



Research article

Evaluation of storage stability of refrigerated buffalo meat coated with hydrothermally treated potato starch incorporated with thyme (*Thymus vulgaris*) and ginger (*Zingiber officinale*) essential oil

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Abstract: The present study was carried out to prepare thyme essential oil (TEO) and ginger essential oil (GEO)-incorporated edible starch coating on buffalo meat to extend its refrigerated shelf-life. Edible coatings incorporated with antimicrobials can act as an active packaging system for the preservation of meat using biopolymers and plant-based essential oils. Buffalo meat samples were coated by hydrothermally treated starch solution incorporated with thyme and ginger essential oil at five different proportions (total of 2.5% of starch solution). A total of five treatments (S1, S2, S3, S4, and S5) along with two controls (S6 and S7) were subjected to microbiological [total viable count (TVC), *Staphylococcus aureus* count, psychrotrophic bacteria count (PTC), and coliform count] and physico-chemical analyses such as thiobarbituric acid reactive substance (TBARS) value, total volatile basic nitrogen (TVBN) content, extract release volume (ERV), metmyoglobin (Met-Mb), pH, weight loss, and water activity at 0, 3, 6, 9, and 12 days of storage. These metrics were compared between days and between treatments. Compared with the uncoated control (S7), S4 decreased TVC by 2.60

log, and S5 decreased PTC, *Staphylococcus aureus*, and coliform by 4.71 log, 1.18 log, and 3.01 log, respectively, in 12 days. S4 reduced TBARS and TVBN by 46.14% and 27.86%, respectively, while S5 increased the ERV by 40.94% in 12 days when compared to S7. Metmyoglobin content, pH, ERV, and TVBN were found to have a high correlation with TVC, while pH was found to have a high correlation with TVBN and ERV. It can be concluded that the increase in TEO concentrations on starch coating increases the ability of buffalo meat to resist microbiological as well as chemical spoilage.

Keywords: buffalo meat; edible coating; ginger essential oil; thyme essential oil; storage stability

1. Introduction

Water buffalo (*Bubalus bubalis*) is the domesticated animal with the greatest promise and possibility for the production of meat [1]. Due to its lower fat, lower cholesterol, and other healthy qualities, use of buffalo meat has grown in a number of Southeast and Middle Eastern Asian nations as well as in Africa. The most appealing qualities of buffalo meat include its red color, low fat and cholesterol content with weak marbling, lack of connective tissue, good texture, water-holding capacity, high protein content, myofibrillar fragmentation index, and emulsifying ability [2]. Many value-added meat products, including burger patties, sausages, meat loaves, and corned buffalo meat, have all been made with buffalo meat [3].

Meats provide the optimum habitat for microorganism growth due to their high nutritional content. Thus, farmers and processors have been interested in preserving the meat to improve its stability [4]. Meat, as well as its products, are known to be perishable [5]. Meat is highly prone to oxidation due to its chemical composition and complex physical structure [6]. It is well known that the growth of microorganisms and their activity on the product's surface are the primary causes of sensorial changes in meat [7]. Since it does not contain hormones or stimulants, buffalo meat is regarded as a healthy substitute for other meat sources. In addition, compared to skinless chicken, hog, and beef meats, it has lower fat and cholesterol contents and is higher in iron and protein. It is also important to note that the extremely low levels of intramuscular fat in buffalo meat give it a beautiful red color. Buffalo meat is highly perishable, similar to other fresh meat varieties. Furthermore, the safety, sanitary quality, and shelf life of buffalo meat may be impacted by the presence of pathogenic microbes and rotting on its surface. Moreover, lipid oxidation and subsequent color deterioration may occur in buffalo meat, both raw and cooked [8]. Consumers today seek high-quality foods without chemical additives that have a long shelf life [9]. Thus, ensuring food safety as well as its quality while maintaining its storage life for a longer period is a significant concern in the meat industry.

Edible films and coatings added with natural antimicrobials are a promising preservation technology for raw and processed meats because they provide a good barrier against spoilage and pathogenic microorganisms [9]. Essential oils (EOs) from oregano, rosemary, thyme, clove, balm, ginger, basilica, coriander, marjoram, and basil have demonstrated a greater potential to be used as an antimicrobial agent with regard to meat and meat products [10]. It is generally recognized that phenolic compounds such as eugenol, shogaols, zingerone, gingerdiols, and gingerols are major constituents in ginger essential oil (GEO) [11], while thyme essential oil (TEO) mainly contains thymol, carvacrol, p-cymene, and β -caryophyllene [12]. According to previous reports, GEO significantly inhibits the majority of both Gram-positive and Gram-negative bacteria [13]. GEO has long been utilized in anti-

inflammatory treatments in experimental rheumatoid arthritis. Similarly, when compared to other plant essential oils, GEO can be employed in a variety of distinctive phytopharmaceutical and medical applications [14]. Both ginger and thyme essential oils have significant bacteriostatic effects [15]. Over 60 components make up TEO, and the majority of them have significant positive benefits, such as anti-cancer, carminative, anti-microbial, anti-oxidant, and anti-septic qualities [16]. *Thymus vulgaris* L. essential oil (EO) and plant-based thymol possess significant antibacterial properties, making them suitable for use as bio-preservatives in meat and meat products. Many Gram-positive and Gram-negative species, such as *Salmonella enteritidis*, *Salmonella typhimurium*, *Escherichia coli* O157:H7, *Escherichia coli*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Shigella flexneri*, *Shigella sonnei*, and *Staphylococcus aureus*, were inhibited in their growth by the EO of *Thymus vulgaris*. When applied to fungi like *Absidia*, *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Mucor*, *Penicillium*, *Rhizopus*, *Stachybotrys*, *Trichoderma*, and *Ulocladium* spp., the oil showed a potent antagonistic impact [17].

There is actually a lot of interest in edible coatings because of their biocompatibility, biodegradability, extensive application potential, and use as carriers of functional chemicals [18]. Adding antimicrobial agents directly to meat and meat products may result in partial deactivation of the active ingredients due to interaction with the product's components and is thus anticipated to have little impact on the surface bacteria [19]. The meat sector may profit from employing antimicrobial edible coatings by preventing moisture loss, preventing changes in texture, flavor, and color, and generating a large economic impact by raising the saleable weight of products. Additional benefits include less dripping, which improves product presentation, and less need for absorbent pads at tray bottoms. Low oxygen permeability reduces the burden of spoilage and pathogenic microbes, partially inactivates deteriorative proteolytic enzymes at the surface of coated meat, and inhibits the oxidation of lipids and brown color-causing myoglobin. Edible films and coatings can limit the amount of volatile flavor loss and foreign aromas that meat, poultry, or seafood absorb. Direct meat treatment can also involve the addition of chemicals like antimicrobial agents [20].

Environmental concerns are currently driving a trend toward the use of antimicrobial edible films and coatings. With their many advantages, antimicrobial edible films and coatings that incorporate essential oils and natural herbal extracts are regarded as an advancement in active biodegradable packaging. To reduce or avert the growth of microorganisms on the surface of food products, edible films and coatings with antimicrobial properties have been created. In addition to being environmentally benign and biodegradable, edible coatings made from natural polymers like proteins and carbohydrates also maintain quality, improve storage stability, and reduce moisture and volatile nutraceutical loss [21].

Food additives such as organic acids, enzymes, bacteriocins, fungicides, natural extracts, and vitamins can also be commercially added to edible coatings; hence, an active packaging system is created to increase their shelf life by lowering the potential of pathogen growth on the food's surface and to enhance the sensory quality of the product when it is packaged or coated [22]. In fact, their direct use is frequently constrained due to their powerful flavor. To solve this issue and enhance food quality and safety, essential oils could be incorporated into such edible coatings suggested as an alternative to traditional food packaging [18]. Being consumed worldwide, a lot of studies have been done on antimicrobial edible coatings for beef, pork, chicken, fish, and seafood, whereas buffalo meat only has regional preferences, especially in Southeast and Middle East Asia [20]. Limited studies have been done to extend the shelf-life of buffalo meat. It is expected that this study will provide a simple hurdle technology for the preservation of buffalo meat with minimal or no use of chemical

preservatives. Hence, this study focuses on utilizing herbs such as thyme and ginger to develop an antimicrobial edible coating for buffalo meat.

2. Materials and methods

2.1. Materials

Thyme (*Thymus vulgaris*) and ginger (*Zingiber officinale*) were collected from the market of Itahari, Sunsari. Buffalo (*Bubalus bubalis*) meat was purchased from the local market of Dharan, Sunsari. Potato starch (brand name: Kamatsu Katakuri Ko, Japan) was used. All the necessary chemicals, apparatus, and instruments were provided by the Central Department of Food Technology, Dharan, Sunsari.

2.2. Experimental setup

The experiment started with the extraction of essential oils of thyme and ginger. Starch was subjected to hydrothermal modification, and a 5% starch coating suspension was prepared with the addition of sorbitol (45% of dry weight of starch) as a plasticizer, Tween 20 (30% v/v of essential oil) as an emulsifying agent, and five different combinations of thyme essential oil (TEO) and/or ginger essential oil (GEO) as antimicrobials (total of 2.5% w/v of starch suspension). In previous studies, 0.5%–1% of GEO was incorporated in nanoparticles for refrigerated chicken fillets [23], 1%–2% GEO was incorporated in cellulose nanofibers to extend the shelf-life of ready-to-cook barbecued chicken [24], and 5% v/v GEO was incorporated in sodium-alginate agar coating to extend the shelf-life and enhance the quality of beef [25]. Regarding TEO, 0.5% and 2% can be incorporated into chicken breast meat and raw beef meat, respectively, to preserve them [26]. Also, 1%–2% TEO was incorporated in chitosan-based coating emulsion to prepare edible coatings for the preservation of Karish cheese [27]. Buffalo meat of the thigh area (round) was bought from the market and coated with starch-based coatings with antimicrobials as well as with starch coatings without antimicrobials and was stored in the refrigerator at 4 °C for further analysis. Coatings were applied to buffalo meat of dimension 50 mm × 50 mm × 10 mm. Five different treatments (essential oil–incorporated starch coating), namely S1, S2, S3, S4, and S5, and two controls, S6 (starch coating without essential oil incorporated) and S7 (uncoated sample), were used for analysis and comparison. One sample was prepared per treatment per storage time. Each prepared sample was coated evenly. Total viable count (TVC), *Staphylococcus aureus* count, coliform count, psychrotrophic bacteria count (PTC), pH, TBARS value, weight loss (%), water activity (a_w), extract release volume (ERV), total volatile basic nitrogen (TVBN) value, and metmyoglobin (%) were analyzed at 0, 3, 6, 9, and 12 days of storage at 4 °C. These values were compared between uncoated and starch-coated buffalo meat. The experimental design shown in Table 1 was prepared using Design Expert software with *Optimal (custom) Design*. A quadric model was used with two lack-of-fit points.

Table 1. Thyme and ginger essential oil concentrations used in starch coating suspension.

Treatments	Ginger EO concentration (% v/v of coating suspension)	Thyme EO concentration (% v/v of coating suspension)
S1	2.5	0
S2	1.875	0.625
S3	1.25	1.25
S4	0.625	1.875
S5	0	2.5

Two control samples were used, namely S6 (starch coating without essential oil incorporated) and S7 (uncoated sample).

Note: 5% starch coating suspension was prepared with the addition of sorbitol (45% of the dry weight of starch) as a plasticizer, and Tween 20 (30% v/v of essential oil) as an emulsifying agent.

2.3. Extraction of essential oil by hydro-distillation

Thyme and ginger essential oils were extracted by the hydro-distillation method as described by Abd Aziz et al. [28] and Lucchesi et al. [29] with some modifications. 100 g of thyme/ginger was mixed with 500 mL of water and extracted until no more essential oil was obtained in the Clevenger apparatus. The essential oil was collected, dried with anhydrous sodium sulfate, packed in a brown bottle, and stored at 4 °C. Essential oil was extracted from the single batch of raw materials brought from the market.

2.4. Modification of starch by hydrothermal treatment

Starch (100 g) was adjusted to 28% moisture, pH 6.7, equilibrated at 4 °C overnight (refrigerated condition), and placed in a hot air oven for 3 h at 110 °C. The sample was shaken occasionally for an even distribution of heat. The sample was cooled to room temperature (approximately 30 °C) and dried at 45 °C, equilibrated for 4 h, and sealed in polyethylene bags until use as described by Collado et al. [30].

2.5. Preparation of coating solutions

The starch suspensions were prepared according to the method described by Müller [31]. The coating was prepared by slow addition of sorbitol and hydrothermally treated starch in water at 45% dry starch and 5% (w/v), respectively, with simultaneous heating to avoid lump formation. Sorbitol was used as a plasticizer [32]. The mixture was heated to boiling temperature on a hot plate with constant stirring for 10 min by a magnetic stirrer. The mixture was cooled until any bubbles vanished. After cooling, thyme and/or ginger essential oil were added at different concentrations (shown in Table 3), and Tween 20 was added as an emulsifier at 30% (v/v) of essential oil to starch suspension [33].

2.6. Application of coating on buffalo meat

An edible coating solution was applied to buffalo meat using the method described by Fernández-Pan [33]. Using a meat slicer, homogeneous 50 mm × 50 mm × 10 mm thick buffalo meat slices were prepared and coated following three steps: dipping, draining, and drying. To ensure that the initial microbiological population of the samples was not changed, the three steps were carried out in a

laminar flow hood under sterilized conditions. Edible coating was formed over the buffalo meat slices by immersion in coating suspension for 2 min. Excess coating was drained for 30 s, and coatings were formed by exposing meat slices to a cool air stream for 45 s on each side. The coated samples were placed in trays without any other packaging applied and stored at 4 °C for 12 days. Each treatment was done in triplicate. Adhesiveness over buffalo meat slice surfaces, continuity, and homogeneity in coatings were visually examined. Microbiological and physiochemical evaluation of fresh samples as well as coated and uncoated stored samples at days 0, 3, 6, 9, and 12 was performed.

2.7. Physiochemical analysis

2.7.1. Thiobarbituric acid reactive substances value measurement

Lipid oxidation was measured based on Yoon et al.'s [34] description of the TBARS method. Initially, 3 g of sample was homogenized at $3,220 \times g$ for 30 s in 9 mL of distilled water and 60 μL of 6% (w/v) butylated hydroxytoluene in ethanol. The TBA reagent [20 mM 2-thiobarbituric acid in 15% trichloroacetic acid (TCA) solution] was then mixed with 2 mL of the homogenate. In an 80 °C water bath, the mixture was heated for 15 min. After chilling in ice water, the samples were centrifuged at $2000 \times g$ for 10 min. The supernatant was filtered with Whatman filter paper No. 1, and absorbance was measured using a spectrophotometer at 532 nm. Using the equation below and a molecular extinction coefficient of $1.56105 \text{ M}^{-1} \text{ cm}^{-1}$, the malondialdehyde (MDA) concentration was converted to TBARS value.

$$\text{TBARS value (mg MDA/ kg of meat)} = A_{532} \times 2.77 \quad (1)$$

2.7.2. pH measurement

The method of Zhang et al. [35] was used to estimate pH. 100 mL of distilled water and 10 g of meat slice were homogenized for 10 s at 13,000 rpm. Then, the pH of the obtained homogenate was measured using a digital pH meter. The pH meter was calibrated using buffers of 4, 7, and 10 as suggested in the manual.

2.7.3. Extract release volume

Extract release volume was determined using the method described by Anandh and Lakshmanan [36]. For determining the extract release volume (ERV), 15 g of minced stored samples and 60 mL of distilled water were mixed in a homogenizer, and the homogenate was quickly transferred into a funnel with Whatman filter paper no. 1. The ERV of the respective sample corresponded to the amount of filtrate that was collected in the first 15 min.

2.7.4. Total volatile basic nitrogen measurement

The total volatile basic nitrogen (TVBN) was calculated using the technique by Shadman et al. [37]. 50 mL of distilled water was added to 10 g of ground meat. After adding 2 g of MgO, the mixture was transferred with 200 mL of distilled water into a 500 mL round-bottom flask. The mixture was then distilled in the Kjeldahl apparatus. The distillate receiver was a 250 mL Erlenmeyer flask

with 25 mL of a 3% aqueous solution of boric acid and 0.04 mL of a mixture of methyl red and methylene blue indicators for the titration of ammonia. Distillation continued until a final volume of 125 mL of distillate was achieved. When the distilled TVBN made the boric acid solution alkaline, it turned the solution green. An aqueous 0.1 N hydrochloric acid solution was used to titrate this until the color of the distillate changed to pink, indicating complete neutralization. The amount of TVBN in mg N/100 g of meat was estimated using the volume of HCl consumed (V) and concentration (C) of hydrochloric acid added as follows:

$$\text{TVBN (mg N/100 g meat)} = (V \times C \times 14 \times 100)/10 \quad (2)$$

2.7.5. Weight loss (%) determination

Weight loss (%) was calculated using the method described by Marcinkowska-Lesiak et al. [38]. The difference in the weight of the meat before and after storage was used to calculate the weight loss of the meat using the following formula:

$$\text{Weight loss \%} = (\text{Initial weight} - \text{Final weight}) / (\text{Initial weight}) \times 100\% \quad (3)$$

2.7.6. Water activity measurement

Water activity of meat was determined using Pawkit water activity (Decagon Devices, Inc., USA) meter at 5 °C [39].

2.7.7. Metmyoglobin (%) determination

The metmyoglobin concentration in each sample was determined using the method by Fernández-López et al. [40]. For analysis, 5 g of minced meat was taken. Cold 0.04 M phosphate buffer, pH 6.8, and a sample to buffer ratio of 1:10 were used to extract myoglobin. A grinder machine was used to homogenize the samples for 15 s. The homogenates were then centrifuged for 30 min at 5 °C. The absorbance of the filtered supernatant (using Whatman filter paper No. 1) was measured at 525, 572, and 730 nm. Metmyoglobin percentage was calculated using the following formula:

$$\text{Met-mb \%} = [1.395 - \{(A_{572} - A_{730}) / (A_{525} - A_{730})\}] \times 100\% \quad (4)$$

2.8. Microbiological analysis

Inoculum was prepared using the method described by Alizadeh et al. [41] and Koirala et al. [42] with some modifications. To determine changes in the microbial load of meat slices during the study, meat slices and 0.1% peptone water were mixed in a ratio of 1:9 and ground in a sterilized grinder machine. 1 mL of supernatant was taken from the resulting slurry and mixed with 9 mL of 0.1% peptone water in a test tube to make a 10^{-2} dilution. From the 10^{-2} dilution, 1 mL was taken and mixed with 9 mL of 0.1% peptone water to make a 10^{-3} dilution; the process was subsequently diluted up to 10^{-8} , and each dilution was inoculated into Petri plates containing the corresponding culture media. Petri dishes were incubated at a suitable temperature for a suitable period, and the number of colonies was counted using

a colony counter. The microbial load obtained was expressed in log CFU/g of meat using the formula:

$$\text{Log CFU/g} = \text{Log10}\{\text{CFU} / (\text{dilution factor} \times \text{aliquot})\} \quad (5)$$

2.8.2. Total viable count

Total viable count was determined by the method described by Alizadeh et al. [41] with some modifications. To determine the total viable count (TVC), 1 mL of prepared inoculum was inoculated by the pour plate technique into Petri plates containing plate count agar and incubated at 37 °C for 48 h. After that, the microbial load was counted.

2.8.3. Psychrotrophic bacteria count

Psychrotrophic bacteria count (PTC) was determined by the method described by Alizadeh et al. [41] with some modifications. To determine psychrotrophic bacteria count (PTC), 1 mL of prepared inoculum was inoculated by the pour plate technique into Petri plates containing plate count agar and incubated at 7 °C for 10 days. After that, the microbial load was counted.

2.8.4. *Staphylococcus aureus* count

Staphylococcus aureus count was determined by the method described by Alizadeh et al. [41] with some modifications. To determine the *Staphylococcus aureus* count (TVC), 0.1 mL of prepared inoculum was inoculated by the spread plate technique into Petri plates containing mannitol salt agar and incubated at 37 °C for 24 h. After that, *Staphylococcus aureus* count was obtained.

2.8.5. Coliform count

Coliform count was determined by the method described by Koirala et al. [42] with some modifications. To determine the coliform count, 1 mL of prepared inoculum was inoculated by the pour plate technique into Petri plates containing violet red bile agar and incubated at 37 °C for 48 h. After that, the coliform count was obtained.

2.9. Statistical analysis

All the analyses were performed in triplicate. For comparison between treatments, analysis of variance was used for parametric data, while the Kruskal–Wallis test was used for non-parametric data. Tukey test was applied to determine the significance difference between the treatments. All of them were performed using the IBM SPSS Statistics 25 software. Statistical analysis was run with a confidence level of 95%. All the graphs were generated using Excel software (Office 16).

3. Results and discussion

Coatings were prepared using a hydrocolloid of starch at 5% concentration in water, incorporated with a total of 2.5% essential oils in five different proportions. This allowed the evaluation of the effects of five different treatments on buffalo meat. Total viable count (TVC), psychrotrophic bacteria

count (PTC), *Staphylococcus aureus* count, coliform count, metmyoglobin (%), thiobarbituric acid reactive substances (TBARS) value, total volatile basic nitrogen (TVB-N) content, pH, weight loss (%), extract release volume (ERV), and water activity (a_w) were analyzed and compared between treatments.

3.1. Composition of thyme essential oil (TEO)

The composition of thyme essential oil (TEO) extracted by the hydro-distillation method is shown in Table 2.

Table 2. Essential oil composition of thyme extracted by the hydro-distillation method.

S.N	Components	% of total essential oil			
		A*	B*	C*	D*
1.	Thymol	40.02	41.04	55.3	48.1
2.	Carvacrol	18.31	2.77	8.7	5.5
3.	Cymene(p)	16.78	10.50	11.2	11.7
4.	Linalool	4.84	2.80	1.7	4.4
5.	Γ-Terpinen-7-al	4.16	12.06	3.4	6.1
6.	Borneol	2.67	-	2.3	2.2
7.	Caryophyllene	2.10	0.43	-	-
8.	Thymol acetate	1.47	-	-	-
9.	Caryophyllene (E)	1.23	-	4.2	2.3
10.	α-Terpinene	1.10	9.22	1.0	0.8
11.	α-Pinene	0.97	-	0.4	2.1
12.	Myrcene	0.89	0.04	0.7	1.0
13.	Terpinen-4-ol	0.65	0.65	1.1	-
14.	Camphene	0.60	-	0.4	0.7
15.	Limonene	0.46	-	0.4	1.3
16.	Carvacrol acetate	0.40	-	-	-
17.	Caryophylla-4-(12),8(13)-dien-5-β-ol	0.31	-	-	-
18.	α-Thujene	0.27	0.22	0.5	0.5
19.	Sabinene hydrate	0.22	-	-	0.2
20.	α-Humulene	0.18	-	0.1	-
21.	β-Pinene	0.16	-	-	0.9
22.	α-Terpineol	0.15	1.10	0.1	0.5
23.	Trans-linalool oxide	0.14	-	-	Trace
24.	α-Phellandrene	0.14	-	-	0.1
25.	Aromadendrene	0.10	-	0.3	-
26.	Piperitone	0.10	-	-	-
27.	Caryophyllene oxide	-	-	-	0.3

Where: A*: [43]; B*: [44]; C*: [12]; D*: [45].

3.2. Composition of ginger essential oil (GEO)

The composition of ginger essential oil (GEO) extracted by the hydro-distillation method is shown in Table 3.

Table 3. Essential oil composition of ginger extracted by hydro-distillation method.

S.N	Components	% of total essential oil			
		A [*]	B [*]	C [*]	D [*]
1.	Zingiberene	28.57	0.16	15.20	35.65
2.	Ar. Curcumene	14.22	4.27	5.60	12.04
3.	Geranyl acetate	13.29	1.05	-	-
4.	Geranial	9.16	7.24	-	1.38
5.	β-sesquiphellandrene	5.97	5.64	6.96	-
6.	α-Farnesene	5.41	6.37	7.04	4.52
7.	β-Bisabolene	5.30	-	-	2.88
8.	α-Eudesmol	3.65	0.73	-	-
9.	Camphene	3.53	4.22	7.69	-
10.	Germecrene	3.26	0.18	-	-
11.	Nerolidol	2.81	1.61	-	-
12.	γ-Cardinene	2.10	-	-	-
13.	Trans-Ocimene	1.38	-	-	-
14.	Endo Borneol	0.54	-	-	0.28
15.	Cymene	0.52	-	-	-
16.	Cis-Ocimene	0.29	-	-	-
17.	Bornyl acetate	Trace	0.06	-	-
18.	Ethyl cinnamate	Trace	-	-	-
19.	Ethyl acetate	Trace	-	-	-
20.	γ-Terpinene	Trace	-	-	-
21.	Linalool	Trace	3.05	-	-
22.	β-caryophyllene	Trace	-	-	-
23.	α-Pinene	Trace	1.09	-	-

Where: A^{*}: [46]; B^{*}: [47]; C^{*}: [48]; D^{*}: [49].

3.3. Total viable count (TVC)

On day 0, all the samples had a TVC of 2.89 log CFU/g of meat. At the end of day 12, TVC was highest on S7 at 7.94 log CFU/g and lowest on S4 at 5.34 log CFU/g, which later brought a reduction of 2.60 log CFU/g. For each sample, TVC differed significantly between all days. The uncoated sample S7 reached the threshold value of 10⁷ CFU/g [50] on day 9, and sample S6 reached that threshold value on day 12 of storage. None of the treated samples reached the threshold value until the final day of analysis.

The application of thyme essential oil as a coating on meat was reported to produce comparable results in Shaltout and Koura [6], Harpaz et al. [51], and Khare et al. [52]. The TVC of meat samples was found to be comparatively lower with a higher concentration of TEO, while GEO exhibited only a minimal influence. TEO presented stronger antimicrobial effects than GEO on reducing TVC. This result could probably be due to some key components of TEO, such as thymol, carvacrol, and eugenol. According to Gutierrez, Barry-Ryan, and Bourke [53], substances containing phenolic compounds, such as carvacrol and thymol, are extremely effective against bacteria. Carvacrol and thymol are known to disintegrate the outer membrane of Gram-negative bacteria [54]. Kachur and Suntres [55] further said that the cytoplasmic membrane is expected to suffer structural and functional damage as a result of the antibacterial action of phenolic chemicals like thymol and carvacrol. The oxygen

permeability of starch film is lower than that of normal plastics [56]. Also, the oxygen permeability of starch film plasticized with sorbitol is much lower than that of normal starch films [57]. So, starch might have acted as a barrier against oxygen transfer, inhibiting the growth of aerobic microbes. Table 4 shows the changes in TVC during the storage period.

Table 4. Changes in microbial count of the samples along with the increase in storage day.

Parameter	Day	S1	S2	S3	S4	S5	S6	S7
Total viable count	0	2.89 ± 0.02 ^a						
	3	3.66 ± 0.02 ^d	3.89 ± 0.01 ^c	3.39 ± 0.04 ^c	3.26 ± 0.01 ^b	3.15 ± 0.02 ^a	4.26 ± 0.01 ^f	4.39 ± 0.04 ^g
	6	5.02 ± 0.02 ^c	5.06 ± 0.01 ^c	4.90 ± 0.02 ^b	4.38 ± 0.06 ^a	5.01 ± 0.02 ^c	5.63 ± 0.02 ^d	5.79 ± 0.02 ^e
	9	5.71 ± 0.02 ^d	5.83 ± 0.02 ^c	5.39 ± 0.00 ^c	4.87 ± 0.01 ^a	5.23 ± 0.01 ^b	6.63 ± 0.03 ^f	7.73 ± 0.03 ^g
	12	6.86 ± 0.02 ^e	6.64 ± 0.02 ^d	6.27 ± 0.01 ^c	5.34 ± 0.05 ^a	5.80 ± 0.01 ^b	7.73 ± 0.03 ^f	7.94 ± 0.03 ^g
<i>Staphylococcus aureus</i> count	0	4.21 ± 0.06 ^a						
	3	4.57 ± 0.03 ^b	4.55 ± 0.06 ^b	4.49 ± 0.03 ^b	4.43 ± 0.04 ^b	4.30 ± 0.06 ^a	4.80 ± 0.04 ^b	4.76 ± 0.06 ^b
	6	4.66 ± 0.02 ^c	4.79 ± 0.02 ^c	4.54 ± 0.05 ^b	4.45 ± 0.02 ^b	4.32 ± 0.05 ^a	4.96 ± 0.02 ^c	5.28 ± 0.05 ^c
	9	4.97 ± 0.02 ^d	4.90 ± 0.04 ^c	4.80 ± 0.05 ^c	4.74 ± 0.03 ^c	4.81 ± 0.04 ^b	5.73 ± 0.04 ^d	5.96 ± 0.02 ^d
	12	5.92 ± 0.02 ^e	5.77 ± 0.04 ^d	5.45 ± 0.03 ^d	5.28 ± 0.05 ^d	5.23 ± 0.09 ^c	6.32 ± 0.08 ^e	6.41 ± 0.02 ^e
Coliform count	0	3.55 ± 0.05 ^a						
	3	4.78 ± 0.03 ^b	4.61 ± 0.04 ^b	4.47 ± 0.09 ^b	4.33 ± 0.13 ^b	4.17 ± 0.12 ^b	5.35 ± 0.06 ^b	5.48 ± 0.03 ^b
	6	5.74 ± 0.05 ^c	5.69 ± 0.04 ^c	5.31 ± 0.11 ^c	5.19 ± 0.03 ^c	5.16 ± 0.07 ^c	6.51 ± 0.04 ^c	6.29 ± 0.15 ^c
	9	5.96 ± 0.04 ^d	5.74 ± 0.06 ^c	5.10 ± 0.05 ^d	5.23 ± 0.11 ^c	5.31 ± 0.03 ^c	7.25 ± 0.06 ^d	7.50 ± 0.03 ^d
	12	6.77 ± 0.05 ^e	6.57 ± 0.08 ^d	6.37 ± 0.05 ^e	5.50 ± 0.06 ^d	5.47 ± 0.02 ^d	8.52 ± 0.06 ^e	8.58 ± 0.04 ^e
Psychrotrophic count	0	3.72 ± 0.02 ^a						
	3	3.71 ± 0.01 ^a	3.80 ± 0.01 ^b	3.82 ± 0.01 ^b	3.72 ± 0.02 ^a	3.68 ± 0.01 ^a	5.17 ± 0.02 ^c	5.23 ± 0.01 ^d
	6	4.58 ± 0.03 ^d	4.51 ± 0.04 ^d	4.36 ± 0.02 ^c	4.18 ± 0.01 ^b	4.06 ± 0.01 ^a	6.30 ± 0.04 ^e	6.67 ± 0.01 ^f
	9	5.19 ± 0.01 ^e	5.10 ± 0.01 ^d	4.80 ± 0.01 ^c	4.48 ± 0.01 ^b	4.15 ± 0.01 ^a	7.50 ± 0.02 ^f	7.94 ± 0.01 ^g
	12	6.12 ± 0.01 ^e	5.92 ± 0.01 ^d	5.48 ± 0.01 ^c	4.92 ± 0.01 ^b	4.43 ± 0.02 ^a	8.70 ± 0.01 ^f	9.14 ± 0.01 ^g

Note: Mean bars with different superscript letters are significantly different ($P < 0.05$). Values after \pm are the standard deviation (S.D) values.

3.4. Psychrotrophic bacteria count (PTC)

PTC on days 0 and 3 exhibited no significant difference ($P > 0.05$) in S1 and S4; all other treatments showed a significant difference on PTC ($P < 0.05$). Starting from 3.72 log CFU/g on day 0, S7 reached the highest PTC at 9.14 log CFU/g on day 12. S5 had the lowest count at 4.43 log CFU/g on the same day, showing a total of 4.71 log reduction in PTC. Psychrotrophic bacteria count followed a similar pattern as TVC: it also decreased with an increase in the concentration of TEO on the coating solution and was less affected by the presence of GEO. TEO has stronger antimicrobial effects compared to GEO on reducing psychrotrophic bacteria count [10,58]. This could also be due to the presence of thymol, carvacrol, and eugenol. Changes in PTC during the storage period are shown in Table 4.

3.5. Staphylococcus aureus count

On the final day, S4 and S5, S1 and S2, and S6 and S7 exhibited no significant differences ($P > 0.05$). S1 had significant differences in count between days ($P < 0.05$). S6 and S7 also showed similar

results, while S3 and S4 had no significant difference ($P > 0.05$) on their days 3 and 6. On days 6 and 9, S2 showed no significant differences ($P > 0.05$), while there was no significant increase ($P > 0.05$) in *S. aureus* count up to the first 6 days in the case of S5. On the first day, starting from 4.21 log CFU/g, S7 had the highest load at 6.41 log CFU/g while S5 had the lowest count at 5.23 log CFU/g on day 12. TEO had a stronger antimicrobial effect compared to GEO on reducing *Staphylococcus aureus* count [10,58]. This effect could also be due to the presence of thymol, carvacrol, and eugenol. Changes in *Staphylococcus aureus* count during the storage period are shown in Table 4.

3.6. Coliform count

On days 9 and 12, the coliform count differed significantly on each treatment ($P < 0.05$). On day 0, coliform count on all samples was 3.55 log CFU/g of meat, but at the end of day 12, it reached 8.58 log CFU/g on the uncoated sample S7. S5 showed a 3.01 log reduction in 12 days of refrigerated storage. Similar individual results were found by Salem, Amin, and Afifi [59] and Shaltout and Koura [6], with the use of GEO and TEO, respectively. This might be due to the additive effect of components of TEO and GEO but with a greater influence of TEO. Changes in coliform count during the storage period are shown in Table 4.

3.7. Thiobarbituric acid reactive substances (TBARS) value

S4 and S5, and S6 and S7, exhibited no significant difference ($P > 0.05$) in TBARS values between days. Within each treatment, a significant difference ($P < 0.05$) was found when comparing the TBARS values with their respective value on other days.

On the first day of analysis, i.e., day 0, the TBARS value was 0.43 mg MDA/kg of meat. The uncoated sample S7 presented 1.45 mg MDA/kg of meat, while S4 exhibited the lowest TBARS at 0.781 mg MDA/kg of meat at the end of day 12, with a 46.14% reduction compared to S7. S1, S6, and S7 reached the threshold of TBARS, i.e., 1 mg MDA/kg of meat [60] on day 9, while the remaining samples did not cross that threshold value. TBARS was found to decrease with an increase in TEO concentration in starch coatings.

Bacterial action was not the only reason for the fluctuations in TBARS value [61]. Oxygen permeability may raise the value of TBARS [62]. Carvacrol, a major phenolic component of TEO, has good antioxidant activity [63], which might decrease lipid oxidation if used in food. Thyme has a significantly greater antioxidant impact than ginger [64]. Moreover, TEO has the ability to chelate iron [44], which is regarded as the initiator for lipid oxidation. GEO also has an iron-chelating effect [65]. Furthermore, polysaccharide films and coatings can be utilized to keep meat from drying out, going rancid, or turning brown on the surface. Oxygen is selectively permeable through polysaccharide coatings [9]. Starch films plasticized with sorbitol had substantially reduced oxygen permeability than regular starch films [57, 66]. As a result, starch may have served as a barrier to the passage of oxygen. Changes in TBARS value during the storage period are shown in Table 5.

Table 5. Changes in the physicochemical properties of the samples along storage days.

Parameter	Day	S1	S2	S3	S4	S5	S6	S7
TBARS value	0	0.43 ± 0.003 ^a						
	3	0.67 ± 0.006 ^b	0.62 ± 0.004 ^b	0.58 ± 0.002 ^b	0.52 ± 0.004 ^b	0.51 ± 0.004 ^b	0.71 ± 0.003 ^b	0.72 ± 0.004 ^b
	6	0.88 ± 0.004 ^c	0.82 ± 0.003 ^c	0.76 ± 0.007 ^c	0.62 ± 0.003 ^c	0.62 ± 0.005 ^c	0.96 ± 0.004 ^c	0.97 ± 0.005 ^c
	9	1.07 ± 0.003 ^d	0.93 ± 0.004 ^d	0.88 ± 0.004 ^d	0.74 ± 0.004 ^d	0.73 ± 0.000 ^d	1.22 ± 0.004 ^d	1.23 ± 0.006 ^d
	12	1.25 ± 0.004 ^e	0.97 ± 0.003 ^c	0.92 ± 0.002 ^c	0.78 ± 0.003 ^c	0.78 ± 0.006 ^c	1.45 ± 0.002 ^c	1.45 ± 0.006 ^c
TVBN content	0	8.18 ± 0.28 ^a						
	3	10.97 ± 0.47 ^b	10.97 ± 0.47 ^b	10.57 ± 0.09 ^b	10.44 ± 0.28 ^a	10.37 ± 0.38 ^b	10.71 ± 0.09 ^b	11.17 ± 0.19 ^b
	6	13.97 ± 0.94 ^c	13.97 ± 0.94 ^c	14.30 ± 0.47 ^c	13.90 ± 1.03 ^b	13.97 ± 0.94 ^c	14.63 ± 0.00 ^c	13.97 ± 0.94 ^c
	9	17.62 ± 0.47 ^d	17.09 ± 0.28 ^d	15.30 ± 0.94 ^c	16.29 ± 0.47 ^c	15.96 ± 0.00 ^d	19.62 ± 0.47 ^d	21.61 ± 0.47 ^d
	12	20.95 ± 0.47 ^e	20.15 ± 0.28 ^c	19.62 ± 0.47 ^d	18.95 ± 0.47 ^d	19.09 ± 0.28 ^c	25.60 ± 0.47 ^c	26.27 ± 0.47 ^c
ERV	0	24.65 ± 0.64 ^d						
	3	21.67 ± 0.61 ^c	21.94 ± 0.23 ^c	22.31 ± 0.27 ^c	22.88 ± 0.31 ^c	22.80 ± 0.28 ^c	20.35 ± 0.35 ^c	20.99 ± 0.72 ^c
	6	19.90 ± 0.42 ^{bc}	19.73 ± 0.32 ^b	20.36 ± 0.20 ^b	21.34 ± 0.37 ^c	20.58 ± 0.40 ^b	17.62 ± 0.54 ^b	16.45 ± 0.49 ^b
	9	18.20 ± 0.42 ^{ab}	18.35 ± 0.35 ^{ab}	19.15 ± 0.50 ^{ab}	19.66 ± 0.36 ^b	19.80 ± 0.42 ^b	15.65 ± 0.64 ^{ab}	14.55 ± 0.64 ^{ab}
	12	16.58 ± 0.60 ^a	17.10 ± 0.57 ^a	17.65 ± 0.64 ^a	17.69 ± 0.27 ^a	17.93 ± 0.39 ^a	15.00 ± 0.71 ^a	12.65 ± 0.64 ^a
Metmyoglo bin %	0	14.90 ± 1.79 ^a						
	3	26.38 ± 3.55 ^b	29.90 ± 0.37 ^b	25.61 ± 0.27 ^b	24.88 ± 3.23 ^b	20.51 ± 2.26 ^a	34.00 ± 0.96 ^b	37.10 ± 1.72 ^b
	6	41.04 ± 0.71 ^c	37.78 ± 1.23 ^c	34.24 ± 2.57 ^c	33.34 ± 1.21 ^c	36.12 ± 0.06 ^b	44.90 ± 2.07 ^c	46.39 ± 1.05 ^c
	9	51.40 ± 1.35 ^d	47.56 ± 0.39 ^d	43.62 ± 1.66 ^d	42.35 ± 0.82 ^d	43.55 ± 0.82 ^c	58.49 ± 0.76 ^d	60.35 ± 0.67 ^d
	12	61.62 ± 0.49 ^e	53.88 ± 1.08 ^e	53.98 ± 0.93 ^e	49.01 ± 0.21 ^d	53.15 ± 1.55 ^d	67.94 ± 0.26 ^e	73.36 ± 0.25 ^e
pH	0	5.977 ± 0.006 ^a						
	3	6.103 ± 0.006 ^b	6.053 ± 0.006 ^b	6.060 ± 0.01 ^b	6.077 ± 0.006 ^b	6.093 ± 0.006 ^b	6.110 ± 0.010 ^b	6.147 ± 0.006 ^b
	6	6.157 ± 0.006 ^c	6.107 ± 0.006 ^c	6.123 ± 0.006 ^c	6.117 ± 0.006 ^c	6.130 ± 0.010 ^c	6.210 ± 0.010 ^c	6.317 ± 0.006 ^c
	9	6.210 ± 0.010 ^d	6.193 ± 0.006 ^d	6.220 ± 0.010 ^d	6.237 ± 0.006 ^d	6.210 ± 0.010 ^d	6.383 ± 0.006 ^d	6.613 ± 0.006 ^d
	12	6.503 ± 0.006 ^e	6.497 ± 0.006 ^e	6.483 ± 0.006 ^e	6.467 ± 0.006 ^e	6.547 ± 0.006 ^e	6.717 ± 0.006 ^e	6.987 ± 0.006 ^e
Weight loss %	0	0.00 ± 0.00 ^a						
	3	2.92 ± 0.01 ^b	2.59 ± 0.02 ^b	2.31 ± 0.04 ^b	2.23 ± 0.04 ^b	2.32 ± 0.04 ^b	3.72 ± 0.05 ^b	4.08 ± 0.06 ^b
	6	4.08 ± 0.03 ^c	4.04 ± 0.04 ^c	3.76 ± 0.03 ^c	3.69 ± 0.05 ^c	3.86 ± 0.07 ^c	5.49 ± 0.04 ^c	6.07 ± 0.06 ^c
	9	5.55 ± 0.09 ^d	5.47 ± 0.03 ^d	5.36 ± 0.06 ^d	5.35 ± 0.06 ^d	5.50 ± 0.04 ^d	6.81 ± 0.04 ^d	7.99 ± 0.05 ^d
	12	5.97 ± 0.06 ^e	5.82 ± 0.06 ^e	5.87 ± 0.06 ^e	5.61 ± 0.04 ^e	5.77 ± 0.04 ^e	8.19 ± 0.04 ^e	9.12 ± 0.05 ^e
Water activity (a _w)	0	0.99 ± 0 ^a						
	3	0.99 ± 0 ^a	0.98 ± 0 ^b	0.97 ± 0 ^c				
	6	0.98 ± 0 ^b						
	9	0.97 ± 0 ^c	0.97 ± 0 ^c	0.98 ± 0 ^b	0.98 ± 0 ^b	0.98 ± 0 ^b	0.96 ± 0 ^c	0.95 ± 0 ^d
	12	0.97 ± 0 ^c	0.98 ± 0 ^b	0.95 ± 0 ^d	0.93 ± 0 ^e			

Note: Mean bars with different superscript letters are significantly different ($P < 0.05$). Values after \pm are the standard deviation (S.D) values.

3.8. Metmyoglobin% (Met-mb%)

Treatment-wise, S1, S2, S3, S6, and S7 presented significant differences ($P < 0.05$) on met-mb% every other day. There was no significant difference ($P > 0.05$) on days 9 and 12 for S4.

Day-wise, on day 12, S2, S3, and S5 showed no significant differences ($P > 0.05$). On the first day of analysis, met-mb% meat was 14.9%; at day 12, the highest value was reached at 73.36% for the

uncoated sample S7 and the lowest at 49.01% for sample S4. S1, S6, and S7 reached the threshold of 40% [67] on day 6, while the rest of the samples reached that level on day 9. Met-mb% change followed a similar trend to that of microbial counts.

A strong correlation was found between microbial load, lipid oxidation, and protein oxidation (Met-Mb), showing that microbial growth was actually mostly responsible for these unfavorable processes occurring with meat constituents [67]. Increased oxygen consumption caused by bacterial contamination raises the amount of metmyoglobin in beef. One of the most common bacteria in meat, *Pseudomonas*, has been shown to promote the synthesis of metmyoglobin. It has been demonstrated that inoculating beef with *Pseudomonas fragi* and *Pseudomonas fluorescens* increases the amount of metmyoglobin in the meat [68, 69]. As bacteria grow, oxygen tension at the surface of meat decreases, which causes metmyoglobin levels to rise. Particularly during the logarithmic phase of *Pseudomonas*, *Achromobacter*, and *Flavobacterium*, metmyoglobin is generated [70]. The co-occurrence of protein oxidation and microbial growth was also found by Guyon and de Lamballerie [71]. Changes in metmyoglobin content during the storage period are shown in Table 5.

3.9. Total volatile basic nitrogen (TVBN)

No significant differences ($P > 0.05$) were found in TVBN on day 12 for S1, S2, and S3, S2, S3, S4, and S5, and S6 and S7. On the other hand, for S1, S2, S3, S6, and S7, the TVBN value was found to be significantly different between days ($P < 0.05$). However, for S3, the TVBN value on days 6 and 9 presented no significant differences ($P > 0.05$); for S4, no significant differences were found for TVBN on days 0 and 3. S7 reached the threshold value of 20 mg N/100 g meat [72] on day 9, while S1, S2, and S6 reached it on day 12. S3, S4, and S5 did not cross the threshold within the 12 days of the study.

On the initial day of analysis, TVBN was found to be 8.18 mg N₂/100 g meat, increasing on each day of analysis; the highest TVBN value was 26.27 mg N/100 g meat for the uncoated sample, and the lowest TVBN value was 18.95 mg N/100 g meat for treatment S4. This later decreased by 27.86% compared to uncoated meat. A similar pattern to the bacterial counts was also found, with lower TVBN values for higher TEO concentrations.

In accordance with the comparatively low initial TVC, the initial TVBN value of 8.18 mg of N/100 g meat indicates that the buffalo meat was of high quality [73]. A similar pattern of TVBN to TVC on different treatment samples was probably due to TEO. Jouki et al. [73] also found a similar result. The protein content of buffalo meat decreased throughout the course of storage days [74]. Such a decrease in protein content was caused by the enzymatic activities of psychrotrophic microbial growth, which led to the denaturation, breakdown, and deamination of proteins and amino acids [75]. Strong off flavors are frequently caused by protein breakdown and the release of nitrogenous volatile chemicals [61]. Changes in TVBN content during the storage period are shown in Table 5.

3.10. pH

pH was found to be significantly different on each day of analysis for all the samples. On day 12, no significant differences ($P > 0.05$) were found between S2 and S3, and between S1 and S2. On day 0, the pH of meat was 5.98, increasing until the end of the experiment, with the highest value (i.e., 6.99) for the uncoated sample S7 and the lowest (6.47) for S4. pH showed an increasing pattern throughout

storage, which somehow followed the pattern of TVBN values. Samples coated with higher TEO concentrations exhibited a comparatively lower pH.

Salem, Amin, and Afifi [59] and Shaltout and Koura [6] found similar results. The pH value of meat rises with increasing storage times [76]. Bacterial activity, producing ammonia, amines, and other alkaline compounds, is likely the cause [77]. The pH value of meat was raised by autolysis and an increase in microbial load [78]. pH and TVBN demonstrated a significant positive correlation [75]. Lower pH with higher TEO concentration was probably due to the effect of TEO as an antimicrobial agent causing protein hydrolysis. Changes in pH during the storage period are shown in Table 5.

3.11. Water activity (aw)

On the first day of analysis, aw of meat was found to be 0.99. Lower values were found for the uncoated sample S7 (0.93), the sample coated with starch only (S6) (0.95), and for S1, S2, S3, S4, and S5 (0.97). On day 12, no significant differences ($P > 0.05$) were found between essential oil-incorporated samples, while there was a significant difference ($P < 0.05$) between samples incorporated with essential oil and samples without essential oil. This might be due to the higher loss of moisture in non-treated samples due to the expulsion of water from the intra-myofibrillar space due to proteolysis by microbes and autolysis. Treated samples contain more hydrophobic constituents in their coating. Water vapor transfer often takes place through the hydrophilic section of films; the addition of essential oil improved the meat's water activity [79]. Water vapor permeability is therefore dependent on the hydrophilic-hydrophobic ratio of the film components from the meat's surface. Changes in water activity during the storage period are shown in Table 5.

3.12. Extract release volume (ERV)

Treatment-wise, S1, S2, S3, S6, and S7 exhibited no significant differences ($P > 0.05$) in ERV on days 9 and 12. Day-wise, on day 12, S1, S2, and S6 showed no significant differences ($P > 0.05$), while S1, S2, S3, S4, and S5 showed no significant differences ($P > 0.05$) on days 9 and 12.

ERV on day 0 was 24.65 mL for all meat samples; after treatment, S5 had the highest ERV at 17.9 mL, and S7 had the lowest at 12.7 mL at the end of day 12, exhibiting a similar pattern to TVC. S5 showed a 40.94% increase in ERV. Meat can be regarded as acceptable if the ERV is at least 17 mL [80]. S7 reached this threshold only on day 6, while S6 and S1 reached it on days 9 and 12. S2, S3, S4, and S5 did not reach this threshold within the study period. This result is comparable to that of Khare et al. [80], who found that the decrease in ERV values during storage was due to the rise in microbial load. The ERV of every sample decreased with storage duration. The ERV of buffalo meat also decreased with storage duration in chilled conditions [78]. Also, a decrease in ERV is a result of an increase in pH [81]. Meat with a lower ERV value may have a higher total plate count [82] and a higher pH [81]. Changes in ERV during the storage period are shown in Table 5.

3.13. Weight loss%

Day-wise, no significant differences ($P > 0.05$) were found between S1, S2, S3, S4, and S5 for day 9; likewise, for day 12 in S1, S2 and S3, S2, S3, and S5, and S4 and S5. Treatment-wise, weight loss was found to be significantly different ($P < 0.05$) between each day for every sample. Starting

from 0% on day 0, weight loss for the uncoated sample S7 was highest at 9.15%, while S4 exhibited the lowest weight loss at 5.63%, which somehow followed a similar increasing pattern as did microbial count. Meat samples with higher TEO concentrations exhibited lower moisture loss and vice versa. This loss in moisture can be attributed to moisture evaporation from meat stored in the refrigerator. Moisture loss in non-treated samples may be attributed to protein degradation due to proteolysis, which results in water expulsion from intra-myofibrillar spaces [83]. Changes in weight loss during the storage period are shown in Table 5.

3.14. Correlation between different parameters

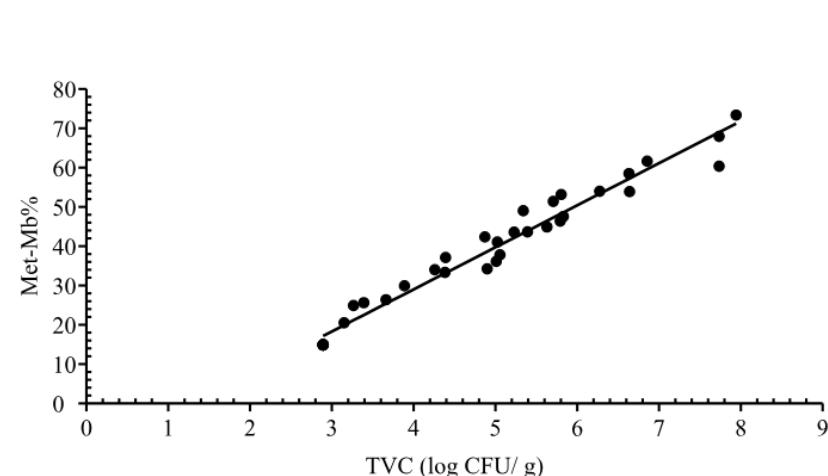
A highly positive correlation was found between TVC and the metmyoglobin content of meat, as shown in Figure 1(a). Metmyoglobin formation has been linked to aerobic bacteria including *Pseudomonas aeruginosa*, *Pseudomonas geniculata*, *Pseudomonas fluorescens*, and *Achromobacter facians*, which lower the oxygen tension at the flesh surface [68]. As bacteria grow, oxygen tension at meat's surface decreases, which causes metmyoglobin levels to rise. Particularly during the logarithmic phase of *Pseudomonas*, *Achromobacter*, and *Flavobacterium*, metmyoglobin is generated [70].

A highly negative correlation of ERV with TVC and pH was found, as shown in Figure 1(b) and Figure 1(c), respectively. The decrease in ERV of fresh meat during microbiological deterioration was brought on by increased hydration of protein due to the meat's gradual pH increase [82].

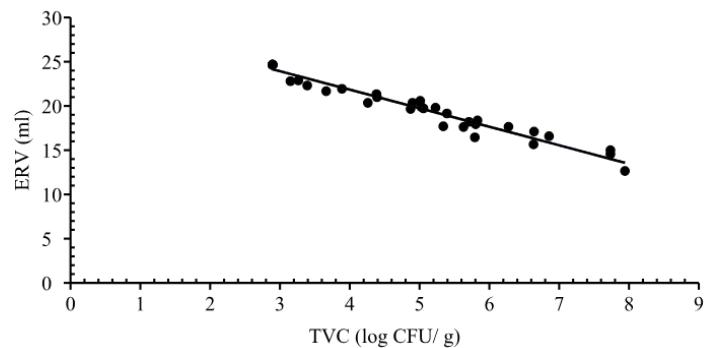
A highly positive correlation was found between TVC and TVBN during the study period, as shown in Figure 1(d). Throughout storage, buffalo meat's protein level decreased [74]. The enzymatic activities of psychrotrophic microbial growth, which caused the denaturation, disintegration, and deamination of proteins and amino acids, resulted in a decrease in the protein content [75]. Nitrous volatile chemicals and protein degradation are common causes of strong bad odors [61].

A highly positive correlation was found between TVC and the pH, as shown in Figure 1(e). The bacterial activity that produced ammonia, amines, and other alkaline compounds was likely what caused the pH to rise [77]. The pH value of meat was raised by autolysis and an increase in microbial load [78]. pH and TVBN demonstrated a significant positive correlation [75].

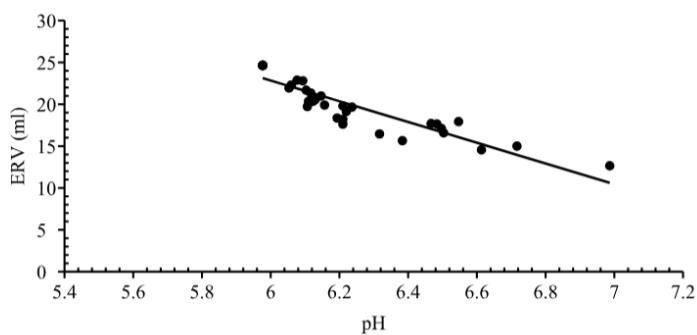
A highly positive correlation was found between TVBN and the pH of meat, as shown in Figure 1(f). The bacterial activity that produced ammonia, amines, and other alkaline compounds was likely what caused the pH to rise [77].



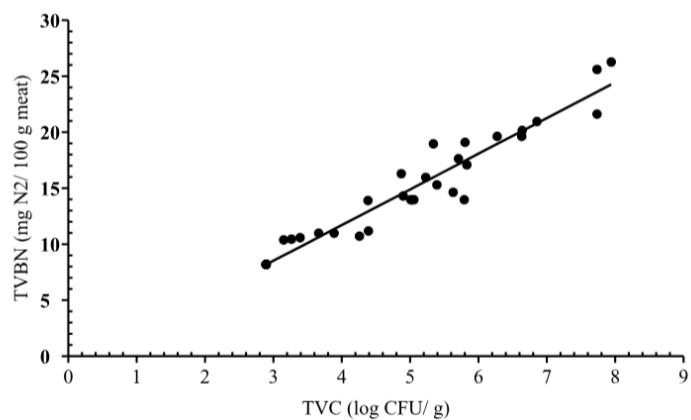
b)



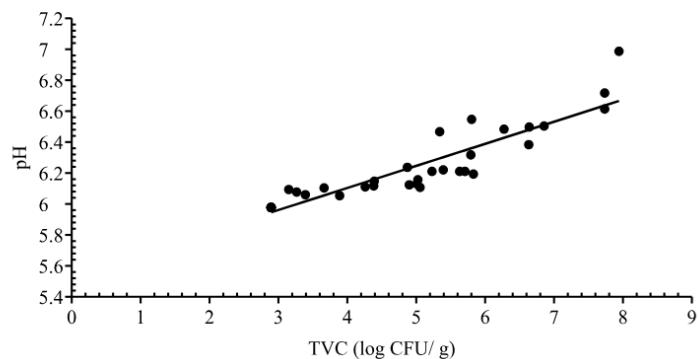
c)



d)



e)



f)

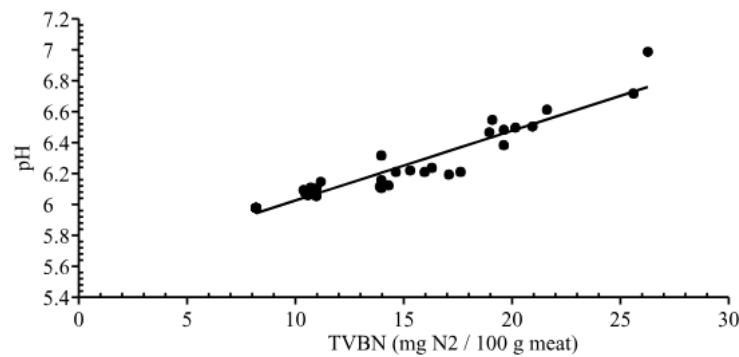


Figure 1. Correlation between a) TVC and metmyoglobin content, b) TVC and ERV, c) ERV and pH, d) TVC and TVBN, e) TVC and pH, and f) TVBN and pH.

4. Conclusions

Buffalo meat has shown increasing demand as it has low fat, low cholesterol, an appealing red color, weak marbling, and high protein content. Edible coatings incorporated with higher TEO concentrations, i.e., S4 and S5, improved the refrigerated shelf-life of buffalo meat in every aspect analyzed. During 12 days of refrigerated storage, compared to the uncoated sample, treatment S4 showed a 2.6 log reduction in TVC, a 46.14% reduction in TBARS value, and a 27.86% reduction in TVBN, while treatment S5 had a 4.43, 1.18, and 3.01 log reduction in PTC, *Staphylococcus aureus* count, and coliform count, respectively. S5 also showed a 40.94% increase in ERV compared to the uncoated sample after 12 days. Effects on other meat spoilage and pathogenic microorganisms such as *Pseudomonas aeruginosa* and *Salmonella* can be analyzed in the future.

Declaration for the use of AI tools

All the authors declare that no Artificial Intelligence (AI) tools were used for the preparation of this article.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

S.D.: Conceptualization, Methodology, Software, Resources, Investigation, Formal Analysis, Validation, Data Curation; B.K.R.: Supervision, Methodology, Resources, Validation, Project administration. A.D.: Software, Writing-original draft, Writing- review & editing, Visualization, Validation; K.R. and P.T.: Software, Writing-original draft, Writing- review & editing; R.K., S.C., P.D. and T.B.: Software, Resource; A.M.G.: software, resources, writing—review and editing, visualization, supervision, project administration.

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