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# EFFECT OF ORANGE PEEL ESSENTIAL OIL ON THE PROPERTIES OF CHITOSAN: GELATIN CASTED FILMS PREPARED FOR ACTIVE PACKAGING

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## *Highlights*

- Bioloogically active films were prepared for food packaging
- OEO improved physicochemical properties positively
- Composite films showed inhibitory activity on *E. coli* and *S. aureus*

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**ABSTRACT:** Food packaging is a passive barrier that protects food against environmental factors such as ultraviolet light, oxygen, water vapor, pressure, heat, chemical, and microbiological contaminants. In a changing and developing world, consumers now want to reach healthier, fresher, and more diverse foods. In response to consumer demands and expectations, the food sector has focused on developing active and intelligent packaging. The purpose of active packaging is to protect the properties of the food by interacting with the coating material and prolonging the shelf life. In this context, it is aimed to prepare active package films by integrating bioactive agents into films prepared based on biodegradable polymers. It is an important point that is determined how the characteristics of the films such as morphology, molecular structure, surface property, and antimicrobial activity, will shift depending on the type and quantity of bioactive agent addition. Based on this, packaging films loaded with different concentrations of orange peel essential oil (OEO) (25, 50 and 100% of total polymer weight) were produced on the basis of chitosan and gelatin natural polymers. The changes that occur in the active films as a result of the increasing amounts of oil were revealed by determining the molecular structure, surface property, morphological characteristics, solubility quality, and antibacterial activity. The solubility of the films, which is an effective parameter in the evaluation of the environmental impact of the films that will be released as waste after use, varied between 20% and 25% at the end of 48 hours. The 100OEO@CH:GEL film showed the highest antibacterial properties against *Escherichia coli* and *Staphylococcus aureus*.

***Keywords:*** *Chitosan, Gelatin, Orange Peel Essential Oil, Solvent Casting, Food Packaging*

## 1. INTRODUCTION

Food packaging plays a primary role in protecting the food product from external environmental effects (germs, insects, light, heat, oxygen, water vapor, odours, dirt, dust, etc.). The main purpose of packaging is to ensure food safety and extend the shelf life of food by minimizing environmental impacts to meet both industrial and consumer needs [1]. Plastic packaging has been widely used in the food industry for many years due to its advantageous properties. They are economical, functional, lightweight and versatile as they can be prepared in rigid (bottles, jars, cans), thermoformed (trays) or flexible (woven mesh, multilayer films) forms. Therefore, they have until recently replaced other traditional food packaging materials such as glass, metals (aluminium, laminated, tin and steel), paper and cardboard [2]. On the other hand, this extensive use of plastics has caused serious environmental problems, as most of these materials are petroleum derivatives, cannot be degraded and pollute the environment during their production and disposal [3]. In order to keep up with current trends and meet the demands of consumers, the food industry market has recently demonstrated new technological breakthroughs in the form of

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environmentally friendly packaging with novel packaging solutions. As a result of these advances, work on developing active and smart packaging has been initiated.

Active packaging is a new method used to extend the shelf life of perishable foods, to protect or improve the quality and safety of prepared foods due to their interaction with the product. Furthermore, active packaging can replace active compounds in foods, reduce particle movement from packaging materials to food, and eliminate industrial processes that can introduce pathogenic microorganisms into the product. This packaging system also has an advantage in reducing foodborne illness outbreaks, and food recalls [4]. Active packaging systems can be divided into active cleaning systems (absorbers) and active release systems (emitters). The first is the removal of unwanted compounds such as moisture, carbon dioxide, oxygen, ethylene or odor from the food or its environment, while the second one is the release of compounds such as antimicrobials and antioxidants into the packaged food or the air space inside the packaging [5]. When the studies conducted in recent years are examined, it shows that there is more interest in the production of packages that can release antioxidants. In this context, there is increasing relevance in incorporating natural antioxidant agents such as plant extracts and essential oils into polymer-based active packaging materials.

Essential oils are the volatile liquids collected from diverse plant parts such as flowers, leaves, seeds, peels, fruits and barks. These oils, which are the most characteristic features of plants and are directly related to many vital functions of the plant, are valuable compounds that are generally known for their distinctive odor and colorless structure. It is considered as one of the important herbal products because it contains a meager amount of the total mass. They are known for their potential benefits to human health due to their biological properties such as anticancer, anti-inflammatory, antidiabetic, antiulcerogenic, antidepressant, anti-anxiety and high antioxidant and antimicrobial activities. These include basil (*Ocimum basilicum* L.) [6], chamomile (*Matricaria chamomilla* L.) [7], peppermint (*Mentha piperita* L.) [8] and rosemary (*Rosmarinus officinalis* L.) [9] essential oils which have been approved by the Food and Drug Administration (FDA) as food additives. In addition, they have been recognized as safe (GRAS) for use in the food packaging industry [10]. It is possible to produce packaging material by dispersing these oils in a polymeric film. The films in active packaging technology are preformed thin sheets and can be applied directly on the product as a wrap or cover [11]. With this packaging, it is aimed to extend the shelf life of the food while preserving the nutritive and sensory quality of the food. Active packaging combined with essential oils has so far been applied to a variety of foods such as beef, butter, ham, chicken, dairy products and seafoods [10].

Chitosan is produced from seafood waste and is biocompatible, biodegradable, has a high adsorption capacity, and antibacterial properties, making it suitable for food packaging materials [12]. In recent years, many scientists have been interested in gelatin, a water-soluble protein product formed from the partial hydrolysis of collagen, because of its remarkable properties. These include its biodegradability, edibility, non-toxic and good film-forming capability. So, it is a feasible alternative for producing films or coatings included in food packaging.

Within the scope of the study, essential oil incorporated polymeric films were prepared. Chitosan and gelatin were selected to obtain the main polymeric structure of films. Since both polymers have been proven to be biocompatible and biodegradable in other studies carried out so far, they are suitable for safe use in packaging material production [13]–[15]. Orange peel essential oil (OEO) was chosen as the essential oil because of its potent antibacterial properties. Polymer film based on chitosan and gelatin and with and without OEO-integrated active films were produced using the solvent-casting technique. The films were characterized with employed chemical, morphological and biological analyses, the effect of OEO enrichment on the films was discussed, and the usability of films as food packaging material was assessed in the light of the findings.

## 2. MATERIAL and METHOD

### 2.1. Material

Chitosan with medium molecular weight, gelatin for microbiology, glacial acetic acid, glycerol for molecular biology and tween 20 were purchased from Sigma Aldrich (USA). OEO was obtained from a herb market in Mersin Province, Turkey. Distilled water was used in all solution preparation and washing steps. The strain of *Staphylococcus aureus* CECT 435 (ATCC 25923) and *Escherichia coli* O157:H7 (ATCC 35150) were obtained from from KWIK STIK™. Tryptone Soy Broth (TSB), Tryptone Soy Agar (TSA) and Mueller Hinton agar (MHA) were purchased from Conda (Spain).

### 2.2. Preparation of films

The chitosan:gelatin (CHI:GEL) film and different amounts of OEO added films (OEO@CHI:GEL) were prepared using a traditional solvent-casting method. First, to obtain the polymer solution, CHI and GEL solutions were prepared separately. For CHI solution, the calculated amount of CHI (2%, w/v) was weighed and dissolved in 4% (v/v) acetic acid solution. For GEL solution, the certain amount of GEL (6%, w/v) was dissolved in distilled water at 65°C. Afterwards, a final solution was obtained by mixing both solutions, tween 20 and glycerol by using a magnetic stirrer at room temperature for 30 min. The 2 mL of solution was poured into glass Petri dishes coated with Teflon tape with a diameter of 5 cm and spread over carefully to result in a film. The polymeric film was solidified by evaporating the solvent at 60°C during overnight using a temperature-set oven. After 24 h incubation, the films were pealed from the Petri dishes. Each film was cut into small pieces of 1x1 cm appropriate for further tests and kept in a desiccator (with approximately 20-30% relative humidity provided by the use of silica gel as a conventional desiccant) until use.

To prepare the OEO@CHI:GEL films, different amounts of OEO were added to the 2 mL of the final solution based on the total polymer weight (25, 50 and 100% of polymer weight). The essential oil-added solutions were stirred continuously until a homogeneous mixture was obtained. After preparing the composite solution, the OEO loaded films were carried out by following all the other steps described above for CH:GEL film. The experimental steps of film preparation are summarized in Figure 1. Film samples prepared from only polymer solutions named as CH:GEL, and film samples integrating 25, 50 and 100% OEO were coded as 25OEO@CH:GEL, 50OEO@CH:GEL and 100OEO@CH:GEL, respectively.

### 2.3. Morphology

For determination of surface morphology of the CH:GEL film as control and films incorporated with different amounts of OEO, microscopic analysis was performed using an optical microscopy (Zeiss, Eco 40, Germany) at 4x magnification.

### 2.4. Fourier Transform Infrared spectroscopy (FTIR)

The chemical structure of CH:GEL film and OEO@CHI:GEL films was characterized by Fourier Transform Infrared (FTIR) spectrum obtained by using a spectrometer (Perkin-Elmer Spectrum 1000, USA), equipped with an attenuated total reflection (ATR) accessory. The FTIR spectra were performed from wavenumber 400-4000 $cm^{-1}$ with a resolution of 4 $cm^{-1}$ and 128 scans.

### 2.5. Solubility

The solubility was tested in water based on the weight loss of films. First, each film was cut into pieces, and the initial dry weights were measured. In order to test the solubility of the films in water, the cut samples were placed in Eppendorf tubes, and 10 mL of water was added to them. After the tubes were

kept at room temperature for 48 hours, the samples were removed and dried in an oven at 60°C. The completely dried samples were weight again. The solubility in water was calculated by using the following equation:

Solubility (%) = [final weight/initial weight] × 100

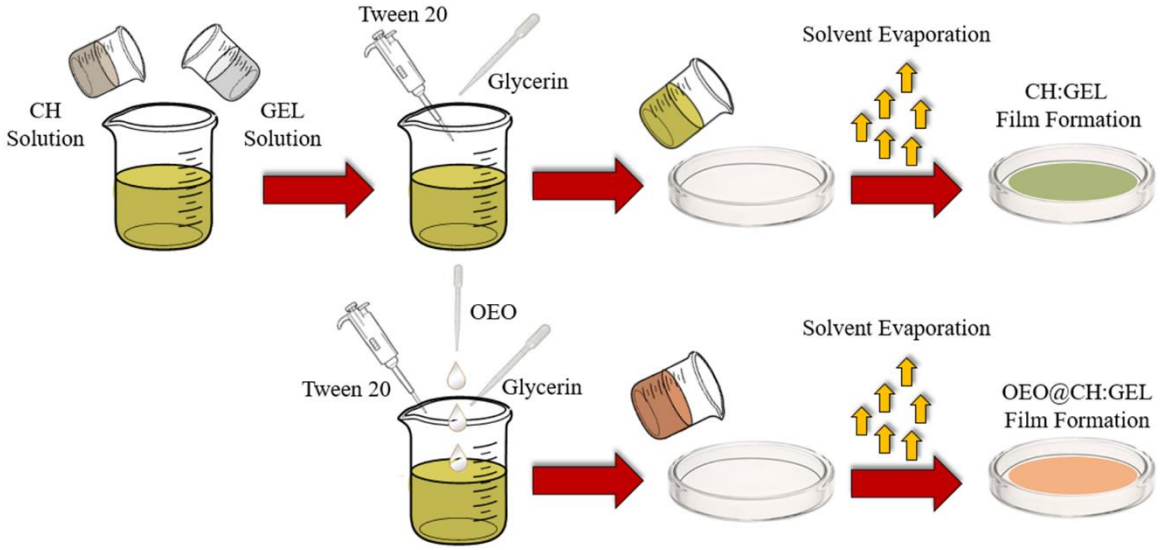


**Figure 1.** Experimental steps of the formation of CH:GEL and OEO@CHI:GEL film using solvent-casting method

### 2.6. Antibacterial Activity

The antibacterial activity was first determined by the agar disc diffusion method, according to Clinical and Laboratory Standards Institute. Briefly, 100 µL of bacterial cell suspensions ($10^8$ CFU $mL^{-1}$) were spread on the surfaces of MHA using sterile cotton swabs. Films were cut as disc shapes, and they were sterilized under UV light for 30 mins. Then sterile film disk samples (8 mm) were placed on MHA's surface and incubated overnight at 37 °C. After 24 h incubation, the diameter of the resulting inhibition zone was measured using a micrometer and expressed in mm. Experiments were done in triplicate.

### 2.7. Statistical Analysis

All experiments were performed in triplicate and all data were statistically analyzed. Data are expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) was performed using SPSS 16.0 (SPSS, Chicago, IL, USA) to compare means by Duncan test with a significance level of 0.05.

## 3. RESULTS and DISCUSSIONS

The films for active food packaging were successfully produced using chitosan and gelatin polymers and the addition of different amounts of OEO to these polymers. The photographs of the produced films are given in Figure 2. As can be seen, the films were easily peeled off from Teflon-coated petri dishes (Ø=5 cm) in one piece without any tearing. In particular, it can be said that the film prepared with the highest oil content (100OEO@CHI:GEL) has a more uniform and homogeneous structure visually. In addition, all films generally exhibited a transparent structure. Transparency is a crucial intrinsic parameter for the shelf-life evaluation of food packaging materials [16]. Therefore, photographs of films were taken to show optical properties (Figure 2). The addition of increasing concentration OEO into the polymer structure

decreased the transparency. is decrease in the transparency rate of OEO@CH:GEL films can possibly be due to the presence of pigments of the OEO.

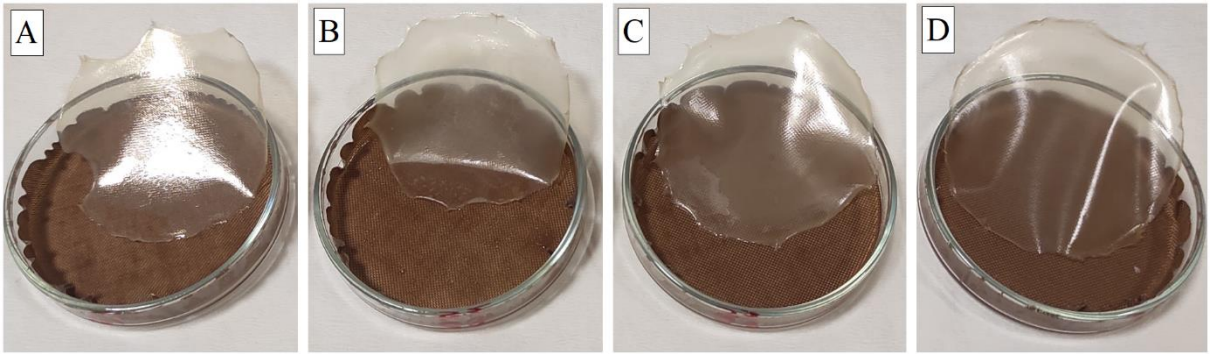


**Figure 2.** Images of films. A) CH:GEL, B) 25OEO@CHI:GEL, C) 50OEO@CHI:GEL and D) 100OEO@CHI:GEL

The films were examined with an optical microscope to confirm the changes in morphology with the addition of increased OEO amounts. Figure 3 shows the images of the films obtained at the same magnification. As is seen from the figure, morphology of 100OEO@CHI:GEL film was observed as smooth, compact and without any crack or pores. Nevertheless, CH:GEL film exhibited irregular forms with many bulges, tiny particles and droplets on the surface. 50OEO@CHI:GEL film also exhibited a heterogeneous structure, but it had lower bulges. An increase in the viscosity of the final solution was observed with the addition of essential oil to the polymer solution. With increasing viscosity, the resulting surface may have been smoother as the evaporation of solvent from the mixture was delayed [17]. This result created a more stable structure especially in the film sample with the highest oil content (100OE@CHI:GEL). In the daylight photographs in Figure 2 above, it can be easily seen with its circular peeling without any deformation from the petri dish due this flexibility and stability. According to other similar studies, essential oil incorporation could improve the elasticity of biofilms' matrix by their possible plasticizing effect [12], [18], [19] and adding essential oil to the polysaccharide structure improved the functional properties of edible films and coatings [20].

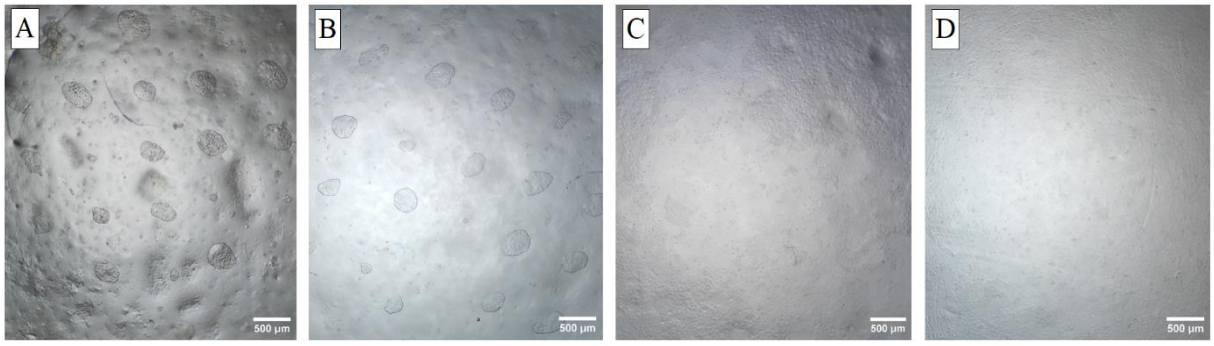


**Figure 3.** Optical microscopy images of films. A) CH:GEL, B) 25OEO@CHI:GEL, C) 50OEO@CHI:GEL and D) 100OEO@CHI:GEL

In order to study the interaction between chitosan, gelatin and essential oil and identify any changes in the molecular structure that could have occurred with varying OEO amount, FTIR spectrum of films were measured. Figure 4 displays FTIR spectra of the CH:GEL film (considered as control) and films loaded with different amounts of OEO. The broad absorption band between 3000 $cm^{-1}$ to 3650 $cm^{-1}$ corresponds to O-H and N-H stretching vibrations of hydrogen-bonded O-H and N-H functionalities (Amide A). The band duo seen at wavenumbers of 2929/2873 $cm^{-1}$ are related to the asymmetric and symmetric stretching vibrations of the $CH_3$ and $CH_2$ functionalities (Amide B) [12]. In the spectrum of the

films, C=O stretching at 1647cm$^{-1}$ (Amide I), N–H stretching at 1552 cm$^{-1}$ (Amide II), and C-N and N-H stretching at 1240 cm$^{-1}$ (Amide III) peaks are associated with the functional structure of both chitosan and gelatin polymers [15], [21]. In addition, the bands in the range of 1033 cm$^{-1}$ is attributed to the saccharide structure of the polymers. With the addition of OEO, no change was observed in the FTIR spectrum of films, showing absorption bands similar to the CH:GEL film spectrum. However, some difference in the positions of the absorption peaks was observed depending on the added OEO levels. In detail, at the highest amount of OEO, the peaks of Amide A was moved to 3290 cm$^{-1}$ and the Amide B absorption peak was moved to 2925 cm$^{-1}$. In addition, a new small peak (at 1742 cm$^{-1}$) was observed in relation to the carbonyl C=O stretching vibration from the ester linkage of triacylglycerol [22].

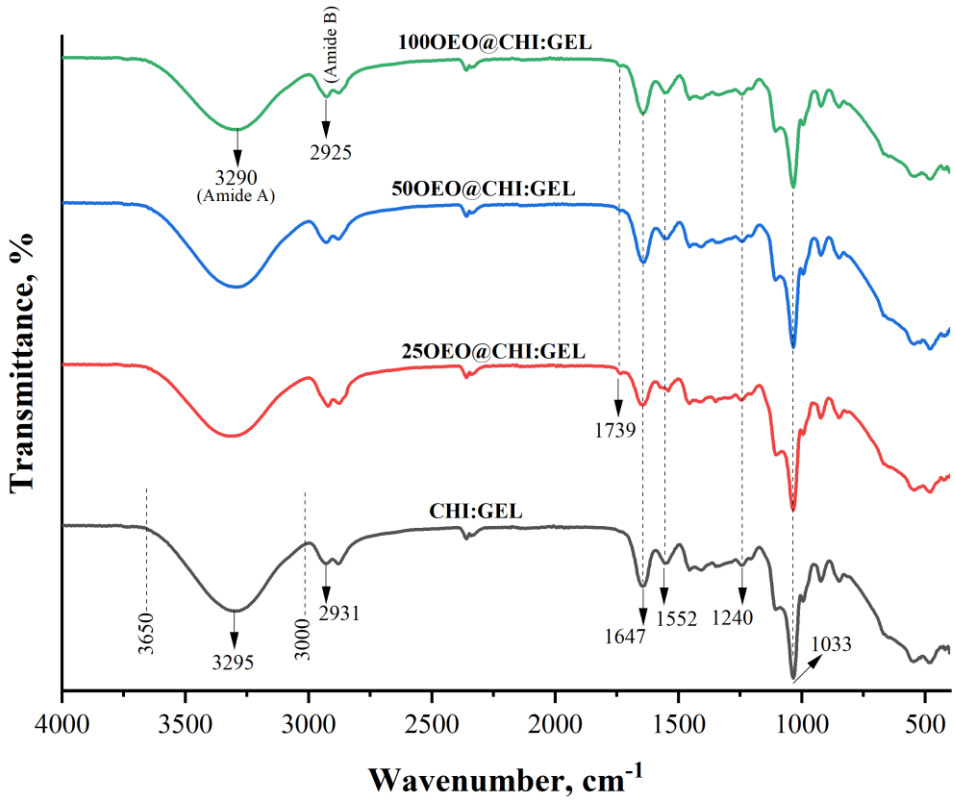


**Figure 4.** FTIR spectrums of CH:GEL and different concentrations of OEO incorporated into OEO@CH:GEL films.

A key parameter for food packaging, film solubility is a measure of the film's resistance to liquids. In our study, the solubility of films was carried out with distilled water for 48 h, and the results are displayed in Figure 5. The solubility (% wt.) values are seen to vary between 20% and 25%. The increasing amount of OEO into the polymer solution resulted in decreased water solubility from 25.06% to 20.14%. The decline in water solubility of OEO@CH:GEL films should be attributed to the hydrophobic effect of oil molecules. The interaction between the polymers and phenols (in the structure of the oil) reduced the availability of hydroxyl and amino groups in the structure of chitosan and gelatin. With OEO present, the hydrogen bond interactions among chitosan, gelatin, and water were disrupted, emerged leading to a decrease in solubility. A similar result was observed in the water solubility of fennel and peppermint essential oils loaded active chitosan films as previously reported by Liu et al. [23].

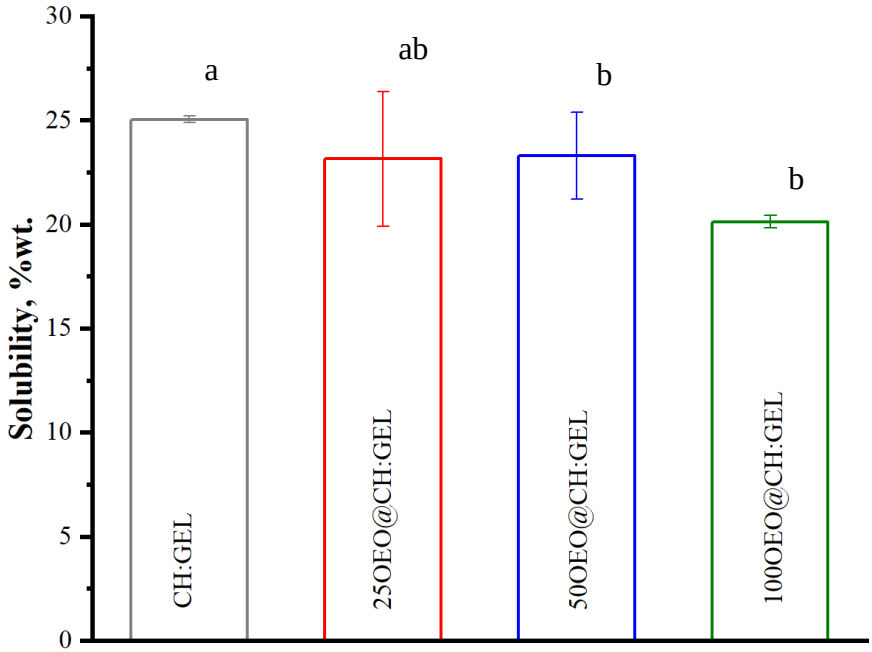


**Figure 5.** The solubility of films against water for 48 h. Different letters in the bar indicate a significant difference ($p < 0.05$).

The antibacterial activity of the films against Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli O157:H7*) was also carried out via the disk-diffusion method (shown in Table 1). The results for the inhibition zones (mm) of microorganisms shown that Gram-positive bacteria were more sensitive than the Gram-negative ones. For instance, CH:GEL, 25OEO@CH:GEL, 50OEO@CH:GEL, 100OEO@CH:GEL indicated inhibition zones between 19.74 and 24.14 mm for *E.coli*, while inhibition zones of these films were not not measured due to partial growth of bacteria in the petri dish for *S. aureus*. These films exhibited more sensitive properties against *S. aureus* than *E.coli*. Chitosan has bactericidal properties, making it particularly appropriate for food packaging materials [24]. So results of all films showed that these films had inhibitory activity on E. coli and S. aureus, obviously. Increasing the concentration of EO in the films were emerged rising of the inhibitory activity. The anti *Escherichia coli* O157:H7 activity of CH:GEL film showed significance differences from 50 and 100% OEO loaded CH:GEL samples. Li et al. reported similarly that orange peel essential oil inhibited *S. aureus* and *E.coli* [18]. The results of the current study suggest that the addition of OEO (50 and 100%) significantly increased the antibacterial activity of the CH:GEL film.

**Table 1.** Results antibacterial activity of films against *Escherichia coli* O157:H7 ve *Staphylococcus aureus* via disc diffusion method.

| Sample | Inhibition zone (mm) | |
|---|---|---|
| | *Escherichia coli* O157:H7 | *Staphylococcus aureus* |
| CH:GEL | 17.74±2.40[a] | ZN |
| 25OEO@CH:GEL | 20.29±1.00[ab] | ZN |
| 50OEO@CH:GEL | 23.35±1.99[b] | ZN |
| 100OEO@CH:GEL | 24.14±2.81[b] | ZN |

[1]ZN: Zone measurement was not performed; growth of bacteria was partial in the petri dish. Mean±standard deviation. Different letters in the same column indicate a significant difference ($p < 0.05$).

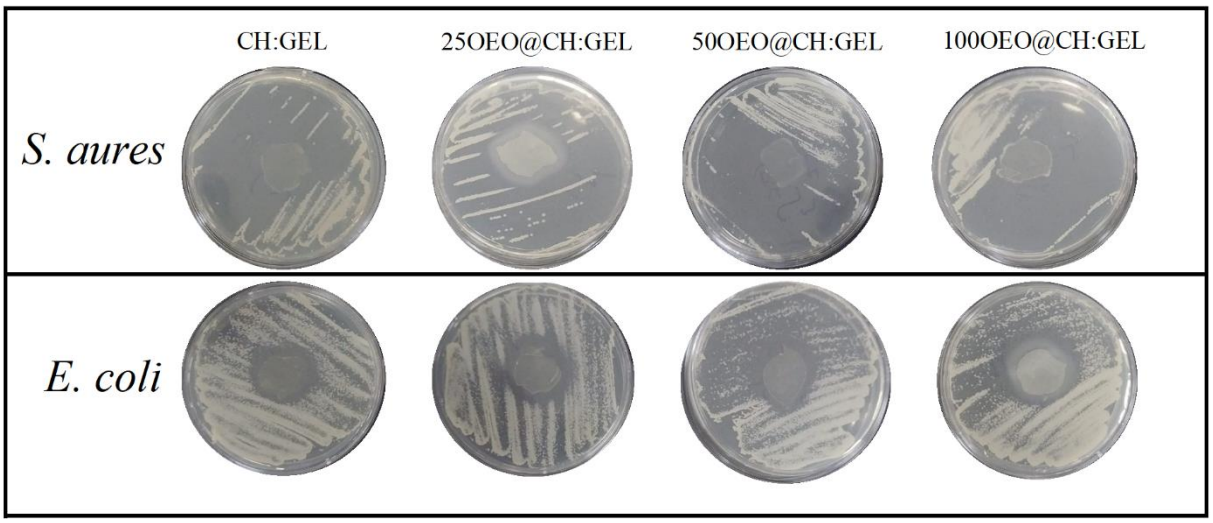


**Figure 6.** Antibacterial activity of the packaging films against to *S. aureus* and *E. coli* via disc diffusion method.

## 4. CONCLUSION

In this research, a novel biocomposite film was developed using polymers from sustainable and low-cost sources (chitosan and gelatin) and incorporated with OEO. The incorporation of OEO could positively improve morphological, molecular structure, physical and antibacterial properties of the films, linked on the determined concentrations. Addition of OEO (25, 50 and 100%, w/w) into the CH:GEL films greatly assisted solubility, and light barrier properties while boosting their antibacterial activity, compared with the control film without OEO. Analyses of FTIR spectra indicated the presence of multiple CH-GEL-OEO interactions. Solubility properties decreased, while antibacterial activities increased with increasing concentration of OEO addition to CH:GEL. In conclusion, CH:GEL films enriched with OEO could be provided for an efficient coating that extends the shelf life and preserves the quality attributes of perishable foods.

### Declaration of Ethical Standards

The authors declare that the materials and methods used in this study do not require ethical committee permission.

### Credit Authorship Contribution Statement

FD carried out the experiments related to film production and characterization studies of the produced films. GG performed antimicrobial analysis and contributed to the writing of the manuscript. DD conceived the original idea, processed the experimental data, supervised the project and performed writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships.

**Acknowledgement**

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**Data Availability**

The data used to support the findings of this study are included within the article.

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