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Investigating the effectiveness of electrospun polyvinyl alcohol nanofibers containing eucalyptus essential oil in increasing shelf life and improving the quality of ground beef

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ABSTRACT

The objective of the current study was to encapsulate eucalyptus essential oil (EEO) within polyvinyl alcohol (PVA) nanofibers using the electrospinning technique. This approach aimed to enhance the shelf life and improve the quality of minced beef during a 12-day refrigerated storage period. Scanning electron microscope (SEM) images of the produced PVA nanofibers showed that all the nanofibers were thin, well-defined, smooth, and without any knot-like structures. Fourier transform infrared spectroscopy (FTIR) results indicated the absence of chemical interactions between EEO and the PVA polymer matrix, and it was determined that the essential oil was well encapsulated in the nanofibers. PVA/EEO nanofiber film showed a significant reduction in microbial growth of total bacteria (42.73%), psychrophilic bacteria (52.92%), and chemical parameters peroxide (48.51%), total volatile nitrogenous bases (25.4%), and thiobarbituric acid (56.76%) in minced meat compared to the control group during the storage period, which resulted in an increase in the shelf life of minced meat up to 12 days. Furthermore, the results indicated that the PVA/EEO treatment showed better color characteristics (L*, a*, b*) than the other groups during storage. On average, the PVA/EEO treatment showed an improvement of 18.46% in color parameters compared to the control samples. These findings suggest that biopolymer-based active packaging materials, such as PVA/EEO nanofibers, may be effectively used in meat products to preserve and improve quality.

1-INTRODUCTION

Meat, especially minced meat, is an excellent sources of high-quality protein, iron, zinc, vitamin B12, and essential amino acids, contributing significantly to a healthy daily diet [1]. However, due to the higher surface-to-volume ratio in minced meat, this product is susceptible to lipid and protein oxidation as well as microbial spoilage [2]. Microbial spoilage is one of the most important factors in reducing the quality of minced meat, which can lead to unpleasant odors and tastes and a decrease in product quality. For this reason, to ensure the safety, quality, and increase the shelf life of minced meat, it is essential to use packaging containing natural antioxidants and antibacterial agents during the production, storage, and transportation stages of this product (Khezrian and Shahbazi, 2018). The three main types of minced meat packaging include aerobic packaging, vacuum packaging, modified atmosphere packaging, as well as more innovative types such as functional packaging including active or smart packaging (active or smart packaging) [3]

Active packaging (AP) is an advanced food packaging system that releases active ingredients such as antimicrobial agents, antioxidants, and flavors while absorbing undesirable substances. This type of packaging significantly improves product quality and shelf life by reducing microbial growth, reducing oxidation, and preventing moisture loss [4]. Antimicrobial packaging also increases food safety and extends shelf life by using compounds such as enzymes, bacteriocins, and metal ions [5]. The meat processing industry has concluded that the use of active packaging materials containing natural compounds is an effective strategy to ensure the safety and increase the shelf life of ground meat. For instance, Haghighat Panah et al (2022) used a composite film made of mung bean protein isolate and pullulan fortified with marjoram essential oil to increase the shelf life of ground beef [6]. Moradinejad, Hedayati, and Ansarifar produced an antimicrobial package that was prepared by combining Shirazi thyme essential oil with polyvinyl alcohol nanofibers using an electrospinning technique [7].

Plant essential oils are part of natural compounds that are widely used in the food industry due to their antimicrobial and antioxidant properties [8]. Eucalyptus essential oil, extracted from eucalyptus trees, is used in food preservation, aromatherapy, and various medicinal purposes. The plant contains bioactive compounds such as eucalyptol, which is known for its aroma and medicinal properties. EEO has antimicrobial and antioxidant properties that allow its use in active packaging systems, thereby increasing shelf life [9]. It is not possible to use eucalyptus essential oil alone in the food industry due to its sensitivity to light, heat, oxygen, and insolubility in water. For this reason, microencapsulation methods are used to combine this essential oil with other polymers to protect them from environmental factors. This capability can inhibit the growth of spoilage microorganisms and enhance the sensory quality of food. Its natural origin is in line with consumer demand for sustainable food preservation methods [10].

Encapsulation is a technique in which active and sensitive compounds are enclosed by a coating or wall, forming a capsule that protects them from degrading and environmental factors [11]. Microencapsulation techniques include electrospinning, spray drying, freeze drying, extrusion, co-crystallization, rotary suspension separation, surface polymerization, liposome entrapment, and molecular inclusion [12].

Electrospinning is a popular microencapsulation method for creating antimicrobial nanofibers that has attracted much attention due to its significant porosity, high surface-to-volume ratio, and flexibility [13]. Electrospinning involves applying an electric field to thin fibers that are affected by electrostatic forces, allowing the formation of a continuous jet that is collected on a collector in nano- or micro-diameters [14].

Polyvinyl alcohol is a non-toxic, biocompatible, and heat-resistant synthetic biopolymer produced through the hydrolysis of polyvinyl acetate [15]. The hydrophilic properties of polyvinyl alcohol make it suitable for various industries such as papermaking, adhesives, textiles, packaging, and medicine [16]. Also, the high elasticity, chemical resistance, and mechanical properties of PVA make it ideal for film formation in food packaging [17].

Given the high perishability of minced meat and the necessity of using active packaging to increase its shelf life, the present study investigated the effect of polyvinyl alcohol nanofiber film containing eucalyptus essential oil in minced meat packaging. The properties of electrospun polyvinyl alcohol/eucalyptus essential oil nanofibers were analyzed using Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM. In addition, color parameters (L*, a*, b*), and chemical characteristics (peroxide, total volatile nitrogenous bases, and thiobarbituric acid) and microbial analysis (total bacteria and psychrophilic bacteria) of the control, PVA, and PVA/EEO treatments were evaluated at three-day intervals (0, 3, 6, 9, 12) during refrigerated storage.

2. Materials and methods

2.1 Characterization of nanofibers

Solution preparation

A solution of polyvinyl alcohol (PVA, Mw = 130,000 Da, Aldrich Chemical) was created by dissolving 10% w/v PVA polymer in distilled water, which was stirred for 2 hours at 80 °C to achieve a homogeneous mixture. EEO (Tabib daroo Co.) (EEO) (2% w/v) was incorporated into the PVA solution and allowed to mix for 24 hours

Electrospinning

An electrospinning machine equipped with a high-voltage power supply (ES-1000, Nanoscale Technologists Co., Iran) was utilized to generate nanofibers. The voltage of the device was connected to a stainless steel capillary needle and the polymer solution was placed in a 10 ml syringe. The solution was then transferred to the needle through a thin tube using a digitally controlled pump. The collector of the device consisted of a metal plate covered with aluminum foil. The parameters of the electrospinning device, including the applied voltage, solution feed rate, and distance between the needle and the collector plate, were set to 15 kV, 0.75 mL/h, and 15 cm, respectively [18].

Scanning electron microscopy (SEM)

The morphology of the electrospun fibers was analyzed using a scanning electron microscope (Leo 1450VPSEM) operating at a voltage of 20 kV [14].

Fourier Transform Infrared Spectrometry (FTIR)

Fourier transform infrared spectroscopy (Tensor27, Bruker GmbH, Ettlingen, Germany) was used to investigate chemical changes and potential interactions between the nanofiber structure components and EEO in the produced samples. This experiment was performed using the potassium bromide tablet method at room temperature in the frequency range of 400-4000 cm with an average resolution of 14 cm−1, with an average of 32 scans per measurement. In this method, before the experiment, the sample (3 mg) is mixed with potassium bromide and made into a tablet. The reason for using potassium bromide is its lack of absorption in the infrared region [19].

2.2 Minced Beef preparation and active packaging

Ground beef was purchased from a local store in Birjand city, South Khorasan province, Iran and stored in insulated polystyrene boxes containing ice for transportation to the laboratory within one hour of purchase. A structured factorial design was designed to evaluate the effect of PVA nanofiber films, with and without eucalyptus essential oil (EEO), on the shelf life of ground beef during refrigerated storage.

Minced meat was stored in polyethylene containers (measuring 11.5 × 9.5 × 6.2 cm) that were thoroughly sterilized, divided into three groups with lids securely fitted:

1- Control group: Minced meat was placed in containers with lids
2- PVA group: Minced meat meat was placed in active packaging with film (nanofiber without essential oil) installed on the lid (its inner part)
3- PVA+EEO group: Minced meat meat was placed in active packaging with film (nanofibers containing essential oil) installed on the lid (its inner part)

Minced meat packages were categorized into three groups and stored under refrigerated conditions (4 ± 0.5°C and 85% relative humidity). The quality parameters of the minced meat were evaluated on days 0, 3, 6, 9, and 12 of storage, with assessments conducted independently in triplicate.

2.3 Attributes evaluated in minced meat:

peroxide value (PV)

To measure the peroxide index (PV), approximately 50 grams of the sample was weighed in a 500 ml Erlenmeyer flask, and a 200 ml flask was used for fat extraction. The flasks were shaken for one hour at medium speed, then filtered. The solvent was evaporated using a rotary machine, and the remaining oil's weight was determined. Subsequently, a mixture consisting of chloroform, acetic acid solution, saturated potassium iodide, distilled water, and 1% starch was added to the oil. Finally, the amount of released iodine was titrated with a 0.01 N sodium thiosulfate solution [20].

Total volatile nitrogen bases (TVB-N)

To determine the total volatile nitrogen bases (TVB-N), a mixture of 10 g of minced beef, 2 g of magnesium oxide, and 300 ml of distilled water was prepared in a Kejaldal Flask and connected to the Kjaldal apparatus. A 250 ml Erlenmeyer flask with 25 ml of 2% boric acid solution (containing a few drops of methyl red reagent) was attached to the apparatus's end. Following a

45-minute distillation process, the boric acid solution changed color to yellow, indicating the absorption of volatile nitrogen bases. Subsequently, titration was conducted using 0.1 N sulfuric acid until the asolution reverted to a red hue. The TVB-N content was then calculated in milligrams per 100 grams of meat [21] .

Thiobarbituric acid (TBA)

To measure the thiobarbituric acid (TBA) index, first 200 g of minced meat was poured into a 25 ml volumetric flask and then made up to the final volume with 1-butanol solution. After that, 5 ml of the sample and 5 ml of TBA reagent were added into dry, capped tubes. These tubes were placed in a hot water bath at 95°C for 2 hours and then allowed to cool to room temperature. Finally, the absorbance of the samples was read by spectrophotometer at 530 nm and the results were calculated in milligrams of malonaldehyde per kilogram of meat [22].

Color parameters

To examine the impact of treatments on the surface color alterations of minced meat samples, the following methodology was adopted: The procedure encompassed image capture, color profiling and calculation, and conversion of images into L*, a*, b* units. Uniform sample lighting conditions were maintained in line with a prior study [23]. Adobe Photoshop was utilized to enhance background contrast and facilitate segmentation. Subsequently, the images were transformed into L*, a*, b* units to ensure consistent color perception and approximate the Euclidean distance between colors. Image assessment was conducted using Image J software (National Institutes Health, Bethesda, MD, USA) version 1.40 g.

Microbial analysis

During each sampling instance, 10 grams of minced beef were combined with 90 milliliters of sterile 0.1% peptone water and homogenized using a laboratory mixer (stomacher 400, Interscience, France) for a duration of 2 minutes. Subsequently, decimal dilutions were prepared in a 0.1% peptone water solution, and these dilutions were either spread or poured onto the suitable agar medium. Microbial assessments encompassed the determination of total viable count (TVC) and psychrotrophic count (PTC), which were evaluated on count agar at 37°C for 24 to 48 hours and at 4°C for a period of 10 days, respectively [24].

Statistical analysis:

The data was analyzed using a completely randomized design to verify the results obtained from various tests. Initially, the data's normality was assessed through the Kolmogorov-Smirnov test. To ascertain variations among the mean values (with a minimum of three replicates for each experiment), Tukey's multiple range test was conducted following analysis of variance at a significance level of $P<0.05$. In cases where the data did not exhibit normal distribution, nonparametric tests were employed as alternatives. Statistical analysis was carried out using the SPSS statistical software program, version 22.

3. Result and Discussion

3.1 Characterization of Nanofibers

3.1.1 SEM

Morphological study of PVA/EEO nanofibers produced by electrospinning (Figure 1) shows the formation of nanofibers with a uniform structure, smooth surface, and free of defects (without grains and knots) [25]. SEM image analysis shows that the average diameter of PVA/EEO nanofibers is approximately 325±27 nm. This value is in comparison to the data of a previous study, where the diameter of PVA nanofibers alone was reported to be in the range of 104–162 nm [18]. A significant increase in nanofiber diameter indicates that it can be attributed to the presence of eucalyptus essential oil in the polymer matrix. This increase in diameter is probably due to mechanisms such as a decrease in the electrical conductivity of the polymer solution due to the addition of essential oil or a change in the viscosity and rheological properties of the polymer solution, which leads to a shortening of the length of the polymer jet after applying voltage, which is consistent with reports from other studies in this field [18, 26].

Ardakani et al. (2019) also confirmed the increase in diameter of electrospun nanofibers by adding different concentrations of Shirazi thyme essential oil to the PVA solution. This phenomenon can be attributed to the increase in fiber density due to the formation of interfiber bonds after crosslinking [14, 26]. In a similar study, Rahmatinia, Aran, Miri, and Ramadan, investigated electrospun zein nanofibers as carriers of eucalyptus essential oil. The results of SEM images showed smooth fibers without spheres from eucalyptus essential oil [27].

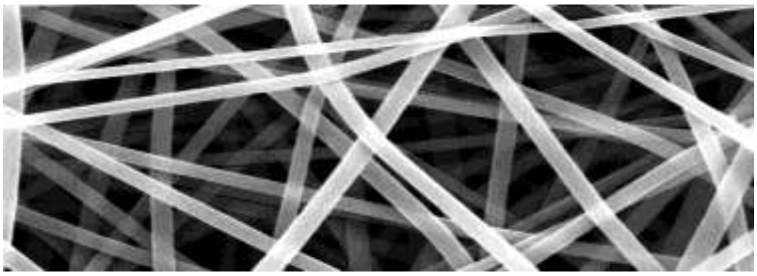
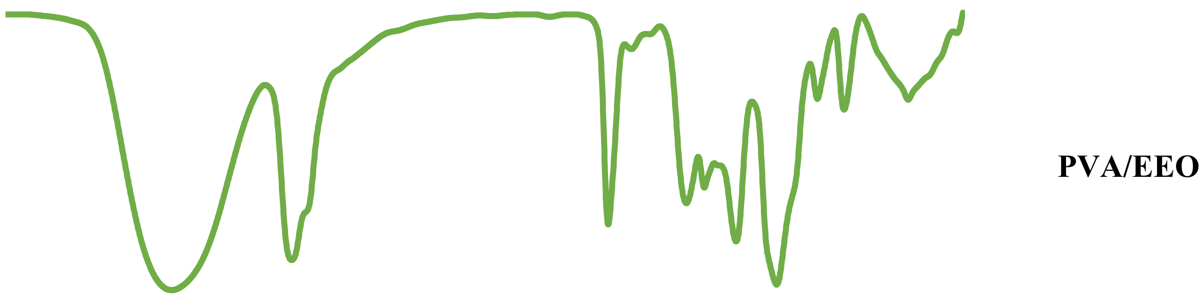


Fig 1. SEM image of PVA/EEO (Polyvinyl alcohol nanofibers / eucalyptus essential oil) electrospun nanofibers

3.1.2 FTIR

FTIR is a convenient tool to observe the presence of various functional groups and molecular interactions within the polymer structure [15]. The FTIR spectra of PVA, EEO, and PVA+EEO are shown in Fig 2. The broad band at 3300 cm^{-1} in the PVA spectrum is associated with the stretching vibrations of hydroxyl (-OH) groups. This broadness is indicative of hydrogen bonding between hydroxyl groups, which is common in PVA due to its hydrophilic nature. The bands at 2912 cm^{-1} correspond to C-H stretching vibrations, reflecting the presence of methylene ($-CH_2-$) groups in the polymer backbone. A broad band near 1720 cm^{-1} observed, which is attributed to carbonyl (C=O) stretching vibrations, especially if there are any acetyl or carbonyl functionalities present due to partial oxidation. The other important band at 1085 cm^{-1} shows broad bands related to C-O stretching vibrations, reflecting the ether and alcohol functional groups present in PVA [17]. In the EEO spectrum, a broad band observed at 3472 cm^{-1} is associated with the stretching vibrations of hydroxyl (OH) groups. This band indicates the presence of phenolic compounds and alcohols in eucalyptus oil, which contribute to its characteristic aroma and therapeutic properties. Bands in the range of 2726-2967 cm^{-1} may represent C-H stretching vibrations, primarily from aliphatic hydrocarbons present in the oil. These peaks are indicative of the saturated and unsaturated carbon chains found in eucalyptus oil. The spectrum in the range of 2352-2016 cm^{-1} is related to the stretching of C≡C and C≡N groups. The band at 1607 cm^{-1} was also derived from the stretching vibrations of carbonyl (C=O), especially if ketones or aldehydes are present in the composition of eucalyptus oil [11]. When EEO was added to PVA matrices, the infrared spectrum of EEO-loaded PVA nanofibers contained all the characteristic peaks of the two, indicating that EEO could be effectively encapsulated in PVA nanofibers. Compared with PVA, the spectrum of PVA/EEO nanofiber film showed more peaks at around 919 cm^{-1} and 1043 cm^{-1}, which could be attributed to CH2 and C-O-C γ-terpene and 1.8-cineole, respectively [28]. In the study by Rahmatinia et al, (2022) FTIR test results indicated the reaction between eucalyptus essential oil and zein nanofibers due to hydrophobic interactions between the two [27]. In another study, Ghaderi et al, (2021) investigated the fabrication and characterization of biocomposite films based on carboxymethyl cellulose/polyvinyl alcohol/fish gelatin for food packaging purposes. The FTIR results of their study indicated the interaction of polyvinyl alcohol, gelatin, and carboxymethyl cellulose through the interaction between the polar groups of gelatin and the hydroxyl groups of carboxymethyl cellulose and polyvinyl alcohol through the formation of hydrogen bonds with each other, and it was found that these three easily mix together [29].



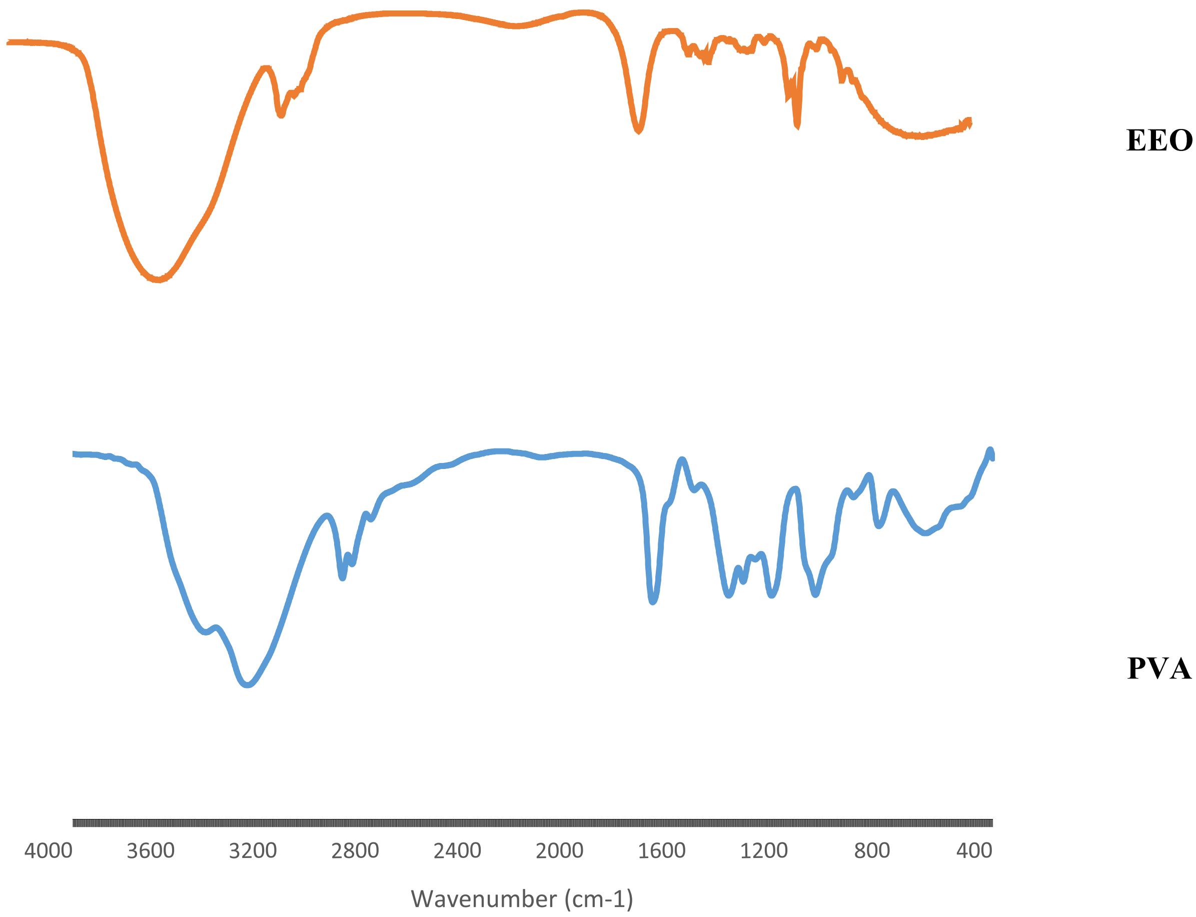


Fig 2. The FTIR spectra of PVA, EEO and PVA/EEO electrospun nanofibers.

PVA: Contains polyvinyl alcohol, EEO: Contains eucalyptus essential oil, PVA/EEO: Contains polyvinyl alcohol/eucalyptus

3.2 Assessment of minced beef meat quality

3.2.1 The peroxide value (PV)

Peroxide value (PV) is commonly used to measure the total content of hydroperoxides (primary products of lipid oxidation) and to monitor lipid oxidation during storage of minced beef [30]. For this reason, the PV value of minced meat samples was measured during refrigerated storage for 12 days (Figure 3). The results showed that the PV parameter increased significantly ($P<0.05$) in all treatments. The initial peroxide value for all treatments was on average 0.2 meq/kg. At the end of 18 days of storage at 4°C, the PV values for the control sample (2.22 meq/kg) were higher than those of the PVA sample (1.9 meq/kg) and the PVA/EEO sample (1.24 meq/kg). The performance of the PVA/EEO active packaging was significantly ($P<0.05$) better in reducing the peroxide parameter. Because this sample showed a higher antioxidant effect in inhibiting hydrogen peroxide with gradual release of essential oil from nanofibers during storage. This effect may be due to the presence of bioactive metabolites such as 1,8-cineole present in EEO which reacts with free radicals in the film and the environment [31]. Similarly, in a study, the antioxidant capacity of chitosan films was increased when essential oils rich in etheric compounds (peppermint, clary sage, melaleuca quinquefolia, and eucalyptus globulus) were combined with the surface of pure chitosan films [32]. Furthermore, it has been stated that the interaction between biodegradable films and polyphenolic compounds present in essential oils plays a vital role in modifying the film properties and increasing its antioxidant capacity. Higher antioxidant activity of the film is crucial for reducing oxidative stress and inhibiting the formation of free radicals. In a similar study, Chen et al [4], showed that PVA film loaded with clove oil (CO) played an effective role in reducing lipid oxidation and consequently reducing PV in Japanese horseman fish. In another study, Khazarian and Shahbazi [24], showed that the use of chitosan and carboxymethyl cellulose film containing natural preservative compounds of Kakuti kohi in minced camel meat delayed the increasing trend of PV value of the samples.

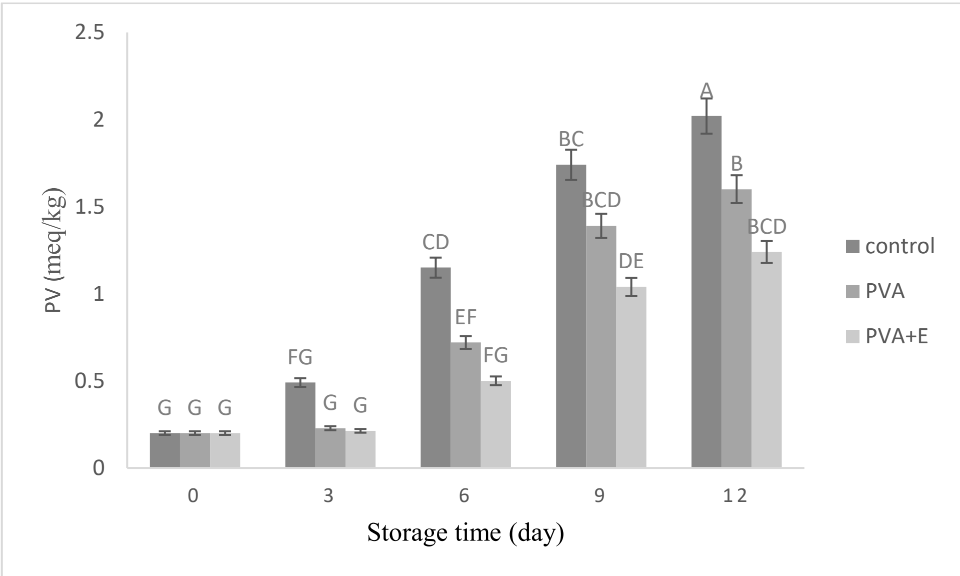


Fig 3. Changes in PV values in control, PVA, PVA/EEO treatments during 12 days of storage at 4°C (meq/kg)

Different letters in each day indicate a significant difference between the data ($p<0.05$), control: without polyvinyl alcohol and eucalyptus essential oil, PVA: Contains polyvinyl alcohol, PVA/EEO: contains polyvinyl alcohol / eucalyptus

3.2.2 Total volatile nitrogen bases (TVB-N)

Total Volatile Base Nitrogen (TVB-N) refers to the formation of amines and ammonia during the spoilage and oxidation process of meat proteins. These compounds are toxic and play a key role in determining the shelf life of meat and meat products [33]. The results of the TVB-N index in different treatments during storage at 4°C are shown in Figure 4. As can be seen, the TVB-N index increased significantly during storage (P<0.05). The initial TVB_N content on day 0 for all treatments was 12.37 mg/100g on average. After 12 days of storage at 4°C, this content was 46.2, 43.31, and 37.61 mg/100g for the control, PVA, and PVA/EEO samples, respectively. PVA/EEO treatment had the lowest increase significantly (P<0.05), which can be attributed to the antioxidant role of EEO against protein oxidation process [34]. In addition, Mirzaei, Joorshari, Jananshir, Nouri and Mahdavi [35], acknowledged that essential oils, especially eucalyptus, have strong antibacterial properties due to the abundance of compounds such as 1,8-cineole, which can delay the chemical spoilage process. They also investigated the use of pH-sensitive films made from κ-carrageenan (CG) containing quercetin (QUE) or eucalyptus leaf extract (ELE) to control the spoilage of chicken meat. Their study showed that films containing QUE and ELE had lower total volatile basic nitrogen (TVB-N) levels compared to the control group. Another similar finding was reported by Dehlaghy, Ekrami, Djomeh and Yousefi [36], for rainbow trout fillets coated with electrospun PVA nanofibers containing Marshmallow (AO) extract. In this study, TVB-N levels in control samples increased significantly during storage, while coating fillets with multifunctional smart packaging effectively reduced TVB-N.

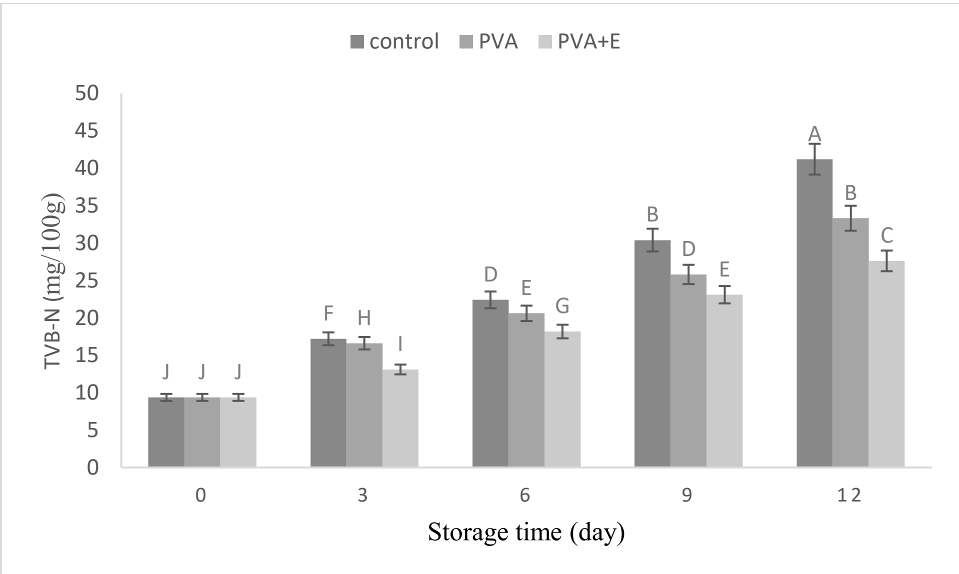


Fig 4. Changes in TVB_N values in control, PVA, PVA/EEO during 12 days of storage at 4°C (mg /100g)

Different letters in each day indicate a significant difference between the data (p<0.05), control: without polyvinyl alcohol and eucalyptus essential oil, PVA: Contains polyvinyl alcohol, PVA/EEO: contains polyvinyl alcohol / eucalyptus

3.2.3 Thiobarbituric acid (TBA)

Lipid oxidation is another factor affecting the spoilage of meat and its products, during which a series of compounds such as hydroperoxides as well as secondary oxidation products such as malonaldehyde are formed. Malonaldehyde reacts with thiobarbituric acid (TBA) as a colorimetric indicator, so the TBA indicator plays an effective role in measuring the quality of minced meat [37]. As can be seen, the TBA index increased significantly during storage ($P<0.05$). The initial TBA value on day 0 for all treatments was 0.185 mg MDA/kg on average. After 12 days of storage at 4°C, this value was 0.770, 0.564, 0.438 mg MDA/kg for the control, PVA, and PVA/EEO samples, respectively. The PVA/EEO treatment had the lowest increase ($P<0.05$). This could be due to the ability of EEO to reduce the oxidation of unsaturated fatty acids in meat due to the presence of phenolic compounds such as gallic, catechol, chlorogenic, p-coumaric, and gentisic [38]. In a similar study, Khazarian and Shahbazi [24], showed that the use of chitosan nanofibers and carboxymethyl cellulose film containing the essential oil of the Kakuti mountain plant in minced camel meat reduced TBA compared to the control sample at the end of the storage period. Another study was conducted by Zanela et al.(2024) with the aim of designing a food simulant packaged with starch and polybutylene adipate terephthalate (PBAT-poly) films containing EEO essential oil [39]. The results showed that these films have a positive effect on food preservation, especially fatty or non-acidic foods, due to the presence of essential oil antioxidant compounds. In a similar study, Najafi et al. [38], conducted on the antimicrobial and antioxidant properties of eucalyptus extract and its application in the production of potato starch film for meat packaging. Their results showed that the addition of eucalyptus extract delayed the increase in TBA levels in meat samples. In another study, Ceylan, Kotlu, Meral, Ekin, and Kovus [40], showed a decrease in TBA levels in samples coated with PVA nanofibers containing grape seed oil compared to the control.

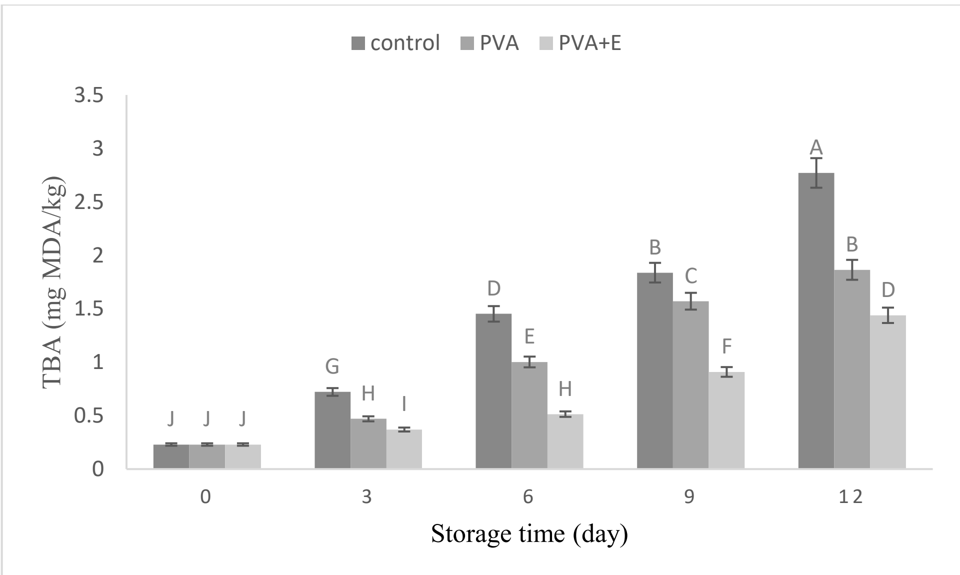
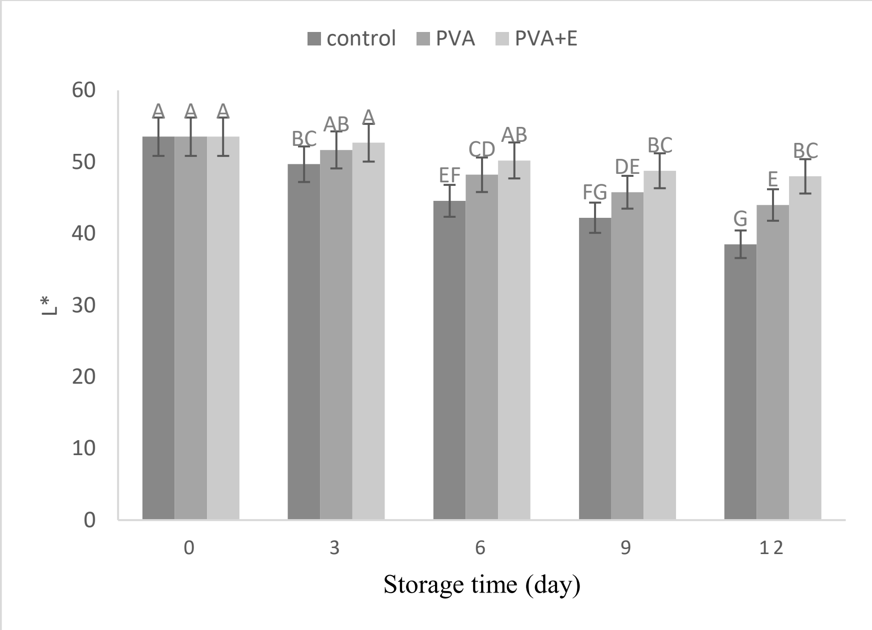


Fig 5. Changes in TBA values in control, PVA, PVA/EEO treatments during 12 days of storage at 4°C (mg MDA/KG)

Different letters in each day indicate a significant difference between the data ($p<0.05$), control: without polyvinyl alcohol and eucalyptus essential oil, PVA: Contains polyvinyl alcohol, PVA/EEO: contains polyvinyl alcohol / eucalyptus

3.2.4 Color parameters

Color is an important parameter in indicating the spoilage process of meat and significantly affects the marketability of the product. Bright red color is often associated with freshness, as it reflects the presence of myoglobin and oxymyoglobin, which are indicators of high quality meat [41]. During storage, myoglobin can be oxidized to metmyoglobin, resulting in a color change from red to brown [42]. The results of color properties including L* (lightness), a* (green to red), b* (blue to yellow) values during storage of minced meat samples are shown in Figure 6. All three color markers showed a decreasing trend in samples from day 0 to 12 ($P<0.05$). L* values, which indicate brightness, decreased throughout the storage period. This decrease was greater in the control samples compared to the PVA and PVA/EEO samples ($P<0.05$). The a* values or redness, a key indicator for judging the freshness and oxidation of meat, were the lowest in the control samples ($P<0.05$), while the highest values were in the PVA/EEO treatments ($P<0.05$). The b* values were shown in the treatments with nanofibers with the highest values and almost the same ($P<0.05$). The color change of minced meat is affected by factors such as lipid oxidation and microbial spoilage, which is why the color parameters of the samples decrease during refrigerated storage. According to Figure 6, PVA/EEO treatments had the best color parameter results compared to other samples. Najafi et al.(2020) acknowledged that EEO, due to its content of 1,8-cineole, has strong antioxidant and antimicrobial properties against oxidation and microbial spoilage, thus having a positive effect on color parameters during storage [2]. Similarly, the study by Göksen et al.(2021) showed that in chicken breast fillets wrapped with electrospun PVA fibers with bay leaf (LEO) and rosemary (REO) essential oils, there was a decreasing trend in L*, a* and b* values. Also, this study reported that the reason for the decrease in a* values in samples wrapped with fibers containing natural essential oils was due to the reaction between the essential oil and flesh pigments [43]. In another study, Zhang et al.(2020) showed that when cold meat packaging (black pork fillet) was coated with Cordelan (CD) and PVA film containing thyme essential oil, the a* values in samples packaged with composite films containing different amounts of thyme essential oil were significantly higher than those in control samples during storage ($P<0.05$) [44]. This increase is attributed to the antioxidant properties of natural essential oils, which help reduce oxidation and spoilage in meat.


control
PVA
PVA+E
L*
60
50
40
30
20
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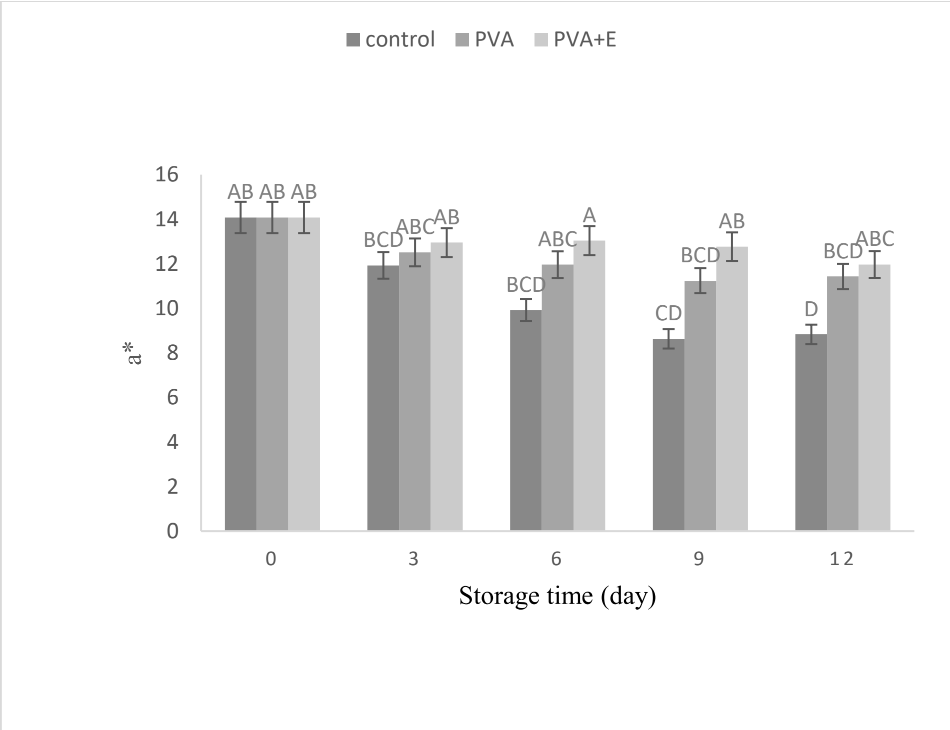
0
A A A
BC AB A
EF CD AB
FG DE BC
G E BC
0
3
6
9
12
Storage time (day)

(A)


control
PVA
PVA+E
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16
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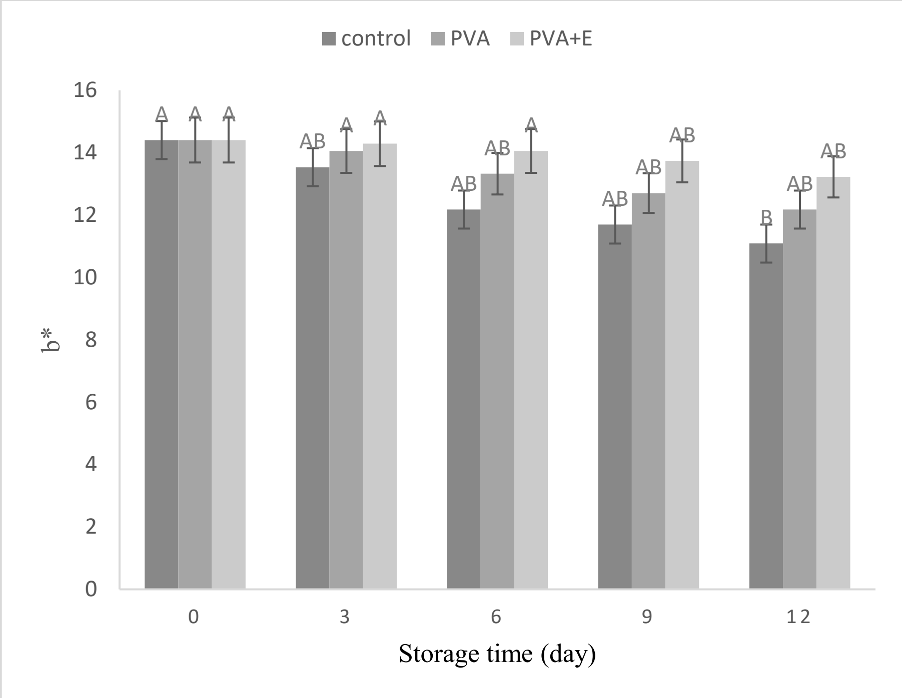
Storage time (day)

(B)



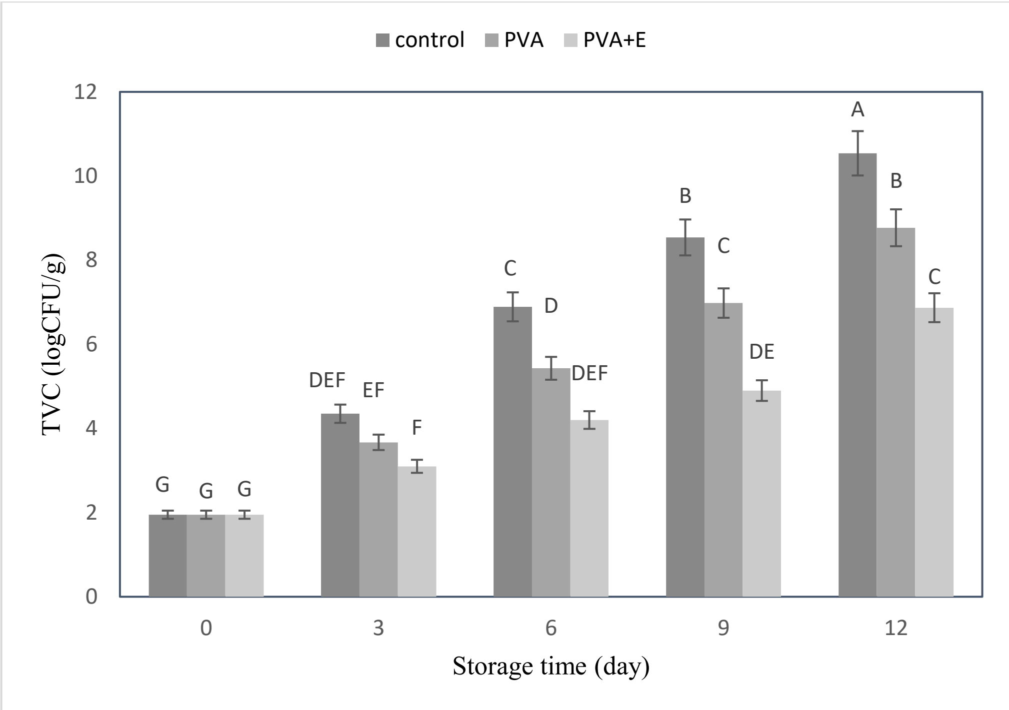
(C)

Fig 6. Changes in Color measurement of L * (A), a * (B), and b *(C) values in control, PVA, PVA/EEO treatments during 12 days of storage at 4°C (mg /100g)

Different letters in each day indicate a significant difference between the data (p<0.05), control: without polyvinyl alcohol and eucalyptus essential oil, PVA: Contains polyvinyl alcohol, PVA/EEO: contains polyvinyl alcohol / eucalyptus

3.2.5 Microbial analysis

The changes in total bacterial count (TVC) and pyrethroid bacterial count (PTC) of ground beef stored at refrigerated temperature (4°C) and the control, PVA, and PVA/EEO treatments over a 12-day period are shown in Figure 7 , respectively. The results showed a significant decrease in microbial population (TVC, PTC) of PVA and PVA/EEO treatments compared to the control group (P < 0.05). The initial TVC and PTC values on day 0 for all treatments were on average 1.95 logCFU/g and 2.14 logCFU/g, respectively. After 12 days of storage at 4°C, the TVC values for the control, PVA, and PVA/EEO samples were 10.54, 8.77, and 6.87 logCFU/g, respectively, and the PTC values for the control, PVA, and PVA/EEO samples were 11.4, 8, and 6.5 logCFU/g, respectively. PVA/EEO treatment had significantly (P < 0.05) the lowest increase in microbial load compared to other treatments. This can be attributed to the antimicrobial properties of eucalyptus essential oil due to the presence of compounds such as cineole and alpha-pinene [27]. In a similar study, Ahmadi et al. [45], showed that a combination of essential oils or various plant extracts in edible films or coatings can effectively prevent the proliferation of psychrophilic bacteria in meat and seafood products. The effectiveness of EEO against a wide range of microorganisms is attributed to its hydrophobic nature, which facilitates its incorporation into cell membranes and mitochondrial lipids, thereby increasing permeability and causing leakage of cellular contents. In a similar study, Cheng, Min, Luo, Zhang, and Yu [46], showed that microencapsulation of 1,8-cineole in cyclodextrin and electrospun PVA/chitosan nanofibers imparted antimicrobial properties that could extend the shelf life of food products such as strawberries. Furthermore, a similar study by Diaz et al. [9] investigating the antimicrobial properties of electrospun zein fibers with EEO and β-cyclodextrin showed that Gram-positive bacteria (Staphylococcus aureus and Listeria monocytogenes) showed greater sensitivity to eucalyptus essential oil compared to Gram-negative bacteria (Salmonella typhimurium and Escherichia coli).



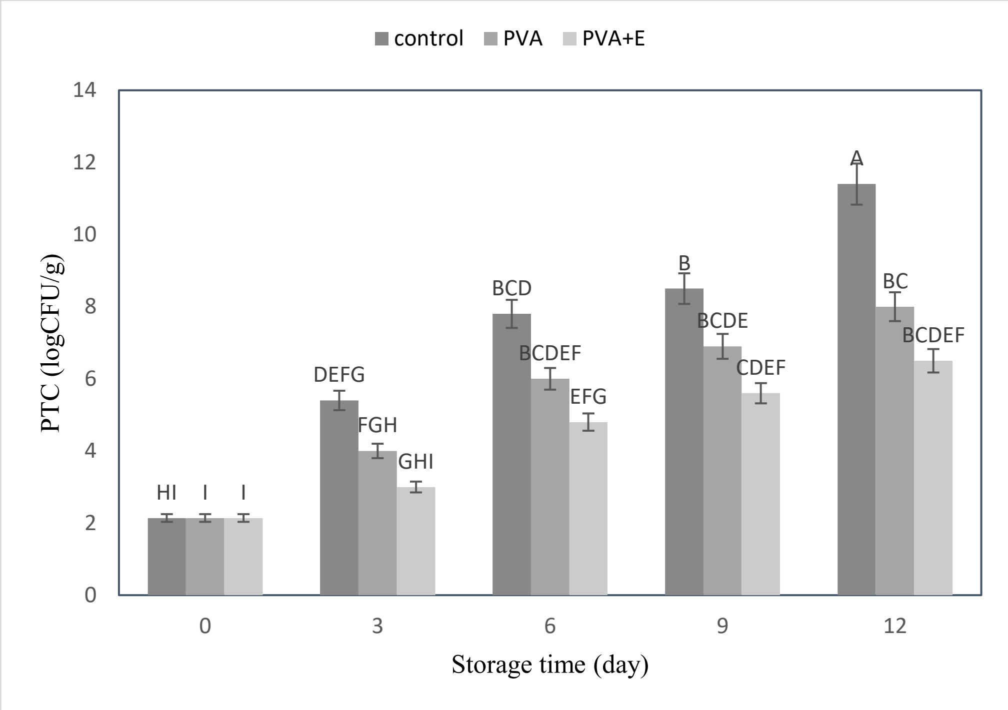


Fig 7. Changes in viable count (TVC) and psychrotrophic bacterial count (PTC) values in control, PVA, PVA/EEO treatments during 12 days of storage at 4°C (logCFU/g)

Different letters in each day indicate a significant difference between the data ($p<0.05$), control: without polyvinyl alcohol and eucalyptus essential oil, PVA: Contains polyvinyl alcohol, PVA/EEO: contains polyvinyl alcohol / eucalyptus

4. Conclusion

In this study, the production of polyvinyl alcohol nanofibers containing eucalyptus essential oil was investigated with the aim of increasing the shelf life and maintaining the quality of ground beef. The findings showed that the incorporation of EEO into the PVA nanofiber matrix not only enhanced the antioxidant and antimicrobial properties of the packaging, but also provided controlled release of the essential oil. The results of FTIR analysis showed favorable compatibility of eucalyptus essential oil in microencapsulated PVA nanofibers and no reaction between EEO and the PVA polymer matrix. According to the results, it was found that the use of PVA/EEO film in minced meat packaging significantly reduced microbial growth (TVC and PTC), lipid oxidation, and spoilage indicators such as PV, TVB-N, and TBA compared to the control treatment. In addition, the evaluation of color parameters showed that PVA/EEO treatment delayed the loss of color parameters in ground beef over a 12-day refrigerated storage period. The findings indicate that polyvinyl alcohol film containing eucalyptus essential oil can maintain the quality and increase the shelf life of ground beef compared to samples without film. Hence, the use of these biopolymer-based active packaging materials is promising for application in meat products.

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7-REFERENCES

[1] Beya, M.M., et al., Plant-Based Phenolic Molecules as Natural Preservatives in Comminuted Meats: A Review. Antioxidants (Basel), 2021. **10**(2).

[2] Najafi, M., et al., Evaluation of Antimicrobial and Antioxidant Properties of Eucalyptus Extracts in Zein Films to Improve of Minced Sheep Meat Packaging Shelf Life. 2020. **11**: p. 119–126.

[3] Goudarzi, J., H. Moshtaghi, and Y. Shahbazi, Kappa-carrageenan-poly (vinyl alcohol) electrospun fiber mats encapsulated with Prunus domestica anthocyanins and epigallocatechin gallate to monitor the freshness and enhance the shelf-life quality of minced beef meat. Food Packaging and Shelf Life, 2023. **35**: p. 101017.

[4] Chen, C., et al., Properties, vapour-phase antimicrobial and antioxidant activities of active poly(vinyl alcohol) packaging films incorporated with clove oil. Food Control, 2018. **88**: p. 105–112.

[5] Li, X., et al., Active packaging for the extended shelf-life of meat: Perspectives from consumption habits, market requirements and packaging practices in China and New Zealand. Foods, 2022. **11**(18): p. 2903.

[6] Haghighatpanah, N., et al., Effect of mung bean protein isolate/pullulan films containing marjoram (Origanum majorana L.) essential oil on chemical and microbial properties of minced beef meat. International Journal of Biological Macromolecules, 2022. **201**: p. 318–329.

[7] Moradinezhad, F., S. Hedayati, and E. Ansarifar, Assessment of zataria multiflora essential oil—incorporated electrospun polyvinyl alcohol Fiber mat as active packaging. Polymers, 2023. **15**(4): p. 1048.

[8] Dias Antunes, M., et al., Antimicrobial electrospun ultrafine fibers from zein containing eucalyptus essential oil/cyclodextrin inclusion complex. International Journal of Biological Macromolecules, 2017. **104**: p. 874–882.

[9] Limam, H., et al., Variation in chemical profile of leaves essential oils from thirteen Tunisian Eucalyptus species and evaluation of their antioxidant and antibacterial properties. Industrial Crops and Products, 2020. **158**: p. 112964.

[10] Azadbakht, E., et al., Development and structural characterization of chitosan films containing Eucalyptus globulus essential oil: Potential as an antimicrobial carrier for packaging of sliced sausage. Food Packaging and Shelf Life, 2018. **17**: p. 65–72.

[11] Matche, R.S. and O.O. Adeogun, Physicochemical characterisations of nanoencapsulated Eucalyptus globulus oil with gum Arabic and gum Arabic nanocapsule and their biocontrol effect on anthracnose disease of Syzygium malaccense Fruits. Scientific African, 2022. **18**: p. e01421.

[12] Gharsallaoui, A., et al., Applications of spray-drying in microencapsulation of food ingredients: An overview. Food Research International, 2007. **40**(9): p. 1107–1121.

[13] Lamarra, J., et al., Electrospun nanofibers of poly(vinyl alcohol) and chitosan-based emulsions functionalized with cabreuva essential oil. International Journal of Biological Macromolecules, 2020. **160**: p. 307–318.

[14] Costa, L.M.M., et al., Bionanocomposites from electrospun PVA/pineapple nanofibers/Stryphnodendron adstringens bark extract for medical applications. Industrial Crops and Products, 2013. **41**: p. 198–202.

[15] Estevez-Areco, S., et al., Release kinetics of rosemary (Rosmarinus officinalis) polyphenols from polyvinyl alcohol (PVA) electrospun nanofibers in several food simulants. Food Packaging and Shelf Life, 2018. **18**: p. 42–50.

[16] Xu, L., et al., Preparation and Performance of Radiata-Pine-Derived Polyvinyl Alcohol/Carbon Quantum Dots Fluorescent Films. Materials, 2020. **13**(1): p. 67.

[17] Lan, W., et al., Electrospun polyvinyl alcohol/d-limonene fibers prepared by ultrasonic processing for antibacterial active packaging material. Molecules, 2019. **24**(4): p. 767.

[18] Ansarifar, E., et al., Encapsulation of Jujube Extract in Electrospun Nanofiber: Release Profile, Functional Effectiveness, and Application for Active Packaging. Food and Bioprocess Technology, 2022. **15**(9): p. 2009–2019.

[19] Safari, J., et al., Fabrication of chitosan/pectin/pva nanofibers using electrospinning technique. Nanoscience & Nanotechnology-Asia, 2020. **10**(2): p. 134–141.

[20] Azarifar, M., et al., The effects of gelatin-CMC films incorporated with chitin nanofiber and Trachyspermum ammi essential oil on the shelf life characteristics of refrigerated raw beef. International Journal of Food Microbiology, 2020. **318**: p. 108493.
[21] Zheng, K., et al., Effect of chitosan coating incorporated with oregano essential oil on microbial inactivation and quality properties of refrigerated chicken breasts. LWT, 2023. **176**: p. 114547.
[22] bagheri, r. and P. aryaee, The Effect of Chitosan along with Basil Seed Gum and Nettle Essential Oil on the Microbial, Chemical, and Sensory Properties of Hamburger. Food Engineering Research, 2022. **21**(1): p. 75–92.
[23] Mohebbi, M., et al., Suitability of Aloe Vera and Gum Tragacanth as Edible Coatings for Extending the Shelf Life of Button Mushroom. Food and Bioprocess Technology, 2012. **5**(8): p. 3193–3202.
[24] Khezrian, A. and Y. Shahbazi, Application of nanocompostie chitosan and carboxymethyl cellulose films containing natural preservative compounds in minced camel's meat. International Journal of Biological Macromolecules, 2018. **106**: p. 1146–1158.
[25] Prahasti, G., A. Zulfi, and K. Khairurrijal, Synthesis of fiber membranes from polyvinyl alcohol (PVA)/shell extract of melinjo (SEM) using electrospinning method. Materials Today: Proceedings, 2021. **44**: p. 3400–3402.
[26] Ardekani, N.T., et al., Evaluation of electrospun poly (vinyl alcohol)-based nanofiber mats incorporated with Zataria multiflora essential oil as potential wound dressing. International Journal of Biological Macromolecules, 2019. **125**: p. 743–750.
[27] rahmatinia, n., et al., Electrospun zein nanofibers as a nanocarrier of Eucalyptus essential oil: Characterization, and antimicrobial properties. Journal of food science and technology(Iran), 2022. **18**(121): p. 81–91.
[28] Dzimitrowicz, A., et al., Comparison of the characteristics of gold nanoparticles synthesized using aqueous plant extracts and natural plant essential oils of Eucalyptus globulus and Rosmarinus officinalis. Arabian Journal of Chemistry, 2019. **12**(8): p. 4795–4805.
[29] Ghaderi, J., et al., Fabrication and characterization of biocomposite films based on carboxymethyl cellulose/polyvinyl alcohol/fish gelatin for food packaging exploits. Innovative Food Technologies, 2021. **8**(3): p. 383–398.
[30] Guyon, C., A. Meynier, and M. de Lamballerie, Protein and lipid oxidation in meat: A review with emphasis on high-pressure treatments. Trends in Food Science & Technology, 2016. **50**: p. 131–143.
[31] Ningrum, A., et al., Characterization of Tuna Skin Gelatin Edible Films with Various Plasticizers-Essential Oils and Their Effect on Beef Appearance. Journal of Food Processing and Preservation, 2021. **45**(9): p. e15701.
[32] Mouhoub, A., et al., Development of Food Packaging with Desirable Properties and Activities Using Chitosan and Mentha piperita, Salvia officinalis, Melaleuca quinquenervia, and Eucalyptus globulus EOs. Food and Bioprocess Technology, 2023. **16**(12): p. 3033–3044.
[33] Min, B. and D.U. Ahn, Mechanism of lipid peroxidation in meat and meat products -A review. Food Sci. Biotechnol, 2005. **14**: p. 152–163.
[34] Lu, H., et al., Antimicrobial activity of eucalyptus essential oil against Pseudomonas in vitro and potential application in refrigerated storage of pork meat. International Journal of Food Science and Technology, 2016. **51**(4): p. 994–1001.
[35] Mirzaei, A., et al., Colorimetric pH-sensitive hydrogel film based on kappa-carrageenan containing quercetin or eucalyptus leaf extract for freshness monitoring of chicken meat. Food Chemistry: X, 2024. **22**: p. 101307.
[36] Roshani-Dehlaghi, N., et al., Intelligent real-time multi-functional packaging of rainbow trout fillet containing anthocyanin-rich Althaea Officinalis extract encapsulated with poly (vinyl alcohol) electrospun nanofiber mats. 2024.
[37] Trabelsi, I., et al., Incorporation of probiotic strain in raw minced beef meat: Study of textural modification, lipid and protein oxidation and color parameters during refrigerated storage. Meat Sci, 2019. **154**: p. 29–36.
[38] Habibi Najafi, M., et al., Evaluation of the antimicrobial and antioxidant properties of eucalyptus and application in the production of potato starch for meat packing. Journal of food science and technology(Iran), 2021. **18**(113): p. 211–224.
[39] Zanela, J., et al., Active Biodegradable Starch/PBAT-poly(butylene adipate-co-terephthalate) Film with Eucalyptus citriodora Essential Oil Incorporation. Foods, 2024. **13**(13): p. 2104.
[40] Ceylan, Z., et al., Protective effect of grape seed oil-loaded nanofibers: Limitation of microbial growth and lipid oxidation in kashar cheese and fish meat samples. Food Bioscience, 2021. **42**: p. 101076.
[41] Kanatt, S.R. and S.H. Makwana, Development of active, water-resistant carboxymethyl cellulose-poly vinyl alcohol-Aloe vera packaging film. Carbohydrate Polymers, 2020. **227**: p. 115303.

[42] Faustman, C., et al., Myoglobin and lipid oxidation interactions: Mechanistic bases and control. Meat Science, 2010. **86**(1): p. 86–94.

[43] Göksen, G., et al., Biodegradable active food packaging structures based on hybrid cross-linked electrospun polyvinyl alcohol fibers containing essential oils and their application in the preservation of chicken breast fillets. Food Packaging and Shelf Life, 2021. **27**: p. 100613.

[44] Zhang, Y., et al., Preparation and characterization of curdlan/polyvinyl alcohol/ thyme essential oil blending film and its application to chilled meat preservation. Carbohydrate Polymers, 2020. **247**: p. 116670.

[45] Ahmadi, S.S., et al., Application of electrospun zein/polyvinyl alcohol nanofibers incorporating thymoquinone and electrosprayed resveratrol nanoparticles on the shelf life of fresh rainbow trout fillet and inoculated Escherichia coli O157:H7. Food Control, 2024. **155**: p. 110089.

[46] Cheng, C., et al., Electrospun polyvinyl alcohol/chitosan nanofibers incorporated with 1,8-cineole/cyclodextrin inclusion complexes: Characterization, release kinetics and application in strawberry preservation. Food Chemistry, 2023. **418**: p. 135652.