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Synthesis and Characterization of Microcellulose from Red Algae *Gracillaria longissima* and Its Effect on the Properties of Composite Films from Avocado Seed Starch

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macroalgae;
red seaweed.

ABSTRACT. Red algae are abundant worldwide, and in recent years, their use to make more valuable products has grown significantly. The present study used red algae *Gracillaria longissima* as raw material to produce microcrystalline cellulose to strengthen avocado seed-based film. Microcrystalline cellulose was obtained by chemically treating the red algae with alkali, bleaching, and acid hydrolysis. The rough and irregularly shaped microcrystalline cellulose was successfully isolated at the micrometric scale with an average particle size of 44.1 µm. The as-extracted microcrystalline cellulose was used as filler to produce avocado seed-based composite films with improved tensile and barrier properties. Adding 4 wt% microcrystalline cellulose into the avocado seed matrix increased tensile strength by 152% and reduced elongation by 63%. Additionally, the barrier properties of avocado seed composite films were similar to those of cellulose derivatives utilized in food packaging. Adding 4 wt% microcrystalline cellulose into the avocado seed matrix reduced the water vapor transmission rate by 43% of the neat starch value. Considering these findings, microcrystalline cellulose-containing starch film is suggested as a biodegradable substitute for applications in food packaging.

INTRODUCTION

Research on food packaging has attracted considerable interest due to the increasing impact of conventional plastic waste on the environment, consumer awareness of foods with extended shelf life, and environmental awareness regarding limited natural resources. Polysaccharide-based natural biopolymers have been studied as potential alternatives to conventional plastics in order to solve the environmental issues brought on by non-biodegradable plastics. Several biopolymers from various natural materials are used to manufacture biodegradable films. Among biodegradable polymers, starch meets all the main aspects because starch is renewable, inexpensive, and can be decomposed entirely without toxic residues. Therefore, starch has a high potential as an environmentally friendly material in the future, as a substitute for non-biodegradable polymers.

Polymers based on starch are transparent, odorless, non-toxic, and semipermeable to oxygen, carbon dioxide, water vapor, and flavoring ingredients. However, starch films have several disadvantages, including difficulty in processing, high water absorption, and low mechanical strength. Since films based on starch have brittle mechanical properties, several studies have been conducted to improve the films' thermal, resistance, and mechanical properties by modifying starch and adding reinforcements to the film matrix (Bangar and Whitesode, 2021).

Starch/cellulose composites are among the most promising composites because the matrix and the reinforcement possess the same glucose chemical bonds, leading to a compatible interface without requiring modification or additional materials. Because of the lower cost and biodegradability, starch/cellulose composites have a lot of potential for use in food packaging (Fu *et al.*, 2024). Recently, cellulose fibers, including microcrystalline cellulose (MCC) and nanocrystalline cellulose (CNC), have been investigated for potential use as functional materials in many industries, including agriculture, food, medicine, and pharmaceuticals. These micro-

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and nano-scale cellulose exhibited better film performance when combined with starch (Bangar and Whitesode, 2021). The addition of cellulose materials into a biopolymer matrix resulted in composite materials with superior characteristics, such as high mechanical, optical, thermal, and barrier properties. This is due to the unique structure, morphology, low density, high crystallinity, high tensile strength, large specific surface area, and high elastic modulus of nano-cellulose materials (El Achaby *et al*., 2018).

Applications for starch films are numerous and include both food and non-food items. Because starch-based materials have poorer mechanical performance, improving their mechanical properties is a constant challenge. Other drawbacks of starch-based films are their hydrophilic property and water sensitivity due to the many hydroxyl groups in the polymer chain. Modifying and adding reinforcements represent the most widely used methods to overcome these drawbacks. Originally, different types of cellulose were used to enhance the tensile strength of materials made from starch. However, significant changes were obtained using nano-scale cellulose reinforcements, based on the nanocomposites principle.

Fruit seeds are a potential source of starch, but they are still considered waste and are usually discarded. Using avocado seed starch as a composite biofilm can be an alternative starch source derived from fruit waste. Lubis *et al*. (2018) conducted a study on the effect of adding microcrystalline cellulose sourced from palm sugar palm fiber on the mechanical properties of bioplastics from avocado seed starch. However, variations in cellulose diameter differences do not significantly affect mechanical properties, based on research by Fu *et al*. (2024). Thinner fibers from okara show a slightly higher modulus. In addition, they also found that nano-scale cellulose slightly enhanced the potato starch films' elongation, while macroscale cellulose had the opposite result, which was a reduction in elongation. To improve the mechanical properties of avocado seed starch films, cellulose is required. Nanocrystalline cellulose (CNC) from red algae (RA) has been successfully synthesized by El Achaby and coworkers (El Achaby *et al*., 2018) along with its application as a reinforcement in polyvinyl alcohol (PVA)-based films. To the author's knowledge, no study has reported the effect of adding microcrystalline cellulose from red algae on the characteristics and performance of avocado seed starch-based films.

RESEARCH METHODS

Fresh red algae (*Gracillaria longissima*) were obtained from Pangandaran beach, West Java, Indonesia. The species name has been identified by the Biology Faculty, Gadjah Mada University. Avocado (*Persea americana*) seeds were obtained as fruit residues from a local fruit juice shop in Sleman, Yogyakarta, Indonesia. All chemicals used, such as 3000 ppm sodium metabisulfite($Na_2S_2O_5$), sodium hydroxide (NaOH) pellet, 8% sodium hypochlorite (NaOCl), 2% acetic acid p.a. (CH_3COOH), and 98 % sulfuric acid (H_2SO_4), were purchased from CV Sentra Teknosains Indonesia in Sleman, Yogyakarta, Indonesia.

Cellulose fiber production

Microcrystalline cellulose (MCC) was successfully isolated from RA through alkali and bleaching treatments, then an acid hydrolysis procedure, as detailed in previous research by El Achaby and colleagues (El Achaby *et al*., 2018). Before being ground with a grinder, the fresh RA samples were cut into tiny pieces (about 2 cm). After that, distilled water was used to sterilize the ground RA fibers for one hour at 60 °C while being mechanically stirred. Following sterilization, the RA fibers underwent three alkali treatments using a 5 wt% sodium hydroxide solution at 80 °C for two hours while being stirred. The alkali-treated red algae (ATRA) were bleached three times with a solution made up of equal parts of 2% (v/v) acetic acid and 8% (v/v) sodium chlorite solutions, producing cellulose fibers that are pure white in color, which are described as bleached red algae (BRA). Alkali treatment (delignification) and the bleaching process aimed to remove amorphous components from the surface of the RA fibers (El Achaby *et al*., 2018). Figure 1 shows the general procedures for MCC extraction nd digital pictures of each product produced.

Isolation of MCC

To isolate MCC, the bleached red algae (BRA) were hydrolyzed with sulfuric acid. The acid hydrolysis was carried out with mechanical stirring and a 50% sulfuric acid solution at 50 °C for 40 minutes. The hydrolysis process plays a role in obtaining crystalline cellulose (de Vilhena *et al*., 2024). After stopping the reaction with ice cubes (the tube sample was sealed and placed in a container filled with ice cubes), the mixture was centrifuged several times at 6000 rpm for 20 minutes and dialyzed against distilled water until the pH was neutral. The resulting MCC aqueous suspension was then homogenized for five minutes using a probe-type ultrasonic homogenizer.

After that, MCC aqueous suspensions were obtained in white gels, as shown in Figure 1. Finally, the homogenized MCC suspension was freeze-dried to obtain the MCC in a solid form for characterizations (Figure 1).

Figure 1. Step-by-step procedures of MCC extraction and the representative digital images of the obtained products.

Extraction of avocado seed starch

Starch extraction was carried out based on the method carried out by Martins *et al*. (2022) with slight modifications. First, the avocado skin was removed from its pit. Then it was cut into small pieces and put into a sodium metabisulfite solution (3000 ppm) for 24 hours. Starch was extracted by crushing avocado seeds with the sodium metabisulfite solution in a mixer. After homogenization, decantation was carried out for 24 hours. Filtration was then carried out to obtain the starch, while the supernatant was discarded. The starch obtained was then dried in an oven at 60 °C for 4 hours and sieved through a 100 mesh sieve.

Processing of composite films

The MCC samples were utilized as fillers in the solvent casting process to obtain starch-MCC composite films with varying MCC contents (0, 1, 2, 3, and 4 wt%). For each composite formulation, the desired amount of MCC and 2 g of dry starch were dissolved in distilled water using a ratio of starch:distilled water = 1:20 (w/v). The starch solution was heated at 50 °C and stirred on a hot plate for 15 minutes. A mixture of chitosan with acetic acid was then introduced into the starch solution. Glycerol 2% (v/v) was then added to the starch solution and continued stirring for 10 minutes. After that, the mixture was poured into a flat acrylic plate (18 × 18 cm) and dried in an oven at a temperature of 70 °C for 8 hours. The film obtained was then peeled from the plate and stored for further analysis.

Characterization Techniques

The characterization carried out consisted of three parts, namely 1) Characterization of red algae MCC; 2) Analysis of mechanical properties of biocomposite films; and 3) Characterization of biocomposite films.

Red algae MCC Morphology and Particle Size

Surface morphology and particle size analysis of red algae MCC were examined using Scanning Electron Microscopy (SEM) and Particle Size Analyzer (PSA), respectively. SEM was used to examine the particle structure and was conducted at the Integrated Research and Testing Laboratory, Gadjah Mada University (LPPTD UGM), while particle size analysis by PSA was conducted at the UII Integrated Laboratory.

Fourier Transform Spectroscopy of MCC and Composite Films

Fourier-transform infrared spectroscopy (FTIR) with an attenuated total reflectance (ATR) device was used to identify the functional groups found in MCC powders and their composite films. Infrared (IR) analysis was carried out in the 4000 – 500 cm^{-1} wavelength range.

Optical Properties of Film

Visible and UV light barriers were among the films' optical characteristics. To test these characteristics, quartz cells were filled with cut rectangular films. Using a UV-Visible spectrophotometer, the percentage of transmission (%T) between 200 and 700 nm was measured to determine the light barrier, and UV radiation blocking was calculated for the UV-A (315 – 400 nm) and UV-B (280 – 315 nm) regions using Equations 1 and 2, respectively (Grisales-Mejía *et al*., 2024). The films' opacity was determined by dividing their absorbance at 600 nm by their thickness (mm) (Grisales-Mejía *et al*., 2024).

$$UV - A\ radiation\ barrier\ (\%) = 100 - T_{UV-A} \quad (1)$$

$$UV - B\ radiation\ barrier\ (\%) = 100 - T_{UV-B} \quad (2)$$

where $T_{UV\text{-}A}$ and $T_{UV\text{-}B}$ represent the typical transmittance value in their respective regions.

Mechanical Properties of Bio-composite Films

A universal testing machine was used to measure the mechanical properties of films with and without the addition of MCC. The mechanical properties of bio-composite films were analyzed through thickness, tensile strength, and elongation at break.

Water Vapor Transmission Rate (WVTR) of film

The films were also analyzed for their Water Vapor Transmission Rate (WVTR) following the procedure from (Ningrum *et al*., 2021). The film is cut into circular shapes and attached to perforated aluminum foil. The size of the holes in the aluminum foil is 10% of the surface area of the distilled water. Next, the edible film-aluminum foil was used as a cover for petri dishes filled with 30 ml of distilled water. The covered Petri dish was weighed, and its mass was recorded. Then the Petri dish is placed in an oven at a temperature of 30°C and weighed every hour for 5 hours. Equation 3 was used to calculate WVTR.

$$WVTR\ (g\ s^{-1}m^{-2}) = \frac{\text{Lost water mass (gr)}}{time\ (second) \times Surface\ area\ (m^2)} \quad (3)$$

RESULTS AND DISCUSSION

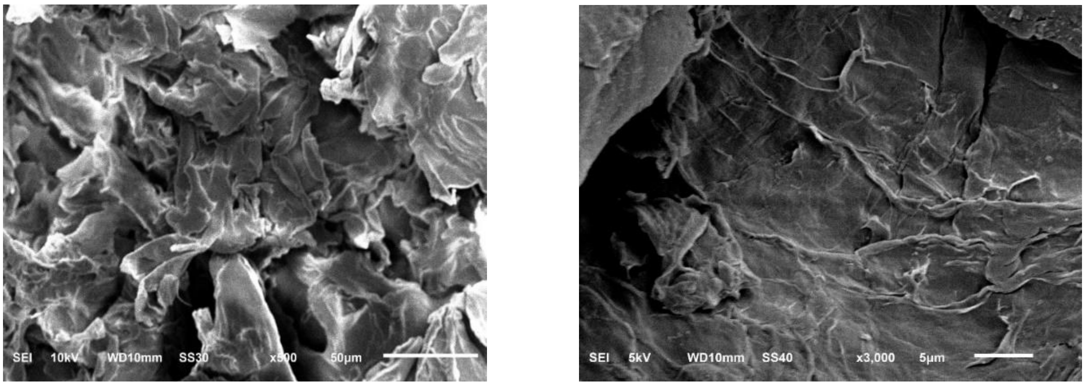
Isolation of MCC

Gracillaria longissima, a red algae (RA), was successfully used to isolate MCC. Figure 1 summarizes the procedure and physical characteristics of the cellulosic materials acquired at various treatment stages. As seen in Fig. 1, the RA's red color was maintained after it was dried and crushed. Following a hot water wash, the ground RA fibers were treated with an alkali to remove the non-cellulosic compounds (lignin, hemicellulose, and pectin). This produced alkali-treated red algae (ATRA) fibers that were grey in color (Figure 1). Certain alkali-labile bonds between lignin monomers or between lignin and polysaccharides broke, causing these ATRA fibers to partially dissociate (El Miri *et al*., 2015). The remaining lignin and contaminants from the alkaline treatment were then eliminated by bleaching the ATRA fibers. The bleaching process may result in complete defibrillation of the fibers into tiny microfibrils with pure bleached cellulose, which are distinguished by their extremely white color, suggesting that the bleaching process most likely removed the non-cellulosic substances (Figure 1). To eliminate the amorphous domains of cellulose chains, the BRA fibers were bleached and then hydrolyzed with sulfuric acid for 30 minutes. As seen graphically in Figure 1, this produced MCC bundles with micrometric dimensions with a yield of roughly 0.9% of the initial raw materials (RA) in the form of clearly white powdered forms produced following separation from water using a freeze-drying process.

Characterization

Particle size and SEM observations of MCC

The mean particle size of the obtained MCC sample was measured using a Particle Size Analyzer and found to be 44.15 µm. Microcrystalline cellulose (MCC) obtained from the sulfuric acid process usually produces powder with particle sizes ranging between 10 and 50 µm in diameter (Bangar *et al*., 2023). The morphology of the freeze-dried MCC sample was examined by SEM observations (Figure 2). Thin plates of algal cellulose with cracks and a rough surface were formed due to the sulfuric acid treatment, as seen in Figure 2. The removal of the cellulose's amorphous portion is responsible for the particles' rough surface, which results in many gaps and irregularly shaped particles (Zhao *et al*., 2018). The characteristic structures of natural cellulosic materials, including rough and irregular surfaces, are also found in nanocrystalline cellulose from brown algae (Bogolirsyn *et al*., 2024) and microcrystalline cellulose from carrot pomace (Meral and Demirdöven , 2024).



(a) (b)

Figure 2. SEM images of MCC with (a) 500× magnification, (b) 3000× magnification.

FTIR Analysis of Cellulosic Materials

Figure 3 shows the FTIR analyses of the RA, ATRA, BRA, and MCC samples. Since cellulosic materials are hydrophilic, the hydrogen bond of OH stretching and bending vibrations of the adsorbed water were generally ascribed to the broad peaks at 3338 – 3283 cm^{-1} seen in all samples (Chen *et al*., 2016). Furthermore, OH stretching vibrations from cellulose molecules are linked to the peaks at 3338 – 3283 cm^{-1} (Trache *et al*., 2016). According to Tan and coworkers (Tan *et al*., 2015), the single bond –OH bending of the absorbed water was responsible for the peaks at 1640 – 1631 cm^{-1}. The skeletal vibration of the C–O–C pyranose ring skeleton in cellulose fiber is responsible for the peak at 1060 – 1047 cm^{-1} in all samples under study reported by Chen *et al*., 2016.

The presence of β-glucosidic linkages between the anhydroglucose rings in the cellulose chains is linked to the observed peak at 893 cm^{-1} in the MCC sample (Chen *et al*., 2016). This peak shows that after alkali, bleaching, and acid hydrolysis treatments, the sample's cellulose content increased (El Achaby *et al*., 2018). Furthermore, the peaks at 1427 and 1314 cm^{-1} in the MCC sample were primarily linked to the parent chain of cellulose (El Achaby *et al*., 2018). These results clearly show that MCC was successfully separated from raw RA, and the outcomes are consistent with previous research.

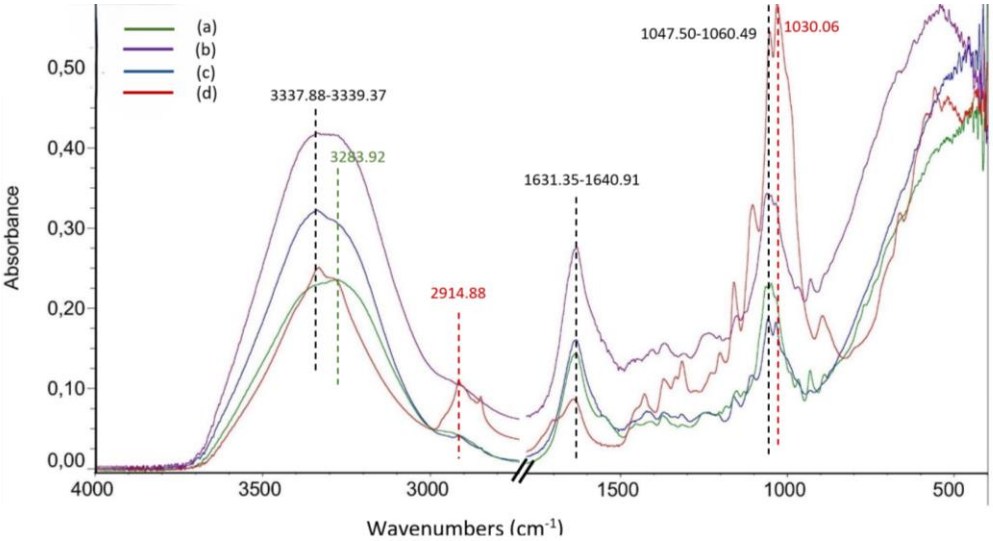


Figure 3. FTIR spectra of: (a) RA, (b) ATRA, (c) BRA, and (d) MCC samples.

FTIR Analysis of Film Composites

The FTIR spectra of neat starch and starch-MCC composite films are shown in Figure 4. It is commonly known that the hydroxyl band in FTIR spectra can be forced to a shifted wave number due to its sensitivity to hydrogen bonding (El Achaby *et al*., 2018). In this case, however, adding MCC to the starch matrix did not affect the wave number of the hydroxyl bands in the 3500 – 3000 cm^{-1} range (roughly 3272 cm^{-1} for all samples). This could indicate weak hydrogen bonding interactions between the hydroxyl groups on the starch polymer's macromolecular chains. When MCC was incorporated into carrageenan film, no significant changes in functional groups or peak positions were observed, as noted by Park *et al*. (2024).

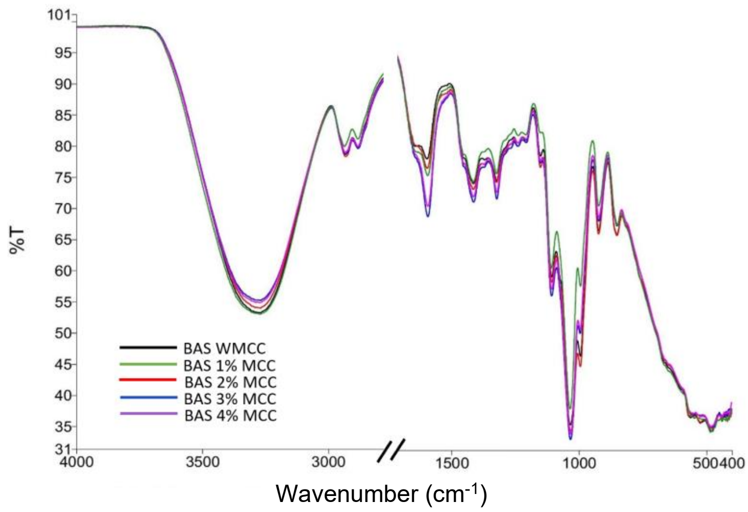


Figure 4. FTIR spectra of neat starch and its composite films.

Optical Properties of Films

The UV-vis spectroscopy was used to assess the degree of transparency of the resultant composite films as well as the dispersion of MCC within the starch matrix. The UV-vis transmittance spectra and values for both neat starch and starch-MCC composite films at λ = 700 nm are displayed in Figure 5. The addition of varying MCC contents had little effect on the starch polymer's transparency level. For all samples, the UV-vis transmittance of the resulting starch composite was measured in the 38 – 69% range, which could be attributed to its orange coloration. All film samples presented transmittance values under 80%. Since the transmittance values obtained from the current study were between 10 and 80%, the films are considered as translucent materials (Guzman-Puyol *et al*., 2022a). To accurately evaluate transparency, paper printed with the word "TEXT" was placed under the film to demonstrate the difference in film transparency. As shown in Figure 6, "TEXT" can be seen clearly, even with "TEXT" printed in different thicknesses of black letters, demonstrating qualitatively good transparency in the resulting composite film. Furthermore, the opacity values were between 1.24 – 5.01 nm/mm (Table 1). The opacity of all the film samples is lower than 5, which indicates that all the films can clearly display packaging content and condition (Lei *et al*., 2021). Therefore, all films can be considered transparent films.

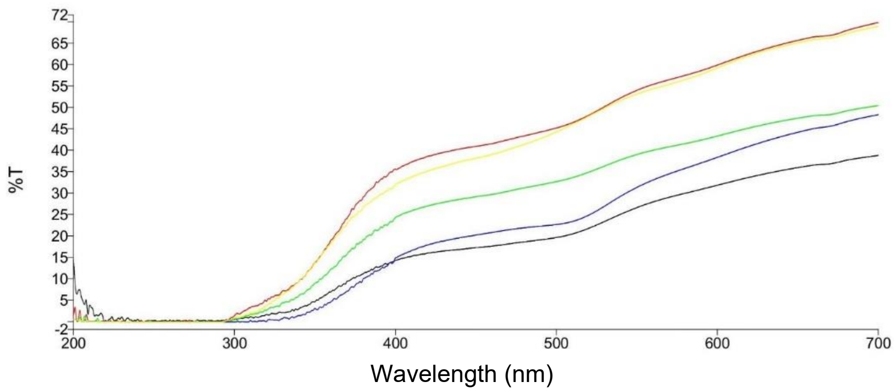


Figure 5. UV-Vis transmittance of neat avocado seed starch and its starch-MCC composite films.

(a) (b) (c) (d) (e)

Figure 6. The apparent transparency of the avocado seed composite film which qualitatively demonstrated by the appearance of "TEXT" printed on the paper behind the films: (a) starch, (b) starch-MCC-1, (c) starch-MCC-2, (d) starch-MCC-3, and (e) starch-MCC-4.

Table 1. The opacity and the UV spectrum blocking percentage of avocado seed starch-MCC composite films.

Samples	Opacity (nm/mm)	UV-A block (%)	UV-B block (%)
Starch	1.24	81.4	98.6
Starch-MCC-1	5.01	93.0	98.9
Starch-MCC-2	2.54	87.9	99.4
Starch-MCC-3	2.77	94.6	100.0
Starch-MCC-4	1.85	82.8	99.2

In recent years, there has been a lot of interest in producing biodegradable films with light-barrier properties, especially for use in food packaging. Food matrices can be damaged by photo-oxidation and photo-degradation when the visible (Vis) and ultraviolet (UV) spectrums penetrate them. This can lead to the loss of certain compounds of interest, the appearance of unwanted flavors and odors, and even a decrease in nutritional value (Guzman-Puyol *et al.*, 2022b). In this study, the avocado seed-derived composite films demonstrated good light-barrier properties. It was found that the composite films could block approximately 82.8 – 94.6% of the UV-A spectrum's wavelengths and 98.9 – 100% of the UV-B spectrum's wavelengths (Table 1). According to Guzma-Puyol and colleagues (Guzman-Puyol et al., 2022b), the presence of cyclic organic molecules in avocado seed starch, such as tannins, flavonoids, and phenols, may lead to this phenomenon. These molecules can absorb UV radiation and produce materials that effectively block UV transmission from the outside. Similar behavior was also reported by previous research (Grisales-Mejía *et al.*, 2024) and (Merino *et al.*, 2021). Although there were no observable trends in the current study, adding MCC into the avocado seed matrix increased the value of UV-A and UV-B blocks.

Thickness and Tensile Properties of Composite Films

The thickness of films obtained in this study ranged from 0.099 to 0.180 mm (Table 2). These results correspond with the thickness of avocado seed films obtained by Grisales-Mejía *et al*. (2024), who reported the film's thickness was around 0.087 to 0.118 mm. Tensile tests were used to characterize the tensile behavior of starch-MCC composite films. The values of tensile strength and elongation at break are compiled in Table 2 to assess the strength and flexibility of these composite films. The ultimate tensile strength was defined as the maximum stress value applied to the material, whereas the elongation at break represents the strain required to break the material.

Table 2. Tensile strength and elongation at break of neat starch and starch-MCC composite films.

Samples	Thickness (mm)	Tensile strength (MPa)	Elongation at break (%)
Starch	0.180 ± 0.016	0.439 ± 0.077	138.060 ± 1.740
Starch-MCC-1	0.099 ± 0.008	0.647 ± 0.080	135.265 ± 16.24
Starch-MCC-2	0.143 ± 0.005	0.718 ± 0.035	116.483 ± 6.295
Starch-MCC-3	0.150 ± 0.014	1.717 ± 0.070	63.910 ± 5.473
Starch-MCC-4	0.123 ± 0.009	1.105 ± 0.175	51.004 ± 5.222

These data demonstrated that the tensile properties of composites containing MCC were better than those of neat starch polymer, indicating that adding MCC improves the composite films' tensile properties. The neat starch film exhibits an ultimate tensile strength of 0.439 MPa and elongation at break of 138%. Compared to the neat avocado seed starch, composite films containing 1, 2, 3, and 4 wt% MCC exhibit an ultimate tensile strength of about 48, 64, 292, and 152% increases, respectively. These results were previously reported for various polymer composites filled with cellulose (El Achaby *et al*., 2018). Meanwhile, the elongation at break was reduced with the increase in MCC contents, which corresponds to 2, 16, 54, and 63% decrease for films containing 1, 2, 3, and 4 wt% MCC, respectively, compared to the neat starch (138%). This is related to the reinforcing capacity of rigid particles and has also been noted in previously published cellulose-filled polymer composites (El Achaby *et al*., 2018).

Barrier Properties

The avocado seed composite films' water vapor barrier properties were evaluated by measuring the water vapor transmission rate (WVTR) (Figure 7). With WVTR values of 0.108 g/m^{2}s for neat starch and 0.062 g/m^2·s for Starch-MCC-4, the values of WVTR generally decrease as the MCC content increases, resulting in a 43% decrease from the initial WVTR value. This tendency can be explained by the lack of free space for vapor diffusion in the cellulose matrix and the presence of MCC molecules, which prevent water vapor molecules from passing through the films (Guzman-Puyol *et al*., 2022a). The WVTR values from the current study are similar to the results from cellulose-based bioplastics obtained by Guzman-Puyol *et al*. (2022a).

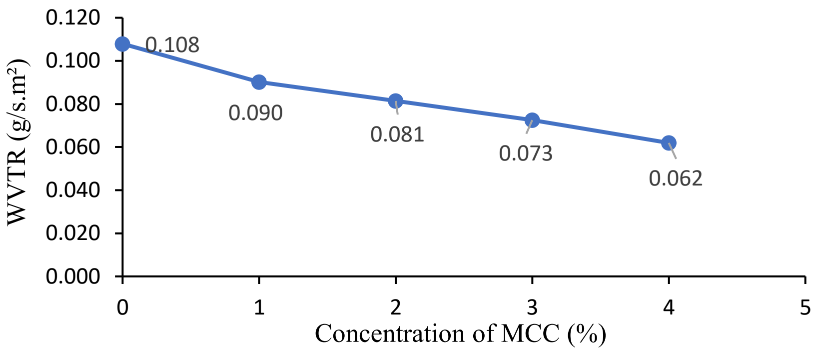


Figure 7. Water vapor transmission rates of avocado seed composite films.

CONCLUSION

In this work, microcrystalline cellulose (MCC) has been extracted from red algae *Gracillaria longissima* through alkalization, bleaching, and acid hydrolysis processes. The as-produced MCC was utilized as a reinforcing filler to develop composites, followed by characterization of the MCC. To achieve this, the solvent casting method was used to produce starch-MCC composite films by dispersing various MCC contents (1, 2, 3, and 4 wt%) into an avocado seed starch matrix. Characterization results showed that the particle size of MCC obtained was 44.1 µm with rough and irregular surface morphology. The properties of the composite films have improved as a result of MCC incorporation into the starch polymer. The produced composite films demonstrated strong UV blocking properties against UV-A, particularly UV-B rays, and good transparency. It was found that the composite films could block 82.8–94.6% of the UV-A wavelengths and 98.8–100% of the UV-B wavelengths. The starch-MCC composites show enhanced tensile characteristics. With the incorporation of 4 wt% MCC into a starch matrix, the tensile strength was increased by 152% compared to neat starch. Meanwhile, the elongation at break was decreased by 63%. Finally, the barrier properties of avocado seed composite films were similar to those of cellulose derivatives used in food packaging applications. Adding 4 wt% MCC into the avocado seed starch matrix reduced the water vapor transmission rate by 43% of the neat starch value. Therefore, the resulting composite films from the current work could be an alternative for food packaging applications.

CONFLICT OF INTEREST

There is no conflict of interest in this article.

AUTHOR CONTRIBUTION

MPP: Investigation, Methodology, Project Administration, Resources, Validation, Visualization, Manuscript Drafting and Editing; IP: Conceptualization, Data Analysis, Funding Acquisition, Manuscript Review, Supervision; DD: Conceptualization, Manuscript Review, Supervision; YY: Data Analysis, Validation, Visualization, Manuscript Review.

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