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Isolation of cellulose from *Gracilaria* sp. for the development of biodegradable edible film: A sustainable approach for eco-friendly food packaging

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ABSTRACT

The isolation of cellulose from *Gracilaria sp.* seaweed offers a sustainable approach for developing biodegradable and edible food packaging materials. This study aimed to isolate cellulose from *Gracilaria sp.* and evaluate its applicability in edible film fabrication. Cellulose was isolated through sequential washing, hydrolysis, delignification, and bleaching processes, and its lignocellulosic composition was analyzed using the Van Soest method, while chemical functional groups were identified by Fourier transform infrared (FTIR) spectroscopy. The results demonstrated a substantial enhancement in cellulose content accompanied by effective removal of hemicellulose and lignin, confirming the efficiency of the isolation process. Edible films were subsequently prepared using *Gracilaria sp.* cellulose in combination with chitosan and glycerol as a plasticizer. The developed films exhibited acceptable thickness, high water solubility, improved tensile strength with increasing glycerol concentration, and glycerol-dependent surface morphology as observed by SEM analysis. FTIR confirmed the presence of characteristic cellulose functional groups within the film matrix. In addition, the edible films demonstrated antimicrobial activity against *Escherichia coli*, highlighting their potential as active food packaging materials. Overall, this study confirms that cellulose derived from *Gracilaria sp.* is a viable and sustainable raw material for the development of functional, biodegradable, and antimicrobial edible films for food packaging applications.

Keywords: *Gracilaria sp.,* cellulose isolation, edible film, biodegradable materials, Van Soest method.

INTRODUCTION

Global plastic production has reached an alarming rate, exceeding 359 million tons annually (Pilapitiya & Ratnayake, 2024). Indonesia produces 5.4 million tons of plastic waste annually, making it the second largest contributor to household waste (Kibria et al., 2023). This plastic waste is primarily derived from primary and secondary food packaging for fresh (Ncube et al., 2021) and processed products (Maskun et al., 2023), raising serious concerns about consumer safety (Zhang et al., 2021) and health risks. Non-biodegradable plastics present critical environmental challenges and long-term health hazards (Chow et al., 2018). In Indonesia, the problem of plastic waste is increasingly pressing in urban and rural areas, causing significant ecological imbalances through soil and water contamination (Meijer et al., 2021). Inadequate waste management infrastructure exacerbates the plastic pollution crisis (Lestari & Trihadiningrum, 2019), highlighting the urgent need for an effective waste management system. Food packaging is critical in the distribution chain, using paper, styrofoam, plastic, glass, and cans (Irawan et al., 2018). Despite extending the shelf life of products, its non-edible and

non-biodegradable nature creates new environmental challenges (Borelle et al., 2022). While plastic offers advantages such as versatility, light weight, flexibility, resistance to moisture, durability, and cost-effectiveness, its environmental impact is of increasing concern. The global production of plastic-based products and unsustainable usage patterns have resulted in severe environmental degradation (Setyowati & Mulasari, 2013) requiring urgent innovative solutions. Biodegradable plastics have been developed as an alternative, allowing microorganisms to decompose naturally into environmentally friendly compounds. However, most plastic packaging comprises synthetic materials, contributing to environmental pollution. Therefore, research on biodegradable packaging materials is fundamental and urgent. Edible films have emerged as a promising alternative for biodegradable packaging (Widodo et al., 2019). These films consist of three main components: hydrocolloids (including proteins, polysaccharides, and alginates), lipids (fatty acids, natural glycerol, and waxes), and composites formed from hydrocolloid-lipid mixtures (Rodríguez et al., 2006). The abundant availability of seaweed in Sulawesi represents a valuable yet underutilized resource for such applications. Seaweed offers several advantages over terrestrial plants: it requires no fertilization, is non-toxic, exists as an abundant natural resource, provides a relatively short harvest cycle of approximately three months, and contains valuable polysaccharides, including cellulose, making it ideal for environmentally friendly plastic production (Khalil et al., 2017). Indonesia's rich biodiversity strategically positions it to develop cellulose-based bioplastics, with seaweed as an alternative source of readily biodegradable cellulose. Developing seaweed-based edible films offers a sustainable solution for food packaging (Janowicz et al., 2023).

This application is particularly relevant in Takalar Regency, South Sulawesi, a production centre for *Gracilaria* sp. The region benefits from optimal seaweed cultivation characteristics (Nursidi et al., 2021; Saleh et al., 2023), ease of access, and direct processing into natural polymers, specifically as a cellulose source. The innovative aspect of this research lies in the unique combination of abundant natural polymers with chitosan, enhanced through plasticizer addition, to create an effective food packaging material. This approach utilizes locally available natural resources while meeting the growing demand for sustainable packaging solutions.

One effort to overcome plastic waste is to develop alternative packaging, especially edible film. Edible film is a thin layer primarily used for packaging food ingredients, made from consumable components (Indarti et al., 2022). This layer acts as a barrier against oxygen, water vapor, and dissolved substances in food without altering the product's original form. Unlike conventional packaging, edible film can be consumed with the packaged product (Ding & Eldridge, 2019), offering advantages that traditional packaging systems lack. Besides being edible, these films are biodegradable, making them a sustainable packaging solution. Developing biodegradable plastics is currently a significant focus for reducing environmental pollution, as microorganisms can naturally decompose them into more environmentally friendly compounds. These plastics are generally produced from natural polymer materials such as starch, cellulose, chitin, and modified synthetic polymers designed to accelerate decomposition.

Biodegradable plastics can decompose within weeks or months when disposed of in the environment, without leaving harmful residues (Barboza et al., 2018). Consequently, biodegradable plastics can help reduce plastic waste problems and microplastic pollution. Natural polymer sources with the potential to produce cellulose as raw material for biodegradable plastics have been most extensively researched in terrestrial plants, such as oil palm empty fruit bunches (Souhoka & Latupeirissa, 2018). Marine sources have primarily been studied for their carrageenan content, particularly *Eucheuma cottonii* and *Eucheuma spinosum* (Nurmilla et al., 2021). However, no studies have yet thoroughly explored the utilization of cellulose content in seaweed. Seaweed offers distinct advantages over terrestrial plants: it requires no fertilization, is non-toxic, exists as an abundant natural resource, has a harvest cycle of approximately three months, and contains valuable polysaccharides, including cellulose, that can be applied in environmentally friendly plastic production (Khalil et al., 2017). Polymers derived from seaweed are renewable, environmentally friendly, and relatively economical, as seaweed represents an abundant fishery commodity in Indonesia.

Gracilaria sp. cultivation represents one of Southeast Asia's leading agricultural programs, particularly in South Sulawesi, where it produces

superior raw materials containing 30-35% cellulose (Wadi et al., 2019). Takalar Regency is one of South Sulawesi's regions with significant potential for *Gracilaria sp.* production (Agustang et al., 2021). This seaweed serves not only as a food source but also provides raw materials for various industries, including pharmaceutical, cosmetic, textile, beverage, and toothpaste manufacturing. Its applications extend broadly across the biotechnology and microbiology fields. Seaweed is preferred as a cellulose source compared to terrestrial plants because plant cellulose contains lignin, which impacts processing costs. In contrast, most seaweed species contain no true lignin, resulting in cleaner cellulose fractions. Seaweed contains a significant proportion of cellulose, which is often underutilized or discarded as waste during agar extraction processes (Trache et al., 2020), which is often unused or discarded as waste in agar production. Agar processing waste generally contains relatively high cellulose content. *Gracilaria sp.,* a red alga (Rhodophyta) known by various regional names such as sango-sango, rambu kasang, dayang jenggot, dongi-dongi, bulung embulung, coral agar, jahe agar, and bulu sangu, represents a promising cellulose source for biodegradable plastic production.

Plasticizers are essential additives in food packaging film production. These low-molecular-weight organic substances are added to reduce polymer rigidity while enhancing flexibility and extensibility. Glycerol is a commonly used plasticizer (Charles et al., 2022), proving particularly effective in improving plastic film properties due to its low molecular weight (Basiak et al., 2018). The commercial use of chitosan adds natural preservative qualities to food products. Chitosan is frequently incorporated into bioplastic formulations (Dewi et al., 2023), creating packaging materials that are easily degradable, environmentally friendly, and possess antimicrobial properties, extending food preservation when used as a packaging material. Chitosan represents an ideal biopolymer for food coating film production due to its biocompatibility, non-toxicity, biodegradability, and film-forming capabilities when applied to foods (Karimnezhad et al., 2017). Edible films coat food products, resulting in packaging that can be consumed along with the product. Food products' high protein nutritional content (Fahmi & Romadhon, 2023) makes them susceptible to pathogenic microbial attacks that cause product deterioration. Preventing such deterioration requires specialized handling, specifically through developing edible film layers to extend food product shelf life, where chitosan-based edible films can be created as active packaging solutions (Arif et al., 2022)

Plastic packaging materials cannot be sustained for widespread use because they are difficult to degrade, so researchers are interested in developing biodegradable edible film plastic packaging from *Gracilaria* sp. with glycerol and chitosan applied to food materials. The approach and problem-solving strategies that have been formulated focus on isolating cellulose from *Gracilaria* sp., determining the effect of adding chitosan and plasticizers on the ED value of *Gracilaria sp*. edible film. In addition, the impact of adding chitosan and plasticizers on the water absorption of *Gracilaria sp.* edible film as food packaging will be studied. This research presents an innovation in biodegradable edible films using cellulose isolated from *Gracilaria* sp., combined with chitosan and glycerol as plasticizers. The study thoroughly analyzes the impact of this combination on the film's energy density and water absorption to enhance its performance. Additionally, the natural antimicrobial properties of chitosan are employed to extend food shelf life, providing an eco-friendly active packaging alternative to conventional plastics.

MATERIALS AND METHODS:

Chemicals and materials

The materials used in this study were cellulose from *Gracilaria* sp, chitosan (Sigma-Aldrich), glycerol (Merck), citric acid (HNO_3) (Merck), sodium hydroxide (NaOH) (Merck), hydrogen peroxide (H2O2) (Sigma-Aldrich), 2% acetic acid (CH3COOH) (Sigma-Aldrich), Potato Dextrose Agar (PDA) media (Oxoid), Nutrient Agar (NA) media (Oxoid), Staphylococcus aureus, and Escherichia coli. The tools used include a Scanning Electron Microscope (SEM JSM6510LV, JEOL, Japan), Fourier Transform Infrared Spectroscopy (IRTracer-100, Shimadzu, Japan), Tensile Strength ZP recorder 50 N Imada (Imada Inc), grinder (Iwaki), sieve (Iwaki), Petri dish (/Iwaki), basin (Iwaki), oven (PT. Elo Karsa Utama), wooden spoon (Iwaki), analytical balance (Thermo Fisher Scientific), scale (Iwaki),

thermometer (Iwaki), 10mL measuring pipette (Pyrex), suction ball (Iwaki), 100 mL measuring cup (Iwaki), hot plate (Thermo Fisher Scientific), magnetic stirrer (Thermo Fisher Scientific), vernier caliper, 100 mL volumetric flask (Pyrex), Erlenmeyer (Iwaki), glass funnel (Pyrex), desiccator (Pyrex), filter paper (PT. Laborindo Sarana), ent case (PT. Laborindo Sarana).

Methods

The edible film developed in this study was systematically prepared and characterized to evaluate its suitability for biodegradable food packaging applications. The characterization focused on physical, mechanical, structural, and antibacterial properties to ensure that the film was not only successfully formed but also functionally evaluated.

Raw material preparation

Gracilaria sp. was collected from the coastal area of Takalar Regency, South Sulawesi, Indonesia. Fresh seaweed was washed thoroughly with running water to remove physical dirt such as sand, epiphytes, and other marine debris. A final rinse was carried out using distilled water to ensure the sample was clean and free from potential contaminants that could interfere with downstream processing. The cleaned *Gracilaria* sp. was cut into small segments (about 1–2 cm) to facilitate drying and grinding. The samples were dried in direct sunlight for approximately four days until the water content was sufficiently reduced, resulting in a brittle texture suitable for grinding. The dried seaweed was ground using a laboratory grinder to obtain a fine powder. To ensure uniformity and increase the surface area during subsequent chemical treatments, the powder was sieved using a 60-mesh shaker sieve. This process produced *Gracilaria* sp. powder with a consistent particle size, which was then stored in a closed container at room temperature for cellulose extraction.

Preparation of cellulose from *Gracilaria* sp.

Cellulose isolation was initiated by hydrolysis, which was done by mixing 50 g of dry *Gracilaria* sp. powder with 500 mL of 3.5% (v/v) nitric acid (HNO_3). The mixture was heated at 90 °C with constant stirring for 2 hours to remove hemicellulose. After hydrolysis, the suspension was filtered using filter paper, and the residue was washed repeatedly with distilled water until it reached a neutral pH. The residue was then delignified by adding it to a 2% (w/v) sodium hydroxide (NaOH) solution with a ratio of 1:10 (w/v), and the mixture was autoclaved at 120 °C for 1 hour. After delignification, the sample was filtered and rewashed with distilled water until it reached a neutral pH. Furthermore, the sample was bleached using 10% (v/v) hydrogen peroxide (H_2O_2) in a water bath at 60°C for 1 hour to remove residual pigments and further purify the cellulose. The mixture was then filtered, and the cellulose was thoroughly rinsed with distilled water to remove any remaining chemicals. The final cellulose product was dried in an oven at 50–60 °C until it reached a constant weight. The dried cellulose was then stored in a desiccator for further characterization. Fourier transform infrared spectroscopy confirmed the isolated cellulose's chemical structure

Determination of lignocellulose content

The determination of lignocellulose content was carried out using the Van Soest method, which involves several stages, including the determination of acid detergent fibre (ADF), neutral detergent fibre (NDF), hemicellulose, lignin, insoluble ash, and cellulose content (Rizkiansyah et al., 2023)

Acid detergent fiber content

A total of 0.3 g of the sample (a gram) was placed into a test tube, followed by the addition of 45 mL of ADF solution, and the tube was tightly sealed. The mixture was heated in boiling water for 1 hour. After completion, filtration was carried out using a vacuum pump and a pre-weighed sintered glass crucible (b gram). The retained residue was washed thoroughly with acetone and hot water. Subsequently, the residue was dried in an oven at 105 °C for 8 hours or left overnight. After drying, the residue was cooled in a desiccator and then reweighed (c gram). The ADF content was calculated using Equation (1).

$$ADF\ content = \frac{c-b}{a} \times 100\% \quad (1)$$

where: a – weight of dry sample (g), b – weight of empty sintered crucible (g), c – weight of sintered glass crucible + dried residue after oven drying (g).

Determination of neutral detergent fiber content

A total of 0.3 g of the sample (a) was weighed and placed into a 250 mL Erlenmeyer flask, followed by the addition of 30 mL of NDF solution. The flask was tightly sealed, and the mixture was heated in boiling water for 1 hour with occasional shaking. After heating, the mixture was filtered using a pre-weighed sintered glass crucible No. 1 (b) with the aid of a vacuum pump. The residue obtained was washed with 100 mL of boiling distilled water until the foam disappeared, followed by washing with 50 mL of ethanol. The washed residue was then dried in an oven at 100 °C for 8 hours. After drying, the residue was cooled in a desiccator for 30 minutes before being reweighed (c). The calculation of NDF and hemicellulose content is described in Equations 2 and 3.

$$\textit{NDF content}\ (\%) = \frac{c-b}{a} \times 100\% \quad (2)$$

$$\textit{Hemiselulosa content}\ (\%) = \text{NDF} - \text{ADF} \quad (3)$$

where: a – weight of sample (g), b – weight of empty sintered glass crucible (g), c – weight of sintered glass crucible + dried residue after oven drying (g).

Determination of lignin and cellulosa content

The sintered glass containing the ADF residue (c) was placed on a Petri dish and added with 20 mL of 72% H_2SO_4. The mixture was stirred periodically to ensure that the sample was thoroughly wetted with H_2SO_4, and then left to stand for 2 hours. Afterwards, the mixture was filtered using a vacuum pump while being rinsed with hot water until completely clean. The dried solid was placed in a desiccator and weighed (d). The solid was then heated in a muffle furnace at 500 °C for 2 hours, partially cooled, and subsequently transferred into a desiccator for 30 minutes before being weighed again (e). The calculations for lignin content, insoluble ash, and cellulose content are described in Equations 4, 5, and 6.

$$\textit{Lignin content}\ (\%) = \frac{e-f}{a} \times 100\% \quad (4)$$

$$\textit{Insoluble ash content}\ (\%) = \frac{f-b}{a} \times 100\% \quad (5)$$

$$\textit{Cellulosa content}\ (\%) = \text{ADF} - \textit{Lignin} - \textit{Insoluble ash} \quad (6)$$

where: a – weight of sample (g), b – weight of empty sintered glass crucible (g), c – weight of sintered glass + dried sample after oven and desiccator (g), e – weight of sintered glass + sample after muffle furnace (g).

Preparation of edible film

A total of 0.5 g of cellulose extracted from *Gracilaria sp.* was accurately weighed and placed into a beaker. Subsequently, 10 mL of a 2% (w/v) chitosan solution (prepared by dissolving chitosan in 1% acetic acid) was added under continuous stirring to ensure uniform dispersion of the cellulose. Glycerol was then incorporated as a plasticizer at concentrations of 10%, 15%, and 20% (v/v) relative to the total solution volume. The mixture was heated and stirred using a magnetic stirrer at 80 °C for 40 min until a homogeneous film-forming solution was obtained. During this process, the pH was maintained between 4.0 and 5.0 to promote chitosan solubility and stable film formation. The resulting warm solution was cast onto a clean, flat Petri dish (90 mm diameter) to control film thickness and ensure uniform spreading. The cast solution was initially dried in an oven at 60 °C for 2–3 h until a semi-dry, non-liquid surface was formed. Subsequently, the Petri dishes were transferred to a dust-free environment and air-dried at room temperature (25–27 °C) for 24–48 h to allow gradual solvent evaporation and improve film flexibility. After complete drying, the edible films were carefully peeled from the Petri dishes and stored in a sealed polyethylene bag or desiccator at room temperature prior to further physical, mechanical, structural, and antibacterial characterization.

Film thickness test

The thickness of the edible film is determined by taking measurements at five different random points using a digital caliper. The thickness value of the edible film is obtained from the average of the five measurement points

Edible film solubility test

The samples were cooled in a desiccator for 30 minutes and weighed as the initial weight of the sample. The samples were placed in a container containing ± 50 mL of water and incubated for 24 hours at 25 °C. After 24 hours, the water was

filtered, and the dried film was dried in an oven for 24 hours at 50 °C. After heating, the sample was cooled in a desiccator for 30 minutes and weighed as the final weight of the sample.

$$\text{solubility (\%)} = \frac{(Before\ weight - final\ weight)}{Before\ weight} \times 100\% \quad (7)$$

Tensile strength and percent elongation test

Tensile strength is the maximum tension a material can withstand when stretched or pulled, tested using the ASTM American Standard Testing Method (1993) using an MPY testing machine. The film sheet size is 2 × 5 cm within 48 hours. The instrument was set at an initial gripping distance of 50 mm, a crosshead speed of 50 mm/min, and a load cell of 50 kg. Elongation was calculated by dividing the length increment of the film piece at the tear.

$$Tensile\ strength\ (\text{MPa}) = \frac{F}{A} \quad (8)$$

where: F – tensile strength force (N), A – cross-sectional area (mm).

Percent elongation is calculated by dividing the increase in the length of the film piece when torn. Percentage elongation is calculated using the equation:

$$\%\ Elongation = \frac{A-B}{B} \times 100\% \quad (9)$$

where: A – length after breaking, B – length before breaking.

Biodegradable test

Samples were prepared for immersion in soil at a depth of 15 cm and soaked for 10 days. The samples were analyzed for their percentage mass loss every 2 days.

Scanning electron microscope (SEM)

Cut the film sheet with a 1 × 1 cm width, then place it on the preparation and observe the structure of the resulting edible film.

Antibacterial activity test

Muller-Hinton Agar (MHA) media is sterilized and then cooled to 50°C. Staphylococcus aureus and Escherichia coli bacteria cultured for 24 hours are inoculated into Muller-Hinton Agar media. The volume and area of MHA media are adjusted so that the thickness reaches 4–5 mm. Then, the edible film is cut with a diameter of 5 mm and attached to the agar surface. The samples are incubated at 37 °C for 24–48 hours with the dish in an inverted position.

RESULTS AND DISCUSSION

Preparation of *Gracilaria* sp. seaweed

The preparation of *Gracilaria sp.* seaweed as the raw material was successfully carried out to support efficient cellulose isolation for edible film production. *Gracilaria sp.*, which is abundantly available in Takalar Regency, South Sulawesi, Indonesia, underwent a series of preparatory steps to ensure sample cleanliness, reduced moisture content, and increased surface area. The seaweed was thoroughly washed to remove impurities and subsequently sun-dried to significantly reduce its moisture content, resulting in a brittle texture suitable for size reduction. The dried material was then ground and sieved to obtain a uniform powder, which is essential for enhancing the effectiveness and consistency of the subsequent cellulose isolation process. These preparatory steps played a crucial role in determining the quality of the extracted cellulose and, consequently, the performance of the resulting edible film.

Cellulose isolation from *Gracilaria* sp. seaweed

Cellulose isolation from *Gracilaria* sp. is a complex process involving several steps to separate pure cellulose from other components in algal biomass. This process begins with the initial preparation of the raw material, namely *Gracilaria* sp., which is dried to reduce its water content and then cut or chopped into smaller sizes, ideally in the range of 40–60 mesh, to increase the surface area and facilitate the penetration of chemicals during the following process. The hydrolysis stage is critical in this process, where the prepared *Gracilaria* sp. is reacted with nitric acid at a concentration of 3.5% (v/v). Acid hydrolysis aims to remove hemicellulose and other non-cellulose components bound to cellulose (Rahmi et al., 2020). The hydrolysis reaction is carried out at high temperatures between 90–100 °C for 2 hours to ensure effective

decomposition of the polysaccharide matrix. After hydrolysis, the mixture is filtered to separate the solid (pulp) from the filtrate containing the hydrolysis products. The solid obtained is then washed repeatedly with distilled water until the pH of the filtrate reaches neutral, indicating that the remaining nitric acid has been completely removed. The next stage is delignification, which aims to remove lignin, a complex polymer that provides rigidity to the cell walls of plants and algae (Abolore et al., 2024). This process involves using a 2% sodium hydroxide solution as a solvent at a temperature of 90 °C for 1 hour. Bleaching is carried out using 10% hydrogen peroxide (H_2O_2) in a water bath at 60–80 °C for 1–2 hours. The bleaching process aims to whiten the cellulose and remove pigments that may still be present after the delignification process. Figure 1 presents the cellulose isolation process from *Gracilaria* sp. seaweed.

Fourier transform infrared spectroscopy

The cellulose isolated from *Gracilaria sp.* was subsequently compared with standard cellulose and characterized using FTIR spectroscopy to identify its functional groups. The FTIR spectra of *Gracilaria sp.* cellulose powder and standard cellulose are presented in Figure 3.

Based on Figure 2, the FTIR spectra of two samples, standard cellulose and isolated cellulose from *Gracilaria sp.*, are presented. These spectra illustrate the presence and positions of the main functional groups within the cellulose chemical structure, as indicated by their wavenumbers (cm^{-1}). In general, both spectra exhibit similar patterns, suggesting that the cellulose isolated from *Gracilaria sp.* possesses a chemical structure comparable to that of standard cellulose. The detailed functional groups identified within each wavenumber range are summarized in Table 1.

Based on Table 1, the absorption band in the range of 3200–3600 cm^{-1} corresponds to hydroxyl groups (–OH), a primary characteristic of cellulose due to extensive hydrogen bonding. The slight shift between standard cellulose (3379.88 cm^{-1}) and *Gracilaria sp.* cellulose (3406.72 cm^{-1}) suggests variations in hydrogen bonding, possibly influenced by crystallinity or moisture content. Bands in the 2800–3000 cm^{-1} region are attributed to aliphatic C–H stretching, while the absorption near 1733.49 cm^{-1} indicates the presence of carbonyl groups (C=O), likely from residual hemicellulose or lignin. The 1350–1470 cm^{-1} and 1000–1300 cm^{-1} regions correspond to aliphatic C–H bending and C–O stretching, respectively, with a strong band at 1035.13–1049.29 cm^{-1} confirming

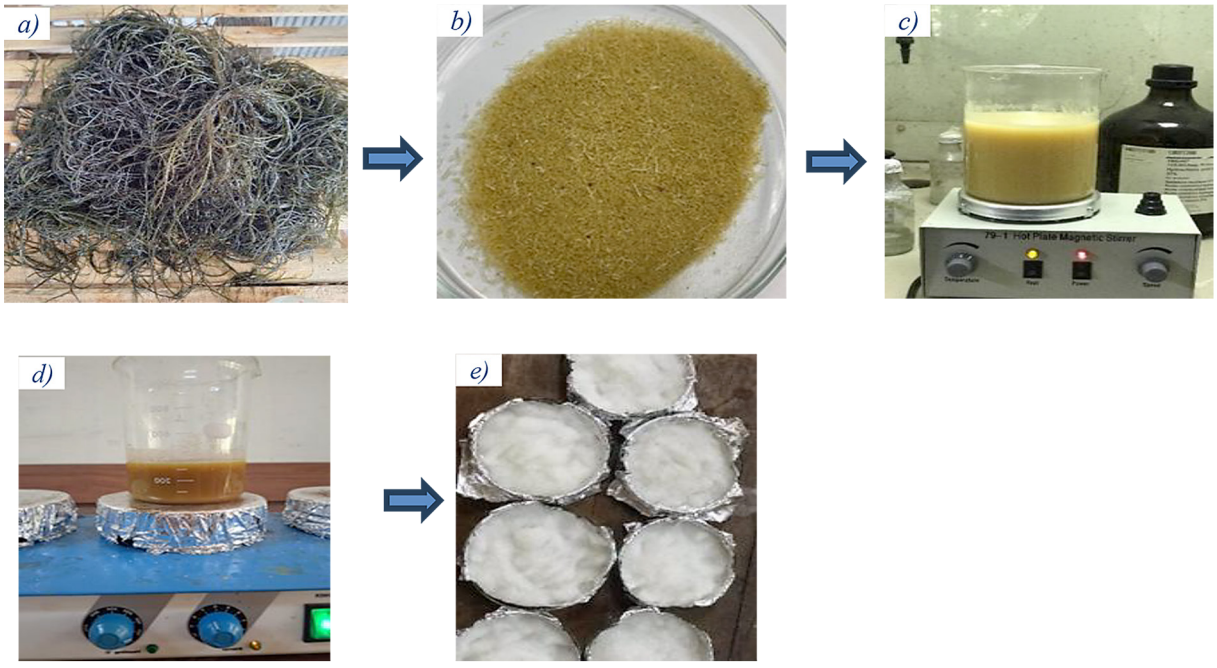

Figure 1. Outlines the cellulose isolation process from *Gracilaria* sp. seaweed: (a) *Gracilaria* sp., (b) *Gracilaria* sp. seaweed powder, (c) hydrolysis, (d) delignification, (e) bleaching

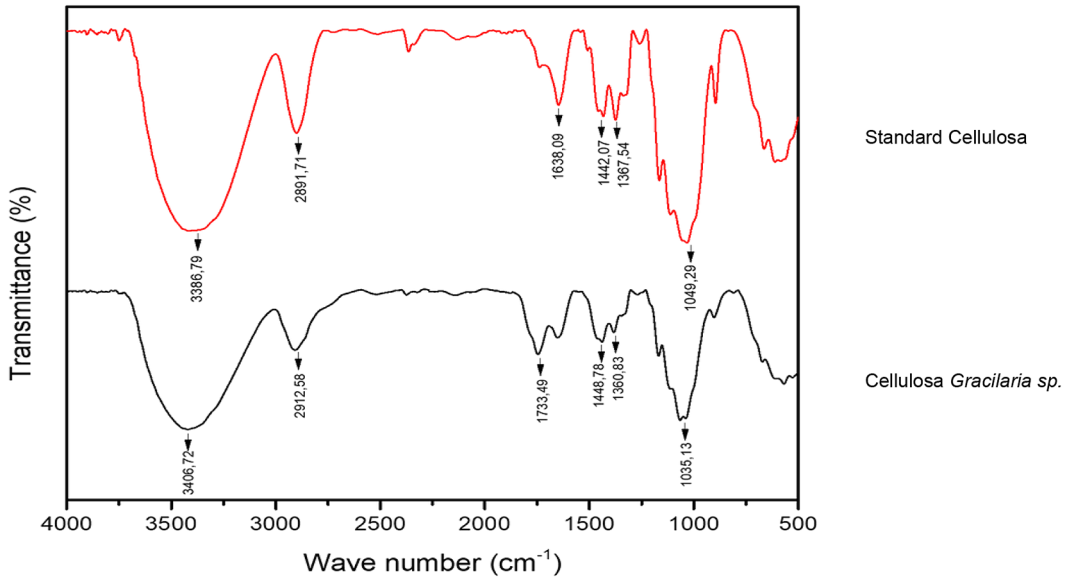

Figure 2. FTIR spectra of standard cellulose and, *Gracilaria sp.* cellulose.

Table 1. Comparison of functional groups between standard cellulose and cellulose isolated from *Gracilaria sp.* based on FTIR spectra

Standard cellulosa	Cellulosa *Gracilaria sp.*	Wave number (cm^{-1})	Funcional group
3379.88	3406.72	3200-3600	Hydroxyl (O-H)
2891.71	2912.58	2800-3000	Aliphatic (C-H)
1638.09	1733.49	1600-1750	Carbonil (C=O)
1442.07	1448.78	1350-1470	Aliphatic (C-H)
1367.54	1360.83	1000-1300	C-O (Polysaccharide)
1049.29	1035.13	1000-1150	C-O-C (Ether)

ether linkages (C–O–C). Similar findings were reported by (Bhutiya et al., 2018), associating the 1046 cm^{-1} peak with pyranose ring C–O–C stretching in cellulose fibres. Overall, the FTIR spectra indicate that the isolated cellulose from *Gracilaria sp.* shares structural similarity with standard cellulose, although minor shifts suggest the possible presence of residual hemicellulose. When compared to FTIR results reported for cellulose and cellulose-derived films from other seaweeds (*Ulva*, *Gracilaria*), the spectral patterns and absence of strong sulphated polysaccharide signals indicate effective removal of non-cellulosic fractions during pretreatment, an essential confirmation because residual phycocolloids markedly alter film hydrophilicity and barrier behaviour (Muthukumar & Chidambaram, 2022). This result is consistent with (Krishnan et al., 2024), who reported that isolated cellulose typically exhibits O–H, C–H, and C–O functional groups.

The lignocellulose content of *Gracilaria* sp.

The lignocellulosic composition of *Gracilaria sp.* was examined in both dried biomass and isolated cellulose to assess the efficiency of the isolation process and its suitability as a precursor for edible film development. Acid Detergent Fibre (ADF), comprising cellulose and lignin, is widely recognised as a robust indicator of structural polysaccharides resistant to chemical degradation. Before isolation, *Gracilaria sp.* exhibited a moderate ADF content of 11.93%, reflecting the predominance of soluble fractions such as hemicellulose and proteins. Following cellulose isolation via the Van Soest method, ADF content markedly increased to 76.60%, indicating that the remaining material was predominantly pure cellulose with minimal lignin residues. These findings align with previous reports by (Kustantinah et al., 2023), which documented seaweed ADF values typically ranging

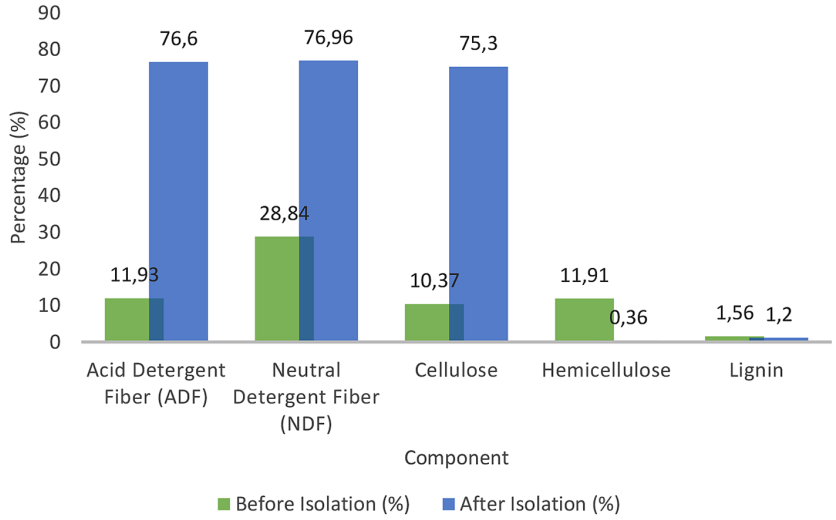

Figure 3. Comparison of lignocellulose content before and after isolation

from 5–15%, contingent upon growth environment and harvest season.

Based on Figure 3 neutral detergent fibre (NDF), comprising cellulose, hemicellulose, and lignin, represents the overall structure of plant cell walls that is insoluble in neutral detergent solutions. The initial *Gracilaria sp.* powder exhibited a relatively low NDF content (23.84%), which markedly increased to 76.96% following isolation using the Van Soest method, indicating successful removal of soluble components and enrichment of the cellulose fraction. This result is consistent with (Sismaini et al., 2022), who reported approximately 42% NDF in the synthesis of cellulose acetate from the same seaweed species. Thereby concentrating on cellulose as the primary target for bioplastic-based edible film production. The increase in NDF further highlights the effectiveness of the Van Soest method in eliminating hemicellulose and non-structural compounds (Variables & Methods, 2022). Comparative analysis of cellulose content across different seaweed species, presented in Table 3, demonstrates that the isolated cellulose content of *Gracilaria sp.* in this study is higher than that reported in international literature.

The isolated cellulose from *Gracilaria* sp. (75.3% ADF) significantly exceeds the typical cellulose content of other seaweeds (0.85–34%), highlighting its potential as a high-quality cellulose source (Table 2). The isolation process, comprising washing, hydrolysis, delignification, NaOH bleaching, and drying, yielded pale yellow-white cellulose powder. Cellulose yield, calculated as the ratio of dry cellulose to initial dry seaweed, demonstrates both the efficiency and consistency of the extraction process. The cellulose yield calculated from five replicates is presented in Table 4. These yield values indicate the efficiency of the cellulose extraction process from *Gracilaria sp.* biomass and demonstrate the consistency of results obtained throughout the isolation procedure (Table 3).

Table 2. Comparison of cellulose content in seaweeds

Seaweed species	Cellulose content (%)	Reference
Gracilaria sp. (isolated)	75.3%	Present study
Green seaweeds (Chlorophyta)	1.5–34 (average 9.7)	(Simatupang, 2021)
Red seaweeds (Rhodophyta)	0.85–18 (average 4.75)	(Simatupang, 2021)
Brown seaweeds (Phaeophyceae)	2.2–10.2 (average 7.88)	(Simatupang, 2021)
Ulva lactuca (Green alga)	30.1 (biomass); 90.3 after cellulose extraction	(Tamori & Yamada, 2023)
Caulerpa lentillifera (Green seaweed, Indonesia) *Chaetomorpha aerea* (and other green species)	31.13 36.5–41 (cellulose content range in green seaweeds)	(Romadhan & Pujilestari, 2018) (Machado et al., 2024)
Seaweed cellulose	55.16% (After Delignification)	(Dibyasti et al., 2025)

Table 3. Cellulose yield from *Gracilaria* sp. seaweed

Initial dry seaweed weight (g)	Cellulose weight after isolation (g)	Yield (%)
50.0059	14.0756	28.14
50.0107	17.0487	34.09
50.0130	20.2378	40.45
50.0050	13.5679	27.13
50.0072	18.7890	37.57
Average		33.48

Based on Table 3 cellulose yield from *Gracilaria* sp. ranged from 27.13% to 40.45%, with an average of 33.48%, indicating efficient extraction from the seaweed biomass. The highest yield was obtained in the third replicate, while minor variations among samples likely reflect differences in raw material composition or procedural factors. Overall, the results demonstrate the reproducibility and consistency of the isolation process, producing high-purity cellulose. Several recent investigations have reported wide variability in cellulose recovery depending on species and extraction protocol, with conventional single-step extractions typically producing lower recoveries (10–30%), while integrated biorefinery or optimized alkali/bleaching sequences can boost cellulose recovery into the 30–40% range a pattern that aligns with the yields observed here and suggests efficiency of our extraction conditions (Machado et al., 2024).

Preparation of edible film

The edible film was prepared using cellulose isolated from *Gracilaria sp.*, combined with a 2% (w/v) chitosan solution and various glycerol concentrations (10%, 15%, and 20%) as a plasticizer. The heating and continuous stirring process at 80 °C for 40 min resulted in a homogeneous and viscous film-forming solution, indicating effective dispersion and interaction among the film components. The film-forming solution was cast onto a clean, flat Petri dish (90 mm diameter) to ensure uniform thickness and consistent film formation. The cast solution was initially dried in an oven at 60 °C, followed by air drying at room temperature to allow gradual solvent evaporation. After the drying process, the film was easily peeled off from the surface of the Petri dish, demonstrating good film-forming ability and sufficient mechanical integrity for handling and further testing. Visual observation revealed that the resulting edible films exhibited a yellowish and translucent appearance. An increase in film flexibility was observed with increasing glycerol concentration. Films prepared with lower glycerol content (10%) appeared stiffer and more brittle, whereas higher glycerol concentrations (15% and 20%) produced films that were more flexible and elastic. This behavior is consistent with the role of glycerol as an effective plasticizer, which reduces intermolecular hydrogen bonding between polysaccharide chains, thereby enhancing molecular mobility and film flexibility (Yanti et al., 2023). These observations provide preliminary qualitative evidence of the influence of glycerol concentration on the physical properties of the edible film, which was further quantified through mechanical and structural characterization. The overall procedure for edible film preparation is illustrated in Figure 4.

Edible film characterization

The edible films produced from cellulose isolated from *Gracilaria* sp. were successfully formed and subjected to comprehensive characterization. The evaluation of physical, mechanical, and antibacterial properties was conducted to establish the relationship between film structure, composition, and functional performance. These measurements were conducted to assess the functionality and performance of the film for active food packaging applications.

The thickness of edible film

Film thickness is critical in determining the suitability of edible film as food product packaging because thickness significantly affects other physical and mechanical properties of edible film, such as tensile strength, elongation, solubility, and water vapor permeability. A

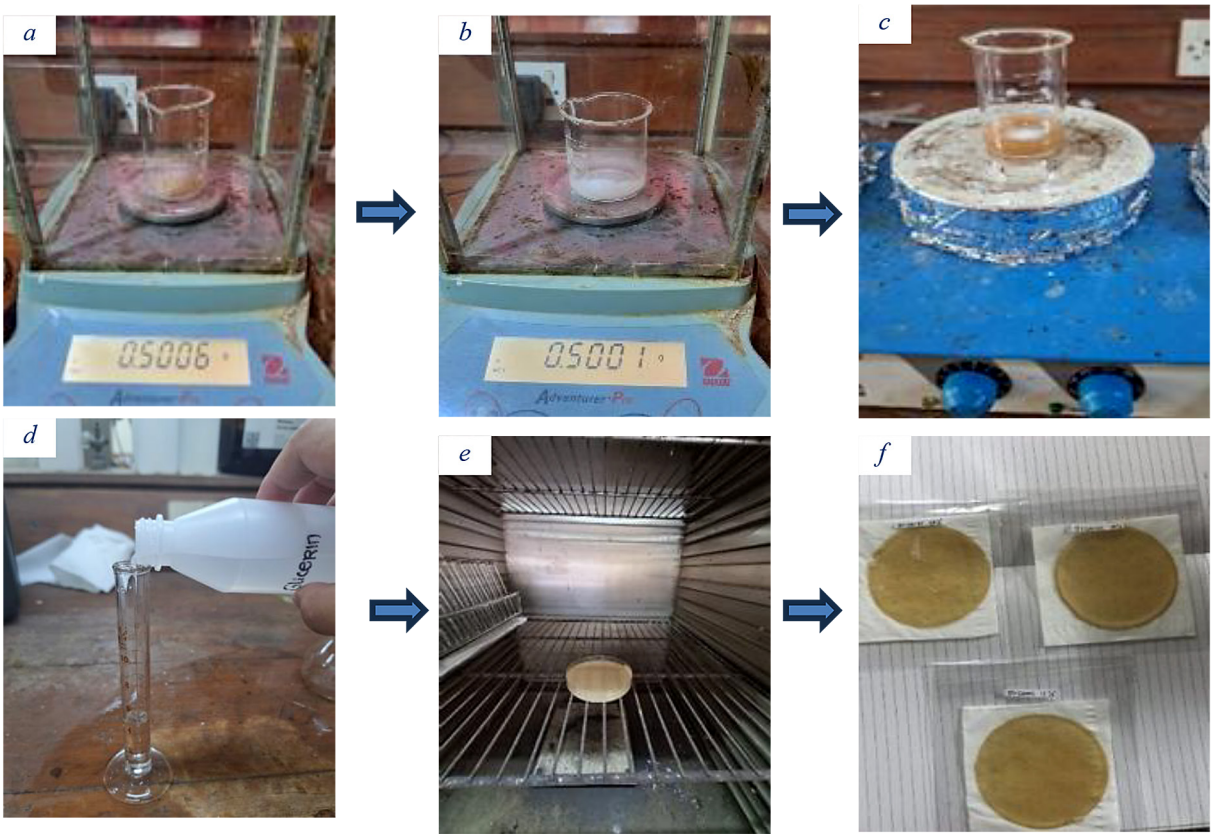

Figure 4. Edible film preparation process: (a) weighing of cellulose, (b) weighing of chitosan, (c) heating of the cellulose–chitosan mixture, (d) addition of glycerol, (e) further heating of the film-forming solution, and (f) resulting edible film

thicker edible film will better protect packaged food products, but its water vapor permeability will be lower. Thickness is a characteristic of edible film influenced by the uniformity of solution volume, mold size, and number of solids. Thickness determination is carried out using a caliper on the five sides of the sample as a representative size of the entire sample. Based on the Japanese Industrial Standard (JIS), the maximum thickness of edible film is 0.25 mm. Film thickness significantly affects tensile strength and elongation, whereas a thick edible film will better protect packaged food products (Widodo et al., 2019).

Increasing the concentration of chitosan and glycerol tends to affect the increase in the thickness of the edible film. Increasing glycerol concentration in the manufacture of edible film causes an increase in dissolved solids in the solution forming the edible film, so the resulting thickness increases. The mold size and volume of the sample poured into the mold are uniform, so these factors do not affect the thickness of the edible film obtained. Based on the data in the Figure 5, it shows that the thickness of the edible film produced using several concentrations of glycerol, for a concentration of 10%, a thickness of 0.152 mm was obtained, a concentration of 15% glycerol obtained a thickness of 0.174 mm, a concentration of 20% obtained an edible film thickness of 0.186 mm. These thicknesses meet JIS standards, so the application of edible film will be more effective.

Solubility of edible film

The solubility of edible film is significant. Solubility is a benchmark for edible films to dissolve when consumed. Film solubility in water is an essential property in determining food products; films with low water solubility are needed to provide waterproof properties and can increase the shelf life of food products. The resulting edible film is expected to have low absorption, so using supporting materials, namely chitosan and plasticizers, chitosan significantly affects solubility (Arif et al., 2022). Mostly in food product applications,

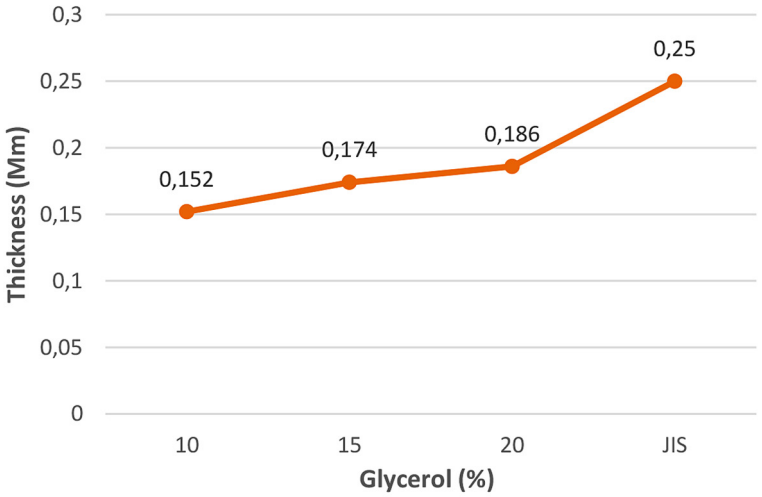

Figure 5. Thickness of edible film

films with low water solubility are needed to provide waterproof properties and can increase the shelf life of food products. Conversely, packaging films are designed to dissolve easily in water for some food products before consumption (Sanyang et al., 2016) (Figure 6).

The solubility of the *edible film* produced in this study is relatively high, around 92.58% for a glycerol concentration of 15%. The solubility for a glycerol concentration of 10% is around 80.17%, and for a glycerol concentration of 20%, it is around 91.24%. It was reported that the increase in film solubility with increasing plasticizer concentration is due to the hydrophilic nature of the plasticizer, which can increase the solubility of the film in water (Bourtoom, 2008). The high solubility value of the Edible film produced indicates that the *edible film* degrades quickly in nature and can be used as primary packaging for ready-to-eat foods with low water activity, because when consumed, the *edible film* is easily dissolved.

Tensile strength of edible film

Tensile strength is a measure of the maximum tensile force that a material can withstand when stretched or pulled before the film breaks or tears. A higher applied force indicates a greater tensile strength. Edible films with high tensile strength are able to effectively protect packaged products from mechanical damage (Sismaini et al., 2022). The results of the tensile strength test are presented in Figure 3 and 4. The optimum tensile strength of the edible film with the addition of chitosan and glycerol was obtained at a glycerol concentration of 10%, yielding a tensile strength of 0.46 MPa. At glycerol concentrations of 15% and 20%, the tensile strength decreased to 0.28 MPa and 0.15 MPa, respectively. Cellulose plays an important role in determining the tensile strength due to its good interfacial adhesion, which promotes the formation of strong hydrogen bonds between the hydroxyl (O–H) groups of starch and the hydroxyl (O–H) groups of cellulose.

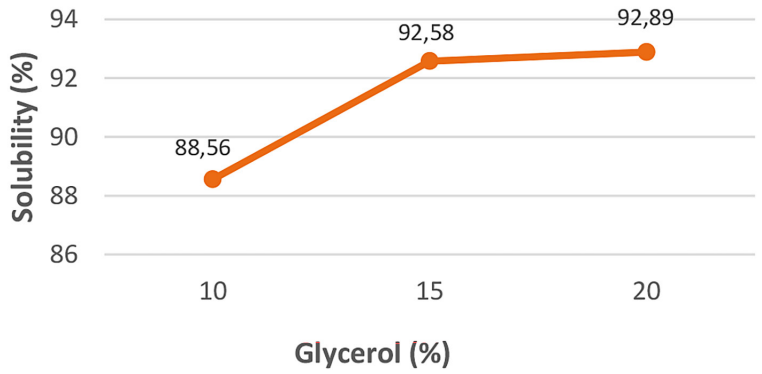

Figure 6. Solubility of edible film

Based on Figure 7 the addition of glycerol as a plasticizer functions to enhance the flexibility of the film. However, increasing the glycerol concentration enlarges the distance between polymer chains, which leads to a reduction in intermolecular interactions (such as hydrogen bonding) within the film matrix. Consequently, the tensile strength of the film decreases.

Percent elongation

Percent elongation on edible film aims to determine the ability of edible film elongation, where the results are in the form of a percentage that shows changes in the length of the edible film when pulled until it breaks. The higher the elongation value, the more flexible the edible movie will be. The results of the percent elongation of edible film based on variations in glycerol concentration are shown in Figure 6.

Based on Figure 8 a significant effect of glycerol concentration on the elongation at break of the edible film was observed. The highest elongation value was obtained at a glycerol concentration of 20%, reaching 29.21%, followed by 10% with 26.38%, while the lowest elongation was recorded at 15% glycerol with a value of 17.53%. The increase in elongation at the 20% glycerol concentration indicates that glycerol effectively acts as a plasticizer, enhancing the flexibility of the polymer network. Glycerol reduces intermolecular attractive (Kajla et al., 2024) forces within the polymer matrix, resulting in a more flexible film structure that is better able to withstand deformation before failure. The increase in elongation percentage with increasing glycerol concentration up to a certain level has been widely reported in studies on biopolymer-based edible films, including those incorporating cellulose and chitosan (Kajla et al., 2024). Glycerol is able to reduce the intermolecular attractive forces among cellulose polymer chains by inserting itself between these chains, thereby creating intermolecular spacing and rendering the film more flexible and less prone to cracking. These results are consistent

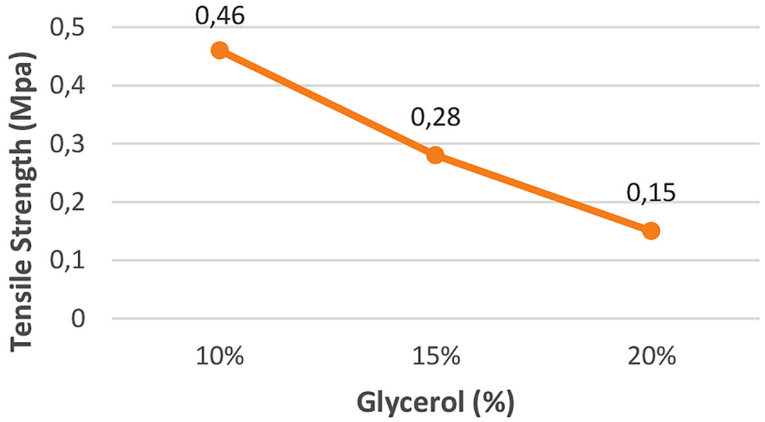

Figure 7. Tensile strength of edible film

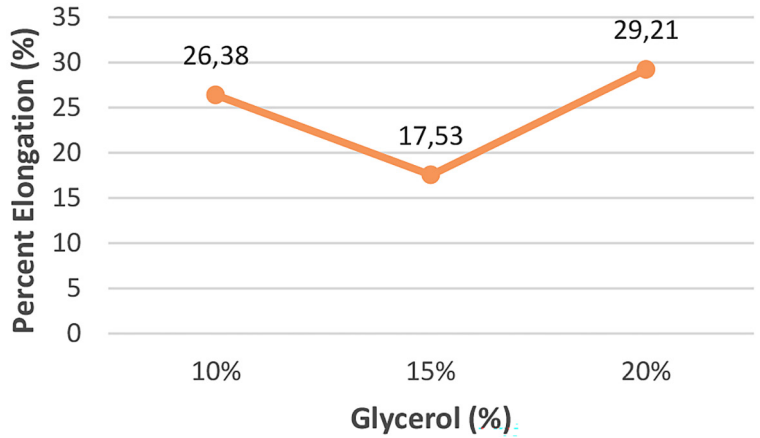

Figure 8. Percent elongation of edible film

with the findings of (Wahab et al., 2023) who also investigated the characteristics of edible films with glycerol addition using carrageenan as the base material. In their study, an elongation value of 27.57% was reported. Compared to that study, the elongation value of the *Gracilaria* sp.-based edible film at a glycerol concentration of 20% in the present study demonstrates superior flexibility performance. This indicates that the use of cellulose derived from *Gracilaria* sp., modified with glycerol, produces a more plastic film structure than carrageenan-based edible films. Overall, the edible film developed in this study not only meets the minimum elongation standard specified by the Japanese Industrial Standard (JIS), namely >10%, but also exhibits high potential for application as a flexible packaging material for food products.

Biodegradability of edible film

Biodegradability is conducted to determine how quickly and efficiently the film can be biodegraded by microorganisms. The selected biodegradability test uses soil as an adjuvant to the degradation process. The ability to degrade the film in the environment is one of the characteristics of edible film as a biodegradable packaging. The degradation process of the sample was carried out using visual sensory observation every day until the edible film was utterly degraded. The standard for measuring biodegradability analysis is that the faster the plastic decomposes, the better the quality of the edible film. The results of the biodegradability analysis of *Gracilaria* sp cellulose with the addition of chitosan and glycerol are shown in Figure 9.

The biodegradability results of edible films composed of *Gracilaria sp.* cellulose, chitosan, and varying glycerol concentrations (10%, 15%, and 20%) are shown in Figure 8. Biodegradability increased with increasing glycerol concentration, from 8 days at 10% glycerol to 9 days at 15%, and reaching 11 days at 20%. This enhancement in biodegradability is attributed to the plasticizing effect of glycerol, which weakens intermolecular interactions within the polymer matrix, resulting in a more flexible structure with increased porosity. The more open film structure facilitates the penetration of microorganisms and degrading enzymes, thereby accelerating biological degradation. Similar effects have been reported for chitosan–cellulose-based edible films (Luchese et al., 2018) Morphological changes observed after biodegradation testing included surface roughness, cracking, pore formation, soil penetration, and discoloration, consistent with general biodegradation behavior of bioplastics Additionally, glycerol increased film hydrophilicity and water absorption, leading to higher moisture content that promotes microbial activity. The combined presence of cellulose, chitosan, and glycerol thus enhances the biodegradability of the edible film, highlighting its potential as an environmentally friendly food packaging material.

Fourier transform infrared

Functional group testing was performed with an infrared spectrophotometer (Figure 10). This analysis aims to compare the functional groups from the cluster test to identify the functional groups of the constituent materials contained in the edible film.

The results of the identification of functional groups on the graph of almost all samples show the same spectrum, based on the results of absorption at wavelengths 3800–2700 cm^{-1}, identical to the hydroxyl O-H functional group, this

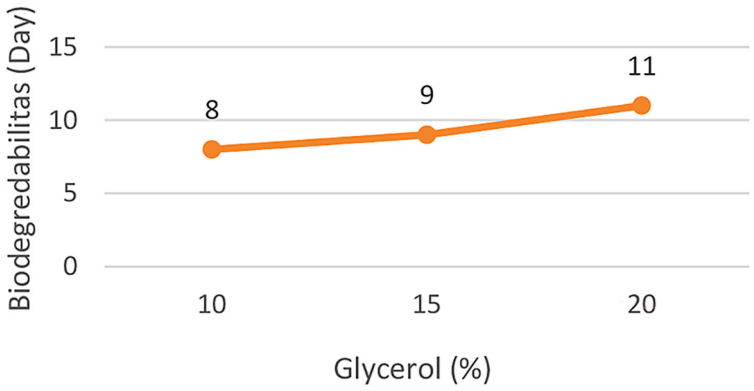

Figure 9. Biodegradability of edible film

group plays a vital role in the formation of hydrogen bonds, which are crucial in the formation of polymer matrix and mechanical properties of the film. 3000–2850 cm^{-1} wavelength absorption is identical to the aliphatic C-H functional group, indicating the presence of carbonyl groups (Table 4). These groups may come from amide groups in chitosan or other contaminants. The 1850–1600 cm^{-1} wavelength absorption is identical to the C=O carbonyl functional group, and the 1300–1800 extended absorption with the C-O carboxyl functional group; this group is likely to come from free carboxyl groups in chitosan or the oxidation of hydroxyl groups. The wavelength value obtained from the FTIR results shows the suitability of the research (Vázquez et al., 2021). The main groups of cellulose constituents are O-H, C-O, and C-H groups, which show typical absorption. Adding chitosan and glycerol solution causes a shift in wavelength, indicating the presence of polymer content through physical interactions (Zhou et al., 2022).

Scanning electron microscope

SEM characterization aims to determine the surface morphological structure and pore size formed on the edible film. A good edible film is an edible film that has a homogeneous surface. The homogeneity of edible film affects its physical and mechanical properties. Testing the characteristics of edible film with scanning electron microscopy (SEM JSM6510LV, JEOL, Japan) is used to analyze the surface and morphology of the edible film. The results can be seen in the picture of edible film morphology, where each cellulose-chitosan and glycerol ratio is very uneven; this is due to the edible film solution being less homogeneous because cellulose does not dissolve in organic solvents, resulting in an inhomogeneous plastic surface.

SEM test results on the surface of edible film with the addition of cellulose and chitosan 0.5 g without the addition of plasticizer show an uneven surface (Figure 11a); the presence of crystals formed due to cellulose not entirely dissolved during stirring. This is because the edible film solution from the mixture of each component produced is still less homogeneous. Figure 11b, with a cellulose composition of 0.5 g with the addition of 2% chitosan and glycerol with a concentration of 10%, shows that the Edible film surface is less smooth and porous, and the number of indentations causes the solution to be less homogeneous during stirring. Figure 11c, with a cellulose composition of 0.5 g with the addition of 2% chitosan and 15% glycerol concentration, shows many indentations and bubbles caused by the lack of homogeneity in the mixing process, where stirring, drying time, and temperature are less precise. Meanwhile, Figure 11d shows that the edible film surface is getting tighter due to adding more glycerol concentration, i.e., 20% glycerol molecules enter the crevices of the chitosan polymer

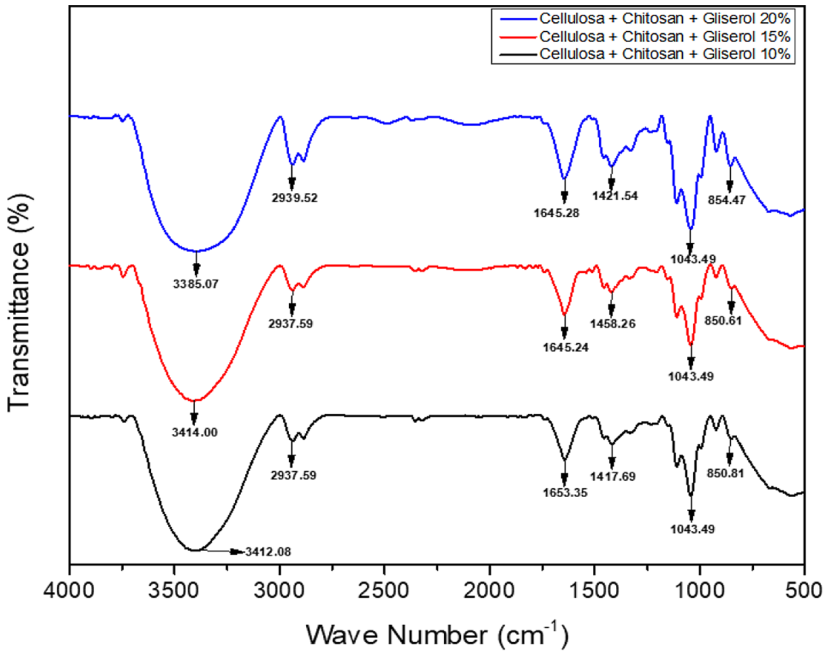

Figure 10. Comparison of FTIR spectra of edible film

Table 4. Results of functional group analysis of cellulose+chitosan and glycerol

Function group	Wave number (cm^{-1})			
	Wave number range (cm^{-1})	Cellulose + chitosan + glycerol 10%	Cellulose + chitosan +glycerol 15%	Cellulose + chitosan +glycerol 20%
O-H	3800-2700	3412.08	3414.00	3385.07
C-H	3000-2850	2937.59	2937.59	2939.52
C=O	1850-1600	1653.35	1645.24	1645.28
C-O	1300-1800	1447.69	1458.26	1218.48

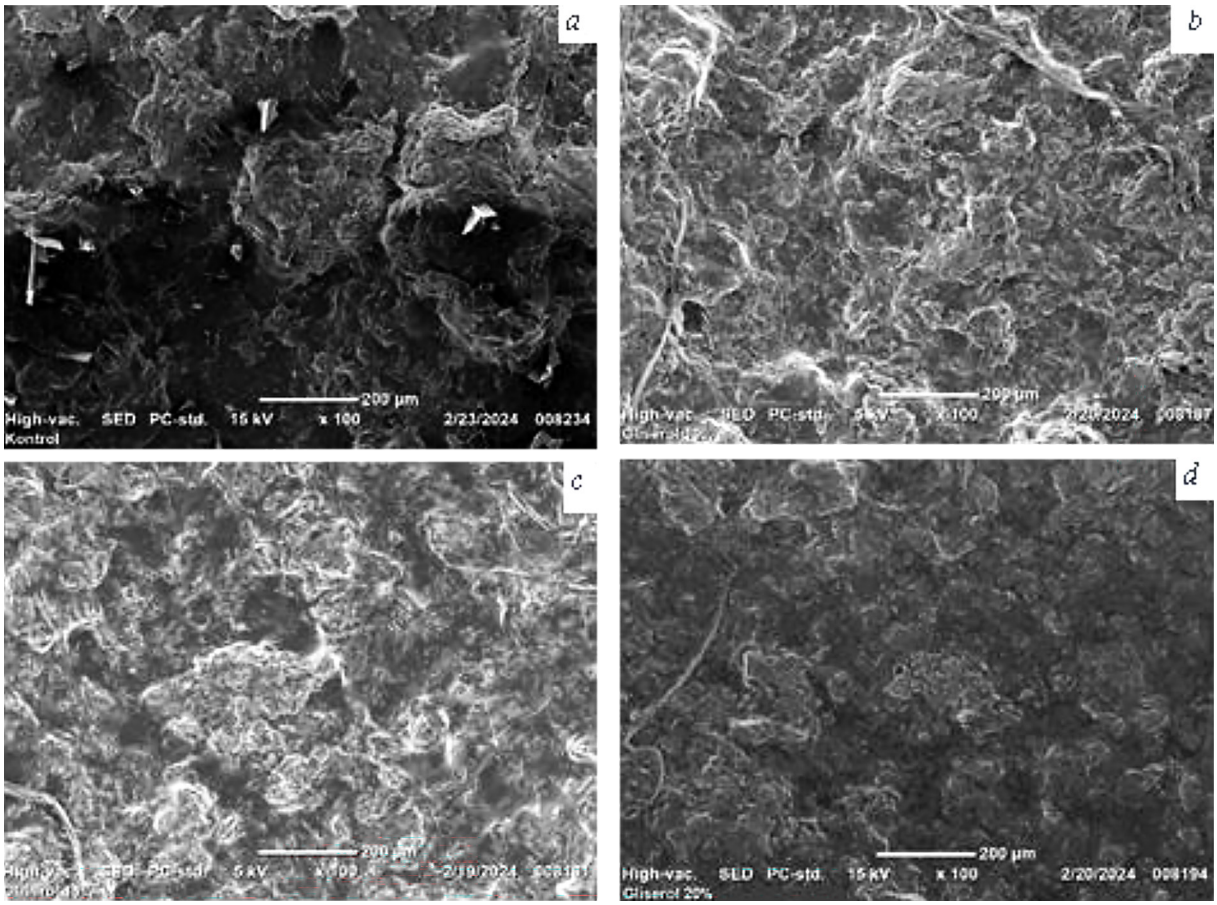

Figure 11. Surface morphology of (a) control (cellulose+chitosan), (b) edible film with cellulose+chitosan+glycerol 10%, (c) edible film with cellulose+chitosan+glycerol 15%, (d) edible film with cellulose+chitosan+glycerol 20%

molecular chain evenly. The addition of 20% glycerol shows that, in addition to glycerol functioning as a gap filler between polymer molecular chains, it also functions to form a layer on the surface of the resulting edible film.

The water-insoluble nature of cellulose results in a less homogeneous surface. There are cracks on the surface of the edible film, indicating that the cellulose size is still too large, so the cellulose particles in the starch matrix are not tightly bound. Research states that the absence of a tight structure in these cracks causes more water to be absorbed. It is also seen that there are still bubbles on the surface of the edible film. This can also occur due to clumps of cellulose that do not dissolve in the process. The presence of indentations and bubbles results in the ease of water entering the edible film sample, making it more straightforward to decompose and easier regarding water absorption. Indentations will also result in a smaller tensile strength and elongation value because cavities in the edible film cause the centre of the edible film to be easily detached (Dewati et al., 2023).

Antibacterial activity of edible film

Antibacterial properties refer to the ability of a substance or material to inhibit or kill bacterial growth (Tampubolon et al., 2023). Cellulose contained in seaweed is not intrinsically antibacterial (Putri et al., 2020). However, seaweed can have antibacterial properties due to the presence of particular compounds (Azzahra & Trimulyono, 2023). Several studies have shown that some types of seaweed contain bioactive compounds such as polysaccharides, polyphenols, and antimicrobial peptides that have potential antibacterial activity against several types of bacteria. The antibacterial test in this study used a variety of concentrations, using a positive control of chloramphenicol, to obtain data and documentation in the following Table 5 and Figure 12.

Based on Table 5 the antibacterial activity test results of the edible film show an increase in the diameter of the inhibition zone, particularly against *Escherichia coli*, reaching 14.8 mm at a concentration of 20,000 ppm. This value exceeds that of chloramphenicol used as the positive control (10.0 mm). The positive control confirms the reliability of the testing procedure, while negative controls – such as plastic and solvent (2% acetic acid) – were employed to ensure that bacterial inhibition originated from the active components of the film rather than from the matrix or solvent (Glebko et al., 2025). These findings support the potential of the edible film as an effective antimicrobial active packaging solution for extending the shelf life of food products (Ahmed et al., 2024). Several studies have reported that certain types of seaweed contain bioactive compounds, including polysaccharides, polyphenols, and antimicrobial peptides, which exhibit antibacterial activity against various bacterial strains (Asmawati et al., 2023). Mechanism of antibacterial action of chitosan. Chitosan exhibits multiple mechanisms in inhibiting bacterial growth. Low-molecular-weight chitosan can penetrate bacterial cells, bind to DNA and RNA, and inhibit transcription and protein synthesis (Amorim et al., 2022). Adding chitosan improved the film matrix structure, enhanced mechanical properties, and introduced antimicrobial functionality (Pardan et al., 2025). Films containing chitosan generally display enhanced antibacterial activity, as chitosan is known to disrupt bacterial cell membrane integrity and increase membrane permeability. In addition, chitosan can bind to electronegative substances within bacterial cells, interfere with physiological activities, and ultimately lead to bacterial cell death. Consequently, all chitosan-based films demonstrate antibacterial activity (Abral et al., 2021). Overall, the role of glycerol in antibacterial activity is primarily supportive rather than acting as an antibacterial agent itself. Moreover, cellulose-based films have been shown to exhibit antimicrobial activity, particularly when combined with other biopolymers such as chitosan (Yanti et al., 2023; Yanti et al., 2023). Glycerol enhances the effectiveness of antibacterial components (e.g., chitosan and cellulose) by improving the physical properties of the film, facilitating the release of active compounds, and ensuring that the film remains effective against bacterial growth.

Table 5. Antibacterial activity test in the inhibition of *E. coli*

Sampel	Inhibition zone diameter
	E. coli
Control (+) kloramfenikol	10.0
Control plastik	0.0
Kontrol (-) CH_3COOH 2%	7.6
Edible film 10.000 ppm	11.5
Edible film 15.000 ppm	14.6
Edible film 20.000 ppm	14.8

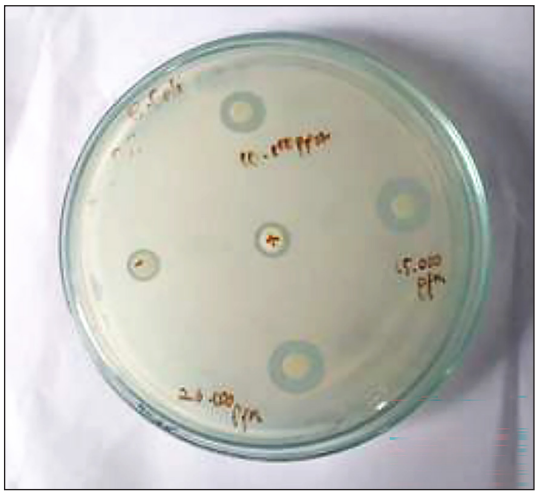

Figure 12. Antibacterial activity of the edible film against *E. coli*

CONCLUSIONS

Cellulose was successfully isolated from *Gracilaria sp.* through hydrolysis, delignification, and bleaching processes, as confirmed by FTIR analysis and Van Soest fractionation, demonstrating a substantial increase in cellulose content and effective removal of non-cellulosic components. These results confirm the feasibility of *Gracilaria sp.* as a sustainable and renewable source of cellulose for edible film production. The developed edible films exhibited favorable physical, mechanical, and functional properties, including acceptable thickness, enhanced tensile strength and elongation after formulation optimization, high water solubility, and complete biodegradability within a short period. Structural analysis confirmed the successful incorporation of cellulose and chitosan within the film matrix, while antimicrobial testing demonstrated activity against *Escherichia coli*, highlighting the potential of the films as active food packaging materials. Overall, this study provides clear evidence that edible films derived from *Gracilaria sp.* cellulose, in combination with chitosan and glycerol, meet essential requirements for eco-friendly food packaging applications and offer a promising alternative to conventional synthetic packaging materials. Future research should focus on expanding the range of seaweed sources and evaluating the performance of these films in real food packaging systems to further optimize their industrial applicability.

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