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Formulating cellulose-based edible films from seaweed with chitosan fortification as antimicrobial food packaging test innovation

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ABSTRACT

Edible film is the latest innovation in food packaging that is both consumable and environmentally safe. Edible film is crucial in food safety because it can protect against oxidation, moisture loss, and microbial contamination, thereby extending the shelf life of food. The characteristics of edible film will determine the final quality of its application to food products. The purpose of this study was to determine the characteristics of edible films made from seaweed cellulose with varying concentrations (3%, 5%, and 7%) and the addition of chitosan. This research was conducted in two stages. The first stage was the isolation of cellulose from seaweed. The isolated cellulose was then analyzed using FTIR. At this stage, the best cellulose was obtained as the basic material for edible film. The second stage is the manufacture of edible film with variations in cellulose concentration (3%, 5% and 7%) and the addition of glycerol and chitosan. The edible film obtained was then characterized in terms of its thickness, solubility, water vapor transmission rate, biodegradability, and organoleptic and antimicrobial properties. In the antimicrobial test, the test bacteria used were *Escherichia coli* and *Staphylococcus aureus*, while in the antifungal test, the test fungus used was *Aspergillus flavus*. The results of this study showed that the cellulose obtained exhibited the presence of hydroxyl groups (-OH), a characteristic of cellulose. The edible film characteristics showed optimal thickness at a cellulose concentration of 7%. Regarding solubility and water vapor transmission rate, the optimal concentration is 3% cellulose. Furthermore, in the biodegradation test, the observation results show that it takes between 12 days for all edible films to degrade. The antimicrobial test demonstrated activity that inhibited the growth of bacteria and fungi on the edible film.

Keywords: edible film; cellulose; seaweed; chitosan; antimicrobial.

INTRODUCTION

Plastic waste is a significant contributor to environmental pollution in most metropolitan areas (Zhou et al., 2023). An imbalance in waste management leads to environmental contamination and other detrimental effects (Růžičková et al., 2023). An effective method for minimizing plastic waste commonly associated with food packaging is the development of edible films composed of biodegradable materials (Sheikh et al., 2023). These films represent one of the safest options for food packaging, as they are naturally degraded by microbes into environmentally benign substances (Capar, 2023).

Improving the quality of edible films requires careful selection of appropriate base materials. Research has demonstrated that various natural polymers, including proteins, polysaccharides, lipids, and composite materials, have

been effectively utilized as base ingredients in the production of edible films (Ebrahimzadeh et al., 2023). Seaweed is a common polysaccharide used in edible films due to its low cost, availability, biodegradability, and non-toxic nature (Chaudhary, 2023). *Eucheuma* sp. is a type of seaweed commonly found in Indonesia, contains a substantial amount of cellulose in its biomass. Previous studies have reported that the cellulose content in *Eucheuma* sp. ranges from 35.8% to 38% of its dry weight (Smith et al., 2021). In addition, the residual biomass remaining after carrageenan extraction has been found to contain more than 50% crystalline cellulose (Kim et al., 2022), highlighting its strong potential as a sustainable cellulose source for biopolymer development (Rahmawati et al., 2023). This cellulose is an ideal material for producing edible films because of its biodegradability (Jumadi et al., 2023). Recent developments in edible film research have focused on utilizing cellulose derived from sustainable natural products, including *Eucheuma* sp. seaweed as a base materials (Romao, et al., 2022; Kong, et al., 2024).

Additional components, such as chitosan, are required to enhance the flexibility and durability of edible films. Chitosan has been recognized for its strength, durability, and resistance to tearing (Yuan et al., 2021). In addition, its antimicrobial properties, including both antibacterial and antifungal activities, are vital in enhancing the overall quality of the edible films produced (Elsherif et al., 2024).

The addition of plasticizers is essential for enhancing the elasticity and durability of edible films (Mukherjee et al., 2024). Glycerol, a commonly used plasticizer, reduces internal hydrogen bonding and interacts with amylopectin molecules, providing the desired flexibility due to its low molecular weight (Latif et al., 2024).

Edible films are primarily used as food packaging in the food industry. In south Sulawesi, *wajik*, a traditional delicacy, is typically wrapped in plastic, dried banana leaves, or corn husks. Previous research has demonstrated the potential of using edible films made from potato starch for *dodol* traditional food packaging to extend its shelf life and offer a more environmentally friendly alternative (Agustini et al., 2023). Therefore, researchers are interested in utilizing *wajik* as one of the traditional food products that will be packaged using edible film.

Given these insights, researchers must focus on developing a product that utilizes cellulose from *Eucheuma* sp. seaweed, fortified with chitosan, and possesses both antifungal and antibacterial properties. This innovative edible film would serve as an eco-friendly food packaging solution while maximizing the utilization of seaweed resources in Indonesia, particularly in south Sulawesi.

MATERIALS AND METHODS

This research is divided into four stages: producing edible film from cellulose extracted from seaweed and the addition of chitosan; analyzing the characteristics of the edible film; performing FTIR spectrophotometric analysis; conducting SEM morphological analysis; evaluating the biodegradability of the edible film; and analyzing the antimicrobial properties of the edible film.

Sampling

Eucheuma sp. was collected in Ujung Baji village, Sanrobone district, Takalar regency, south Sulawesi, Indonesia, during the rainy season. The species identification was carried out by the Center of Excellence for Development and Utilization of Seaweed, Hasanuddin University (CEDUS-UNHAS), and was registered under voucher ID No. 03/UN4.PUI-P2RL/AD/III/2024.

Preparation of seaweed

The preparation of *Eucheuma* sp. for cellulose isolation began by thoroughly washing the fresh seaweed, followed by sun-drying for approximately six days. The dried *Eucheuma* sp. was then cut into small pieces, ground into powder, and sieved using a 100-mesh screen to obtain seaweed powder (Jumadi et al., 2023).

Isolation of cellulose from seaweed

About 50 g of dried seaweed powder was weighed and hydrolyzed using 3.5% HNO_3 at 120 °C, followed by neutralization to produce a precipitate (Tang et al., 2024). The precipitate was then delignified using a 2% NaOH solution in a 1:10 ratio at 120 °C for 1 hour in an autoclave (Ni et al., 2024). Afterwards, the precipitate was bleached with 10% H_2O_2, heated, and

then neutralized with distilled water. The final product was dried and characterized using FTIR analysis (Li et al., 2023).

Preparation of edible film

Chitosan 5% (w/v) and glycerol solution 15% (v/v) were mixed and stirred with a magnetic stirrer for 10 minutes. Cellulose from seaweed was added in variations of 3%, 5%, 7% (w/v) and then homogenized. The film solution was poured as much as 30 mL into a 25 × 250 mm petri dish and dried for 24 hours at 50 °C to obtain an edible film (Cazón et al., 2024).

Characteristics of edible film

The characteristics of edible films can be evaluated through various tests, including thickness measurement, solubility, water vapor transmission rate, FTIR, SEM, and biodegradability (Yang et al., 2024).

Thickness analysis

The thickness of the sample was measured using a caliper at seven different points, with three measurements taken at each location. The recorded values for each test sample were documented according to the caliper scale, and the results are expressed in millimeters (mm) (Natsir et al., 2022). Each sample was tested in triplicate.

Solubility analysis

This study aimed to assess the solubility of the film in water. The percentage of film solubility indicates the portion of the film that dissolves in water after being immersed for 24 hours. Samples measuring 3 × 3 cm were placed in pre-dried aluminum cups that had been weighed beforehand. Subsequently, the film samples were dried in an oven at 100 °C for 30 minutes (Jannatamani et al., 2024). The initial dry weight (W0) was recorded. After this, the samples were immersed in water for 24 hours. Any undissolved film was subsequently removed, dried in an oven at 100 °C for 2 hours, and then stored in a desiccator for 10 minutes. Finally, the weight of the dry sample after soaking (W1) was measured with analytical balance (De Moura et al., 2011; Mojo-Quisani et al., 2024). Each sample was tested in triplicate. The solubility percentage of the sample in water was calculated using Equation 1.

$$S = (W_0\text{-}W_1)/W_0 \cdot 100\%\text{S} = \frac{\text{W0-W1}}{\text{W0}} \cdot d \quad (1)$$

where: S – sample solubility, W_0 – initial sample weight, W_1 – final sample weight

Water vapor transmission rate analysis

The water vapor transmission rate (WVTR) was evaluated using the gravimetric method. Edible film samples measuring 6 × 6 cm were affixed to glass containers holding 2 g of silica gel using double-sided tape. These containers were then placed in a desiccator containing a 1 N NaOH solution for 6 days. After the exposure period, the containers were weighed using an analytical balance. The increase in the weight of the silica gel, resulting from absorbed moisture, was used to calculate the WVTR of the edible film. (Rawat and Saini, 2024). Each sample was tested in triplicate. The water vapor transmission rate was calculated using Equation 2.

$$WVTR = (Weight\ B - Weight\ A)/(A \times t) \quad (2)$$

where: *Weight A* – initial weight of edible film (gram), *Weight B* – weight of beaker glass + silica gel after deviation (gram), A – surface area of edible film (m^2), t – time (minutes).

Statistical analysis

The characteristics of edible film were evaluated using data presented as the mean ± standard deviation (SD) from three replicates (n = 3). Before conducting statistical analysis, the normality of the data was examined using the Shapiro-Wilk test to determine whether the data followed a normal distribution. If the data were normally distributed, a one-way analysis of variance (ANOVA) was carried out. In contrast, if the data did not meet the normality assumption, the non-parametric Kruskal-Wallis test was used. A p-value less than 0.05 was considered statistically significant (Li et al., 2025).

FTIR analysis

FTIR spectrum analysis of the sample was carried out using a SHIMADZU Fourier Transform Infrared Spectrometer. Measurements were taken in the spectral range of 4000–5000 cm^{-1},

and the resulting data were analyzed using Omnic 8.1 software (Qi et al., 2024a).

Morphology analysis with scanning electron microscope

Morphological analysis of the top cross-section of the edible film was performed using scanning electron microscope (SEM) (JEOL JSM-6360LA). The edible film sample was mounted on the holder using double-sided adhesive and coated with gold in a vacuum chamber. The sample was then placed in the SEM, and a topographic image was captured at 5000x magnification (Qi et al., 2024a).

Biodegradability analysis of edible film

The biodegradability of the edible films was evaluated by burying 3 × 3 cm samples at a soil depth of approximately 15 cm under uniform conditions for all film variations. Decomposition was observed visually each day, and biodegradability was determined based on the point at which the edible films were fully degraded in the soil. (Capar, 2023).

Application of edible film as a packaging for Wajik typical food of south Sulawesi

The preparation of wajik began by soaking glutinous rice in clean water overnight. The soaked rice was then cleaned and drained before being steamed. Once cooked, the glutinous rice was set aside. Grated coconut was gradually roasted while being stirred, followed by the addition of brown or palm sugar, which was stirred until aromatic and evenly mixed. Granulated sugar was then added and mixed thoroughly. The cooked glutinous rice was subsequently added to the coconut mixture and combined evenly. The small portion of dough was wrapped by edible film prepared beforehand.

Organoleptic analysis

Organoleptic tests were carried out on edible films made from seaweed, with test parameters including color, aroma, and texture, assessed using a hedonic rating method. The hedonic test employed a 5-point scale with the following criteria: (1) dislike, (2) less like, (3) neutral, (4) like, and (5) highly like (Agustini et al., 2023). The test samples were presented randomly. Panelists evaluated the samples, assigned scores, and recorded their assessments accordingly.

Analysis of antibacterial activity of edible film

A total of 3 mL of each sample, including positive controls (Chloramphenicol and commercial plastic), negative control (2% CH_3COOH solution), edible film at 10.000 ppm (before packaging), edible film at 15,000 ppm (before packaging), edible film at 10.000 ppm (after packaging), and edible film at 15,000 ppm (after packaging), was taken using a micropipette. Each sample was prepared in triplicate and mixed with Mueller Hinton Agar (MHA) media until solidified (Hamed et al., 2024). The extraction was then pipetted as much as 30 μL on each positive control (Chloramphenicol), positive control (commercial plastic), negative control (2% CH_3COOH solution), edible film 10.000 ppm (before packaging), edible film 15,000 ppm (before packaging), edible film 10.000 ppm (after packaging) and edible film 15,000 ppm (after packaging) and placed on the disk, then allowed to stand for 5 minutes. After that, the disk on the surface of MHA media using sterile tweezers. Then, the sides of the Petri dish are sealed using a seal or plastic wrap. Then, the samples were incubated at room temperature and observed for 24 hours. Observe and measure the zone of inhibition from triplicate testing (Semsari et al., 2024; Wu et al., 2024).

Analysis of antifungal activity of edible film

The antifungal activity test of edible film begins with marking the Petri dish according to the substance used. potato dextrose agar (PDA) sterilized at 40 °C is poured into 2 Petri dishes provided, each as much as 20 mL, and the PDA solution is allowed to stand for a while so as not to overheat. *A. flavus* fungal suspension was put into each petri dish that already contained 0.2 mL of PDA solution using a 1 cc syringe, and this action was carried out until it reached the 2nd petri dish. Next, the petri dish was shaken to spread and homogenize the suspension with the PDA solution. The petri dish was left to evaporate for a few minutes at room temperature to remove any condensation water, and then it was allowed to stand until solid. Wells were made using 6 mm diameter preparations, a total of 7 wells were aseptically prepared, and the distance between

wells was arranged so that one well was far apart from another well. Wells were made using a preparator with a diameter of 6 mm, a total of 7 wells aseptically, and the distance was arranged so that one well with another was far apart. Positive control (ketoconazole), positive control (plastic), negative control (2% CH_3COOH solution), edible film 10.000 ppm (before packaging), edible film 15,000 ppm (before packaging), edible film 10.000 ppm (after packaging) and edible film 15,000 ppm (after packaging) were put as much as 1 mL into each well in each Petri dish. Incubation was carried out for 2 × 24 hours at 30–37 °C and observed every day. The diameter of the clear zone produced in each well was measured using a caliper. All steps were carried out aseptically, and each sample tested for triplicate (Das et al., 2024).

RESULTS AND DISCUSSION

Sample preparation

To standardize the seaweed's size and increase its surface area for absorption throughout the analytical procedure, it was chopped into tiny pieces and sun-dried. In addition, according to (Bilba et al., 2023), the smaller the sample size, the more that can be extracted and the higher the cellulose content obtained. Samples that have become powder were analyzed for cellulose content first and obtained about 22.97%.

Cellulose isolation from seaweed

Cellulose isolation from seaweed was conducted through several stages. The seaweed underwent hydrolysis with 3.5% HNO_3 to yield lignocellulose, composed of lignin and cellulose. Delignification was then performed to separate lignin from cellulose using 2% NaOH solutions. The bleaching stage utilized 10% H_2O_2, followed by washing with distilled water and drying, resulting in purified cellulose.

Seaweed cellulose content was extracted using the Van Soest method. The computed cellulose, lignin, and hemicellulose compositions are presented in Table 1. The results of the cellulose separation procedure from seaweed indicate that the 2% NaOH delignification process increased the levels of cellulose and hemicellulose while reducing lignin content. Compared to other studies on cellulose isolation – which reported a purity level ranging from 35.8% to 38% these findings suggest that the cellulose obtained from *Eucheuma* sp. has a relatively lower degree of purity (Bilba et al., 2023). The step-by-step process of cellulose isolation from *Eucheuma* sp. is illustrated in Figure 1.

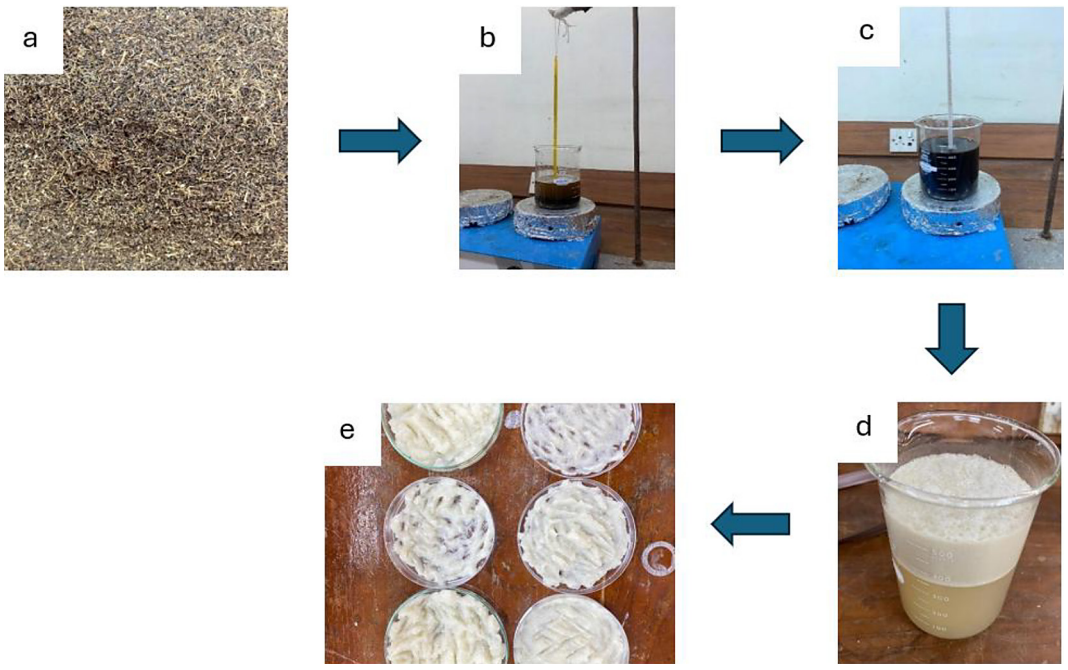

Figure 1. (a) *Eucheuma* sp. seaweed after drying, grinding, and sieving to a particle size of 100 mesh; (b) hydrolysis process; (c) delignification process; (d) bleaching process; e) cellulose isolation result

Table 1. Average value of cellulose content of seaweed

Seaweed composition	Before delignification	After delignification
Cellulose (%)	22.97	55.16
Hemicellulose (%)	3.67	5.82
Lignin (%)	21.39	1.38

Using FTIR to detect characteristic absorption and functional groups present in the material, cellulose produced from seaweed was detected. Figure 2 displays the findings of the cellulose's spectra profile study.

The FTIR spectrum of cellulose extracted from seaweed exhibits several significant peaks when compared to the standard cellulose spectrum (Figure 2). The peaks at wavenumbers 3729.106 cm^{-1} and 3454.114 cm^{-1} indicate the presence of hydroxyl (-OH) groups, which are characteristic of cellulose. The peak at 2911.955 cm^{-1} is associated with C-H stretching vibrations from methyl and methylene groups, while the peak at 1662.567 cm^{-1} represents amide I vibrations, likely due to residual proteins from the extraction process. Additionally, the peak around 1033.961 cm^{-1} indicates C-O stretching, which is a typical characteristic of the cellulose structure. The similarity between the sample spectrum and the standard cellulose spectrum suggests the successful isolation of cellulose from seaweed, with the distinctive cellulose peaks clearly identified. Functional group identification and chemical components for each spectrum, based on peak intensity, are provided in Table 2.

The results presented in Table 2 confirm the successful isolation of cellulose from Seaweed. The comparison between the standard cellulose and the extracted cellulose shows similarities in the spectral peaks, indicating the presence of characteristic cellulose functional groups, such as O-H stretching, C-O-H bending, and C-O stretching in both samples (Raza et al. 2024). The wavenumber values identified through FTIR analysis are consistent with previous research (Purwandi et al. 2022), which also observed similar peaks, such as O-H, C-H, and C-O. Therefore, it can be concluded that the basic structure of cellulose from Seaweed closely resembles that of standard cellulose.

Characterization of edible film

Edible films were produced using varying concentrations of cellulose extracted from *Eucheuma* sp. seaweed (3%, 5%, and 7%). Each formulation was supplemented with 0.5 g of chitosan and a glycerol-based plasticizer to improve mechanical strength and flexibility. The physical

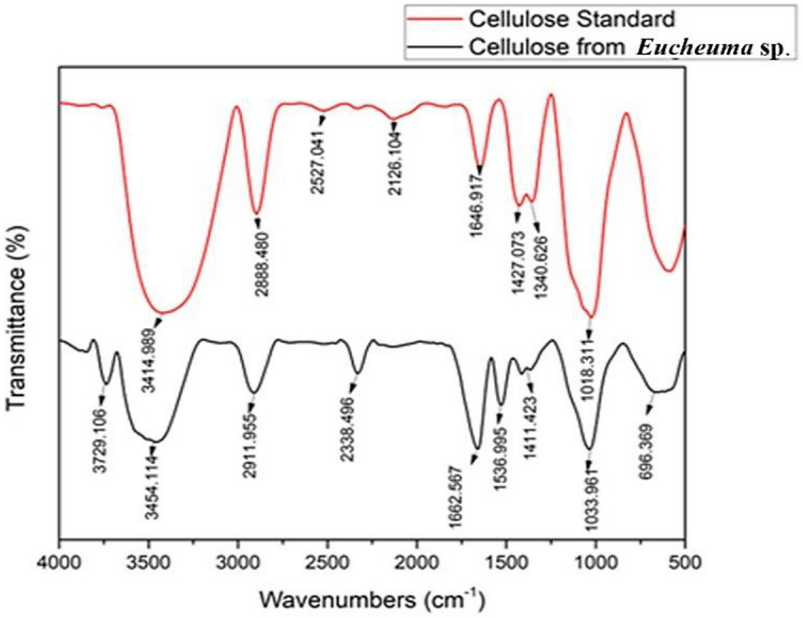

Figure 2. Comparison of seaweed with ordinary cellulose's FTIR spectra

Table 2. Analysis of functional groups from FT-IR spectra of cellulose from seaweed and cellulose standard

Wavenumbers (cm^{-1})		Range (cm^{-1})	Functional group interpretation
Cellulose from seaweed	Cellulose standard		
-	3729.106	3800–3700	O-H (hydroxyl, free)
3414.989	3454.114	3400–3200	O-H strethching vibration
2888.480	2911.955	3000–2850	Sp^3 C-H strethching
2527.041	2338.496	2500–3300	O-H (Carboxylic Acids)
1646.917	1662.567	1680–1620	C=O (Amide I band)
-	1536.995	1600–1450	C=C aromatic aromatic ring
1427.073	1411.423	1440–1000	C-O-H bending
1340.626	1033.961		
1018.311	-	1300–1000	C-O stretching vibration
-	696.369	900–690	=C-H stretchingaromatic ring

and functional properties of the films were evaluated through several characterization techniques, including measurements of thickness, solubility, water vapor transmission rate (WVTR), and biodegradability. Visually, the resulting edible films exhibited a transparent to slightly yellowish appearance, with higher cellulose concentrations producing films that appeared denser and more structurally uniform. The visual results of the edible films derived from *Eucheuma* sp. cellulose can be seen in Figure 3.

Figure 3. Edible film from cellulose of *Euchema* sp.

Thickness

A crucial element impacting gas permeability is the thickness of the edible layer. The thicker the edible layer, the better it preserves the packaged food and the less gas permeability it has (Rawat and Saini, 2024). Figure 4 and Table 3 shows the outcomes of the thickness test of edible film.

According to Figure 4, adding 7% of seaweed cellulose concentration results in the highest level of edible film thickness, while adding 3% concentration results in the lowest level of edible film thickness. These findings are based on experimental data obtained from triplicate measurements. These observations are further supported by the data presented in Table 3, which shows a consistent increase in film thickness with increasing cellulose concentration.

Prior to statistical analysis, the data were first assessed for normality using the Shapiro-Wilk test. The results revealed that the thickness data from all three cellulose concentrations (3%, 5%, and 7%) were not normally distributed ($p < 0.05$) (Grzebieniarz et al., 2023). Therefore, a non-parametric Kruskal-Wallis test was conducted to evaluate whether there were significant differences in film thickness among the formulations (Rahmasari and Yemiş, 2022). The analysis yielded a Kruskal-Wallis H statistic of 31.28 with a p-value of 1.61×10^{-7}, indicating a statistically significant difference in thickness across the different cellulose concentrations. Thus, the null hypothesis (H_0), which states that there is no significant difference in film thickness among varying cellulose concentrations, was rejected. The results support the alternative hypothesis (H_1), affirming that cellulose concentration significantly affects the thickness of the edible films.

This result aligns with the findings of (Natsir et al., 2022), who reported that higher seaweed

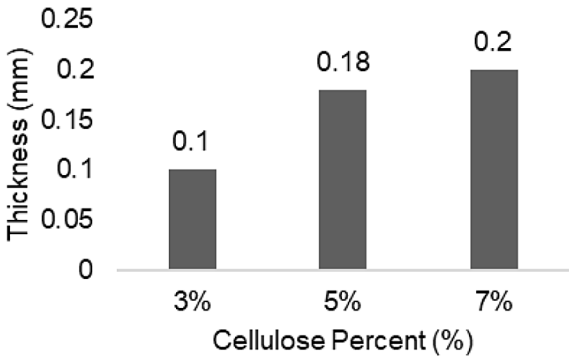

Figure 4. Impact of seaweed cellulose concentration of edible film thickness

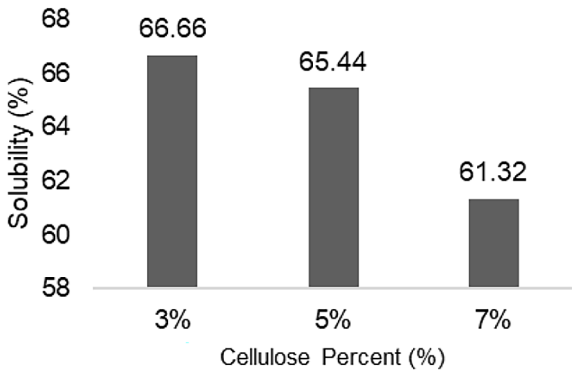

Figure 5. Impact of seaweed cellulose concentration of edible film solubility

cellulose content contributes to increased edible film thickness. Moreover, the variation in thickness also influenced the visual appearance of the films, where thicker films tended to be more opaque and less transparent due to greater material density (Cai et al., 2025).

Solubility of the edible films

One crucial factor in assessing the degree of biodegradation in edible films is solubility. Figure 5 displays the solubility test results of the edible films. To determine whether the differences in solubility across various cellulose concentrations were statistically significant, a one-way ANOVA test was conducted. The results of the analysis, as presented in Table 4.

According to Figure 5, adding 3% seaweed cellulose extraction results in the highest level of edible film solubility, while a concentration of 7% results in the lowest level of edible film solubility. These results were obtained from triplicate measurements conducted to ensure the accuracy and validity of the data. This is consistent with studies conducted by (De Moura et al., 2011), where hydroxyl groups (-OH) are abundant in cellulose, a polymer capable of forming hydrogen bonds. Increasing cellulose concentration enhances the density of hydrogen bonding interactions, thereby reinforcing the film matrix and reducing its water solubility.

Before conducting statistical evaluations, the solubility data were assessed for normality using the Shapiro-Wilk method to verify whether the data followed a normal distribution pattern. Once the normality assumption was satisfied, a one-way ANOVA was applied to analyze differences among the groups (Table 4). The analysis showed a significant variation in solubility across the different cellulose concentration levels, with a p-value of 0.001 ($p < 0.05$). These results suggest that changes in cellulose concentration notably affected the solubility properties of the edible films (Li et al., 2025). Therefore, the null

Table 3. Thickness analysis of edible film from cellulose seaweed *Euchema* sp. based on the Kruskal-Wallis statistical analysis

Samples		Thickness							Average	Mean ± SD	P-value	Note
Cellulose		Side 1	Side 2	Side 3	Side 4	Side 5	Side 6	Side 7				
3%	Single	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.114	0.11 ± 0.008	1.61×10^{-7}	Significant difference
	Duplicate	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.100			
	Triplicate	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.114			
5%	Single	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.214	0.195 ± 0.032		
	Duplicate	0.2	0.1	0.1	0.2	0.2	0.2	0.1	0.200			
	Triplicate	0.2	0.1	0.1	0.3	0.1	0.2	0.2	0.171			
7%	Single	0.2	0.2	0.3	0.2	0.2	0.2	0.1	0.200	0.200 ± 0.054		
	Duplicate	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.200			
	Triplicate	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.200			

Table 4. Solubility analysis of edible film from cellulose seaweed *Euchema* sp. based on the ANOVA statistical analysis

Sample (Edible Film)		EF Dry Weight (g)	EF Wet Weight (g)	Solubility %	Mean ± SD	*P-value*	Note
3%	Single	0.098	0.289	66.18%	66.66% ± 0.011	0.001	Significant difference
	Duplicate	0.099	0.245	65.83%			
	Triplicate	0.095	0.341	67.98%			
5%	Single	0.174	0.496	65.01%	65.44% ± 0.006		
	Duplicate	0.167	0.510	66.17%			
	Triplicate	0.179	0.498	65.15%			
7%	Single	0.252	0.567	61.31%	61.33% ± 0.001		
	Duplicate	0.201	0.601	61.38%			
	Triplicate	0.230	0.598	61.28%			

hypothesis (H_0), which assumes no significant difference in solubility among the various cellulose concentrations, was rejected. Conversely, the alternative hypothesis (H_1), indicating that cellulose concentration plays a significant role in influencing solubility, was statistically supported.(Cai et al., 2025).

Water vapor transmission rate (WVTR) of the edible films

WVTR can be used to determine a product's shelf life. A lower WVTR indicates better barrier properties, which may contribute to extending the product's shelf life (Ebrahimi et al., 2024). When a product experiences less water vapor migration through the edible film coating, the film is more effective in preserving the product over time (Yasmeen et al., 2023). Figure 6 displays the WVTR values of the edible films. Furthermore, the results of the one-way ANOVA analysis, as presented in Table 5.

The WVTR test results indicate that the water vapor transmission rate decreases with increasing cellulose concentration. This occurs because the addition of cellulose enhances the film's resistance to water vapor permeation. The data were obtained from three replicate measurements to improve the accuracy and reliability of the results. Similar to the findings of (Ebrahimi et al., 2024), cellulose possesses a dense and fibrous structure that can restrict the movement of water vapor molecules through the film matrix, thereby reducing the WVTR and improving the film's ability to retain moisture.

Based on the Kruskal-Wallis analysis (Table 5), the differences in WVTR among films with varying cellulose concentrations were statistically significant ($H = 7.20$, $p = 0.0273$; $p < 0.05$). This result indicates that cellulose concentration had a significant influence on the WVTR of the edible films (Grzebieniarz et al., 2023). In this context, the null hypothesis (H_0), which states that there is no significant difference in WVTR between edible films with different cellulose concentrations, was rejected. Conversely, the alternative hypothesis (H_1), which posits that cellulose concentration significantly affects WVTR, was supported by the statistical evidence (Rahmasari and Yemiş, 2022).

The statistical significance observed may be attributed to the cumulative impact of cellulose content on the film matrix structure, possibly affecting its density and permeability. Higher concentrations of cellulose could enhance the compactness of the polymer network, thereby influencing its barrier properties against water vapor transmission. Although only three replicates were used per group, the differences were large enough to be detected by the non-parametric test, which is robust even with small sample sizes and data that deviate from normality assumptions. This finding highlights the measurable role of cellulose concentration in modulating WVTR characteristics of the developed edible films.(Kumar et al., 2025).

Functional group analysis of edible film

FTIR spectroscopy was used to characterize the edible film made from the cellulose of seaweed with chitosan fortification to assess its success. Figure 7 displays the findings of the spectrum profile study of the edible film that was created.

The FTIR analysis of the edible films based on cellulose with varying concentrations of 3%, 5%, and 7%, along with the addition of chitosan,

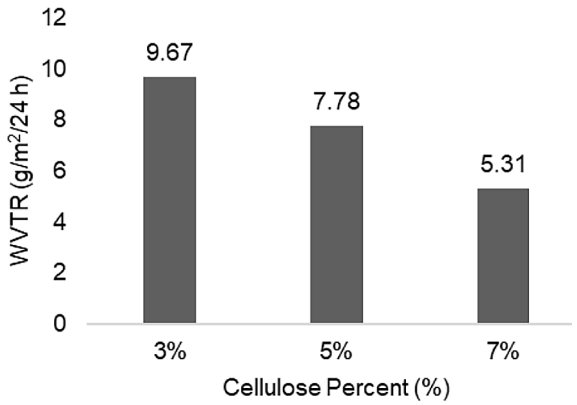

Figure 6. Impact of seaweed cellulose content on the rate at which water vapor diffuses through edible films

revealed differences in several key functional groups. The O-H vibration appeared consistently across all concentrations, with a slight shift at the 7% concentration, indicating an increase in hydrogen bonding. The C-H sp^3 vibration remained constant across all concentrations, while shifts in the C-O peak indicated changes in the interactions between cellulose and chitosan. Differences were also observed in the C-O-H and N-H groups, suggesting alterations in the structure and chemical interactions within the films. The identification of functional groups and chemical components for each spectrum, based on the number and intensity of wave peaks, can be found in Table 6.

Table 6 shows the presence of functional groups O-H, C-H, C-O, C-O-H, and N-H, which indicate chemical interactions between cellulose and chitosan in the edible film. The O-H group represents hydrogen bonding, signifying the interaction between cellulose and chitosan. The C-H group indicates the presence of aliphatic carbon chains within the structure of cellulose and chitosan. The C-O group demonstrates the presence of ether or alcohol bonds, which are part of the cellulose structure. Furthermore, the N-H group is a characteristic feature of chitosan, indicating the presence of amine groups (Qi et al., 2024). The presence of each functional group reinforces the evidence of strong chemical interactions in the formulation of the edible film.

Surface morphology analysis of edible film with SEM

The morphological study of edible films with the highest and lowest permeability values was done using SEM (scanning electron microscopy). The SEM results for edible films under investigation are shown in Figure 8.

The morphological form of 3%, 5%, and 7% edible films, which feature surface indentations and bubbles, is depicted in the above figure. The indentations and bubbles observed are attributed to the uneven dispersion of cellulose, chitosan, and glycerol during the mixing process of the edible film. Water can more readily enter the edible film sample due to bubbles and indentations, which hasten the water absorption rate and eventual degradation (Zhang et al., 2022). Due to cavities in the edible film that allow the film's center to become readily detached, indentations will also cause reduced elongation and tensile strength values. There is also less smoothness in the image. The uneven surface suggests that there is less homogeneity in the film (Qi et al., 2024).

Table 5. WVTR analysis of edible film from cellulose seaweed *Euchema* sp. based on the Kruskal-Wallis statistical analysis

Stored	Observation (g)									*P-value*	Note
(Day)	3%			5%			7%				
1	28.604	28.622	28.612	28.319	28.306	28.298	28.706	28.714	28.699	0.027	Significant difference
2	28.807	28.765	28.812	28.518	28.543	28.486	29.010	28.987	29.001		
3	28.815	28.807	28.824	28.635	28.665	28.646	29.117	29.105	29.107		
4	28.893	28.893	28.903	28.718	28.753	28.775	29.138	29.135	29.144		
5	28.982	28.982	28.975	28.753	28.773	28.797	29.382	29.525	29.289		
6	29.000	29.000	29.005	28.986	28.981	28.951	29.660	29.677	29.457		
WVTR g/24 hour. m^2	5.312	5.301	5.320	7.773	7.811	7.779	9.699	9.712	9.609		
Mean ± SD	5.311 ± 0.145			7.788 ± 0.226			9.673 ± 0.325				

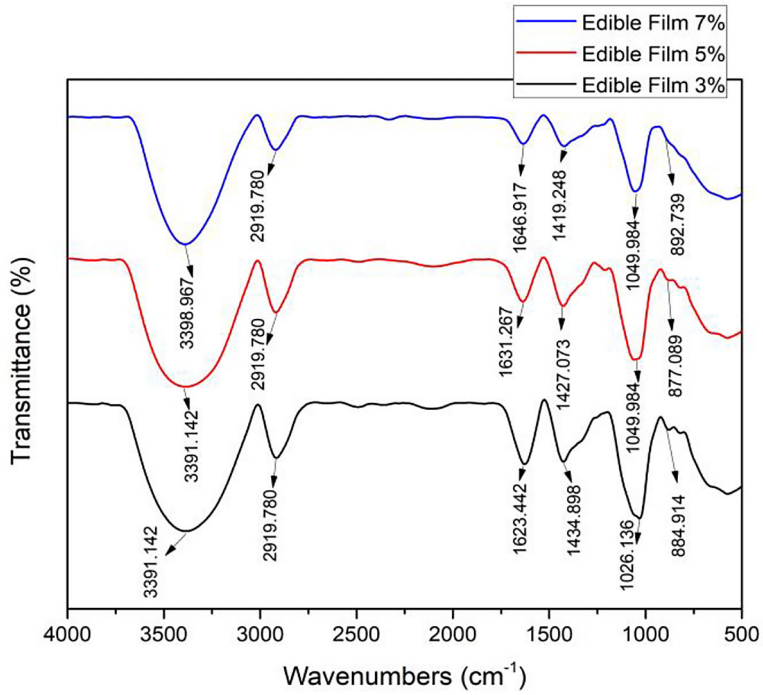

Figure 7. Comparing the FTIR spectra of edible films with varying amounts of 3%, 5%, and 7% cellulose from seaweed

Table 6. Analysis of functional groups from FTIR spectra of 3%, 5% and 7% cellulose edible film

Wavenumbers (cm^{-1})			Range (cm^{-1})	Functional group Interpretation
Edible film 3%	Edible film 5%	Edible film 7%		
3391.142	3391.142	3398.967	3400-3200	O-H strethching vibration
2919.780	2919.780	2919.780	3000-2850	Sp^3 C-H strethching
1623.442	1631.267	1646.917	1650-1630	C-O
1434.898	1427.073	1419.248	1440-1000	C-O-H bending
1026.136	1049.984	1049.984	1300-1000	C-O stretching vibration
884.914	877.089	892.734	900-650	N-H (amine)

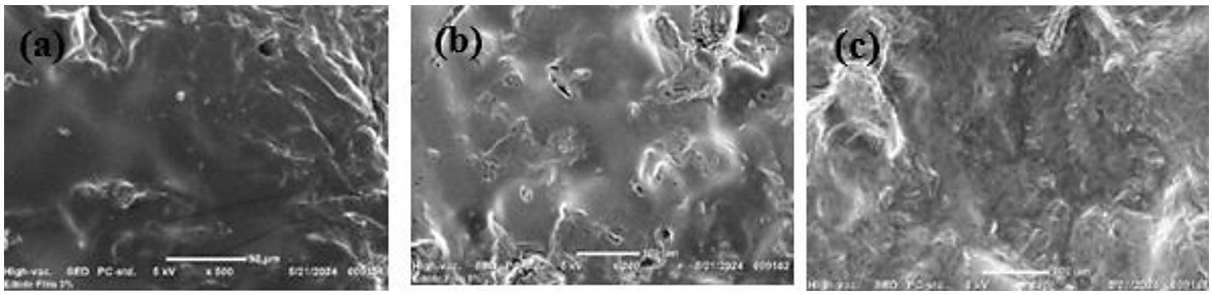

Figure 8. Scanning electron micrograph of edible film with (a) 3% cellulose content (b) 5% cellulose content and (c) 7% cellulose content

Biodegradability analysis of the edible films

The edible film samples were placed on soil collected from FMIPA Hasanuddin University's yard to assess the edible film's biodegradation or capacity to decompose. The biodegradation test results, derived from the observation results, showed that it took between 12 days for all the edible film to degrade. Comparing the biodegradation results from this study to those of Chaudhary

(2023), which require 14 days for 100% decomposition, the results are nearly identical. The edible film has a smooth, white surface and is translucent. However, throughout the biodegradation test, the edible film's color and surface shape altered, which may have something to do with microbial growth or morphological changes. Figures 9, 10, and 11 show this change in surface morphology and color, illustrating the type of degradation that occurs when soil is buried.

During soil burial studies, the percentage of weight loss was used to assess the biodegradation behavior of edible films containing 3%, 5%, and 7% cellulose. All samples showed complete degradation by day 12. However, the edible film with 7% cellulose concentration exhibited a slower degradation rate during the observation period compared to the 3% and 5% formulations, indicating greater resistance to microbial breakdown. This suggests that a higher cellulose concentration improves the structural integrity and slows the degradation process of the film (Latif et al., 2024). These results demonstrate better biodegradation performance than previously reported by Pawle et al. (2025), in which complete degradation of edible films occurred after 14 days of soil burial. External factors such as moisture, temperature, and microbial activity may also influence the rate of decomposition (Capar, 2023).

Organoleptic analysis

The objective of organoleptic analysis using the hedonic test method is to ascertain the degree of public acceptability or like for *wajik* (Sulawesi traditional food) goods that are packaged in edible films manufactured from cellulose seaweed with chitosan. The evaluation of 10 panelists from 1 to 5 is the basis for the standard measurement of organoleptic characteristics. Comparing the F value of the sample with the F count ratio at the 1% and 5% levels reveals the degree of departure from each feature of the test sample (edible film). The results of the organoleptic examination are shown in Table 7 for *wajik* packed in edible film made of chitosan and cellulose from seaweed.

According to data in Table 7, consumers find *wajik* packed in edible film highly appealing. Each characteristic's evaluation falls within the overall range of good ratings, averaging 4.65 (almost very like). In the calculation of organoleptic characteristics by comparing the F count data of the sample, it was found that the aroma and

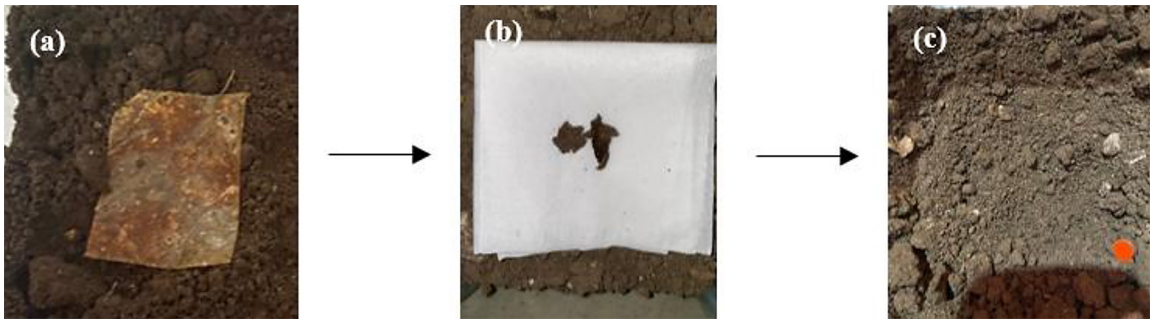

Figure 9. Biodegradation study of 3% edible film using soil burial method (a) edible film day 1st; (b) edible film day 6th; and (c) edible film day 12th

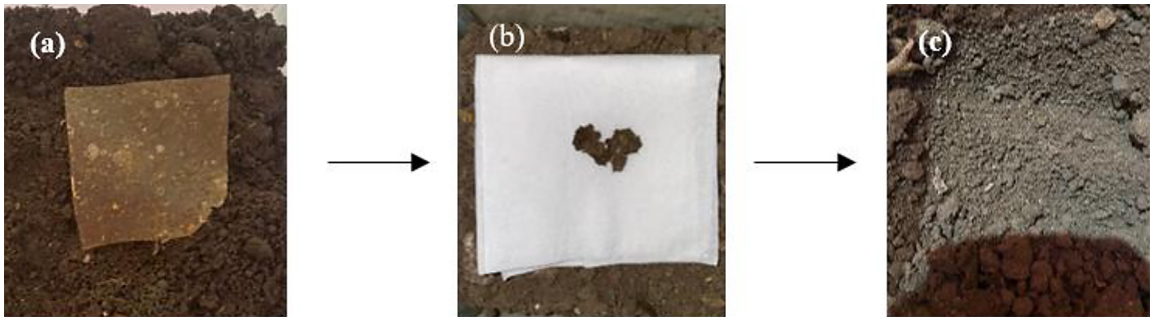

Figure 10. Biodegradation study of 5% edible film using soil burial method (a) edible film day 1st; (b) edible film day 6th; (c) edible film day 12th

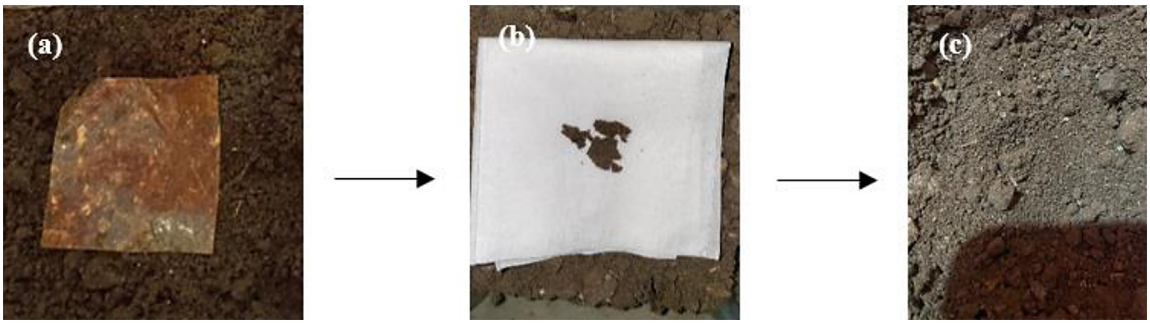

Figure 11. Biodegradation study of 7% edible film using soil burial method (a) edible film day 1st; (b) edible film day 6th; (c) edible film day 12th.

Table 7. Results of the organoleptic analysis on *wajik* packed with edible films with variations in cellulose concentration of 3%, 5%, and 7%

Organoleptic characteristics	Sample	Total assessment	F count sample	F count ratio 1%	F count ratio 5%
Colors	W (EF3%)	42	6.00	6.01	3.55
	W (EF5%)	46			
	W (EF7%)	46			
Aroma	W (EF3%)	49	0.23		
	W (EF5%)	49			
	W (EF7%)	48			
Texture	W (EF3%)	46	0.47		
	W (EF5%)	46			
	W (EF7%)	47			

Note: W (EF3%) = *Wajik* packed with 3% edible film, W (EF5%) = *Wajik* packed with 5% edible film, W (EF7%) = *Wajik* packed with 7% edible film.

texture categories were not significantly different at the 1% and 5% ratio levels because the F count value of the sample was smaller than the F count at the 1% and 5% ratios. The color category has a significant difference at the 1% and 5% ratio levels because the F value of the sample count is smaller than the F count at the 5% ratio. The edible film's color category varies significantly since the amount of cellulose it adds determines how transparent it becomes. This leads to a wider range of assessments in the color category.

Antibacterial analysis of edible film

The results of antibacterial activity testing by measuring the clear zone of edible film added with cellulose from seaweed with the addition of chitosan against *E. coli* bacteria and *S. aureus* bacteria. The antibacterial test in this study used variations of 10,000 ppm and 15,000 ppm concentrations before and after application on the *wajik*, using positive control chloramphenicol and commercial plastic and negative control 2% CH_3COOH solution until the data were obtained in Figure 12, Table 8, and Table 9.

Based on the available data (Table 8 and Table 9), edible films with concentrations of 10% and 15% demonstrated the ability to inhibit the growth of *E. coli* and *S. aureus*, as indicated by the formation of inhibition zones ranging from approximately 10.2 mm to 12.3 mm. The Kruskal-Wallis test revealed a statistically significant difference in inhibition zone diameters among the tested samples against *E. coli* ($H = 19.69$, $p = 0.003$) and *S. aureus* ($H = 19.58$, $p = 0.003$). This indicates that the type of film and treatment conditions had a significant effect on antimicrobial activity (Rahmasari and Yemiş, 2022). Notably, the edible films, especially those with higher cellulose concentrations, demonstrated measurable zones of inhibition compared to the negative and commercial plastic controls.

The main antibacterial inhibitory mechanisms are damage to the cell wall, suppression of protein, DNA, or RNA bio-synthesis, and damage to the cell membrane. The most common

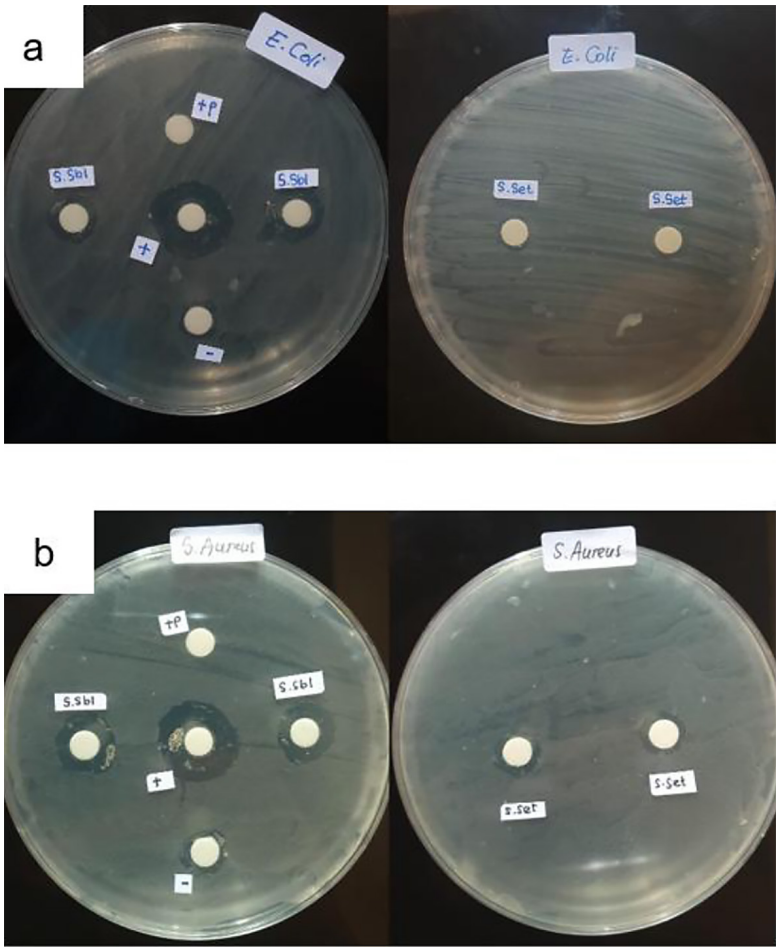

Figure 12. (a) Antibacterial analysis using *E. coli* test bacteria; (b) antibacterial analysis using *S. aureus* test bacteria

Table 8. Analysis of edible film activity in inhibiting *E. coli* bacteria growth based on the Kruskal-Wallis statistical analysis

Sample	Inhibition zone diameter (mm)					Note
	E. coli			Average	P-value	
	Single	Duplicate	Triplicate			
Control (+) chloramphenicol	14.5	14.2	13.6	14.1 ± 0.4	0.003	Significant difference
Control (+) commercial plastic	0	0	0	0		
Control (-) CH_3COOH 2%	7.7	7.1	7.2	7.3 ± 0.3		
Edible film 10% (before packaging)	11.8	11.6	11.7	11.7 ± 0.1		
Edible film 15% (before packaging)	12.2	12	12.1	12.1 ±0.1		
Edible film 10% (after packaging)	10.5	10.3	10	10.2 ± 0.2		
Edible film 15% (after packaging)	10.8	10.6	10.7	10.7 ± 0.1		

inhibitory mechanism by antibacterial compounds is cell wall destruction. However, some antibacterial compounds, such as cellulose, have different inhibitory mechanisms against target bacteria (Wang et al., 2022). Certain substances can prevent the growth of bacteria through a variety of processes. For example, chitosan can harm bacterial cell walls, microsomes, and lysosomes by interacting with bacterial DNA (Hamed et al., 2024).

Antifungal analysis of edible film

The antifungal efficacy of the edible film against *A. flavus* was evaluated using the well diffusion method. The inhibitory effect was

Table 9. Analysis of edible film activity in inhibiting *S. aureus* bacteria growth based on the Kruskal-Wallis statistical analysis

Sample	Inhibition zone diameter (mm)					Note
	S. aureus			Average	P-value	
	Single	Duplicate	Triplicate			
Control (+) chloramphenicol	14.2	14.1	13.7	14 ± 0.2	0.003	Significant difference
Control (+) commercial plastic	0	0	0	0		
Control (-) CH_3COOH 2%	7.8	7.5	7.6	7.6 ± 0.1		
Edible film 10% (before packaging)	12	11.9	11.9	11.9 ± 0.05		
Edible film 15% (before packaging)	12.6	12	12.4	12.3 ± 0.3		
Edible film 10% (after packaging)	11	10.9	10.8	10.9 ± 0.1		
Edible film 15% (after packaging)	11.2	11	11.1	11. 1 ± 0.1		

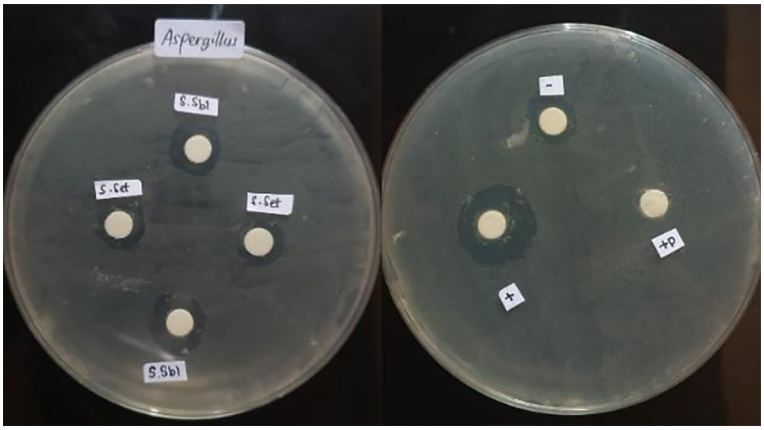

Figure 13. Antifungal analysis using *A. flavus* test fungi

Table 10. Analysis of edible film antimicrobial activity in the inhibition against *A. flavus* fungus based on the Kruskal-Wallis statistical analysis

Sample	Inhibition zone diameter (mm)				*P-value*	Note
	Single	Duplicate	Triplicate	Avarage		
Control (+) ketoconazole	14.4	13.9	14	14.1 ± 0.1	0.993	Not significant difference
Control (+) Commercial plastic	0	0	0	0		
Control (-) CH_3COOH 2%	7.9	7.6	7.6	7.7 ± 0.1		
Edible film 10% (before packaging)	11.4	11.3	11.2	11.3 ± 0.3		
Edible film 15% (before packaging)	11.8	12	11.9	11.9 ± 0.1		
Edible film 10% (after packaging)	10.5	10.3	10.2	10.3 ± 0.3		
Edible film 15% (after packaging)	10.7	10.5	10.6	10.6 ± 0.1		

determined by observing the formation of a clear zone around the wells containing the edible film solution. Variations in concentrations of 10.000 ppm and 15,000 ppm were used for the antifungal test, both before and after application on the wajik. Plastic and ketoconazole served as the positive control, while a 2% CH_3COOH solution was used as the negative control. The results of the experiment are presented in Figure 13 and Table 10.

The results of the antifungal activity analysis of the edible film against *A. flavus* showed that variations in chitosan concentration influenced the diameter of the inhibition zone (Table 9). As the chitosan concentration increased, the diameter of the inhibition zone also tended to grow. This is likely due to the increasing amount of active compounds present in higher concentrations of chitosan, which enhances antifungal activity.

This finding aligns with previous research by (Torrijos et al., 2022), which stated that the concentration of antifungal compounds is one of the key factors influencing efficacy. However, the Kruskal-Wallis test revealed a statistically significant difference in the inhibition zone diameters among the tested samples against *A. flavus* ($p = 0.003$), indicating that the type of edible film and treatment conditions significantly influenced their antifungal activity (Rahmasari and Yemiş, 2022). Notably, the edible films especially those with higher cellulose concentrations produced clear zones of inhibition compared to the negative and commercial plastic controls.

CONCLUSIONS

This study successfully developed a biodegradable and antimicrobial edible film using cellulose extracted from *Eucheuma* sp., a seaweed source that remains underutilized in food packaging applications. The cellulose extraction was confirmed by the presence of hydroxyl (-OH) groups through FTIR analysis. Statistical tests showed significant differences in film thickness and WVTR across cellulose concentrations based on the Kruskal-Wallis test ($p < 0.05$), while solubility varied significantly according to one-way ANOVA ($p = 0.001$). The 3% cellulose film had the highest solubility and thickness, whereas the 7% film exhibited the highest WVTR. Antimicrobial testing demonstrated that the addition of chitosan enhanced both antibacterial and antifungal activity, with a significant difference in antifungal activity against *A. flavus* ($p = 0.003$). Among all treatments, the 5% cellulose film provided the most balanced performance in terms of physical and antimicrobial properties, contributing effectively to the extended shelf life of *wajik*, a traditional food product. This research offers a novel contribution by introducing *Eucheuma* sp. cellulose as a functional film-forming material for active packaging.

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