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The Effect of Nanoliposomes of *Achillea millefolium* Essential Oil on the Shelf Life of Fresh Beef at Refrigerated Storage

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ABSTRACT

Nanoencapsulation of essential oils (EOs) improves their solubility, stability, and bioavailability. This research aimed to prepare nanoliposomes containing 1% and 2% yarrow (*Achillea millefolium*) EO and their effect on the shelf life of fresh beef at refrigerated storage. Nanoliposome properties, including encapsulation efficiency, particle size, zeta potential, morphology, Fourier transform infrared (FTIR) spectroscopy, and thermal properties, were evaluated. The nanoliposomes were characterized by an appropriate nanosize scale (91.3–139 nm) and a negative charge (−27.35 mV to −22.64 mV) that successfully encapsulated the EO with high efficiency (> 86%). Scanning electron microscopy (SEM) showed a homogeneous structure in which the EO was well dispersed in the nanoliposome matrix. The FTIR spectrum indicated the successful incorporation of the EO into the nanoliposomes without changing their chemical structure. Differential scanning calorimetry (DSC) findings confirmed that the glass transition (T_g) and melting (T_m) temperatures increased about 2°C with nanoliposomes, resulting in an improvement in thermal stability. The nanoliposomes containing 2% yarrow EO (NLP-2% EO) with the smallest particle size and highest encapsulation efficiency, zeta potential, antioxidant properties, and thermal stability were selected as the best treatment and used to preserve fresh beef for 10 days. During the meat storage, pH values, total volatile nitrogen (TVB-N), thiobarbituric acid reactive substances (TBARS), and total mesophilic and psychrophilic bacteria increased significantly ($p < 0.05$), indicating meat spoilage. Regarding chemical and microbial indices, the control treatment (C) and the treatment containing free EO were acceptable for less than 5 days and 5–10 days, respectively, while the incorporation of EO into nanoliposomes (NLP-EO) extended the shelf life of fresh beef up to 10 days at 4°C. Therefore, the encapsulated yarrow EO in nanoliposomes was suggested for beef preservation.

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1 | Introduction

Meat is a valuable source of high biological value protein, minerals, and vitamins, which play an important role in human health [1].

Fresh meat is highly perishable due to its high water activity and fat content. Meat spoilage is mainly caused by microbial spoilage and lipid oxidation can lead to quality defects such as discoloration, sour taste, decrease in tenderness, and pH changes, ultimately leading to consumer rejection and economic losses [2]. The primary method for detecting meat spoilage is microbiological testing by counting the total bacteria or specific spoilage organisms. Sensory analyses are based on discoloration, off-odor, and sliminess. Off-odor is attributed to the accumulation of volatile compounds, especially sulfur-containing compounds, biogenic amines, and other low-molecular-weight volatile compounds, which are mainly produced by microbial activities [3].

The chemical antimicrobials as additives and preservatives to enhance the shelf life of meat have a negative impact on public health, especially in the long term. Therefore, in recent years, there has been increasing interest in plant bioactive compounds due to their potential to reduce the risk of some diseases such as cancer, cardiovascular diseases, and neurological disorders. Plants can be used in the food industry as a major source of functional and bioactive substances such as vitamins, antioxidants, minerals, and phytochemicals, that is, essential oils (EOs), flavonoids, phenols, glycosides, alkaloids, tannins, organic acids, coumarins, and saponins, which are considered antioxidants [4].

In addition, special attention has been paid to plants that are rich sources of natural antioxidants and antimicrobials. One of these plants is yarrow (*Achillea millefolium*), which belongs to the Asteraceae family and is native to Europe, North America, and Asia. The genus *Achillea* contains more than 100 species in the world and 19 species in Iran, which are mostly aromatic [5]. Achillea has been used in popular medicine for retarding the growth of cancer cells, analgesic, anti-inflammatory, and antidiabetic effects [6]. Yarrow EO, as a natural antioxidant and preservative, can prevent the oxidation and growth of pathogenic and spoilage bacteria *in vitro* conditions [5] or in food matrices [7–10].

Although yarrow EO extends the shelf life of food by preventing oxidation and microbial growth, its direct incorporation into food is challenging due to high volatility, water insolubility, low absorption in the gastrointestinal tract, sensitivity to environmental conditions such as light, pH, and temperature, and strong organoleptic properties. Therefore, its applications may be limited. These problems can be overcome by encapsulation [2]. Encapsulation is a popular way to increase bioavailability, stability, and protection of bioactive agents. The encapsulated compounds can be used as functional agents in food products. Furthermore, the antimicrobial effect of encapsulated EO is greater than that of free EO [10, 11].

Some factors such as insolubility, unpleasant taste, and astringency prevent the applications of polyphenols. Therefore, encapsulation can be a beneficial way of masking the unacceptable and distinctive flavor, as well as preserving the bioavailability and stability of these volatile ingredients. Encapsulation can also protect the polyphenolic compounds from internal and external degradation and retard the chemical reactions. Additionally, encapsulation techniques significantly affect the stability, encapsulation efficiency (EE), morphology, and release mechanism of the encapsulated phenols [12].

Studies have shown that encapsulation protects yarrow phenolics from degradation during gastrointestinal digestion and enhances their delivery to the colon [13]. Encapsulation of yarrow EO does indeed enhance its antibacterial activity against *S. aureus* and *E. coli* compared to using the free EO and can protect the active components of yarrow EO from the damaging effects of sunlight [10, 14].

Nikzadeh et al. [15] confirmed that the nanoencapsulated *Mentha pulegium* EO enhances the preservation of meat quality. Gorzin et al. [16] successfully encapsulated *Oliveria decumbens* and basil EOs using maltodextrin/gum Arabic and revealed that the encapsulated EOs enhanced beef quality.

One of the encapsulation methods is the lipid-based carrier systems, including nanoliposomes. Liposomes, with particle sizes ranging from 10 nm to several micrometers, are composed of spherical vesicles with a phospholipid bilayer. They enhance the bioavailability of bioactive compounds through their bilayer morphology. In addition, van der Waals interactions and hydrogen bonding between phospholipids and water molecules provide unique functionality for carrying hydrophobic and hydrophilic molecules [17].

Given that the advantages of application of yarrow EO are demonstrated in various studies [5–10], it seems that the development of yarrow EO-loaded nanocarriers can improve its effects [11].

Emtiazi et al. [11] studied the preparation and characterization of nanoliposomes/nanoniosomes containing *A. millefolium* EOs and concluded that the lipid-based nanosystems show promise as carriers for EOs, potentially enhancing their effectiveness in treating breast cancer and exhibiting antimicrobial properties.

Although the antimicrobial and antioxidant properties of *A. millefolium* EO have been previously reported, its encapsulation into nanoliposomes and application for meat preservation have not yet been investigated. Therefore, the present study is the first to develop and characterize yarrow EO-loaded nanoliposomes and evaluate their effectiveness in extending the shelf life of meat products.

2 | Materials and Methods

2.1 | Materials

Fresh beef was purchased from a local market (Isfahan, Iran). Lecithin and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich Co. (UK). All chemicals and microbial culture media were purchased from Merck Co. (Darmstadt, Germany).

2.2 | Yarrow EO Extraction

A. millefolium was obtained from the Medicinal Plants Center (Isfahan, Iran), and after approval by the Botanical Herbarium, dried and powdered in a miller (Mulinex, Spain); 100 g of powder was extracted with a Clevenger apparatus (Ashk Shishe, Iran) for 4 h, yielding 0.86%. The EO was stored in dark and sealed vials after dehydration with sodium sulfate [5, 10].

2.3 | Yarrow EO Encapsulation

The EO-loaded nanoliposomes (NLP-EO) were prepared at different EO concentrations (1% and 2% w/v) and soy lecithin (2% w/v). First, the required amounts of EO and lecithin were weighed and dissolved in a solution of dichloromethane/methanol (1:1) to create a homogeneous solution. The mixtures were stirred for 30 min at 30°C (1000 rpm). Next, a rotary evaporator (Heidolph Hei-VAP, Germany) was used to separate the solvent (dichloromethane/methanol) at 30°C–50°C under vacuum conditions, then hydrated by adding 20 mL of deionized water and 3% v/v of glycerol, followed by the addition of 40 mL of 0.01 M phosphate buffer (pH 7.4). Then, the liposomal suspensions were subjected to sonication (1 min, 1 s on and 1 s off) using the probe sonicator (20 kHz, maximum nominal power 500 W, Helisher-up200s, Germany) at 80% of total power. During sonication, the temperature was controlled in a double-wall circulation cell at 30 ± 1.0°C. Subsequently, the suspension was centrifuged at 16,000 rpm for 15 min at room temperature to remove the aggregated particles, and the supernatant was collected. The empty nanoliposomes were also prepared using the same procedure without EO. The prepared formulations were maintained at 4°C [12, 18].

2.4 | Nanoliposome Characterization

2.4.1 | EE

The suspension was centrifuged at 4200 × g for 20 min. The nonencapsulated particles were separated from the liposomal vesicle. The lower layer contained the encapsulated EO, and the upper layer contained the nonencapsulated EO; 1 mL of the supernatant was mixed with sodium carbonate (1 mL) and 5 mL of Folin Ciocalteu reagent (200 μL) for 5 min. The samples were incubated for 24 h. After filtration with Whatman filter paper (No.4), it was kept at room temperature for 24 h, then the absorbance was measured by a UV/Vis spectrophotometer (Jasco V-550, Japan) at 750 nm. The EE was calculated from equation (1) [12]:

$$\mathrm{EE} = \frac{\text{total phenols} - \text{phenol in nonencapsulated EO}}{\text{total phenols}} \times 100. \quad (1)$$

2.4.2 | Particle Size

Dynamic light scattering (DLS, Horiba sz-100, Japan) was used to determine the particle size distribution of nanoliposomes. The suspensions were diluted with distilled water (50:1), and the ζ-potential was determined using a zeta-sizer (Horiba sz-100, Japan) [12, 19].

2.4.3 | Antioxidant Properties

First, 100 μL of nanoliposomes containing yarrow EO were mixed with DPPH solution (2.5 mg of DPPH in 5 mL of methanol) and shaken for 30 min in a dark place. After that, the absorbance (A) was read at 517 nm using a UV/Vis spectrophotometer, and the percentage of the radical scavenging was calculated from equation (2). The methanolic DPPH solution was used as a blank [12].

$$\text{DPPH scavenging activity}(\%) = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100. \quad (2)$$

2.5 | Scanning Electron Microscopy (SEM)

The surface of the nanoliposome with and without EO was fixed on aluminum stubs with double adhesive tape and covered with a thin layer of gold-palladium on the SEM tubes (2 min) and was examined using a scanning electron microscope (Philips XL 30 FEG, the Netherlands) at 10 kV and in vacuum [12, 19].

2.6 | Differential Scanning Calorimetry (DSC)

The free EO and nanoliposomes containing 1% and 2% EO were placed in an aluminum pan, and a thermal program was performed from 30°C to 300°C at a heating rate of 10°C/min with a nitrogen flow of 15 mL/min. The glass transition (T_g), melting (T_m), and crystallization (T_c) temperatures were measured using DSC (Mettler Toledo, Switzerland). An unoccupied aluminum pan was considered as a reference [12].

2.7 | Fourier Transform Infrared (FTIR) Spectroscopy

The *FTIR spectra* of free EO and nanoliposomes containing 1% and 2% EO were recorded by an FTIR spectrometer (PerkinElmer Spectrum 65, USA) *using KBr* pellets in the range of 400–4000 cm^{-1} [12].

2.8 | Meat Treatment

The nanoliposomes containing 2% EO were selected as optimal based on the highest EE and smallest particle size. The fresh beef meat was cut into uniform portions (100 ± 1 g). Before treatment, the surface of the meat was blotted with sterile paper to remove excess moisture. The free EO and optimal nanoliposomes containing 2% EO (NLP-EO) were used for meat treatment. Each treatment was applied using a manual spray method to ensure uniform coating; 2 mL of each EO or original suspension of NLP-EO was sprayed directly onto the surface of the meat using a sterilized spray bottle. The solutions were applied evenly from a distance of approximately 10 cm to prevent localized pooling. After spraying, the meat samples were gently massaged for 30 s to ensure uniform distribution of the treatment over the whole surface. A sample without free or encapsulated EO was selected as a control (C). The samples were stored at 4°C, and their chemical and microbial properties were analyzed for 10 days (intervals 0, 5, and 10).

2.9 | Chemical Analysis of Meat

2.9.1 | pH

Beef samples (5 g) were homogenized in 50 mL of distilled water for 1 min, and pH values were measured with a pH meter (Metrohm, Switzerland).

2.9.2 | TVB-N

The Kjeldahl method was used to determine the TVB-N content in beef meat; 10 g of sample was mixed with 50 mL of distilled water, 2 g of magnesium oxide, and 2 drops of methyl red, and dissolved in 200 mL of distilled water. Then, hydrochloric acid (0.1 N) was titrated into the solution until the green color changed to pink. TVB-N was calculated from equation (3) [2, 15].

$$\mathrm{TVB-N} = \frac{\text{Amount of hydrochloric acid consumed} \times 1.4 \times 100}{\text{sample weight}}. \quad (3)$$

TABLE 1 | Physicochemical properties of nanoliposomes.

Nanoliposomes	EE (%)	Particle size (nm)	ζ-potential (mV)	PDI	DPPH (%)
NLP-1% EO	86.37 ± 0.11^{b}	139 ± 0.17^{a}	-27.35 ± 0.02^{b}	0.42 ± 0.12^{a}	86.18 ± 0.16^{a}
NLP-2% EO	92.81 ± 0.12^{a}	91.3 ± 0.13^{b}	-22.64 ± 0.07^{a}	0.29 ± 0.12^{b}	92.62 ± 0.14^{b}

Note: Mean ± SD. Different letters in the column indicate a significant difference ($p < 0.05$).

2.9.3 | TBARS

Briefly, 0.5 g of the beef meat was mixed with 5 mL of the thiobarbituric acid solution. The solution was placed in boiling water for 10 min to obtain a pink color and then centrifuged for 10 min at 7000 g. The absorbance of the solution was recorded at 532 nm, and TBARS was expressed as mg of malonaldehyde/kg of sample [2].

2.10 | Microbial Analysis of Meat

To assess bacterial counts, 10 g of beef meat was homogenized with 90 mL of sterile 0.1% peptone water under aseptic conditions to prepare a 1:10 dilution. Serial dilutions were performed, and microbial groups were enumerated [15].

The aerobic mesophilic bacteria were counted using Plate Count Agar (PCA, Merck, Germany) and incubated for 48 h at 37°C. Incubation for 7–10 days at 7°C was carried out to enumerate the psychrophilic bacteria, and the number of microorganisms was reported as log CFU/g [2].

2.11 | Statistical Analysis

All experiments were performed in triplicate ($n = 3$), and all data were presented as mean ± SD. The two-way analysis of variance (ANOVA) and Duncan's test were performed using SPSS software (ver. 22) at a 95% confidence level ($p < 0.05$).

3 | Results and Discussion

3.1 | Nanoliposome Characterization

The physicochemical properties of nanoliposomes are presented in Table 1. The highest EE was observed in the NLP-2% EO ($p < 0.05$). The high EE indicated the suitability of nanoliposomes for the encapsulation of yarrow EO. In other words, less than 8% of the EO was nonencapsulated. High EE affects particle size, oxidative stability, and morphology. Liposome membrane disruption, EO leakage, or capsule degradation likely decreased EE in the NLP-1% EO [20].

In agreement with our findings, nanoliposomes containing yarrow EO [11] and nettle extract [21] were produced with high EE > 80%. Mehaya et al. [22] stated the high EE (89.1%) for oregano EO using biopolymers.

The smallest and largest particle sizes belong to NLP-2% EO and NLP-1% EO, respectively ($p < 0.05$). The lower particle size improves EE and oxidative stability [23]. *Lower levels of oxidation products have been reported with decreasing particle size* [24].

Ahmadi et al. [12] reported that the particle size of tea extract nanoliposomes was 100 nm, which is close to the data obtained in the present study.

The smaller polydispersity index (PDI) in NLP-2% EO indicates a more homogeneous particle size distribution compared to NLP-1% EO ($p < 0.05$).

A high PDI (> 0.3) in NLP-1% EO indicates a heterogeneous size distribution, polydisperse system, and presence of multiple liposome populations (e.g., small unilamellar vesicles mixed with larger multilamellar vesicles or aggregates) [25].

In general, nanoliposomes with smaller droplet diameters and lower PDIs are more suitable for food shelf life because the EO can be dispersed more uniformly throughout the nanoliposomes.

The negative zeta potential (> −30 mV) observed for the nanoliposomes indicates desirable stability and good dispersion quality of yarrow EO nanoliposomes. This surface charge generates electrostatic repulsion between droplets, preventing aggregation and thereby improving the long-term stability of the nanoparticles [26].

The decrease in zeta potential was consistent with the increase in particle size. Auwal et al. [20] also reported lower zeta potential with larger particle size. The nanoliposomes of *A. millefolium* EO in the research of Emtiazi et al. [11] were characterized by nanosize and negative charge. Tea extract nanoliposomes had a zeta potential of −56 mV, a diameter of 82.20 nm, and a PDI of 0.69 [12]. The particle size and zeta potential of nanoliposomes containing nettle extract were reported to be 269.4 nm and −20.7 mV, respectively [21].

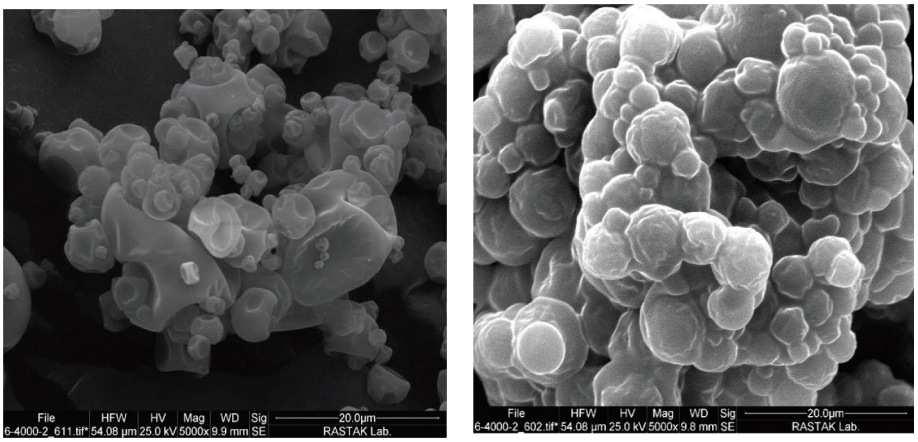
A direct relationship was observed between the concentration of yarrow EO and the radical scavenging effect of nanoliposomes, and with increasing EO concentration, the antioxidant effects increased ($p < 0.05$). This could be due to the presence of phenolic compounds of yarrow EO [5]. Ahmadi et al. [12] showed that nanoliposomes inhibited 77.65% of free radical activity due to the phenolic compounds of tea extract.

3.2 | SEM

The microstructure of liposomal particles is shown in Figure 1. The nanoliposomes containing yarrow EO formed a spherical and regular structure. The EO was well dispersed in the nanoliposome matrix and showed a homogeneous structure. SEM micrographs of empty nanoliposomes revealed an irregular structure, characterized by nonuniform and distorted vesicles with undefined spherical morphology, uneven surfaces, and noticeable size heterogeneity. The increase in particle size after encapsulation confirmed the presence of yarrow EO in the liposomes [12].

3.3 | FTIR Spectroscopy

FTIR spectroscopy was used to identify the functional groups in the nanoliposomes (Figure 2). The absorption bands were



(a) (b)

FIGURE 1 | SEM images of the nanoliposomes. Empty nanoliposomes (a) and nanoliposomes containing yarrow essential oil (b).

observed in the range of 2994–2944 cm^{-1} corresponding to the symmetric and asymmetric stretching vibrations of the CH_3 and CH_2 groups. The narrow peak in this region indicates the formation of stronger hydrogen bonds in the formation of nanoliposomes. A sharp peak was observed in this region in the spectrum of pure EO. The stretching vibration of the C=O bond led to the formation of an absorption band at 1747 cm^{-1}, which was narrow in the spectrum of pure EO and sharp in the other two spectra. The asymmetric and symmetric bending vibrations of the C–H bonds were shown at 1451 cm^{-1} and 1360 cm^{-1}, respectively. The band at 1180 cm^{-1} is attributed to the asymmetric P–O–C stretching vibrations, and the band at 1079 cm^{-1} is related to the polar interaction between the PO_2 group of lecithin and the polar side chains of liposomes [1].

The increase in the vibrational frequency of PO_2 indicates a dehydrated phosphate group due to the absence of hydrogen bonding, while its decrease is related to the hydrated phosphate group and the presence of hydrogen bonding [20].

These spectral changes can be attributed to the location of the carbonyl groups (C=O) in the phospholipid bilayer due to the EO trapping in the phospholipid bilayer [21].

The similarity of the spectrum of pure EO and encapsulated EO in the nanoliposomes indicates the successful incorporation of EO into the nanoliposomes. Since the EE was high and the lower EO was nonencapsulated, slight changes were observed in the *peak* position of each sample. Therefore, the peaks overlapped and there was no noticeable change in their intensity. It can be said that the addition of yarrow EO did not change the chemical structure of the nanoliposomes.

3.4 | DSC

In all DSC thermograms, T_g is about 60°C–65°C, immediately followed by a small hysteresis peak. Above 100°C, the crystallization exothermic peak is visible. Finally, melting occurs at 150°C (Figure 3).

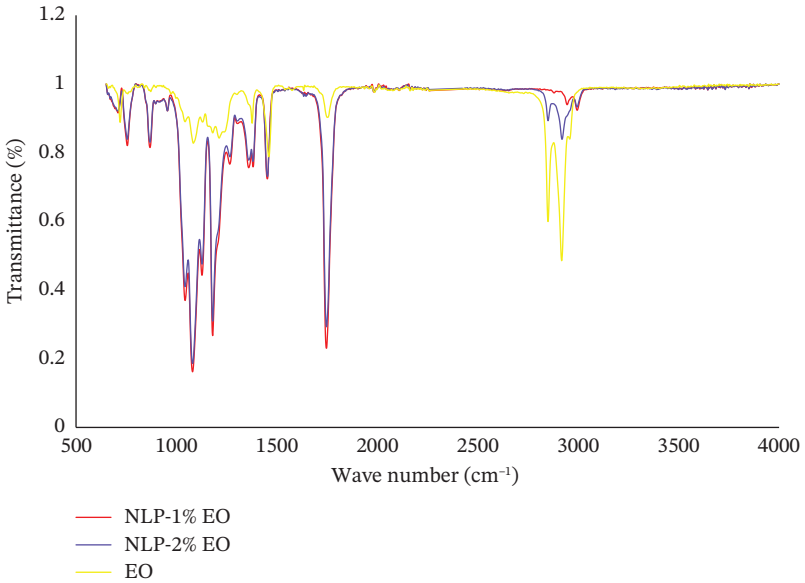


FIGURE 2 | FTIR spectrum of the nanoliposomes.

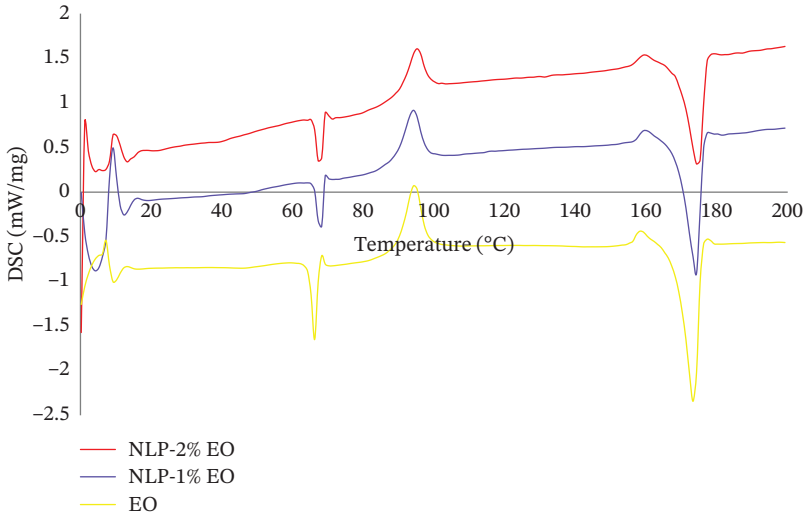


FIGURE 3 | DSC thermograms of the nanoliposomes.

The increase in T_g with the encapsulation of EO confirms their antiplasticizing effect in the formation of nanoliposomes. In all curves, an endothermic melting peak was observed. The increase in T_g and T_m can be attributed to the formation of more complete crystalline regions due to the interaction between EO and nanoliposomes and strong hydrogen interactions between the hydroxyl groups, which reduces mobility and flexibility. The decrease in crystallization temperature (T_c) can be attributed to the high crystallization of nanoliposomes in the presence of EO [27].

The melting enthalpy (ΔHm) increased from 6.96 J/g in empty nanoliposomes to 18.55–26.91 J/g after incorporation of EO. This increase may be attributed to enhanced molecular interactions, such as hydrogen bonding and hydrophobic interactions between the EO components and the lipid bilayer, leading to a more ordered and compact liposomal structure. Consequently, the EO-loaded nanoliposomes exhibited improved thermal stability [12, 28].

3.5 | Chemical Analysis of Meat

The nanoliposomes containing 2% yarrow EO (NLP-2% EO) with the smallest particle size and highest EE, antioxidant activity, and thermal stability are the optimal treatment used for the treatment of meat.

Figure 4 shows the chemical characteristics of meat during storage at 4°C. The pH and TVB-N are used as parameters of meat freshness [31].

At the beginning of the experiments (day 0), the pH of meat samples was not significantly different ($p > 0.05$) but increased significantly during storage ($p < 0.05$), indicating meat spoilage [15, 32]. At the end of the storage, pH values in the control treatment (C), the treatment containing free yarrow EO, and the treatment containing nanoliposomes of yarrow EO (NLP-EO) reached 7.12 ± 0.04, 6.15 ± 0.02, and 5.87 ± 0.02 (Figure 4(a)).

The pH increase during storage can be attributed to the microbial and biochemical activity, protein degradation, the formation of ammonia and volatile amines, catabolism of nitrogen compounds and amino acids, as well as the reactions of secondary metabolites [15, 29]. The final pH > 6 in meat is considered spoiled [33]. In this regard, the control treatment was acceptable for less than 5 days at 4°C. The treatment containing EO was acceptable for 5–10 days, and the treatment containing NLP-EO was acceptable for up to 10 days.

Similarly, in the research of Nikzadeh et al. [15], the pH levels of beef meat samples showed an increasing trend throughout the 20-day storage period. In contrast, samples treated with *M. pulegium* EO and its nanoencapsulated EO maintained lower pH values (6.1 by day 20, compared to 7.0 in the control), indicating a suppressive effect on microbial metabolism.

At the beginning of the storage, the initial TVB-N content in the meat treatments was 18.11 ± 0.40 mg/100 g, indicating the freshness and high quality of meat [2]. The amount of TVBN in all studied groups increased during storage ($p < 0.05$) and after 10 days reached 36.19 ± 2, 27.11 ± 2, and 24.48 ± 1.61 mg N/100g in the control, EO, and NLP-EO treatments, respectively (Figure 4(b)).

The increase in TVB-N is due to the growth of microorganisms and the effect of microbial proteolytic enzymes on protein and nonprotein nitrogenous compounds of meat [15]. The increase in microbial population and the chemical indicators of spoilage, including pH and TVBN, have been previously reported [2, 15, 30].

The permissible limit of TVB-N in meat is 25 mg N/100g [29]. In some sources, TVB-N levels of 13 mg/100 g for fresh meat and ≤ 17 mg/100 g are considered acceptable [30].

Thus, the control treatment was storable for less than 5 days at 4°C, after which its TVB-N content reached above the permissible limit (25 mg N/100g), and the meat spoiled. The EO treatment was slightly spoiled but suitable 5–10 days. The NLP-EO treatment was fresh for up to 10 days, and its TVBN content did not exceed the permissible limit. Therefore, nanoliposomes, due to the presence of yarrow EO and the gradual release of EO, increased the shelf life of meat and reduced spoilage during storage [2, 10].

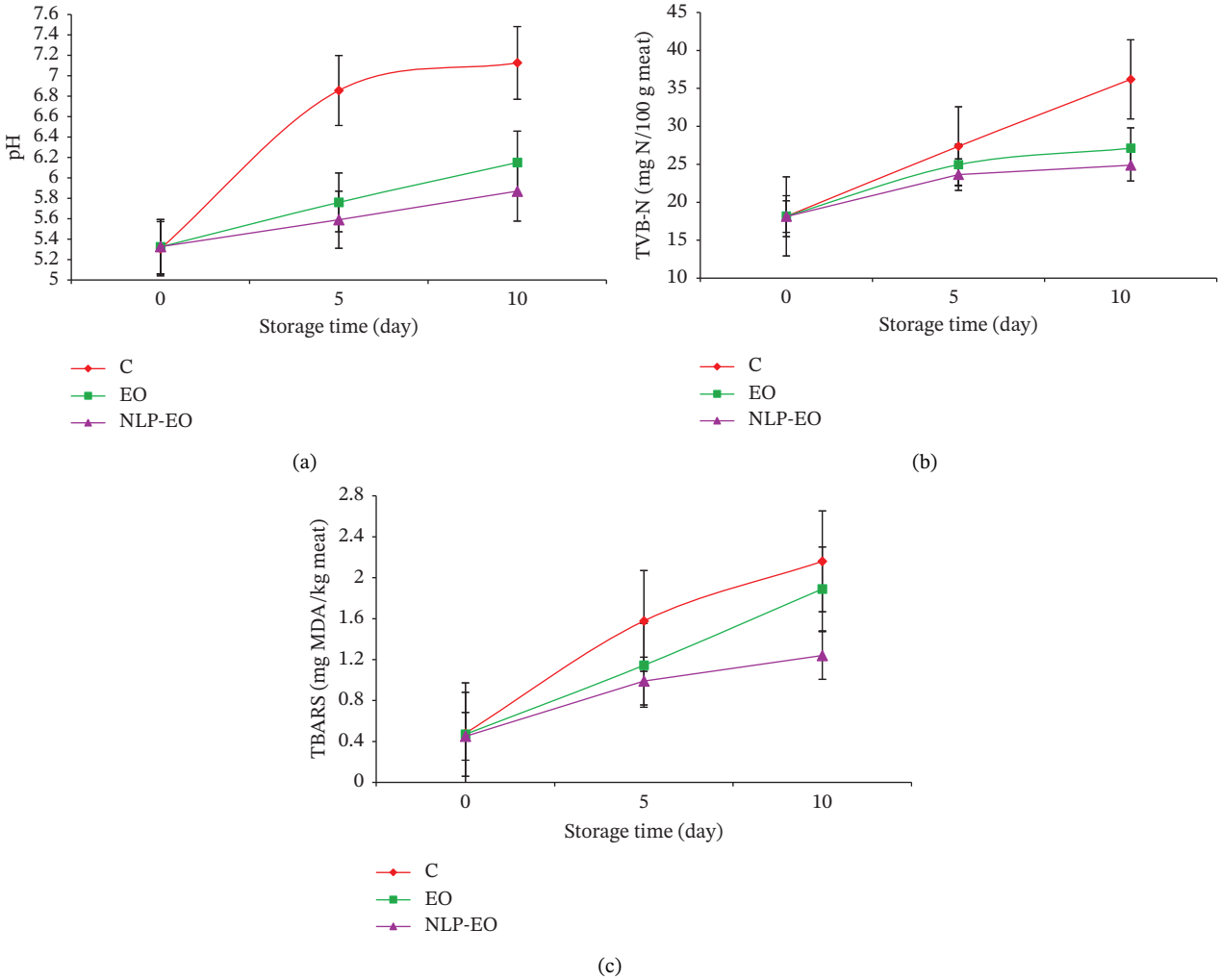


FIGURE 4 | Chemical analysis of meat during storage at 4°C. Error bars indicate a significant difference ($p < 0.05$).

Similarly, Nikzadeh et al. [15] observed the TVB-N content increased over time during storage of beef meat, but decreased when meats were coated with free and nanoencapsulated *Mentha pulegium* EOs with reductions of 6.2-fold and 2.1–3.8-fold, respectively, compared to the control.

Mehaya et al. [22] reported that the control beef burger samples exceeded the permissible TVB-N limits on the 12th day of refrigerated storage, whereas the burger samples mixed with encapsulated oregano EO remained within the acceptable range until the 12th day.

Due to the high fat content, lipid peroxidation occurs during meat storage, resulting in off-flavor and off-odor [34]. As can be seen in Figure 4(c), at the beginning of the tests (day 0), the TBARS of the meat samples did not show a significant difference ($p > 0.05$), but it increased significantly over time ($p < 0.05$), and at the end of the storage, it reached 2.16 ± 0.07, 1.89 ± 0.05, and 1.24 ± 0.03 mg MDA/kg in the control and treated samples, respectively.

The increase in TBARS values during meat product storage may be attributed to the formation of oxidized byproducts such as aldehydes and ketones during the lipid autooxidation process [22].

The permissible limit of TBA in meat has been reported as 2 mg MDA/kg [22, 35]. In general, the permissible TBARS levels between 5 and 8 MDA eq/kg are suitable for human consumption, between 3 and 5 MDA eq/kg is good quality, and less than 3 MDA eq/kg is excellent quality [36].

In this regard, the control treatment was suitable for less than 10 days, and the other treatments showed good to excellent quality. The lower oxidation rate in the treated meat is due to the antioxidant properties of yarrow EO, which increases the shelf life of meat [7, 9]. EOs, as natural antioxidants, react with free radicals and inactivate them, breaking the oxidation chain [5, 10].

Phenolic compounds such as eugenol, thymol, and methyl chavicol present in yarrow EO play a key role in inhibiting lipid oxidation [5]. These polyphenols act through multiple mechanisms, including chain-breaking during free radical propagation, decomposition of hydroperoxides, and chelation of pro-oxidant metal ions. By scavenging free radicals, they interrupt the oxidation cycle; they can readily donate hydrogen atoms to reactive species, forming more stable radicals, or neutralize radicals via electron transfer. Additionally, phenolics inhibit enzymes involved in reactive oxygen species (ROS) generation and chelate transition metals, thereby reducing oxidative damage [5, 7, 10].

The results are in agreement with other researchers who have also reported an increase in lipid peroxidation of beef meat and the levels of TBARS during storage, but were reduced by EO-based materials [15].

Mehaya et al. [22] reported that the beef burger samples incorporated with 0.75% and 1% encapsulated oregano EO showed a significant increase in TBA values on the 12th day of storage, recording 2.11 and 1.96 mg MDA/kg, respectively. However, the control sample recorded TBA values of 0.49, 1.32, 3.01, and 5.91 mg MDA/kg on the 4th, 8th, 12th, and 16th day of storage, respectively. These results are in agreement with previous research that highlights the potential use of oregano EO to preserve meat products from lipid peroxidation [37, 38].

3.6 | Microbial Analysis of Meat

The total mesophile (TMB) and psychrophile (TPB) bacteria counts were selected to assess the meat spoilage over time at 4°C (Figure 5).

At day 0, the microbial population of meat did not show a significant difference ($p > 0.05$) but increased significantly during storage ($p < 0.05$). After 10 days, TMB count in the control treatments and treatments containing free and encapsulated EOs was 8.35 ± 0.16, 7.73 ± 0.14, and 5.54 ± 0.16 Log CFU/g, respectively (Figure 5(a)). At the end of storage, TPB count in untreated and treated meat was reported to be 8.66 ± 0.26, 7.18 ± 0.23, and 6.55 ± 0.22 Log CFU/g, respectively (Figure 5(b)).

The increase in TMB and TPB was consistent with pH and TVB-N increasing. In other words, meat freshness is related to microbial spoilage and TVB-N production and pH changes [2, 31, 33].

In general, when the TMB exceeds 6 log CFU/g, the meat is considered a low-quality product [30]. Therefore, meat quality is considered acceptable if the TMB is $\leq 10^7$ cfu/g [2]. 7 Log CFU/g has also been proposed as the acceptable microbiological limit for TPB in meat products [22, 34].

Therefore, based on the microbial counts, the control treatment for 0–5 days, the EO treatment for 5–10 days, and the NLP-EO treatment due to the gradual release of EO for up to 10 days were fresh and acceptable [2, 10].

A 10-day shelf life at 4°C is generally viable for fresh beef with active packaging with yarrow EO nanoliposomes and is comparable to that reported for conventional chemical preservatives (~5–7 days). This indicates that nanoliposome encapsulation of yarrow EO can serve as a natural and effective alternative to synthetic preservatives, providing comparable or enhanced preservation while avoiding chemical additives.

In nanoliposome-loaded systems, EO molecules are either embedded within or associated with the lipid bilayer, resulting in delayed diffusion into the surrounding medium. This structural confinement allows for sustained release of EO, while simultaneously protecting its bioactive components from oxidation, light, and thermal degradation. Free EO, however, lacks such protection and is quickly depleted due to evaporation and rapid diffusion, which limits its long-term preservative efficacy.

Encapsulation can modify both the efficacy and release kinetics of active compounds, thereby influencing the overall stability of the final product. The effective release of volatile antimicrobial constituents from NLP-EO, together with the large surface area associated with its fine droplets, resulted in a greater reduction in microbial populations compared with EO [2].

The lower microbial growth in the treated groups is due to the antimicrobial properties of yarrow EO and the presence of secondary metabolites, including terpenes such as 1,8-cineol, camphor, eugenol, p-cymene, thymol, carvone, and terpinene [5], which are known for their antimicrobial properties. Terpenes can interact with and disrupt the lipid components of microbial cell membranes, leading to increased membrane permeability, protein dysfunction, and loss of cellular integrity, which ultimately results in cytoplasmic leakage and cell death [5, 8, 10].

EOs cause the death of microorganisms through various mechanisms, including disruption of cellular metabolism, inhibition of DNA and RNA activity and protein synthesis, inhibition of respiratory enzymes, and membrane leakage [5].

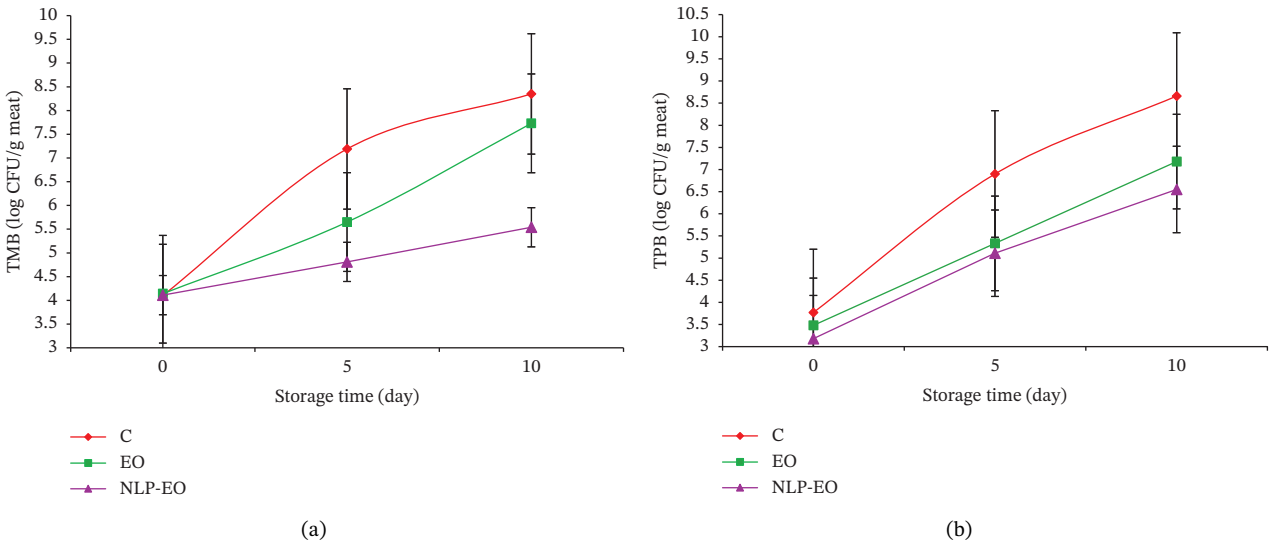


FIGURE 5 | Microbial analysis of meat during storage at 4°C. Error bars indicate a significant difference ($p < 0.05$).

Phenolic and other hydrophobic components of EOs interact with the lipid constituents of microbial cell membranes, leading to metabolic disruption, altered membrane permeability, and ultimately cell death. These effects are largely attributed to the hydrophobic nature of phenolic compounds, which enables them to insert into the lipid bilayer and disturb membrane integrity. This disruption can cause leakage of cellular contents and interfere with essential membrane-associated functions [8, 10].

Similarly, Emtiazi et al. [11] confirmed the antimicrobial properties of yarrow EO nanoliposomes.

Nikzadeh et al. [15] observed that nanoencapsulated EOs at 16, 80, and 160 ppm decreased the population of aerobic mesophilic bacteria by 10%, 41%, 29%, and 20%, respectively. Mehaya et al. [22] reported that beef burger samples mixed with encapsulated oregano EO exhibited insignificant microbial growth until the 8th day. For instance, the bacterial count in burgers mixed with 1% encapsulated oregano EO increased by 1.05 log on the 8th day, compared to 2.08 log for the control sample. The addition of encapsulated oregano EO at 1% significantly reduced the bacterial count until the 12th day compared to other levels. Tsitsos et al. [39] found that oregano EO maintains the microbiological quality and prolongs the shelf life of mutton meat during storage.

4 | Conclusion

Nanoliposomes containing 2% yarrow EO presented a smaller size and higher zeta potential and also were able to encapsulate the EO in a higher percentage. Yarrow EO was well dispersed in the nanoliposome matrix and did not change the chemical structure of the nanoliposomes, and increased the thermal stability. The nanoliposomes were effective in increasing the shelf life of fresh meat at refrigerated temperatures and reducing the chemical and microbial spoilage indicators of meat. The antioxidant and antimicrobial effects of liposomal EO were greater than those of free EO. NLP-EO extended the shelf life by up to 10 days compared to 5 days for the control at 4°C. Consequently, they were introduced as a natural preservative in meat products. They offer a high potential for desirable antimicrobial effects and can be used to delay oxidation in meat, making them a potential alternative to synthetic antioxidants and preservatives. A detailed compositional analysis of the encapsulated EOs will be conducted in future studies further to elucidate the effects of nanotechnology on their chemical profile. Also, the lack of sensory analysis and EO release studies are limitations of the present study that can be addressed in the future.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

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