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Evaluation of the Impact of Alginate Coating Infused With Nanoemulsified *Eryngium Campestre* Essential Oil on the Physicochemical and Microbial Quality of Ostrich Thigh Meat

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ABSTRACT

The aim of this study was to evaluate the impact of a sodium alginate (SA) coating incorporating *Eryngium campestre* (zulang) essential oil (EO) nanoemulsion (EON) regarding the preservation characteristics of ostrich meat under cold storage condition. The primary constituents of the EO were germacrene D, α-bisabolol, campestrolide, and salvial-4(14)-en-1-one. The formulated nanoemulsions exhibited a zeta potential of −31 mV, an average droplet size of 76 nm, and a polydispersity index (PDI) of 0.288, indicating good stability and uniformity. Ostrich meat samples were coated with SA alone and SA combined with 1%, 2.5%, or 5% EON, then stored at 4°C for 12 days. Comprehensive physicochemical analyses—including free fatty acid (FFA), peroxide value (PV), pH, total volatile basic-nitrogen (TVB-N), drip loss, and texture—alongside microbial assessments were conducted throughout storage. Samples treated with SA containing 2.5% or 5% EON exhibited significantly reduced FFA, PV, and TVB-N levels and counts of mesophilic, Enterobacteriaceae, and psychrotrophic bacteria compared to those coated with pure SA or untreated controls. Furthermore, these treatments resulted in lower shear force values, indicating enhanced tenderness, as well as reduced drip loss. The antimicrobial efficacy and preservation of chemical and physical attributes ranked as SA with 5% EON > SA with 2.5% EON > SA with 1% EON > SA alone. The integration of EON into the SA matrix appeared to improve dispersion and may have supported a more gradual release of active compound, thereby providing a more consistent antimicrobial effect and improved preservation of meat quality.

1 | Introduction

Due to their perishable nature, meat products are particularly susceptible to microbial spoilage, which significantly increases the risk of foodborne infections. The proliferation of pathogenic microorganisms, coupled with enzymatic and biochemical transformations, contributes to their deterioration [1]. In recent years, the popularity of ostrich meat has grown substantially relating to its nutritional composition and health advantages, which have led to increased consumer interest. Ostrich (*Struthio camelus*), a member of the ratite family and the world's largest flightless bird, is now raised globally for its meat [2].

Compared to other red meats, ostrich meat is increasingly viewed as a healthier and high-quality alternative. Its organoleptic features, particularly flavor and tenderness, are similar to beef,

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which enhances its acceptability among consumers. Moreover, ostrich meat is remarkably lean and nutrient-dense, containing lower levels of fat, saturated fatty acids, and collagen when compared to poultry such as chicken, duck, goose, and turkey. Because of its lean composition with little collagen, ostrich meat is well-suited to preservation techniques that rely on natural additives [2].

A notable feature of ostrich meat is its tenderness, attributed to its low collagen and saturated fat per unit of protein. This composition not only contributes to a desirable texture and chewing experience but also promotes easier digestion. In culinary applications, ostrich meat retains moisture effectively, resulting in a crisp yet juicy texture postcooking. Interestingly, the thigh is the principal edible part of the ostrich carcass, and variations in lipid profile, including cholesterol and fatty acid content across different muscle groups, are minimal [3].

Under traditional refrigeration and packaging, ostrich meat has a shelf life of approximately 3 days. To extend freshness and maintain quality, modern food preservation methods such as packaging with modified atmosphere and active packaging systems have been introduced. Among these, using edible coatings and films infused with natural preservatives has gained traction both in scientific research and the food [4].

Recently, plant-derived essential oils (EOs) and botanical extracts have emerged as promising antimicrobial agents and natural preservatives due to their dual antioxidant and antibacterial properties. However, due to the potential toxicity and sensory changes associated with uncontrolled additive use, there is growing interest in edible coatings that facilitate the controlled release of active agents. Such techniques prolong the shelf-life period of food stuffs without compromising sensory qualities. Edible coatings can be administered in two principal ways: by direct application over the food surface or through preformed films that envelop the product [5, 6].

EOs, known for their rich terpene content, are typically obtained via distillation processes and are derived from various parts of aromatic and medicinal plants [7]. One such plant is *Eryngium campestre* (commonly known as zulang), indigenous to the northern regions of Iran. This medicinal herb is known for its pharmacological and food-related applications. Its EO is rich in compounds such as polyphenols, monoterpenes, and flavnoids—components responsible for its protective properties against microbes and oxidation [8, 9].

Studies have shown that zulang EO has an extensive antimicrobial property, demonstrating inhibitory effects against common foodborne pathogens [8, 10]. The pronounced antioxidant capability of this EO is significantly related high polyphenol and flavonoid content. In addition to its preservative efficacy, the safety of zulang EO must be considered for food applications. Previous toxicological and phytochemical studies have reported that zulang EO is rich in sesquiterpenes such as germacrene D, bisabolol, and campestrolide, compounds that are commonly found in edible and medicinal plants and have demonstrated low toxicity at concentrations used in food preservation. Moreover, many constituents of this EO are listed as generally recognized as safe (GRAS) when used at low levels in food systems [11, 12].

Despite their benefits, EOs face several limitations like low solubility in water, high volatility, high aromatic intensity, and limited environmental stability. These factors can diminish their bioactivity and hinder their broad adoption in food matrices. However, advancements in nanoemulsion technology have offered solutions to a great deal of these problems. Nanoemulsions, owing to their small droplet size and enhanced surface area, provide higher dispersion and stability for EOs in water. They also modulate the release of active compounds, diminish sensory perception issues, and promote better interaction with microbial cells [13].

For instance, research by Parham et al. [14] illustrated that coatings based on nanoemulsified EOs provided more uniform distribution and more persistent antimicrobial activity than their conventional counterparts [14]. As such, nanoemulsion-based delivery systems offer a compelling strategy to enhance the performance of EOs in food applications [15].

Alginate is a polysaccharide, comprising beta-D-mannuronic acid and alpha-L-glucuronic acid (glucuronan) units consisting of the sodium or potassium salt of alginic acid, which is extracted from brown seaweed. Alginate with unique colloidal properties possesses strong gelation potential [16, 17]. Alginate is used as a coating on the surface of plant foods, meat, poultry, and seafood to inhibit the growth of microorganisms and chemical deterioration. Articles on the applications and usage of natural polymers to encapsulate bioactive compounds on meat, chicken, and marine products have been published [18–22].

Nevertheless, no prior research has specifically addressed the use of alginate coating incorporating zulang EO nanoemulsions (EONs) for the preservation of ostrich thigh meat. A previous study by Moadab and Azizkhani [15] investigated the effect of chitosan-based edible coating enriched with *E. campestre* EONs on ostrich meat [15]. However, due to the distinct structural, chemical, and functional properties of chitosan and alginate, their interactions with EO and efficacy in preservation may vary significantly. The simultaneous use of sodium alginate (SA) and EON was designed to exploit the synergistic effects of a physical oxygen barrier and a bioactive antimicrobial/antioxidant agent. While alginate alone can reduce moisture and oxygen transfer, it has limited antimicrobial activity. Conversely, EOs exhibit strong bioactivity but suffer from volatility and rapid degradation. Incorporating EO in a nanoemulsion within an alginate matrix improves its stability, controlled release, and contact with the meat surface, thereby enhancing preservation efficacy compared to using alginate or EO alone [23].

Although numerous studies have investigated edible coatings containing EOs for meat preservation, most have focused on commonly used oils such as thyme, oregano, clove, and rosemary, primarily applied to beef, chicken, or fish. To the best of our knowledge, no study has reported the use of *Eryngium campestre* EO in nanoemulsion form incorporated into an alginate coating for the preservation of ostrich meat. Ostrich meat possesses distinct physicochemical properties, including low fat content and high susceptibility to oxidative deterioration, which require tailored preservation strategies. Therefore, the present study introduces a novel application of *Eryngium campestre* EON within a biopolymer matrix (SA) and evaluates its effectiveness on the quality maintenance of ostrich thigh meat through

comprehensive microbial, oxidative, and sensory analyses during refrigerated storage.

2 | Materials and Methods

2.1 | Chemicals and Culture Media

All chemicals and culture media used in this study were purchased from Merck company (Germany).

2.2 | Plant Preparation and EO Extraction

Aerial parts of zulang were procured fresh from a local market in Sari (Mazandaran Province) and transported to the Food Laboratory at Amol University of Special Modern Technologies. The leaves were washed dried in at 40°C (Oven HOT-789 model, GHI Technologies, France). EO extraction was then performed using a Clevenger-type system (Model CLEV-456, DEF Scientific Equipment, USA) over a period of 4 h. The resulting EO was kept in a dark bottle till further analysis.

2.3 | Identification of EO Compounds

The chemical composition of *Eryngium campestre* EO was analyzed using gas chromatography coupled with mass spectrometry (GC–MS; Thermo Quest 2000, UK). Separation was performed on a capillary column (e.g., DB-5 MS, 30 m × 0.25 mm i.d., film thickness 0.25 μm). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injector temperature was set at 250°C, and 1 μL of the EO sample, diluted in n-hexane (1:100 *v*/*v*), was injected in split mode (split ratio 1:50). The oven temperature program was as follows: initial temperature of 60°C (held for 3 min), increased to 220°C at a rate of 3°C/min, then raised to 280°C at 10°C/min and held for 10 min. The mass spectrometer operated in electron impact (EI) mode at 70 eV with a mass scan range of 40–450 m/z. The identification of EO constituents was performed by comparing their mass spectra with those in the Wiley 275.L and Wiley7n.L libraries and by comparing their calculated retention indices (RIs) with literature values [24]. RIs were determined using a homologous series of n-alkanes (C8–C24) analyzed under the same chromatographic conditions. Quantitative data were obtained by peak area normalization without the use of correction factors. The representative GC–MS spectra are part of the raw instrumental data and are available from the authors upon request.

2.4 | Preparation of SA Solution Containing EON

For preparing nanoemulsion, Tween 80, as a surfactant, was added to EO in a 1:1 (*v*/*v*) ratio, along with ethanol (acting as a cosurfactant at 10% *v*/*v* relative to Tween 80). This mixture was subjected to high-speed homogenization at 10,000 rpm for 5 min using an Ultra-Turrax device. Subsequently, the emulsion underwent ultrasonic treatment (UP400S model, Hielscher Co., Germany) operating at 1.5 kW power, 80% amplitude, and a 0.5 s pulse duration for 10 min. The zeta potential, particle size, and polydispersity index (PDI) were measured by a Nano Zetasizer (ZEN 3600, Malvern Instruments, UK) at 363 nm wavelength to confirm the formation of a stable nanoemulsion. To determine encapsulation efficiency (EE), the prepared EON was centrifuged (10,000 rpm, 20 min) to separate the free (nonencapsulated) EO from the nanoemulsion droplets. The supernatant containing free EO was collected, and its concentration was quantified spectrophotometrically using a previously prepared calibration curve of the EO at its maximum absorbance wavelength. EE was then calculated using the equation:

$$\mathrm{EE}(\%) = [(\text{Total EO} - \text{free EO})/\text{total EO}] \times 100$$

where total EO is the amount of EO initially used for nanoemulsion preparation and free EO is the amount detected in the supernatant [25].

The concentration of 2% *w*/*v* of SA was selected as the coating matrix due to its excellent film-forming ability, oxygen barrier properties, biodegradability, nontoxicity, and GRAS status in food applications [26]. SA solution was prepared by dissolving SA powder in distilled water with continuous stirring at 50 ± 1°C until a homogeneous solution was achieved. pH was set to 4.5–5.0 using 1 M NaOH or HCl, as needed. To infuse the EON into the SA matrix, EON was mixed with the SA solution, at concentrations of 1%, 2.5%, and 5% (*v*/*v*) separately, and stirred 10 min at 200 rpm. This process was performed for nanoemulsion ensuring uniform dispersion within the SA matrix [27]. The selection of EON concentrations (1%, 2.5%, and 5% *v*/*v*) was based on preliminary screening tests and previous studies investigating the antimicrobial efficacy of zulang EO in edible coatings [15]. These concentrations were chosen to represent low, moderate, and high levels of EO incorporation, allowing evaluation of dose-dependent effects while avoiding potential sensory alterations and excessive EO usage. Similar concentration ranges have been reported as effective and safe in meat preservation studies using EONs within biopolymer matrices.

2.5 | Sample Treatment With SA Containing EON

The ostrich thigh meat used in this study was obtained from a licensed commercial slaughterhouse in Semnan Province, Iran, where animals were slaughtered for routine food production under the supervision of the Iranian Veterinary Organization in accordance with national animal welfare regulations. No animals were slaughtered specifically for the purpose of this research. Therefore, according to institutional and national guidelines, this study did not require approval from an animal ethics committee, as the samples were procured postmortem from the food supply chain. Ethical procurement and handling of meat samples were strictly followed throughout the study. Fresh meat was transported to laboratory under chilled conditions in an ice-filled container. It was sectioned into 200 g portions, each approximately 10 cm × 5 cm × 5 cm in size, and divided into five experimental groups as follows:

Control: No coating treatment

F1: 2% (*w*/*v*) SA and 2% (*w*/*v*) sodium tripolyphosphate solution

F2: SA solution + 1% (*v*/*v*) EON

F3: SA solution + 2.5% (*v*/*v*) EON

F4: SA solution + 5% (*v*/*v*) EON

Each sample portion was treated through immersion in the solution for 15 min to allow uniform coating, then transferred to a sterile metal rack and left (10 min) to drain. After coating, the samples were individually vacuum-sealed in sterile zip-lock pouches and stored at 4 ± 1°C for a period of 12 days. The analyses were carried out every 3 days throughout the storage period.

2.6 | Chemical Tests

2.6.1 | Components of the Ostrich Thigh Meat

The chemical composition of meat sample was analyzed to measure fat, protein, monounsaturated, and polyunsaturated fatty acids, as well as vitamin B12, using standard procedures outlined by the AOAC [28]. These analyses were independently performed in this study, although the results were consistent with our earlier report [15] due to uniform sourcing and species-specific consistency.

2.6.2 | pH Value

For pH determination, only the surface layer (~2–3 mm) of the meat samples, which was directly in contact with the coating, was carefully excised using a sterile scalpel. A 10 g portion of each sample was then homogenized (UltraTurrax T25 homogenizer, IKA-Werke GmbH & Co. KG, Germany) with 25 mL of neutral distilled water to ensure that the measured pH reflected the surface conditions influenced by the edible coating rather than the internal muscle tissue. It was left at ambient temperature (10 min) and the pH of the resulting extract was measured using a digital pH meter (HI98103 model, Hanna Instruments, USA) [29].

2.6.3 | Free Fatty Acid (FFA) Content

FFA value was determined according to the method of Rahman et al. [30]. Five grams of fillet sample was homogenized in 30 mL chloroform at 11,000 × *g* for 1 min and then filtered using Whatman filter paper Number 1 to remove fillet particles from the filtrate. After adding 4–5 drops of ethanolic phenolphthalein (1%) as an indicator, the filtrate was titrated with ethanolic potassium hydroxide solution and FFA content on basis of oleic acid was calculated as follows:

$$\mathrm{FFA}(\%) = (V \times N \times 28.2)/W,$$

where V is the volume of titration (mL) with KOH, N is the normality of the KOH solution, and W is the sample weight (g) [30].

2.6.4 | Total Volatile Basic-Nitrogen (TVB-N)

TVB-N is consisted of amines (primary, secondary, and tertiary) in the form of volatile compounds and toxic nitrogen which show degradation of proteins and spoilage. These compounds have considerable adverse effects on the organoleptic properties and acceptability of meat products and increase during the storage period of the product. To determine TVB-N, 10 g of fillet sample, 2 g magnesium oxide (MgO), and 500 mL of distilled water were transferred into a balloon and volatile nitrogen compounds were accumulated in a solution of boric acid (2%) and methyl red (as the indicator). Titration of the solution was carried out with sulfuric acid and the results were reported as mg TVB-N/100 g of chicken fillet according to the following equation [31]:

$$\mathrm{TVN} = \text{Sulfuric acid} \times 14.$$

2.6.5 | Peroxide Value (PV)

PV serves as an indicator of hydroperoxides produced during the intial stages of lipid oxidation. High PV reflects initial oxidative deterioration in meat samples. The PV determination followed the procedure described by Rahman et al. [30]. Ten grams of the samples were weighed in a 250 mL Erlenmeyer flask and heated at 60°C in for 3 min in a water bath to melt the fat. Then, 30 mL acetic acid–chloroform solution (3:2 *v*/*v*) was added and the flask was thoroughly agitated for 2 min to dissolve the fat. To separate tissue particles from the liquid part, the suspension was filtered using Whatman filter paper Number 1; 0.5 mL of saturated potassium iodide solution and 4–5 drops of starch solution (as the indicator) were added to the filtrate. The solution was titrated against the standard solution of sodium thiosulfate. PV was expressed as milliequivalent peroxide per kilogram of the sample and calculated by the following equation:

$$\mathrm{PV}\,(\mathrm{meq/kg}) = (V \times N)/W \times 100,$$

where V is the volume of titration (mL), N is the normality of sodium thiosulfate solution, and W is the sample weight (g) [30].

2.6.6 | Thiobarbituric Acid Reactive Substances (TBARSs)

TBARSs analysis was used to quantify secondary lipid oxidation products, primarily malondialdehyde (MDA), which are responsible for rancidity and off-flavors in meat during storage. TBARS is, therefore, considered a reliable indicator of advanced lipid degradation. The assay was performed according to the method described by Govindaiah et al. [32] with slight modifications. In brief, 5 g of each meat sample was homogenized with 15 mL of 7.5% (*w*/*v*) trichloroacetic acid (TCA) solution to precipitate proteins and release bound MDA. The homogenate was then centrifuged at 4000 rpm for 10 min at room temperature. The clear supernatant was carefully collected and filtered through Whatman Number 1 filter paper to remove residual particles. Two milliliters of the filtrate were mixed with 2 mL of 0.02 M thiobarbituric acid (TBA) solution prepared in distilled water. The mixture was then heated in a boiling water bath (95–100°C) for 15 min to allow the formation of a pink MDA–TBA complex under acidic conditions. After heating, the tubes were cooled to room temperature under running tap water. The absorbance of the resulting chromogen was measured at 532 nm using a UV–visible spectrophotometer against a blank containing TCA and TBA solutions without sample. The TBARS value was expressed as mg MDA per kg of sample using a standard calibration curve prepared with 1,1,3,3-tetraethoxypropane (TEP) as the MDA precursor. [32]:

$$\mathrm{TBARS}\,(\mathrm{mg\,MDA/kg\,sample}) = (C \times V)W,$$

where C is the absorbance at 532 nm, V is the total volume of extract (mL), and W is the sample weight (g).

2.7 | Microbial Tests

For the preparation of decimal dilutions, 10 g of sample and 90 mL of peptone water (0.1%) were evenly mixed to ensure uniform suspension.

2.7.1 | Total Mesophilic Bacteria

Mesophilic bacteria were quantified by inoculating the prepared decimal dilutions onto Plate Count Agar, which were then incubated at 37°C for 24 h using an INCU-Line incubator (Abtron Equipment Ltd., UK). The bacterial counts were recorded as $\log_{10}$ colony-forming units per gram (cfu/g) [33].

2.7.2 | Psychrotrophic Bacteria

To assess psychrotrophic bacterial growth, the decimal dilutions were plated onto PCA and incubated at 7°C for 10 days. Results were expressed as $\log_{10}$ cfu/g [33].

2.7.3 | Enterobacteriaceae

Enterobacteriaceae was cultured and counted on VRBG agar after incubation at 37°C for 24 h. The results were calculated as $\log_{10}$ cfu/g [33].

2.8 | Drip Loss

Drip loss, representing the amount of water released from raw meat during a determined length of time, was assessed by individually keeping 50 g samples in suspended form inside polyethylene bags, ensuring no contact with the bag surfaces. The samples were stored at 4°C for 24 h. Afterward, each sample was carefully removed, gently blotted to remove surface moisture, and reweighed. Drip loss was calculated as the percentage of weight loss relative to the initial weight [30].

2.9 | Texture Analysis

Texture analysis of the samples was performed to assess firmness using a texture analyzer (TA.XTplusC, Stable Microsystems, Surrey, UK). A total of 10 samples ($n = 10$) from each treatment group were analyzed [34].

2.10 | Sensory Evaluation

Sensory assessment was conducted to evaluate the color, flavor, odor, tenderness, and overall acceptability of ostrich thigh meat samples during storage in refrigerator. A 9-point hedonic scale was utilized, as 1 (dislike extremely), 2 (very unacceptable), 3 (unacceptable), 4 (lightly unacceptable), 5 (neither acceptable nor unacceptable, borderline), 6 (slightly acceptable), 7 (acceptable (good quality, slight changes noticeable), 8 (very acceptable), and 9 (like extremely), in accordance with standardized sensory evaluation protocols. A trained panel consisting of 10 persons, selected based on their prior experience, participated in the evaluations. Prior to testing, panelists underwent training sessions to calibrate their use of the hedonic scale and to standardize their understanding of key sensory attributes using reference samples. The samples (cubes of 2 cm × 2 cm × 2 cm) were cooked using an electric grill to the internal temperature of 75 ± 1°C, then cooled slightly before serving. Panelists were provided with water to cleanse their palates between samples to minimize sensory carryover [15].

2.11 | Statistical Analysis

All experiments were conducted in triplicate, and the results are reported as mean values ± standard deviation. A two-way analysis of variance (ANOVA) was employed to assess the effects of treatment and storage time, along with their interaction. Tukey's honest significant difference (HSD) post hoc test was conducted for multiple pairwise comparisons to determine statistically significant differences among treatment groups. All statistical analyses were conducted at a 95% confidence level ($p < 0.05$) using SPSS software (Version 26.0, IBM, USA).

3 | Results and Discussion

3.1 | Ostrich Meat Composition

Ostrich thigh meat exhibits a commendable nutritional profile, marked by its low lipid content (~1% *w*/*w*) and elevated protein concentration (higher than 21% *w*/*w*). Furthermore, it is a rich source of mono-unsaturated (39.03% of total fat) and poly-unsaturated fatty acids (27.61% of total fat), along with a significant vitamin B12 concentration (12.9 μg/kg). When evaluated against traditional meat sources, ostrich meat demonstrates several nutritional advantages. For instance, beef typically possesses a higher fat percentage, varying between 10% and 20%, but chicken, though generally considered leaner, exceeds ostrich in fat content, particularly when consumed with skin. In addition, ostrich meat supplies comparable or greater levels of bioavailable heme iron and B12 vitamin relative to beef, but with a more favorable lipid profile. These attributes position ostrich meat as a health-conscious alternative to conventional red meats [35].

3.2 | Composition of Zulang EO

The EO was freshly extracted and analyzed in this study. While Table 1 shows a chemical composition consistent with our prior findings [15], minor variations in some constituents confirm independent extraction and characterization. The primary constituents identified included germacrene D, campestrolide, salvial-4 (14)-en-1-one, and α-bisabolol. The dominant constituents belong predominantly to the terpene and terpenoid classes, alongside a number of aromatic and aliphatic molecules. Campestrolide, an uncommon naturally occurring lactone with conjugated acetylenic bonds, was previously characterized in zulang EO by a previous study [36]. The lower level of germacrene D observed in our study could be linked to variables such as the plant's geographical origin, surrounding environmental factors, timing of harvest, or the extraction technique used—all of which can alter the composition of EOs. Such variations may impact the biological activity of the EO, particularly its antimicrobial and cytotoxic properties [36, 37].

3.3 | Nanoemulsion Droplet Size and Zeta Potential

The functional performance of nanoemulsions is highly dependent on their physicochemical parameters, including droplet size,

TABLE 1 | Chemical compounds of zulang essential oil.

Compounds	Amount (%)	Retention index	Compounds	Amount (%)	Retention index
β-Pinene	Tr	973	Sesquicineole	0.56	1507
Myrcene	1.63	982	β-Curcumene	0.43	1510
p-Cymene	Tr	1011	δ-Cadinene	Tr	1513
Limonene	Tr	1020	δ-Cadinene	0.4	1515
(Z)-β-ocimene	Tr	1026	(E)-α-bisabolene	1.13	1533
δ-Terpinene	Tr	1045	β-Elemol	Tr	1534
Nonan-2-one	Tr	1077	7-epi-trans-Sesquisabinene hydrate	Tr	1546
Terpinolene	Tr	1080	Salvial-4 (14)-ene-1,5-epoxide	0.93	1549
Nonanal	Tr	1084	Germacrene B	0.18	1551
Decanal	Tr	1173	Spathulenol	0.66	1536
α-Copaene	0.49	1379	Caryophyllene oxide	0.81	1571
β-Elemene	2.85	1391	Salvial-4 (14)-en-1-one	4.60	1578
β-Ylangene	0.73	1420	Ledol	0.46	1604
δ-Elemene	Tr	1429	1,10-di-epi-Cubenol	1.35	1611
(E)-β-farnesene	2.32	1450	α-Cadinol	1.57	1646
α-Curcumene	0.51	1473	α-Bisabolol	4.22	1666
Germacrene D	37.20	1480	14-Hydroxy-α-muurolene	0.7	1760
β-Selinene	0.13	1485	14-Hydroxy-δ-cadinene	0.74	1785
α-Muurolene	2.08	1504	Campestrolide	18.54	2144
β-Bisabolene	Tr	1504	Total	84.32	—

Abbreviation: Tr, trace.

uniformity, morphology, and surface charge. These characteristics play crucial roles in modulating the release dynamics of encapsulated compounds, influencing the rheological behavior of colloidal dispersions, and governing cellular internalization efficiency [38]. Previous studies, such as Zhu et al. [39], have described the uptake of nanoparticles by microbial membranes as a two-phase mechanism involving initial adherence to the cell wall, followed by intracellular transport. The efficiency of this interaction is largely dictated by the electrostatic characteristics of the nanoparticles, particularly their surface charge. The interaction of nanoparticles with the cell membrane is largely influenced by the surface charge of the emulsion droplets [39]. In this study, droplet size, PDI, zeta potential, and EE were determined for each EON formulation prior to incorporation into the SA solution (Table 2). The nanoemulsion corresponding to the coating containing 1% EO exhibited the smallest droplet size (76 nm), lowest PDI (0.288), and highest zeta potential magnitude (−31 mV), indicating a more uniform and stable system. As the EO concentration increased to levels later used for 2.5% and 5% coatings, droplet size and PDI increased, while the magnitude of zeta potential decreased, reflecting reduced stability due to higher oil content. After mixing these nanoemulsions with SA to prepare coating formulations, the system no longer represented a true nanoemulsion but rather a viscous polymeric matrix. Therefore, particle size and zeta potential measurements were not applicable at this stage, as dynamic light scattering analysis is not reliable in polymer-based coating solutions. The encapsulation efficiencies (82.55%, 72.69%, and 65.25%) correspond exclusively to the entrapment of EO within the nanoemulsion droplets prior to their incorporation into the alginate matrix. No visual signs of instability, such as creaming phase separation, or sedimentation, were detected during a 12-day storage period at $4 \pm 1°C$,

TABLE 2 | Physicochemical characterization of EO nanoemulsion before incorporation into sodium alginate.

EO level	Droplet size (nm)	PDI	Zeta potential (mV)	Encapsulation efficiency (%)
1% EO	76 ± 3.25^{a}	0.288 ± 0.05^{a}	-31 ± 1.19^{a}	82.55 ± 5.11^{a}
2.5% EO	91 ± 4.10^{b}	0.342 ± 0.06^{b}	-28 ± 1.37^{b}	72.69 ± 3.60^{b}
5% EO	118 ± 5.42^{c}	0.401 ± 0.08^{c}	-24 ± 1.52^{c}	65.25 ± 2.74^{c}

Note: Data are presented as mean ± SD. Superscript letters indicate statistically significant differences ($p < 0.05$) between means within each column.

indicating a high degree of dispersion stability. The zeta potential is a key indicator of electrostatic interactions among dispersed particles, influencing phenomena such as dispersion, aggregation, and flocculation. Consistent with the reviews of Soni and Sharma [38], systems exhibiting a stronger negative zeta potential have been shown to promote enhanced cellular uptake compared to those with lower or positive surface charges [38]. Furthermore, our observations align with prior reports indicating that nanoemulsions with highly negative surface charges and low PDIs tend to exhibit extended shelf stability and consistent release profiles [40, 41].

3.4 | Chemical Evaluation Results

3.4.1 | pH

Considerable differences were observed between the control and plain SA coated samples and all treatments containing SA combined with EON (F2–F4; $p < 0.05$; Figure 1). Samples coated with pure SA exhibited higher surface pH compared to the control, likely due to SA's neutral-to-slightly alkaline nature. This aligns with previous observations that SA forms solutions in the pH 6.5–7.5 range [42]. Among the treatments, F4 (SA + 5% EO) recorded the lowest pH throughout storage ($p > 0.05$). While all SA–based EO treatments showed slight pH decreases toward the end of storage, uncoated control and SA coated samples experienced a pH increase. The pH decline in EO-enriched treatments, especially at higher EO concentrations, can be attributed to organic acids present in the oils and ongoing conversion of muscle glycogen to lactic acid under low-oxygen conditions. Moreover, refrigerated storage may cause minor acidification via carbonic acid formation from microbial CO_2 metabolism [43].

Hu et al. [44] reported that SA coatings with cinnamon EO microcapsules effectively restrained pH elevation in refrigerated chicken breast, indicating better stability compared to uncoated controls [44]. Similar to reports by these authors, our SA/EON coatings maintained more stable pH values, demonstrating superior efficacy in pH control compared to alginate alone. Also, an investigation using SA–agar coatings containing ginger EO (GEO) on beef demonstrated a slower pH increase during cold storage, highlighting the acidifying contribution of GEO and the protective barrier provided by the SA matrix [45, 46]. Pure SA coatings tend to increase surface pH, as seen in F1 and supported by Zhang et al. [45]. In contrast, SA/EONs balance this effect; the acids in EOs moderate the alkalinity and actively suppress pH rise during storage.

3.4.2 | FFA Content

Release of FFAs results from enzymatic lysis or microbial breakdown of lipids. In Figure 2, treatments involving SA coatings with 2.5% and 5% zulang EON showed significantly lower FFA accumulation compared to both SA–coated and uncoated control samples ($p < 0.05$). Throughout storage, treated samples exhibited a gradual increase in FFAs, while the control experienced a much steeper rise. Specifically, SA coatings supplemented with 2.5% and 5% EONs had FFA levels of approximately 0.08%–0.09%, significantly lower than the 0.11% observed in samples with pure SA ($p < 0.05$). Among all formulations, the SA + 5% EON coating resulted in the lowest FFA value ($p < 0.05$), followed by the SA + 2.5% EON (difference not statistically significant; $p > 0.05$). Our recent work has also demonstrated that combining zulang EON with chitosan coatings exerts synergistic antioxidant effects [15]. The FFA value in the samples in the storage period was as follows: Control > SA > SA/1% EON > SA/2.5% EON > SA/5% EON. Laein et al. [47] found that combining SA with oregano EO significantly slowed FFA accumulation in refrigerated chicken, with a 30% reduction observed versus control after 12 days—a trend that was similarly observed in our study when comparing 2.5% and 5% EO concentrations [47].

3.4.3 | TVB-N

TVB-N content, a key indicator of meat freshness, rises during refrigerated storage due to spoilage microorganisms and endogenous enzymes. In our ostrich meat study, TVB-N steadily increased over 12 days at 4°C in the control samples, reaching 33.7 mg/100 g. Samples coated with SA showed significantly

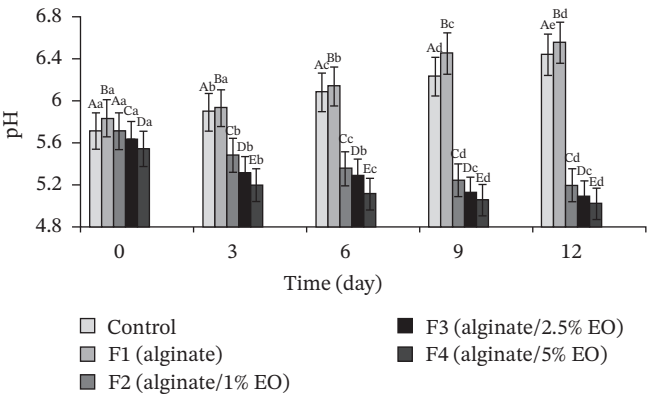


FIGURE 1 | pH values of the ostrich thigh meat coated with sodium alginate and nanoemulsion of zulang essential oil during storage in refrigerator. Different uppercase letters (A–E) in the same row indicate significant differences during storage within the same treatment ($p < 0.05$), according to two-way ANOVA, followed by Tukey's test. Different lowercase letters (a–e) in the same column indicate significant differences between treatments at the same storage time ($p < 0.05$).

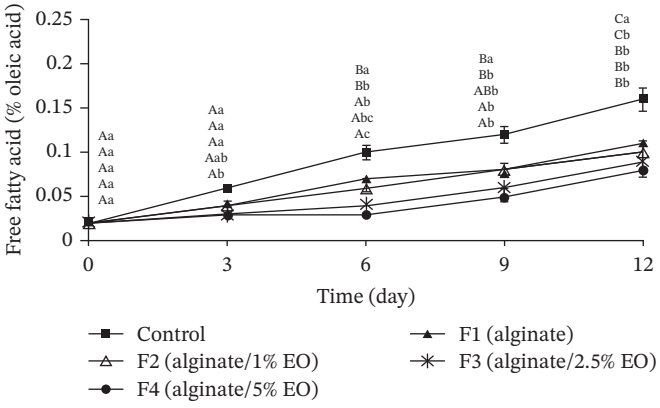


FIGURE 2 | Free fatty acid content of the ostrich thigh meat coated with sodium alginate and nanoemulsion of zulang essential oil during storage in refrigerator. Different lowercase letters (a–e) in the same column indicate significant differences between treatments at the same storage time ($p < 0.05$). Different uppercase letters (A–E) in the same row indicate significant differences during storage within the same treatment ($p < 0.05$), according to two-way ANOVA, followed by Tukey's test.

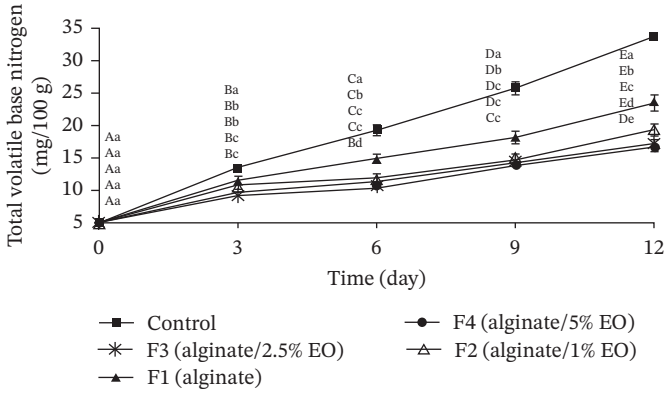


FIGURE 3 | TVB-N content of the ostrich thigh meat coated with sodium alginate and nanoemulsion of zulang essential oil during storage in refrigerator. Different lowercase letters (a–e) in the same column indicate significant differences between treatments at the same storage time ($p < 0.05$). Different uppercase letters (A–E) in the same row indicate significant differences during storage within the same treatment ($p < 0.05$), according to two-way ANOVA, followed by Tukey's test.

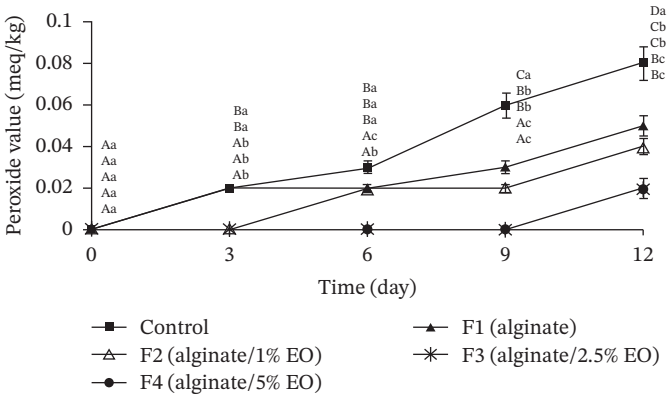


FIGURE 4 | Peroxide value of the ostrich thigh meat coated with sodium alginate and nanoemulsion of zulang essential oil during storage in refrigerator. Different lowercase letters (a–e) in the same column indicate significant differences between treatments at the same storage time ($p < 0.05$). Different uppercase letters (A–E) in the same row indicate significant differences during storage within the same treatment ($p < 0.05$), according to two-way ANOVA, followed by Tukey's test.

lower TVB-N: pure SA reached 23.5 mg/100 g, while SA coatings containing EONs decreased this further—to 19.3 mg/100 g in F2, 17.3 mg/100 g in F3, and 16.7 mg/100 g in F4 ($p < 0.05$; Figure 3). The SA + EON (F4) maintained the lowest TVB-N throughout storage, although its improvement over F3 was modest in practical terms, suggesting diminishing returns at higher EO concentrations. Although F4 showed a modest enhancement in preservation, the advantage of utilizing a higher EO concentration must be balanced against economic feasibility. Further optimization is required to identify the most effective and efficient formulation. These results are in accordance with recent studies that SA coatings enriched with cinnamon EO nanocapsules and nisin kept beef TVB-N below the spoilage threshold (20 mg/100 g) for up to 15 days [48], while an agar–SA bilayer film with GEO maintained beef TVB-N under approximately 15 mg/kg over 9 days [49]. Moreover, SA films loaded with resveratrol and thymol applied to cooked sausages demonstrated strong antimicrobial and antioxidant effects, significantly reducing spoilage microbial counts and delaying TVB-N accumulation in 40-day storage at 4°C [50]. Altogether, these results confirm that SA coatings—especially when combined with antimicrobial antioxidants—can substantially inhibit nitrogenous spoilage compounds in meat, markedly extending shelf life. In our case, while the highest EO concentration (F4) offered marginally better TVB-N control, balancing effectiveness and cost suggests that moderate EO levels (e.g., similar to F3) are most practical. Future optimization should explore synergistic additive combinations and varying EO dosages to maximize preservation efficacy.

3.4.4 | PV

Peroxide index which indicates the presence of hydroperoxides—the initial products of lipid oxidation—is a crucial marker of rancidity development, as their breakdown leads to off-flavors and odors. According to Figure 4, in our refrigerated ostrich meat samples, the uncoated control exhibited a significant increase in PV during storage ($p < 0.05$), whereas samples treated with SA coatings—both plain (F1) and loaded with EON—showed significantly lower PV in comparison with control ($p < 0.05$), and no significant differences among the SA–treated groups ($p > 0.05$). This suggests that SA itself has notable antioxidant properties, potentially masking the additional effects of incorporated EOs at early stages of oxidation, or alternatively, that EO release may occur later in storage, partially diminishing their detectable impact on PV. These observations are consistent with recent reports that SA films enriched with thymol nanoemulsions significantly reduced lipid oxidation in chicken samples stored at 4°C, achieving consistently lower PV compared to plain SA films [51]. Furthermore, studies of SA coatings combined with cinnamon EO nanocapsules and nisin demonstrated delayed oxidation in beef slices, maintaining low PV levels over 15 days [48]. Additionally, composite coatings of SA and di-1-p-menthene effectively slowed lipid oxidation, as reslted in decreased peroxide and TBARS in treated samples [52]. The inherent film barrier function of SA—which limits oxygen diffusion—also contributes to delaying lipid oxidation during cold storage [53]. Together, these results affirm that SA coatings, even without added antioxidants, significantly impede oxidative deterioration, and that their effectiveness can be further enhanced through strategic incorporation of EONs; however, timing and dosage play critical roles in maximizing antioxidant impact.

3.4.5 | TBARSs

In our study, the release of TBARS—a marker of secondary lipid oxidation—was considerably elevated in the uncoated samples compared to treated ones ($p < 0.05$; Figure 5). In contrast, samples treated with SA coating containing 2.5% and 5% EON showed no detectable TBARS throughout refrigerated storage (Figure 5). At the end of the storage period, TBARS levels in samples coated with plain SA (F1) and SA with 1% EON (F2) were significantly different ($p < 0.05$), indicating that low EO concentrations did not markedly augment SA's inherent antioxidant effects. The elevated TBARS in the control confirm progressive lipid oxidation under cold storage conditions. Meanwhile, the complete suppression of TBARS in F3 and F4 shows that higher EO contents in SA coatings strongly inhibit secondary lipid peroxidation, possibly

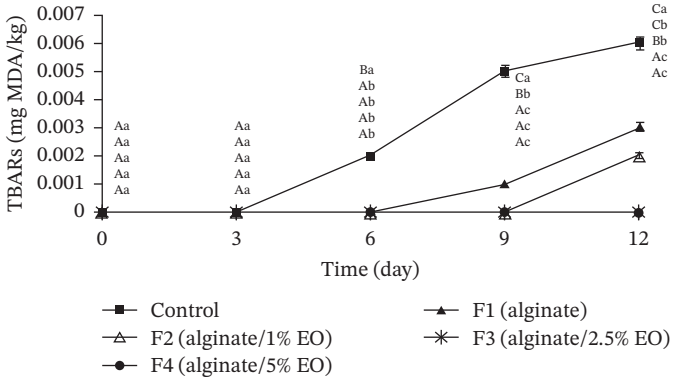


FIGURE 5 | TBARS of the ostrich thigh meat coated with sodium alginate and nanoemulsion of zulang essential oil during storage in refrigerator. Different lowercase letters (a–e) in the same column indicate significant differences between treatments at the same storage time ($p < 0.05$). Different uppercase letters (A–E) in the same row indicate significant differences during storage within the same treatment ($p < 0.05$), according to two-way ANOVA, followed by Tukey's test.

related to potent antioxidant compounds released from the nanoemulsion. These data are consistent with previous studies in which SA coatings infused with GEO in agar–SA bilayer films achieved TBARS values below approximately 1.1 mg MDA/kg in beef during storage and SA coatings loaded with origanum or cinnamon EONs have been shown to effectively suppress TBARS formation in treated meat products [54, 55]. In brief, this evidence demonstrates the synergistic interaction of biopolymer and EON in mitigating lipid oxidation, with sufficiently high EO concentrations being pivotal to achieving complete TBARS inhibition.

3.5 | Microbiological Evaluation

3.5.1 | Mesophilic Bacteria Count

The inherent chemical composition of ostrich meat makes it highly susceptible to microbial spoilage during storage, as evidenced by a sharp increase in mesophilic bacterial counts in uncoated samples, reaching 8.55 log cfu/g by day 12. In contrast, samples coated with SA–based formulations, particularly those incorporating EONs, demonstrated significantly reduced microbial loads ($p < 0.05$), with the SA/5% EON treatment (F4) showing the most substantial inhibition (1.42 log cfu/g). According to the international microbiological standards for fresh meat, the acceptable limit for total mesophilic count is generally considered to be 7 log CFU/g, beyond which meat is regarded as microbiologically spoiled and unacceptable for consumption [56, 57]. As reported in Table 3, the mesophilic count in control and plain SA treated samples was higher than 7 log cfu/g while other samples' count was within the standard range. The population of mesophils was the lowest in F4 and the highest in control. This enhanced antimicrobial performance appears to stem from the synergistic relation between the bioactive inrdients in the EO and the barrier properties of the biopolymer matrix. Our results are supported by those of Hu et al. [44] demonstrated that alginate coatings with cinnamon EO microcapsules inhibited bacterial growth in chicken meat. Borhani et al. [58] reported the superior antibacterial activity of SA–thymol nanoemulsion coatings in reducing microbial counts in chilled chicken. Moura-Alves et al. [59] confirmed that SA films incorporated with olive leaf extracts and laurel effectively prolonged the shelf life of beef by limiting microbial growth. The oxygen-limiting properties of biopolymer coatings may contribute to the observed bacterial growth inhibition. These researches support our work's results and reinforce the potential of SA coatings combined with zulang EONs as an effective method to improve the microbial safety and quality of ostrich meat during refrigerated storage.

3.5.2 | Psychrotrophic Bacteria

At low temperatures, gram-negative psychrotrophic bacteria are recognized as the primary agents of meat spoilage. According to international standards, counts above 6–7 log cfu/g for psychrotrophic bacteria are associated with spoilage during refrigerated storage [56, 57]. In this study, a significant reduction in psychrotrophic bacterial counts was observed in ostrich meat samples coated with SA enriched with 2.5% and 5% zulang EON, particularly on days 3, 6, and 9 of refrigerated storage (Table 4). These counts were approximately 6 log cfu/g lower than those in the control and SA-only samples, with the greatest antibacterial effect recorded in treatments F3 and F4 on day 12 ($p < 0.05$). In contrast, no significant difference was found between the F1 group (SA without EO) and the control, suggesting that SA alone does not possess strong antimicrobial activity. The results of this study confirm that SA–based edible coatings enriched with EONs can significantly improve the microbial stability of meat products

TABLE 3 | Changes in mesophilic population (log cfu/g) in ostrich meat coated with sodium alginate and nanoemulsion of zulang essential oil during storage in refrigerator.

	Time (day)				
Treatments	**0**	**3**	**6**	**9**	**12**
Control	2.64 ± 0.03^{Aa}	4.01 ± 0.10^{Ba}	5.80 ± 0.00^{Ca}	6.65 ± 0.01^{Da}	8.55 ± 0.09^{Ea}
Alginate	2.64 ± 0.03^{Aa}	3.80 ± 0.15^{Bb}	5.45 ± 0.05^{Cb}	6.03 ± 0.15^{Cb}	8.20 ± 0.07^{Db}
Alginate + 1% EO	2.64 ± 0.03^{Aa}	2.90 ± 0.13^{Bb}	2.65 ± 0.10^{Cc}	2.68 ± 0.29^{Cc}	5.03 ± 0.05^{Bc}
Alginate + 2.5% EO	2.64 ± 0.03^{Aa}	2.32 ± 0.03^{Bc}	2.16 ± 0.09^{Bd}	2.11 ± 0.20^{Bd}	2.08 ± 0.11^{Bd}
Alginate + 5% EO	2.64 ± 0.03^{Aa}	1.68 ± 0.10^{Bd}	1.51 ± 0.21^{Ce}	1.46 ± 0.22^{Ce}	1.42 ± 0.05^{Ce}

Note: Data are presented as mean ± SD. Different lowercase superscript letters (a–e) in the same column indicate significant differences between treatments at the same storage time ($p < 0.05$). Different uppercase superscript letters (A–E) in the same row indicate significant differences during storage within the same treatment ($p < 0.05$), according to two-way ANOVA, followed by Tukey's test.

TABLE 4 | Changes in psychrotrophic population (log cfu/g) in ostrich meat coated with sodium alginate and nanoemulsion of zulang essential oil during storage in refrigerator.

	Time (day)				
Treatments	**0**	**3**	**6**	**9**	**12**
Control	2.38 ± 0.12^{Aa}	4.35 ± 0.10^{Ba}	5.40 ± 0.15^{Ca}	6.62 ± 0.07^{Da}	7.52 ± 0.05^{Ea}
Alginate	2.38 ± 0.12^{Aa}	4.16 ± 0.08^{Bb}	5.60 ± 0.11^{Cb}	6.46 ± 0.20^{Db}	7.41 ± 0.17^{Eb}
Alginate + 1% EO	2.38 ± 0.12^{Aa}	3.51 ± 0.09^{Bc}	4.97 ± 0.30^{Cc}	5.23 ± 0.10^{Dc}	6.60 ± 0.14^{Ec}
Alginate + 2.5% EO	2.38 ± 0.12^{Aa}	2.13 ± 0.38^{Bd}	1.93 ± 0.05^{Cd}	1.75 ± 0.24^{Dd}	1.58 ± 0.01^{Dd}
Alginate + 5% EO	2.38 ± 0.12^{Aa}	2.09 ± 0.06^{Bd}	1.76 ± 0.10^{Cd}	1.36 ± 0.15^{De}	1.14 ± 0.20^{Ee}

Note: Data are presented as mean ± SD. Different lowercase superscript letters (a–e) in the same column indicate significant differences between treatments at the same storage time ($p < 0.05$). Different uppercase superscript letters (A–E) in the same row indicate significant differences during storage within the same treatment ($p < 0.05$), according to two-way ANOVA, followed by Tukey's test.

during refrigerated storage. The incorporation of zulang EO into the alginate matrix exhibited strong antimicrobial effects, particularly against psychrotrophic bacteria. Hu et al. [44] reported that SA coatings containing cinnamon EO microcapsules effectively reduced bacterial growth on poultry meat and extended its shelf life. Similarly, Borhani et al. [58] demonstrated that SA–thymol nanoemulsions significantly inhibited spoilage microorganisms in chilled chicken meat. Ghafari et al. [60] also showed that a nanochitosan–tragacanth gum composite film enriched with zulang EO effectively suppressed psychrotrophic bacterial growth in goat meat. Together, these studies highlight the synergistic antimicrobial effects of combining biopolymer coatings with EOs and support the use of such formulations for improving the microbial safety and shelf life of meat products during cold storage.

3.5.3 | Enterobacteriaceae Count

Enterobacteriaceae counts exceeding 4 log cfu/g indicate poor hygienic quality and potential safety concerns [56, 57]. In the present study (Table 5), the control sample and samples coated with plain SA exceeded these limits during storage, whereas samples coated with SA containing EON remained below the microbial acceptability thresholds for a significantly longer period ($p < 0.05$). The antimicrobial efficacy increased in the order F2 < F3 < F4. Notably, formulations F3 (2.5% EO) and F4 (5% EO) delivered the most potent inhibition, the latter demonstrating significantly stronger effects ($p < 0.05$), suggesting that 5% EO provides maximum antimicrobial activity. These results align with recent studies demonstrating the effectiveness of SA/EO composite coatings in limiting Enterobacteriaceae proliferation in meat. For instance, pullulan/SA films embedded with thyme EO microemulsions effectively reduced bacterial growth in chilled pork over 10 days at 4°C [61]. Similarly, SA coatings incorporating oregano EO via zein–Arabic gum nanoparticles significantly inhibited microbial growth, including Enterobacteriaceae, in chilled pork [62]. Moreover, alginate films containing cinnamon and rosemary EOs markedly suppressed Enterobacteriaceae counts in chicken meat throughout 15 days of refrigerated storage [63]. It was confirmed that alginate-based edible coatings combined with EONs represent an effective, natural strategy for controlling spoilage bacteria and enhancing meat safety during cold storage.

3.6 | Drip Loss

At the first day of the experiment, the drip loss of fresh ostrich thigh meat was obtained around 5%, but over the storage period, the control samples showed a significant increase in drip loss ($p < 0.05$), whereas samples coated with SA coatings containing EONs exhibited notably lower moisture loss, particularly those with 2.5% and 5% EO (Figure 6). In contrast, samples treated with pure SA (without EO) showed a minor reduction in drip loss compared to the control, confirming the role of EO in enhancing water retention. By day 12, EO–enriched coatings consistently exhibited significantly lower drip loss than earlier

TABLE 5 | Changes in Enterobacteriaceae population (log cfu/g) in ostrich meat coated with sodium alginate and nanoemulsion of zulang essential oil during storage in refrigerator.

	Time (day)				
Treatments	**0**	**3**	**6**	**9**	**12**
Control	1.11 ± 0.05^{Aa}	2.76 ± 0.20^{Ba}	4.59 ± 0.08^{Ca}	5.12 ± 0.18^{Da}	7.15 ± 0.32^{Ea}
Alginate	1.11 ± 0.05^{Aa}	2.43 ± 0.16^{Bb}	4.54 ± 0.11^{Cb}	5.07 ± 0.09^{Db}	6.95 ± 0.25^{Eb}
Alginate + 1% EO	1.11 ± 0.05^{Aa}	1.80 ± 0.13^{Bc}	2.15 ± 0.22^{Cc}	3.41 ± 0.10^{Dc}	4.02 ± 0.12^{Ec}
Alginate + 2.5% EO	1.11 ± 0.05^{Aa}	1.09 ± 0.06^{Ad}	1.65 ± 0.19^{Ad}	1.95 ± 0.07^{Bd}	2.08 ± 0.01^{Bd}
Alginate + 5% EO	1.11 ± 0.05^{Aa}	1.07 ± 0.10^{Ad}	1.34 ± 0.00^{Bd}	1.78 ± 0.07^{Bd}	1.90 ± 0.00^{Cd}

Note: Data are presented as mean ± SD. Different lowercase superscript letters (a–e) in the same column indicate significant differences between treatments at the same storage time ($p < 0.05$). Different uppercase superscript letters (A–E) in the same row indicate significant differences during storage within the same treatment ($p < 0.05$), according to two-way ANOVA, followed by Tukey's test.

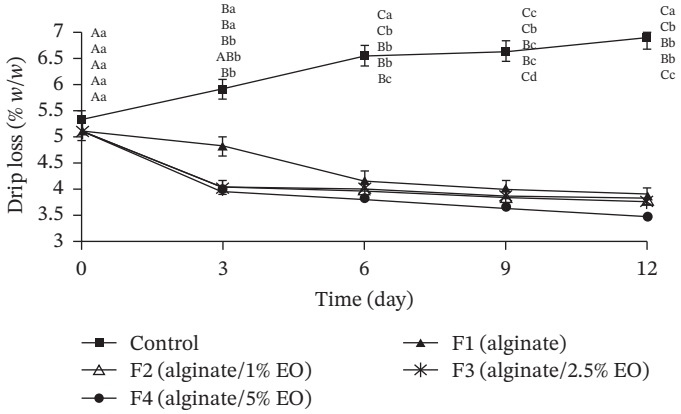


FIGURE 6 | Drip loss percentage of the ostrich thigh meat coated with sodium alginate and nanoemulsion of zulang essential oil during storage in refrigerator. Different lowercase letters (a–e) in the same column indicate significant differences between treatments at the same storage time ($p < 0.05$). Different uppercase letters (A–E) in the same row indicate significant differences during storage within the same treatment ($p < 0.05$), according to two-way ANOVA, followed by Tukey's test.

time points (days 3, 6, and 9; $p < 0.05$), underscoring their sustained effectiveness in preserving muscle integrity and water-holding capacity. These observations are consistent with several recent studies on SA/EO coatings. For example, Zhang et al. [48] reported that SA coatings with cinnamon EO nanocapsules and nisin reduced weight loss and improved texture retention in refrigerated beef slices [48]. Likewise, Cao et al. [62] found that SA films embedded with oregano EO via zein–Arabic gum nanoparticles significantly decreased water loss and delayed spoilage in chilled pork [62]. In another study, alginate-based edible coatings containing oregano and rosemary EOs demonstrated reduced water loss and lipid oxidation in lamb patties during refrigerated display [64]. Our findings together with the reports above indicate that SA has excellent moisture-holding capacity due to its hydrophilic and film-forming properties, which help reduce water evaporation from meat surfaces. When applied as an edible coating, alginate creates a semipermeable barrier that retains internal moisture and minimizes drip loss. This protective layer preserves the texture and juiciness of meat during storage. Additionally, combining alginate with EOs enhances its ability to maintain water retention while offering antimicrobial benefits [65].

3.7 | Texture

Shear force analysis is a common method for evaluating meat tenderness, where lower shear values indicate a softer and more desirable texture. In the current study, the initial shear force of ostrich meat samples was about 65.5 N mm, and this value significantly decreased over the storage period in all treatments ($p < 0.05$). Coated samples, especially those treated with SA combined with 2.5% and 5% zulang EON (F3 and F4), demonstrated a more pronounced reduction in firmness compared to the control group, suggesting enhanced tenderness ($p < 0.05$). This softening effect may be related to better moisture retention and slower protein degradation in the coated meat. Similar achievements have been reported by Zhang et al. [48], who showed that beef treated with SA coatings enriched with cinnamon EO nanocapsules retained better texture quality during storage. Cao et al. [62] also observed improved tenderness in pork coated with alginate films containing oregano EO–loaded nanoparticles, attributing the effect to reduced moisture loss and inhibition of structural breakdown. SA coatings help maintain meat tenderness by reducing moisture loss and forming a protective barrier against oxidative and microbial degradation. This moisture retention prevents the drying and toughening of muscle fibers. Additionally, alginate can create a humid microenvironment that slows down protein and collagen breakdown, helping preserve the meat's natural softness and tenderness during storage [66].

3.8 | Sensory Scores

The sensory assessment of the samples coated with SA and nanoemulsions of zulang EON showed noticeable differences over the 12-day refrigerated storage period (Table 6). Initially, all treatments had high odor scores, with the SA/5% EO group recording the highest (9.0 ± 0.00), matching the pleasant aroma of the control. As storage progressed, odor scores declined across all samples, but the control dropped more sharply, reaching 6.5 ± 0.01 by day 12, while SA/5% EO maintained a higher score of 8.0 ± 0.01. This suggests that incorporating EO into SA coatings helped preserve freshness, likely due to its antimicrobial and antioxidant effects [45]. A similar trend was found for flavor: the SA/5% EO group started with a peak score of 9.0 ± 0.00 and sustained superior flavor throughout storage, whereas the control's score dropped to 7.0 ± 0.02 by day 12, reflecting potential spoilage. EO's bioactive compounds likely contributed to this flavor preservation by minimizing microbial degradation. In terms of color, all samples began with high scores (around 9.0), but the SA/EO–coated meats—especially those with 2.5% and 5% EO—retained better color (8.0 on day 12), while the control declined to 6.5 ± 0.01, indicating the coatings delayed pigment oxidation [67]. Tenderness scores in the control rose from 5.5 ± 0.00 to 7.5 ± 0.01, while the SA/5% EO samples reached 9.0 ± 0.01 by the end of storage, supporting instrumental data from shear force measurements (Figure 7). The greater tenderness may result from better moisture retention provided by the SA matrix, which helps preserve meat texture [45]. Finally, overall acceptability of the consumers mirrored the other sensory traits, with the SA/5% EO group maintaining the highest ratings (from 8.5 ± 0.00 to 7.5 ± 0.01), while the control group's acceptability dropped significantly. These findings indicate that the

TABLE 6 | Changes in sensory attributes of ostrich meat coated with sodium alginate and nanoemulsion of zulang essential oil during storage in refrigerator.

	Time (day)				
Treatments	**0**	**3**	**6**	**9**	**12**
Odor					
Control	9.0 ± 0.00^{Aa}	9.0 ± 0.03^{Aa}	8.0 ± 0.00^{Bb}	7.5 ± 0.00^{Ca}	6.5 ± 0.01^{Da}
Alginate	9.0 ± 0.00^{Aa}	8.5 ± 0.00^{Ba}	8.0 ± 0.00^{Ca}	7.5 ± 0.01^{Da}	6.5 ± 0.00^{Eb}
Alginate + 1% EO	9.0 ± 0.02^{Ab}	9.0 ± 0.02^{Ab}	8.5 ± 0.01^{Bb}	8.0 ± 0.01^{Cb}	7.0 ± 0.04^{Dc}
Alginate + 2.5% EO	9.0 ± 0.00^{Ac}	9.0 ± 0.00^{Ac}	8.5 ± 0.01^{Bc}	8.0 ± 0.00^{Cc}	7.5 ± 0.00^{Dd}
Alginate + 5% EO	9.0 ± 0.00^{Ac}	9.0 ± 0.00^{Ac}	9.0 ± 0.00^{Ad}	8.5 ± 0.00^{Bd}	8.0 ± 0.03^{Ce}
Flavor					
Control	8.0 ± 0.03^{Aa}	8.0 ± 0.02^{Aa}	7.5 ± 0.01^{Ba}	7.0 ± 0.00^{Ca}	7.0 ± 0.02^{Ca}
Alginate	8.0 ± 0.00^{Aa}	8.0 ± 0.00^{Aa}	8.0 ± 0.05^{Ab}	7.5 ± 0.02^{Bb}	7.0 ± 0.01^{Ca}
Alginate + 1% EO	8.5 ± 0.02^{Ab}	8.5 ± 0.01^{Ab}	8.5 ± 0.01^{Ac}	7.5 ± 0.00^{Bb}	7.0 ± 0.00^{Ca}
Alginate + 2.5% EO	8.5 ± 0.01^{Ab}	9.0 ± 0.00^{Cc}	9.0 ± 0.01^{Cd}	9.0 ± 0.01^{Cc}	8.0 ± 0.00^{Cb}
Alginate + 5% EO	8.5 ± 0.00^{Ab}	9.0 ± 0.00^{Bc}	9.0 ± 0.00^{Bd}	9.0 ± 0.00^{Bc}	9.0 ± 0.00^{Bc}
Color					
Control	9.0 ± 0.00^{Aa}	8.0 ± 0.00^{Ba}	7.5 ± 0.00^{Ca}	7.0 ± 0.00^{Da}	6.5 ± 0.05^{Ea}
Alginate	9.0 ± 0.00^{Aa}	8.5 ± 0.02^{Bb}	8.0 ± 0.04^{Cb}	7.5 ± 0.01^{Db}	7.0 ± 0.01^{Eb}
Alginate + 1% EO	9.0 ± 0.00^{Aa}	8.5 ± 0.01^{Bb}	8.0 ± 0.00^{Cc}	7.5 ± 0.00^{Db}	7.5 ± 0.00^{Db}
Alginate + 2.5% EO	9.0 ± 0.00^{Aa}	9.0 ± 0.00^{Ac}	8.5 ± 0.01^{Bd}	8.5 ± 0.02^{Bc}	8.0 ± 0.02^{Cc}
Alginate + 5% EO	9.0 ± 0.00^{Aa}	9.0 ± 0.0^{Ac}	8.5 ± 0.00^{Bd}	8.5 ± 0.00^{Bc}	8.0 ± 0.00^{Cc}
Tenderness					
Control	5.5 ± 0.00^{Aa}	5.5 ± 0.00^{Aa}	6.0 ± 0.01^{Ba}	6.5 ± 0.0^{Ca}	7.5 ± 0.00^{Da}
Alginate	6.5 ± 0.00^{Ab}	7.5 ± 0.01^{Bb}	7.5 ± 0.05^{Bb}	8.0 ± 0.01^{Cb}	8.0 ± 0.00^{Cb}
Alginate + 1% EO	7.0 ± 0.01^{Ac}	7.5 ± 0.00^{Bb}	7.5 ± 0.00^{Bb}	8.0 ± 0.00^{Cb}	8.0 ± 0.02^{Cb}
Alginate + 2.5% EO	7.5 ± 0.01^{Ad}	8.0 ± 0.04^{Bc}	8.0 ± 0.0^{Bc}	8.0 ± 0.00^{Bb}	8.5 ± 0.00^{Cc}
Alginate + 5% EO	8.0 ± 0.01^{Ae}	8.5 ± 0.00^{Bd}	8.5 ± 0.00^{Bd}	9.0 ± 0.00^{Cc}	9.0 ± 0.00^{Cd}
Overall acceptability					
Control	7.5 ± 0.00^{Aa}	7.0 ± 0.01^{Ba}	6.0 ± 0.0^{Ca}	5.5 ± 0.00^{Da}	5.5 ± 0.00^{Da}
Alginate	8.0 ± 0.00^{Ab}	7.5 ± 0.00^{Bb}	7.0 ± 0.00^{Cb}	6.5 ± 0.01^{Db}	5.0 ± 0.00^{Ea}
Alginate + 1% EO	8.5 ± 0.00^{Ac}	7.5 ± 0.04^{Bb}	7.5 ± 0.02^{Bc}	7.0 ± 0.00^{Cc}	6.5 ± 0.05^{Db}
Alginate + 2.5% EO	8.5 ± 0.00^{Ac}	8.0 ± 0.02^{Bc}	8.0 ± 0.05^{Bd}	7.5 ± 0.00^{Cd}	7.0 ± 0.02^{Dc}
Alginate + 5% EO	8.5 ± 0.00^{Ac}	8.5 ± 0.00^{Ad}	8.5 ± 0.00^{Ae}	7.5 ± 0.01^{Bd}	7.5 ± 0.01^{Bd}

Note: Data are presented as mean ± SD. Different lowercase superscript letters (a–e) in the same column indicate significant differences between treatments at the same storage time ($p < 0.05$). Different uppercase superscript letters (A–E) in the same row indicate significant differences during storage within the same treatment ($p < 0.05$), according to two-way ANOVA, followed by Tukey's test.

incorporation of EON not only delayed microbial and oxidative spoilage but also preserved overall acceptability which is directly perceived by consumers. The absence of undesirable off-flavors at effective concentrations suggests that the coating system could be acceptable in real market conditions. Therefore, this approach has promising potential for consumer-friendly preservation of ostrich meat without negatively affecting sensory quality.

Although the nanoemulsion levels incorporated into the alginate solution were 1%, 2.5%, and 5% (*v*/*v*), it is important to clarify that these values do not represent the actual EO concentration applied to the meat surface. The nanoemulsion itself contains only a small fraction of EO, and it was further diluted within a 2% (*w*/*v*) alginate matrix. Therefore, the final EO content in contact with the meat was considerably lower than 1%. This low

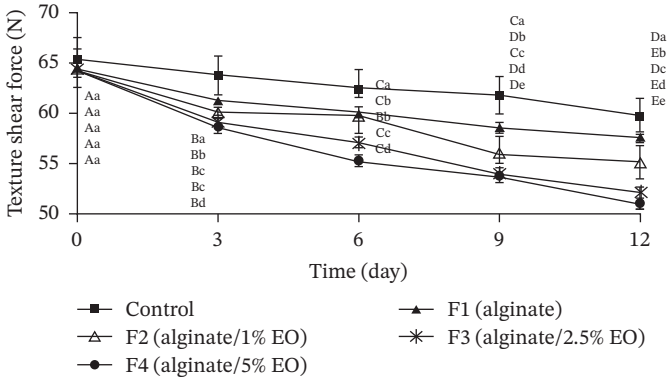


FIGURE 7 | Texture shear force of the ostrich thigh meat coated with sodium alginate and nanoemulsion of zulang essential oil during storage in refrigerator. Different lowercase letters (a–e) in the same column indicate significant differences between treatments at the same storage time ($p < 0.05$). Different uppercase letters (A–E) in the same row indicate significant differences during storage within the same treatment ($p < 0.05$), according to two-way ANOVA, followed by Tukey's test.

effective EO concentration, combined with the controlled release provided by the nanoemulsion–alginate system, minimizes potential sensory and safety concerns while maintaining preservative efficacy. This is further supported by the sensory evaluation results above, where coated samples remained acceptable throughout storage.

In our previous study [15], a chitosan-based coating containing *E. campestre* EON was used to preserve ostrich meat, while the present study evaluates the same EON embedded in a SA matrix. Although both biopolymers improved meat preservation by reducing microbial load, lipid oxidation, and quality degradation during refrigeration, some differences were observed. Notably, the chitosan-based coating showed stronger initial antimicrobial activity, likely due to chitosan's inherent positive charge and known antimicrobial properties. In contrast, alginate coatings demonstrated greater effectiveness in moisture retention and texture preservation, which may stem from their hydrophilic nature and higher water-binding capacity. While both systems exhibited similar trends in TVB-N, FFA, and TBARS reduction, the alginate + EON coating offered more uniform sensory stability and better retention of tenderness overtime. These findings suggest that alginate is more effective in preserving physical and sensory attributes, whereas chitosan offers enhanced microbial control, especially in the early stages of storage. This comparative perspective highlights the importance of selecting coating materials based on the specific preservation goals and product characteristics.

3.9 | Antimicrobial and Antioxidant Mechanisms

Although a direct comparison between free EO and nanoemulsified EO was not performed in this study, previous research has demonstrated that nanoemulsion systems significantly enhance the functional performance of EOs in food preservation [68, 69]. Therefore, the use of nanoemulsified *E. campestre* EO in the present work was based on this established scientific rationale. The antimicrobial and antioxidant effects observed in this study can be explained by the combined action of the EO constituents, nanoemulsion characteristics, and the barrier properties of SA. EOs are rich in hydrophobic terpenes and oxygenated sesquiterpenes such as germacrene D, bisabolol, and caryophyllene oxide, which are known to interact with microbial cell membranes. These compounds penetrate the phospholipid bilayer, disrupt membrane integrity, increase permeability, and cause leakage of intracellular components, ultimately leading to microbial cell death [70, 71]. From an oxidative perspective, these bioactive compounds act as hydrogen or electron donors, neutralizing free radicals and interrupting the lipid oxidation chain reaction, thereby reducing the formation of primary (peroxides) and secondary (MDA) oxidation products, as reflected in the lower PV and TBARS values [68, 72].

The nanoemulsion system further enhances these effects by reducing droplet size to the Nanometer range, which increases surface area and improves the dispersion, stability, and interaction of EO compounds with microbial cells and lipid substrates. This facilitates a more efficient delivery of active compounds compared to coarse emulsions or free EO [68, 69]. In addition, the SA coating acts as a semi-permeable barrier to oxygen and moisture transfer, limiting oxygen availability at the meat surface and slowing oxidative reactions while also restricting microbial access to nutrients [66]. Therefore, the preservation effect observed in this study is the result of a synergistic mechanism involving chemical (EO activity), physical (alginate barrier), and colloidal (nanoemulsion delivery) factors.

3.10 | Industrial Applicability, Regulatory Considerations, and Cost-Effectiveness

From an industrial perspective, the application of SA coatings containing EONs presents a feasible strategy for meat preservation. Alginate is already widely used in the food industry due to its low cost, availability, biodegradability, and GRAS status. The coating process used in this study (simple immersion) is compatible with existing meat processing lines and does not require sophisticated equipment, making scale-up technically achievable.

Regarding cost-effectiveness, although EOs may appear expensive, the nanoemulsion system allows the use of relatively low concentrations while maintaining high antimicrobial and antioxidant efficacy. Moreover, the extension of shelf life, reduction of spoilage losses, and potential replacement of synthetic preservatives can economically compensate for the additional cost of EO incorporation.

From a regulatory standpoint, the use of EOs in edible coatings for meat products must comply with national and international food safety regulations. Many EOs and alginate are classified as safe food additives; however, approval for specific applications, concentration limits, and labeling requirements may vary across countries. Therefore, before commercial implementation, regulatory evaluation and sensory acceptability studies at industrial scale are recommended.

3.11 | Study Limitations and Future Perspectives

Despite the promising findings, this study has certain limitations. The experiments were conducted under laboratory-scale conditions, and industrial validation was not performed. Additionally, only one type of meat cut (ostrich thigh) and one storage

temperature were evaluated. The long-term sensory perception of EO coatings and consumer acceptance were not assessed beyond trained panel evaluation. Also, the migration of EO compounds into the meat matrix was not quantified using analytical techniques such as GC–MS. Future research should focus on pilot-scale trials in real meat processing environments, evaluation of different meat types, and detailed cost–benefit analyses. Investigating the synergistic effects of EONs with other natural preservatives and studying consumer acceptance will further support the practical implementation of this preservation approach in the food industry.

4 | Conclusions

In this work, the effect of SA combined with a nanoemulsion of zulang EO on the quality of refrigerated ostrich thigh meat was investigated in this study. The developed formulation significantly enhanced antioxidant capacity, reduced lipid oxidation, and effectively kept the meat's physicochemical and microbiological integrity during storage, indicating its potential as a green substitute to chemical preservatives in the poultry industry. Our findings demonstrated that edible coatings composed of SA enriched with EON provided stronger preservation effects than SA alone. Treatments containing 2.5% and 5% EONs showed marked improvements in extending shelf life, preserving texture, sensory attributes, and minimizing drip loss. These coated samples exhibited notably lower free FFA, PV, TVB-N, and microbial loads compared to those coated with pure SA or left untreated. The preservation effectiveness ranked as follows: SA with 5% EON > 2.5% EON > 1% EON > SA alone. It can be suggested that incorporating EON into the SA matrix enhances its interaction with the meat surface, promoting more uniform distribution of active compounds and boosting overall preservation performance.

4.1 | Transparency and Ethical Compliance Statement

The authors confirm that this manuscript is an original work and has not been published elsewhere. A prior study by the authors investigated the effect of *Eryngium campestre* EON in a chitosan-based coating on ostrich meat preservation [15]. In the present study, we examine the same EON within a SA matrix, offering a novel scientific perspective on matrix-dependent preservation effects. All experimental data in this manuscript were generated independently. Any reused background data (e.g., EO composition or meat nutritional profile) are properly cited. This disclosure is provided to ensure adherence to ethical publishing practices and to maintain full transparency. Also, the meat samples used in this study were obtained from a commercial slaughterhouse as part of the routine food production chain. No animals were sacrificed specifically for this research. All procedures complied with national regulations for animal welfare and food hygiene.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available upon request from the corresponding author.

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