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ORIGINAL PAPER

Development and characterization of antimicrobial films from gums obtained from cold-pressed flaxseed oil by-product

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Abstract

In this study, antimicrobial edible films incorporated with allyl isothiocyanate in free form (FG-EO) and nanoemulsion form (FG-NE) were produced using the gum obtained from a cold-pressed flaxseed oil by-product (FG). All films were characterized in terms of physicochemical, barrier, mechanical, biodegradability, thermal, and molecular properties. The results showed that integrating allyl isothiocyanate into the films improved the thermal and barrier properties of the films and decreased the tensile strength. According to the molecular characterization, the allyl isothiocyanate antimicrobial compound showed a homogeneous distribution in the film. The FG-EO films and FG-NE films provided strong antimicrobial effect against *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, and *Staphylococcus aureus* pathogenic bacteria at rates ranging from 42 to 87%. Furthermore, during 15-day storage period of fresh-cut meat samples which were packaged with FG-Control, FG-EO, and FG-NE films, significant ($P < 0.05$) decrease in the number of total mesophile bacteria (TMB), total psychrophile bacteria, and coliform bacteria compared to control fresh-cut meat samples was observed. As a result, it could be said that allyl isothiocyanate incorporated FG-based edible films have the potential as an alternative packaging material to improve the quality of foods and extend the shelf life of fresh-cut meat.

Keywords Antimicrobial film · Cold press linen oil by-product · Gum · Edible film

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Introduction

In recent years, there has been a substantial increase in consumer demand for high-quality food products [1]. Due to increasing global health and economic concerns, the packaging industry has undertaken many important responsibilities such as reducing costs, reducing the use of materials produced from petroleum, and designing packaging environmentally friendly. Accordingly, the industry has been focusing on the production of edible, biodegradable, and environmentally friendly packaging to meet consumer demand in recent years [2, 3]. Edible packaging is a type of packaging that is produced from based natural materials, such as protein, lipid, and polysaccharide, and applied to the food product as a thin layer to protect the food against physicochemical effects and microbial spoilage, thus prolonging its shelf life [4]. Edible films must have sufficient mechanical strength and extensibility to resist stress factors during food processing, packaging, and storage [5].

Edible films based on polysaccharides are obtained from substances such as pectin, chitin, alginate, chitosan, seed gums, starch, and algae. Polysaccharide-based films provide a good moisture barrier as they have low gas permeability, thus keeping the moisture content of the food stable [6, 7]. By-products from the manufacturing and processing of food, such as husks, seeds, offal, leaves, and gums, include significant amounts of fiber and plant proteins including starch, cellulose, and pectin. Food by-products may then be recycled and used for high-value applications, which reduces environmental pollution and promotes sustainable green development [8].

Flaxseed gum (FG) is a heteropolysaccharide that has been used in the food industry as an emulsifier, thickener, and stabilizer [9, 10]. The FG has functional properties similar to that of Arabic gum and guar gum, and it could be utilized to improve emulsion stability by increasing viscosity and decreasing interfacial tension [11, 12]. The flaxseed by-product, which contains a high amount of gum, is the main waste material remaining after cold press oil extraction [13]. The gum to be extracted from the flaxseed by-product have the potential to be used as a raw material for the production of polysaccharide-based edible films in the food industry. In the literature, there have already been several studies on the production of edible films from flaxseed gum, which is extracted from the seed directly [14]. However, according to our best knowledge, no edible film studies have been conducted on the flaxseed gum obtained from cold press oil waste. In addition, no study has been found in the literature on the production of antimicrobial packaging using this by-product gum and its use as packaging material in food products. For this reason, it is thought that this study will be an example for future studies on the evaluation of waste in the food industry and that these studies will contribute to the circular economy through waste management.

Meat is one of the most perishable food groups, and proper packaging, in addition to enhancing shelf life, helps to reduce waste and improve public health by minimizing pollution produced by the use of unhygienic and improper items. Because of its high water activity (aw) and fat content, meat rotting is mostly

caused by microbial degradation and lipid oxidation [15]. When previous studies are examined, the use of hydrocolloid in meat products, particularly gums, can result in increased functional characteristics and compensation for the undesirable effects of lowering salt and fat content, freezing and melting process, WHC, and acceptable adhesiveness [16].

In light of all this information and reasons, it was aimed to produce antimicrobial edible films incorporated with allyl isothiocyanate in free form (FG-EO) and nanoemulsion form (FG-NE) using the gum obtained from a cold-pressed flaxseed oil by-product (FG) and to characterize in terms of physicochemical, barrier, mechanical, biodegradability, thermal, and molecular properties, as well as, it was aimed to determine physicochemical and microbial quality of the antimicrobial FG film-coated fresh-cut meat samples during the storage period of 15 days.

Material and methods

Material

Escherichia coli O157:H7, *Salmonella* spp., *Listeria monocytogenes*, and *Staphylococcus aureus* pathogenic bacteria were obtained from Yildiz Technical University Food Engineering Department (Istanbul, Turkey). Cold-pressed flaxseed oil by-product was obtained from ONEVA Food Co. (Istanbul, Turkey). Ethanol, albumin (from bovine serum), and allyl isothiocyanate were purchased from Sigma-Aldrich (Darmstadt, Germany). MRS Agar, MRS Broth, Nutrient agar, and glycerol (85%) were provided by Merck (Darmstadt, Germany).

Methods

Gum extraction from cold-pressed flaxseed oil by-product

The gum was extracted from cold-pressed flaxseed oil by-products according to the procedure described by Naji-Tabasi, Razavi [17]. Firstly, 500 g of cold-pressed flaxseed by-product was added to 10 L of water and mixed with a magnetic stirrer at 80 °C for 5 h to obtain gel structures. The extracted gum solution was passed through to the sieve and then purified by mixing with 96% ethanol which was stored at 4 °C (w/v) to precipitate gum. The purified gum was frozen at −80 °C for 2 h and then dried using a freeze dryer (Martin Christ, Beta 1–8 LSCplus) for 3 days.

Film preparation

In this study, three types of film samples were prepared: (i) Control film (FG-Control), (ii) essential oil-containing film (FG-EO), and (iii) nanoemulsion-containing film (FG-NE).

To prepare FG-Control, 2% of flaxseed gum (w/v) was dissolved in distilled water and stirred for 1 h at 70 °C using a magnetic stirrer. This mixture was kept in a

refrigerated incubator at 4 °C overnight. Then, 1.5% of glycerol (w/v) was added into the mixture as a plasticizer and held in a water bath at 70 °C for 30 min. 30 g of film solution was then poured into each sterile Petri plate (90×17 mm) and dried for 24 h in a ventilated incubator at 35 °C.

To prepare FG-EO, 2% of flaxseed gum (w/v) was dissolved in distilled water and stirred for 1 h at 70 °C using a magnetic stirrer. This mixture was kept in a refrigerated incubator at 4 °C overnight. Then, 1.5% of glycerol (w/v) and 1% of essential oil (v/v) were added to the mixture and held in a water bath at 70 °C for 30 min. 30 g of film solution was then poured into each sterile Petri plate (90×17 mm) and dried for 24 h in a ventilated incubator at 35 °C.

To prepare FG-NE, 2% of flaxseed gum (w/v) was dissolved in distilled water and stirred for 1 h at 70 °C using a magnetic stirrer. This mixture was kept in a refrigerated incubator at 4 °C overnight. To form nanoemulsion, 0.1% of albumin (w/v) was dissolved in distilled water, and 1% of essential oil (v/v) was added and mixed using an ultraturax for 3 min at 40 revolutions. The particle size of the nanoemulsion was measured using a Zeta Sizer (Malvern) and found approximately 79.21 nm with a PDI value of 0.402. Then, 1.5% of glycerol (w/v) was added into the mixture as a plasticizer and held in a water bath at 70 °C for 30 min. 30 g of film solution was then poured into each sterile Petri plate (90×17 mm) and dried for 24 h in a ventilated incubator at 35 °C.

Thickness and moisture content

The thickness of the film samples was determined with a digital micrometer (Fowler Digitrix Mark 2, Chicago, USA). Main values and standard deviations were calculated using five measurements at different points for each sample.

The moisture contents (%) of the film samples were calculated by the gravimetric method. The film samples (approximately 1–3 g) were dried in a conventional oven at 105 °C for 24 h. Then, the weight losses of the samples were determined and the moisture contents were calculated [18].

Optical properties

The surface color parameters (L^*, a^*, and b^*) of film samples were determined by a Konica Minolta Chroma Meter (CR-400, Minolta Co. Ltd., Osaka, Japan) [19]. The color measurements were performed by placing the film specimens over a colorimeter with at least three points of each sample selected randomly to measure the color parameters of the films. Total color differences (ΔE) of film samples are calculated with Eq. (1).

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \tag{1}$$

where $\Delta L = L_{\text{standard}} - L_{\text{sample}}$, $\Delta a = a_{\text{standard}} - a_{\text{sample}}$, and $\Delta b = b_{\text{standard}} - b_{\text{sample}}$

Mechanical properties

Mechanical properties of film samples which are tensile strength (TS) and elongation at break ($E\%$) values were identified according to the standard method of ASTM D882-12 [19] by using a texture analyzer (TA. XT Plus StableMicro Systems, Surrey, UK) with a load cell of 5 kg. The film samples were cut into rectangular shapes (10 cm × 2 cm) in which the initial distance between the grips and crosshead speed was set to 50 mm and 4 mm/s, respectively. At least five replications were performed to determine the TS and $E(\%)$. TS and E (%) values are calculated with Eq. (2) and Eq. (3).

$$\mathrm{TS} = \frac{\text{maximum force}}{\text{initial specimen cross} - \text{sectional area}} \tag{2}$$

$$E(\%) = \frac{d_{\text{after}} - d_{\text{before}}}{d_{\text{before}}} \times 100 \tag{3}$$

where d is the distance between grips holding the specimen before or after the break of the specimen.

Water vapor permeability

Water vapor permeability (WVP) was measured according to the method described by Jahit, Nazmi [20]. Briefly, silica gel was put into glass tubes, and the tubes were held at 105 °C for 24 h to remove the humidity. The top of the tubes was covered with the film samples, and the tubes were stored in desiccators containing distilled water at 25 °C for 24 h. Non-film-covered silica gel tubes were used as a control. Then, the weight of the tubes was measured daily for every 3 h during the day. WVP is calculated by Eq. (4).

$$\mathrm{WVP} = \frac{w}{t} \times \frac{x}{\Delta P \times A} \tag{4}$$

where w/t: mass change over time (g), t: time (h), X: thickness (mm), ΔP: partial vapor pressure difference of the atmosphere with silica gel and distilled water (kPa, at 25 °C), and A: film area (m^2).

Oxygen permeability

The oxygen permeability of the films (O_2P) was measured according to the method used by Memiş, Tornuk [21]. Firstly, 15 mL of antioxidant-free sunflower oil was filled in conical flasks (25 mL), and the flasks were covered with the film samples. After storing at 60 °C for 9 days, the peroxide value of the oil samples was determined by sodium thiosulfate titration.

Differential scanning calorimetry (DSC)

The thermal behavior of the films was determined according to the method of Llana-Ruiz-Cabello, Pichardo [22] with a slight modification using differential scanning calorimetry (DSC Q20, TA Instruments, Inc., USA). Briefly, 5 mg of the film sample was weighed in an aluminum pan and then heated from 20 to 300 °C at a rate of 10 °C min^{-1} (N_2 flow rate: 20 mL/min). An empty aluminum pan was used as a reference.

Molecular characterization by Fourier transform infrared spectroscopy (FTIR)

Molecular characterization of the film samples was performed using an FTIR tool (Bruker Tensor 27 spectrometer equipped with the DLa TGS detector, Bremen-Germany). Measurements were realized in the wavelength spectral range of 4000–400 cm^{-1} [23].

Film biodegradability

The film biodegradation test referred to the soil burial method previously used by Jahit, Nazmi [20]. Film samples (2 cm^2) were dried in a desiccator until their weight was constant (initial weight). Then, these samples were buried in 100 g of soil, and daily weight changes were recorded for five consecutive days. Each sample was then dried until its weight became constant (final weight). The percentage weight loss (W%) is calculated using Eq. 5:

$$W(\%) = \left[\frac{(\text{initial weight} - \text{final weight})}{\text{initial weight}}\right] \times 100 \tag{5}$$

Antimicrobial activity

The antimicrobial activity of the films was determined according to the method described by Lian, Sun [24]. *Staphylococcus aureus* ATCC 29,213, *Escherichia coli* O157:H7 ATCC 25,150, *Listeria monocytogenes* ATCC 13,932, and *Salmonella typhimurium* ATCC 14,028 pathogenic bacteria were used as test pathogens. Firstly, pathogens were activated in nutrient broth, and the number of microorganisms was standardized to be approximately 10^6 cfu/mL. 5 mg of the film samples sterilized under UV light were added to the bacterial suspensions, and all solutions were incubated for 24 h at 37 °C with constant shaking speed. The absorbance was then measured at 625 nm. The bacterial inhibition rate (%) was calculated using the absorbance values.

Determination of physicochemical and microbial quality of edible antimicrobial film-coated fresh-cut meats during storage

Coating of red meat samples with films Red meat samples were purchased from a local market on the initial day of storage under a cold chain and divided into four

groups under aseptic conditions. Film solutions were prepared according to the methods described in "Film Preparation" section, and the meats were immersed in these solutions and kept for 10 min to provide film adhesion. The meat to be used as a control was dipped in sterile distilled water. At the end of the waiting period, the meats were removed from the solutions and placed on blotting papers and kept in a sterile incubator at 25 °C for 60 min to ensure penetration and dry the films on the surface. Then, the samples were taken into sterile packages, vacuum packed, and stored at 4 °C for 15 days. There were four groups of products: Control-Meat group (CM), FG-Control-coated meat group (FGCM), FG-EO-coated meat group (FGEOM), and FG-NE-coated meat group (FGNEM).

pH analysis A digital pH meter (HI, Hanna, USA) was used to determine the pH change of meat samples during storage. For this purpose, 10 g of the sample was homogenized with 90 ml of distilled water, and pH values were measured.

Color analysis The change in optical properties of meats during storage was determined according to the method specified in "Optical properties" section.

TBA analysis The method applied by Tarladgis, Watts [25] was used to determine the thiobarbituric value in meats. For this purpose, 10 gr sample was homogenized with 50-ml distilled water and transferred to a Kjeldahl tube. 47.5 mL of water was added to it, and the pH value was adjusted to 1.5 using HCl. 5 mL of the distillate obtained by distillation of the mixture was taken and mixed with 5 mL of TBA reagent. The mixture was boiled for 35 min. The absorbance was measured at 532 nm after cooling. Results were expressed as mg/MDA/kg [25].

Microbiological analysis Total mesophilic bacteria (TMB), psychrophilic bacteria (PB), and total coliform bacteria (TCB) counts were analyzed to determine the microbiological quality of the meats. For this purpose, 25 gr of the sample was weighed under aseptic conditions and homogenized in 225 mL of 0.1% (w/v) sterile peptone water for 3 min using a stomacher. Then, serial dilutions were prepared and inoculated on PCA-containing media by spreading method and incubated at 35 °C for 24 h and at 7 °C for 7 days to determine TMB and PB, respectively. For TCB, the inoculated VRB agar containing Petri dishes were incubated at 37 °C for 24 h. At the end of the incubation periods, the colonies formed on the Petri dishes were counted, and the bacterial amount was expressed as log CFU/g meat sample.

Sensory analysis Meat samples were evaluated by ten panelists on the 0, 5, 10, and 15th days during 15 days of storage, based on color, odor, texture, and general taste, and scored over 5 points.

Statistical analysis

Statistical analysis was carried out by a Windows-based statistical analysis program (IMB SPSS 25). One-way analysis of variance (ANOVA) was performed, and the

statistical differences between the means were evaluated at the significance level of 95% using the Tukey test. The analyses were performed in triplicate.

Result and discussion

Moisture content, thickness, and optical properties

The amount of moisture is a parameter that significantly affects the physical, mechanical, and barrier properties of edible films. As seen in Table 1, the moisture contents of FG-Control, FG-EO, and FG-NE films ranged from 5.72 to 7.19%. Since allyl isothiocyanate is a hydrophobic character, there was no interaction between free-form water molecules and EO in FG-EO film samples. Therefore, the addition of allyl isothiocyanate in free form to the film did not cause a significant ($P > 0.05$) change in moisture level. On the other hand, its addition in nanoemulsion form caused a significant decrease in moisture content ($P < 0.05$) due to the interaction between protein, EO, and free water molecules during nanoemulsion formation. Differences in the moisture content of edible films could occur due to changes in their hydrophilic properties, which affect water permeability during the drying process. It has been reported that moisture level could also be affected by drying conditions, where longer drying times result in a rough film surface [26]. Moreover, to avoid microbial growth, the moisture content and water activity must be kept below approximately 10% and 0.60–0.65, respectively [27]. It can be said that all film samples obtained in this study were safe in terms of microbial growth.

Film thickness is another factor that has an important role in the optical, mechanical, and barrier properties of edible films [28]. The thickness of FG-Control, FG-EO, and FG-NE films was determined as 0.17 ± 0.01, 0.15 ± 0.02, and 0.11 ± 0.00 mm, respectively. As seen in Table 1, essential oil (EO) incorporation into the film matrix did not affect the thickness significantly ($P > 0.05$), while the nanoemulsion form of EO caused a significant decrease ($P < 0.05$). In a similar research, Bahram, Rezaei [29] fabricated an active whey protein-based edible film incorporating cinnamon essential oil and found that the incorporation of EO did not affect the film thickness significantly ($P > 0.05$). On the other hand, in another study conducted by Acevedo-Fani, Salvia-Trujillo [30], they found that

Table 1 Moisture, thickness, and color values of the films

Films	Moisture (%)	Thickness (mm)	Color parameter		
			L*	a*	b*
FG-Control	5.72 ± 0.96^{a}	0.17 ± 0.01^{a}	70.76 ± 0.83^{a}	4.90 ± 0.41^{a}	17.90 ± 0.85^{a}
FG-EO	7.19 ± 1.03^{a}	0.15 ± 0.02^{a}	70.38 ± 1.04^{a}	4.88 ± 0.25^{a}	18.32 ± 0.77^{a}
FG-NE	3.45 ± 0.57^{b}	0.11 ± 0.00^{b}	70.76 ± 0.83^{a}	4.90 ± 0.41^{a}	17.90 ± 0.85^{a}

FG-Control control film, *FG-EO* film containing essential oil, *FG-NE* film containing nanoemulsion form of essential oil, *a–b* the different lowercases within the same column show that the results are significantly different ($P < 0.05$)

the thickness of the films containing EO in nanoemulsion form decreased significantly ($P < 0.05$) compared to the control film. These results could be related to droplet size in the nanoemulsion and probable oil phase losses during film formation, which could reduce the total quantity of solids concentration in the film matrix.

Color is an important parameter that affects preferability for consumers. The color values of FG-EO, FG-NE, and FG-Control films are shown in Table 1. There was no significant difference ($P > 0.05$) in optical properties depending on the incorporation of EO into the film samples.

Mechanical properties

Edible films used in the packaging of foods must have a certain mechanical strength and flexibility in order not to be damaged during processing and storage [31]. Tensile strength (TS) and elongation at break (EAB) parameters are commonly used to describe the mechanical properties of edible films. TS stands for tension force resistance, and EAB stands for film stretching capacity [30]. The TS and EAB results of the films are given in Table 2. TS values were determined as 0.47, 0.60, and 0.69 MPa for FG-EO, FG-Control, and FG-NE, respectively. The presence of free EO caused a significant decrease from 0.60 to 0.47 MPa ($P < 0.05$) in the TS value of the films. Many studies showed that the addition of EO to the film formulation tends to weaken the film by reducing the cohesive forces within the structure [32–34]. On the other hand, FG-NE film was more stretchable (EAB: $51.08 \pm 4.57\%$), whereas FG-Control film (EAB: $33.89 \pm 2.18\%$) and FG-EO film (EAB: $38.22 \pm 3.77\%$) did not have significant difference ($P > 0.05$). The flexibility in the FG-NE film occurred with the effect of the electrical charge of the nanoemulsions in the film structure. As the repulsive forces between molecules with the same charge increase the distance between polymers, a plasticizing effect can occur in the charged polymeric film structure [30, 34]. In similar researches, Souza, Goto [35], Kavoosi, Rahmatollahi [36] and Boonruang, Chinsirikul [37] reported that the addition of EO into the edible

Table 2 Mechanical properties of films

Films	Tensile strength (MPa)	Elongation at break (%)	WVP (g mm h^{-1} m^{-2} kPa^{-1})	PV (meq/kg)
Blank				78.28 ± 3.43^{a}
FG-control	0.60 ± 0.03^{ab}	33.89 ± 2.18^{b}	1.981 ± 0.014^{a}	70.75 ± 4.13^{ab}
FG-EO	0.47 ± 0.03^{b}	38.22 ± 3.77^{b}	1.246 ± 0.027^{c}	67.00 ± 4.12^{ab}
FG-NE	0.69 ± 0.08^{a}	51.08 ± 4.57^{a}	1.467 ± 0.073^{b}	57.11 ± 8.14^{b}

FG-Control control film, *FG-EO* film containing essential oil, *FG-NE* film containing nanoemulsion form of essential oil, *WVP* water vapor permeability, *PV* peroxide value, *a–c* the different lowercases within the same column show that the results are significantly different ($P < 0.05$)

films based on starch, gelatin, and PLA decreased significantly the TS value and increased the E value, respectively.

Barrier properties

Water vapor permeability (WVP) and oxygen permeability (O_2P) values of the films are determined to describe the barrier properties which are used for estimating the shelf life of the packed food products [38, 39]. As shown in Table 2, WVP significantly decreased ($P < 0.05$) with the addition of EO to the films. The presence of lipid molecules in film structure improves water barrier qualities by increasing tortuosity, which provides resistance to water vapor passing through the film. It has been reported that tortuosity enhances when the oil phase ratio in the film increases or the oil particle size decreases [30, 40]. On the other hand, several factors related to film structure, namely thickness, water sensitivity, and crystallinity, can influence the WVP of the films. In general, highly crystalline polymers are less permeable to water vapor due to their ordered structure [38, 41]. Zhou, Wu [42] reported that the incorporation of cinnamon EO into the cassava starch film caused a significant decrease in the WVP value of the films. Moreover, a similar trend was observed in *Oregano vulgare* L. EO-loaded gelatin/chitosan films fabricated by Hosseini, Rezaei [43] and *Zataria multiflora* EO-incorporated gelatin films fabricated by Kavoosi, Rahmatollahi [36].

The O_2P values of the films were expressed as peroxide values (PV) in Table 2. The PV values of the films were found lower with the rate ranging from 57.11 to 70.75 meq/kg compared to the blank (78.28 meq/kg). Although free EO incorporation into the film did not significantly ($P > 0.05$) affect the O_2 permeability, adding it in nanoemulsion form significantly ($P < 0.05$) improved the O_2 barrier capacity. This can be due to the emulsification process, which allows the EO to exist better in the film matrix and prevents phase separation in the system. Moreover, the conformity between the film matrix and the EO forms a compact structure that prevents nonpolar oxygen molecules from entering the film [42, 44, 45].

DSC

Intermolecular structural changes caused by temperature changes were measured by DSC analysis, and results are given in Table 3 and Fig. 1. The melting temperatures of FG-Control, FG-EO, and FG-NE films were found as 92.89 °C, 77.33 °C, and 102.10 °C, respectively. In general, heat flow changes at thermal transitions between 80 and 100 °C are mainly related to water evaporation associated with hydrophilic groups in the film structure [46–49]. The addition of free EO into the film matrix caused a significant decrease ($P < 0.05$) in T_m value, while the nanoemulsion form of EO caused a significant increase ($P < 0.05$). A similar trend was also observed in the enthalpy values of the samples. It could be said that this is due to the EO integration, which diffused between the amorphous and crystalline regions in the polymer chain, providing molecular

Table 3 Thermal behavior of the films

Films	T_m (°C)	ΔH_m (J/g)	T_d (°C)
FG-control	92.89 ± 0.04^{b}	245.70 ± 0.08^{b}	307.44 ± 0.12^{b}
FG-EO	77.33 ± 0.06^{c}	210.90 ± 0.06^{c}	307.89 ± 0.15^{a}
FG-NE	102.10 ± 0.03^{a}	277.40 ± 0.04^{a}	307.89 ± 0.18^{a}

T_m (°C) melting point temperature, ΔH_m (J/g) melting enthalpy and T_d (°C) depolymerization temperature, *FG-Control* control film, *FG-EO* film containing essential oil, *FG-NE* film containing nanoemulsion form of essential oil, *WVP* water vapor permeability, *PV* peroxide value, *a–c* the different lowercases within the same column show that the results are significantly different ($P < 0.05$)

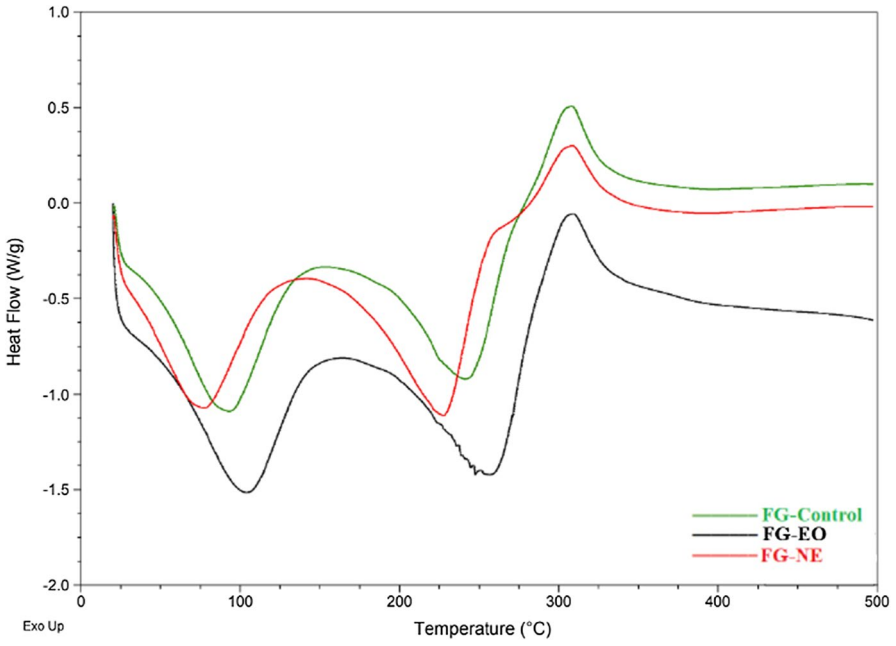

Fig. 1 DSC thermogram of the films

mobility [48]. Polymer crystallinity and melting temperature can be lowered by adding inorganic components and EO. The temperature decrease in the FG-EO films may be because of higher EO contents loosening the molecular structure of the films and disrupting the growth of polymer crystals. On the other hand, in FG-NE films, the temperature was found higher because the crystallinity was higher compared to FG-EO films due to the strong interaction between the nanoemulsion and the polymer [50, 51]. In similar research, it was observed that

the T_m value decreased in PLA films with the addition of CLO essential oil [49]. On the other hand, there was no significant difference ($P > 0.05$) between the depolymerization temperatures of the film samples.

FTIR

In order to determine the molecular structure of the film samples, the FTIR profile of the films was investigated and is given in Fig. 2. While almost a similar spectrum profile was observed in all film samples, different bands of 2095 cm^{-1} and 2166 cm^{-1} were detected in the FG-EO samples. These bands are thought to be related to the organosulfur groups in the essential oil composition. In addition, similar spectra of FG-Control and FG-NE samples showed that the essential oil forms a very homogeneous structure with flaxseed gum in nanoemulsion form. In FG-EO films, because of the hydrophobic nature of EO, it was observed physical bounding between polymer and EO. On the other hand, with the formation of nanoemulsion, the hydrophobicity of EO was reduced and protein-water–oil nanoemulsion interacted with the polymer during the film formation process. Thus, 2095 cm^{-1} and 2166 cm^{-1} bands, which are thought to be associated with organosulfur groups in the EO composition, could not be observed in the spectrum of FG-NE films. Other findings related to film characterization in the manuscript support this conclusion. Mahcene, Khelil [38] obtained similar FTIR spectra in the film samples, which they produced with sodium alginate and various essential oils.

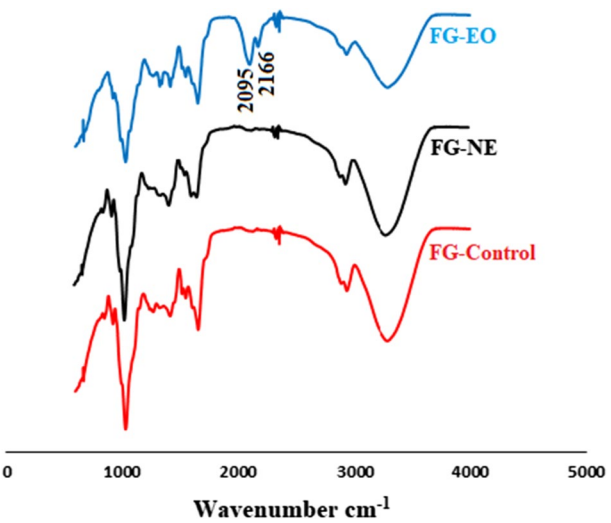

Fig. 2 FTIR spectrum of the films

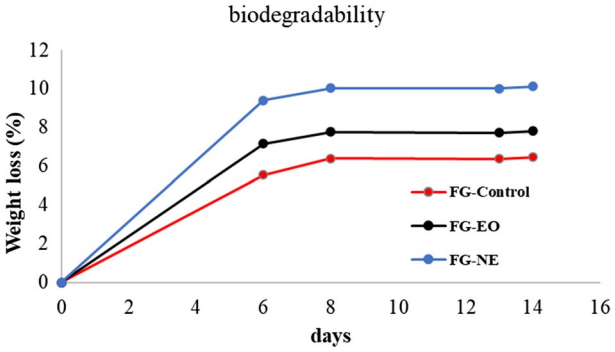

Fig. 3 Biodegradability of the films

Film biodegradability

The biodegradability of the films was carried out by measuring the % weight loss of the films which were buried in the soil for 15 days, and the results are given in Fig. 3. As seen in the figure, a significant weight loss occurred in all film samples over 15 days. While the most weight loss was seen in the FG-NE film, it was seen the least in the FG-Control film. This is an indication that films can degrade in nature under certain environmental factors. Crystallinity also correlated positively with biodegradability in the present study. According to DSC results, nanoemulsion integration into the film contributed to its crystallinity, thus increasing the film's biodegradability compared to other films. The diffractograms accounted for this crystalline fragility, which easily ruptures at low tensile strength and hastens biodegradability [20]. Robertson [52] stated that the complete biochemical decomposition of organic molecules by microorganisms is also called biodegradation, which involves mineralization or conversion of C, N, S, and P (organic compound content) into inorganic products. Today, due to increasing concerns about waste management, biodegradability has become a very important quality criterion in film production. Similar to this study, Jahit, Nazmi [20] and Debandi, Bernal [53] also showed in their studies that polysaccharide-based films are easily biodegradable.

Antimicrobial activity of films

The antimicrobial effect of the films on the test pathogens is given in Table 4. As seen in the table, the control film sample showed no effect on the test pathogens, while FG-EO and FG-NE films had a strong antimicrobial activity with a rate ranging from about 49% to 87%. These results supported that FG-EO and FG-NE films have strong inhibitory effects on both gram (+) and gram (−) pathogenic bacteria. According to various studies, the antimicrobial action of such oils is attributed to monoterpenes, phenolic compounds, and organosulfur compounds [54, 55]. Wattananawinrat, Threepopnatkul [55] stated that these bioactive components disrupt

Table 4 Antimicrobial activity of the films

Films	Bacterial inhibition %			
	S. aureus ATCC 29,213	*E. coli* O157:H7 ATCC 25,150	*Salmonella typhimurium* ATCC 14,028	*L. monocytogenes* ATCC 13,932
FG-Control	00.00 ± 0.00^{Ca}	00.00 ± 0.00^{Ca}	00.00 ± 0.00^{Ca}	00.00 ± 0.00^{Ca}
FG-EO	54.70 ± 3.45^{Bab}	41.60 ± 8.17^{Bb}	49.50 ± 4.66^{Bb}	61.30 ± 4.48^{Ba}
FG-NE	69.70 ± 5.32^{Aa}	74.60 ± 6.24^{Aa}	77.70 ± 9.12^{Aa}	87.40 ± 5.83^{Aa}

FG-Control control film, *FG-EO* film containing essential oil, *FG-NE* film containing nanoemulsion form of essential oil, *A–C* different uppercases in the same column indicate significant difference ($P < 0.05$) between the results, *a–b* the different lowercases within the same line show that the results are significantly different ($P < 0.05$)

the cellular integrity of microorganisms, thereby limiting respiration activity and ion transport. Many studies have shown that the antimicrobial activity of oils can be attributed to their hydrophobicity and components, which allow them to split bacterial cell membranes and mitochondrial lipids, disrupt cell structures and make them more permeable after membrane distraction. Antimicrobial activity depends not only on the chemical composition of essential oils, but also on their lipophilic properties and the potency of functional groups or solubility in water [41, 56].

pH changes of meat samples during storage

The pH change during storage is an important factor in sensitive foods with high water content such as meat. The pH changes of the film-coated meat samples during 15 days of storage are given in Table 5. Initial pH values of the samples were found in the range of 6.09–6.43 in all meat groups and the pH decreased in all samples during storage. pH results are related to changes in meat quality characteristics. Physicochemical properties and microbial load changes in meat during storage caused a decrease in pH values. In a study carried out by Eker [57],

Table 5 pH changes in meat samples during storage

Films	Sampling days			
	0	5	10	15
CM	6.09 ± 0.08^{Ba}	5.94 ± 0.01^{ABb}	5.82 ± 0.01^{Abc}	5.69 ± 0.05^{Ac}
FGCM	6.08 ± 0.03^{Ba}	5.98 ± 0.01^{Aa}	5.72 ± 0.07^{Ab}	5.67 ± 0.03^{Ab}
FGEOM	6.43 ± 0.04^{Aa}	5.87 ± 0.03^{Cb}	5.74 ± 0.05^{Ac}	5.50 ± 0.04^{Bd}
FGNEM	6.31 ± 0.01^{Aa}	5.90 ± 0.00^{BCb}	5.74 ± 0.06^{Ac}	5.67 ± 0.03^{Ac}

Control-Meat uncoated meat sample, *FGCM* meat sample coated with FG-Control, *FGEOM* meat sample coated with FG-EO, *FGNEM* meat sample coated with FG-NE, *A–C* different uppercases in the same column indicate significant difference ($P < 0.05$) between the results, *a–d* the different lowercases within the same line show that the results are significantly different ($P < 0.05$)

minced meatballs were covered with films containing gelatin and thyme essential oil, and the pH change was monitored during cold storage. Similarly, a decrease was observed in the pH values of the minced meat samples with initial pH values of 6 during storage. In another study conducted by İncili, Karatepe [58], the pH change of chicken fillets covered with films containing chitosan and antimicrobial agents was found to be almost similar when the sample groups were compared, while a decrease was observed in all samples during 15 day storage.

Color change of meat samples during storage

While no significant change was observed in the *L** values (brightness) of all sample groups during storage, there were statistically significant changes in the *a** and *b** values (Table 6). While *a** (red-green) values of all samples decreased during storage, the greatest decrease was observed in the Control-Meat and FG-Control-Meat samples. This is an indication that the red color reduction in the meats in Control-Meat and FG-Control-Meat samples is greater than the samples in FG-EO-Meat and FG-NE-Meat samples; in other words, they are more rapidly exposed to microbial or physicochemical change. On the other hand, while *b** (yellow-green) values of Control-Meat samples decreased during storage, *b** values were increased in other groups. This is thought to be due to the interaction between the film components and the meat components in the film-coated samples.

Table 6 Color changes of meat samples during storage

Films	Sampling days			
	0	5	10	15
L*				
CM	37.85 ± 0.61^{Aa}	36.87 ± 0.79^{Aa}	37.74 ± 0.55^{Aa}	36.77 ± 2.02^{ABa}
FGCM	35.6 ± 1.36^{ABa}	36.29 ± 0.98^{Aa}	35.40 ± 4.44^{Aa}	37.14 ± 1.31^{Aa}
FGEOM	33.29 ± 0.94^{Ba}	32.32 ± 0.31^{Ba}	32.10 ± 0.57^{Aa}	33.45 ± 1.40^{Ba}
FGNEM	34.53 ± 1.77^{Ba}	36.27 ± 0.71^{Aa}	36.57 ± 0.55^{Aa}	35.85 ± 0.26^{ABa}
a*				
CM	18.37 ± 0.42^{Aa}	13.14 ± 3.57^{Bb}	12.08 ± 0.52^{Bb}	11.63 ± 1.41^{ABb}
FGCM	15.75 ± 1.14^{Ba}	12.38 ± 2.34^{Bab}	12.30 ± 0.88^{Bb}	9.55 ± 0.95^{Bc}
FGEOM	18.39 ± 1.06^{Ab}	20.24 ± 0.58^{Aa}	7.24 ± 0.75^{Cd}	13.69 ± 0.37^{Ac}
FGNEM	19.75 ± 0.90^{Aa}	15.18 ± 1.72^{Bb}	16.48 ± 0.21^{Ab}	13.64 ± 0.03^{Ac}
b*				
CM	3.77 ± 0.52^{ABa}	3.1 ± 0.49^{ba}	2.96 ± 0.89^{Ca}	2.57 ± 2.06^{Da}
FGCM	2.99 ± 0.49^{Bb}	3.21 ± 0.88^{Bb}	3.70 ± 0.88^{BCb}	5.41 ± 0.58^{Ca}
FGEOM	4.17 ± 0.49^{Ac}	7.69 ± 0.23^{Ab}	4.81 ± 0.21^{Bc}	8.83 ± 0.41^{Aa}
FGNEM	5.22 ± 1.07^{Ab}	7.24 ± 1.19^{Aab}	7.83 ± 0.14^{Aa}	7.81 ± 0.20^{Ba}

Control-Meat uncoated meat sample, *FGCM* meat sample coated with FG-Control, *FGEOM* meat sample coated with FG-EO, *FGNEM* meat sample coated with FG-NE, *A–C* different uppercases in the same column indicate significant difference ($P < 0.05$) between the results, *a–d* the different lowercases within the same line show that the results are significantly different ($P < 0.05$)

TBA change of meat samples during storage

The thiobarbituric (TBA) value is a parameter used to detect lipid oxidation in food products containing animal fat. In good-quality meat, the TBA number should be less than 3 mg MA/kg [59]. As seen in Fig. 4, the TBA amount remained below 2 mg MA/kg during the storage period in all samples. It could be due to removing the oxygen to a large extent by vacuum packaging. On the other hand, there was a rapid increase in TBA values in CM and FGCM samples during storage, while this increase was slower in FGEOM and FGNEM samples. Similarly, in the study carried out by İncili, Karatepe [58], it was determined that the TBA value was much lower in chicken meat samples coated with postbiotic chitosan film during 15 days of storage compared to control samples.

Microbial quality change of film-coated meat samples during storage

Microbial change is another important factor to determine food quality during the storage period. Table 7 shows the change in total mesophyll bacteria (TMB), psychrophilic bacteria (TPB), and total coliform bacteria (TCB), respectively, during 15 days of storage. At the beginning of storage, the highest TMB count was determined at the rate of 4–5 log cfu/g in CM and FGCM samples. On the other hand, there was a significant decrease ($P < 0.05$) in the amount of TMB in FGEOM and FGNEM samples compared to control samples. At the end of storage, the TMB value reached an acceptable max limit value for consumption in CM and FGCM samples, while there was no significant increase in the TMB number in FGEOM and FGNEM samples. In addition, the lowest TMB count was observed in the FGNEM samples during storage. This is thought to be due to the increase in essential oil stability with nanoemulsion formation. In a similar study, fish fillets covered with films prepared by adding carvacrol essential oil to sodium alginate and flaxseed gum were stored, and at the end of storage, the TMB amount in fillets covered with films containing carvacrol was found to be quite low, while this value was found to be quite high (approximately 7–8 log cfu/g) in the control group [60].

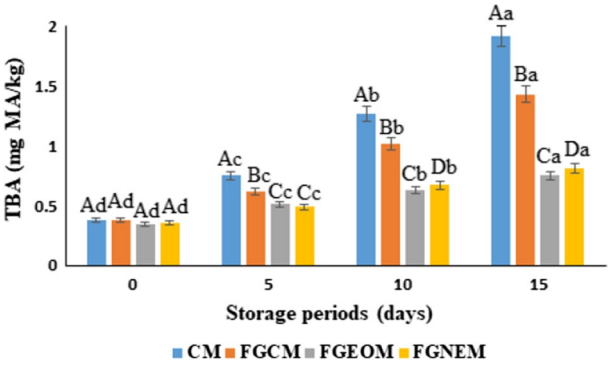

Fig. 4 TBA (mg MA/kg) change in meat samples during 15 days of storage

Table 7 Microbial quality change of meat samples during storage

Films	Sampling days			
	0	5	10	15
Total Mesophyll Bacteria (TMB)				
CM	4.95 ± 0.14^{Ac}	5.32 ± 0.17^{Ac}	6.98 ± 0.25^{Ab}	8.15 ± 0.18^{Aa}
FGCM	4.73 ± 0.25^{Ad}	5.41 ± 0.07^{Ac}	6.77 ± 0.33^{Ab}	7.65 ± 0.09^{Ba}
FGEOM	2.57 ± 0.18^{Cc}	3.14 ± 0.03^{Bc}	3.85 ± 0.46^{Bb}	4.68 ± 0.17^{Ca}
FGNEM	3.24 ± 0.15^{Bb}	3.37 ± 0.09^{Bab}	3.65 ± 0.12^{Ba}	3.71 ± 0.20^{Da}
Total Number of Psychrophilic Bacteria (TPB)				
CM	4.12 ± 0.17^{Ad}	4.64 ± 0.05^{Ac}	5.27 ± 0.08^{Ab}	6.81 ± 0.33^{Aa}
FGCM	4.26 ± 009^{Ac}	4.73 ± 0.17^{Ac}	5.45 ± 0.33^{Ab}	6.99 ± 0.20^{Aa}
FGEOM	2.42 ± 0.04^{Ba}	2.55 ± 0.09^{Ba}	2.71 ± 0.15^{Ba}	2.84 ± 0.28^{Ba}
FGNEM	2.30 ± 0.47^{Ba}	2.35 ± 0.12^{Ba}	2.44 ± 0.05^{Ba}	2.51 ± 0.33^{Ba}
Total Coliform Bacteria (TCB)				
CM	2.19 ± 0.07^{Ad}	2.45 ± 0.33^{Bc}	2.73 ± 0.28^{Ab}	3.82 ± 0.15^{Ba}
FGCM	2.15 ± 0.09^{Bd}	2.57 ± 0.12^{Ac}	2.69 ± 0.07^{Bb}	3.85 ± 0.05^{Aa}
FGEOM	$< 1^{Ca}$	$< 1^{Ca}$	$< 1^{Ca}$	$< 1^{Ca}$
FGNEM	$< 1^{Ca}$	$< 1^{Ca}$	$< 1^{Ca}$	$< 1^{Ca}$

Control-Meat uncoated meat sample, *FGCM* meat sample coated with FG-Control, *FGEOM* meat sample coated with FG-EO, *FGNEM* meat sample coated with FG-NE, *A–D* different uppercases in the same column indicate significant difference ($P < 0.05$) between the results, *a–d* the different lowercases within the same line show that the results are significantly different ($P < 0.05$)

Similarly, TPB counts were significantly higher in the CM and FGCM samples during storage compared to the FGEOM and FGNEM samples. At the same time, the increase in the number of TPB was again faster in these two groups during the storage period. Due to the antimicrobial activity of the EO in free form or nanoemulsion form, the rate of increase in TPB number remained almost constant during storage in FGEOM and FGNEM samples. In a similar study, chicken fillets covered with chitosan film containing antimicrobial postbiotics were stored in vacuum packaging, and a rapid increase was observed in the number of TPB in control samples during storage, while there was no significant increase in the number of TPB in samples containing postbiotics [58].

The total amount of coliform (TCB) was found to be around 2 log cfu/g in CM and FGCM samples, while it was < 1 log cfu/g in FGEOM and FGNEM samples. This indicates that essential oil has a higher inhibitory effect on coliform bacteria. At the end of storage, a significant increase ($P < 0.05$) was observed in the coliform bacteria counts in CM and FGCM samples, and there was no significant increase in FGEOM and FGNEM samples.

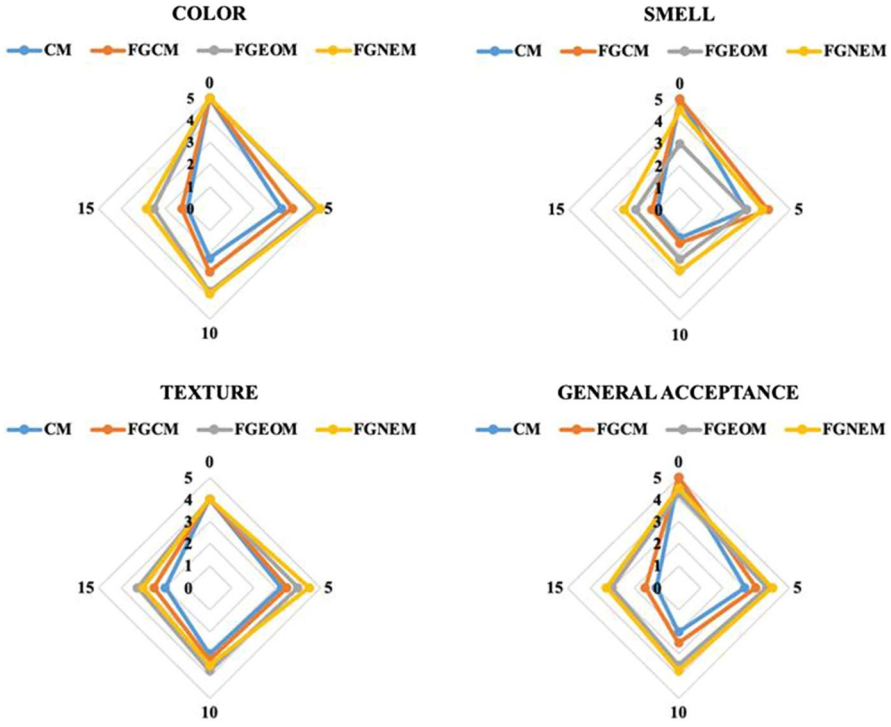

Fig. 5 Sensory evaluation of meat samples during 15 days of storage period

Sensory evaluation of meat samples during storage

Sensory evaluation of the samples was performed by ten panelists in terms of color, odor, and texture during 15 day storage (Fig. 5). Initially, the general appreciation of all samples was high and close to each other. However, in the later periods of storage, although there was a decrease in the scores in all groups, the color, odor, texture, and general taste scores of the CM and FGCM groups were found to be significantly lower compared to the scores of the FGEOM and FGNEM samples. While the panelists did not find the CM and FGCM groups suitable for consumption at the end of storage, they stated that the FGEOM and FGNEM samples were still consumable with sensory properties.

Conclusion

This study showed that flaxseed gum obtained from linseed oil by-product is an alternative biodegradable material to design active antimicrobial films that can improve the quality properties of foods and extend their shelf life. Allyl isothiocyanate was successfully integrated (free form or nanoemulsion form) into the films to gain antimicrobial properties in the food packaging application. The nanoemulsion

form of the allyl isothiocyanate improved the mechanical, physicochemical, biodegradable, barrier, and thermal properties compared to other film samples. Both films that included free form or nanoemulsion form of allyl isothiocyanate had a strong inhibitory effect on food spoilage and pathogenic microorganisms. In addition, it has been considered that these antimicrobial films can be used as an alternative packaging material in meat products, as they make a significant contribution to the shelf life of red meat samples**.** According to quality and sensory analysis results, the edible films containing essential oil in nanoemulsion form preserved the quality properties of red meat for a longer period compared to other produced film samples. Considering all the results from this study, it was concluded that the gums, which are found in significant amounts in the flaxseed oil by-product can be used as a promising environmentally friendly biodegradable packaging material and that allyl isothiocyanate can be integrated into these films to improve the quality properties and extend the shelf life of the foods.

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