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[1]Department of Animal Science, Bangladesh Agricultural University, Mymensingh-2202

Research Article

Influence of aerial and vacuum packaging on the quality and shelf life of beef treated with extra virgin olive oil

MA Hashem[1]*, FA Himo[1], MS Arafath[1], AA Numan[1], MAK Azad[1]

***Corresponding Author**

MA Hashem
E-mail: hashem_as@bau.edu.bd

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Abstract

The aim of this study was to evaluate the effects of olive oil as a natural preservative and aerial and vacuum packaging on the quality and shelf life of raw beef. Meat samples were divided into four treatments i.e. T_0= with 1% olive oil and aerial packaged, T_1= without olive oil and aerial packaged, T_2 = with 1% olive oil and vacuum packaged and T_3 = without olive oil and vacuum packaged. All samples were kept at refrigerated temperature. Days of interval were 0, 5, 10 and 15. The samples were tested for sensory properties (color, flavor, tenderness, juiciness and overall acceptability), physicochemical characteristics (water holding capacity, cooking loss, pH); biochemical properties (TBARS), and microbiological counts (TVC, TCC and TYMC). During the whole storage process, the sensory and instrumental color value were considerably higher ($P<0.05$) in T_0 and T_2 treatments. WHC in treatment T_2 was higher, CL was lower in T_0 treatment and pH in treatment of T_0, T_1 were considerably higher ($P<0.05$) than in the T_2 and T_3 group. The values of TBARS, viable count, coliform count, and yeast-mold count were significantly lower in T_0 and T_2 group. Based on the findings of this study, it is possible to conclude that olive oil treated beef in aerial and vacuum packaging storage technique can be used in the future for meat preservation, providing antioxidant and antimicrobial agents as a value addition by inhibiting lipid oxidation and extending the shelf life of beef.

Introduction

Bangladesh is mainly an agricultural country which is adorned with different agricultural and livestock products. Livestock plays potential role in the national economy being a vital component of agriculture. In Bangladesh most of the farmers are interested in rearing cattle for meat purpose. Beef fattening is an emerging sector for employment and income generation of the rural poor, especially landless, destitute and divorced women (Hasan et al., 2022 and 2023; Islam et al., 2022). Meat is recognized as a highly nutritious food and also contains essential amino acids which are helpful for human life. Generally, in Bangladesh, people are highly interested in eating cattle meat (Beef). Knowledge about the origin, chemical and nutritional components of beef are important tools to aware in the selection of beef in their daily diet (Islam et al., 2023). Spices and herbs, like many other agricultural products, are susceptible to microbial contamination both before and after harvest. Such contamination may occur during processing, storage, distribution, and sale and/or use (Hossain et al., 2022a and 2022b; McKee, 1995).

Oxidation in meat results in rancid taste, off flavors, texture and color changes, which adversely affect consumer acceptability and limits meat shelf life, causing issues in marketing and distribution (Singh et al., 2015). Extension of the shelf-life of meat is one of the technologies needs to meet the demands of consumers. In this respect, increasing attention is put on packaging techniques. Modified atmosphere packaging (MAP) and vacuum packaging (VP) are recent innovations that have been gaining importance as preservation techniques to improve the shelf-life of meat. Vacuum packaging removes the oxygen from the meat. This reduces the activity of bacteria that require oxygen and significantly increases shelf life. Removing the oxygen drastically slows the meat maturation process and the meat quality is maintained for a longer period. Meat generally lasts 6 months in a refrigerator when stored with conventional methods. But a vacuumed sealed meat lasts 2-3 years in a freezer. Antioxidants can be applied in meat processing to preserve meat quality and extend its storage time (Banerjee et al., 2012; Sajad et al., 2017). Even low concentrations of antioxidants (ppm) added to meat products can slow down the oxidation of meat lipids and proteins, therefore increasing the storage time of meat by saving them from chemical oxidation (Karre et al., 2013). However, the application of synthetic antioxidants in meat has declined recently because of consumer concern over possible toxicity (Al-Sherick et al., 2005). Many plant-derived sources that have been suggested to contain high level of antioxidant ingredients to inhibit oxidative deterioration have been studied, such as bearberry, cranberry, grape seed extract, pine bark extract, plum, pomegranate, rosemary, and oregano (Karre et al., 2013). Fresh meat is also highly perishable product due to its biological composition (Zhou et al., 2010). In addition, meat and poultry products have frequently been found to be contaminated with microorganisms during the butchering and manufacturing process. These microorganisms produce

undesirable quality changes in meats, especially in relation to lactic acid bacteria, a major bacterial group associated with meat spoilage (Devatkal et al., 2010). Color, microbial growth and lipid oxidation are important factors for the shelf life and consumer acceptance of fresh meat (Jakobsen and Bertelsen, 2000). Lipid oxidation, which is initiated in the unsaturated fatty acids fraction in subcellular membranes, is a major cause of the deterioration and reduced shelf-life of meat products (Devatkal et al., 2010).

The use of natural antioxidants, especially from plants, was reported to be of greater use for the acceptance, palatability, stability of meat products as they contain a number of valuable antioxidants and antimicrobial properties that are capable of prevention or reduction not only of oxidation of lipids, fats and oils but also enhancement of shelf life (Shahidi and Zhong, 2010). In the last few years, the identification and development of phenolic compounds or extracts from different plants has become a major area of health- and medical-related research (Das et al., 2022). The polyphenolic compounds extracted from leaves and olive fruits are excellent antimicrobial and antioxidant agents. The most abundant phenolic component is oleuropein which gives the bitter taste to olive and olive oil. Olive oil are rich in biophenols (BPs), such as oleuropein, verbascoside, ligostroside, tyrosol or hydroxyl tyrosol. These compounds have shown several biological activities such as antioxidant and antimicrobial, and consequently can be used in food application (Mukhtar et al., 2014). Health benefits of this compound have been extensively investigated. It has been reported that oleuropein, and related compounds such as tyrosol, verbascoside, ligostroside, and dimethyleuropein, act as antioxidants by preventing the formation of free radicals by its ability to chelate metals such as copper and iron, which catalyze free radical generation reactions such as lipid oxidation (Mokhtar et al., 2012). In addition, it lowers the risk of coronary diseases, several cancers, and could have antimicrobial and antiviral activity. Synthetic antioxidants, such as butylated hydroxyl toluene (BHT) and sodium erythorbate were extensively used to delay, retard, or prevent the lipid oxidation by scavenging chain carrying peroxyl radicals or suppressing the formation of free radicals. Therefore, the objective of this study was to evaluate the effects of aerial and vacuum packaging on the quality and shelf life of beef treated with extra virgin olive oil.

Materials and Methods

Place of Experiment

The experiment was conducted at the laboratory of the Department of Animal Science, Faculty of Animal Husbandry, Bangladesh Agricultural University.

Collection of Raw materials

The samples of beef were collected from Kamal Ronjit-market (K.R. Market) of Bangladesh Agricultural University, Mymensingh. The samples were taken only the muscle part, avoiding bone part. After that, the meat sample was immediately transferred to the Animal Science Laboratory. Extra virgin olive oil was collected from a super shop, Chorpara, Mymensingh.

Preparation of Jar and other Instruments

With hot water and detergent powder, all essential tools and jars were properly cleaned and dry before the experimental activities started.

Sample preparation

With the assistance of a knife, all visible fat and connective tissue of the flesh were clipped off as far as possible, and the sample was chopped into small pieces. Following that, the half of the sample were mixed with 1% extra virgin olive oil Four treatment having T_0 (with 1% olive oil and aerial packaged), T_1(without olive oil and aerial packaged), T_2 (with 1% olive oil and vacuum packaging), T_3 (without olive oil and vacuum packaged) analyzed for different sensorial, Instrumental color, physicochemical; biochemical and microbiological properties.

Experiment design

Experimental design was a 4^2 factorial experiments replicated three times in Completely Randomized Design (CRD). Sensory, Instrumental color, Physicochemical, Biochemical and Microbiological parameters were studied at 0,5,10 and 15 days of intervals.

Sensory properties of Beef

Each meat sample was evaluated by a trained 3-member panel. Panelists were chosen from among department personnel and students. The sensory evaluation for color, flavor, tenderness, juiciness, and overall acceptability was performed using 9-point Hedonic Scale which is the most widely used scale for food acceptability. Here 9=like extremely, 8=like very much, 7=like moderately, 6=like slightly, 5=neither like or dislike, 4=dislike slightly, 3=dislike moderately, 2=dislike very much, 1=dislike extremely. At least 5 expert penalists will evaluate the sensory parameters for meat acceptability.

Instrumental Color measurement

The physiochemical change in color of was determined using L*a*b* specified Konica Minolta Chromameter (CR-400, Konica Minolta Inc., Tokyo, Japan). Uncooked meat sample was taken in a petri dish to measure the reflectance. The color measurement was made by touching the outer surface of the meat by the chromameter. The measures taken for color was L* for lightness, a* for redness and b* for yellowness.

Physico-chemical parameter analysis

Water holding capacity

Water holding capacity of meat was determined as per centrifugal method. For this 1g of meat sample was minced properly. In a centrifugal tube cotton was inserted first. Then the minced meat sample was inserted into the tube and weight was taken. Then the centrifugal tubes were centrifuged in a centrifuge machine at 10000 rpm for 10 min. After the cotton was removed and weight of the tube with minced meat was taken again. WHC was calculated as follows:

WHC= (W2\W1) *100

Where,

W1= weight of tube, cotton and meat before centrifugation

W2= weight of tube and meat after centrifugation:

Cooking loss

To determine cooking loss, 20 g of samples was weighed and wrapped in a heat-stable foil paper and kept in water bath at 70° C for 30 minutes. Samples surface were dried and weighed. Cooking loss was practiced at day 0, 5th day, 10th day and 15th day. Cooking loss was calculated after draining the drip coming from the cooked meat as follows:

Cooking loss (%) = [(W1-W2) \ W1] x 100;

Where,

W1 = meat weight before cooking and

W2= meat weight after cooking.

pH analysis

The pH was determined with HANNA HACCP Quality pH meter. For this, the electrode was placed in the buffer with a pH value of 7 and started reading. Then the "measure" or calibrate button was pressed to begin reading the pH once the electrode was placed in the buffer. Then the pH reading was allowed to stabilize before letting it sit for approximately 1-2 minutes. After that the electrode was placed in the meat samples and waited 1-2 minutes for measuring the pH of the meat samples.

Biochemical properties

Lipid oxidation was assessed in triplicate using the 2-thiobarbituric acid (TBA) method. Beef samples (5g) were blended with 20% trichloro acetic acid solution (200 g/L of trichloro acetic acid in 135ml/L phosphoric acid solution) in a vortex machine for 60s.The homogenized sample was filtered with Whatman filter paper number 4 and 2 ml of filtrate was added to 2 ml of 0.02 M aqueous TBA solution (3g/L) in a test tube. The test tube were incubated at 100ºC for 30 min and cooled with tap water. The absorbance was measured at fixed wavelength of 532 nm using a UV-VIS spectrophotometer (UV-1200, Shimadzu, Japan). The TBA value was expressed as mg malonaldehyde per kg of beef sample.

Equipment and reagents

2- thiobarbaturic acid (TBA), 25ml of 20% trichloro acetic acid solution (200g/L of trichloro acetic acid in 135ml/L phosphoric acid solution), Whatman filter paper number -4, 2 ml of 0.02M aqueous TBA solution (3g/L), 532 nm using a UV-VIS spectrophotometer (UV-1200, Shimadzu, Japan), pestle and mortar, beaker, test-tube, graduated cylinder, temperature-controlled water-bath, centrifuge machine, vortex machine, cold water, spectrophotometer and distilled water**.**

Microbial analysis

From each sample 10 gm meat from each sample had taken for microbial analysis. The samples were analyzed for Total Viable Count (TVC) on a Plate Count Agar after incubation for 3 days at 35°C, Total Coliform Count (TCC) by MacConkey Agar (MA) after incubation for 1-2 days, yeast-mold count (TYMC) by Potato Dextrose Agar after incubation at 25°C for 3-5 days.

The procedures that were used to determine these parameters are described below:

Preparation of samples for TVC, TCC and Yeast-Mold count

Total viable counts (TVCs), total coliforms, and sampling counts were listed according to American Public Health Association (APHA) procedures (APHA, 1984). Some 10 g of the material were mixed with 90 mL sterile water for 0.1% of peptone and pestle, as per the International Organization for Standardization's guideline serial dilutions were produced (ISO, 1995). Thereby the samples were diluted by 1:10. After that, many serial dilutions between 10-2 and 10-6 were produced in accordance with the usual procedure instructions (ISO, 1995).

Media and reagent for bacteriological study

Solid media and reagents

Plate count agar (PCA), MacConkey agar (MA), and potato dextrose agar (PDA) were used in these microbiological analyses. The commercial media were produced according to the manufacturers' instructions. Appendix A details the media preparation composition and techniques. 0.1 percent peptone water was utilized as a diluent in the experiment.

Glass wares and other appliances

Different types of glass wares and appliances were used during the course of the experiment. These included test tubes (with or without Durham's fermentation tube and stopper), pipette, a conical flask, Petridishes (1 mL, 5 mL, 10 mL, and 25 mL volumes), a glass rod mixer, a test tube holder, a pestle and mortar, a spiny mixture machine, blender machine, water bath, incubator, refrigerator, sterilizing instruments, hot air oven, ice boxes, electronic balance, electronic pH meter etc.

Preparation of media

In two separate conical flasks, 11.50 g of PCA agar and 15.6 g of MA agar were dissolved in 500 mL and 300 mL of cold distilled water, respectively, and then heated to boiling for dissolving the components thoroughly. In the instance of PDA, 200 g of peeled and sliced potato was cooked for an hour in 1000 mL of purified water. After boiling, the mixture was sieved using clean cheesecloth. To disintegrate the components thoroughly, 20 g of industrial dextrose and 15 g of agar were added to the potato infusion solution and heated to boiling. The media were then sterilized in an autoclave at 121°C (6.795 kg pressure/sq. inch) for 15 minutes. The pH of the final reaction was set at 7.0 ± 0.1. The agar was now ready to be poured. The medium was maintained at 45°C in a water bath before pouring.

Enumeration of total viable count (TVC)

0.1 mL of every ten times dilution was transferred and distributed to triplicate PCA agar by a sterile pipette on every dilution for the measurement of the total number of bacteria. The samples were diluted as soon as possible with a sterile glass spreader over the surface of the plate. Each plate was fitted with a sterile spreader. They were then maintained for 24-48 hours in an incubator at 35°C. Following incubation, plates exhibiting 30-300 colonies were counted. With the use of a colony counter, the colonies were numbered. The average number of dilution colonies was multiplied by the dilutive factor to achieve the total viable number. The overall feasible number has been determined by ISO (1995).

Enumeration of total coliform count (TCC)

0.1 mL was sent and distributed across a triple dilution of every tenfold. MA agar using a sterile pipette for each dilution to determine total coliform counts. The samples were diluted as soon as possible with a sterile glass spreader over the surface of the plate. Each plate was fitted with a sterile spreader. They were then maintained for 24-48 hours in an incubator at 35°C. Following incubation, plates exhibiting 30-300 colonies were counted. The samples were diluted as soon as possible with a sterile glass spreader over the surface of the plate. Each plate was fitted with a sterile spreader. They were then maintained for 24-48 hours in an incubator at 35°C. The total coliform count was calculated according to ISO (1995). The results of the total coliform count were expressed as the number of organism of colony forming units per gram (CFU/g) of meat samples.

Enumeration of Yeast-Mold count (TYMC)

For the calculation of the number of yeasts and molds, a sterile pipette was used to transfer 0.1 mL of each tenfold dilution to duplicate PDA agar. The samples were diluted as soon as possible with a sterile glass spreader over the surface of the plate. Each plate was fitted with a sterile spreader. The plates were maintained for 48-72 hours in an incubator at 25°C. Following incubation, plates exhibiting 30-300 colonies were counted. With the use of a colony counter, the colonies were numbered. The dilution factor for yeast and mold count increased the average number of colonies at a specific dilution. The yeast and mold count was calculated according to ISO (1995). The results of the yeast and mold count were expressed as the number of organism of colony forming units per gram (CFU/g) of meat samples.

Statistical model and analysis

The proposed model for the planned experiment was factorial experiment with two factors A (Treatments) and B (Days of Intervals) is: $y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + \varepsilon_{ijki}$ $= 1,\ldots,a;\ j = 1,\ldots,b;\ k = 1,\ldots,n$

Where, y_{ijk}= observation k in level i of factor A and level j of factor B μ = the overall mean

A_i = the effect of level i of factor A B_j= the effect of level j of factor B

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

Sensory Evaluation

Color

The color score of different treatments with days of intervals are shown in Table 1. For all treatments there were significant differences ($p<0.01$) of color of all treatments. The most preferable color was observed in T_2 group and the lowest color from T_1 group. The color of different treatments was decreased with increased storage period. There were significant ($p<0.01$) differences of color of all days. Similar value was observed in color is one of the major causes of quality deterioration (Krishnan et al., 2014) because it can negatively affect sensory attributes such as color, texture, and flavor as well as the nutritional quality of the product.

Flavor

The flavor score of different treatments with days of intervals are shown in Table 1. For all treatments there were significant differences ($p<0.01$) of flavor of all treatments. The most preferable flavor was observed in T_2 group and the lowest flavor from T_1 group. The flavor of different treatments was decreased with increased storage period. There were significant ($p<0.01$) differences of flavor of all days. Decline in flavor scores of meat products during storage was also reported by Zhou et al. (2014), Muthu et al. (2014) in different meat products.

Tenderness

The tenderness score of different treatments with days of intervals are shown in Table 1. For all treatments there were significant differences ($p<0.01$) of tenderness of all treatments. The most preferable tenderness was observed in T_2 group and the lowest flavor from T_1 group. The tenderness of different treatments was decreased with increased storage period. There were significant ($p<0.01$) differences of flavor of all days. The result of this experiment is related to Lui et al. (2010) findings. The results were in accordance with findings of Krishnan et al. (2014) and Chidanandaiah et al. (2009) who also reported a decline in the juiciness scores of different meat products during refrigerated storage.

Juiciness

The juiciness score of different treatments with days of intervals are shown in Table 1. For all treatments there were significant differences ($p<0.01$) of juiciness of all treatments. The most preferable juiciness was observed in T_2 group and the lowest flavor from T_1 group. The tenderness of different treatments was decreased with increased storage period. There were significant ($p<0.01$) differences of flavor of all days. Some of the early studies found that different fat levels play an important role in juiciness and acceptability (Chaijan et al., 2005). Thus, intramuscular fat, mostly in the form of marbling, has been found to contribute to the juiciness of meat (Guilcin et al., 2003).

Overall acceptability

The overall acceptability score of different treatments with days of intervals are shown in Table 1. For all treatments there were significant differences ($p<0.01$) of flavor of all treatments. The most preferable flavor was observed in T_2 group and the lowest flavor from T_1 group. The flavor of different treatments was decreased with increased storage period. There were significant ($p<0.05$) differences of flavor of all days. Similar value was observed in flavor is one of the major causes of quality deterioration (Huiyun et al., 2007) because it can negatively affect sensory attributes such as color, texture, and flavor as well as the nutritional quality of the product (Ibrahim et al., 2011).

Table 1. Effect of aerial and vacuum packaging of beef treated with extra virgin olive oil on sensory properties (Mean ± SE) stored at 4±1ºC temperature

Parameters	DI	Treatments					Level of significance		
		T_0	T_1	T_2	T_3	Mean	Treat.	DI	T× DI
Color	0	9.00±0.00	9.00±0.00	9.00±0.00	9.00±0.00	**9.00^{a}±0.00**	<0.001	<0.001	<0.001
	5	8.00±0.00	7.00±0.00	9.00±0.00	8.00±0.00	**8.00^{b}±0.00**			
	10	7.00±0.00	6.00±0.00	7.00±0.00	7.00±0.00	**7.00^{c}±0.00**			
	15	5.00±0.00	5.00±0.00	6.00±0.00	5.00±0.00	**5.00±0.00**			
	Mean	**7.250^{a}±0.00**	**6.75^{b}±0.00**	**7.75^{c}±0.00**	**7.25^{a}±0.00**				
Flavor	0	9.00±0.00	9.00±0.00	9.00±0.00	9.00±0.00	**9.00^{a}±0.00**	<0.001	<0.001	<0.001
	5	8.00±0.00	7.00±0.00	9.00±0.00	7.00±0.00	**8.75^{b}±0.00**			
	10	7.00±0.00	5.00±0.00	7.00±0.00	6.00±0.00	**5.75^{c}±0.00**			
	15	6.00±0.00	4.00±0.00	6.00±0.00	5.00±0.00	**5.00^{d}±0.00**			
	Mean	**7.25^{a}±0.00**	**6.25^{b}±0.00**	**7.75^{c}±0.00**	**6.75^{d}±0.00**				
Tenderness	0	9.00+0.00	9.00+0.00	9.00+0.00	9.00+0.00	**9.00^{a}±0.00**	<0.001	<0.001	<0.001
	5	7.00+0.00	7.00+0.00	8.00+0.00	8.00+0.00	**7.25^{b}±0.00**			
	10	6.00+0.00	5.00+0.00	6.00+0.00	6.00+0.00	**5.75^{c}±0.00**			
	15	5.00+0.00	4.00+0.00	5.00+0.00	4.00+0.00	**4.25^{d}±0.00**			
	Mean	**6.75^{a}±0.00**	**6.25^{b}±0.00**	**7.00^{c}±0.00**	**7.00^{c}±0.00**				
Juiciness	0	9.00±0.00	9.00±0.00	9.00±0.00	9.00±0.00	**9.00^{a}±0.00**	<0.001	<0.001	<0.001
	5	7.00±0.00	6.00±0.00	8.00±0.00	8.00±0.00	**8.00^{b}±0.00**			
	10	6.00±0.00	5.00±0.00	6.00±0.00	6.00±0.00	**7.00^{c}±0.00**			
	15	4.00±0.00	4.00±0.00	5.00±0.00	4.00±0.00	**5.00^{d}±0.00**			
	Mean	**6.50^{a}±0.00**	**6.00^{b}±0.00**	**7.00^{c}±0.00**	**6.75^{d}±0.00**				
Overall acceptability	0	9.00+0.00	9.00+0.00	9.00+0.00	9.00+0.00	**9.00^{a}±0.00**	<0.001	<0.001	<0.001
	5	8.00+0.00	7.00+0.00	8.00+0.00	7.00+0.00	**8.00^{b}±0.00**			
	10	6.00+0.00	6.00+0.00	7.00+0.00	7.00+0.00	**7.00^{c}±0.00**			
	15	5.00+0.00	5.00+0.00	6.00+0.00	6.00+0.00	**6.00^{d}±0.00**			
	Mean	**7.00^{a}±0.00**	**6.75^{b}±0.00**	**7.50^{c}±0.00**	**7.25^{d}±0.00**				

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 (Olive oil and aerial packaged), T_1 (without olive oil and aerial packaged), T_2 (Olive oil and vacuum packaged), T_3 (without olive oil and vacuum packaged), treat =treatment, DI= days of interval, T×DI= Interaction of treatment and days of interval.

Instrumental color value

The instrumental color value different treatments with days of intervals are shown in Table 2. In case of lightness (L*) of beef, the most preferable color was observed from T_2 (48.12) and less preferable color was observed from T_0 (41.20) group among all four treatments. The most preferable color was observed at T_2 at 0 day and less preferable color was observed at 15 day. The L* values were non significantly differed at different treatment groups, days of interval, and the interaction between treatments and days interval. In case of redness (a*) of beef, the most preferable color was observed from T_2 (16.59) and less preferable color was observed from T_1 (13.45) group among all four treatments. The most preferable color was observed from T_2 at 5 day (20.08) and less preferable at 10 day (14.41). The a* values were significantly differed at different treatment groups, days of interval, and the interaction between treatments and days interval.

In case of yellowness (b*) of beef, the most preferable color was observed from T_2 (10.85) and less preferable color was observed from T_0 (9.36) group among all four treatments. The most preferable color was observed from T_2 at 15 day (13.23) and less preferable at 0 day (8.01). The b* values were non significantly differed at different treatment groups, days of interval, and the interaction between treatments and days interval. The meat color is qualitative trait that most influences the choice of the consumer to purchase or reject the product. L*, a*and b* value of treatment T_2 were found higher compared to T_0, T_1 and T_3 treatments. All these values were found significantly differed. L*, a*and b* values decreased of increasing storage period. Gradual decline in color scores of meat stored at refrigeration conditions at 4ºC might be due to pigment and lipid oxidation resulting in non-enzymatic browning between lipids and amino acids. A similar result was reported by Kumar et al. (2011) in ground mustard incorporated chicken meat nugget.

Table 2. Effect of aerial and vacuum packaging of beef treated with extra virgin olive oil on instrumental color value (Mean ± SE) stored at 4±1ºC temperature

Parameters Instrumental color value	DI	Treatments					Level of significance		
		T_0	T_1	T_2	T_3	Mean	Treat.	DI	T×DI
	0	38.16±8.17	47.29±3.45	43.40±2.13	48.63±3.10	**44.37^a±4.21**	> 0.10	> 0.82	> 0.24
L	5	38.32±10.55	38.60±11.17	47.56±4.23	47.24±12.80	**42.93^a±9.69**			
*	10	47.83±5.64	45.46±1.69	48.74±3.32	40.11±6.62	**45.53^a±4.56**			
	15	40.50±10.12	40.38±0.14	52.79±3.20	41.83±6.47	**43.87^a±4.98**			
	Mean	**41.20^a±8.62**	**42.19^a±4.11**	**48.12^a±3.22**	**44.45^a±7.25**				
a*	0	16.57±1.96	13.89±3.07	15.00±1.88	13.67±2.09	**14.78^a±2.25**	<0.006	>0.15	<0.003
	5	13.24±2.25	12.25±2.49	20.08±3.40	17.58±0.98	**15.78^a±2.28**			
	10	15.27±2.74	16.61±1.22	14.41± 1.97	15.01±3.43	**15.32^a±2.34**			
	15	9.25±3.02	11.06±0.75	16.86±3.98	16.92±1.70	**13.52^a±2.36**			
	Mean	**13.58^b±2.49**	**13.45^b±1.88**	**16.59^a±2.80**	**15.79^a±2.06**				
b*	0	8.76±1.07	7.32±2.38	8.01±2.00	9.25±1.71	**8.33±1.79**	>0.14	<0.007	>0.27
	5	8.89±1.82	9.82±3.50	12.15±2.61	13.03±1.79	**10.97^a±2.43**			
	10	10.50±1.37	10.27±0.53	10.03±1.39	8.92±2.28	**9.93^a±1.39**			
	15	9.31±2.65	10.16±0.87	13.23±2.11	11.64±2.14	**11.08^a±1.94**			
	Mean	**9.36^a±1.73**	**9.39^a±1.82**	**10.85^a±2.03**	**10.71^a±1.98**				

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 (Olive oil and aerial packaged), T_1 (without olive oil and aerial packaged), T_2 (Olive oil and vacuum packaged), T_3 (without olive oil and vacuum packaged), treat =treatment, DI= days of interval, T×DI= Interaction of treatment and days of interval.

Physicochemical Quality

For the physicochemical study, four types of fresh beef samples were prepared. These were with 1% olive oil and aerial packaged, without olive oil and aerial packaged, with 1% olive oil and vacuum packaged, without olive oil and vacuum packaged. After determining the pH on the 0 day, all samples were kept at 4°C for 15 days and tested on the 5th, 10th, and 15th days.

Water holding capacity

Table 3 shows the WHC of beef combined with olive oil and in aerial and vacuum packaged. On days 0, 5, 10 and 15 there was a substantial variation between the different treatments. The range of overall observed WHC from the meat was from 86.11 to 92.11 at different treatment group. The range of over treatments groups the T_0 was significantly higher than other groups. The value of T_0 was highest at 0 day (93.6016) and lowest at 10 day (91.826). The highest value is most preferable to consumer. Huff-Lonergan et al., (2005) reported that early post-mortem events including rate and extent of pH decline, proteolysis and even protein oxidation are key in influencing the ability of meat to retain moisture. WHC is related to the sensory parameters (flavor, tenderness, juiciness) after cooking. De Huidobro et al. (2003) reported that water-holding capacity (expressed as percentage of expelled water) increased in heifer meat. Instrumental texture measures (texture profile analysis, TPA) showed a decrease in hardness, springiness and chewiness in bull raw meat. Sensory analysis showed that assessors perceived a decrease in hardness and in springiness in bull meat and a decrease in juiciness and in chewiness (number of chewings before swallowing) in heifer meat.

Cooking loss

Table 3 shows the cooking loss of beef combined with olive oil and in aerial and vacuum packaged. On days 0, 5, 10 and 15 there was a substantial variation between the different treatments. The range of overall observed CL was from 35.89 to 36.14 at different treatment group. The range of overall observed CL of different days of interval was 36.28 to 35.3. Among all four treatments groups the T_1 was significantly higher than other groups. The lowest value is most preferable for consumer's health. Cooking loss refers to the reduction in weight of meat during the cooking process (Jama et al., 2008). Major components of cooking losses are thawing, dripping and evaporation. Thawing loss refers to the loss of fluid in meat resulting from the formation of exudates following freezing and thawing (Jama et al., 2008; Muchenje et al., 2009). Such losses are lower following a rapid freezing compared with slow freezing. This is because of small crystallization formed by the rapid freezing. Dripping is the cooking loss in meat cuts is important for maintaining an attractive retail display of meat.

pH Value

The pH changes in aerial and vacuum packaged beef treated with extra virgin olive oil during refrigerated (4°C) storage are shown in Table 3. Throughout the storage periods, the pH of beef samples indicated a significant difference (P<0.01) among treatments. The various superscripts seen on the 0th, 5th, 10th, 15th days of observation revealed a substantial difference between these four days of observation. Throughout the storage period, T_3 had lowest pH values than T_1 and T_2 samples. The pH value of meat in all treatments gradually decreased as the storage period extended. The accumulation of lactic acids from microbial secretions and thaw loss of meat were likely to blame for the lowering pH trend. Bacteria and mold have a tendency to diminish as storage duration increases, and they release pH lowering components. Similar findings were observed by Singh et al. (2014). The rise in the pH (P<0.05) at 15th day may be caused by bacterial consumption of acids produced during the breakdown of proteins due of the depletion of the stored glucose. The last increase in pH levels might have been caused by release of ammonia molecules from endo protease or proteolytic microbial flora in the raw meat by Mokhtar et al. (2012).

Table 3. Effect of aerial and vacuum packaging of beef treated with extra virgin olive oil on the physicochemical characteristics (Mean ± SE) stored at 4±1ºC temperature

Parameters	DI	Treatments					Level of significance		
		T_0	T_1	T_2	T_3	Mean	Treat.	DI	T×DI
WHC (%)	0	93.01±1.46	89.44±2.71	91.06±1.54	93.33±0.48	**91.71^{a}±1.55**	<0.001	<0.009	<0.008
	5	91.82±1.47	80.01±3.67	91.16±0.13	89.89±1.54	**88.22^{b}±1.70**			
	10	91.47±3.54	86.18±4.54	89.12±1.58	92.17±3.84	**89.74^{b}±3.37**			
	15	92.10±3.78	91.88±1.83	90.21±2.47	90.58±0.78	**91.2^{a}±2.21**			
	Mean	**92.11^{a}±2.56**	**86.88^{b}±3.19**	**90.39^{a}±1.43**	**91.5^{a}±1.66**				
CL (%)	0	36.54±1.15	35.73±1.42	36.33±1.87	36.50±3.63	**36.28^{a}±2.02**	>0.75	>0.54	>0.96
	5	37.19±1.70	38.96±1.36	39.66±0.95	35.67±0.95	**37.88^{a}±1.24**			
	10	38.02±2.74	38.52±19.05	39.03±1.20	35.53±2.31	**37.78^{a}±6.32**			
	15	31.80±1.03	38.61±1.96	35.91±0.74	36.85±1.64	**35.32^{a}±1.34**			
	Mean	**35.89^{a}±1.16**	**37.46^{a}±5.94**	**36.14^{a}±1.19**	**36.94^{a}±2.13**				
pH	0	6.45±0.66	6.46±0.42	6.34±0.20	6.18±0.05	**6.36^{a}±0.33**	<0.004	<0.009	<0.02
	5	6.58±0.08	6.28±0.01	6.04±0.05	6.07±0.05	**6.24^{b}±0.05**			
	10	6.37±0.17	6.19±0.04	6.18±0.01	6.20±0.01	**6.23^{b}±0.05**			
	15	6.14±0.02	6.19±0.05	6.14±0.01	6.17±0.01	**6.16^{b}±0.02**			
	Mean	**6.39^{a}±0.23**	**6.28^{a}±0.13**	**6.18^{c}±0.06**	**6.15^{c}±0.11**				

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 (Olive oil and aerial packaged), T_1 (without olive oil and aerial packaged), T_2 (Olive oil and vacuum packaged), T_3 (without olive oil and vacuum packaged), treat =treatment, DI= days of interval, T×DI= Interaction of treatment and days of interval.

Biochemical properties

TBARS value

Table 4 shows that there were significant differences in all treatments, days of interval and interaction between treatments and days of interval for TBARS parameter. The ranges for mean value of TBARS were 0.16-0.52 for all group. Among these four treatments, the most preferable value was observed from T_2 group. The lowest value indicate the product is most preferable for consumer health. Similar findings were reported by Sharma et al. (2009) in meat patties. The TBARS values of all samples increased significantly with the extension of the storage period. In all the treatments, TBARS value significantly increased throughout the storage period as concluded by Biswas et al. (2012). Shan et al. (2009) also revealed that out of clove, cinnamon, oregano, grape seed and pomegranate peel, clove exhibited strongest antioxidant activity in terms of TBARS value in raw pork at room temperature. TBARS analysis identifies the development of secondary lipid oxidation products, primarily malondialdehyde, which may contribute to the off-flavor of oxidized fat. Perumalla and Hettiarachchy (2011) stated that the antioxidant activity of phenolic compounds has been attributed to assorted mechanisms. These include preventing the start of the radical chain, the binding of transition metal ion catalysts, the decomposition of peroxides and the interaction with free radicals. It is stated that moringa leaf are a rich source of ascorbic acid, flavonoids, phenolics, carotenoids, proteins, calcium, potassium, etc. by Sreelatha and Padma, (2009); Das et al. (2022). So, this could be the reason for the decrease in oxidation in treated meat. In the case of oxygen in aerobic storage, the increase in TBARS value of every sample throughout a storage time might be caused by lipid oxidation and volatile metabolites (Domínguez et al., 2018; Prabakaran et al., 2018).

Table 4. Effect of aerial and vacuum packaging of beef treated with extra virgin olive oil on the biochemical characteristics (Mean±SE) stored at 4±1ºC temperature

Parameters	DI	Treatments					Level of significance		
		T_0	T_1	T_2	T_3	Mean	Treat.	DI	T×DI
TBARS (mg MDA/kg)	0	0.10±0.00	0.94±0.02	0.108±0.00	0.93±0.02	**0.52^{a}±0.12**	<0.0003	<0.0001	<0.0005
	5	0.08±0.00	0.08±0.00	0.116±0.00	0.09±0.00	**0.09^{b}±0.00**			
	10	0.08±0.00	0.10±0.00	0.093±0.00	0.09±0.00	**0.09^{b}±0.00**			
	15	0.37±0.48	0.97±0.017	0.371±0.45	0.38±0.48	**0.52^{a}±0.36**			
	Mean	**0.16^{b}±0.12**	**0.52^{a}±0.012**	**0.12^{b}±0.11**	**0.38^{a}±0.12**				

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 (Olive oil and aerial packaged), T_1 (without olive oil and aerial packaged), T_2 (Olive oil and vacuum packaged), T_3 (without olive oil and vacuum packaged), treat =treatment, DI= days of interval, T×DI= Interaction of treatment and days of interval.

Microbiological assessments

At different days of interval, the presence of micro-flora (TVC) and food-borne pathogens (Coliform and Yeast-Mold). These were with 1% olive oil and aerial packaged, without olive oil and aerial packaged, with 1% olive oil and vacuum packaged, without olive oil and vacuum packaged four types of samples were kept at 4±1°C for observation on the 0, 5, 10 and 15th day of observation**.**

Total Viable Count (TVC)

Table 5 shows the total viable count of aerial and vacuum packaged beef combined with extra virgin olive oil (with 1% olive oil and aerial packaged, without olive oil and aerial packaged, with 1% olive oil and vacuum packaged, without olive oil and vacuum packaged) 15 days of refrigerated storage. As natural preservative and aerial and vacuum packaged were used, total viable counts decreased considerably (P<0.01) when compared to the without preservative. The increase in the number of microorganisms in the preservative treated samples was substantially (P<0.01) lower than in the without preservative treated

samples. The initial value of TVC for fresh meat was 5.49 log10CFU/g, indicating good quality meat. The different superscript was observed from different treatments indicated that there were significant differences of TVC values among these four treatment groups. However, the lowest microbial load at the end of storage was showed by T_2. This suggests olive oil and vacuum packaging are efficient in inhibiting microbial growth during olive oil was able to suppress the growth of major spoilage microorganisms in intermediate moisture foods and vacuum packaging seal the O_2 results in lower micro-organisms count. The progressive decrease in TVC in treated meat might be caused by increased extract bioactive components that are responsible for antibacterial activities in olive oil. Because Oleaeuropaeacontain several types of bioactive chemicals, including flavonoids, saponins, tannins and other antimicrobial acids (Fahey, 2005). These components can thus be ascribed in the treated mucus patties to reduced bacterial counts. In the cold store of various meat products the use of MOLE also reduces the overall bacterial count (Falowo et al., 2016).

Total Coliform Count (TCC)

Table 5 shows the total coliform count of raw beef combined with natural preservatives (with 1% olive oil and aerial packaged, without olive oil and aerial packaged, with 1% olive oil and vacuum packaged, without olive oil and vacuum packaged) after 15 days of refrigerated storage. On days 0, 5, 10, 15, there were a substantial variation between the different treated batches. There was a significant increase ($P<0.01$) in number of coliforms in all treated samples across the treatment during the period of 15 days. During the storage period T_2 (1% Olive oil and vacuum packaged) batch had lower coliform counts than the others. Masniyom et al. (2002), who found that ground clove, fresh garlic, and red chilli had the strongest antimicrobial systems in a broth model systems. The presence of coliform in both treated and untreated samples, however, might be attributable to contaminated equipment and utensils, as well as sloppy handling and cross contamination during processing. A decreasing trend had been noticed on the treated groups compared to control groups. Similar effects have been found with the use of moringa seed powder and OLE in the cold storage of chicken and beef patties (Elhadi et al., 2017).

Total yeast-mold count

Table 5 shows the total yeast-mold count of raw beef combined with natural preservatives (These were with 1% olive oil and aerial packaged, without olive oil and aerial packaged, with 1% olive oil and vacuum packaged, without olive oil and vacuum packaged) after 15 days of refrigerated storage, there was a substantial variation between the different treated batches. Among four treatments, the yeast and mold counts in the T1 (3.75 log10CFU/g) was significantly higher than in other groups. The lowest value was found in the T_2 group (3.6 log10CFU/g). TYMC was gradually raised during storage in various treatments as storage days increased. The antibacterial action of CP inhibited fat deterioration and prevented bacteria from metabolizing fat. The lower TYMC of the treated meat sample may be attributed by the antifungal properties of moringa leaf extract. Moringa leaf extract contains several low weight proteins and peptides which are responsible for the antibacterial and antifungal activity. Fernández-López et al. (2005) reported on the results of a research study related to antimicrobials in mutton meatballs. They noted that the presence of mold and yeasts was not detected in any cooked meatball samples. A dichloromethane root extract of Caudatusshowed antifungal activity against Cladosporiumcucumerinumand Candida albic ansin bioautographic assay on thin layer chromatograph. Although no specific mold was identified in the quail meatballs, these reports support the suggestion that mold develops in quail meatballs. Recently, some researchers have reported the efficacy of plant EOs as antimicrobial agents against food- borne pathogens and spoilage microflora in meat (Bhat et al., 2008).

Table 5. Effect of aerial and vacuum packaging of beef treated with extra virgin olive oil on microbiological parameters (Mean ± SE) stored at 4±1ºC temperature

Parameters	DI	Treatments					Level of significance		
		T_0	T_1	T_2	T_3	Mean	Treat.	DI	T×DI
TVC (logCFU/g)	0	5.12±0.06	5.20±0.02	5.29±0.02	5.33±0.02	**5.24^{d}±0.03**	<0.0001	<0.0001	<0.001
	5	5.41±0.02	5.45±0.01	5.49±0.015	5.54±0.02	**5.48^{c}±0.01**			
	10	5.62±0.01	5.65±0.01	5.70±0.015	5.75±0.02	**5.69^{b}±0.01**			
	15	5.81±0.02	5.83±0.01	5.82±0.045	5.86±0.03	**5.83^{a}±0.02**			
	Mean	**5.49^{d}±0.02**	**5.54^{c}±0.01**	**5.48^{b}±0.023**	**5.62^{a}±0.02**				
TCC (logCFU/g)	0	3.76±0.04	3.68±0.05	3.59±0.132	3.67±.07	**3.68^{c}±0.07**	<0.003	<0.0001	<0.007
	5	3.85±0.04	3.71±0.02	3.66±0.072	3.71±0.46	**3.74^{b}±0.14**			
	10	3.84±0.04	3.79±0.02	3.92±0.064	3.85±0.07	**3.85^{a}±0.05**			
	15	3.90±0.01	3.92±0.04	3.83±0.051	3.89±0.01	**3.89^{a}±0.03**			
	Mean	**3.74^{a}±0.03**	**3.78^{b}±0.03**	**3.75^{b}±0.079**	**3.78^{b}±0.07**				
TYMC (logCFU/g)	0	3.22±0.01	3.19±0.02	3.24±0.07	3.32±0.05	**3.24^{d}±0.04**	<0.0001	<0.0001	<0.001
	5	3.37±0.04	3.45±0.03	3.56±0.02	3.58±0.01	**3.49^{c}±0.02**			
	10	3.64±0.05	3.72±0.01	3.75±0.05	3.81±0.01	**3.73^{b}±0.03**			
	15	3.88±0.02	3.91±0.01	3.93±0.01	3.93±0.02	**3.91^{a}±0.017**			
	Mean	**3.53^{d}±0.01**	**3.75^{c}±0.02**	**3.62^{b}±0.04**	**3.66^{a}±0.02**				

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 (Olive oil and aerial packaged), T_1 (without olive oil and aerial packaged), T_2 (Olive oil and vacuum packaged), T_3 (without olive oil and vacuum packaged), treat =treatment, DI= days of interval, T×DI= Interaction of treatment and days of interval.

Conclusions

From this study it can be concluded that olive oil treated beef in aerial and vacuum packaging storage technique can be used in the future for meat preservation, providing antioxidant and antimicrobial agents as a value addition by inhibiting lipid oxidation and extending the shelf life of beef.

Conflicts of Interest

The authors declare no potential conflict of interest.

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