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RESEARCH ARTICLE

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Peppermint oil-infused polylactic acid films: A novel approach for antimicrobial and biodegradable food packaging

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Abstract

Extensive research is currently dedicated to creating biodegradable packaging materials that match the qualities of traditional synthetic packaging materials. Among these options, polylactic acid (PLA) is noteworthy. PLA is a renewable-source-derived thermoplastic polymer with excellent barrier properties, mechanical strength, and a strong safety profile. To enhance food product shelf life, active packaging materials, incorporating functional ingredients like antimicrobials, have gained prominence. Peppermint essential oil is one such active ingredient, offering potential improvements in preserving food freshness and safety. This study's objective is to craft antimicrobial, biodegradable food packaging materials by blending peppermint oil into PLA films. Various peppermint oil concentrations (1.25%, 1.875%, and 2.5% w/v) were blended with PLA to assess their impact on opacity, water vapor permeability (WVP), mechanical and thermal properties, and antimicrobial characteristics. Higher peppermint oil concentrations increased opacity, making them advantageous for light-sensitive food items. These films reduced WVP without affecting PLA's thermal stability. Antimicrobial effectiveness was evaluated against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*), showing inhibition with 1.875% and 2.5% w/v peppermint oil concentrations. Compared to control, PLA films with peppermint oil extended the shelf life of packaged chicken breast meats from 3 to 8 days. As a result, incorporating peppermint oil into PLA films presents a promising solution for advanced antimicrobial and biodegradable food packaging.

Highlights

- The incorporation of peppermint oil resulted in higher opacity values.
- Peppermint incorporated PLA films showed antimicrobial activity.
- Peppermint oil addition decreased water vapor permeability of PLA films.

- Peppermint oil added PLA films increased the shelf life of chicken meat up to 8 days.

KEYWORDS

antimicrobial package, chicken breast, peppermint oil, polylactic acid (PLA), shelf life

1 | INTRODUCTION

Packaging plays a crucial role in safeguarding products against external factors, acting as a protective shield that upholds quality, safety, and overall integrity.[1] Presently, the predominant composition of packaging films relies on non-renewable petroleum-based materials, accounting for approximately 90% of the market.[2] However, the non-biodegradable nature of these materials necessitates diligent recycling practices to mitigate their environmental impact, which can impose significant economic burdens. Notably, plastic packaging constitutes a substantial portion of global solid waste, comprising 40% of its weight.[2] In response to mounting environmental concerns, there has been a notable surge in research efforts aimed at developing biodegradable packaging materials that can match the performance of their synthetic counterparts.[2,3] Continuous research endeavors are focused on enhancing the mechanical and barrier properties of biodegradable films to meet the standards set by conventional packaging materials.[4] To this end, diverse material combinations and preparation methods are being explored to achieve optimal outcomes.

Polylactic acid (PLA), a widely recognized biodegradable thermoplastic polymer, has garnered significant attention. This hydrophobic polyester is derived from renewable resources and boasts commendable barrier and mechanical properties.[5] In terms of water vapor permeability (WVP), PLA outperforms commonly utilized protein and polysaccharide-based edible films and coating materials, and also offers superior protection against oxygen permeation compared to synthetic polymers such as polypropylene.[6,7] Leveraging its remarkable biocompatibility and ease of processing, PLA finds extensive utility across diverse sectors, including packaging, textiles, 3D printing, agriculture, and healthcare.[8] PLA also exhibits a notable advantage in terms of human safety, as its consumption results in the production of carbon dioxide and water as final metabolites. Furthermore, lactic acid, an intermediate metabolite of PLA digestion, is a natural byproduct of glycolysis, a crucial process in glucose metabolism within human cells. Such characteristics have led to PLA being recognized as the second biodegradable polymer material approved by the US Food and Drug Administration (FDA) for human use.[6,9] Consequently, PLA holds considerable appeal for food packaging applications, given its compatibility with regulatory standards.

Nonetheless, the utilization of PLA presents inherent challenges due to its inferior mechanical toughness, low crystallinity, high cost, limited heat resistance, low durability, and inadequate hydrophilicity, all of which impede its broad application.[8,10] To overcome these limitations, one of the prevailing approaches involves blending PLA with other biodegradable polymers and/or additives. Through the incorporation of diverse materials such as various starch types, polycaprolactone, and polyester, superior thermal stability, mechanical strength, and barrier properties have been achieved in blended films.[11–14] This strategy offers promise in enhancing the overall performance of PLA-based materials and expanding their potential applications.

Active packaging materials have emerged as a promising approach to enhance the quality and prolong the shelf life of food products.[15] These materials utilize a range of functional ingredients, including antimicrobials, antioxidants, light screeners, transport inhibitors, and texture modifiers, which are incorporated into biopolymer films.[16–18] Essential oils, derived from various plants, have been recognized for their potent antimicrobial and antioxidant properties.[16–18] These oils can be effectively integrated into biopolymer solutions, offering antibacterial and anti-mold functionalities in packaging materials.[19] While previous studies have focused on incorporating essential oils from thyme, lemongrass, and sage into alginate-based biopolymer films,[15] there exists a wide range of other essential oils and biopolymers that have been explored for similar applications.[15,19]

Peppermint oil (PO), extracted from fresh peppermint leaves (*Menthae piperita*), stands as a prominent essential oil (EO) renowned for its extensive applications in the food, cosmetics, and pharmaceutical industries. Its widespread use can be attributed to its robust antimicrobial, antiviral, and antifungal properties.[20] Essential oils, in general, consist mainly of non-polar constituents with relatively low water solubility, necessitating their incorporation into aqueous systems through the process of emulsification.[18,21,22] In the study by Karagözlü et al.,[23] significant antimicrobial activity against *E. coli* O157:H7 and *S. typhimurium*, two common pathogens found in lettuce and purslane, was observed with the application of peppermint and basil essential oils during chilled storage. Peppermint essential oil exhibited superior antimicrobial efficacy compared to basil essential oil, displaying stronger inhibitory effects on the survival of the pathogens.[23]

However, the antimicrobial activity exhibited by peppermint oil in its pure form does not necessarily guarantee the same behavior when it is incorporated into a polymer network within a packaging system. One of the main challenges in active packaging is the effective incorporation and controlled release of active compounds, such as antimicrobial agents into the packaging material.[24,25] These active substances are designed to interact with the packaged product, modifying its properties and providing additional functionalities. However, the successful development of active packaging systems requires careful consideration of factors such as compatibility between the active compound and the packaging material, stability of the active compound over time, and rate of release of active agents to ensure the desired effects.[24–26] Hence, whether these active compounds retain their active properties within the package itself must be investigated.

This study aims to explore the potential of peppermint oil both as an antimicrobial agent and as an additive to enhance the properties of biodegradable packaging materials derived from PLA. Specifically, the effect of incorporating peppermint oil into PLA films and its impact on film quality are investigated, with the goal of determining the suitability of this combination for producing antimicrobial packaging materials that can effectively extend the shelf life of food products. For this purpose, PLA films were prepared by blending different concentrations of peppermint oil (1.25%, 1.875%, and 2.5% w/v). Films were analyzed to evaluate the effects of peppermint oil incorporation on the opacity, WVP, mechanical properties, thermal properties, and antimicrobial properties of the PLA films. Furthermore, to assess the antimicrobial potential of the films, a real packaging system was employed by applying them to chicken breast meat, and the impact on the shelf life of the food samples was observed.

2 | MATERIALS AND METHODS

2.1 | Materials

PLA was bought in pellet form from Filamon Company, Kayseri, Turkey. Peppermint oil was provided from Arifoglu Ltd. Sti. and chloroform was purchased from Sigma-Aldrich Chemie GmbH (Darmstadt, Germany). Mueller-Hinton Agar, buffer peptone water, and plate count agar were bought from Condalab to conduct antimicrobial analyses. Fresh chicken breast meat was purchased from a local market in Canakkale, Turkey.

2.2 | Film preparation

To eliminate surface moisture, PLA pellets were pre-dried in an oven at 50°C. A 5% (w/v) PLA solution was prepared by dissolving 5 g of PLA in 100 mL of chloroform using a magnetic stirrer operating at 750 rpm for 6 h. Subsequently, different concentrations of peppermint oil (1.25%, 1.875%, and 2.5% w/v) were added to the PLA-chloroform solution, followed by stirring the mixture at 1200 rpm for 2 h until a homogeneous solution was obtained. The obtained homogeneous solution (20 mL) was evenly distributed onto glass petri plates with a diameter of 10 cm. The plates were then left to dry for a period of 8 h under ambient conditions. The resulting PLA films, distinguished by their varying concentrations of peppermint oil, were labeled as PLA_M1.25, PLA_M1.875, and PLA_M2.5, representing the ascending order of peppermint oil concentrations. Additionally, a PLA film without peppermint oil was designated as PLA for comparative purposes.

2.3 | Film thickness and opacity

The thickness measurements of each film were determined using a digital micrometer (Rohs norm 2011/65/eu) at four randomly selected points. Subsequently, rectangular pieces measuring $1 \times 4\ \text{cm}^2$ were cut from the film and affixed to the wall of a cuvette. The opacity of the films was assessed at a wavelength of 600 nm using a UV–visible spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). Opacity values were calculated using Equation (1)[27]:

$$\text{Opacity} = \frac{A_{600}}{x} \tag{1}$$

where A_{600} and x referred to the absorbance at 600 nm and thickness of the film (mm), respectively.

2.4 | Mechanical properties

Tensile strength (TS) and elongation at break (EAB) of the films were determined using a Tensile Tester (PCM, 305) (Ankara, Turkey). For testing, the films were cut into rectangular strips of 50 mm × 10 mm and securely mounted between grips with a grip-to-grip separation of 25 mm, extending the films at a constant speed of 20 mm min^{-1} using a load cell of 100 N. Each measurement was performed at least in triplicate.

2.5 | Water vapor permeability

The water vapor permeabilities (WVPs) of the films were determined following the method described by Aydogdu et al. (2018).[28] Cylindrical polyacetal (Delrin) test cups with an internal diameter of 40 mm were employed for the measurement. Each cup was filled with 35 mL of distilled water. After measuring the film thickness, the film was inserted between the cup and cap, and securely fastened. The initial weight of the cup was recorded before placing it in a desiccator maintained at a relative humidity of approximately 15%. Weight changes of the cups were periodically recorded over the course of a day. Throughout the measurements, the relative humidity and temperature inside the desiccator were monitored using a humidity/temperature logger (EBI20-TH1, EBRO, Ingolstadt, Germany).

Subsequently, the WVPs of the films were calculated using the following equation:

$$\mathrm{WVP} = \frac{\mathrm{WVTR} \times \Delta x}{S \times (R_1 - R_2)} \tag{2}$$

where WVTR is the steady slope of weight loss versus time data, Δx references the thickness of the film, S is the saturated water vapor pressure at given temperature. R_1 and R_2 refers to the humidity values inside the cups (100%) and desiccator, respectively.

2.6 | Antimicrobial activity of the films

The antimicrobial activity of the films against *E. coli* and *S. aureus* microorganisms was evaluated using the agar diffusion assay, following the methodology described by Wen et al. (2016).[29] In this assay, a bacterial suspension (1×10^8 CFU mL^{-1}) consisting of 100 μL was evenly spread on the surface of nutrient agar. Subsequently, circular films with a diameter of 0.5 cm were placed onto the inoculated agar medium. Following incubation at 37°C for 24 h in an incubator (Min 55, Mikrotest, Turkey), the diameter of the zone exhibiting no bacterial growth was measured using a digital caliper. A larger diameter of the inhibition zone indicated a higher level of antimicrobial activity. Each sample was tested in duplicate to ensure the reliability of the results.

2.7 | Application of antimicrobial films on fresh chicken breast meat

Fresh chicken breast meat was acquired from a local market and subsequently aseptically sliced into 10 g portions. Each meat portion was then positioned between the PLA active films exhibiting the highest antimicrobial activity. To simulate realistic food packaging conditions, the films were hermetically sealed using a constant heat sealer from Taiwan. Additionally, for the assessment of the antimicrobial efficacy of the PLA antimicrobial film, films labeled as PLA_0 were employed as negative controls. All samples were stored at a temperature of 4°C for a duration of 8 days. Microbiological analyses of the samples were performed at 0, 1, 3, 5, 7, and 8 days of storage.

To initiate the microbiological analysis, the samples packed within the PLA films were homogenized in 90 mL of sterile 0.1% peptone water using a homogenizer. Following serial dilution, 100 μL of the diluted sample was cultured on plate count agar and subsequently incubated at 30°C for a period of 48 h. The total viable bacteria were enumerated and expressed as the logarithm of the number of colony-forming units per sample (log CFU g^{-1}). The analysis was conducted twice for each PLA package to ensure the reproducibility of the results.

2.8 | Statistical analysis

The data analysis was conducted using MINITAB software (version 16, State College, PA, USA). Analysis of variance (ANOVA) was performed to assess the significance of differences among the values. Tukey's Multiple Comparison Test was employed for posthoc analysis, with a significance level set at $p \leq 0.05$, to identify specific variations between the groups.

3 | RESULTS AND DISCUSSION

3.1 | Film thickness and opacity

The surface and internal heterogeneity of a film's structure play a significant role in shaping its optical properties, making them reliable indicators of the film's microstructure.[30] Moreover, as packages serve as the primary means of presenting products to consumers, transparency becomes a vital parameter that greatly influences consumer acceptance.[31] Opacity values can be seen in Table 1. The opacities of the films were found to range from 1.593 ± 0.091 to 3.502 ± 0.044 mm^{-1}. As the concentration of peppermint oil increased, the films demonstrated a corresponding increase in opacity. This phenomenon can

TABLE 1 Physical characteristics of PLA and PLA/peppermint oil blend films.

Film sample	Opacity (mm^{-1})	Thickness (µm)	Tensile strength (MPa)	Elongation at break (%)	WVP ($\mathrm{ng\ m}^{-1}\ \mathrm{s}^{-1}\ \mathrm{Pa}^{-1}$)	Diameter of inhibition zone (mm)	
						S. aureus	***E. coli***
PLA	1.593 ± 0.091^{c}	129.0 ± 2.3^{c}	18.9 ± 3.6^{a}	464.7 ± 88.0^{a}	0.1628 ± 0.0154^{a}	No zone of inhibition	No zone of inhibition
PLA_M1.25	2.295 ± 0.1488^{b}	158.1 ± 2.6^{b}	21.5 ± 2.6^{a}	224.5 ± 57.6^{b}	0.0902 ± 0.085^{b}	No zone of inhibition	No zone of inhibition
PLA_M1.875	3.379 ± 0.154^{a}	160.6 ± 1.7^{b}	20.4 ± 3.7^{a}	279.0 ± 14.5^{b}	0.0833 ± 0.0833^{b}	8.5	8
PLA_M2.5	3.502 ± 0.044^{a}	202.3 ± 3.5^{a}	15.7 ± 1.4^{a}	289.0 ± 46.1^{b}	0.0593 ± 0.0218^{b}	10	9

^{a,b,c}Different letter superscripts on the same line indicate a statistically significant difference ($p \le 0.05$).

be attributed to the enhanced light scattering caused by the dispersed oil droplets within the film matrix, thereby reducing the overall transparency of the films.[1] Similar findings have been reported by other researchers in the context of emulsion films,[1,32–34] further corroborating these observations.

It is worth mentioning that the control films made solely from PLA exhibited a significantly lower opacity compared to polyethylene (PE) films ($4.26\ \mathrm{mm}^{-1}$), which serve as the established commercial standard for food packaging.[33] However, the film with the highest concentration of peppermint oil exhibited an opacity level that closely approached that of PE films. Overall, the films made in this study were highly transparent and offered excellent visibility, making them a favorable choice for packaging applications where consumers prefer to see the contents of the food packaging.

Film thicknesses ranged from 129.0 ± 2.3 to 202.3 ± 3.5 µm (Table 1). These values are consistent with findings reported by other researchers in studies involving biopolymer films. For instance, Gomaa et al. reported thicknesses ranging from 107 to 143 µm[35] in alginate-chitosan edible films, while Meira et al. observed thicknesses between 162 and 223 µm in antimicrobial-added corn starch films.[36] Similarly, Lee et al. found thicknesses ranging from 162 to 223 µm in chitosan-based films containing clove oil.[32] The dispersion of peppermint oil within the film matrix and the increase in its concentration led to an increase in film thickness, which aligns with the findings of previous studies on emulsion films.[32,36] The incorporation of more oil into the film matrix may disrupt molecular interactions among the horizontally aligned PLA chains, resulting in a looser molecular structure and a less dense matrix. This disruption could contribute to an overall increase in film thickness. Additionally, the dispersion of peppermint oil may have increased the spacing between these chains, further contributing to the observed increase in film thickness. Localized areas of oil agglomerations and the formation of air-filled pockets could also play a role in the increase in film thicknesses. An analogous finding was reported in the author's previous study.[37]

3.2 | Thermo-gravimetric analysis

Low thermal stability can hinder a polymer's application. Processing PLA often involves high temperatures, and the polymer needs to exhibit sufficient stability at these elevated temperatures to maintain its desired properties. The thermal degradation of PLA initiates at temperatures above 200°C, leading to accelerated processes such as hydrolysis, lactide reformation, inter- or intramolecular transesterification, and oxidative main chain scission.[38,39] These degradation mechanisms cause a reduction in the polymer's molecular weight, consequently altering its physical characteristics.[38]

Thermogravimetric analysis (TGA) and derivative thermogravimetry (DTG) curves are given in Figure 1A,B, respectively. DTG curves help to distinguish and analyze individual peaks more effectively.[40]

As seen in Figure 1, there are two thermal degradation peaks. The initial weight loss is observed at around 100°C (with an onset temperature T_{on1} of 96°C and a peak temperature T_{p1} of 111°C). This peak is evident in all samples, including the PLA-only films, indicating that it can be attributed partially to the evaporation of water adsorbed on the film surface. Unlike many biopolymer-based films that use water as a solvent, PLA is not soluble in water. Although the film formulation does not contain water, the films were exposed to a relative humidity (RH) of 50% during drying and analysis, facilitating water adsorption on the polar sites present on the film surface. Consequently, a relatively small weight loss was observed. A similar finding was reported by Rizal et al. in their investigation of PLA-reinforced cellulose nano-fibrillated fiber biocomposites.[42] The absence of weight loss at the boiling point of chloroform (boiling point of 61.1°C)[41] indicates successful removal of the casting solvent from the films,

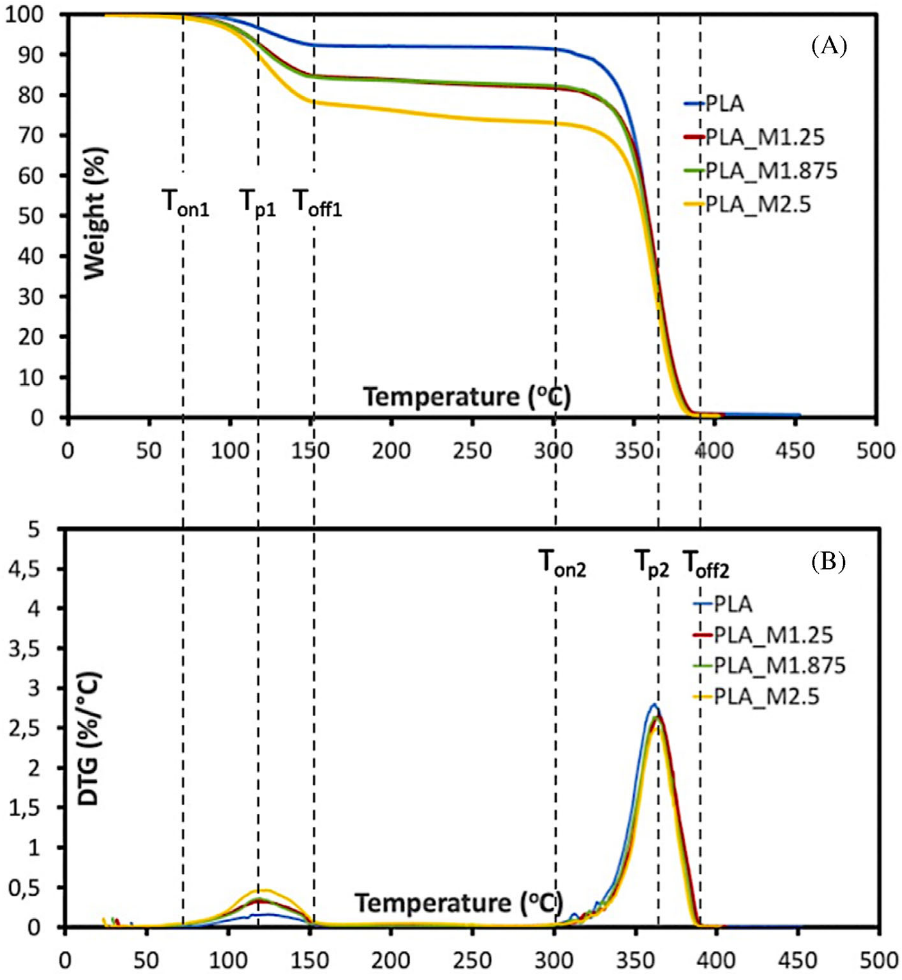

FIGURE 1 Thermogravimetric profiles of PLA and PLA/peppermint oil blend films.

which is crucial as the films come into contact with food substances.

TGA curves exhibit an increased weight loss in the presence of peppermint oil in the samples when exposed to temperatures ranging from 96°C (T_{on1}) to 148°C (T_{off1}). While a portion of this weight loss can be attributed to water evaporation, the higher concentration of peppermint oil in the samples contributes to an overall greater weight loss indicating that the volatile essential oils present in peppermint oil undergo evaporation within this temperature range. Đurović et al. (2022) conducted a study on the thermal properties of peppermint oil and reported that peppermint oil displays a DTG evaporation peak within a temperature range of 101–110°C.[43] These findings align with our observations, suggesting that the PLA polymer film network does not fully encapsulate the peppermint oil, allowing it to evaporate and maintain its functional properties. The retention of essential oil content in the films, even at temperatures as high as ∼150°C, is a noteworthy observation. This retention highlights the film's ability to preserve the volatile components of the essential oil, which is advantageous considering the intended temperature range for the film's application.

The second DTG peak observed in the TGA curves have similar peak size and location for all samples. This peak corresponds to the degradation of the common component present in all specimens, which is the PLA polymer. The TGA curve of PLA alone reveals that the onset of PLA degradation occurs at 311.9°C (T_{on2}), and the degradation process rapidly accelerates. A sharp decline in weight is observed starting from 330.1°C and ending at 393.3°C (T_{off2}), with the maximum degradation temperature (T_{p2}) occurring at 366.9°C. Several studies have investigated the thermal degradation of PLA and reported a single-step degradation process with T_p values around 370°C.[6,7,42,44,45] These findings highlight the characteristic thermal behavior of PLA and its well-defined degradation profile at elevated temperatures.

3.3 | Mechanical properties

The primary function of food packaging is to ensure the continuous mechanical protection of food, thereby preserving its structural integrity. Consequently, films that exhibit good mechanical resistance and provide food with resistance against deformation are preferred for various applications.[46] The limited commercial use of biopolymer-based films compared to synthetic polymers can be attributed to their inferior mechanical properties.[47] TSs ranged from 15.7 ± 1.4 to 21.5 ± 2.6 MPa (Table 1), indicating that the films possess high strength and resistance to breakage. These TSs surpass the recommended threshold of 4 MPa, which is considered acceptable for food packaging applications.[48] Remarkably, the films exhibited exceptionally high EABs, ranging from 224.5% to 464.7%. This result is noteworthy considering the much lower characteristic range of EAB values (ranging around 5%–100%) typically observed in biopolymer-based films.[1,15,33,34,36,49,50] The observed high elasticity provides the films with increased resistance to rupture. The high EAB makes the studied films particularly suitable for wrapping irregularly shaped foods, enhancing their versatility and practicality in food packaging applications. Such high EAB values were encountered in multiple previous studies that formed PLA films with solvent casting method using chloroform as the solvent.[51–53] For films composed of PLA only, Andrade et al.[52] reported EAB values between 260.1% and 432.9%. Similarly, Briassoulis et al.[53] reported EABs of ∼260.0% for PLA films. When chloroform is used as solvent, it seems to help overcome the inherent brittleness of PLA, as confirmed by the results of the authors' previous study which used dichloromethane (DCM) as the solvent.

Peppermint oil addition had a significant influence on film mechanical properties by reducing film EAB by 50% at the highest concentration. At the same concentration of peppermint oil, TS is 20% lower than the control, however this change is not statistically significant ($p > 0.05$). Oil addition is usually associated with a plasticizing effect.[50] The hydrogen bonds established between the ∼OH groups in oil and water molecules are expected to increase the mobility of polymer chains leading to enhanced flexibility. The greater degree of disruption of the polymer matrix with oil introduction supports this behavior. The oil acts as a lubricant and eases the movement of stacked polymer chains on one another. Numerous studies have reported the plasticizing role that oil plays in a hydrocolloid matrix.[33,54–57]

However, contrary to expectations, our study reveals a significant decrease in EAB when incorporating peppermint oil into the film. This decline suggests a structural break within the film. Previous research has linked similar findings to the formation of pinholes and cracks upon the dispersion of a secondary phase into the polymer matrix of the film.[37,58] These structural weak points act as stress concentrators, initiating tears and leading to premature failure of the film. While measures such as degassing might potentially prevent the formation of air-filled holes during film solution homogenization, cracks and other structural imperfections may be a result of the oil dispersion, which might be difficult to prevent. In general, in polymer blends, when the continuous film matrix effectively transfers mechanical stress to the dispersed phase, reducing its own load, the resulting film exhibits improved mechanical properties.[6,7,9,42,59] In short, incorporating oil into films typically softens them, evidenced by decreased TS and increased EAB, characteristic of plasticizers. However, peppermint oil uniquely decreases TS without a proportional increase in EAB. We attribute this to structural integrity disruption during oil dispersion, possibly caused by the dispersion process introducing stress concentrators. Thus, while peppermint oil particles themselves may not act as stress concentrators, their presence during dispersion can create structural imperfections, explaining the observed deviation from typical film behavior.

3.4 | Water vapor permeability

Efficient control of moisture transfer plays a vital role in food packaging as it regulates the interaction between the food and its surrounding environment, including separate components of a multi-component food. This control is especially critical due to the significant impact of water on the rate of deteriorative reactions.[46,60] Consequently, the WVP of edible films has received extensive attention as it represents one of the key properties that has been extensively investigated.[61] WVPs of films are reported in Table 1. The WVP of PLA-only films, measured at 0.1628 ± 0.0154 ng m^{-1} s^{-1} Pa^{-1}, is higher than the range reported in existing literature, which falls between 0.019 and 0.075 ng m^{-1} s^{-1} Pa^{-1}.[62–67] Various factors such as the average molecular weight and crystallinity of the PLA used, differences in the film production method, as well as the environmental and analysis conditions, can contribute to variations in the WVP values.

Comparatively, the WVP value of the control films is ∼1 order of magnitude larger than that of low-density polyethylene films (0.036×10^{-2} ng m^{-1} s^{-1} Pa^{-1}), which are widely employed in food packaging.[68] Nonetheless, PLA exhibits superior water barrier properties compared to functional counterparts like starch or protein-based biopolymer films due to its hydrophobic nature. For instance, the WVP of PLA is lower than that of films made from banana flour (0.200 ng m^{-1} s^{-1} Pa^{-1}),[69] wheat gluten (0.700 ng m^{-1} s^{-1} Pa^{-1}),[70] canola protein isolate (1.194 ng m^{-1} s^{-1} Pa^{-1}),[3] chia seed mucilage (0.188 ng m^{-1} s^{-1} Pa^{-1}),[71] cassava starch (2.40 ng m^{-1} s^{-1} Pa^{-1}),[70] pea starch/polyvinyl alcohol blends (0.611 ng m^{-1} s^{-1} Pa^{-1}),[30] and lentil protein concentrate (0.3095 ± 0.002 ng m^{-1} s^{-1} Pa^{-1}).[72] The hydrophilicity of a material plays a significant role in determining water barrier properties. Although most biopolymer-based films are edible and biodegradable, they inherently consist of hydrophilic materials, posing challenges due to their high-water solubility and permeability.[73] In this context, PLA, as an FDA-approved polymer, emerges as a highly promising alternative for food applications where water transfer plays a crucial role in determining product shelf life.

The addition of peppermint oil to the films resulted in a significant reduction in WVP values, as shown in Table 1. The WVP decreased as much as 63%, from 0.1628 ± 0.0154 to 0.0593 ± 0.0218 ng m^{-1} s^{-1} Pa^{-1}. Water transfer predominantly takes place through the hydrophilic regions within the films. This observation highlights the substantial impact of the hydrophilic–lipophilic ratio of the films on their permeability.[74] The rate of water transfer is closely associated with the tortuosity of the films. Tortuosity refers to the degree of winding or twisting path that water molecules must navigate within the film structure. As the tortuosity of the films increases, the resistance encountered by water molecules also increases, leading to a decrease in the rate of water transfer.[75,76] Manipulating the amount of oil phase or the size of oil droplets within the films offers a means to enhance the tortuosity. By increasing the oil phase content or reducing the size of oil droplets dispersed within the film matrix, the tortuosity of the films can be augmented. These alterations in the film structure create additional obstacles and convolutions for water molecules, thereby impeding their transfer and resulting in reduced WVP.[15] The fact that the theory is in line with our observations demonstrates that mint oil drops are well dispersed within the film matrix. Successful dispersion occurs when there is strong compatibility between the polymer matrix and the dispersed oil phase, ensuring uniform and stable distribution of the oil throughout the film without the formation of clusters or patches. A properly dispersed oil phase significantly enhances the hydrophobicity of the films, resulting in notable improvements in their water barrier properties, as observed in multiple edible emulsion film studies.[34,50,55,75–77]

3.5 | Antimicrobial activity

To assess the antimicrobial efficacy of peppermint oil in the films under investigation, we conducted a test using the four film samples. These samples were placed onto Petri dishes that were inoculated with both Gram-negative (Gr−) bacteria (*E. coli*) and Gram-positive (Gr+) bacteria (*S. aureus*). Subsequently, we measured the diameters of the inhibition zones formed around the film samples, indicating the extent of bacterial growth inhibition. Images of the Petri dishes and sizes of the inhibition zones were shown in Figure 2 and Table 1, respectively.

As anticipated, the control films showed no antimicrobial activity. However, the films containing higher concentrations of peppermint oil (PLA_M1.875 and PLA_M2.5) demonstrated concentration-dependent antimicrobial effects against both Gr− and Gr+ bacteria, as evidenced by the observable inhibition zones depicted in Figure 2. The measured inhibition zone diameters were as high as 10 and 9 mm for Gr+ and Gr− bacteria respectively.

The presence of essential oils like peppermint oil in films has been widely recognized for their antimicrobial properties, as demonstrated in several studies. This antimicrobial effect is attributed to the ability of essential oils to damage cell membranes. In many of these studies, researchers have consistently reported higher antimicrobial activity against Gram-positive (Gr+) bacteria when compared to Gram-negative (Gr−) bacteria. This difference in effectiveness against the two types of bacteria suggests that essential oils have a more pronounced impact on the structural integrity of Gr+ bacterial cells.[1,15,36,78,79] The size of the inhibition zones was not only larger in Gram-positive (Gr+) bacteria but also more pronounced, displaying a clearer appearance with sharper borders. In contrast, the inhibition zones observed in *E. coli* were more diffuse and less distinct in comparison. These observations could potentially indicate the higher effectiveness of peppermint oil against Gr + bacteria and/or variations in the rate and extent of diffusion into the different agars used. Nonetheless, the results collectively highlight the significant contribution of peppermint oil to the films' ability to inhibit the growth of both Gr+ and Gr− bacteria.

3.6 | Shelf-life studies on chicken breast meat

Simply dispersing an antimicrobial material in a package does not guarantee its sustained antimicrobial activity within the package. A major challenge in active packaging lies in effectively incorporating and releasing active compounds, including antimicrobial agents, into the packaging material.[24,25] These active substances are specifically designed to interact with the packaged product, modifying its properties and introducing additional functionalities. However, successful development of active packaging systems requires careful consideration of several factors. These factors include ensuring compatibility between the active compound and the packaging material, achieving complete dispersion of the antimicrobial agent within the package matrix, maintaining the stability of the active compound over time, and controlling the release rate of the active agents to achieve the desired effects.[24–26] Therefore, it is crucial to subject these packages to testing using real food systems to evaluate their antimicrobial activity accurately.

In this study, the main parameter for determining the shelf life was the results of microbiological analysis.

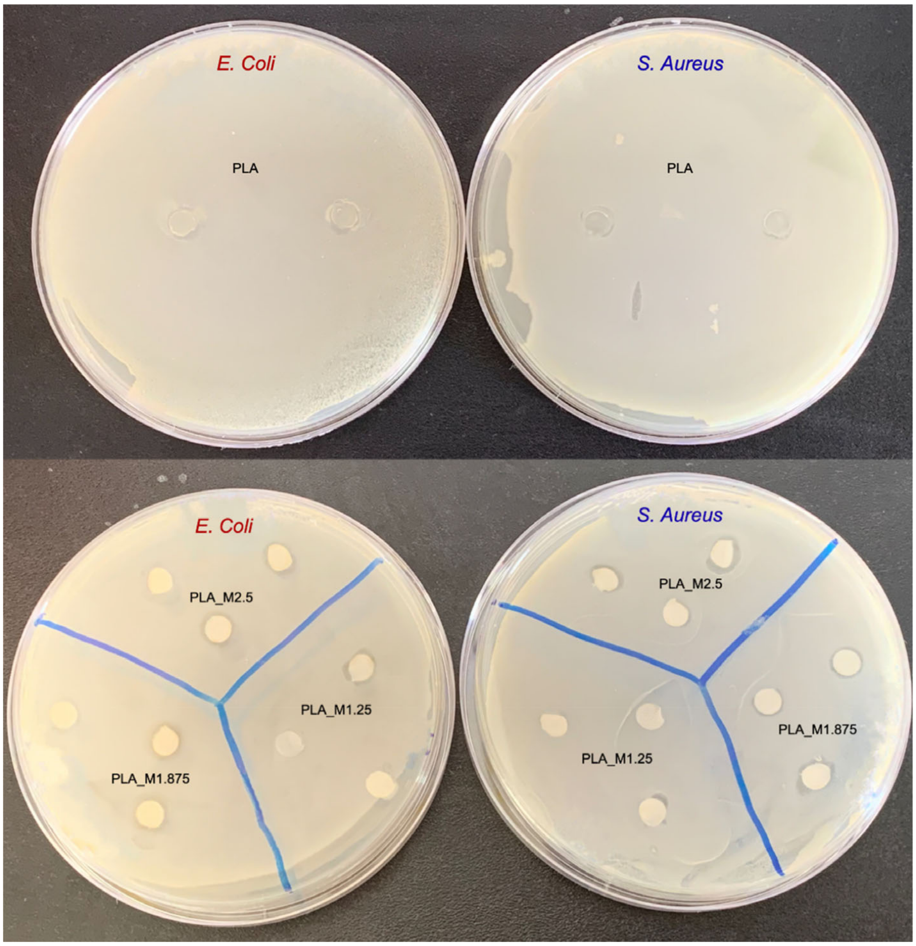

FIGURE 2 Images of *E. coli* and *S. aureus* bacterial culture plates incubated with PLA films (top) and PLA/peppermint oil blend films (bottom).

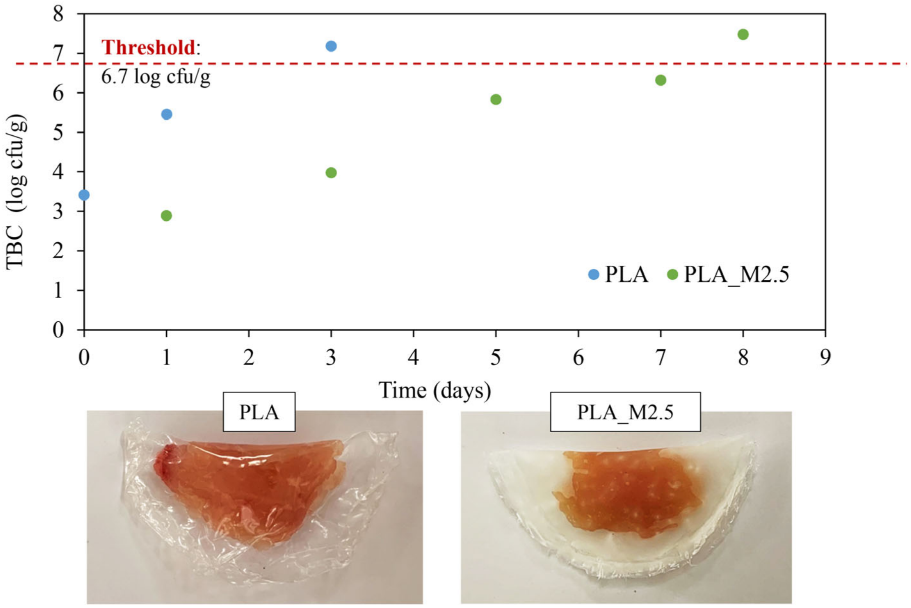

FIGURE 3 Total bacterial count and photos of chicken breast packaged with PLA and PLA_M.25 samples.

Chicken breast meat was subjected to analysis to determine the total bacterial count (TBC) measured in log cfu g^{-1}. The acceptable threshold value for TBC, according to Turkish Standard (TS 24019:2014) and European legislation (EC Regulation 1441/2007, 2007), was determined to be 6.7 log cfu g^{-1}.[80] Figure 3 illustrates the daily changes in TBC and the legislation threshold of 6.7 log cfu g^{-1}. As depicted in Figure 3, chicken breast meat packaged with a PLA film exceeded the threshold within 3 days, with a TBC of 7.18 log cfu g^{-1}. However, when chicken breast meat was packaged with PLA_M2.5, which contains 2.5% w/v peppermint oil, the TBC was significantly lower at 3.97 log cfu g^{-1} on day 3. It took 8 days for the TBC in the same PLA_M2.5 packaged breast meat to exceed the threshold of 6.7 log cfu g^{-1}. Therefore, the addition of peppermint oil resulted in a substantial reduction in microorganism growth and extended the shelf life of the chicken breast meat from 3 days up to 8 days under standard refrigeration conditions.

4 | CONCLUSIONS

The objective of this study was to investigate the potential of peppermint oil as an effective antimicrobial agent to be used in biodegradable packaging materials made from PLA. It is noteworthy that the control films composed solely of PLA exhibited notably lower opacity in comparison to polyethylene (PE) films, which are widely recognized as the established commercial standard for food packaging. This high transparency makes the films visually appealing and suitable for food packaging applications where consumers prefer to observe the contents. However, higher concentrations of peppermint oil increased opacity in the PLA films, rendering them more suitable for light-sensitive food packaging. Results also revealed a substantial reduction in WVP values with the inclusion of peppermint oil. The films exhibited improved water barrier properties, indicating their potential to effectively preserve the quality and freshness of packaged food items. TGA results revealed that the thermal stability of PLA remained unaffected by the inclusion of peppermint oil. Furthermore, the mechanical properties of the films were sufficient to be used as a packaging material, as evidenced by TS values exceeding the acceptable threshold for food packaging. The films displayed remarkable EAB values, granting them superior flexibility and resistance to rupture, particularly beneficial for packaging irregularly shaped food products. Moreover, the presence of peppermint oil in the films imparted a significant concentration-dependent antimicrobial activity against both Gram-positive and Gram-negative bacteria. The observed inhibition zones provided evidence of the films' capability to hinder microorganism growth. Additionally, application of the package on chicken breast meat showed that, the incorporation of peppermint oil was effective in decreasing microorganism growth, effectively extending the shelf life from 3 up to 8 days under refrigeration conditions.

In conclusion, the incorporation of peppermint oil in PLA-based films altered the films physical attributes and bestowed them significant antimicrobial activity. These findings demonstrate the potential of peppermint oil as a promising additive to enhance the performance and sustainability of biodegradable packaging materials. Further optimization and investigation of peppermint oil concentration could lead to the development of advanced eco-friendly packaging solutions, contributing to the advancement of the food industry's packaging practices.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial and non-financial interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, [E. K.], upon reasonable request.

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