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RESEARCH ARTICLE
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Characterization of Whey-Pectin Film Material Encapsulated with Lemon Essential Oil for Biodegradable Food Packaging: A Comprehensive Analysis of Chemical Composition, Morphology and Antimicrobial Activity

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ABSTRACT

The objective of this study is to develop a biodegradable film composed of whey and pectin that encapsulates lemon essential oil, serving as an environmentally friendly food packaging alternative with antibacterial properties. The incorporation of lemon essential oil, characterised by its bioactive compound limonene, is intended to enhance the film's efficacy against microbial organisms. The film was fabricated using the solvent-casting method, incorporating lemon essential oil and employing encapsulation techniques to protect volatile compounds from degradation. The experimental study employed a randomised design, encompassing the following treatments: the three solutions are labelled P1, P2, and P3 and consist of whey-pectin, whey-pectin with lemon essential oil, and whey-pectin with encapsulated lemon essential oil, respectively. The findings of the Fourier Transform Infrared (FTIR) and EDS (Energy Dispersive X-ray Spectroscopy) analyses demonstrated significant chemical interactions between whey, pectin, and lemon essential oil. In addition, morphology observations indicated enhanced film stability and homogeneity. The antimicrobial activity of the essential oil was evaluated through in vitro experimentation against *Escherichia coli* and *Staphylococcus aureus*. The findings demonstrated that encapsulating lemon essential oil led to a substantial increase in the inhibition zone against both gram-negative and gram-positive bacteria. The findings of this study suggest that this combination of natural materials has the potential to be utilised as effective, biodegradable food packaging that extends the shelf life and maintains food product safety, while supporting the sustainability of the food industry.

Keywords: Biodegradable, Encapsulation, Packaging, Pectin, Whey.

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INTRODUCTION

The heightened awareness of environmental and sustainability issues has precipitated the development of environmentally friendly food packaging as an alternative to synthetic plastics that are difficult to break down. A promising approach is the utilisation of biodegradable films derived from biopolymers, such as whey protein and pectin polysaccharides. Protein- and polysaccharide-based films offer the advantages of biodegradability and flexibility for the integration of bioactive ingredients, rendering them promising candidates for active food packaging applications (Silva et al., 2016). The combination of these materials is an attractive candidate due to its ability to form thin layers with relatively good mechanical properties, high solubility, and ecological compatibility, making it potentially suitable for applications in the food industry (Yaashikaa et al., 2023; Urango et al., 2025).

The use of biopolymer-based films is often accompanied by inherent limitations, namely their high permeability to water vapour and gases, as well as a conspicuous lack of antimicrobial properties. These functional properties can be enhanced by incorporating bioactive compounds, with lemon essential oil as one example. Lemon essential oil has been shown to contain active components, including limonene, β-pinene, and linalool, which have been identified as natural antimicrobial agents (Song et al., 2018; Socaciu et al., 2021; Budiarto et al., 2024).

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The incorporation of essential oils into film matrices has been extensively investigated as a method to augment the active functionality of packaging, particularly in terms of antimicrobial activity (Chen et al., 2019; Barbosa et al., 2021; Almajed et al., 2024; Brenda et al., 2024; Rasheed & Aljohani, 2024; Fahrullah et al., 2024, 2025). However, the volatility, susceptibility to oxidation, sensitivity to oxidation, and strong sensory effects of essential oils often pose challenges to their application, rendering them prone to reduced effectiveness when applied directly (Yi et al., 2022). To address this challenge, encapsulation techniques are employed to protect active compounds from degradation and enable controlled release over time (Riseh et al., 2021; Sharma et al., 2022; Wen et al., 2016). A plethora of studies have demonstrated that encapsulating essential oils in a biopolymer matrix can enhance thermal stability, homogeneity of distribution, and antimicrobial activity (Prakash et al., 2018; Antonino et al., 2024; Said & Lee, 2025). The objective of this study is to conduct a comprehensive analysis of the characterisation of lemon essential oil-encapsulated whey-pectin films, including their chemical composition, morphology, and antimicrobial activity, to develop biodegradable food packaging with potential for the sustainable food industry.

MATERIALS & METHODS

Lemon Essential Oil Extraction

The extraction of lemon essential oil was carried out using a Heidolph Rotary Evaporator Hei-VAP Value Digital G3 (Heidolph Instruments GmbH & Co. KG, Germany). The grated lemon was subsequently placed in a rotary flask containing ethanol. The flask was connected to an evaporator unit, whose temperature was maintained at 40-60°C using a water bath. The utilisation of a vacuum pump is instrumental in reducing pressure, thereby facilitating evaporation and enabling more efficient separation of the essential oil. It is imperative to note that during this process, the solvent evaporates, allowing the essential oil to be collected in a suitable container. The essential oil obtained is then stored in dark glass bottles in a cool place to maintain its quality.

Pectin Extraction

Pectin is extracted from the waste residue produced during the extraction of lemon essential oil. This is achieved by an acidification method that uses an acid solution. The extraction process is conducted in a hot hydrochloric acid solution under strictly controlled conditions at 45°C and pH 1.25. This process is undertaken to achieve the hydrolysis and dissolution of the pectin. After the extraction process, ethanol is added to reduce the solubility of pectin in the acid solution, thereby enabling precipitation and separation from the liquid phase. The precipitated pectin is collected through filtration, then washed with ethanol to remove impurities, and finally dried.

Encapsulation of Bioactive Compounds in Lemon Essential Oil

The encapsulation process is undertaken in accordance with the established methodology (Bezerra et al., 2016; Manaf et al., 2018), which involves a two-stage procedure. The preliminary stage of this process is preparing an essential oil emulsion using a gelatin and maltodextrin solution. A total of 100mL of gelatin solution was mixed with 8mL of lemon essential oil mixture and 6mL of Tween 80, then homogenized at room temperature at a speed of 700 revolutions per minute for 30min. Subsequently, 100mL of maltodextrin solution was added and homogenized again for 30min. The second stage of the process was complex coacervation, which commenced with the addition of 2mL of a 10% CH_3COOH solution until the pH had decreased to 4, followed by homogenization for 60min. The emulsion was left at a temperature of ±10ºC for 30min. Subsequently, 20mL of a sodium tripolyphosphate solution is added as a cross-linking agent, and the mixture is homogenized for an additional 60min. Subsequently, the pH was adjusted to 9 with 20% NaOH. Following this adjustment, the homogenization process was carried out for 2 hours. The emulsion was left to settle at ±10°C for 16 hours before being dried using a freeze dryer.

Preparation of Film Solutions for Antimicrobial Testing

The whey-pectin-based film was produced using the solvent casting method. A mixture of 15 grams of whey and 1 gram of pectin is prepared by dissolving these ingredients in 100mL of distilled water. The solution is subjected to continuous stirring at a temperature of 60°C for a duration of 30min, employing a hot plate stirrer. Subsequently, lemon essential oil and lemon essential oil capsules are added to the solution, each constituting 5% of the total. Subsequently, antimicrobial activity testing was conducted. Modification (Fahrullah et al., 2020).

Fourier Transform Infrared Analysis

The testing method employed was the PerkinElmer Spectrum Two ATR-FTIR instrument (Thermo Fisher Scientific, USA). The sample was then placed on an optical window above the ZnSe crystal, after which it was subjected to a pressure of 50 (force gauge) to ensure optimal contact between the sample and the crystal. The experiment was conducted in accordance with the established protocol for IR spectrophotometers, encompassing a wavelength range from 4000 to 550cm^{-1}. The experimental procedure involved acquiring 6 repetitions of readings with a resolution of 4cm^{-1}.

Morphology

The morphology was examined using a JEOL JCM-7000 scanning electron microscope (JEOL Ltd, Japan). The prepared samples were then mounted on an aluminium stub and observed under a scanning electron microscope (SEM) to ascertain their morphology.

Energy Dispersive X-ray Spectroscopy (EDS)

The specimen is meticulously prepared and assured to fit within the SEM chamber, thereby preserving the specimen's smooth surface and preventing contamination. The specimen is then mounted in the SEM, where the film's morphology is observed in detail. The specific areas selected for EDS analysis become the primary focus. The

EDS measurement process begins with detector activation, followed by electron beam focusing and measurement of the emitted X-ray energy. The X-ray data are then converted into a spectrum, which is analysed to identify elements based on energy peaks and to quantify them by comparing peak intensities (Newbury & Ritchie, 2014).

Antimicrobial Activity

The method for testing antimicrobial activity using paper discs consists of two main phases: preparing the test bacteria and assessing antimicrobial effectiveness. In the initial phase, pure cultures of test bacteria are prepared by making a series of MacFarland standard solutions and equalising the turbidity of the bacterial suspension in Buffered Peptone Water (BPW) medium. The antimicrobial potential is then evaluated through the diffusion of antimicrobial compounds from paper discs into agar media inoculated with test microbes, in accordance with established methodology. This phase involves preparing Nutrient Agar (NA) media in sterile Petri dishes and subsequently inoculating the test bacterial suspension using the spread plate method. The primary experiment involved placing paper discs impregnated with antimicrobial agents or negative controls on NA media inoculated with test bacteria, followed by incubation for 24 hours. The evaluation of the results was conducted by measuring the diameter of the cloudy and clear zones around the paper discs. This method was employed to assess the antimicrobial potential of the tested compounds (Moghadam et al., 2020).

Data Analysis

The FTIR, morphology and EDS testing were analysed descriptively, while the antimicrobial activity testing was analysed using Analysis of Variance (ANOVA). If significant results were obtained, they were followed up with Duncan's Multiple Range Test (DMRT). The applications employed for data processing were SPSS 25 and OriginPro 2018.

RESULTS AND DISCUSSION

FTIR Analysis

FTIR analysis is a technique used to identify and analyse functional groups present in materials. This is achieved by measuring infrared absorption at various frequencies. In this context, the primary components employed in the manufacturing of lemon essential oil films can be utilised to analyse the chemical interactions between elements and the structural changes that occur.

As illustrated in Fig. 1, the FTIR spectrum of whey displays an absorption peak at 3274.60cm^{-1}, which is associated with the N–H stretching of the amide bond. This finding indicates the presence of proteins, particularly amide groups. The peak at 2922.13cm^{-1} is indicative of C-H stretching of methylene groups (–CH_2), suggesting the presence of lipid components or aliphatic organic compounds. The peak at 1743.78cm^{-1} indicates C=O stretching of carbonyl or ester groups, thus confirming the presence of lipids or triglycerides. Concurrently, the peak at 1016.13cm^{-1} is associated with C-O stretching from

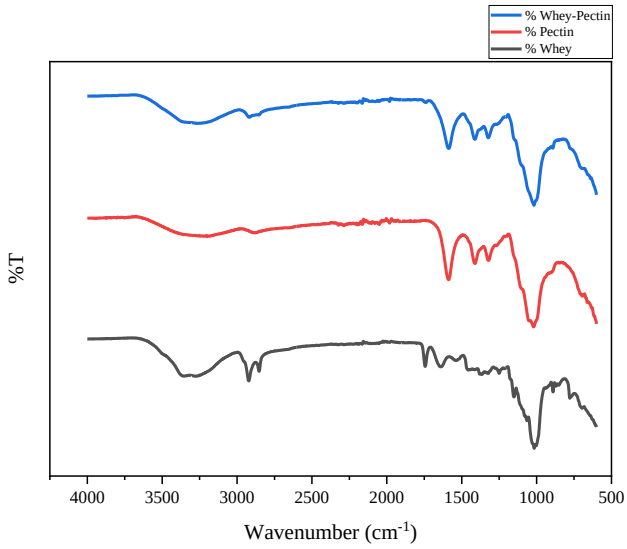

Fig. 1: ATR-FTIR spectra of whey, pectin, and whey-pectin.

alcohols or carbohydrates, indicating the presence of carbohydrates or glycoproteins. In the FTIR spectrum of pectin, the absorption peak at 1587.65cm^{-1} is associated with C=C stretching in aromatic bonds or carboxylate groups, indicating the presence of carboxylate groups, a characteristic feature of pectin. The peak observed at 1020.14cm^{-1} is indicative of C-O stretching in alcohols or polysaccharides, confirming the presence of C-O bonds within the pectin structure. As demonstrated by several studies, whey proteins (particularly dominant fractions such as β-lactoglobulin) and charged polysaccharides (pectin) have the capacity to form soluble complexes or coacervates through electrostatic (ionic) interactions and attractive (hydrophobic) forces, as well as hydrogen bonds (Du et al., 2022; Wang et al., 2022). The formation of coacervates in the protein–pectin system is intertwined through electrostatic interactions, which can also be observed using FTIR techniques (Drusch et al., 2024). In a study of whey–pectin microcapsule systems encapsulating phenolic compounds (grape extract), FTIR spectroscopy analysis demonstrated that interactions occurred without forming new covalent bonds, instead via non-covalent forces such as hydrogen and electrostatic interactions (Oprea et al., 2022; Cruz-Molina et al., 2023). The FTIR spectrum of the whey and pectin mixture exhibits a peak at 3266.14cm^{-1}, indicative of N–H stretching from amides or proteins. This finding suggests that whey proteins remain identifiable following mixing, thereby affirming their persistence in the mixture. The peak at 1587.08cm^{-1} corresponds to the C=C stretching or carboxylate group, indicating the presence of pectin. In contrast, the peak at 1412.19cm^{-1} corresponds to C–H and C–O stretching, which is common in polysaccharide structures. Furthermore, the peak at 1017.65cm^{-1} serves to reinforce the indication of the presence of C–O bonds from alcohols or carbohydrates that are derived from pectin. A study on the complexation between anthocyanin-containing pectin and whey protein noted that heating and pH greatly affect the stability of the complex, and that, in the 800–1200cm^{-1} domain, a new band shift appears due to functional group interactions between

pectin and protein (Salleh et al., 2022). Wefers et al. (2018) also reported that conjugation (a chemical reaction between the protein-lysine group and pectin) can occur under certain conditions, thereby enhancing emulsion stability.

As illustrated in Fig. 2, the FTIR spectrum of lemon essential oil displays absorption peaks indicative of the presence of significant compounds, including terpenoids (limonene), with pronounced peaks at 2964.87 and 2918.45cm^{-1}, corresponding to C-H groups. The peak at 1644.51cm^{-1} indicates a C=C bond, characteristic of hydrocarbon monoterpenes, while the peak at 1376.51cm^{-1} supports the presence of terpenoids. The peaks at 1155.26 and 1230.72cm^{-1} are associated with C-O groups, indicating the presence of oxygenated compounds such as linalool and citronellal. The peaks observed at 759.24cm^{-1} and 956.35cm^{-1} are indicative of C-H vibrations present within aromatic rings, while those observed at 886.20cm^{-1} and 1016.77cm^{-1} are consistent with the presence of alcohols or esters. The FTIR spectrum of encapsulated lemon essential oil demonstrates that encapsulation does not alter the primary chemical structure, as evidenced by the C–O peak at 1016.04cm^{-1} remaining identifiable. The spectra of the coating materials, maltodextrin and gelatin, show characteristic peaks indicating the presence of polysaccharides and proteins. The occurrence of non-covalent physical interactions, most notably hydrogen bonds between maltodextrin and gelatin, as well as between maltodextrin and essential oil, indicates a physical encapsulation mechanism. The absence of new peak formation further supports the hypothesis that encapsulation protects volatile components from oxidative degradation.

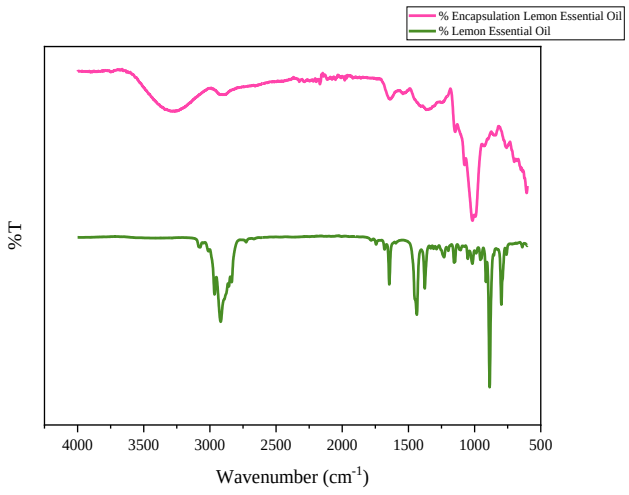

Fig. 2: ATR-FTIR spectra of lemon essential oil and encapsulated lemon essential oil.

The preservation of the characteristic bands of lemon essential oil in the coating matrix is of significant technical and practical importance. Furthermore, the active structure, including aroma-producing compounds and biological activity, is maintained, thus enabling the essential oil to continue to express its important functions, such as aroma, antimicrobial effects, and antioxidants (Mehta et al., 2022; Sousa et al., 2022).

Morphology

The morphology of the base materials utilised in the fabrication of whey, pectin, and whey-pectin films exhibits substantial disparities concerning surface roughness and regularity. In whey (Fig. 3a), a relatively rough structure with irregular particles is visible, indicating that whey contains components that may be separate or aggregated, which can affect the mechanical properties of the film. In contrast, pectin (Fig. 3b) exhibits a more uniform, smooth structure, with smaller, more evenly distributed particles, reflecting the more structured nature of carbohydrate polymers and providing enhanced stability in film production. The combination of whey and pectin (Fig. 3c) displays a more homogeneous morphology pattern, with a smoother and flatter surface than whey alone. This suggests an interaction between the whey and pectin components. This finding indicates that these two materials may form a more stable matrix within the film, which could potentially result in a more integrated and stable product. These morphological disparities are pivotal for elucidating the behaviour of these materials in film-making applications and for the quality of the final product. As illustrated in Fig. 3d, the morphology of encapsulated lemon essential oil is characterised by spherical and elliptical particles the encapsulation process. These particles suggest that the essential oil is entrapped within the formed coacervate structure. However, some particles exhibit cracks, indicating tension during the drying process. Lemon essential oil is highly susceptible to oxidation, a process that can reduce its quality and effectiveness. Coacervation is a process that involves applying a layer of gelatin and maltodextrin to essential oils, thereby protecting them and extending their shelf life while maintaining their stability (Napiórkowska & Kurek, 2022; Kim et al., 2025).

Energy Dispersive X-ray Spectroscopy (EDS)

In the context of food-related research, EDS is a valuable tool for identifying and measuring contaminants, monitoring product quality and safety, and tracing contamination origins. The primary objective of these research endeavours is to enhance the quality and safety control of food products. The EDS analysis results (Fig. 4) reveal the presence of several elements in the main ingredients used to make the film, with the mass and atomic percentages listed in Table 1.

The EDS analysis of film-making materials, namely whey (W), pectin (P), the whey-pectin combination (WP) and encapsulated lemon essential oil (LE), has indicated the presence of several significant elements. The analysis of the whey sample revealed sodium (Na), calcium (Ca), and magnesium (Mg) as the predominant elements, suggesting that these minerals likely originated from the whey sample itself. Furthermore, other elements, including chlorine (Cl), potassium (K), and carbon (C), were identified, suggesting mineral and organic components in whey. In contrast, pectin exhibited analogous components, including oxygen (O), which is associated with the oxygen-containing polymer-carbohydrate structure of pectin. Furthermore, mineral elements, including Ca and Cl, were detected, indicating the incorporation of natural materials

Table 1: The composition of the film's base materials in terms of mass and atomic percentage

Elements	Whey		Pectin		Whey-Pectin		Encapsulation Lemon Oil	
	Mass (%)	Atom (%)	Mass (%)	Atom (%)	Mass (%)	Atom (%)	Mass (%)	Atom (%)
C	34.48±0.43	48.75±0.61	22.87±0.32	33.60±0.47	23.74±0.34	34.59±0.49	18.10±0.34	27.12±0.50
O	41.75±0.87	44.32±0.92	45.09±0.63	49.72±0.70	45.28±0.67	49.53±0.73	56.54±0.92	63.59±1.03
Na	0.85±0.09	0.63±0.07	18.03±0.30	13.84±0.23	17.13±0.31	13.04±0.24	1.27±0.11	0.99±0.09
Mg	0.22±0.05	0.15±0.03	0.12±0.04	0.09±0.03	0.22±0,05	0.16±0.03	0.39±0.06	0.29±0.04
Cl	1.99±0.10	0.95±0.05	0.31±0.04	0.15±0.02	0.29±0.04	0.14±0.02	3.60±0.14	1.83±0.07
K	3.02±0.15	1.31±0.06	0.03±0.03	0.01±0.01	nd	nd	5.17±0.19	2.38±0.09
Ca	2.67±0.15	1.13±0.06	0.04±0.03	0.02±0.01	0.10±0.04	0.04±0.02	3.55±0.17	1.59±0.08
Nb	15.02±0.36	2.75±0.07	15.51±0.30	2.57±0.06	13.24±0.31	2.49±0.06	11.38±0.33	2.20±0.06
Total	100	100	100	100	100	100	100	100

nd: not detected

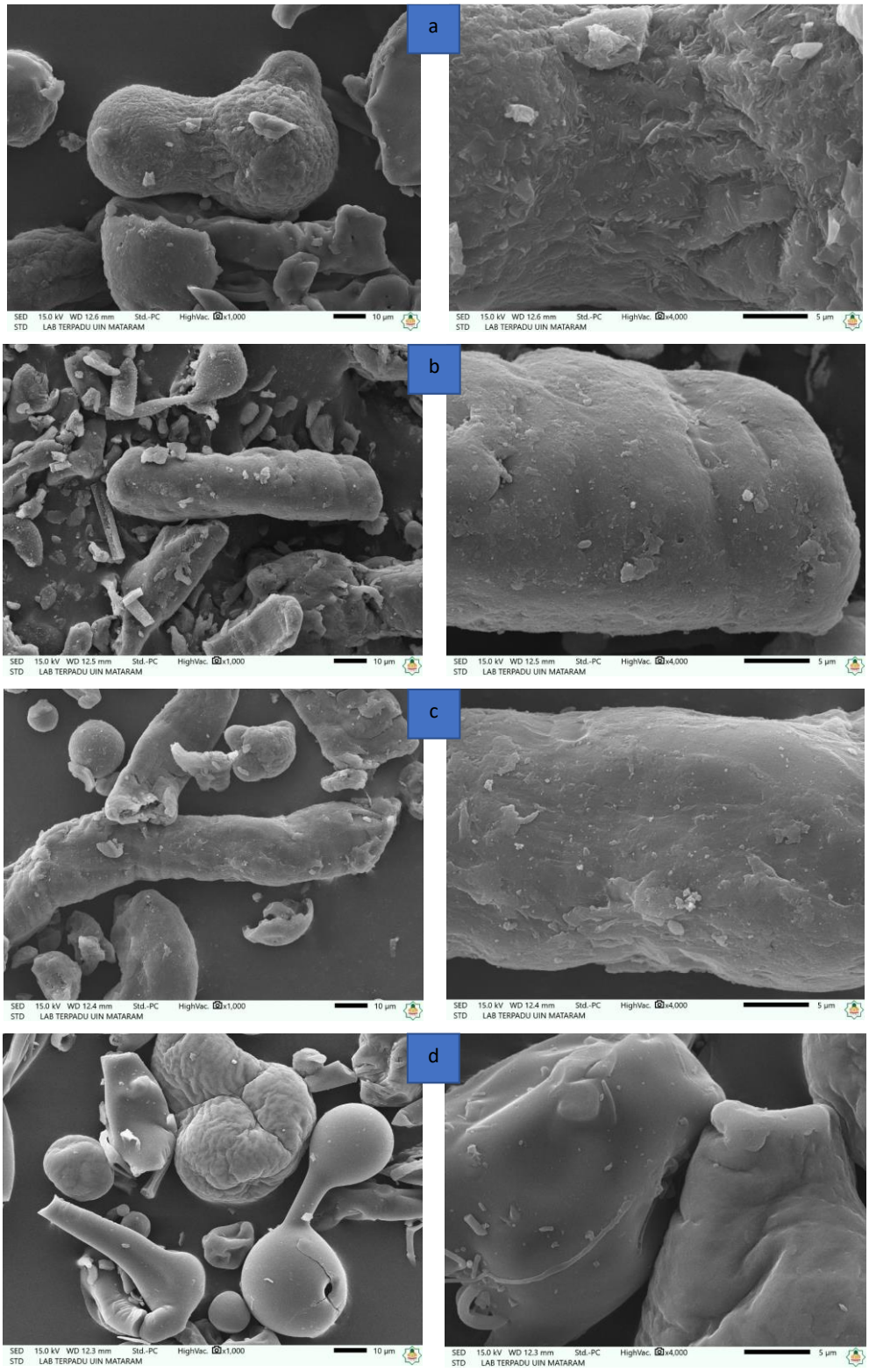

Fig. 3: Morphology of film-forming materials; Note: (a) whey; (b) pectin; (c) whey-pectin; (d) encapsulated lemon essential oil with 1000 and 4000 times magnification.

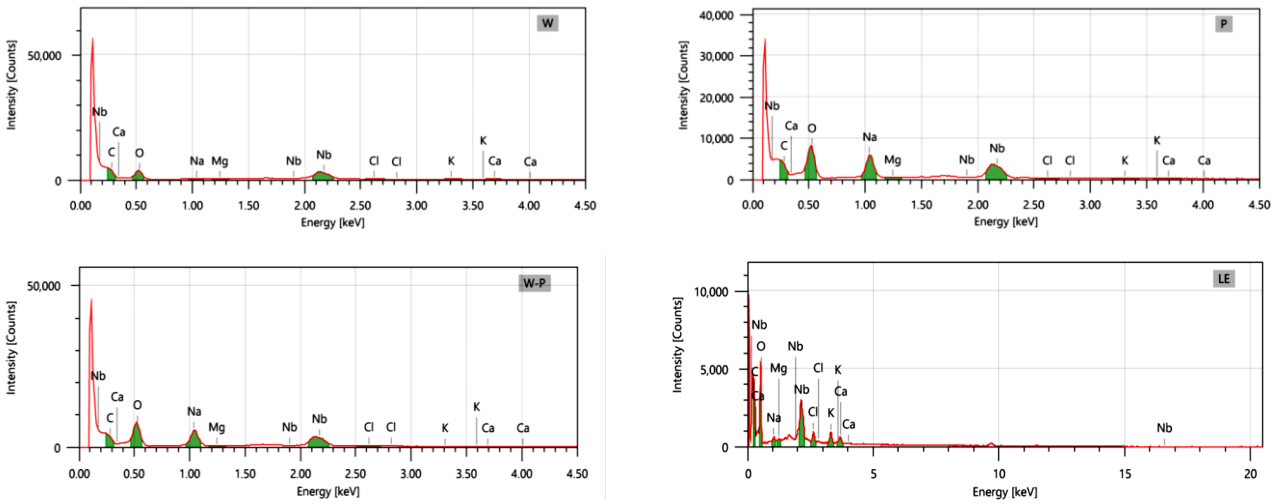

Fig. 4: EDS film-making materials; Note: (W) whey; (P) pectin; (WP) whey-pectin; (LE) encapsulated lemon essential oil.

into the pectin matrix. In the WP combination, the EDS spectrum displays a pattern analogous to the two constituent materials, yet with more uniformly dispersed element peaks. This finding suggests an interaction or combination between whey and pectin within the film. The intensity of elements such as Ca, Na, Mg, K, Cl, and C demonstrates a more balanced composition, which may indicate a more homogeneous matrix formation in the final product. The incorporation of pectin into the whey sample introduces novel components and demonstrates the capacity for chemical interaction between these two materials (Du et al., 2022; Im et al., 2023). The analysis of the lemon essential oil revealed the presence of light elements such as O, Na, and Mg, as evidenced by very high-intensity peaks. This finding indicates that the compounds present in lemon essential oil and in the encapsulation may comprise organic and inorganic molecular components, such as fatty acids, glycerol, and minerals within the encapsulation matrix. At higher energy ranges (5–10 keV), elements such as Ca, K, and Cl become discernible. The presence of these elements indicates the presence of inorganic components in the encapsulation material, such as minerals in gelatine.

Antimicrobial Activity

The findings of the variance analysis demonstrate that the utilisation of lemon essential oil exerted a substantial impact ($p < 0.01$) on the inhibition zones of both *E. coli* and *S. aureus*. As demonstrated in Fig. 5, the solution composed of whey-pectin (P1) exhibited comparatively diminutive inhibition zones, specifically 2.80mm for *E. coli* and 0mm for *S. aureus*. This finding suggests that whey-pectin exhibits limited antibacterial potential, or in some cases, no antimicrobial activity at all. However, the incorporation of lemon essential oil into the second solution (P2) elicited a more pronounced effect, with the inhibition zones for *E. coli* and *S. aureus* increasing to 4.40 and 4.80mm, respectively. Lemon essential oil contains active compounds such as limonene, which has been shown to possess potent antimicrobial properties (Chouhan et al., 2017). In addition, the compounds found in lemon essential oil have been shown to interact with

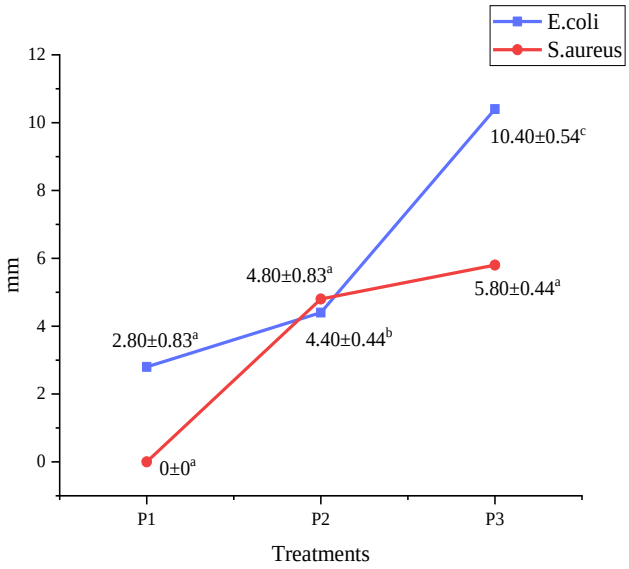

Fig. 5: Inhibition zone (mm) of *E. coli* and *S. aureus*.
Note: P1: whey-pectin; P2: whey-pectin with lemon essential oil; P3: whey-pectin with encapsulated lemon essential oil

lipids in microbial cell membranes (Akachat et al., 2025; Djerri et al., 2025), resulting in the disruption of their structure and the leakage of important cellular components (Li et al., 2019; Zubair et al., 2022). Finally, it was found that solutions containing encapsulated lemon essential oil produced the largest inhibition zones, namely 10.40mm for *E. coli* and 5.80mm for *S. aureus*. This encapsulation technology enhances antimicrobial effectiveness by enabling the gradual release of more stable lemon essential oil, thereby prolonging antimicrobial activity (Gupta et al., 2016; Becerril et al., 2020). The process of encapsulation has been shown to reduce the rate of evaporation of essential oils (Kokina et al., 2019; Mukurumbira et al., 2022). This effect ensures that the antimicrobial compounds contained within the essential oils are released in a controlled and sustained manner, rather than evaporating quickly. The result is a strengthening and prolongation of the inhibitory effect of the essential oils. The increase in the inhibition zone of *E. coli* (Fig. 6) indicates that controlled release helps essential

oils overcome the additional defences of the Gram-negative bacterial cell wall. The sustained release of bioactive compounds has been demonstrated to allow for increased penetration and disruption of cell walls (Zhao et al., 2023; Sheikh et al., 2024).

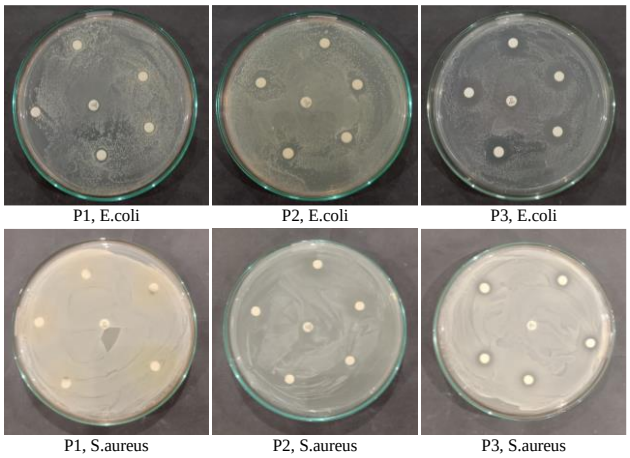

Fig. 6: The inhibition zones (mm) of *E. coli* and *S. aureus* of the whey-pectin film.
Note:
P1: whey-pectin
P2: whey-pectin with lemon essential oil
P3: whey-pectin with encapsulated lemon essential oil

Conclusion

The present study demonstrates that combining whey, pectin and lemon essential oil during film production enhances stability, mechanical properties, and antibacterial activity. The application of FTIR and EDS has been demonstrated to facilitate the analysis of chemical interactions between components. Furthermore, encapsulating lemon essential oil has been shown to provide a protective barrier for volatile compounds, thereby extending their antibacterial activity. Consequently, the resulting film has the potential to be utilised in the field of food packaging, offering additional benefits such as protection against microbes.

DECLARATIONS

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Conflict of Interest: The authors have no conflict of interest to declare.

Data Availability: The data generated and analyzed during this study are fully presented in this article.

Ethics Statement: This research did not require ethics statement approval because there were no human and animal participants.

Author's Contribution: All authors contributed equally in this paper, including: designing the study, conducting the experimental work, analyzing data, writing the manuscript, and reading and approving the final manuscript.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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