



## Development of Protein-Based Films with Essential Oil Incorporation for Edible Packaging Applications

Fahrullah Fahrullah; PhD<sup>\*1</sup>, Djoko Kisworo; PhD<sup>1</sup>, Bulkaini Bulkaini; PhD<sup>1</sup>, Baiq Rani Dewi Wulandani; PhD<sup>1</sup> & Wahid Yulianto; PhD<sup>1</sup>

<sup>1</sup> Department of Animal Science, Faculty of Animal Science, Universitas Mataram, Jl. Majapahit 62, Mataram, Nusa Tenggara Barat 83115, Indonesia.

### ARTICLE INFO

#### ORIGINAL ARTICLE

##### Article history:

Received: 26 Nov 2024

Revised: 17 Mar 2025

Accepted: 21 Mar 2025

##### \*Corresponding author

fahrullah@unram.ac.id

Department of Animal  
Science, Faculty of Animal  
Science, Universitas Mataram,  
Jl. Majapahit 62, Mataram,  
Nusa Tenggara Barat 83115,  
Indonesia.

Postal code: 83125

Tel: +62 82158492338

##### Keywords

Eugenol; Oils;  
Volatile; Whey;  
Edible Films;  
Packaging.

### ABSTRACT

**Background:** The utilization of protein as a fundamental component in active packaging is an effective approach in manufacturing process, as it facilitates the incorporation of additives, such as antimicrobial agents. The objective of this study was to ascertain physical and microbiological characteristics of protein-based bioactive films with incorporating different essential oils. **Methods:** The films were produced using a completely randomized design with four treatments and five replications. The essential oils used were cinnamon (M1), clove (M2), basil (M3), and lemongrass (M4). The variables observed in this study included thickness, gelation time, FTIR analysis, microstructure, Energy dispersive X-ray spectroscopy (EDS) analysis, and antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. Statistical analysis was conducted using Analysis of Variance in SPSS 24.0. **Results:** Incorporating essential oils into whey-gelatin edible films significantly affected ( $P<0.01$ ) thickness, gelation time, and antimicrobial efficacy. Films with cinnamon essential oil exhibited the lowest thickness. Gelation time varied with the chemical composition of oils, with clove essential oil delaying gelation and cinnamon and lemongrass oils accelerating it. FTIR and EDS analyses indicated changes in chemical structure and microstructure, enhancing antimicrobial properties. Clove essential oil demonstrated the highest antimicrobial activity, followed by cinnamon, lemongrass, and basil oils. **Conclusion:** The addition of essential oils markedly affected film characteristics, including thickness, gelation time, microstructure, and antimicrobial activity. FTIR and EDS analyses confirmed structural changes and interactions that enhanced stability and function. Clove essential oil exhibited superior antimicrobial properties, while cinnamon and lemongrass oils accelerated gelation, showcasing essential oils as promising natural additives for improving edible film performance.

### Introduction

In food industry, packaging plays a pivotal role in processing, preservation, supply, and industrial chain of food products (Anukiruthika *et al.*, 2020). The primary function of packaging is to

safeguard packaged food products from deterioration during storage (Motelica *et al.*, 2020). Active packaging represents a packaging technology that is capable of protecting foodstuffs.

This paper should be cited as: Fahrullah F, Kisworo D, Bulkaini B, Wulandani BRD, Yulianto W. Development of Protein-Based Films with Essential Oil Incorporation for Edible Packaging Applications. Journal of Nutrition and Food Security (JNFS), 2025; 10(3): 441-452.

It is defined as an intelligent system that enables packaging components to interact with food or the atmosphere, as well as internal gases, in a manner that fulfils consumer expectations for high-quality, fresh, and safe products (Erkmen and Barazi, 2018). The primary objective of active packaging is the regulation of moisture levels, particularly the reduction of water vapour loss, which can foster an environment conducive to microbial growth. The maintenance of controlled humidity can prolong the shelf life or storage period of the product (Dhandapani *et al.*, 2017).

The utilization of protein as a fundamental component in active packaging is an effective approach in manufacturing process of active packaging. Protein plays a pivotal role in the transportation of additives, including antioxidants and antimicrobial agents (Abdelhedi *et al.*, 2018, Adilah *et al.*, 2018), and exhibits barrier properties against water vapour (Cinelli *et al.*, 2014). A number of studies have been published on the use of whey proteins for packaging applications, with highly promising results as barriers against moisture, oxygen, lipids, and aroma (Schmid, 2013). Alternatively, they can be blended with other polymeric materials (Fahrullah *et al.*, 2021, Fahrullah *et al.*, 2022, Fahrullah *et al.*, 2020). However, packaging developed from whey protein isolate exhibits poor water vapour barrier properties due to its hydrophilic nature (Azevedo *et al.*, 2015, Cardoso *et al.*, 2016, Teixeira *et al.*, 2014). Furthermore, gelatin-based films typically exhibit favorable optical properties and excellent oxygen barrier properties (Nilsuwan *et al.*, 2017), as well as robust mechanical and barrier characteristics against gases, volatile compounds, oils and UV radiation (Kanmani and Rhim, 2014, Tongnuanchan *et al.*, 2016). Proteins with hydrocolloid properties can further improve the mechanical strength and barrier properties of edible films (Akram *et al.*, 2019). Biopolymer-based edible films, including proteins, polysaccharides, and lipids, extend shelf life by offering protection against environmental factors.

A promising application of active packaging involves the use of edible films with essential oil,

which act as moisture barriers and improve physical properties (D Antunes *et al.*, 2012). Essential oils, such as those from cinnamon, clove, and lemongrass, contain bioactive compounds that enhance antioxidant and antimicrobial activities, helping to prevent microbial spoilage (Lee *et al.*, 2015, Ribeiro-Santos *et al.*, 2017). These oils are incorporated into composite films to optimize their performance, making them an effective solution for extending food shelf life (Elsabee and Abdou, 2013). The objective of this study was to ascertain physical and microbiological characteristics of protein-based bioactive films with incorporating different essential oils.

## Materials and Methods

### Materials and instruments

This experiment used whey protein, gelatin (Granology, Indonesia), glycerol (Merck, Germany), NaOH, cinnamon essential oil (Chemika Karya, Indonesia), clove essential oil (Chemika Karya, Indonesia), basil essential oil (Chemika Karya, Indonesia), lemongrass essential oil (Chemika Karya, Indonesia), silica gel, distilled water, label paper, aluminum foil, alcohol, and clean water. The study primary instruments included an Erlenmeyer flask, thermometer, digital balance, magnetic stirrer, hot plate stirrer, desiccator, stopwatch, screw micrometer, electronic blender, ATR-FTIR (Perkin Elmer/Spectrum Two) for infrared spectrum analysis, and scanning electron microscope (SEM JEOL JCM-7000, Japan) for material morphology characterization.

### Sample preparation

Whey (1 g) and gelatin (1 g) were combined with distilled water (15 ml) using an electronic blender, and then heated on a hot plate at 90 °C for 30 minutes. Once the temperature reached 70 °C, 30% glycerol was introduced. The essential oils were found to have reached a concentration of 8% (w/v) at the conclusion of heating process. The solution was neutralized with NaOH to a pH of 7-8, as determined by pH meter measurement, and immediately poured into Petri dishes at a volume of 20 ml. Subsequently, the samples were subjected to a two-day drying process at 50 °C in

an oven. The edible films were stored for a period of 24 hours prior to analysis (Fahrullah *et al.*, 2020).

### **Thickness**

The film thickness was determined using an MDC-25M micrometer (Mitutoyo, MFG, Japan), with measurements taken on five distinct sections of each sample (Maruddin *et al.*, 2018, Sabil *et al.*, 2021).

### **Gelation time**

The gelation time was determined by observing the length of time required for gel formation, recorded in minutes (Fahrullah *et al.*, 2023).

### **Fourier transform infrared spectroscopy (FTIR) analysis**

The sample was positioned within the optical window situated above the ZnSe crystal and subjected to a pressure of 15 (as indicated by the force gauge) in order to guarantee optimal contact between the sample and the crystal. Tests were conducted using the SOP IR spectrophotometer within the wavelength range of 4000 1/cm to 400 1/cm, with 16 readings and a resolution of 4 1/cm.

### **Microstructure**

The microstructures of the edible film was tested using an electron microscope of SEM JEOL JCM-7000. The edible film was prepared with size 0,5 x 0,5 cm and then it coated with carbon and gold. The ready sample was then placed on the Scanning Electron Microscopy (SEM) device for microstructural observations.

### **Energy dispersive X-ray spectroscopy (EDS)**

The edible film was sliced into smaller sections to fit into the SEM chamber, ensuring the sample surface remained flat and uncontaminated for accurate results. The sample was then placed inside the SEM chamber, where its surface morphology was observed. A specific area of the film surface was then chosen for further analysis using EDS. The EDS measurement process began with the activation of the EDS detector on the SEM, followed by focusing the electron beam on the chosen area. The EDS detector then measured the X-ray energy emitted from the sample as the

electron beam interacted with atoms in the material. The detected X-ray data were converted into a spectrum showing energy peaks corresponding to specific elements in the sample. This spectrum was subsequently analyzed to identify elements based on positions of energy peaks, and the quantification of elements was performed by comparing the intensity of resulting peaks (Newbury and Ritchie, 2015).

### **Antimicrobial activity**

The method of testing antimicrobial potential by diffusion using paper discs comprised two principal phases: the preparation of test bacteria and the assessment of their antimicrobial efficacy. In the initial phase, pure cultures of the test bacteria were prepared through the creation of a series of Mac Farland standard solutions and the equalization of turbidity of bacterial suspensions in Buffered Peptone Water (BPW) media. Subsequently, antimicrobial potential was evaluated through the diffusion of antimicrobial compounds from paper discs to agar media inoculated with test microbes, in accordance with the established methodology. This stage comprised the preparation of Nutrient Agar (NA) media in sterile petri dishes and subsequent inoculation of test bacterial suspension in an even manner using the spread plate method. Subsequently, primary test was conducted by placing paper discs impregnated with antimicrobial agents or negative controls onto NA media that had been inoculated with test bacteria, followed by incubation for 24 hours. The results were evaluated by measuring the diameter of turbid and clear zones surrounding paper discs to assess the antimicrobial potential of the tested compounds (Moghadam *et al.*, 2020).

### **Data analysis**

This research was conducted using a Completely Randomized Design with five replications. The treatments consisted of the use of different essential oils, including cinnamon (M1), clove (M2), basil (M3), and lemongrass (M4) essential oils. The data obtained were analyzed using Analysis of Variance (ANOVA) using SPSS 24.0 Software. If the treatment made a difference, the

Duncan Multiple Range Test (DMRT) would be conducted.

## Results

### Thickness

The thickness of edible films is a crucial factor in their characterization and subsequent applications. The thickness of an edible film affects its mechanical properties, permeability, and functional performance. The findings of the study

concerning the thickness of the whey-gelatin edible film with the incorporation of various essential oils are presented in **Table 1**. The results of the analysis of variance demonstrated that the incorporation of diverse essential oils exerted a considerable influence ( $P<0.01$ ) on the thickness value of the whey-gelatin edible film. The mean thickness value produced ranged from 0.129 to 0.177 mm.

**Table 1.** Thickness and gelation time of edible film with the addition of essential oil.

| Parameters             | Treatments               |                          |                          |                          |
|------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|                        | M1                       | M2                       | M3                       | M4                       |
| Thickness (mm)         | 0.129±0.122 <sup>a</sup> | 0.172±0.119 <sup>b</sup> | 0.177±0.008 <sup>b</sup> | 0.171±0.010 <sup>b</sup> |
| Gelation time (minute) | 4.59±0.53 <sup>a</sup>   | 6.70±0.64 <sup>c</sup>   | 5.74±0.48 <sup>b</sup>   | 4.37±0.13 <sup>a</sup>   |

**M1:** Cinnamon essential oil; **M2:** Elove essential oil; **M3:** Basil essential oil; **M4:** Lemongrass essential oil. Means in the same row with different superscripts differ significantly ( $P<0.01$ ).

### Gelation time

The gelation time of an edible film is defined as the period of time required for a film-forming solution to undergo gelation when cooled or when the solvent is evaporated. The findings of the research investigating the gelation time of whey-gelatin edible films with the incorporation of

various essential oils are presented in **Table 2**. The analysis of variance demonstrated that the incorporation of diverse essential oils exerted a considerable influence ( $P<0.01$ ) on the gelation time of whey-gelatin edible films. The resulting gelation time exhibited a range of 4.37 to 6.70 minutes.

**Table 2.** Elements of whey-gelatin-based edible films enriched with essential oils.

| Element | Treatments   |              |              |              |              |              |              |              |
|---------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
|         | M1           |              | M2           |              | M3           |              | M4           |              |
|         | Mass (%)     | Atom (%)     | Mass (%)     | Atom (%)     | Mass (%)     | Atom (%)     | Mass (%)     | Atom (%)     |
| C       | 38.11 (0.93) | 45.60 (1.11) | 39.79 (0.70) | 47.25 (0.83) | 40.41 (0.74) | 47.92 (0.87) | 37.63 (0.79) | 45.00 (0.95) |
| O       | 58.21 (2.17) | 52.29 (1.95) | 57.13 (1.66) | 50.93 (1.48) | 56.44 (1.72) | 50.25 (1.53) | 59.20 (1.90) | 53.14 (1.70) |
| Na      | 2.81 (0.43)  | 1.75 (0.27)  | 2.58 (0.31)  | 1.60 (0.19)  | 2.58 (0.32)  | 1.60 (0.20)  | 2.62 (0.37)  | 1.64 (0.23)  |
| Cl      | 0.87 (0.20)  | 0.35 (0.08)  | 0.33 (0.10)  | 0.13 (0.04)  | 0.58 (0.13)  | 0.23 (0.05)  | 0.54 (0.14)  | 0.22 (0.06)  |

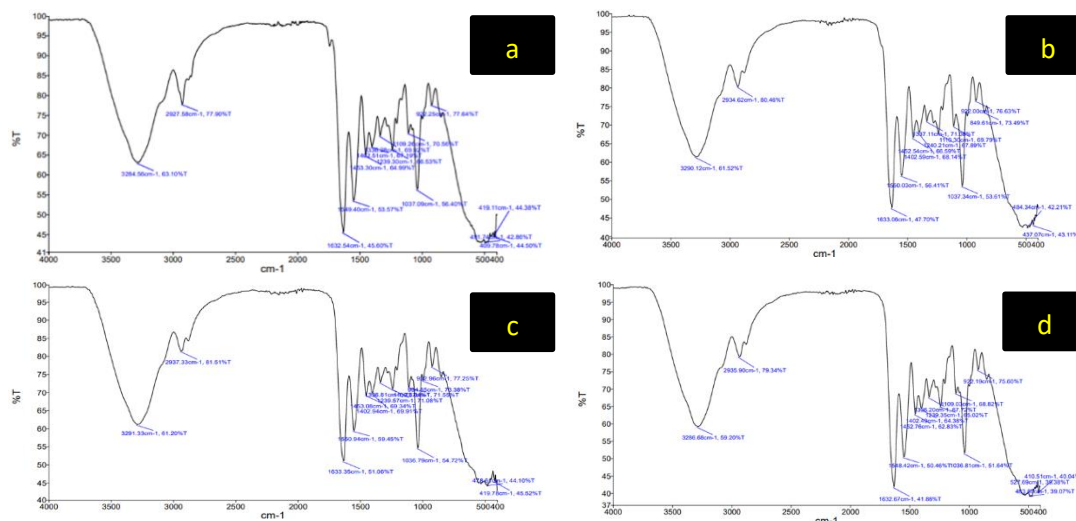
**M1:** Cinnamon essential oil; **M2:** Elove essential oil; **M3:** Basil essential oil; **M4:** Lemongrass essential oil.

### FTIR analysis

FTIR is an invaluable analytical technique for investigating the chemical composition and structure of a vast array of materials, including whey-gelatin edible films. In the context of whey-gelatin edible films, FTIR can provide

information regarding chemical bonds and interactions between principal components, including those between whey protein and gelatin. An overview of FTIR results is provided in **Figure 1**.



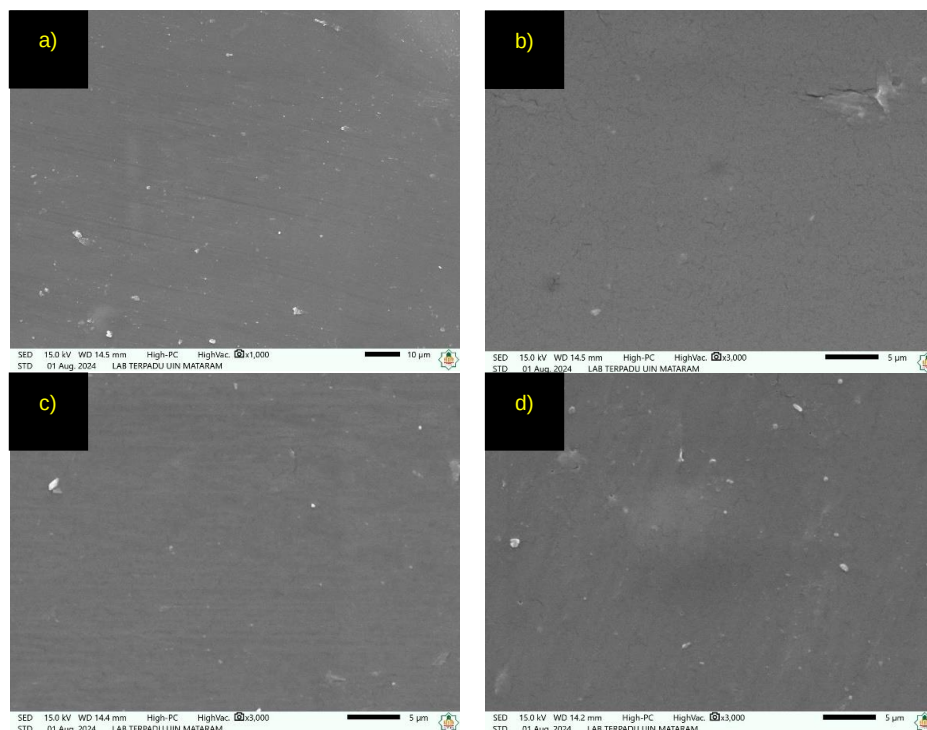


**Figure 1.** FTIR of whey-gelatin edible film with incorporation of (a) cinnamon essential oil; (b) clove essential oil; (c) basil essential oil; (d) lemongrass essential oil.

### Microstructure film

The microstructure of edible films, which is formed by the complex interactions between polymer components and additives, plays a crucial role in determining the functional properties of the film. This structure consists of a polymer network

formed from whey protein, gelatin, or other polymeric materials, which forms a basic matrix with a specific molecular configuration. The results of the study on the microstructure of whey-gelatin edible films with the incorporation of various essential oils are presented in **Figure 2**.

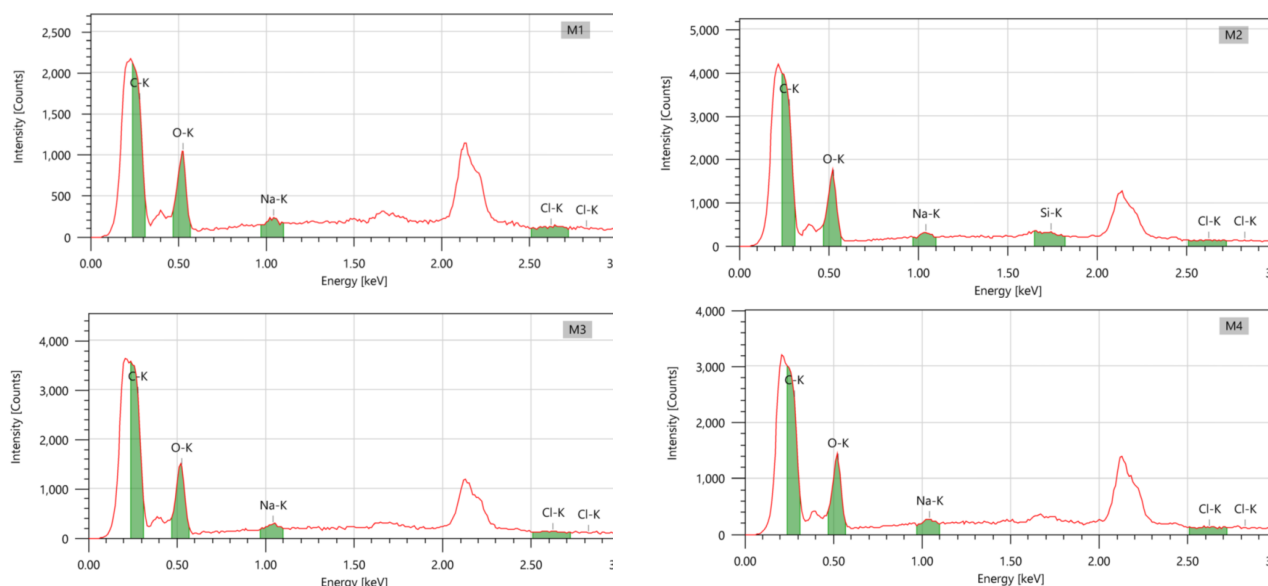


**Figure 2.** Microstructure of whey-gelatin edible film with incorporation of (a) cinnamon essential oil; (b) clove essential oil; (c) basil essential oil; (d) lemongrass essential oil under 3000x magnification.

### EDS

EDS is an analytical technique employed to ascertain the elemental composition of a given sample. In the context of whey-gelatin edible

films, EDS is employed to investigate the distribution and presence of elements within the film. The results of the EDS analysis of the whey-gelatin edible film are presented in **Figure 3**.



**Figure 3.** EDS whey-gelatin edible film with incorporation of (M1) cinnamon essential oil; (M2) clove essential oil; (M3) basil essential oil; (M4) lemongrass essential oil.

### Antimicrobial activity

The antimicrobial properties of essential oils have been recognized for centuries. In recent years, a significant number of herbs, spices and their constituents have been investigated for their antimicrobial properties against food spoilage bacteria. **Table 3** illustrates the antimicrobial activity of the films. All of the tested films demonstrated antimicrobial activity against the *E. coli* and *S. aureus*. The analysis of variance demonstrated that the incorporation of diverse essential oils exerted a considerable impact ( $P < 0.01$ ) on the antimicrobial activity of *E. coli* and *S. aureus*.

### Discussion

#### Thickness

The mean thickness value produced ranged from 0.129 to 0.177 mm, thus edible film thickness value remained within the parameters set forth by the Japanese Industrial Standard (1975), which stipulated a maximum film thickness of 0.25 mm. The highest film thickness was produced by basil

oil, followed by clove oil, lemongrass oil, and the lowest was cinnamon oil. The lowest thickness (0.129 mm) was observed for cinnamon oil, which exhibited the greatest capacity to interact with the whey-gelatin polymer matrix, resulting in the formation of a denser structure or matrix shrinkage during the drying process (Bahram *et al.*, 2014). The application of clove essential oil resulted in a thickness of 0.172 mm. This value was higher than that observed for cinnamon essential oil. The presence of active components, such as eugenol, in clove oil can increase the viscosity of film-forming solution, resulting in a thicker layer after drying process (G. Al-Hashimi *et al.*, 2020). Moreover, the film produced using basil oil exhibited the highest thickness value of 0.177 mm. This suggests that basil oil was responsible for the increase in thickness, which was attributed to the formation of additional hydrophobic interactions within the polymer matrix, thereby enhancing the film density through the strengthening of interactions between oil and protein components (Gahruie *et al.*, 2017).

The thickness of the film produced by lemongrass essential oil was 0.171 mm, which was almost similar to that produced by clove essential oil. This discrepancy is likely attributable to the distinct chemical and physical properties of essential oils, including their capacity to interact with polymer matrix, the effect on the viscosity of the solution, and modification of film-drying process (Sharma *et al.*, 2021).

**Table 3.** The average diameter of the resistance zone (mm) of essential oil against *Escherichia coli* and *Staphylococcus aureus*.

| Treatments | <i>E. coli</i>         | <i>S. aureus</i>       |
|------------|------------------------|------------------------|
| M1         | 14.4±0.74 <sup>b</sup> | 16.4±0.41 <sup>c</sup> |
| M2         | 16.3±1.68 <sup>b</sup> | 17.5±1.36 <sup>c</sup> |
| M3         | 11.3±2.41 <sup>a</sup> | 12.6±0.65 <sup>a</sup> |
| M4         | 11.7±1.82 <sup>a</sup> | 14.5±0.93 <sup>b</sup> |

**M1:** Cinnamon essential oil; **M2:** Elove essential oil; **M3:** Basil essential oil; **M4:** Lemongrass essential oil. Means in the same row with different superscripts differ significantly ( $P < 0.01$ ).

### Gelation time

It has been observed that incorporating essential oils, such as clove oil containing eugenol, reduces the gelation time. This is attributed to the increase in viscosity and the reduction in the rate of formation of polymer networks. The eugenol content of clove oil can increase the viscosity of the whey-gelatin solution, which in turn slows down the diffusion of molecules and the formation of a dense polymer network (Kong *et al.*, 2022). This results in a longer gelation time. Conversely, oils comprising components such as citral (in lemongrass essential oil) or cinnamaldehyde (in cinnamon essential oil) have been observed to accelerate the gelation time, due to their capacity to disrupt or accelerate intermolecular interactions within the whey-gelatin matrix (Asghar *et al.*, 2024, Azizah *et al.*, 2023). This discrepancy demonstrates that the chemical constitution of each essential oil is a significant determinant of the gelation time of the edible film.

### FTIR analysis

The FTIR spectra of whey-gelatin-based edible films enriched with various essential oils reveal

key functional groups and molecular interactions shaping their structural and functional properties. Peaks at 3300 1/cm reflect O-H or N-H groups, suggesting hydrophilic contributions from whey and gelatin, while peaks around 2900 1/cm indicate C-H stretching vibrations, often linked to lipid components from the essential oils. Protein structures are confirmed by amide I and II peaks (1630 1/cm and 1535 1/cm), while C-O interactions (1025 1/cm) and vibrations below 1000 1/cm highlight the involvement of carbohydrates, esters, and lipid components (Meng *et al.*, 2021, Saxton and McDougal, 2021, Xu *et al.*, 2021). Essential oils, such as cinnamon, clove, basil, and lemongrass, contribute active compounds like cinnamaldehyde, eugenol, and terpenoids, which interact with film components through hydrogen bonding and hydrophobic interactions (Sun *et al.*, 2024). These interactions enhance film stability, mechanical strength, and barrier properties, underscoring the functional role of essential oils in edible film development (Zhang *et al.*, 2020, Zhao *et al.*, 2022).

### Microstructure film

The microstructures of whey-gelatin-based edible films enriched with various essential oils, as shown in **Figures 2(a)–2(d)**, highlight the effects of oil incorporation on film homogeneity, structural integrity, and mechanical properties (Cofelice *et al.*, 2019). In **Figure 2(a)**, the cinnamon oil-enriched film exhibits a smooth surface with minimal roughness, indicating a homogeneous blend of whey, gelatin, and cinnamon oil. The presence of small white dots, likely encapsulated oil droplets, reflects efficient emulsion and uniform oil distribution within the matrix. The absence of cracks and phase separation suggests strong intermolecular interactions between protein components, contributing to flexibility and enhanced mechanical properties suitable for food packaging. In contrast, **Figure 2(b)** shows the clove oil-enriched film with minor fissures on its surface, likely caused by internal tensile stress during drying or incomplete interactions between components. While the clove

oil is evenly distributed, the presence of cracks and potential phase separation may weaken the film tensile strength and elasticity, resulting in structural fragility and reduced cohesion of the matrix. **Figure 2(c)** depicts a smoother and more uniform surface for the basil oil-enriched film, suggesting superior blending and reduced surface tension during processing. The absence of substantial fissures enhances mechanical integrity and flexibility, demonstrating the beneficial effects of basil oil on the film structural and functional properties. Finally, **Figure 2(d)** reveals uneven distribution of lemongrass oil particles, with evidence of agglomeration and phase separation that negatively impacts the film homogeneity and bonding strength. The microstructure of a material can be affected by several factors, such as the homogenization method, structural arrangement, and composition of various components formed at the end of drying process (Fahrullah *et al.*, 2024). These observations collectively underscore the critical role of oil selection and distribution in determining the physical and mechanical performance of whey-gelatin-based edible films.

### EDS

**Table 2** illustrates the mass and atomic percentage of several elements identified in this sample, including carbon (C), oxygen (O), sodium (Na), silicon (Si), and chlorine (Cl). The EDS technique is employed to ascertain the fundamental elemental composition (Hu *et al.*, 2022). The EDS spectrum exhibits pronounced peaks for the elements carbon (C), oxygen (O), and sodium (Na), which are integral to structures of whey and chia seeds. The examination of film elements elucidates the consequence of the polymerization process in the formation of polymer matrix.

The EDS spectra of various whey-gelatin-based edible films enriched with essential oils (cinnamon oil, clove oil, basil oil, and lemongrass oil) exhibited similarities in the primary elemental composition, yet exhibited variations that reflect the distinct functional properties that each film may possess. In all spectra, the C-K (Carbon) and O-K (Oxygen) peaks are the most prominent,

indicating that these films are based on an organic matrix with a high carbon and oxygen composition. This is consistent with the characteristics of whey protein and gelatin, as well as the contribution of organic compounds present in essential oils. The presence of Na-K (sodium) in the spectra indicates the presence of residual materials from raw material or production process. The Cl-K (chlorine) peak observed in all spectra signifies the presence of chloride compounds, which may originate from essential oil or contaminants. It is noteworthy that the spectra of films containing clove and basil essential oils also exhibit the presence of Si-K (silicon), which may be attributed to the contamination or addition of additives that enhance mechanical properties of films. The discovery of Si-K (silicon) in films enriched with clove and basil essential oils is also pertinent to studies demonstrating that silicon can enter film formulations from a range of sources, including contamination from processing equipment or as an additive to enhance mechanical properties or film resistance to moisture. The presence of silicone is uncommon in formulations devoid of additives, suggesting the necessity for more rigorous oversight during manufacturing process. In general, despite minor variations in composition, the prevalence of carbon and oxygen and the consistent presence of sodium and chlorine indicate the presence of a robust organic structure in these films. This structure may be significantly affected by essential oils, which could impart antimicrobial, antioxidant, and stability properties to the films.

### Antimicrobial activity

The findings revealed that the whey-gelatin film incorporating clove essential oil exhibited the most pronounced inhibitory activity against *E. coli* (16.3 mm) and *S. aureus* (17.5 mm) in comparison to other essential oils. The significant antimicrobial activity demonstrated in the results of clove-incorporated whey-gelatin films against Gram-positive bacteria is consistent with the findings of previous studies (Elsabee and Abdou, 2013). In general, Gram-positive bacteria are considered to



be more susceptible to antimicrobial compounds than Gram-negative bacteria. This is typically attributed to disparities in their cell wall architecture, as the cell wall of Gram-negative bacteria comprises lipopolysaccharides, which can impede the penetration of active compounds to the cytoplasmic membrane (Abdelhedi *et al.*, 2018). The antimicrobial mechanism of essential oils is based on their ability to disrupt and penetrate the lipid structure of bacterial cell membranes, leading to their destruction. Clove essential oil (M2) demonstrated the most pronounced antimicrobial activity against both bacterial strains, with the inhibition zone diameter reaching 16.3 mm for *E. coli* and 17.5 mm for *S. aureus*. This finding is consistent with previous studies which have demonstrated that eugenol, the primary constituent of clove essential oil, possesses the capacity to damage bacterial cell membranes, resulting in the leakage of cellular contents and subsequent bacterial death (Seow *et al.*, 2014). Similarly, cinnamon oil (M1), which contains cinnamaldehyde compounds, demonstrated high activity. These compounds have been demonstrated to effectively disrupt bacterial metabolic processes (Tyagi and Malik, 2010). In contrast, basil essential oil (M3) demonstrated the lowest antimicrobial activity, with an inhibition zone of 11.3 mm against *E. coli* and 12.6 mm against *S. aureus*. The main components of basil essential oil are linalool and estragole, which possess antibacterial properties; however, they may be less effective than eugenol or cinnamaldehyde in damaging bacterial cell membranes. The lemongrass essential oil (M4) demonstrated moderate activity, exhibiting a slightly higher zone of inhibition against *S. aureus* (14.5 mm) than *E. coli* (11.7 mm). This finding aligns with the existing literature, which indicates that citral, the primary component of lemongrass essential oil, exhibits a broader spectrum of activity against gram-positive bacteria (Liao *et al.*, 2023). The findings indicated that essential oils demonstrated superior efficacy against *S. aureus* compared to *E. coli*. This observation could be attributed to the distinctive cell wall structure of gram-positive and

gram-negative bacteria. Gram-negative bacteria, such as *E. coli*, possess a more intricate outer layer, which may confer enhanced resilience to antimicrobial agents. These findings lend support to the use of essential oils as natural additives in food products, particularly those designed to inhibit the growth of pathogenic bacteria.

**Strengths of the study:** The use of protein-based materials and essential oils is biodegradable and can reduce dependence on conventional plastics. This study combines two functional components: protein films (as structural matrices) and essential oils (as antimicrobial/antioxidant agents), which aligns well with current trends in active and functional food packaging.

**Limitation of the study:** The stability of essential oils is a concern, as they are volatile and prone to degradation by heat and light. Therefore, encapsulation technology or appropriate processing techniques are needed to preserve their effectiveness. Additionally, the presence of essential oils can influence the taste and aroma of the packaged food products.

## Conclusion

The incorporation of essential oils significantly affected thickness, gelation time, microstructure, and antimicrobial activity of whey-gelatin edible films. FTIR and EDS analyses revealed changes in chemical structure and microstructure, with interactions between essential oil components and film matrices enhancing stability and functional properties. Clove essential oil showed the highest antimicrobial activity, while cinnamon and lemongrass oils accelerated gelation, highlighting the potential of essential oils as natural additives to improve edible film performance.

## Acknowledgments

The author would like to express gratitude to the LPPM of Universitas Mataram for providing financial support for this research project through the Lektor Kepala Acceleration Research with grant number: 1269/UN18.L1/PP/2024.

## Authors' contributions

Fahrullah F., Kisworo D., Bulkaini B, Wulandani BRD and Yulianto W designed the

research; Fahrullah F and Wulandani BRD conducted the research; Fahrullah F, Kisworo D, Bulkaini B analyzed the data; Fahrullah F, Kisworo D, Bulkaini B, Wulandani BRD. and Yulianto W wrote the manuscript and had primary responsibility for final content. All authors have read and approved the final manuscript.

### Conflicts of interest

The authors declare no conflict of interest.

### Funding

The present study was funded by the Institute for Research and Community Service at the University of Mataram, with the grant number 1269/UN18.L1/PP/2024.

### References

- Abdelhedi O, et al.** 2018. In silico analysis and molecular docking study of angiotensin I-converting enzyme inhibitory peptides from smooth-hound viscera protein hydrolysates fractionated by ultrafiltration. *Food chemistry*. **239**: 453-463.
- Adilah AN, Jamilah B, Noranizan M & Hanani ZN** 2018. Utilization of mango peel extracts on the biodegradable films for active packaging. *Food packaging and shelf life*. **16**: 1-7.
- Akram MZ, Firincioğlu SY, Jalal H & Doğan SC** 2019. The use of essential oils in active food packaging: a review of recent studies. *Turkish journal of agriculture-food science and technology*. **7 (11)**: 1799-1804.
- Anukiruthika T, et al.** 2020. Multilayer packaging: Advances in preparation techniques and emerging food applications. *Comprehensive reviews in food science and food safety*. **19 (3)**: 1156-1186.
- Asghar L, Sahar A, Khan MI & Shahid M** 2024. Fabrication and Characterization of Chitosan and Gelatin-Based Antimicrobial Films Incorporated with Different Essential Oils. *Foods*. **13 (12)**: 1796.
- Azevedo VM, Silva EK, Pereira CFG, da Costa JMG & Borges SV** 2015. Whey protein isolate biodegradable films: Influence of the citric acid and montmorillonite clay nanoparticles on the physical properties. *Food hydrocolloids*. **43**: 252-258.
- Azizah F, Nursakti H, Ningrum A & Supriyadi** 2023. Development of edible composite film from fish gelatin-pectin incorporated with lemongrass essential oil and its application in chicken meat. *Polymers*. **15 (9)**: 2075.
- Bahram S, et al.** 2014. Whey protein concentrate edible film activated with cinnamon essential oil. *Journal of food processing and preservation*. **38 (3)**: 1251-1258.
- Cardoso GP, et al.** 2016. Selection of a chitosan gelatin-based edible coating for color preservation of beef in retail display. *Meat science*. **114**: 85-94.
- Cinelli P, et al.** 2014. Whey protein layer applied on biodegradable packaging film to improve barrier properties while maintaining biodegradability. *Polymer degradation and stability*. **108**: 151-157.
- Cofelice M, Cuomo F & Chiralt A** 2019. Alginate films encapsulating lemongrass essential oil as affected by spray calcium application. *Colloids and Interfaces*. **3 (3)**: 58.
- D Antunes M, M Gago C, M Cavaco A & G Miguel M** 2012. Edible coatings enriched with essential oils and their compounds for fresh and fresh-cut fruit. *Recent patents on food, nutrition & agriculture*. **4 (2)**: 114-122.
- Dhandapani K, Krishnamurthy R & Raghunathan PP** 2017. Incorporation of Natural Antimicrobials in Edible Films for Food Preservation: A Review. *Asian journal of advanced basic sciences*. **5 (2)**: 54-59.
- Elsabee MZ & Abdou ES** 2013. Chitosan based edible films and coatings: A review. *Materials science and engineering: C*. **33 (4)**: 1819-1841.
- Erkmen O & Barazi A** 2018. General characteristics of edible films. *Journal of food biotechnology research*. **2 (1)**: 3.
- Fahrullah F, Ervandi M & Rosyidi D** 2021. Characterization and antimicrobial activity of whey edible film composite enriched with clove essential oil. *Tropical animal science journal*. **44 (3)**: 369-376.

- Fahrullah F, Kisworo D & Noersidiq A** 2023. Edible film based on whey-chia seed: physical characterization with addition of different plasticizers. *Jurnal penelitian pendidikan IPA*. **9 (10)**: 8554-8562.
- Fahrullah F, Noersidiq A, Kisworo D & Maruddin F** 2024. Evaluating Physicochemical Properties of Whey-Chia Seed Edible Films for Biodegradable Packaging. *Tropical animal science journal*. **47 (4)**: 519-528.
- Fahrullah F, Noersidiq A & Maruddin F** 2022. Effects of glycerol plasticizer on physical characteristic of whey-konjac films enriched with clove essential oil. *Journal of food quality and hazards control*. **9 (4)**: 226-223.
- Fahrullah F, Radiati LE & Rosyidi D** 2020. The physical characteristics of whey based edible film added with konjac. *Current research in nutrition and food science journal*. **8 (1)**: 333-339.
- G. Al-Hashimi A, Ammar AB, Cacciola F & Lakhssassi N** 2020. Development of a millet starch edible film containing clove essential oil. *Foods*. **9 (2)**: 184.
- Gahrue HH, Ziaee E, Eskandari MH & Hosseini SMH** 2017. Characterization of basil seed gum-based edible films incorporated with Zataria multiflora essential oil nanoemulsion. *Carbohydrate polymers*. **166**: 93-103.
- Hu G, et al.** 2022. Isolation, purification and structure identification of a calcium-binding peptide from sheep bone protein hydrolysate. *Foods*. **11 (17)**: 2655.
- Kanmani P & Rhim J-W** 2014. Properties and characterization of bionanocomposite films prepared with various biopolymers and ZnO nanoparticles. *Carbohydrate polymers*. **106**: 190-199.
- Kong I, Degraeve P & Pui LP** 2022. Polysaccharide-based edible films incorporated with essential oil nanoemulsions: Physico-chemical, mechanical properties and its application in food preservation—A review. *Foods*. **11 (4)**: 555.
- Lee SY, Lee SJ, Choi DS & Hur SJ** 2015. Current topics in active and intelligent food packaging for preservation of fresh foods. *Journal of the science of food and agriculture*. **95 (14)**: 2799-2810.
- Liao Z, et al.** 2023. Transcriptomic analyses reveal the potential antibacterial mechanism of citral against *Staphylococcus aureus*. *Frontiers in microbiology*. **14**: 1171339.
- Maruddin F, Ratmawati R, Fahrullah F & Taufik M** 2018. Characteristics of edible film based dangke whey with carrageenan addition. *Jurnal Veteriner*. **19 (2)**: 291-297.
- Meng Y, et al.** 2021. Ultrasonic modification of whey protein isolate: Implications for the structural and functional properties. *LWT-Food science and technology*. **152**: 112272.
- Moghadam M, Salami M, Mohammadian M, Khodadadi M & Emam-Djomeh Z** 2020. Development of antioxidant edible films based on mung bean protein enriched with pomegranate peel. *Food hydrocolloids*. **104**: 105735.
- Motelica L, et al.** 2020. Biodegradable antimicrobial food packaging: Trends and perspectives. *Foods*. **9 (10)**: 1438.
- Newbury DE & Ritchie NW** 2015. Performing elemental microanalysis with high accuracy and high precision by scanning electron microscopy/silicon drift detector energy-dispersive X-ray spectrometry (SEM/SDD-EDS). *Journal of materials science*. **50**: 493-518.
- Nilsuwan K, Benjakul S & Prodpran T** 2017. Properties, microstructure and heat seal ability of bilayer films based on fish gelatin and emulsified gelatin films. *Food biophysics*. **12**: 234-243.
- Ribeiro-Santos R, Andrade M, de Melo NR & Sanches-Silva A** 2017. Use of essential oils in active food packaging: Recent advances and future trends. *Trends in food science & technology*. **61**: 132-140.
- Sabil S, Maruddin F, Wahyuni T & Taufik M** 2021. Edible film characteristics at different casein concentrations. In *IOP conference series: Earth and environmental science*, p. 012115. IOP Publishing.
- Saxton R & McDougal OM** 2021. Whey protein powder analysis by mid-infrared spectroscopy. *Foods*. **10 (5)**: 1033.

- Schmid M** 2013. Properties of cast films made from different ratios of whey protein isolate, hydrolysed whey protein isolate and glycerol. *Materials*. **6** (8): 3254-3269.
- Seow YX, Yeo CR, Chung HL & Yuk H-G** 2014. Plant essential oils as active antimicrobial agents. *Critical reviews in food science and nutrition*. **54** (5): 625-644.
- Sharma S, Barkauskaite S, Jaiswal AK & Jaiswal S** 2021. Essential oils as additives in active food packaging. *Food chemistry*. **343**: 128403.
- Sun Y, et al.** 2024. Natural active products in fruit postharvest preservation: A review. *Food frontiers*. **5** (5): 2043-2083.
- Teixeira B, et al.** 2014. Characterization of fish protein films incorporated with essential oils of clove, garlic and origanum: Physical, antioxidant and antibacterial properties. *LWT-Food science and technology*. **59** (1): 533-539.
- Tongnuanchan P, Benjakul S, Prodpran T, Pisuchpen S & Osako K** 2016. Mechanical, thermal and heat sealing properties of fish skin gelatin film containing palm oil and basil essential oil with different surfactants. *Food hydrocolloids*. **56**: 93-107.
- Tyagi AK & Malik A** 2010. Liquid and vapour-phase antifungal activities of selected essential oils against *Candida albicans*: microscopic observations and chemical characterization of *Cymbopogon citratus*. *BMC complementary and alternative medicine*. **10**: 1-11.
- Xu Yp, et al.** 2021. Evaluation of the properties of whey protein films with modifications. *Journal of food science*. **86** (3): 923-931.
- Zhang Y, et al.** 2020. Preparation and characterization of curdian/polyvinyl alcohol/thyme essential oil blending film and its application to chilled meat preservation. *Carbohydrate polymers*. **247**: 116670.
- Zhao R, Guan W, Zhou X, Lao M & Cai L** 2022. The physiochemical and preservation properties of anthocyanidin/chitosan nanocomposite-based edible films containing cinnamon-perilla essential oil pickering nanoemulsions. *LWT-Food science and technology*. **153**: 112506.