the highest was on day 9. The rise in DM content is mainly attributed to evaporative moisture loss during refrigeration. These findings are consistent with previous research by Modi et al. (2008), Al-Bachir and Zeinou (2014), and Konieczny et al. (2007), who also observed increases in DM content during storage. Additionally, Naveena et al. (2008) reported higher DM content in products stored for longer periods, especially those treated with pomegranate peel extract.

Crude Protein

Table 2 summarizes the crude protein (CP) content across different treatments and storage intervals. The CP content ranged from 19.98% to 20.64% among the treatment groups, with significant differences ($p < 0.01$) observed between groups with natural and synthetic antioxidants. The T3 group exhibited the highest CP content, which is considered more beneficial for consumer health, while the control group (T_0) had the lowest. Regarding storage time, CP content ranged from 20% to 20.43%, with significant differences ($p < 0.05$) observed between storage days (0, 3, 6, and 9). The CP content generally decreased as the storage period lengthened, with the highest values recorded on day 0 and the lowest on day 9, where all treatments declined to 20%. This decline in CP content over time aligns with previous studies, such as Konieczny et al. (2007), who reported a reduction in protein content during frozen storage. Higher CP levels in products, like those treated with antioxidants such as Curcuma, are beneficial for consumers, particularly for children's growth and during periods of increased nutritional demand, such as pregnancy and lactation (Heinz and Hautzinger, 2007). As a result, products with higher CP content can help meet nutritional needs more effectively and potentially reduce meat consumption costs. Overall, the mean CP content across all samples over the

9-day storage period followed this order: T_0 (control) < T_1 < T_2 < T_3. The antioxidants likely helped preserve higher CP levels by inhibiting microbial decomposition in the treated samples.

Ether Extract

Study presents the ether extract (EE) content in beef treated with natural and artificial antioxidants. The EE content ranged from 3.65% to 3.80%, with no significant differences ($p > 0.05$) observed between the control and antioxidant-treated groups. The T_3 group exhibited the most desirable EE content, as lower EE levels are considered more beneficial for consumer health, making T_3 the preferred option, while the control group (T_0) had less favorable EE content. Across different storage intervals, the EE content varied between 3.50% and 4.02%. Significant differences ($p < 0.01$) were observed across the storage days (0, 3, 6, and 9), with the lowest EE content recorded on day 0 and the highest on day 9, where the EE content increased to 4.02% across all treatments. This rise in EE content suggests that storage time affects fat concentration in the meat. Similar trends were reported in previous studies. While Verma et al. (2012) reported similar findings in various meat products. These results indicate that both the type of antioxidant treatment and storage duration influence changes in EE content.

Ash

Table .2 presents the ash content of beef samples treated with antioxidants across different storage intervals. The ash content ranged from 1.19% to 1.31%, with significant differences ($p < 0.05$) observed between the treatment groups. The T_3 group showed the most favorable ash content, while T_1 had lower values, which are considered less desirable for consumer health. Regarding the effect of storage duration, ash content varied from 1.16% to 1.36% across different days (0, 3, 6, and 9). Significant differences ($p < 0.01$) were noted, with ash content increasing as the storage period extended. The lowest ash content was recorded on day 0, while the highest was on day 9, where it reached 1.36% across all treatments. This increase in ash content over time is likely due to changes that occur during frozen storage. Similar trends were found in studies on Malaysian commercial beef meatballs, where ash content ranged from 1.76% to 3.40%, aligning with this study's results. Serdaroglu et al. (2005) observed similar ash content in koefte beef meatballs, and Konieczny et al. (2007) also noted an increase in ash content during prolonged frozen storage.

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

		Treatments					Level of significance		
Parameters	**DI**	**T_0**	**T_1**	**T_2**	**T_3**	**Mean**	**Treat.**	**DI**	**T×DI**
	0	25.41±1.24	25.22±1.05	25.49±1.69	25.94±0.60	25.52^{a}			
DM (%)	3	25.77±1.76	25.27±1.36	25.74±1.34	26.13±2.25	25.73^{a}			
	6	26.32±1.16	25.59±1.24	26.06±1.25	26.37±0.59	26.08^{a}	NS	NS	NS
	9	26.93±1.86	26.02±1.26	26.54±1.53	26.68±1.96	26.54^{a}			
	Mean	26.10^{a}	25.53^{a}	25.96^{a}	26.28^{a}				
	0	20.33±0.08	20.27±0.08	20.37±0.06	20.75±0.12	20.43^{a}			
CP (%)	3	20.12±0.04	20.17±0.03	20.29±0.07	20.70±0.09	20.32^{ab}			
	6	19.85±0.29	20.02±0.42	20.16±0.10	20.60±0.14	20.16^{ab}	**	*	NS
	9	19.64±0.53	19.86±1.08	20.03±0.23	20.49±0.60	20.00^{b}			
	Mean	19.98^{b}	20.08^{b}	20.21^{b}	20.64^{a}				
	0	3.54±0.07	3.48±0.10	3.55±0.42	3.43±0.08	3.50^{c}			
	3	3.68±0.08	3.60±0.47	3.63±0.08	3.58±0.10	3.62^{bc}			
EE (%)	6	3.80±0.08	3.69±0.06	3.70±0.13	3.66 ±0.05	3.71^{b}	NS	**	NS
	9	4.20±0.28	3.93±0.18	4.03±0.23	3.92 ±0.19	4.02^{a}			
	Mean	3.80^{a}	3.68^{a}	3.73^{a}	3.65^{a}				
	0	1.13 ±0.23	1.23 ±0.12	1.16 ±0.08	1.12 ±0.17	1.16^{c}			
	3	1.20 ±0.16	1.28 ±0.12	1.22 ±0.11	1.16 ±0.38	1.21^{bc}			
Ash (%)	6	1.32 ±0.15	1.33 ±0.08	1.27 ±0.19	1.20 ±0.10	1.28^{ab}	*	**	NS
	9	1.44 ±0.08	1.40 ±0.05	1.35 ±0.09	1.27 ±0.09	1.36^{a}			
	Mean	1.27^{ab}	1.31^{a}	1.25^{ab}	1.19^{b}				

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_0 = (Control group), T_1 = (1% olive oil), T_2 = (1 % mustard oil), T_3 = (1% sesame seed oil) and T_4 = (1% soybean oil), 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.

Physicochemical Properties

pH of Raw Meat

Table 3 shows the raw pH values of beef samples treated with natural and synthetic antioxidants across different storage intervals. The pH ranged from 5.59 to 5.90, with significant differences ($p < 0.01$) between the treatment groups. The T_3 group had the most favorable pH, indicating potential health benefits. Over the storage period, pH values increased from 5.30 on day 0 to 6.42 on day 9, with untreated samples showing a more pronounced increase. This rise in pH is linked to microbial activity, as bacteria and mold secrete compounds that raise pH, especially in meat products. The findings are consistent with previous studies, such as Biswas et al. (2004), Akter et al. (2022), which observed similar pH increases in antioxidant-treated pork patties. Maintaining lower pH levels is beneficial for preventing spoilage in dried meat products, as higher pH values result from microbial activity, particularly ammonia accumulation.

Water holding capacity

Study shows the water-holding capacity (WHC) of beef treated with various antioxidants over a 9-day refrigerated storage period (Table 3). Overall, the WHC ranged from 94.57% to 95.13%, with no significant differences observed between treatments at different time intervals (0, 3, 6, and 9 days). However, the control group had a noticeably lower WHC compared to the antioxidant-treated groups, suggesting that antioxidants positively affect WHC. Across all treatments, a gradual decline in WHC was observed over time, which is typical due to moisture loss during storage. Among the antioxidant-treated groups, T_1 maintained the most favorable WHC, indicating it may provide the most benefits for consumers. Higher WHC is associated with better meat quality, and T_1's performance may make it a more advantageous option for meat preservation. This aligns with previous studies, which have shown that a lower pH in meat, especially poultry, is linked to reduce WHC, resulting in increased drip and cooking loss.

Drip loss

The study found significant differences in drip loss across different treatments, storage intervals (Table 3), and their interaction. Drip loss values ranged from 3.14 to 4.06 for the treatments and 2.79 to 4.51 for the intervals. The T_3 group showed the most preferable drip loss value, indicating better juiciness and overall product quality. Drip loss increased significantly during storage in all treatments. These findings are consistent with previous research by Purslow et al. (2014), which examined muscle tissue water-holding capacity during cold storage

Cooking Loss

The study found that cooking loss in beef treated with antioxidants ranged from 27.66% to 28.50%. T_2, which contained 1% orange peel extract, had the lowest cooking loss and was preferred for its ability to retain moisture and nutrients. Over time, cooking loss decreased as storage increased. High cooking loss can negatively impact meat quality and consumer appeal, while antioxidants help reduce this loss. Lower cooking loss improves cooking yield, which is important for the meat industry in predicting product behavior during processing.

Table 3. Effect of different types of anti-oxidants on physicochemical parameters (Mean ± SE) in beef at different day intervals

		Treatments					Level of significance		
Parameters	**DI**	**T_0**	**T_1**	**T_2**	**T_3**	**Mean**	**Treat.**	**DI**	**T×DI**
	0	5.29 ±0.02	5.31 ±0.09	5.33 ±0.10	5.27 ±0.06	5.30^{d}			
	3	5.49 ±0.09	5.43 ±0.05	5.44 ±0.06	5.34 ±0.12	5.43^{c}			
pH	6	5.95 ±0.11	5.77 ±0.12	5.80 ±0.06	5.63 ±0.07	5.79^{b}	**	**	**
	9	6.85 ±0.08	6.46 ±0.17	6.25 ±0.08	6.12 ±0.17	6.42^{a}			
	Mean	5.90^{a}	5.75^{b}	5.71^{b}	5.59^{c}				
	0	96.54 ±0.07	96.33 ±0.62	96.21 ±0.30	95.9 ±0.82	96.25^{a}			
	3	95.27 ±0.41	95.87 ±0.40	95.81 ±0.26	95.63 ±0.71	95.65^{a}			
	6	93.97 ±1.14	94.84 ±0.24	94.80 ±0.47	94.55 ±0.90	94.54^{b}	NS	**	NS
WHC	9	92.48 ±1.10	93.49 ±1.08	93.22 ±1.70	93.36 ±1.14	93.14^{c}			
	Mean	94.57^{a}	95.13^{a}	95.01^{a}	94.86^{a}				
	0	2.96 ±0.15	2.94 ±0.24	2.77 ±0.27	2.48 ±0.18	2.79^{d}			
	3	3.54 ±0.24	3.31 ±0.21	3.04 ±0.15	2.92 ±0.17	3.20^{c}			
	6	4.18 ±0.23	3.78 ±0.35	3.49 ±0.32	3.29 ±0.10	3.69^{b}	**	**	*
Drip loss	9	5.54 ±0.68	4.42 ±0.16	4.22 ±0.10	3.85 ±0.33	4.51^{a}			
	Mean	4.06^{a}	3.61^{b}	3.38^{b}	3.14^{c}				
	0	30.87 ±0.40	30.69 ±1.40	29.53 ±0.39	30.96 ±0.14	30.51^{a}			
	3	28.43 ±0.23	29.88 ±0.35	28.46 ±0.19	29.00 ±0.65	28.94^{b}			
	6	26.75 ±0.27	27.78 ±0.78	26.89 ±0.24	27.53 ±0.30	27.24^{c}	**	**	*
Cooking loss	9	25.45 ±0.60	26.71 ±0.27	25.76 ±0.24	26.51 ±0.16	26.11^{d}			
	Mean	27.88^{b}	28.77^{a}	27.66^{b}	28.50^{a}				

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_0 = (Control group), T_1 = (1% olive oil), T_2 = (1 % mustard oil), T_3 = (1% sesame seed oil) and T_4 = (1% soybean oil), 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 TBARS values in both treatment and control groups increased significantly with longer storage periods, indicating a decline in shelf life. Treatment T_3 showed the lowest TBARS values, suggesting it was the healthiest choice for consumers. TBARS values ranged from 0.09 to 0.66 across storage intervals (Table 4), with significant increases over time. This is consistent with previous studies, such as those by Kim et al. (2013) and Reddy et al. (2013), which showed that natural antioxidants, like plant extracts, reduce TBARS values and improve shelf life in meat products.

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

Parameters	**DI**	**Treatments**					**Level of significance**		
		T_0	**T_1**	**T_2**	**T_3**	**Mean**	**Treat.**	**DI**	**T×DI**
	0	0.10 ±0.02	0.07 ±0.01	0.11 ±0.03	0.09 ±0.03	0.09^{d}			
TBARS (mg	3	0.32 ±0.04	0.13 ±0.06	0.16 ±0.03	0.14 ±0.04	0.19^{c}			
MDA/kg)	6	0.53 ±0.08	0.26 ±0.03	0.34 ±0.04	0.25 ±0.05	0.35^{b}	**	**	**
	9	1.07 ±0.15	0.52 ±0.07	0.58 ±0.04	0.47 ±0.06	0.66^{a}			
	Mean	0.51^{a}	0.25^{c}	0.30^{b}	0.24^{c}				

Microbial Assessment

This study examined the presence of microflora (total viable count, TVC) and foodborne pathogens (coliforms and yeast-mold) in beef samples treated with orange peel extract, stored at 4°C for up to 9 days. The TVC values increased over time, with antioxidant-treated samples showing significantly lower bacterial growth compared to the control. Treatment T_3 had the lowest

TVC, indicating better consumer health benefits. Total coliform counts (TCC) also decreased in treated samples, with T_1 showing the most significant reduction. Yeast and mold counts (TYMC) were lower in antioxidant-treated samples, especially T_3, indicating reduced fungal contamination. Overall, antioxidant treatments effectively inhibited bacterial and fungal growth, improving the shelf life of beef. This aligns with previous studies that have highlighted the antimicrobial properties of natural antioxidants in meat preservation (Azad et al., 2022; Akhter et al., 2022).

Table 5. Effect of different types of anti-oxidants on microbial assessment (Mean ± SE)

In beef at different day intervals

Parameters		Treatments					Level of significance		
	DI	T_0	T_1	T_2	T_3	Mean	Treat.	DI	T×DI
TVC (logCFU/g)	0	5.21 ±0.12	5.31 ±0.09	5.19 ±0.10	5.20 ±0.12	5.21^{c}			
	3	5.41 ±0.10	5.43 ±0.05	5.26 ±0.13	5.25 ±0.04	5.30^{c}			
	6	5.96 ±0.15	5.77 ±0.12	5.43 ±0.06	5.31 ±0.08	5.53^{b}	*	**	**
	9	6.84 ±0.37	6.46 ±0.17	5.52 ±0.09	5.37 ±0.05	5.80^{a}			
	Mean	5.86^{a}	5.75^{b}	5.35^{b}	5.28^{b}				
TCC (logCFU/g)	0	2.42 ±0.06	96.33 ±0.62	2.34 ±0.06	2.36 ±0.08	2.36^{d}			
	3	2.66 ±0.17	95.87 ±0.40	2.42 ±0.10	2.43 ±0.05	2.47^{c}			
	6	2.87 ±0.12	94.84 ±0.24	2.62 ±0.15	2.56 ±0.12	2.64^{b}	**	**	NS
	9	3.08 ±0.18	93.49 ±1.08	2.71 ±0.15	2.65 ±0.18	2.78^{a}			
	Mean	2.76^{a}	95.13^{a}	2.52^{b}	2.50^{b}				
TYMC (logCFU/g)	0	2.47 ±0.05	2.94 ±0.24	2.35 ±0.06	2.28 ±0.08	2.37^{d}			
	3	2.82 ±0.13	3.31 ±0.21	2.44 ±0.13	2.35 ±0.10	2.52^{c}			
	6	3.05 ±0.27	3.78 ±0.35	2.61 ±0.14	2.45 ±0.05	2.69^{b}	**	**	*
	9	3.46 ±0.14	4.42 ±0.16	2.80 ±0.09	2.67 ±0.09	2.96^{a}			
	Mean	2.95^{a}	3.61^{b}	2.55^{b}	2.44^{c}				

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_0 = (Control group), T_1 = (1% olive oil), T_2 = (1 % mustard oil), T_3 = (1% sesame seed oil) and T_4 = (1% soyabean oil), 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 concluded that beef can be effectively preserved for up to 9 days using natural and artificial anti-oxidant. The 0.01% BHT (T_3) group showed highly significant improvements compared to the control group across sensory, physicochemical, biochemical, and microbial assessments. The use of 0.01% BHT not only enhanced consumer acceptability but also maintained satisfactory nutritional quality, making it a recommended preservative for up to 9 days preservation at 4°C.

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