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Preparation and Characterization of Edible Film Derived from Local Black Mulberry Pulp and Leaves

Madihah Nabilah Abdul Samad[1], Hatijah Basri*[1]

[1] *Department of Technology and Natural Resources, Faculty of Applied Sciences and Technology, UTHM Kampus Cawangan Pagoh, Hab Pendidikan Tinggi Pagoh, KM 1, Jalan Panchor, 86400 Pagoh, Muar, Johor, MALAYSIA.*

*Corresponding Author: hatijah@uthm.edu.my
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Abstract

Edible films have gained significant attention as eco-friendly packaging solutions due to their biodegradability and ability to preserve food quality. This study aimed to develop and evaluate the characterization of edible films formulated using pectin extracted from local black mulberry pulp and chlorophyll from its leaves. To enhance flexibility and mechanical strength, xanthan gum and carboxymethylcellulose (CMC) were incorporated. The films were evaluated for their characterization and antimicrobial properties to assess their suitability for food packaging applications. Scanning electron microscopy (SEM) was used to observe morphological properties, Fourier transform infrared (FTIR) spectroscopy to analyse chemical structures, and a texture analyzer to assess tensile strength. A water solubility test was conducted to evaluate dissolution properties, while antimicrobial activity was tested against *Escherichia coli* and *Staphylococcus aureus* using agar diffusion assays. The results indicated that xanthan gum enhanced the films' flexibility while CMC effectively improved tensile strength. FTIR spectra confirmed the successful integration of pectin, chlorophyll, xanthan gum, and CMC. Among all formulations, F5 exhibited the best performance, with SEM analysis revealing uniform morphology and reduced structural imperfections. It also demonstrated the highest tensile strength (29.25 MPa), extensibility (40.669 mm), and the lowest water solubility (40.04%). Antimicrobial assays showed inhibition zones of 3.3 mm for *E. coli* and 5.2 mm for *S. aureus*. This research highlights the potential of local black mulberry pulp as a source of pectin, offering a promising, sustainable solution for food packaging with enhanced functionality and environmental benefits.

1. Introduction

Edible films are thin layers made from edible polymers, such as carbohydrates, proteins, and lipids, and are typically produced using casting, compression moulding, or extrusion techniques [1]. These films, derived from biodegradable natural polymers like animal and vegetable proteins, celluloses, gums, and lipids, serve as moisture barriers and preservation agents when applied to food, such as strawberry fruit, to maintain flavour and freshness [2].

In edible film production, plasticizers are incorporated to improve flexibility, aid in moulding and shaping, reduce brittleness, and enhance resistance against biodegradation, as polysaccharide- and protein-based films

tend to be brittle. Plasticizers are substances that interact with polymers through physical and chemical reactions. Commonly used plasticizers include glycerol, sorbitol, mannitol, and phospholipids [3].

The characterization of edible films involves assessing their morphology, water solubility, mechanical properties, and chemical composition to ensure their effectiveness in food preservation. These characteristics influence the film's barrier properties, strength, flexibility, and compatibility with food products [4,5,6].

Most studies on pectin extraction have focused on fruit peels such as apples, watermelon, orange, and dragon fruit. However, research on its extraction from mulberry pulp remains limited [7,8]. To address this gap, this study explores the potential of local black mulberry pulp as a source for edible films and applies the pectin extraction technique used by [9] to assess its suitability for edible film production.

To enhance the physical and mechanical properties of pectin films, carboxymethylcellulose (CMC) and xanthan gum were incorporated to improve tensile strength and flexibility [10]. This research aims to develop and evaluate the characteristics of edible films derived from local black mulberry pulp and leaves.

2. Materials and Methods

2.1 Materials and Instruments

The materials and instruments used in this study included pectin powder and chlorophyll pigment were extracted from mulberry pulp and leaves, carboxymethylcellulose (CMC) powder (food grade) and xanthan gum (food grade) were purchase from DChemie Malaysia, citric acid, ethanol, glycerol, Hydrochloric acid 1 N, and sodium nitrate were prepared from Merck (Germany), Mueller Hinton agar was prepared from Oxoid (UK), Fourier transform infrared (FTIR) spectroscopy (Agilent Cary 630), scanning electron microscopy (SEM) (COXEM EM-30 Plus), and texture analyzer (Stable Micro System TA. XT Plus), *Escherichia coli* and *Staphylococcus aureus*.

2.2 Methods

2.2.1 Preparation for Pectin Extraction

The sample was prepared by adopting the method described by Íñiguez-Moreno et al. Initially, mulberry fruits were harvested and thoroughly cleaned to eliminate foreign matter. The cleaned fruits were then finely diced into uniform pieces, evenly spread on trays, and dried in an oven at 50 °C for 24 hours or until a consistent weight was reached.

After drying, the mulberry was ground into a fine powder using a grinder and sieved through an 80-mesh sieve to achieve a uniform particle size of 0.35 to 1 mm. The powdered mulberry was stored in airtight, sealable bags and refrigerated until further use. Meanwhile, citric acid solutions with a pH of 2 were prepared as extraction solvents.

For extraction, 5 grams of mulberry powder were mixed with the citric acid solution at a solid-to-liquid ratio of 1:20 (w/v). The extraction was performed at 80 °C for 90 minutes. Once the extraction was complete, the mixture was cooled to room temperature and filtered to obtain the pectin-rich filtrate. To precipitate the pectin, double the volume of 95% (v/v) ethanol was added to the filtrate, and the mixture was stored at 4 °C for 24 hours. The precipitated pectin was collected by filtration using filter paper. The wet pectin was washed twice with 70% (v/v) ethanol and dried in an oven at 50 °C until a constant weight was achieved. All extractions were carried out in duplicate to ensure consistency and reliability [9].

2.2.2 Preparation for Chlorophyll Extraction

Adapted from the study by [10], the leaves were collected from black mulberry trees in Bukit Katil, Malacca, and meticulously cleaned to remove contaminants. They were dried thoroughly at a constant temperature of 50 °C until completely dry. Following this, the dried leaves were finely ground using a mill and sieved through a 50-mesh screen to obtain a uniform powder.

Next, 10 grams of the finely powdered leaves were transferred into an Erlenmeyer flask, to which 90 mL of a 90% ethanol solution was added. The flask was placed on a heater stirrer, and the mixture was stirred with a magnetic stir bar for three hours to facilitate the extraction process.

After the extraction period, the mixture underwent centrifugation at 4000 RPM for 5 minutes, separating the supernatant containing the extracted components from the solid residue. The resulting supernatant was carefully decanted and stored at 4 °C for future use [10].

2.3 Encapsulation Of Chlorophyll and Carboxymethylcellulose

Initially, 100 mL of distilled water was agitated using magnetic stirring to dissolve the carboxymethylcellulose (CMC), as outlined in Table 1. Following this, the extracted chlorophyll, as specified in Table 1, was added to the mixture. To ensure complete dissolution of the blend, a high-speed Ultra-Turrax was employed. Finally, the

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solutions were subjected to sonication in an ultrasonic bath operating at 20 kHz with a power of 150 W for five minutes. This step marked the final stage in the microcapsule preparation process [10].

Table 1 Formulation of films

	F1	F2	F3	F4	F5
Pectin (g)	3	3	3	3	3
Ch (mL)	0	0	1	0	1
Xanthan gum (g)	0	0	0	0.4	0.4
CMC (g)	0	1	0	0	1

2.3.1 Preparation of Films

A stirrer was used to dissolve 3 grams of pectin powder in 100 mL of distilled water for film preparation. The prepared pectin solution was then combined with an encapsulated solution to create a viscous mixture containing both pectin and encapsulated chlorophyll. To ensure uniformity, the mixture was rapidly heated at a rate of 5 °C per minute on a hot plate maintained at a constant temperature of 85 °C, followed by the addition of varying amounts of xanthan gum, as specified in Table 1.

Subsequently, 3 mL of glycerol was added as a plasticizer. The mixture was then subjected to sonication in an ultrasonic bath for 2 hours at 50 °C to enhance homogenization. The resulting solutions were poured into 20 mL petri dishes and dried in an oven at 45 °C for 24 hours. After drying, the films were left at room temperature for an additional 48 hours to stabilize. The final composite films were prepared and photographed, as shown in Fig. 1, before being analysed for their properties.

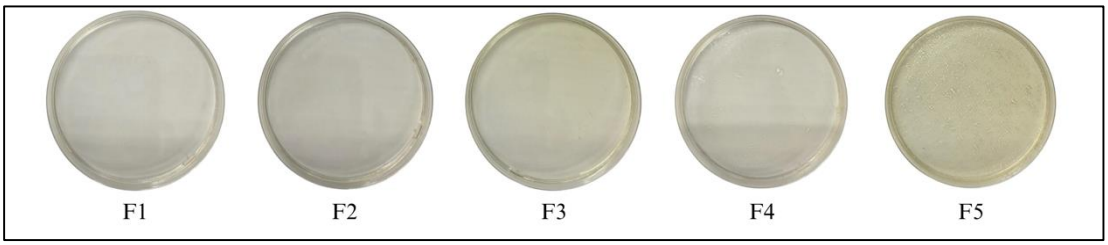

Fig. 1 Image of prepared films

2.3.2 Evaluation of Film Morphology

Scanning electron microscopy (SEM) (COXEM EM-30 Plus), operating at 20 kV, was employed to examine the films' morphology. The samples were cut into 5 mm x 5mm and mounted onto aluminium specimen stubs using double-sided sticky carbon tape, ensuring that the fractured surfaces faced upward. Finally, a thin layer of gold was sputter-coated onto the specimens to enhance conductivity, as described by [11]. The surface's image was taken at magnification of 200x and 1000x [11].

2.3.3 Evaluation of Functional Group of Composite Film

As described by [7], Fourier transform infrared (FTIR) spectroscopy was used to evaluate the functional groups and chemical structure of the edible films. The FTIR analysis was conducted at a resolution of 4 cm^{-1}, covering a wave number range from 4000 to 400 cm^{-1}.

The films were cut into small, uniform pieces with a diameter of approximately 2 cm to ensure consistency. The prepared samples were placed directly onto the sample holder of the FTIR spectrometer, where an Attenuated Total Reflectance (ATR) accessory was employed. This accessory facilitated direct contact between the sample and the ATR crystal, making it an ideal method for analysing solid films with minimal sample preparation. The analysis was performed within the 4000–400 cm^{-1} range to capture the characteristic vibrations of functional groups found in organic compounds, including those in edible films.

After the analysis, the sample was carefully removed from the ATR crystal, which was thoroughly cleaned using ethanol before and after each use to prevent cross-contamination, following the standard cleaning procedure provided by the FTIR manufacturer [7].

2.3.4 Evaluation of Tensile Strength

The mechanical properties of edible films were evaluated using a texture analyzer (Stable Micro System TA. XT Plus). Film samples were cut into rectangular dimensions of 4 × 8 cm to ensure uniformity. Each sample was then

clamped securely in the grips of the texture analyzer. A tensile load of 250 N was applied at a constant speed of 5 mm/min to assess the mechanical properties. The analysis was performed on three replicates from each formulation to ensure the accuracy and consistency of the results. Tensile strength (TS) values were recorded in megapascals (MPa), and extensibility was determined based on the elongation at rupture [12].

2.3.5 Evaluation of Moisture Content and Films' Solubility

Following the method outlined by Chen et al., moisture content (MC) and water solubility were evaluated [13]. Film samples (2 × 2 cm^2) were first weighed (initial weight, m_h) to a precision of ±0.0001 g and then dried in an air-circulating oven at 100 °C for 24 hours. After drying, the samples were weighed again to determine their initial dry matter (m_o), also with a precision of ±0.0001 g. The moisture content was calculated using equation (1).

$$\text{MC (\%)} = \frac{m_{h-}\, m_O}{m_h} \times 100 \quad (1)$$

where m_h represents the initial weight (g) of the samples, and m_o represents the weight (g) of the dry matter.

To determine water solubility, the dried samples were immersed in a beaker containing 30 mL of distilled water and kept in an environmental chamber at 25 °C for 24 hours. After immersion, the samples were carefully retrieved, rinsed with distilled water, and dried in an air-circulating oven at 100 °C until a constant weight (m_f) was achieved. The water solubility was calculated using equation (2).

$$\text{Solubility (\%)} = \frac{m_{o-}\, m_f}{m_o} \times 100 \quad (2)$$

where m_o is the weight (g) of the dry matter, and m_f is the weight (g) of the undissolved residue. Each type of film was tested in triplicate, and the average value was reported for both MC and solubility.

2.3.6 Determination of antimicrobial properties

The antibacterial properties of the films were evaluated using the agar diffusion method, following a modified procedure based on Isik et al. Petri dishes were labelled into five sections, each designated for one sample of each formulation. Müller-Hinton agar was poured into the Petri dishes and allowed to solidify. Using an aseptic technique, the plate cover was partially opened, and E. coli was spread evenly across the agar surface using a sterilized spreader.

A 0.6 cm diameter circular film piece was aseptically cut and placed on Müller-Hinton agar plates that had been inoculated with 0.1 mL of inoculum containing approximately 10^5 CFU/mL of *Escherichia coli* H7 and *Staphylococcus aureus*, using aseptic forceps. Each plate contained five pieces of sample. The plates were then incubated inverted at 37 °C for 24 hours. After incubation, the inhibitory zones were measured using a calliper to determine their radii, and the areas were subsequently recorded [13].

3. Results and Discussion

3.1 Scanning Electron Microscopy (SEM)

SEM imaging (Fig. 2) revealed distinct surface morphologies in the pectin-based edible films, illustrating the influence of incorporated additives on film microstructure. F1 exhibited a rough and heterogeneous surface, characteristic of brittleness and limited flexibility, consistent with the findings by [10]. In contrast, F2 showed a smoother and more homogeneous surface, likely due to interactions between CMC and pectin, which improve film cohesion and network strength. However, the presence of some irregular particles suggests potential incomplete CMC pasting [14].

F3 displayed a relatively consistent surface with small particles dispersed throughout, indicating partial compatibility between chlorophyll and pectin, though further optimization for improved integration may be needed. The surface of F4 was more uniform and continuous, reflecting enhanced mechanical integrity and flexibility, attributed to xanthan gum's ability to increase viscosity and structural cohesion. Finally, F5 exhibited the smoothest surface and most uniform texture, benefiting from the synergistic effects of multiple additives. This multi-component formulation resulted in a well-integrated polymer network, leading to improvements in both mechanical and potentially functional properties.

Overall, the SEM images suggest a progressive improvement in film structure with increasing component complexity, supporting the concept that multi-component systems, such as F5, offer superior properties for potential food packaging applications.

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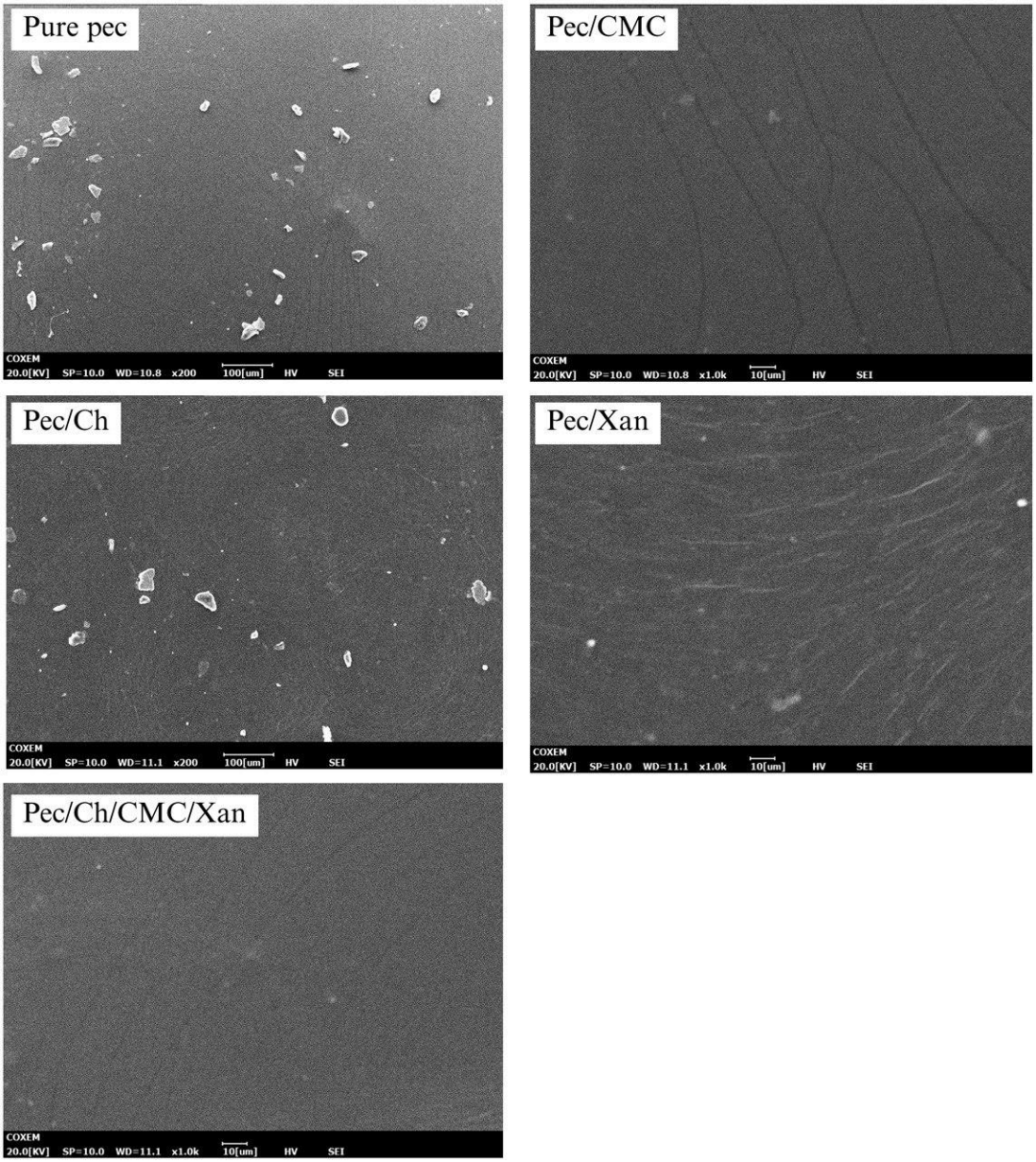

Fig. 2 SEM images

3.2 Fourier Transmission Infrared (FTIR)

The FTIR analysis of the edible film formulations, as shown in Fig. 3, provides significant insights into the chemical interactions between pectin and the added components, including xanthan gum, carboxymethyl cellulose (CMC), and chlorophyll. A broad peak observed around 3200–3400 cm^{-1} corresponds to O-H stretching vibrations, indicating the presence of hydrogen bonding. This is a characteristic feature of polysaccharides such as pectin, xanthan gum, and CMC. The variations in peak intensity across formulations suggest differing levels of hydrogen bonding interactions, with formulation F5 displaying the strongest bonding. This enhanced bonding can be attributed to the combined effect of all additives, resulting in a more cohesive polymer network.

The strong absorption band at 1700–1750 cm^{-1} corresponds to the carbonyl (C=O) group, indicative of ester linkages in the pectin matrix. F5 shows increased intensity in this region, suggesting stronger interactions and a denser matrix due to the combined incorporation of xanthan gum and CMC. Peaks at 1200–1400 cm^{-1} represent the carboxylate (C-O) stretching vibrations, confirming the presence of pectin's carboxylate groups. This region shows increased intensity in F2 and F5, highlighting the role of CMC in enhancing the density of carboxylate groups, contributing to improved mechanical strength [15].

For formulations F3 and F5, peaks near 1500 cm^{-1} are attributed to chlorophyll, representing its aromatic and ester functional groups, which confirm its successful incorporation into the films. Additionally, the fingerprint region between 800–1200 cm^{-1} displays peaks associated with glycosidic linkages and polysaccharide ring

vibrations, demonstrating the structural integrity of pectin and the successful blending of polysaccharides such as xanthan gum and CMC.

These findings align with previous studies by [16] and [17], who reported similar spectral characteristics for polysaccharide-based edible films. Specifically, [16] identified the O-H and C-H stretching vibrations as markers of polysaccharide-based matrices, meanwhile [17] confirmed the role of ester and carboxylate groups in enhancing film mechanical properties. The observed chemical structures validate the functional properties of the films, such as improved tensile strength, flexibility, and antimicrobial potential.

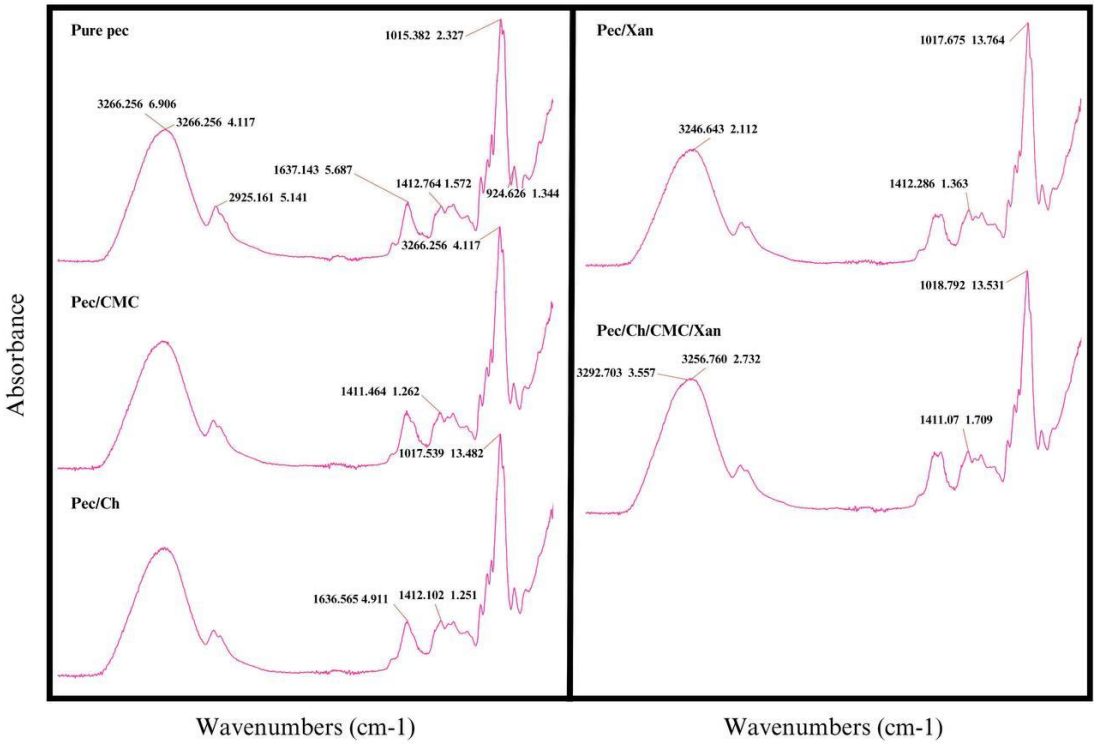

Fig. 3 FTIR result

3.3 Texture Analyzer

Results for the tensile strength and extensibility of the edible film formulations are shown in Table 2. Significant differences were observed, with formulation F5 exhibiting the highest average tensile strength (29.25 MPa) and extensibility (40.67 mm). This represents an increase of 1.27 MPa in tensile strength and 12.17 mm in extensibility compared to the next best formulation, F2. The synergistic effect of xanthan gum and CMC, as supported by [10], likely contributed to this enhancement by improving structural integrity and flexibility [10]. Chlorophyll likely further reinforced the pectin matrix, boosting tensile strength.

Table 2 *Effect of different formulation on mechanical properties*

Sample no	Tensile strength (MPa)			Average	Extensibility (mm)			Average
	1	2	3	(Mpa)	1	2	3	(mm)
F1	22.66	24.49	24.58	23.91	45.315	27.302	33.382	35.333
F2	26.58	29.52	27.84	27.98	35.396	25.482	25.529	28.802
F3	23.87	22.76	23.54	23.39	34.709	44.662	37.115	38.829
F4	20.48	18.98	22.43	20.63	37.075	37.816	39.061	37.984
F5	30.03	28.01	29.71	29.25	39.496	43.689	38.821	40.669

F2 (pectin and CMC) showed the second-highest tensile strength (27.98 MPa) but considerably lower extensibility (28.80 mm) than F5. Meanwhile, CMC improved strength, its impact on flexibility was less pronounced than the combination of xanthan gum and chlorophyll. This aligns with [20], who noted that CMC increases polymer matrix density and uniformity, thus enhancing tensile strength. This trend of increased tensile strength with CMC but decreased elongation is also consistent with [22] work on corn and pea starch films, as well as [11], who found increased tensile strength and elongation with pectin-CMC blends.

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Formulation F1 had moderate tensile strength (23.91 MPa) and higher extensibility (35.33 mm) than F2. Meanwhile, pure pectin films are generally brittle with limited strength, F1's higher extensibility compared to F2 may be due to the absence of additional structural modifiers that could otherwise limit flexibility [10].

Formulation F3 had similar tensile strength (23.39 MPa) to F1 but significantly higher extensibility (38.83 mm). This 3.5 mm increase in extensibility compared to F1 is likely due to the plasticizing effect of encapsulated chlorophyll, which reduces intermolecular forces within the matrix, similar to observations reported by [7].

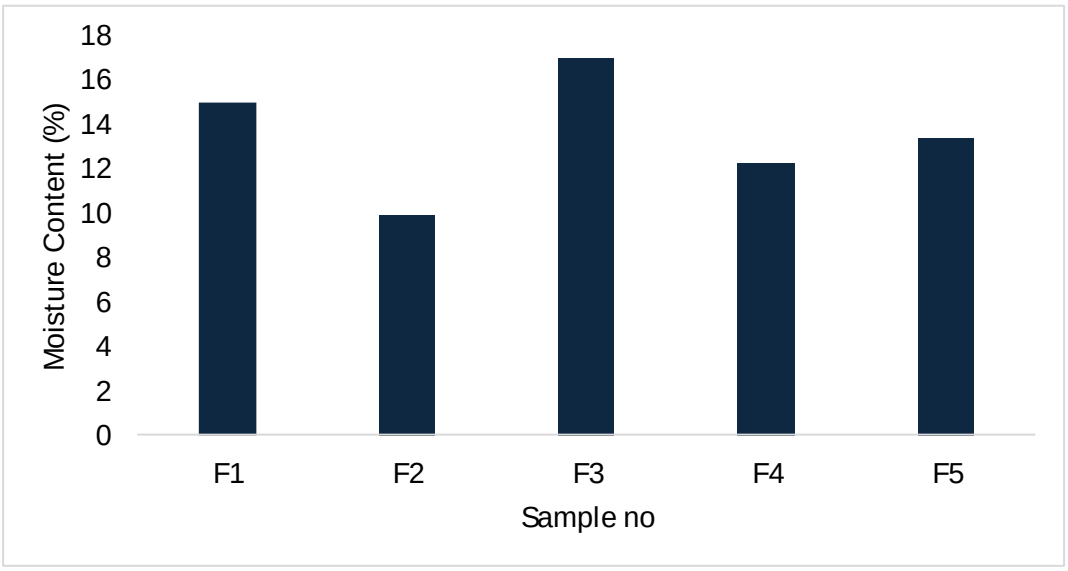
F4 exhibited the lowest tensile strength (20.63 MPa) but relatively good extensibility (37.98 mm). Its tensile strength was significantly lower than F5 (by 8.62 MPa) and F2 (by 7.35 MPa), while its extensibility was higher than F2's (by 9.18 mm). Meanwhile xanthan gum typically enhances flexibility, it may have disrupted the polymer network in F4, reducing tensile strength. This supports by [18], who highlighted xanthan gum's primary role in enhancing extensibility in pectin-based films. However, this contrasts with [23] findings, where xanthan gum increased tensile strength due to interactions between biopolymers and gelatine chain entanglement.

In conclusion, F5, combining xanthan gum, CMC, and chlorophyll, proved to be the best formulation, balancing high tensile strength and extensibility. This makes it promising for food packaging applications, consistent with the importance of these properties in edible films [10].

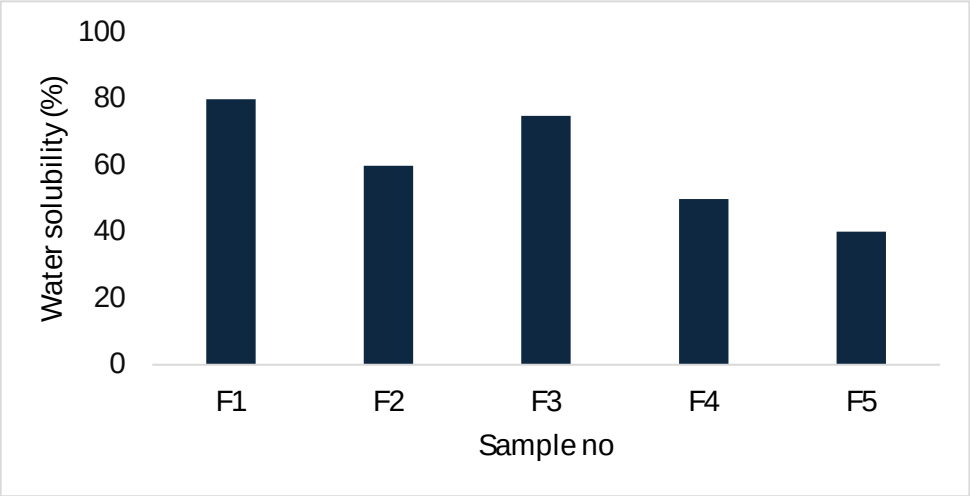
3.4 Moisture content and water solubility

Fig. 4(a) shows that moisture content varied among the film formulations. Formulation F3, comprising pectin and chlorophyll, exhibited the highest moisture content at 17.01%, followed by F1 (pure pectin) at 15.01%. Formulations F5 (pectin, CMC, xanthan gum, and chlorophyll), F4 (pectin and xanthan gum), and F2 (pectin and CMC) had lower moisture contents of 13.41%, 12.25%, and 9.94%, respectively. The difference in moisture content between F3 and F2 represents a decrease of 7.07%. The elevated moisture content observed in F3 suggests that chlorophyll may enhance water retention in pectin films due to its polar nature and its capacity to form hydrogen bonds with pectin. Conversely, the incorporation of CMC and xanthan gum in F2 and F4 appears to limit water retention, likely due to the formation of more structured networks. This observation is consistent with the findings of [8], who reported reduced moisture content in polysaccharide-based films incorporating gelling agents such as CMC and xanthan gum.

Fig. 4(b) shows that water solubility also varied across the formulations. F1 exhibited the highest solubility at 79.93%, followed by F3 at 74.95%. The addition of CMC and xanthan gum resulted in a marked decrease in solubility: F2 at 59.93%, F4 at 49.91%, and F5 at 40.04%. The difference in solubility between F1 and F5 is 39.89%. The inherent water solubility of pectin likely accounts for the higher solubility observed in F1 and F3. The slight reduction in solubility in F3 compared to F1 may be attributed to interactions between chlorophyll and the pectin matrix. The significantly lower solubility observed in formulations containing CMC and xanthan gum (F2, F4, and F5) is likely due to the rigid network structure formed by these gelling agents, which impedes dissolution. This observation aligns with the work by [19], who reported a decrease in water solubility in polysaccharide-based films upon the addition of gelling agents such as CMC and xanthan gum. Xanthan gum, in particular, contributes significantly to this reduced solubility due to its high molecular weight and gel-forming properties.



(a)



(b)

Fig. 4 Figure shown (a) Percentage of moisture content (b) Percentage of water solubility

In summary, the moisture content and water solubility results are consistent with previous research, demonstrating the influence of additives like chlorophyll, CMC, and xanthan gum on film properties. While pectin-based films exhibit higher moisture content and solubility, the addition of gelling agents like CMC and xanthan gum balances these properties by improving mechanical stability and reducing water retention and solubility.

3.5 Antimicrobial properties

Table 3 presents the antimicrobial activity of edible films against *Escherichia coli* and *Staphylococcus aureus* using the agar diffusion method. Among the formulations, F3 demonstrated the strongest antimicrobial activity, with inhibition zones of 3.6 mm against *E. coli* and 5.6 mm against *S. aureus*. This suggests that the combination of pectin and chlorophyll in F3 effectively disrupts microbial cell membranes, as supported by previous studies highlighting chlorophyll's antimicrobial properties. In comparison, F5 also exhibited notable antimicrobial activity, with inhibition zones of 3.3 mm against *E. coli* and 5.2 mm against *S. aureus*, attributed to the synergistic effects of chlorophyll, xanthan gum, and CMC, which enhance both the mechanical properties and antimicrobial release of the film, as observed by [7].

Table 3 Inhibition effect of films against Escherichia coli and Staphylococcus aureus

Sample no	Diameter of Zone Inhibition (mm)	
	Escherichia coli	Staphylococcus aureus
F1	1.3	1.9
F2	2.3	2.8
F3	3.6	5.6
F4	1.0	1.4
F5	3.3	5.2

In contrast, F2 displayed moderate antimicrobial activity, with inhibition zones of 2.3 mm against *E. coli* and 2.8 mm against *S. aureus*, likely due to CMC modifying the film structure to reduce microbial growth. However, the activity was lower compared to F3 and F5, indicating the importance of chlorophyll and xanthan gum. F1 and F4 exhibited the smallest inhibition zones, with F1 showing 1.3 mm and 1.9 mm against the respective bacteria, and F4 showing 1.0 mm and 1.4 mm, suggesting that pectin or pectin-xanthan gum combinations offer limited antimicrobial effects. These findings align with research indicating that pectin-based films typically show weak antimicrobial activity [21]. Overall, the results emphasize that chlorophyll significantly enhances the antimicrobial properties of edible films, particularly when combined with xanthan gum and CMC, confirming the potential of chlorophyll-enriched films as effective antimicrobial agents [10].

4. Conclusion

The results demonstrated that the choice of film-forming components significantly influenced the films' properties. SEM analysis revealed variations in surface morphology, which were dependent on the type and combination of additives used. FTIR analysis confirmed the presence of functional groups and highlighted interactions between the film-forming components, contributing to the films' overall structural integrity. Tensile strength testing showed that films with added CMC and xanthan gum exhibited enhanced flexibility and durability, indicating their suitability for food packaging. Water solubility test highlighted the films' potential biodegradability, with solubility rates varying based on the formulation. Furthermore, films containing encapsulated chlorophyll displayed promising antibacterial activity, suggesting their applicability in extending the shelf life of packaged food products. Based on the findings of this study, it is recommended that further research explore the optimization of the pectin extraction process from local black mulberry pulp to maximize yield and enhance its properties for edible film production. Additionally, further studies could explore the long-term stability and shelf-life of the films under various environmental conditions to assess their practical application for food preservation.

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Conflict of Interest

Authors declare that there is no conflict of interest regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: ***study conception and design:*** *Madihah Nabilah Abdul Samad, Hatijah Basri;* ***data collection:*** *Madihah Nabilah Abdul Samad;* ***analysis and interpretation of results:*** *Madihah Nabilah Abdul Samad;* ***draft manuscript preparation:*** *Madihah Nabilah Abdul Samad, Hatijah Basri. All authors reviewed the results and approved the final version of the manuscript.*

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