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Utilization of Microcrystalline Cellulose Derived from Sugarcane Bagasse (Saccharum officinarum) in Gelatin-Based Edible Film Extracted from Bovine Hide

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Utilization of Microcrystalline Cellulose Derived from Sugarcane Bagasse (*Saccharum officinarum*) in Gelatin-Based Edible Film Extracted from Bovine Hide

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ABSTRACT

Gelatin is known as a good film forming material but has weaknesses in its mechanical properties, thus requiring reinforcement such as microcrystalline cellulose (MCC). The study was conducted in several stages: gelatin extraction from bovine hide, MCC production from sugarcane bagasse, and formulation of edible film with gelatin to MCC ratios of 100:0; 95:5; 90:10; 85:15; 80:20 and 75:25 (%w/w). The results showed that the gelatin produced had a yield of 9.48% and a protein content of 89.36%, while the MCC had a yield of 32.96%, cellulose content of 96%, crystallinity index of 78.3%, and an average particle size of 0.3933 μm. The edible film was tested for tensile strength, elongation at break, water vapor permeability, resistance to water and oil at different temperatures, and was morphologically analyzed and characterized for functional groups using SEM and FT-IR, respectively. The best ratio was obtained with the 15% MCC addition, resulting in a tensile strength of 2.074 MPa, elongation at break of 73.33%, and water vapor permeability of 5.59×10^{-8} g/s·m². The edible film demonstrated faster dissolution at higher temperatures, but remained resistant to water and oil under normal conditions. The films exhibited functional groups corresponding to amide A (N-H), amide I (C=O), amide II (N-H), and amide III (N-H). The surface of the edible film was smoother and more homogeneous at a gelatin to MCC ratio of 85:15 (%w/w). In conclusion, the addition of MCC significantly improved mechanical properties and durability of the edible film at the formulation of 85:15 (%w/w).

Keywords *: Edible film, gelatin, bovine hide, microcrystalline cellulose, sugarcane bagasse*

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1. INTRODUCTION

Edible film is a thin layer of material used to wrap or coat food and is edible. As a type of food packaging, edible film is gaining popularity due to its wide range of benefits, particularly in terms of iproving the practicality of food consumption, enhaning safety, and reducing packaging waste. Future Market Insights reports that this commodity is projected to grow at a compound annual growth rate (CAGR) of 7.60% between 2024 and 2034, with a market value of USD 389.4 million in 2024 to USD 810.1 million in 2034 [1]. The benefits of edible film include providing a more sustainable and practical alternative to conventional non-edible packaging materials while reducing environmental impact and waste. One biopolymer-based raw material used for edible film, in addition to starch and cellulose, is gelatin. Gelatin is a hydrocolloid derived from animal collagen tissues through extraction and hydrolysis processes [2]. Gelatin dissolves in hot water and forms into a gel upon cooling. Currently, the raw materials used to make gelatin are 44% pork skin, 28% beef hide, 27% bone, and 1% other sources [3].

Bovine hide is a potential source of gelatin as a raw material for edible film. The bovine population in Indonesia is projected to reach 11.75 million in 2024, compared to 10.83 million in 2023 [4]. This indicates that the bovine population has increased by approximately 8.5% within one year. Raw bovine hide contains 60-65% water, 30-33% protein, 2-3% fat, and 0.5-1% other compounds [5]. Bovine hide possesses several advantages over hides from other animals, including flexibility, toughness, and a desirable texture [6]. Currently, the use of bovine hide is limited to the food sector, and product diversification remains limited despite its high production volum. The protein content in gelatin makes bovine hide a potential raw material for edible films.

However, gelatin has limitations in its mechanical properties and susceptible to microbial contamination, thereby requiring reinforcement such as microcrystalline cellulose (MCC). This study used microcrystalline cellulose obtained through the hydrolysis of cellulose derived from sugarcane bagasse. As a filler, microcrystalline cellulose, due to its large surface area, can directly improve the material's mechanical properties. The use of microcrystalline cellulose as a filler in edible films has been reported by several researchers [7, 8]. Furthermore, the addition of glycerol as a plasticizer also affects the physical and mechanical properties of the resulting edible film, as glycerol can reduce intermolecular hydrogen bonds and increase the intermolecular distance, which reduces the stiffness of the resulting edible film.

Gelatin from bovine hide has great potential in edible film production. However, it has limitations in mechanical properties and is susceptible to microbial degradation. Therefore, microcrystalline cellulose obtained from sugarcane bagasse was required as a filler. Furthermore, the addition of glycerol as a plasticizer also determines the properties of the resulting edible film. Therefore, the ratio of gelatin to microcrystalline cellulose and the amount of glycerol used will significantly determine the characteristics, physical properties, mechanical properties, and microbial resistance of the edible film [9].

The gelatin extraction process from bovine hide was carried out using water as a solvent. Meanwhile, microcrystalline cellulose was isolated from bagasse using a hydrolysis process with 20% acetic acid. The resulting gelatin was then characterized for its functional groups, while the crystallinity index of microcrystalline cellulose was determined. Edible films were made by mixing gelatin and microcrystalline cellulose and adding glycerol as a plasticizer. Variations in the ratio of gelatin to microcrystalline cellulose ratio and the glycerol concentration were applied to produce the edible film. The edible film was then characterized and tested to determine the best ratio using parameters such as functional groups, morphology, tensile strength and elongation, water and oil solubility, water vapor permeability, and microbial activity.

The use of gelatin as a raw material for edible film production has previously been reported, namely an edible film made from tilapia fish bones with the addition of antimicrobial mangosteen peel extract [10]. Several studies have also been reported on the use of gelatin as an edible film sourced from various natural materials, such as duck bones [11], beef bones [12], salmon bones [13], and broom fish bones [14]. Several modifications have been made to improve the properties of the resulting edible film, such as the addition of chitosan as a filler [15, 16, 17]. In this study, the modification was carried out by adding microcrystalline cellulose as a filler. Microcrystalline cellulose is partially depolymerized cellulose derived from α-cellulose through hydrolysis.

Microcrystalline cellulose is a white, odorless and tasteless powder that is insoluble in water and most organic solvents and has a relatively high surface area, making it a potential reinforcing agent in edible films [18]. Previous studies have shown the characteristics of microcrystalline cellulose and also proven its ability to improve the mechanical properties of bioplastic materials [19, 20]. Microcrystalline cellulose is obtained by isolating cellulose from sugarcane bagasse,

containing 35.2 ± 0.9% cellulose, 24.5 ± 0.6% hemicellulose, 22.2 ± 0.1% lignin, and 20.9 ± 4.3% ash [21]. Previous research [22] reports that microcrystalline cellulose can also play a role in reducing microbial activity in materials. Its dense and compact structure can limit air exchange and reduce the availability of water and nutrients for microbial growth. Several previous studies have been published who added natural extracts as antimicrobials, but this addition reduced the mechanical properties of the resulting bioplastic [23, 24]. The innovation implemented in this research was the combination of bovine hide gelatin with bagasse-derived microcrystalline cellulose as a filler and antimicrobial agent, along with glycerol as a plasticizer, to obtain an edible film with characteristics and that meet JIS 1975 standards.

2. MATERIALS AND METHOD

2.1 Materials

Sugarcane bagasse and bovine hide were obtained from a local market. Glycerol, aquadest, HCl 3%, H_2SO_4 20%, and NaOH 17.5% were obtained from a chemical supplier. The instruments utilized in this study included Erlenmeyer flasks, beakers, a chopper, a hot plate, Fourier Transform Infra-red (FTIR), X-ray Diffraction (XRD), and Scanning Electron Microscopy (SEM).

2.2 Preparation and production process of edible film

2.2.1 The extraction of gelatin from bovine hide

Preparation of raw materials (Figure 1) began with cleaning the bovine hide from its fat and hair through a burning process, followed by removing the hair using a knife. This stage aims to reduce excess fat content and remove dirt adhering to the bovine hide so as not to interfere with subsequent processes. The bovine hide was then cut into 3–4 cm pieces, washed with distilled water until clean, and weight to 1,500 g. Next, the bovine hide was soaked in a 3% HCl solution for 12 hours. After soaking, the bovine hide was washed with running water until it reached a pH 4–5. The gelatin extraction process was carried out by adding distilled water (1,000 mL) and treating it with ultrasound at a temperature (70 ºC, 5 h). The extract was filtered with a filter cloth, then frozen in a refrigerator. The frozen extract was then dried in an oven (72 h). The dry product, in the form of pieces, was ground using a blender to obtain gelatin powder.

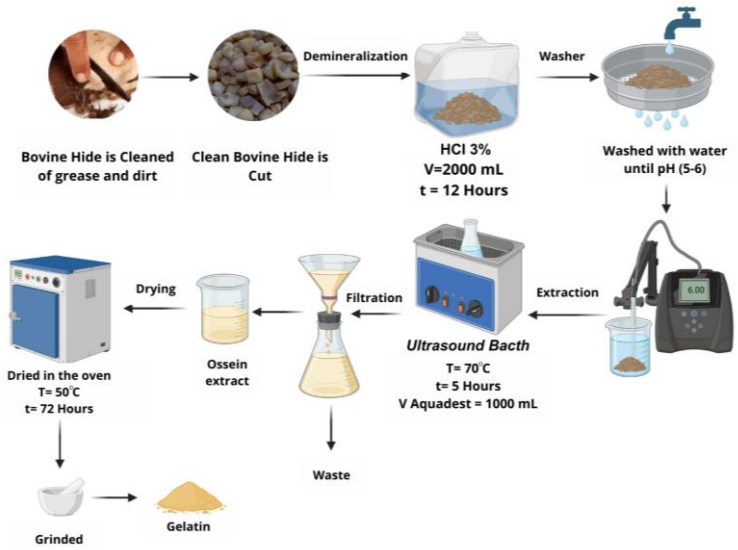


Figure 1. Illustration of extraction gelatin from bovine hide

2.2.2. Preparation microcrystalline cellulose (MCC)

Preparation of MCC (Figure 2), sugarcane bagasse was sun-dried, cut into small pieces, and ground into powder. A total of 100 g of the powder was hydrolysed with 1,000 mL of 3.5% nitric acid (90 ºC, 2 h). The mixture was then washed and filtered. The bleaching stage was carried out using 750 mL of NaOCl (3.5%) at boiling temperature for 10 minutes, followed by alkalisation treatment using 1,000 mL of NaOH (17.5%) at 80 ºC for 30 minutes. The sample was then bleached again with 750 mL of 3.5% NaOCl at 100 ºC for 5 minutes, washed until neutral pH was achieved, and dried at 60 °C. The dried powder was further hydrolyzed with 20% sulfuric acid (1:25 w/v) for 1 h at 45 °C. The process was terminated by adding distilled water at ten times the reaction volume. The suspension was centrifuged at 9,000 rpm for 15 min and washed repeatedly with distilled water until neutral pH was reached. Finally, the obtained cellulose microcrystals were dried at 50 °C.

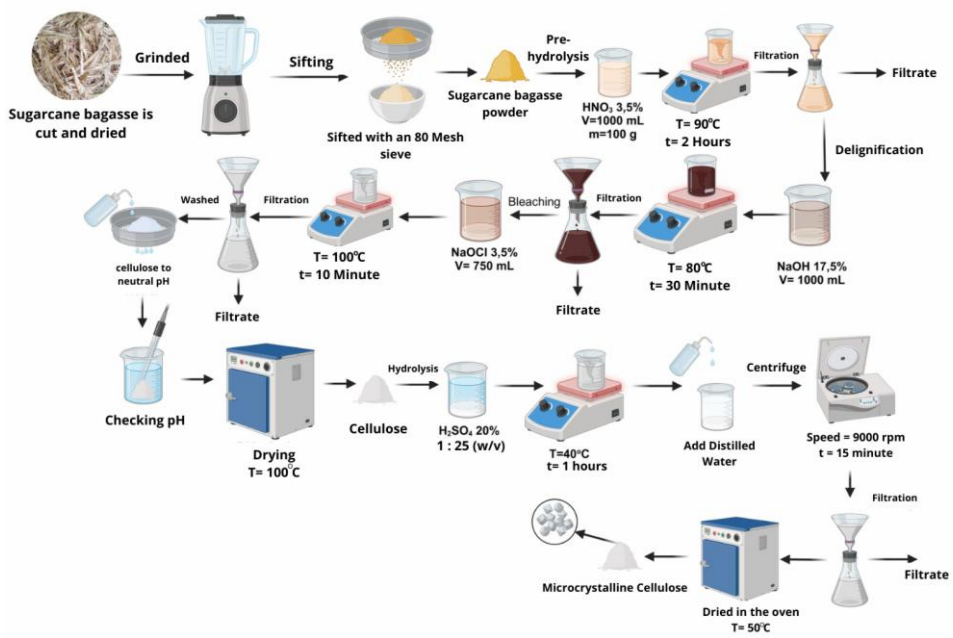


Figure 2. Illustration of extraction microcrystalline cellulose from sugarcane bagasse

2.2.3 Production of edible film

Using a magnetic stirrer set to 300 rpm, the gelatin was dissolved in 100 mL of distilled water until the temperature reached 60 °C. Then, 1% glycerol was added and the solution was stirred for 5 min until a homogeneous mixture was obtained. The solution was then poured into a silicone mold and dried at room temperature for 24 h. The mechanical characteristics of the edible film were then evaluated. Several experimental formulations (Table 1) were prepared using the same procedure (Figure 3).

Table 1. Preparation of edible film with variations of gelatin and MCC

Variations	Gelatin (%w)	MCC (%w)	Aquadest (mL)	Glycerol (mL)
1	100	0	100	1
2	95	5		
3	90	10		
4	85	15		
5	80	20		
6	75	25		

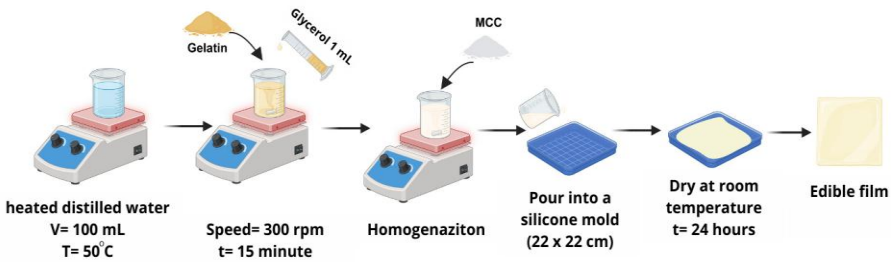


Figure 3. Process of making edible film

2.3 Characterization of gelatin

2.3.1 Yield of gelatin

Yield was calculated as the ratio between the weight of the gelatin solid produced and the weight of fresh bovine hide. All experiments were performed in triplicate and data are presented as mean ± standard deviation. Yield was calculated using equation 1:

$$\text{Yield} = \frac{\text{Weight of gelatin solids}}{\text{bovine hide weight}} \times 100\% \qquad (1)$$

2.3.2 Gelatin functional groups of bovine hide

A Shimadzu IR Prestige-21 FTIR spectrometer was used to analyze the functional groups utilizing the total attenuated total reflectance (ATR) method in the wavenumber range of 650–4,000 cm^{-1} with a resolution of 4 cm^{-1}.

2.3.3 Gelatin protein content from bovine hide using the Kjeldahl method (SNI 01-2891-1992)

The protein content was determined using the Kjeldahl method, which consists of three main steps: destruction (digestion), distillation, and titration. A 0.5 g gelatin sample was weighed and combined with 0.5 g of selenium and 3 mL of concentrated H_2SO_4 in a 100 ml Kjeldahl flask. The mixture was digested at 410 ºC until the solution became clear. The solution was then chilled. Following cooling, the Kjeldahl flask was filled with 50 mL of distilled water and 20 mL of 40% NaOH. Distillation is carried out at 100 ºC. The distillate was collected in a 125 ml Erlenmeyer flask containing 10 mL of 2% boric acid (H_3BO_3) and two drops of methyl red indicator. The distillation process was stopped when the distillate's volume reached 40 mL and turned blue-green. Then distillate was then titrated with 0.1 N HCl until the color changed to pink. The protein content was calculated using the equation:

$$(\%N) = \frac{(\text{mL HCl - mL Blank}) \times 14.007 \times \text{N HCl}}{\text{weight}} \times 100\ \% \qquad (2)$$

$$\%\text{Protein} = \%N \times 6.25 \qquad (3)$$

2.4 Characterization of microcrystalline cellulose (MCC)

2.4.1 Yield of MCC

The yield was calculated as the ratio of the weight of microcrystalline cellulose produced to the weight of the initial raw material. All experiments were performed in triplicate and data are presented as mean ± standard deviation. The yield was calculated using the following equation:

$$\text{Yield} = \frac{\text{Weight of MCC}}{\text{weight of sugarcane baggasse}} \times 100\% \quad (4)$$

2.4.2 MCC function groups from sugarcane bagasse

The functional groups of MCC were analyzed using a Shimadzu IR Prestige-21 to FTIR spectrometer with the ATR method in the wavenumber range of 500–4000 cm^{-1} at a resolution of 4 cm^{-1}.

2.4.3 Index crystallinity of MCC

The MCC crystal pattern was obtained using a Shimadzu Maximaa XRD-7000 X-ray diffractometer with a scanning rate of 1°/min. The degree of crystallinity was calculated as the crystallinity index (CrI).

$$\text{CrI} = \frac{I_{200}-I_{am}}{I_{200}} \times 100\% \quad (5)$$

2.4.4 Particle size analysis of MCC

MCC samples were dispersed in aquadest and sonicated to form a suspension before being analyzed using a laser particle size analyzer (Litesizer 500, Anton Paar, Austria) to determine the particle size distribution.

2.4.5 Cellulose content of MCC

A 1 g sample was refluxed with 150 mL of distilled water at 100 °C for 2 h. The sample was then vacuum-filtered, washed until the filtrate reached 300 mL, and the residue was dried at 100 °C for 12 h until a constant weight was achieved. The residue was then refluxed with 150 mL of 0.5 M H_2SO_4 at 100 °C for 2h, dried, and weighed. Subsequently, the sample was soaked in 10 mL of 72% H_2SO_4 at room temperature for 4 h. Afterwards, 270 mL of distilled water was added to dilute the solution to 0.5 M H_2SO_4, and the mixture was refluxed again hours at 100 °C for 2 h. The residue was then dried and weighed until a constant weight was obtained.

The cellulose content was calculated using Equations 6-8:

$$\text{Hemicellulose content} = \frac{\text{weight b - weight c}}{\text{weight a}} \times 100\% \quad (6)$$

$$\text{Cellulose content} = \frac{\text{weight c - weight d}}{\text{weight a}} \times 100\% \quad (7)$$

$$\text{Lignin content} = \frac{\text{weight d}}{\text{weight a}} \times 100\% \quad (8)$$

Where:

a : Initial oven-dried weight of sample

b : Oven-dried weight of the sample residue after refluxing with hot water

c : Oven-dried weight of the sample residue after refluxing with 0.5 M H_2SO_4

d : Oven-dried weight of the sample residue after refluxing with 72% H_2SO_4 followed by dilution to 0.5 M H_2SO_4

2.5 Characterization of edible film

2.5.1 Tensile properties of edible film

The tensile strength and elongation at break were analyzed by cutting the edible film into 5 cm × 2 cm strips with a thickness of 0.01 mm. The sample was then clamped onto a Universal Testing Machine (UTM RTF 1350), and a tensile load was applied until the film fractured. All experiments were performed in triplicate and data are presented as mean ± standard deviation.

2.5.2 Water vapor permeability of edible film

The water vapor transmission rate was determined using a modified wet cup method based on ASTM E 96-95. Petri dishes were fill with 30 mL of distilled water and covered with aluminum foil perforated with holes corresponding to 10% of the dish diameter. The dishes were weighed, then placed in the oven at 37 ºC. The weight was recorded every hour for 5 h. All experiments were performed in triplicate and data are presented as mean ± standard deviation. The WVTR and WVP were calculated using:

$$WVTR = W/(t \times L) \quad (9)$$

$$WVP = WVTR/(s \times (R_1 - R_2)) \times d \quad (10)$$

Where WVTR is water vapour transmission rate ($g/s{\cdot}m^2$), t is time (s), and L is the film area (m^2)WVP is the water vapour permeability ($g{\cdot}mm/m^2{\cdot}s{\cdot}Pa$), s is the saturated vapor pressure at 37 ºC (Pa), R_1 is the relative humidity of 100%, R_2 is the relative humidity of 81%, and d is the film thickness (mm).

2.5.3 Water temperature resistance time of edible film

The solubility time was deterimined by cutting a 2 cm × 4 cm stips (0.01 mm thick), and soaking it in 50 mL of distilled water at temperatures of 30 ºC, 50 ºC, and 80 ºC. Thetime required for the film to dissolve at each temperature was recorded. All experiments were performed in triplicate and the data are presented as mean ± standard deviation.

2.5.4 Oil temperature resistance time of edible film

The oil resistance time was evaluated by cutting the film into 2 cm × 4 cm strips (0.01 mm thick), then soaking it in 50 mL of oil at temperatures of 30 ºC, 50 ºC, and 80 ºC. The time required for the film to dissolve at each temperature was recorded. All experiments were performed in triplicate and data are presented as mean ± standard deviation

2.5.5 Morphology of edible film

The cross-sectional morphology of the edible film was observed using a Thermo Scientific Prisma E scanning electron microscope (SEM). The cryo-fractured film samples were coated with gold prior to analysis. The micrograph were obtained at 1000× magnification and an accelerating voltage of 10 kV.

3. RESULTS AND DISCUSSIONS

3.1 Characteristics of Gelatin From Bovine Hide

3.1.1 Yield Gelatin

Gelatin production involved the pre-treatment of bovine hide using 3% HCl, followed by soaking for 12 h, which resulted in a gelatin yield of 9.66%. These results indicate that acid hydrolysis of collagen from bovine hide disrupts the helical bonds in the matrix through the action of hydrogen ions. According to Zhang et al. [25], the addition of HCl can effectively break down cross-linked structures, especially when the concentration is sufficient. This break down causes the collagen chains to degrades into smaller peptides, reducing collagen molecular weight, and reducing the amount of hydroxyproline, which are critical for preserving collagen structure and influencing gelaitn yield.

3.1.2 Functional Group of Gelatin

The FT-IR spectrum of gelatin extracted from bovine hide can be seen in Figure 4 and Table 2. Four major amide regions, amide A, amide I, amide II, and amide III, were clearly observed in the spectrum, each representing a specific vibrational mode within the gelatin molecular structure.

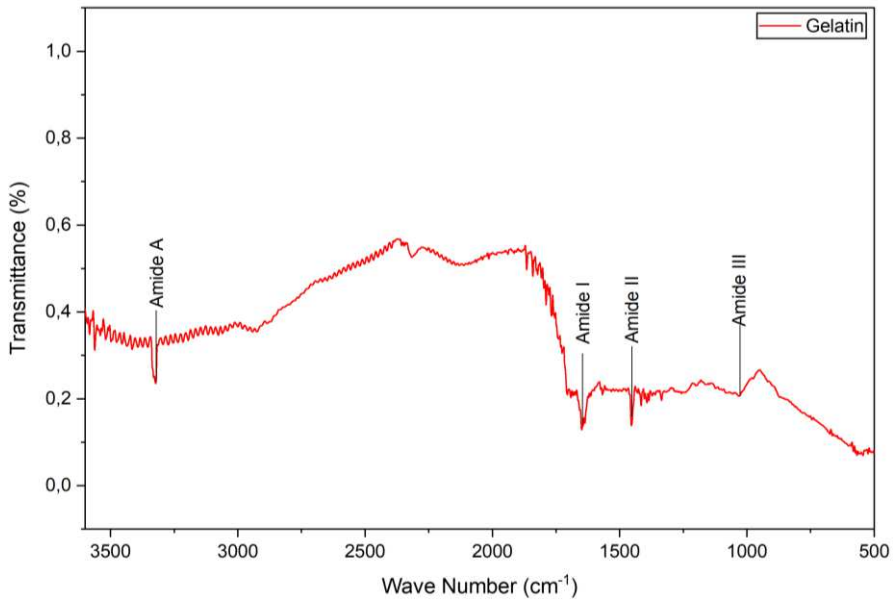


Figure 4. Spectrum of gelatin from bovine hide

Wavenumber of 3316.64 cm^{-1}, amide A, shows the N–H stretching vibrations and indicates the presence of hydrogen bonding within the protein matrix. Amide A is essential to maintain the strength of hydrogen bonds and typically appears in the range of 3600–2300 cm^{-1}. The position of this band in the extracted gelatin suggests that the acid-based hydrolysis treatment did not completely disrupt the hydrogen-bonding network derived from collagen.

Wavenumber of 1637.56 cm^{-1}, amide I, shows the C=O stretching vibrations of the peptide linkage (–CONH–). Amide I is widely recognized as the most important factor for identifying protein secondary structures, particularly the residual of collagen's triple-helix structure. Amide I band indicates that the extraction process partially denatured the original collagen structure, intact essential, and confirming successful conversion into gelatin. The position of this band typically appears in the range of 1661–1636 cm^{-1}. Wavenumber of 1454.33 cm^{-1}, amide II, corresponds to N–H bending and C–N stretching modes. This band reflects the presence of peptide linkages and provides additional evidence of the proteinaceous nature of the extracted material. The peak falls

within the reference range of 1560–1335 cm^{-1}, aligning well with previously reported spectra for gelatin, which indicates that the hydrolysis process largely preserved the primary chain structure of the protein. Additionally, the amide III band, appearing at 1031.29 cm^{-1}, represents a combination of C–N stretching and N–H deformation vibrations. This band is typically associated with the presence of glycine-rich sequences, which dominate the gelatin backbone. The appearance of this peak within the expected range (1240–670 cm^{-1}) confirms the retention of characteristic gelatin features and supports the identification of the product as denatured collagen.

Table 2. Functional groups of gelatin

Functional Group		Wave Number (cm^{-1})	Wave Number Reference (cm^{-1}) [13]
Amide A	O-H (Amide)	3316.64	3600-2300
Amide I	C=O (Carbonyl)	1637.56	1661-1636
Amide II	N-H (Amide)	1454.33	1560-1335
Amide III	N-H (Amide)	1031.29	1240-670

Figure 5 shows the chemical structure of gelatin derived from bovine hide. In the illustration, gelatin is showned as a single polypeptide chain or multiple irregular chains, reflecting the loss of the ordered macromolecular structure originally present in collagen. This denaturation allows gelatin to dissolve in water and form a gel, while also providing excellent film-forming properties. The main functional groups present in the structure includes amide groups (–CONH–), carbonyl groups (C=O) from peptide bonds, N–H groups, and –OH groups from hydroxyproline.

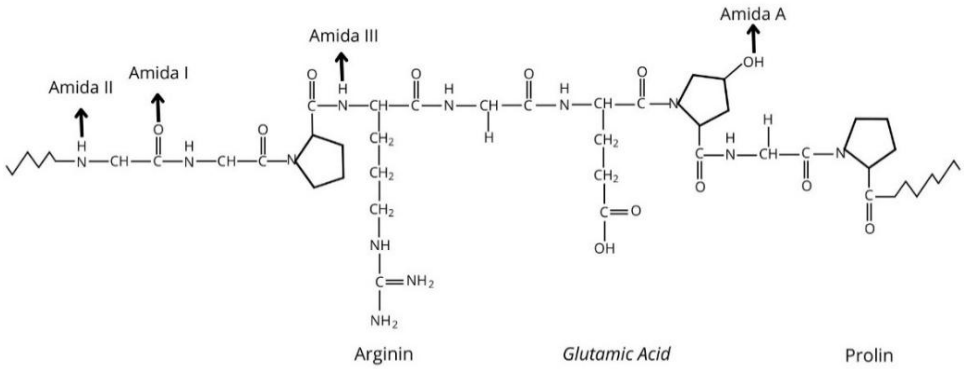


Figure 5. Chemical Structure of gelatin [26]

The FT-IR analysis demonstrated that the gelatin extracted from bovine hide contained all major amide groups typical of protein-based materials. The presence and proper positioning of these functional groups indicated that the extraction method effectively produced gelatin with structural characteristics comparable to commercial and literature-reported gelatin. These findings are consistent with previous reports stating that acid hydrolysis breaks down the triple-helix collagen structure while maintaining the integrity of peptide linkages essential for forming functional gelatin. [27].

3.1.3 Protein Content of Gelatin

The protein content of gelatin extracted from bovine hide was determined using the Kjeldahl method, yielding a value of 89.36%. This result met the Indonesian National Standard for gelatin (SNI 02-3735-1995), which requires a minimum protein content of 87.25%. This requirement is neccessary due to gelatin with a high protein content percentage contains large amount of amino acids forming long polypeptide chains and strong intermolecular protein bonds, resulting in greater water-binding capacity. The combination of appropriate soaking time and extraction time contributed to the production of gelatin with a high amino acid content. High quality gelatin should have high protein content with minimal ash, fat, and other contaminants [28].

3.2 Characterization of Microcrystalline Cellulose (MCC) From Sugarcane Bagasse

3.2.1 Yield MCC

The MCC yield was determined by comparing the weight of the produced MCC with the sugarcane bagasse. The yield obtained in this study was 32.96%. This value was slightly higher than that reported in [29] which was 32%. Acid pretreatment effectively removed hemicellulose content from sugarcane bagasse, as hemicellulose has a less complex structure compared to cellulose and lignin and alkaline treatment using NaOH facilitated the removal of lignin content from sugarcane bagasse [30].

3.2.2 Functional Groups of MCC

Figure 6 and Table 3 present the FTIR spectrum and functional group of MCC respectively. The FTIR spectrum of MCC derived from sugarcane bagasse exhibited characteristic absorption peaks of microcrystalline cellulose. Several distinctive cellulose peaks were observed, confirming the success of the delignification and purification processes in removing lignin and hemicellulose, resulting in high-purity MCC.

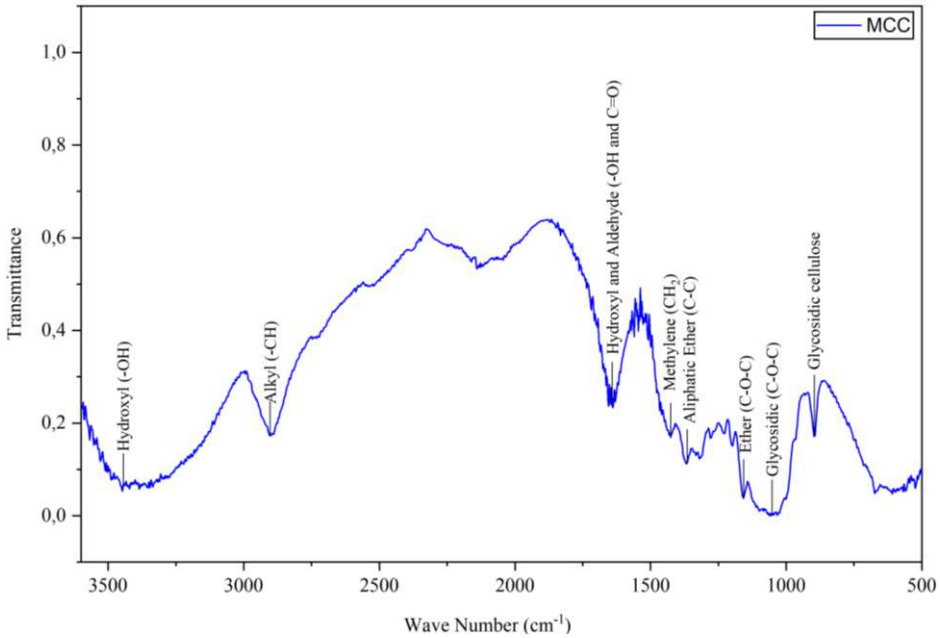


Figure 6. Spectrum of MCC from sugarcane bagasse

The broad peak in the range of 3300–3400 cm^{-1} corresponds to the stretching vibration of –OH groups, a typical feature of cellulose structures rich in hydrogen bonding. The absorption near 2900 cm^{-1} represents C–H stretching from alkyl groups in the glucose units. The absence of lignin-

specific peaks (around 1500–1600 cm^{-1}) and hemicellulose peaks (around 1730 cm^{-1}, C=O ester) confirms that non-cellulosic fractions were effectively removed during the bleaching process. The peaks at 1425 cm^{-1} and 1370 cm^{-1} correspond to CH_2 bending and C–H bending, both characteristic of the crystalline domains of cellulose. Additionally, the strong peak around 1050–1030 cm^{-1} indicates C–O–C stretching of glycosidic linkages, demonstrating that the fundamental cellulose structure remains intact. The peak at 896 cm^{-1} is indicative of β-glycosidic linkages (β-1,4), an important marker confirming that the obtained MCC retains the correct cellulose structure [30]. Overall, the spectrum verifies that MCC derived from sugarcane bagasse exhibits an FTIR profile consistent with standard microcrystalline cellulose, demonstrating that the isolation, bleaching, and acid hydrolysis processes successfully produced high-quality MCC.

Table 3. Functional Groups of MCC

Functional Group		Wave Number (cm^{-1})	Wave Number Refference (cm^{-1}) [31]
Hydroxyl	(-OH)	3446.79	3300-3500
Alkyl	(-CH)	2899.01	2890-2910
Hydroxyl	(-OH)	1637.56	1636-1649
Aldehyde	(C=O)	1637.56	1636-1649
Methylene (Crystalline)	($-CH_2$)	1427.32	1431-1427
Aliphatic	(C-C)	1369.46	1369-1371
Eter (cellulose)	(C-O-C)	1157.29	1155-1165
Glycoside	(C-O-C)	1053.15	1050-1060
Glycoside cellulose	β-1,4-glycosidic	896.90	896-898

The chemical structure of MCC from sugarcane bagasse (Figure 7) illustrates the chemical structure of microcrystalline cellulose (MCC) obtained through delignification, bleaching, and acid hydrolysis of sugarcane bagasse. This structure consists of repeating β-D-glucopyranose units linked by β-(1→4)-glycosidic bonds, forming the linear cellulose polymer chain.

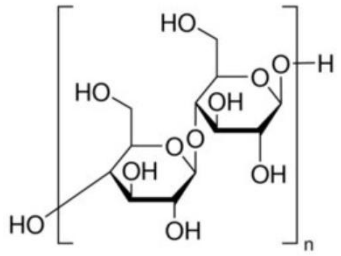


Figure 7. Chemical Structure of MCC from sugarcane bagasse [32]

Each glucose unit contains three hydroxyl (–OH) groups that play a crucial role in forming intra- and intermolecular hydrogen bonds. These hydrogen bonds contribute to the rigidity, ordered arrangement, and high crystallinity of MCC. The crystalline regions are responsible for its superior mechanical strength and thermal stability. The illustration also shows the absence of other functional groups, such as aromatic lignin structures or hemicellulose carbonyl groups. This indicates that the purification process successfully removed non-cellulosic components, resulting in highly crystalline, purified cellulose.

3.2.3 Index Crystallinity of MCC

Crystallinity is one of the parameters that indicates the microcrystalline cellulose produced. Generally, MCC used for industrial applications exhibits a crystallinity degree in the range of 55-85%. Figure 8 presents the XRD pattern of the produced MCC. There are three main peaks from the 2θ diffraction angle at 22.06°; 20.02°; and 43.94°, indicating the presence of cellulose I polymorph. These characteristic peaks confirms that the obtained MCC corresponds to cellulose I crystalline structure with a crystallinity index of 78.2%. The crystallinity index of MCC indicates the amount of crystalline phase in microcrystalline cellulose. A higher crystallinity index indicates a greater proportion of ordered crystalline domains. The obtained result is consistent with previously reported commercial MCC [30], where the crystallinity index of commercial MCC and sugarcane bagasse was comparable and met the standards of existing commercial MCC.

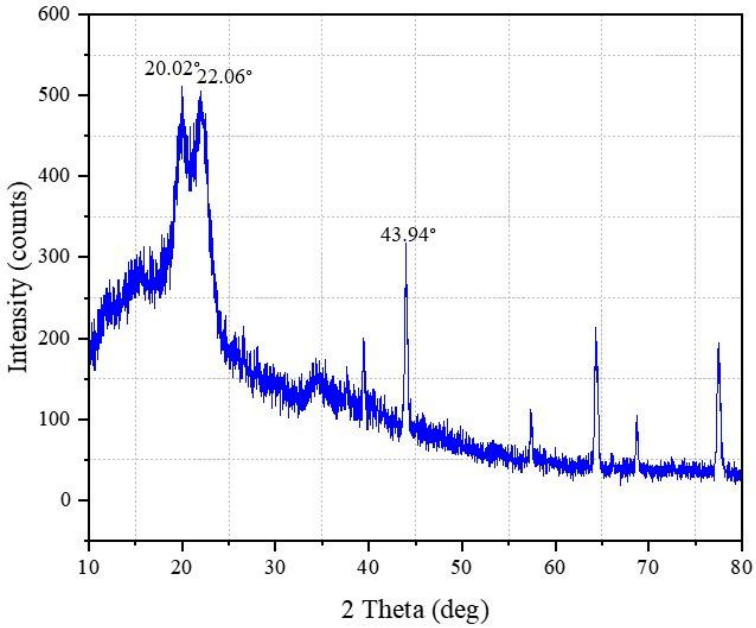


Figure 8. X-Ray Diffractogram of microcrystalline cellulose

3.2.4 Analysis Cellulose Content of MCC

The cellulose content of MCC was determined using the Chesson-Datta method method to verify whether the produced MCC met commercial quality standards. Based on the data, the MCC contained 96% cellulose, 2% hemicellulose, and 1% lignin material. The high celluloes content was attributed to the effective chemical treatment (alkali, acid, and bleaching) that eliminates non-cellulose components. Hemicellulose, a polymer that links lignin and cellulose, may be dissolved by the alkali method that uses NaOH to disrupt its ester and ether linkages. Some lignin is also dissolved by this process. Subsequent acid hydrolysis using sulfuric acid further removed residual hemicellulose, resulting in a higher cellulose purity [33]. FTIR analysis also confirmed the absence of characteristic lignin and hemicellulose functional groups. However, small amounts of hemicellulose and lignin were still detected quantitatively at 2% and 1%, respectively. These value are minimal and consistent with commercial MCC specification, which typically contain trace amounts of lignin.

3.2.5 Particle Size Analysis

Figure 9. shows the particle size distribution of MCC derived from sugarcane bagasse, analyzed using a Particle Size Analyzer (PSA). The PSA curve indicated that the MCC particle size were distributed within the micrometer range with a relatively narrow distribution, indicating a good degree of homogeneity. The average particle diameter was approximately 0.3–0.4 µm, which is consistent with the characteristics of MCC produced through strong acid hydrolysis. This result is in line with conventional MCC, which produces micron-sized particles with a diameter of less than 0.5 µm. Commercially available MCC has a standard-length range of 0.1 to 300 µm.

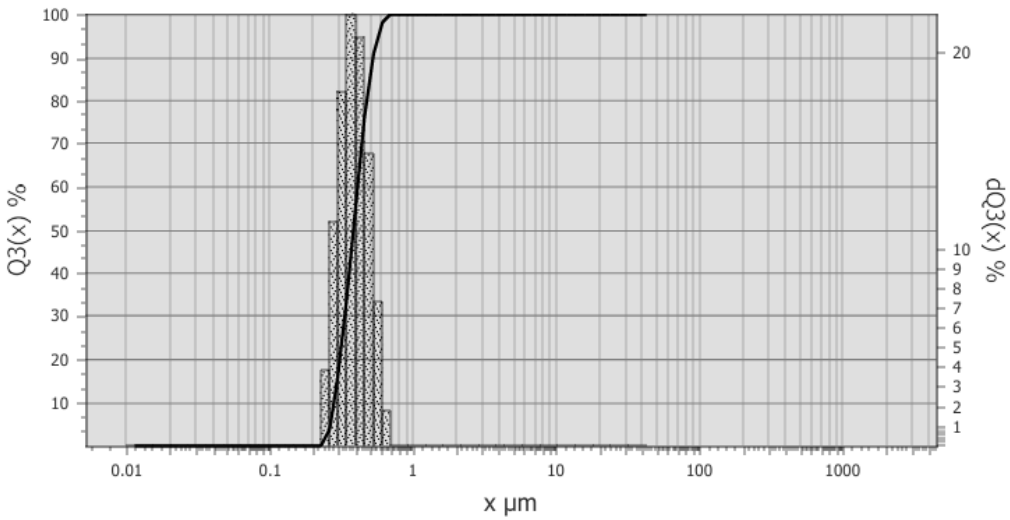


Figure 9. Particle size analyzer MCC

This fine and uniform particle size distribution indicates that the purification and hydrolysis processes were effective, resulting in consistent cellulose fragmentation. The small particle size increases the specific surface area of MCC, thereby enhancing its potential as a reinforcing filler in the gelatin matrix by increasing intermolecular contact and promoting the formation of more hydrogen bonds [34]. Moreover, the curve does not display a secondary peak or excessively broad distribution, suggesting that agglomeration was not significant in the MCC sample. This is important because agglomeration can reduce homogeneity and impair the mechanical performance of composite films.

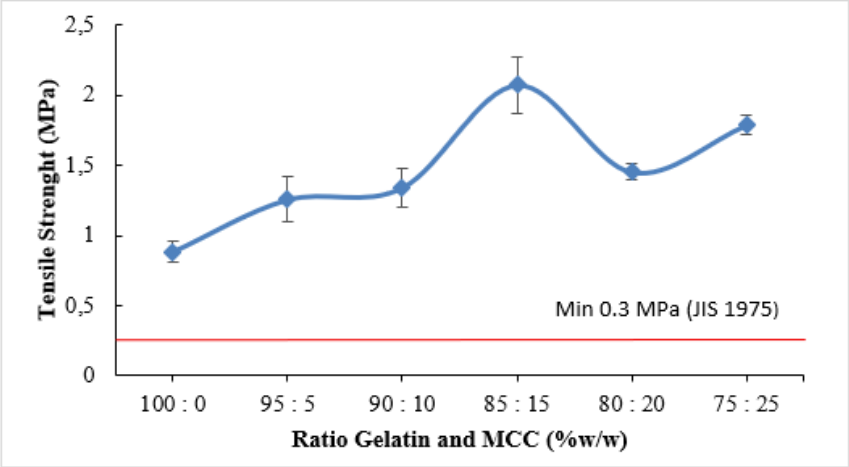
3.3 Characterization of Edible Film

3.3.1 Tensile Properties of Edible Film

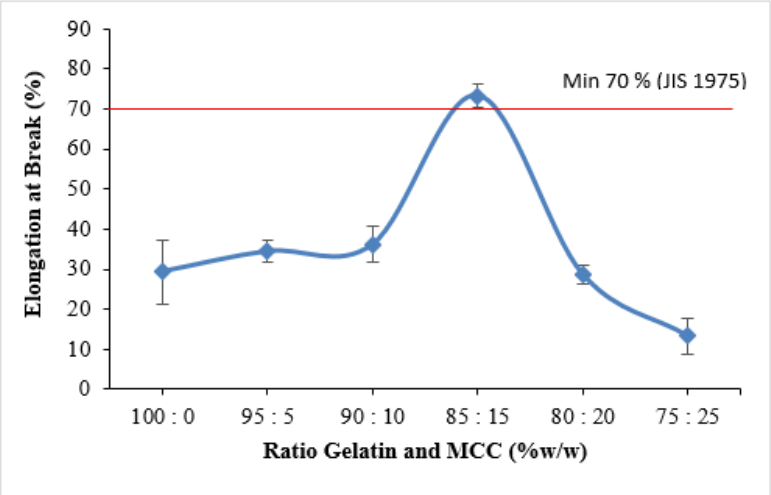
One of the key findings of this study was that increasing the MCC content did not linearly enhance the mechanical performance of the film. Instead, an optimum composition was observed at 85:15 (% w/w) gelatin : MCC ratio. At this ratio, the film demonstrated the highest tensile strength of 2.074 MPa and the best elongation at break of 73.33%. This phenomenon can be explained through the structure–property relationship between MCC and gelatin. Figure 10 (a) shows the effect of MCC variation on tensile strength. According to (JIS) 1975, the minimum requirement for tensile strength for edible film is 0.3 MPa. All formulation produced in this study met this standard. These results are further supported by the hydrogen bonds interaction between gelatin molecules and MCC can strengthen the edible film [35]. In addition, these physical properties are influenced by the high crystallinity of MCC, which acts as a reinforcing phase within the gelatin matrix [36].

The MCC produced in this work has a crystallinity index of 78.3% and an average particle size of 0.3933 µm. Its highly crystalline structure acts as a reinforcing filler capable of forming rigid micro-domains within the gelatin matrix. At moderate concentrations (around 15%), these crystalline domains function as mechanical bridges that enhance tensile strength without restricting polymer chain mobility. This balanced interaction explains the significant increase in tensile strength at the optimal composition.

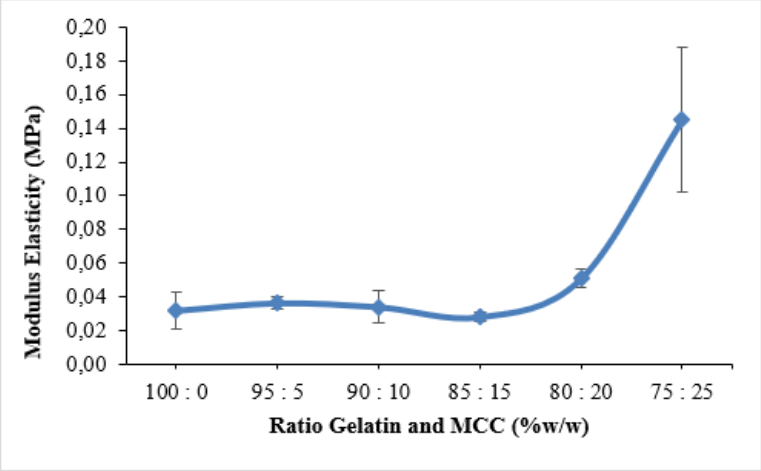
Figure 10 (b) shows elongation at break for each variation of MCC with the gelatin : MCC ratios of 100:0; 95:5; 90:10; 85:15; 80:20 and 75:25 (%w/w). The formulation ratio of 85:15 (%w/w) shows the highest elongation value which is 73.33%. This indicates with ratio 85:15 (%w/w), gelatin and MCC yield the best results because at a certain concentration, MCC forms strong and numerous bonds between the two polymer chains. However, at higher MCC concentrations (> 15%), elongation decreassed. The cellulose properties in MCC enhance the elongation of edible film as the MCC content increases and decrease if excessive MCC is added [37]. In addition, the formation of stronger hydrogen bonds between gelatin molecules and hydroxyl groups resulted in higher chain and molecular bonding, thereby increasing the extensibility of the film [26]. This can be seen from the FTIR results of the edible film in Figure 12, which shows the interaction between the hydrogen bonds of gelatin and the hydroxyl groups of MCC. The relationship between tensile strength and elongation of the film in this study can be concluded to have an inverse relationship and is consistent with existing theory. Additionally, other variations that did not comply with the JIS 1975 standard are due to excessive MCC, which is detrimental to dispersion within the gelatin matrix and causes agglomeration. On the other hand, the film's ability to undergo deformation (elongation) was also highest at this composition due to a balanced interaction between the elastic phase (gelatin) and the rigid phase (MCC). Reinforcement by MCC can improve elongation when MCC forms well-distributed hydrogen bonding interactions without inducing excessive stiffness. At the 85:15 ratio, MCC dispersion remains sufficiently homogeneous, allowing the polymer network to stretch before failure, resulting in the highest elongation. On the other hand, when MCC is present in smaller amounts, there is also less interaction between gelatin and MCC molecules [35].



(a)



(b)



(c)

Figure 10. Analysis physical properties edible film (a) tensile strength, (b) elongation at break, (c) Modulus elasticity

Figure 10 (c) shows the modulus elasticity of the edible film at different MCC variations. The results indicate that the increasing MCC content generally increased the modulus elasticity or stiffness value. This is due to the nature of MCC, which has numerous hydrogen bonds, as observed the FTIR of MCC test results, causing of edible film to become stiffer as more MCC is added. In the MCC addition variation of with ratio 85:15 (%w/w), the modulus elasticity value slightly decreased due to its high elongation value. The high crystallinity index (78.3%) of MCC increased the stiffness of the polymer network by acting as rigid micro-domains capable of hydrogen bonding with gelatin chains, which explains the significant increase in tensile strength at 15% MCC. This is consistent with the theory stating that elasticity is inversely proportional to elongation that reported by [29]. The comprehensive summary of the mechanical properties of the all formulation gelatin : MCC is present by Table 4, is that to confirm that the 85:15 (%w/w) ratio

formulations edible film is the most balanced mechanical performance among the tested composition.

Table 4. Mechanical properties of gelatin–MCC edible films at varying ratios (mean values; different superscript letters within the same column indicate statistically significant differences, Duncan's test, $p < 0.05$)

Gelatin : MCC Ratio (%w/w)	Tensile Strength (MPa)	Elongation at Break (%)	Modulus of Elasticity (MPa)
100 : 0	$0,855^{a}$	$29,33^{bc}$	$0,0321^{a}$
95 : 5	$1,258^{b}$	$34,40^{c}$	$0,0366^{a}$
90 : 10	$1,343^{b}$	$16,67^{ab}$	$0,1458^{b}$
85 : 15	$2,074^{d}$	73.33^{a}	$0,0283^{a}$
80 : 20	$1,454^{b}$	$28,67^{bc}$	$0,0510^{a}$
75 : 25	$1,790^{c}$	$13,27^{a}$	$0,1449^{a}$

According to Table 4, the optimum ratio of gelatin and MCC is 85 : 15 (%w/w). One-way ANOVA demonstrated the influence of MCC concentration on mechanical properties is significant ($p < 0.05$), and the best result test showed that the 85:15 (%w/w) formulation exhibited significantly higher tensile strength compared to other formulations, indicating an optimal balance between polymer chain mobility and filler reinforcement. However, agglomeration begins to occur at the addition of MCC at concentrations greater than 15%, as clearly observed in the SEM results. Agglomeration leads to the formation of defect sites that weaken the interactions between gelatin chains, thereby reducing elongation and making the film more brittle. This is a common behavior when the filler exceeds the critical loading level, where instead of reinforcing the matrix, it begins to deteriorate the mechanical properties.

3.3.2 Water vapor permeability (WVP)

Water vapor permeability (WVP) is a critical parameter for evaluating the barrier performance of edible films, especially those intended for food packaging applications. In biopolymer-based films such as gelatin and MCC composites, WVP is strongly influenced by the film's microstructure, hydrogen bonding interactions, and the distribution of reinforcing fillers within the polymer network. Therefore, analyzing WVP provides essential insight into how the incorporation of MCC modifies the barrier properties of the gelatin film and determines its suitability for practical use in moisture-sensitive food systems. The results of WVP analysis of edible film based on gelatin from bovine hide prepared with MCC from sugarcane bagasse ratios of 100:0; 95:5; 90:10; 85:15; 80:20 and 75:25 (%w/w) are presented in Figure 11.

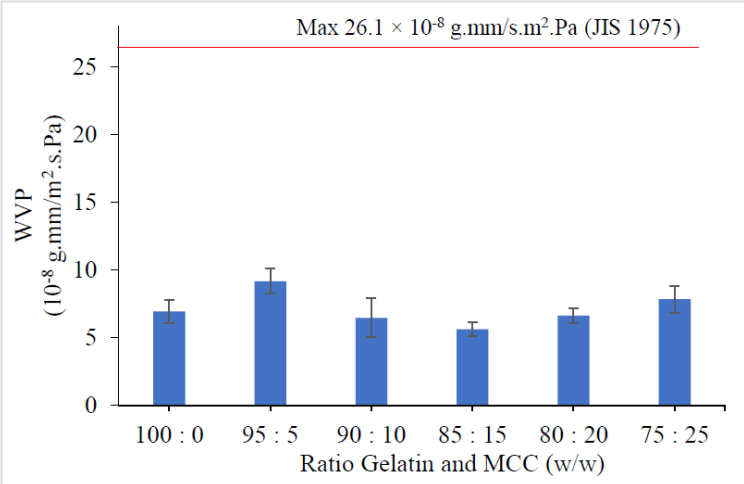


Figure 11. Water Vapour Permeability of edible film

From Figure 11, the lowest WVP value was obtained for the edible film at the 85:15 (%w/w) gelatin : MCC ratio, with a value of $5.59 \times 10^{-8} \pm 0.01$ g/mm·s·Pa. In contrast, the highest value was observed at the 95:5 (%w/w) formulation, reaching $9.15 \times 10^{-8} \pm 0.01$ g/mm·s·Pa. The instability of WVP values influenced by several factors, such as film thickness, MCC content, and glycerol concentration. WVP of edible packaging film is affected by the types of raw materials used and the combination or mixture of constituent materials. Water molecule transport is impeded by the strong molecular interactions between gelatin and MCC at the protein chain and polysaccharide linkages, including hydrogen and hydrophobic bonds, which shorten the distance between molecules in the matrix. Furthermore, cellulose high crystalline and hydrophobic properties have an impact on edible film WVP [38]. Based on the JIS standard the maximum WVP value is $26.1 \times 10^{-8} \pm 0.01$ g·mm/m^2·s·Pa, this shows that the results of the WVP of edible film in this study complies with JIS standard.

The lowest WVP value was observed at the 85:15 (% w/w) composition. This indicates that MCC not only reinforced the film mechanically but also provided a barrier effect against water vapor diffusion. The addition of MCC created a more tortuous diffusion pathway, causing water molecules to require a longer time to pass through the film. Additionally, strong gelatin–MCC interactions reduce the free volume within the polymer network, thereby lowering permeability. However, when MCC is added in excess, agglomeration occurs and generates micro voids, making the diffusion pathway easier for water molecules to traverse. This explains the increase in WVP at compositions exceeding 15%.

3.3.3 Analysis of water and oil temperature resistance time

The dissolution behaviour of gelatin and MCC films in water and oil is an essential parameter for understanding the edible film's stability and suitability for various food packaging applications. Differences in polarity between water and oil, combined with temperature variations, significantly influence the film's structural integrity, hydrogen bonding interactions, and resistance to solvent penetration. This analysis also helps determine the optimal formulation that offers balanced stability in both aqueous and lipid environments. The results are summarized in the following Table 4.

Table 5. The effect of ratio gelatin and MCC dissolve in water and oil at different temperatures

Ratio Gelatin and MCC (%w/w)	Time (Minute)					
	Water Temperature (°C)			Oil Temperature (°C)		
	30	50	80	30	50	80
100 : 0	27.23	19.07	9.30	30.41	22.08	15.58
95 : 5	29.51	16.57	5.43	32.10	19.56	10.05
90 : 10	25.51	15.55	3.47	29.45	16.32	9.30
85 : 15	24.20	13.07	1.36	25.43	14.12	5.14
80 : 20	22.14	12.13	1.10	27.33	15.33	4.17
75 : 25	17.06	9.40	1.01	22.28	12.55	3.55

As seen in Table 5, the lower the MCC content in the edible film, the longer it will take for the edible film to dissolve in water. This is because the addition of MCC causes the hydroxyl rich cellulose chains to bind with water. Cellulose has strong hydrogen bonds and characteristics that make it difficult to bond with water. The absorbent nature of MCC easily absorbs water when there is an excess of cellulose (MCC), causing it to dissolve more quickly in water [36].

Table 5 also shows the solubility time of the edible film in each MCC variation increases as the oil temperature rises and MCC is added. This is because the addition of MCC has an impact on the edible film solubility time, accelerating it during manufacture. Likewise, edible film in oil shrinks more quickly at higher oil temperatures. This is due to the fact that solute solubility rises with solvent temperature. Additionally, edible film that use oil as a medium dissolve slower than those that use water. The following is a Table comparing the solubility of edible film at different temperatures in water and oil. For example, for the ratio of gelatin and MCC of 85:15 (%w/w) for edible film, the solubility time in water at temperatures of 30 °C, 50 °C, and 80 °C was 24.2 minutes, 13.07 minutes, and 1.36 minutes, while the solubility time in oil in 30 °C, 50 °C, and 80 °C, the solubility times was 24.2 minutes, 13.07 minutes, and 1.36 minutes, while solubility in oil at temperatures of 30 °C, 50 °C, and 80 °C yield solubility times of 25.43 minutes, 14.12 minutes, and 5.14 minutes. This is because oil is non-polar, making it difficult to dissolve the edible film even at higher temperatures [26]. In addition, if MCC is below the critical point, it will produce hydrophobic properties, but if the opposite is true, it will be hydrophilic [25].

The differences in dissolution time in water and oil indicate that the hydrophilic/hydrophobic behavior of the film is strongly influenced by the presence of MCC. A higher MCC content leads to faster dissolution in water because MCC readily absorbs water and disrupts the film structure. Additionally, higher temperatures accelerate film dispersion due to the increased kinetic energy of the solvent molecules. However, in oil, the film dissolves slower than in water because the non-polar nature of oil does not interfere with the hydrogen bonding within the film matrix. At the optimum MCC concentration (85:15), the film still maintains a stable mechanical structure, resulting in longer dissolution times in oil compared with formulations containing lower MCC levels.

3.3.4 Morphology edible film

FT-IR testing of edible films to determine how gelatin with MCC affects the intensity and position of absorption peaks in edible films in several functional groups, such as amide and hydroxyl groups, which indicate interactions between the gelatin matrix and MCC fibres. Amide A is an NH-bond extension combined with a hydrogen bond, amide I is a hydrogen bond (C=O) combined with (COO), amide II is an N-H group combined with a (-CN) extension, and amide III

is a (-CN) and NH bond from the amide group or glycine CH_2 group. The following are the FTIR analysis of edible film.

The characteristic peak of hydroxyl group in MCC was a wide peak, which was caused by the interaction of hydroxyl groups between different crystal types in MCC. It could also be explained that hydroxyl groups within or between molecules formed hydrogen bonds, which would lead to the shift/deviation of the characteristic peak of hydroxyl groups. Therefore, the characteristic peaks of hydroxyl groups at different offsets were superimposed to form a wide peak [7].

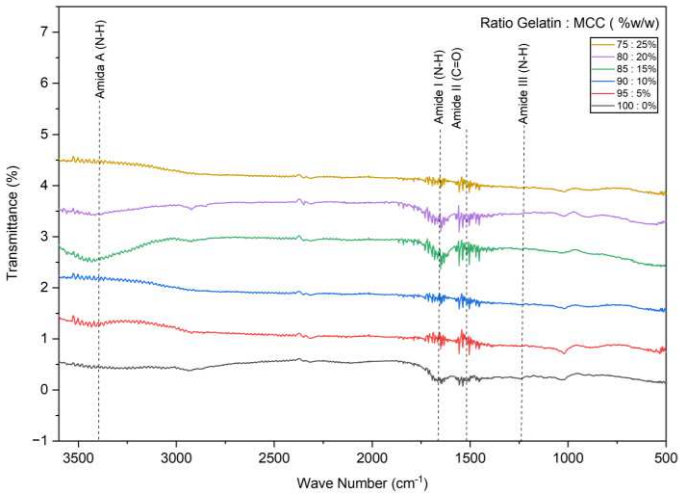


Figure 12. Group Functional of Edible Film

Figure 12. shows the FTIR spectra of the gelatin–MCC composite films exhibit characteristic amide bands, including Amide A (~3300 cm^{-1}), Amide I (~1630 cm^{-1}), Amide II (~1500 cm^{-1}), and Amide III, confirming the presence of protein-based structures. A noticeable shift in the Amide A band toward lower wavenumbers was observed with increasing MCC content, indicating enhanced hydrogen bonding interactions between the hydroxyl groups of MCC and the amide groups of gelatin. In addition, slight changes in the Amide I and Amide II regions suggest modifications in the molecular environment of the gelatin matrix, which can be attributed to intermolecular interactions between gelatin chains and MCC. These interactions contribute to improved structural integrity of the composite films.

3.3.5 Morphology edible film

Scanning Electron Microscopy (SEM) was employed to observe the surface morphology of the gelatin and MCC edible films at various composition ratios. SEM analysis provides important insights into the dispersion of MCC within the gelatin matrix, the formation of microstructural features, and the presence of defects such as pores, cracks, or agglomerates that may influence mechanical strength and barrier performance. The SEM images for each composition are shown in Figure 13.

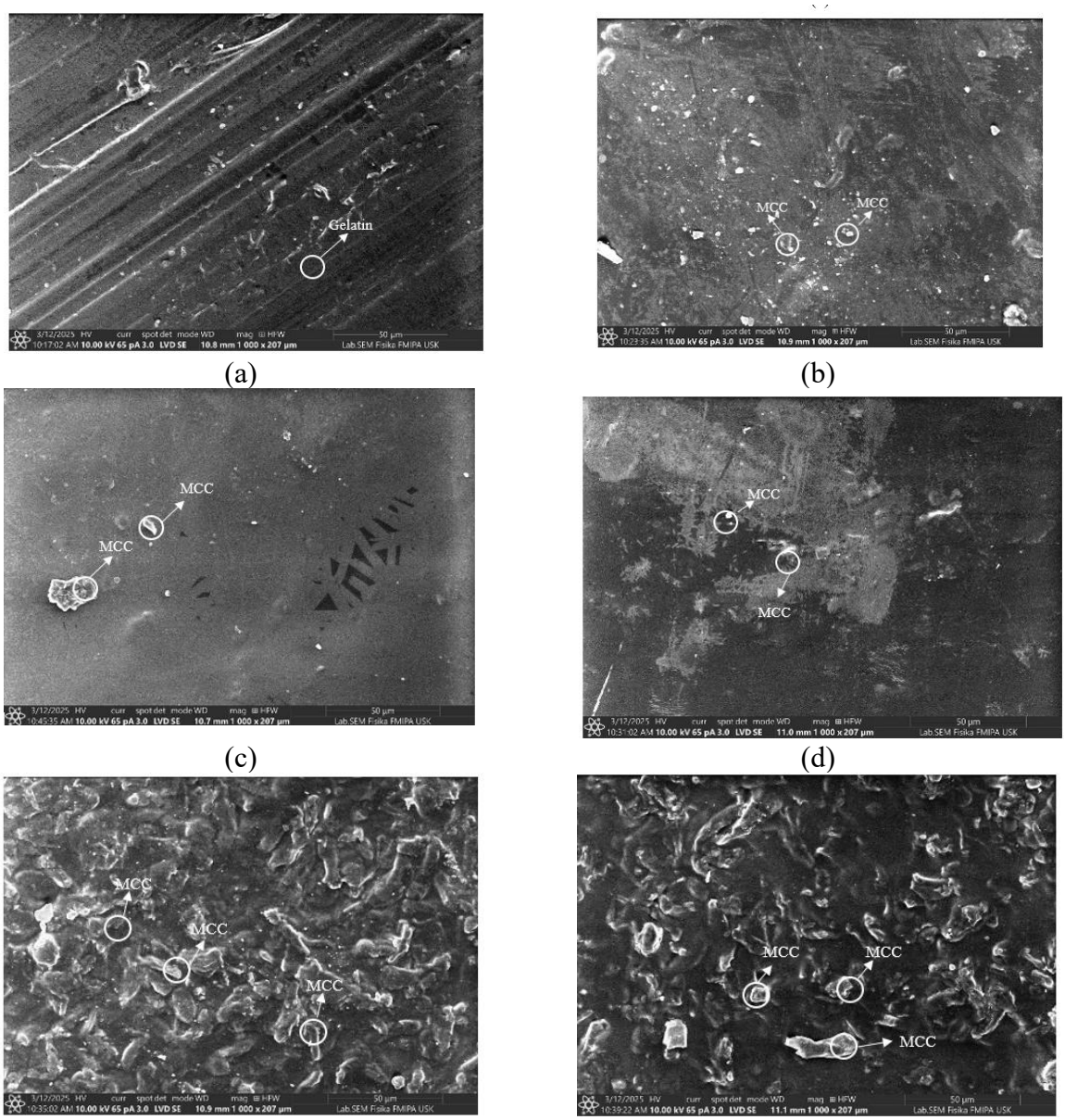


Figure 13. SEM results of edible film for each variation of ratio gelatin and MCC (%w/w): (a) 100:0; (b) 95:5; (c) 90:10; (d) 85:15; (e) 80:20; (f) 75:25

The SEM images revealed significant changes in the surface morphology of the edible films as the ratio of gelatin to MCC varied. Figure 13(a), without MCC addition, the surface appears very smooth and homogeneous, reflecting the structure of pure gelatin without any reinforcing phase. Although this provides flexibility, it also indicates that the film lacks sufficient microstructural support to enhance its mechanical properties. Figure 13(b) shows that the formulation of 95:5 (%w/w) composition exhibits small cellulose particles begin to appear and are fairly well dispersed within the gelatin matrix. The surface remains relatively smooth, but slight textural changes indicate the initial formation of reinforcing domains that may contribute to improved film strength.

Figure 13(c) shows that the 90:10 (%w/w) composition exhibits dispersion becomes more uniformed, with MCC particles appearing more uniformly distributed throughout the matrix. The film surface becomes denser and more compact, suggesting that interactions between gelatin and MCC are becoming more stable. This improved microstructure contributes to enhanced tensile strength, as MCC acts as an effective reinforcing filler. Figure 13(d) shows that the 85:15 (%w/w) composition exhibits the most optimal formulation, where the film surface appears the most homogeneous, smooth, and free of agglomeration. At this ratio, MCC is perfectly dispersed, producing a stable and well-structured microarchitecture. The SEM appearance at this composition aligns with mechanical data showing the highest tensile strength and elongation, indicating that 85:15 (% w/w) is the ideal ratio for forming a compact polymer network.

However, at higher MCC concentration such as 80:20 (% w/w) ratio, shown in Figure 13(e), signs of agglomeration begin to appear. MCC particles form small clusters that disrupt the uniformity of the film surface. These agglomerates create micro voids that act as weak points within the structure, leading to a decline in mechanical performance. Figure 13(f) shows that the 75:25 (% w/w) composition agglomeration becomes much more evident. The film surface appears non-homogeneous, with large MCC clusters interrupting the continuity of the gelatin matrix. The presence of numerous agglomerates makes the film brittle, prone to cracking, and significantly reduced in elongation capability. This indicates that at excessively high MCC concentrations, the filler no longer functions as a reinforcing agent; instead, it deteriorates film quality by surpassing the critical loading threshold [35].

4. Conclusion

The incorporation of MCC into gelatin films enhanced intermolecular hydrogen bonding between cellulose hydroxyl groups and gelatin chains, thereby improving mechanical and barrier properties. The optimal formulation was achieved at the 85:15 (%w/w) gelatin : MCC ratio, which exhibited tensile strength (2.074 MPa) and elongation percentage (73.33%). In dissolution tests, this formulation required 24.2, 13.07, and 1.36 minutes to dissolve in water at 30°C, 60°C, and 80°C respectively. In oil, the formulation required 25.43, 14.12, and 5.14 minutes at the same temperatures. The lowest water vapor permeability was recorded at 5.59×10^{-8} g/s·m·Pa, which complied with the JIS standard requirements. This study demonstrates that valorization of bagasse-derived MCC offers a low-cost and environmentally sustainable reinforcing agent for gelatin-based films. The findings contribute to the development of biodegradable packaging with enhanced mechanical integrity, controlled solubility behavior barrier performance.

Data availability

The raw and processed data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors have not disclosed any personal relationships or interest that might have influence of presented in this publication. The authors declare that there is no plagiarism in the work.

Acknowledgments

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