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Research article

Modification of Nata De Coco Composited with Cow Hoof Keratin as a Candidate for Proton Conducting Membrane

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Abstract

Proton conducting membranes based on nata de coco (bacterial cellulose) and cow hoof keratin were synthesized. Nata de coco was produced by fermentation with *Acetobacter xylinum* bacteria and cow hoof keratin obtained from the hydrolysis of cow hoof using NaOH solvent. Membranes were synthesized with variations in the mass ratios of nata de coco: cow hoof keratin, namely 4.5:0.5, 4.7:0.3. 4.0:1.0. The proton conducting membranes were characterized by functional group analysis with Fourier transform infrared spectroscopy (FTIR), diffractogram patterns were observed using X-ray diffraction (XRD), and proton conductivity was tested at temperatures of 25°C, 40°C, 60°C and 80°C. The degree of swelling and methanol permeability were also analyzed. The FTIR spectrum results showed that physical interactions appeared at the peak of 3000-3500 cm^{-1}. The results of the XRD diffractogram analysis showed that all variations of the composite membrane mass were semicrystalline originating from a combination of the crystalline phase of bacterial cellulose and the amorphous phase of cow hoof keratin. The highest degree of swelling was obtained at a mass ratio of 4.0:1.0 of 33.05% while the lowest methanol permeability was at a mass ratio of 4.5:0.5 of 2.1×10^{-9} mol/cm.s. For the proton conductivity of the composite of bacterial cellulose nata de coco and cow hoof keratin variation 4.0:1.0, the highest proton conductivity was obtained at 2.68×10^{-4} S/cm and at a temperature of 25°C. In short, the results of this study indicate that the composite membrane has the ability to conduct protons and has the potential to be developed as an alternative fuel cell.

Keywords: nata de coco; bacterial cellulose; cow hoof; keratin; conducting membrane

1. Introduction

Coconut water can be fermented into nata de coco, and the use of coconut water to make nata de coco has been the subject of numerous prior research efforts (Nugroho & Aji, 2015; Zhang et al., 2017; Rahmayanti et al., 2019; Herawati et al., 2020). Since cellulose is the primary ingredient of nata de coco, it is frequently referred to as bacterial cellulose in

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scientific contexts. Nata de coco has good mechanical properties, and when modified into a thin film it produces a Young's modulus ranging from 15.2 -18.1 Gpa, Tensile strength 91-260 Mpa, and elongation 0.8-2.1% (Iguchi et al., 2000). Chemically, nata de coco is insoluble in sodium hydroxide, hydrochloric acid, methanol or acetone and can only be dissolved in complex solvents such as cupriethylenediamine. Chemical examinations using FTIR show functional groups contained in cellulose and temperature stability up to 330-335°C. The micro form of nata de coco fibers is irregular, consisting of fine cellulose ribbons called fibrils. The ribbon size is in the range of 50-60 nm and appears as randomly oriented intersecting and overlapping cellulose layers (Halib et al., 2012). This type of cellulose has not only been produced purely from coconut water. Other studies reported the production of bacterial cellulose that did not use coconut water (Fan et al., 2016; Pourjavaher et al., 2017).

In addition, nata de coco has a low production cost and is easy to make, process, and obtain. Initially, nata de coco was used to preserve the remaining coconut water as a jelly-like material. A chewy, translucent, jelly-like substance made from coconut water, nata de coco (also known as "coconut gel") forms a gel when *Acetobacter xylinum* produces bacterial cellulose. Because it is mostly composed of coconut water, nata de coco has a low nutritional profile. However, nata de coco has a lot of dietary fiber because it is composed of cellulose (Tallei et al., 2022). Previous research reports related to bacterial cellulose as a nanofiber material and drug delivery were reported (Barjasteh et al., 2023; Behrouznejad et al., 2024). More research is still needed to determine whether it can be used as a proton conducting membrane.

Cow hoof waste from slaughterhouses has not yet been utilized although cow hoofs contain a lot of keratins that can be extracted and can be used for various purposes. Previous studies on the extraction of keratin from cow hoof reported that keratin filament polypeptides were dissolved from the epidermis of calf hoof in the presence of denaturing and reducing agents and then purified (Sayers et al., 1990). Cow hoof was known to contain 75% insoluble protein in the form of keratin, the fiber of which demonstrated resistance to degradation under pressure and provided good mechanical strength and elasticity (Yilma et al., 2022). Initial research related to keratin from cow hoof waste as a high-performance supercapacitor electrode (Lobato-Peralta et al., 2025) and opportunities for further research for more advanced materials were studied. In addition, keratin is found in hair and nails (Hill et al., 2010). Keratin is also found in chicken feathers and duck feathers, and the fusion of keratin with other materials was studied (Tanabe et al., 2004: Hartrianti et al., 2016). From the abundance of keratin in nature, it is necessary to modify it into a more useful and valuable material.

High community mobility increases fuel demand, as reflected in the use of transportation such as trucks, trains, and ships (Russell et al., 2014; Gohari et al., 2018; Li et al., 2022). In addition, there has been an increasing demand for fuel in the household sector, electronic equipment all requires fuel, especially for fossil fuels in industry and daily life. However, the supply of fossil fuels such as fuel oil is decreasing. Alternative fuels that are environmentally friendly, cheap and safe are needed to overcome these problems. Fuel cells can be used as one of the alternative fuel options (Martin et al., 2009; Luk et al., 2012; Iribarren et al., 2016). Fuel cells are the most effective and promising candidates for zero-emission power sources. Two types of fuel cells, the proton exchange membrane fuel cell (PEMFC) and the direct methanol fuel cell (DMFC) are of interest because they operate at low operating temperatures between 60-80ºC, and demonstrate high efficiency and power density, and good system durability.

The most widely used polymer electrolyte in PEMFC and DMFC applications is the Nafion membrane, but the membrane production cost is very high. During fuel cell operation, the Nafion membrane shows good performance and durability. However, the Nafion membrane has many shortcomings, including limited mechanical strength, low glass transition temperature in the fully hydrated state, and low proton conductivity at high temperatures. The operating range of a fuel cell can be limited by damage to the Nafion membrane. The high permeability, substantial dimensional changes in the fully hydrated state at high temperatures, and high cost of the Nafion membrane further contribute to its poor chemical stability. The durability and commercialization of fuel cells are greatly affected by these characteristics. Therefore, the Nafion membrane can be replaced by cheaper membranes (Jung & Kim, 2012). For example, bio-based materials such as nata de coco offer the advantages of thermal stability up to a temperature of 220ºC, and a glass transition temperature of 44.28ºC. Its chemical structure can absorb water, making it possible to replace Nafion (Mohite & Patil, 2014). Hydrogen besides methanol has been known as one of the alternative energy carriers to generate electricity for household, industrial, and automotive needs (Edwards et al., 2008). PEMFC uses hydrogen as a fuel source, which diffuses through the membrane, while DMFC uses methanol as a fuel source. Although methanol is used as a fuel source in DMFC, hydrogen remains the main component because methanol in DMFC is converted into hydrogen through a catalytic reaction, which then passes through the membrane, where bio-based materials are as membranes.

Fuel cells are electrochemical devices that convert chemical energy from fuel directly into electrical energy. The working principle of the fuel cells is very similar to the working principle of batteries, because both have main components in the form of electrodes and electrolytes. The difference lies in how they work; fuel cells can be used not only as a means of storing electricity, but also as a power generator. Generating electricity with fuel cells is a relatively inexpensive low risk technology, and electricity can be produced from small to large capacities with high system efficiency, and does not cause environmental pollution (Olabi et al., 2021). In the environment, the use of fuel cells in the transportation sector can reduce air pollution in big cities because exhaust emissions from these fuel cells are in the form of water vapor (Hossen et al., 2023).

One of the alternatives in making membranes is the use of natural polymers. One natural polymer that has the potential as membranes and is relatively cheap is nata de coco. Nata de coco is bacterial cellulose that can be used as a polymer-based composite material because it has high mechanical properties and high-water content (98-99%) with good liquid absorption. Lang et al. (2025) reported the attempt of utilizing cellulose as a material for the advanced development of smart stimulus-responsive cellulose-based composites. In general, composite materials consist of reinforcement and matrix. The reinforcement is used to increase the strength of the composite, while the matrix is used to bind the reinforcement. Therefore, the membranes used in this study were bacterial cellulose from nata de coco (which functions as the composite constituent matrix) and natural fibers from cow hoof waste from the slaughterhouse (which functions as the reinforcement). The proton conducting membranes were made based on the mass ratio between nata de coco and cow hoof keratin. In this study, various ratio of wet nata de coco to cow hoof keratin was compared while the total mass of nata de coco and cow hoof keratin was kept constant. The procedure used was modified from previous research reports (Wahid et al., 2019). In general, the research was carried out was to offer information on the characteristics of the proton conduction membrane based on nata de coco and cow hoof keratin.

2. Materials and Methods

The materials used in this study were cow hoof, coconut water, acetone (from Merck), soap, sodium hydroxide (98% from Merck), dimethyl formamide (DMF 99.8% from Merck), distilled water, granulated sugar, ammonium sulfate (98% from Merck), methanol (99% from Merck), glacial acetic acid (99% from Merck), and *Acetobacter xylinum* obtained from the Plant Protection Laboratory of the Faculty of Agriculture, University of Bengkulu. Cow hoof samples were obtained from a cattle slaughterhouse waste disposal site and coconut water samples were obtained from a traditional market, both in Bengkulu City.

2.1 Preparation of cow hoof keratin and making nata de coco

Samples were prepared by washing the cow's hoof thoroughly using water and soap several times, then drying them in an open space. The dried cow hoof was soaked again in acetone for 15 min. Then the cow hoof was dried at room temperature in open space. The dried cow hoof was cut into small pieces using a knife. Next, 50 g of hoof was dissolved in 250 mL of 1 M NaOH solution at a temperature of 75ºC until dissolved. The mixture was stirred using a magnetic stirrer, and glacial acetic acid was added little by little until the pH of the solution became neutral. Then the solution was heated at a temperature of 75ºC until a gel formed. The resulting gel was centrifuged 2 times and sediment, and filtrate were obtained. The sediment from the centrifugation was then heated again at a temperature of 75ºC until dry. The dried sediment was ground using a mortar.

Nata de coco was made following the methods of Sawitri et al. (2020). Three litres of coconut water were filtered and then heated to boiling. After boiling, 300 g of granulated sugar and 15 g of ammonium sulfate were added. After mixing it well, the mixture, which was still hot, was transferred into a plastic container measuring 27 x 21 x 3.5 cm and covered with newspaper. When the mixture was almost cold, 30 mL of glacial acetic acid were added again. After the mixture had completely cooled, 80 mL of *Acetobacter xylinum* bacterial starter was added using aseptic technique. Bacterial fermentation was allowed for 10 days at room temperature. The nata de coco obtained from the fermentation was soaked in water for 24 h and the soaking water was discarded. The nata de coco was soaked again in distilled water at a temperature of between 80-90ºC for 2 h and the distilled water was discarded. This was followed by soaking in 2% NaOH at a temperature of between 80-90ºC for 2 h, discarding of the NaOH solution, and further soaking again in distilled water at a temperature of 80-90ºC for 2 h. Purification of bacterial cellulose by heating at a temperature of 80-90ºC was carried out using a hotplate.

2.2 Membrane synthesis and its characterization

The membranes prepared were nata de coco membranes and proton conducting membranes. The nata de coco/bacterial cellulose membranes were cast in the form of thin sheets with a mass of 5 g in a petri dish and then heated using a hot plate at a temperature of 30ºC to evaporate the water. The preparation of the proton conducting membranes was carried out by mixing the nata de coco that had been made into a slurry and then filtered into the previously prepared cow hoof keratin. In another step, a few drops of DMF solvent were added to the cow hoof keratin. Then added nata de coco slurry, with a mass ratio of bacterial cellulose: cow hoof keratin of three variations, namely 4.5: 0.5; 4.3: 0.7; and 4.0: 1.0. The preparation of the composite membrane was carried out by mixing the ingredients mentioned according to the ratio, stirring until the two ingredients were evenly mixed in each variation for 36 h. Then, poured into a petri dish with a diameter of 5 × 5 cm and heated to evaporate the existing solvent. The membrane thickness was controlled by evaporating the solvent at a constant temperature of 30ºC and the total mass ratio was kept constant. After the solvent evaporated and the dry thin sheet membrane was formed, the resulting membrane was then characterized.

The proton conducting membrane was analyzed using Fourier transform infrared spectroscopy (Bruker Alpha-P, Wismar, Germany), operating in damped total reflectance (ATR) in the range of 4000-400cm^{-1}. This analysis was carried out to determine the functional groups in the cow hoof keratin and composite membranes. The diffractogram patterns were analyzed using an X-ray diffraction instrument (RigakuDMAX2200, Japan) with Cu Ka radiation (λ=1.5406 Å) in the range of 2θ between 0º and 100º. This analysis was conducted to determine the diffractogram pattern of the composite membrane. Methanol permeability was measured using a homemade test cell. The cell was filled with methanol. Methanol vapor in equilibrium with the liquid diffuses along the concentration gradient through the membrane, which is clamped between the mouth of the beaker and its lid (Göktepe et al., 2008; Sen et al., 2008; Sen et al., 2010; Sinirlioglu et al., 2013). Weight loss was recorded as a function of time and data were used for the calculation of permeability (Sen et al., 2008). Determination of water absorption was carried out gravimetrically. This analysis was carried out by cutting a membrane into 1 × 1 cm size, and weighing was carried out until the dry mass (m dry) was obtained. Then the membrane was soaked in distilled water. The membrane surface was dried with a tissue and then weighed, and the wet mass (m wet) was obtained. Measurement of composite material conductivity was carried out using a chemical impedance analyzer IM 3590 HIOKI. The nata de coco-cow hoof keratin composite membrane was made into a size of 1 × 1 cm, then its conductivity was measured with temperature variants of 25ºC, 40ºC, 60ºC and 80ºC. Measurements at several temperatures are related to the performance of proton conductivity at several conditions related to humidity. The research flow that was carried out is shown in Figure 1.

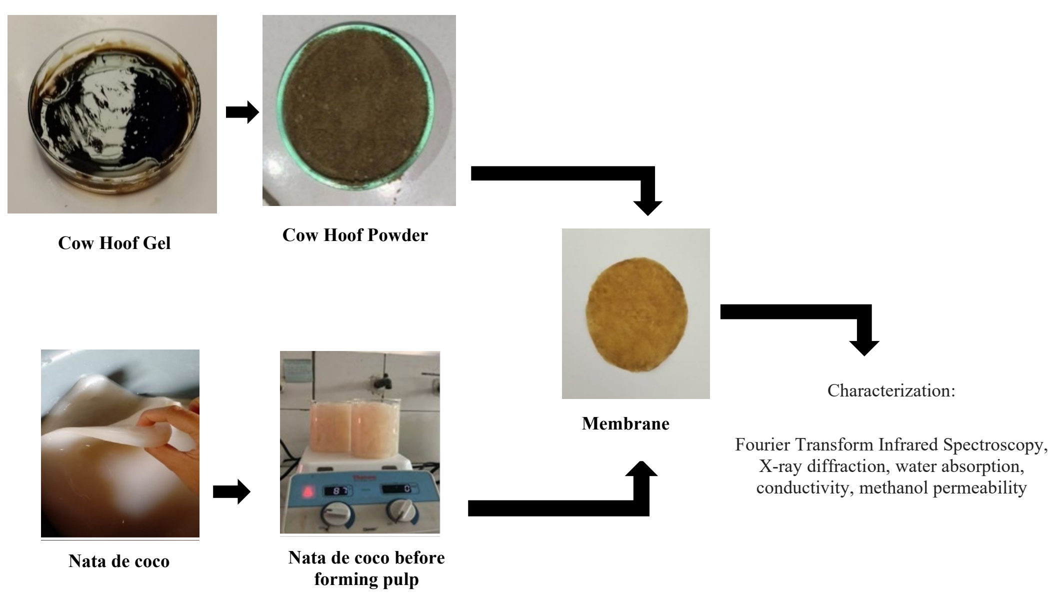

Figure 1. Scheme for synthesis and characterization of membranes

3. Results and Discussion

The prepared cow hoof keratin that was initially in the form of a solution turned thick and blackish green. This was due to the reaction between the strong base NaOH and the weak acid of glacial acetic acid which produced sodium acetate (CH_3COONa) and H_2O. After the solution was heated and stirred until a gel formed, it was then centrifuged to produce 2 phases; the resulting precipitate was taken, and the filtrate was discarded. The precipitate in the form of a gel was heated until dry and then ground using a mortar and pestle until keratin with a brownish green color was obtained, as can be seen in Figure 1. The cow hoof keratin obtained was similar to that reported by Yilma et al. (2022) and was hygroscopic. There are many ways to extract keratin from animals such as cows. Kida et al. (1995) reported the enzymatic hydrolysis of cow and buffalo horns and hooves. Other researchers also reported the extraction and characterization of keratin from bovine hoof, as well as its use as a biofilm (Kakkar et al., 2014; Kumar et al., 2016). The process of making nata de coco as bacterial cellulose through fermentation of *Acetobacter xylinum* bacteria with coconut water as the raw material (Nugroho et al., 2015; Phan et al., 2023). Coconut water is cooked until boiling, a step to remove certain contaminating bacteria in coconut water and to accelerate the dissolution of ammonium sulfate and sugar. The purpose of adding ammonium sulfate is to stimulate the activity of *Acetobacter xylinum* bacteria to increase the viscosity of cellulose. Masaoka et al. (1993) reported that nutrients containing glucose were obtained from coconut water and sugar. *Acetobacter xylinum* bacteria prefer acidic conditions, and according to Verschuren et al. (2000), these bacteria grow well at an optimal pH range of 3-5. The fermentation process is carried out at room temperature because *Acetobacter xylinum* bacteria cannot grow at high temperatures. After the fermentation process was carried out for 10 days, nata de coco obtained was thick and of even texture, as can be seen in Figure 1. The fermented nata de coco gel was soaked in water for 24 h and the soaking water was changed 3 times a day with the aim of removing dirt and mucus in the bacterial cellulose gel.

Nata de coco was soaked using distilled water at a temperature of 80-90°C for 2 h to remove any remaining bacterial in the nata de coco (Nurfajriani et al., 2021). The remaining bacteria cause a decrease in the mechanical strength of the cellulose because the bacteria can still be active and can utilize the nutrients contained in the cellulose gel (Lestari et al., 2014). Klemm et al. (2001) also reported on bacterial cellulose. Composite membranes were produced by mixing nata de coco and cow hoof keratin at several variations with the addition of dimethyl formamide solvent, and heating at a temperature of 30ºC. This was done to prevent the membrane surface shrinking or cracking (Gustian et al., 2022). The proton conducting membranes obtained from the synthesis are shown in Figure 2. The resulting nata de coco membrane was in the form of a white sheet, while the composite membrane was yellowish brown showing the influence of cow hoof keratin on thickness. The thicknesses of the composite membrane ratios of 4.5:0.5, 4.3:0.7 and 4.0:1.0 were 0.33, 0.44 and 0.57 mm, respectively. The thicknesses of the membranes produced in this research were very thick compared to the commercial Nafion membranes, which ranged from 12-60 nm.

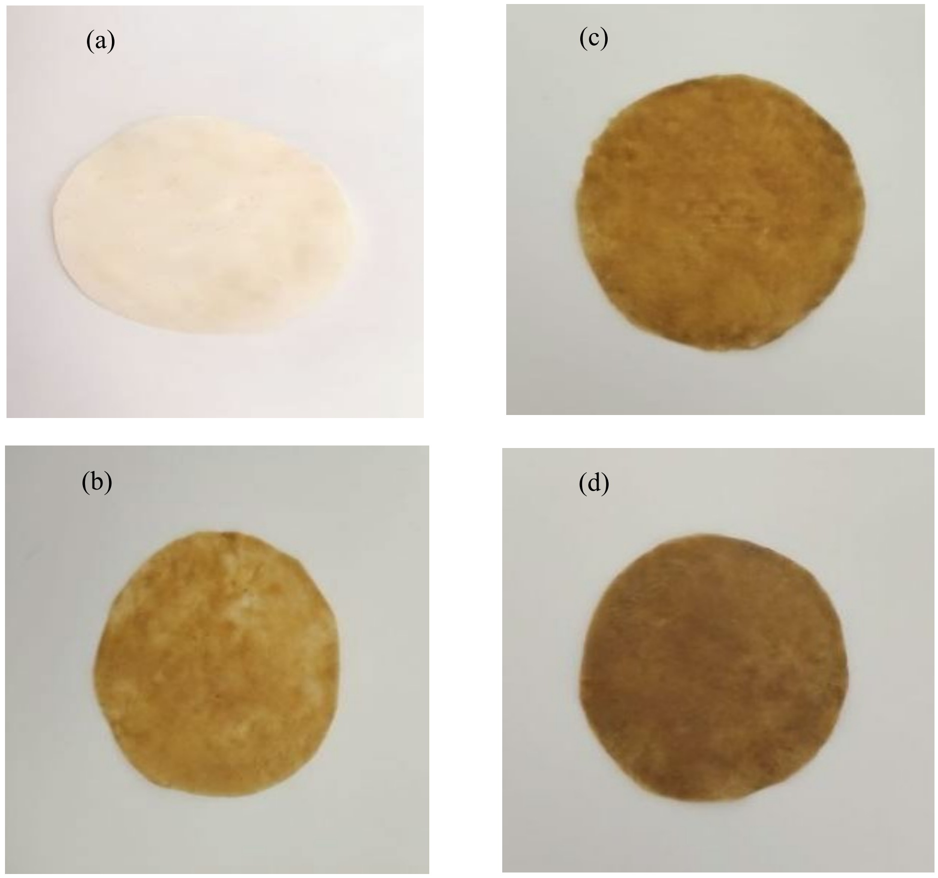

Figure 2. (a) The nata de coco membrane and the proton conducting membranes, (b) 4.5:0.5, (c) 4.7:0.3, and (d) 4.0:1.0

Based on Figure 3, the FTIR spectra of the proton-conducting membrane produced were a combination of bacterial cellulose polymers derived from nata de coco and cow hoof keratin. When compared to the constituent materials, differences in wave numbers are observed. The broadening of the wave number was caused by interactions between the polymers that make up the composite membrane. The main groups that play a role in conducting protons are the hydroxyl group (O-H) from bacterial cellulose and amine group (N-H) in cow hoof keratin. The hydroxyl groups and amine groups can form hydrogen bonds, where hydrogen bonds provide proton transfer from one hydroxyl group to another or amine group, thus facilitating the efficient transfer of protons through the proton conducting membrane. The interaction of keratin with alginate was reported by Tanabe et al. (2004). The interaction between nata de coco as bacterial cellulose and cow hoof keratin as a composite membrane can be seen at wave numbers of 3500 cm^{-1} and 2500 cm^{-1}. This area was emphasized by Aslan et al. (2009), who reported that the presence of band broadening between 3500 cm^{-1} and 2500 cm^{-1} was caused by the hydrogen bond network required for proton conduction. From the analysis of functional groups using FTIR (Figure 3), the nata de coco membrane had distinctive peaks at wave number of 3341 cm^{-1},

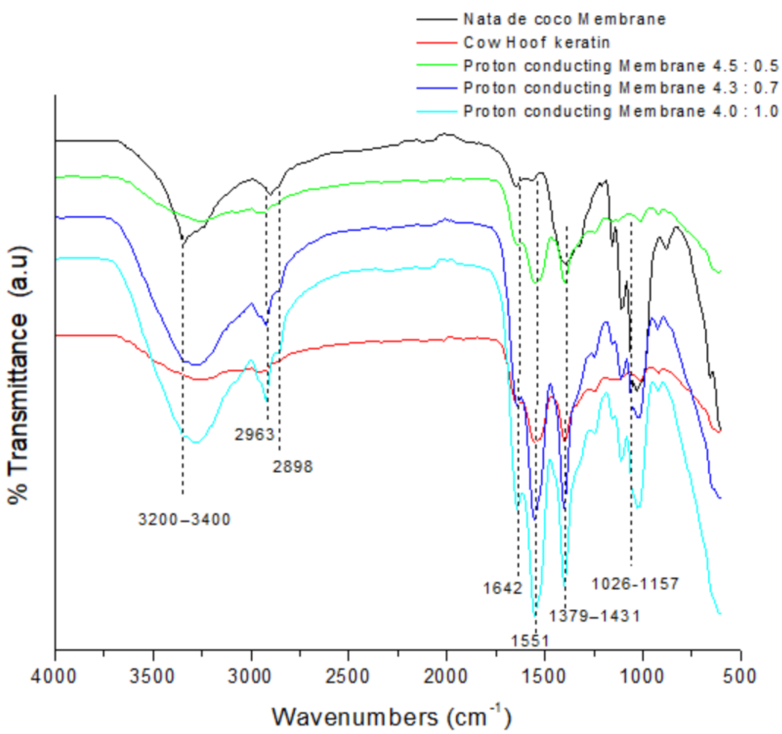

Figure 3. Spectra FTIR nata de coco membrane, cow hoof keratin and the proton conducting membrane; 4.5:0.5, 4.7:0.3, 4.0:1.0

indicating the O-H group. According to Mohite and Patil (2014), the O-H group is indicated by the wave number band of 3200-3400 cm^{-1}. Wave numbers 1026 cm^{-1} - 1157 cm^{-1} indicate a C-O stretching group for C-O-C and C-OH moieties; the absorption in this region indicates the presence of glycosidic bonds in the cellulose ring of nata de coco, which was in accordance with the observation of Muthu and Rathinamoorthy (2021). The wave number 2898 cm^{-1} indicates the presence of C-H groups from alkanes. The wave number range 1379-1431 cm^{-1} indicates vibration (C-H) bending for methylene and methyl groups and at wave number 656 cm^{-1} there is vibration bending of aromatic C-H bonds.

The results of the functional group analysis of cow hoof keratin are shown in Figure 3. From the spectrum, it shows that an absorption peak appears, namely the amine group (N-H) at a wave number of 3275 cm^{-1}. This occurs because hoof keratin is hygroscopic and retains water, allowing it to absorb additional moisture from the environment. While the amine functional group (N-H) indicates the presence of a peptide bond in each protein. A wave number of 2963 cm^{-1} indicates the presence of an alkane functional group (C-C stretching vibration). The peak absorption area of 2900 cm^{-1} to 3000 cm^{-1} indicates the presence of an alkane group. At a wave number of 1642 cm^{-1}, it shows a primary amide and at a wave number of 1551 cm^{-1} it shows a secondary amide. At wave number 1395 cm^{-1}, C–H bending for methyl (-CH) and methylene group (-CH_2-) is indicated and sulfide (vibration C–S) is indicated at wave number 919 cm^{-1}. The reported functional group study of cow hoof keratin was similar to the study reported by Kakkar et al. (2014). Other studies also reported on the functional groups of cow hoof keratin (Kida et al.,1995). The

FTIR spectrum obtained from cow hoof keratin in our studies was also similar to that reported by Hartrianti et al. (2016).

X-ray diffraction (XRD) analysis was carried out to determine the diffractogram patterns of the proton-conducting membranes. The results of the XRD of pure nata de coco bacterial cellulose were semi-crystalline with dominant peaks appearing in the 2θ angle area between 20° - 40°. The nata de coco membrane diffractogram is shown in Figure 4 and is similar to the research conducted by Gustian et al. (2022). The XRD diffractogram of nata de coco in this study shows the formation of a crystalline phase in the 2θ area between 14.5° and 22.7°. Materials with a crystalline structure show sharp peaks and have a more regular and dense structure. While amorphous materials tend to show a wide peak and have an irregular and loose structure (Gustian et al., 2023). The results of the XRD diffractogram of nata de coco show the fiber structure of bacterial cellulose, where the diffraction pattern obtained is crystalline causing the fiber in bacterial cellulose to have high purity. The diffractogram analysis of nata de coco obtained from this study was also similar to that reported by Mohite and Patil (2014) and Muthu and Rathinamoorthy (2021). Based on the diffractogram of cow hoof keratin shown in Figure 4. One peak at an angle of 2θ (22.5°) has an intensity that indicates the contribution of the peak from nata de coco. The diffractogram of cow hoof keratin showed wide peaks resulting in an amorphous to semicrystalline structure type. The amorphous phase results in more empty space which allows more increased ion movement. The diffractogram pattern of keratin shown in this study was similar to that of keratin analyzed by Kida et al. (1995) and Lobato-Peralta et al. (2025).

Figure 4 shows the XRD diffractograms of the proton conducting membrane indicating a semicrystalline phase. This was due to the addition of the amorphous properties of cow hoof keratin into the nata de coco. The proton conducting membrane 4.5: 0.5 showed diffractogram peaks at angles of 17.0°, 22.5° and 29.7°, and had a maximum intensity of 1029.5. In the diffractogram of the membrane with a ratio of 4.3:0.7, peaks at angles of 17.0°, 22.5° and 29.7° had a maximum intensity of 939.6146. The diffractogram of the 4.0:1.0 membrane showed peaks at angles of 17.0° and 22.7° with a maximum intensity of 608.8. Of the three proton conducting membranes, it shows that the higher the amount of cow hoof keratin, the lower the intensity will be. According to Doumeng et al. (2020), the lower the intensity of a diffractogram peak, the more the amorphous phase would increase. This phenomenon was also in line with other studies that were related to the structure and diffraction pattern of keratin starch biocomposite film from chicken feather waste (Oluba et al., 2021). Methanol permeability is a process of measuring the movement of methanol solution through a membrane. High methanol permeability will cause a decrease in membrane performance in fuel cells.

Based on the results of methanol permeability as a function of time in Figure 5, it was revealed that the 4.0:1.0 proton conducting membrane had a methanol permeability of 4.1×10^{-9} mol/cm.s, which was the highest among the membranes. This phenomenon is similar to observations of proton conductivity. In this case, the addition of keratin resulted in the best cross-linking. The hydroxyl groups of nata de coco and the amine groups of keratin form hydrogen bonds, which result in cross-linking and allow methanol to pass through the membrane at many cross-linking sites. Due to insufficient keratin addition, the methanol permeability for other membranes is less than 4.1:1.0. Methanol permeability measurement was carried out at a temperature of 25°C where the rate of methanol evaporation is normally slow. If the measurement is carried out at an operating temperature of 60-80°C, the rate of methanol evaporation will be high, and membrane damage can result. According to Sen et al. (2008), Nafion 112 has a methanol permeability

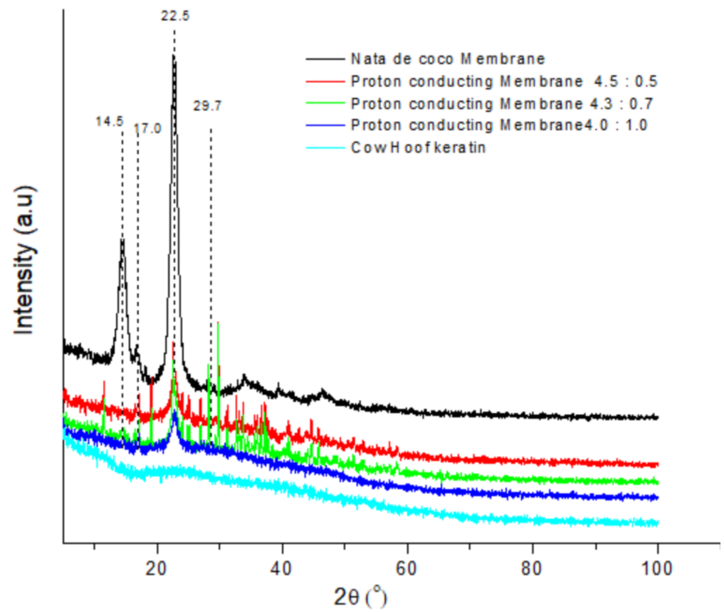

Figure 4. XRD diffractogram of nata de coco membrane, cow hoof keratin and the proton conducting membranes; 4.5:0.5, 4.7:0.3, 4.0:1.0

of 1.89 × 10^{-9} mol/cm.s. This shows that the methanol permeability of the proton-conducting membrane is still in the same order as the Nafion membrane. It can be concluded that the greater the amount of bovine hoof keratin added, the higher the methanol permeability. The high methanol permeability in the 4.0:1.0 membrane was due to the increased number of cross-links within the polymer matrix that facilitate transport. For fuel cell applications, proton conductivity is very influential because if the proton conductivity is low, the membrane cannot be applied to fuel cells. Conversely, if the proton conductivity is high, the quality of the membrane in conducting protons is good in terms of fuel cell efficiency. Membranes that can be used in fuel cell applications have a proton conductivity greater than 1 × 10^{-5} S/cm (Smitha et al., 2006). Figure 6 shows the results of proton conductivity measurement, which indicate that the nata de coco membrane had the highest proton conductivity at a temperature of 25°C of 1.07 × 10^{-5} S/cm.

Figure 5 showed that, in general, with a decrease in temperature there is an increase in proton conductivity. This suggests the membranes tend to work optimally at lower temperatures. When considering the effect of adding cow hoof keratin, the 4.0:1.0 membrane at a temperature of 25°C produced optimal proton conductivity. Under these conditions, it is possible for interactions between the hydroxyl groups of nata de coco and NH in keratin, where more cross-linking occurs through hydrogen bonds as a facilitator of proton movement. For membranes with other variations, the possibility of such interaction was lower, thus the proton conductivity was seen below the 4.0:1.0 membrane. The increase in proton conductivity was caused by the large amount of cow hoof keratin added. The large number of amine groups in the hoof keratin helped the transfer of protons, and the protons were more easily to move from site to site. High

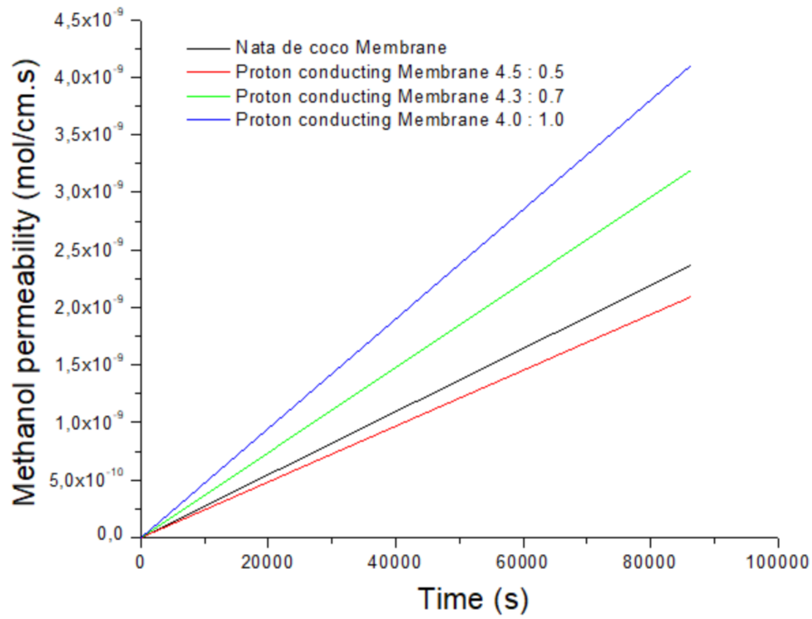

Figure 5. Methanol permeability of nata de coco membrane and the proton conducting membranes; 4.5:0.5, 4.7:0.3, 4.0:1.0

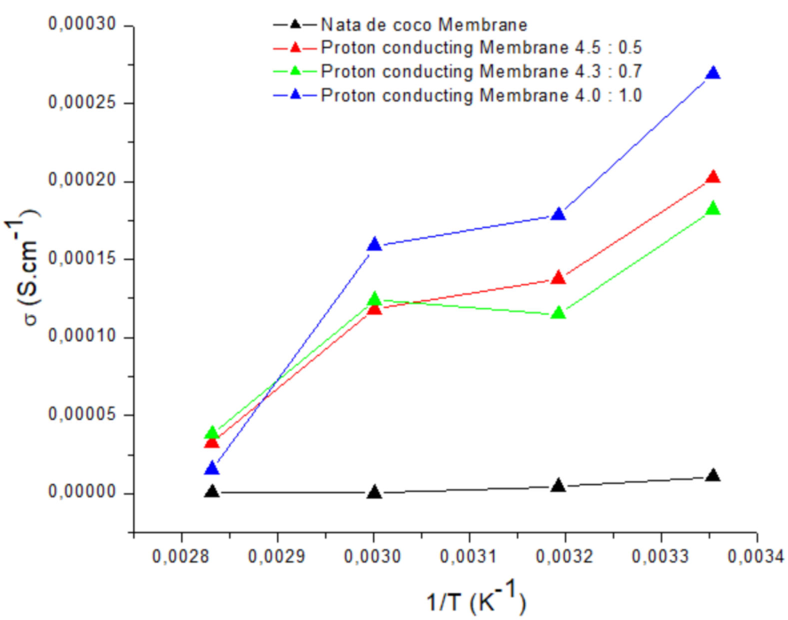

Figure 6. Proton conductivity nata de coco membrane and the proton conducting membrane; 4.5:0.5, 4.7:0.3, 4.0:1.0

proton conductivity is also caused by the highwater retention of the materials used, where hoof keratin has hygroscopic properties that can absorb water molecules from its environment (Smitha et al., 2006). The proton conductivity of the resulting membrane was still low compared to the proton conductivity of the Nafion membrane measured at room temperature of 7.8 X 10^{-2} S/cm (Sone et al., 1996). Proton transfer in hydrated electrolyte membranes can occur through two main mechanisms, namely the proton hopping mechanism and the proton vehicle mechanism. In the proton hopping mechanism, proton transfer involves the formation and breaking of hydrogen bonds in water molecules. Protons will move from one hydrolyzed ionic group such as the $H3O^+$ group to another ionic group along the membrane. While in the proton flow mechanism, hydrated protons ($H3O^+$) diffuse through the water medium due to electrochemical differences. Water molecules function as "vehicles" to transport and move protons, so that they can pass through the membrane. Overall, proton transfer can increase with increasing water content in the membrane. The relationship with the mixing ratio used is that with increasing keratin added, it is possible for cross-linking to occur between nata de coco and keratin. The cross-links facilitate the delivery of protons. The more keratin contained in the membrane, the more cross-links there will be, allowing for greater proton conductivity. The ability of the composite membrane to absorb water also affects proton conductivity. In this study, commercial Nafion membranes were used as a comparison, so that the water absorption capacity of the Nafion membrane became a reference for comparison. Analysis of the percentage of water absorption capacity was carried out by weighing the mass of the membrane after being soaked in distilled water minus the initial mass of the membrane divided by the initial mass and then multiplied by 100%. A membrane that absorbs water plays a very important role as a proton transport medium related to the proton conductivity capacity. Figure 7 shows that the percentage of water absorption capacity of nata de coco membrane was 20%, and the percentage of water absorption capacity of each proton conducting membrane had varying values, i.e. 21.24% for 4.5: 0.5 membrane, 28.84% for 4.3: 0.7 and 33.05% for 4.0:1.0, respectively. It can be seen that the mass ratio of 4.0:1.0 had the highest percentage of water absorption capacity, which was 33.05%. This shows that the more cow hoof keratin added, the higher the percentage of water absorption. This is because cow hoof keratin is hygroscopic so that proton transfer to the membrane increases. According to Inan et al. (2010), high water content in the membrane will facilitate the transfer of protons, but if the membrane absorbs too much water, it will cause the mechanical properties of the membrane to become less stable. Higher water absorption can also prolong the movement of protons, resulting in low proton conductivity. In the results of this study, the air absorption capacity for the composite membrane produced was still close to the air absorption capacity of the commercial Nafion membrane. From Figure 7, it is revealed that the higher the weight ratio of cow hoof keratin added to the proton conducting membrane, the higher the level of cross-linking and thus the higher the percentage of water absorption capacity, indicating an increase in hydrophilicity. This was in agreement with that reported by Hartrianti et al. (2016).

4. Conclusions

The synthesis of composite membranes was successfully carried out using nata de coco and cow hoof keratin at three mass ratios. The resulting proton-conducting membranes were thin sheet-shaped membranes of brownish color with a slight greenish tinge. Characterization of the proton-conducting membranes using FTIR shows interactions at

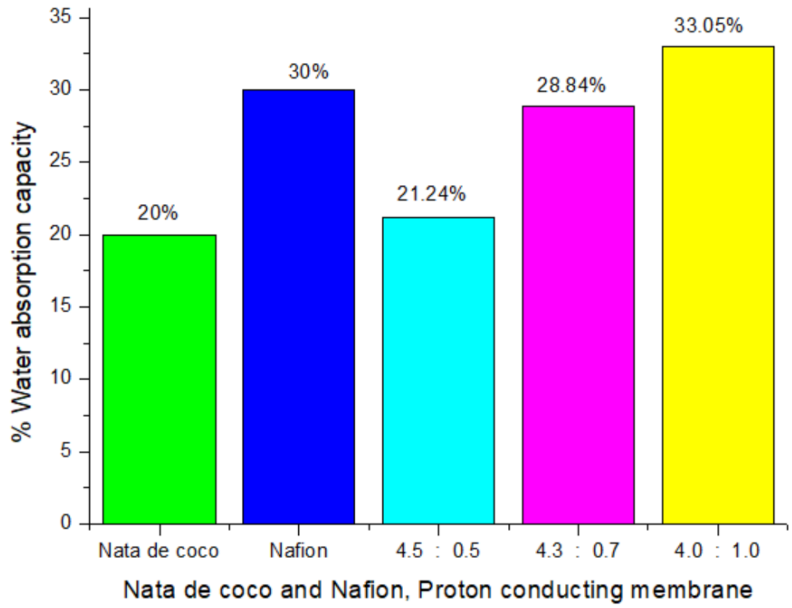

Figure 7. % water absorption capacity nata de coco membrane and the proton conducting membrane; 4.5:0.5, 4.7:0.3,4.0:1.0

the peak of 3000-3500 cm^{-1}. The highest percentage of water absorption capacity of the proton-conducting membrane was 33.05% in the 4.0:1.0 mass ratio membrane. The proton conductivity of the nata de coco membrane was 1.07 × 10^{-5} S/cm at a temperature of 25°C while the highest proton conductivity for the nata de coco: cow hoof keratin membrane was 2.68 × 10^{-4} S/cm in the 4.0:1.0 mass ratio at a temperature of 25°C. The XRD results of the proton conducting membranes show a semicrystalline phase which was influenced by the presence of a crystalline phase in the nata de coco cellulose and an amorphous phase in the cow hoof keratin. The lowest methanol permeability was found in the 4.5:0.5 membrane of 2.1 × 10^{-9} mol/cm.s. The formulation that showed the most promising overall performance was the 4.0:1.0 proton conducting membrane.

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6. Authors' Contributions

Irfan Gustian designed research, wrote the paper, and coordinated research; Gusmi Susriati performed research; Ria Nurwidiyani contributed new reagents/analytic tools; Teja Dwi Sutanto analyzed data, checking the manuscript; and Asdim, analyzed data, checking the manuscript.

7. Conflicts of Interest

The authors declare that they have no conflicts of interest.

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