



# Using Glucose as Protein Remover from Rubber Films with One Pot Method

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## ARTICLE INFO

### Article history:

Received 2 March 2024

Received in revised form 12 June 2024

Accepted 15 August 2024

Available online 2 September 2024

### Keywords:

Deproteinization; High ammonia natural rubber latex; Rubber films; Opacity; Film characteristics

## ABSTRACT

One of Thailand's important agricultural products, particularly in the south, is high ammonia natural rubber latex (HANR) concentrate. Numerous products, including dip-moulded products, rubber films, and foams, are made with this latex. The protein content inside latex is one issue that may happen when using it. This is because those who have contact with rubber products can get allergic reactions as the result of these proteins. Before using latex, these proteins are typically removed in the form of latex. In order to digest these proteins, both chemicals and enzymes may be used. It is very rare to remove protein with a solid rubber product. In this study, raw rubber films were used to deproteinize employing a simple one-pot technique using glucose as protein remover. Glucose was used at a various concentration of 0, 5, 10, 15, and 20 phr by mixing with HANR. The films were subsequently produced by casting, dried, and then submerged in water for glucose leaching. A preliminary analysis of the chemical structure using the FTIR technique demonstrated in removing glucose from the rubber. According to a comparison of deproteinization determined by using total nitrogen analyser between the removal of protein in latex with EDTA solution combined with high-speed centrifugation technique as basic method and the use of glucose, the amount of protein removed in the first method was around 80%, whereas the use of glucose is over 50%. Water absorption, light transmission, as well as opacity and morphological characteristics, are further characteristics that use to compared between rubber film properties made by both deproteinization methods. It was found that the films produced using EDTA performed better than those produced with glucose to remove protein, but not considerably better. Meanwhile, both techniques of protein removal did not significantly affect the produced rubber films mechanical characteristics. The explanation for using glucose to remove protein from rubber as well as the findings will be discussed in more details in this research work.

## 1. Introduction

Natural rubber is a material that is used in a variety of products today, including industrial and medical gloves, saline hoses for medical devices, and shoe soles [1,2]. The primary source of rubber is *Hevea brasiliensis*, commonly known as para rubber. Para rubber is one of Thailand's most

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<https://doi.org/10.37934/araset.51.1.195210>

economically important plant. The area of rubber plantations was 24.47 million Rais (1 Rai = 0.3954 Acres) in 2021, according to the Office of Agricultural Economics, Ministry of Agriculture and Cooperatives of Thailand [3]. The 4.89 million tons of rubber goods were produced during that year. The milky, white liquid known as rubber latex is a primary product of natural rubber. Natural rubber latex has a rubber content of 30–40% by weight that is dispersed as rubber latex particles in water with a diameter of 0.1–1.0  $\mu\text{m}$  [4]. It also contains a few small amounts of minor non-rubber substances such as protein, phospholipids, carbohydrates, sugar, and metal ions. Some are available in forms that are water soluble, whereas others do not dissolve in water [5]. The primary chemical structure of rubber particles is *cis*-1,4-polyisoprene. Although rubber, especially rubber from latex, has been shown valuable in the production of many different types of goods, there is one serious danger associated with its use. When ammonia is added to rubber latex, the protein and phospholipids on the outer surface of the rubber colloid assist stabilize the rubber particle in a colloid form. Rubber is preserved through this technique both during storage and throughout transportation. However, for people who are allergic to latex, the protein on the surface of rubber latex particles might occasionally cause latex allergy [6]. Deproteinization, also known as the removal of protein from rubber latex, is typically requested for rubber industrial products, especially in medical applications, as well as for all human-touchable products like gloves, in order to minimize this danger.

Proteins can be deproteinized from rubber particles using enzymatic techniques, according to reports [7,8]. Based on some reports, the enzymatic deproteinization process works effectively when combined with specific substances, such as urea [9]. However, most of enzymatic protein removal procedures required more chemicals to serve as surfactants, which increased the amount of chemicals needed in the process [9,10]. To remove protein from rubber latex, different types of ethylenediaminetetraacetic acid (EDTA) were used, in addition to sodium dodecyl sulphate (SDS) as a surfactant [5]. With and without the addition of ammonia, the EDTA deproteinization procedure revealed a reduction in the protein concentration of preserved latex. Since for all deproteinization of rubber that has been previously reported, the majority of these methods used rubber latex and required more chemicals and multiple steps in the processing. Although solid types of rubber are also widely used, there were less reports of deproteinization with solid rubber. In this work, a single simple process and a single chemical is employed to remove protein from the rubber film. In this study, glucose (also known as dextrose-glucose or D-glucose) has been used as a protein remover. The deproteinization of latex using EDTA will be compared to the reduction of protein with rubber solid films at various glucose addition ratios. In addition, the mechanism of deproteinization using glucose and the characteristics of their films will be discussed in this research work.

## 2. Methodology

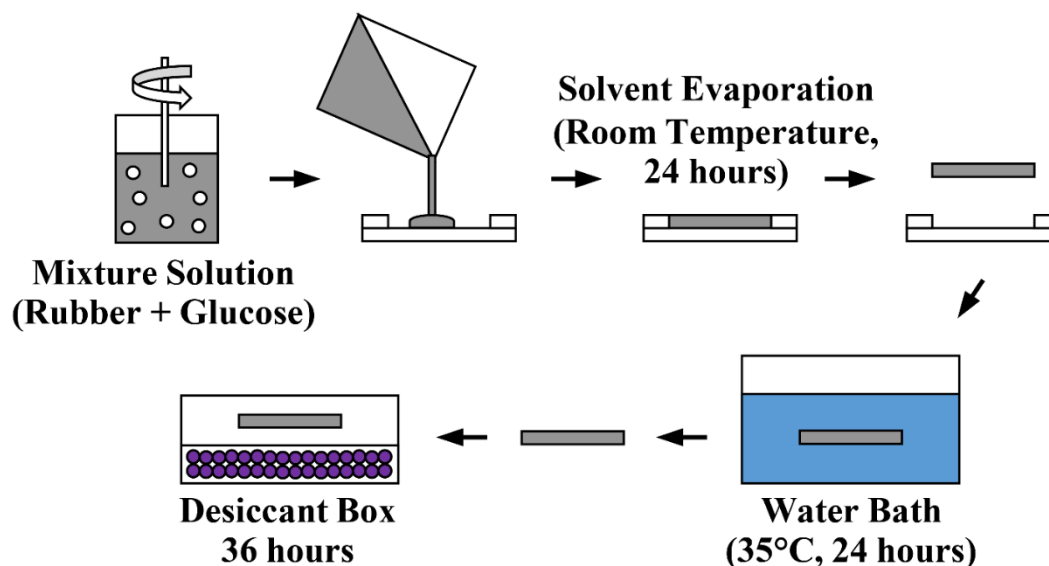
### 2.1 Rubber Films Preparation

High ammonia natural rubber latex (total solid content: 60%) was supplied by Chalong Latex Industry Co., Ltd, Thailand. D(+)-glucose (G) anhydrous ( $\text{C}_6\text{H}_{12}\text{O}_6$  - purity: 99.99%) was purchased from Elago Enterprises Pty Ltd, Australia. The rubber film formulations used in this research were listed in Table 1. HANR and DPNR refer to high ammonia natural rubber latex and deproteinized natural rubber latex, respectively. The HANRXG code refers to the addition of glucose to HANR, where X is the glucose concentration in the mixed latex, expressed in parts per hundred rubbers (phr). The mixed latex solution was prepared by mixing HANR with glucose concentrations as described in Table 1.

**Table 1**  
 Formulation for preparing rubber films

Films	Rubber Concentration (phr)	Glucose Concentration (phr)
HANR	100	0
HANR5G	100	5
HANR10G	100	10
HANR15G	100	15
HANR20G	100	20
DPNR	100	0

The solution was stirred until it achieved homogeneity. The mixed solution was properly weighed, measuring 3 grams, and subsequently transferred into the acrylic mould. It was then uniformly distributed around the mould's surface. The mixed solution was subjected to evaporation and subsequent drying at room temperature for a period of 24 hours. Next, the latex film was removed from the mould and subjected to glucose leaching through immersion in a controlled water bath at a temperature of 35°C for a period of 24 hours. The HDNR film was then dried for 36 hours in the desiccant box prior to conducting any characterization. Figure 1 illustrates a flow chart showing the process of solution casting to form latex film.



**Fig. 1.** Flow chart of rubber latex films preparation by solution casting process

A comparative analysis was carried out to examine the characteristics of the DPNR film compared to the HANRXG films. The DPNR procedure is adapted from the methodology developed by Moonprasith *et al.*, [5]. Figure 2 shows DPNR latex preparing procedure. Briefly, HANR was mixed with ethylenediaminetetraacetic acid tetrasodium (EDTA-4Na, Acros Organics with purity: 99%) and extra pure sodium lauryl sulphate (SLS, Loba Chemie PVT. Ltd.) for 1 hour at room temperature. After that, the mixture was subjected to centrifugation at a speed of 10,000 rpm for 50 minutes (using Refrigerated Universal Centrifuge, Hermle Z326K), while maintaining the room temperature. The fraction of cream from the first centrifugation was redispersed in a solution containing 1% SLS before repeating centrifugation under the same condition. The second portion of cream that underwent centrifugation was then mixed with distilled water and subjected to another round of centrifugation in order to eliminate any remaining chemical residue from the deproteinized latex procedure. The final cream fraction obtained after centrifugation was redissolved in distilled water, resulting in a

latex with a dry rubber content of 60%, called DPNR. The DPNR film was then made using the same process used in the making of HANR and HANRXG films.

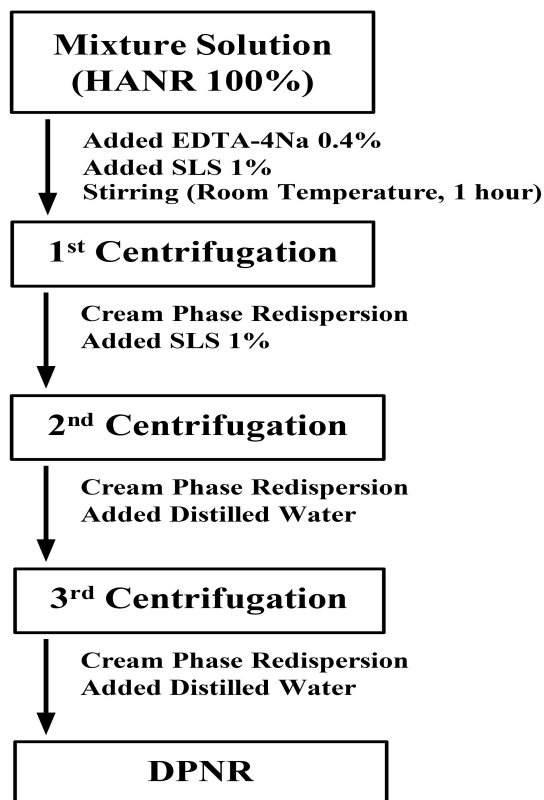


Fig. 2. Schematic for DPNR preparation process

## 2.2 Characterization of Rubber Latex Films

### 2.2.1 Physical and optical characteristic test of films

The film thickness of each sample was initially determined using a digital micrometre. The opacity characteristics of the film samples were determined by measuring the percentage of light transmitted (% light transmittance) within the wavelength range of 600 to 1000 nm, using a UV-Vis spectrophotometer (GENESYS 10UV, Thermo Scientific). The film samples were cut into rectangular pieces and then inserted into the film sample holder, with air serving as the reference medium. The determination of film opacity was conducted using the correlation between Eq. (1) and Eq. (2) based on the principles of the Beer-Lambert law [11,12]. The calculations were carried out in triplicate and the results were presented as the average value together with the corresponding standard deviation.

$$\text{Opacity} = \frac{\text{Abs}_{600}}{\text{Thickness (mm)}} \quad (1)$$

$$\text{Abs}_{600} = -\log_{10} T_{600} \quad (2)$$

Whereas  $\text{Abs}_{600}$  is the absorbance of film measuring at 600 nm while  $T_{600}$  is the transmittance of film measuring at 600 nm.

### 2.2.2 Morphological studies of films by scanning electron microscope (SEM)

The morphological analysis was conducted using a scanning electron microscope (SEM, model SU3900, Hitachi, Japan). The SEM was operated at an accelerating voltage of 15 kilovolts (kV). The specimens chosen for scanning electron microscopy (SEM) analysis, namely HANR, HANR10G, HANR20G, and DPNR, were fractured using liquid nitrogen and subsequently coated with a thin film of gold prior to conducting the tests. The present study employed scanning electron microscopy (SEM) pictures to examine three different areas of films, including the top, bottom, and cross-section areas.

### 2.2.3 Nitrogen content by total nitrogen analyser (TNA)

In order to determine the protein content percentage within rubber films, the Kjeldahl method, as prescribed by the Rubber Research Institute of Malaysia (RRIM) test procedure [5], is used to measure the total nitrogen content. For this experiment, the films that were subjected to testing were cut into size of 6×6 cm<sup>2</sup>.

### 2.2.4 Chemical structure characterization by Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy (VERTEX70, Bruker, Germany) was used to analyse the chemical structure of rubber film both before and after deproteinization, as well as to determine the presence of glucose inside the rubber film. The film specimens that were chosen for analysis were subjected to examination using the attenuated total reflectance Fourier-transform infrared (ATR-FTIR) technique. The samples were scanned across a wavenumber range spanning from 400 to 4000 cm<sup>-1</sup>. The IR transmission spectra were measured to identify the unique peaks.

### 2.2.5 Water absorption determination

The films that completed a drying step after film forming were subsequently cut into smaller pieces of 1×1 cm<sup>2</sup>. Before doing the tests, the specimens were weighted with four digital balances (Model BSA224S-CW, Sartorius). The tested samples were subsequently submerged in distilled water at room temperature for a duration of 24 hours in order to examine the process of water absorption via swelling. Following the last step of testing, the specimens were then taken out, their surfaces were scrupulously dried, and their weights were re-weighted. The water absorption value of the studied films was calculated using Eq. (3) [13]. The test was conducted in triplicate, and the average value together with its corresponding standard deviation was calculated and later on reported.

$$\text{Water absorption value (\%)} = \left[ \frac{(W_s - W_d)}{W_d} \right] \times 100 \quad (3)$$

Whereas  $W_s$  and  $W_d$  are weight (in gram scale) of sample after and before immersion in water, respectively.

### 2.2.6 Tensile property

The tensile qualities were determined using a Universal Testing Machine (Z010, Zwick/Roell, Germany) at room temperature, following the guidelines outlined in ASTM D 412 [14]. A testing crosshead speed of 500 mm/min was employed along with a load cell of 2.5 N. A minimum of three

specimens were used for each measurement. The ultimate tensile strength, elongation at break, and Young's modulus were measured as the tensile characteristics using Eq. (4) to Eq. (6), respectively.

$$\text{Ultimate Tensile Strength } (\sigma) = \frac{F}{A} \quad (4)$$

$$\% \text{ Elongation at Break } (\epsilon) = \left[ \frac{(L_s - L_0)}{L_0} \right] \times 100 \quad (5)$$

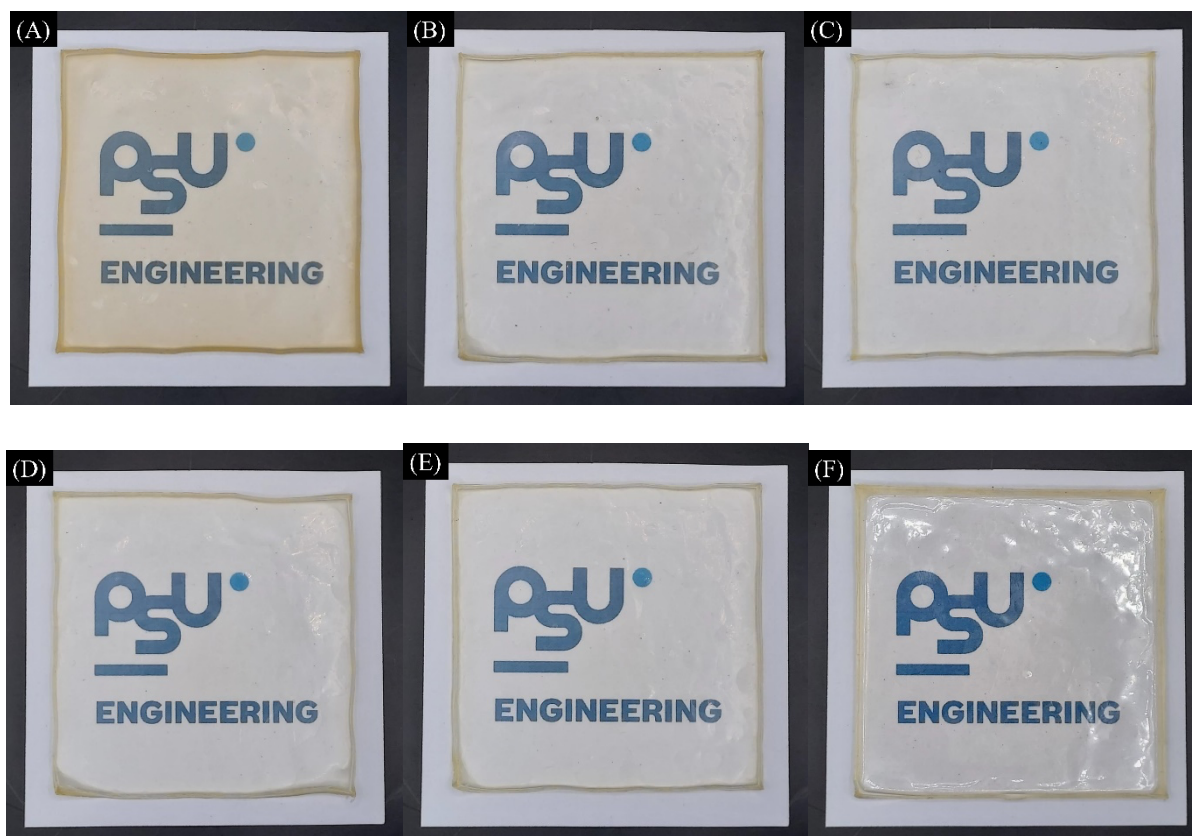
$$\text{Young's modulus } (E) = \frac{\sigma}{\epsilon} \quad (6)$$

Whereas F is breaking load (N), A is cross-section area of sample (mm<sup>2</sup>), L<sub>s</sub> and L<sub>0</sub> are length after and before breaking point (mm), and  $\sigma/\epsilon$  is initial slope of the stress-strain plot within the stretching elastic limit range (MPa). In addition, the modulus (at 100% and 500%) of each sample will be reported in this paper.

### 3. Results

#### 3.1 Physical Observation and Film Opacity

After the solution casting process for forming rubber film, it was observed that the thickness of all film samples was within the range of 278.17±31.35 µm. The main part of the component comes from rubber particles, which compose 60% of the solid rubber composition. Before being immersed in water to remove the glucose, the colour of all of the films was a dark yellow. The presence of non-rubber particles known as carotenoids within natural latex gives it an unusual this colour. According to the literature [15], these molecules are made up of four primary components: lutein, zeaxanthin, beta-carotene, and alpha-carotene. The aggregation of rubber particles that occurred during the production of the film was another contributor to the film's non-uniform coloration in some parts of the films. Figure 3 shows that all of the rubber films can be seen by the naked eyes of the observers after they were all submerged in water at 35°C for 24 hours order to remove the glucose from the rubber latex films. In comparison to HANR, the transparency and colour of all rubber films that included glucose and DPNR were significantly improved. According to this result, the level of transparency typically improves along with an increase in the amount of glucose. These may be from some water-soluble non-rubber particles that leached out along with some remover that was agreed with Rojruthai's work [16], employing enzyme combined with chemicals to remove non-rubber soluble compounds from latex, resulting in a lighter colour of rubber film.



**Fig. 3.** Photographs of rubber films after water immersion as (A) HANR, (B) HANR5G, (C) HANR10G, (D) HANR15G, (E) HANR20G, and (F) DPNR

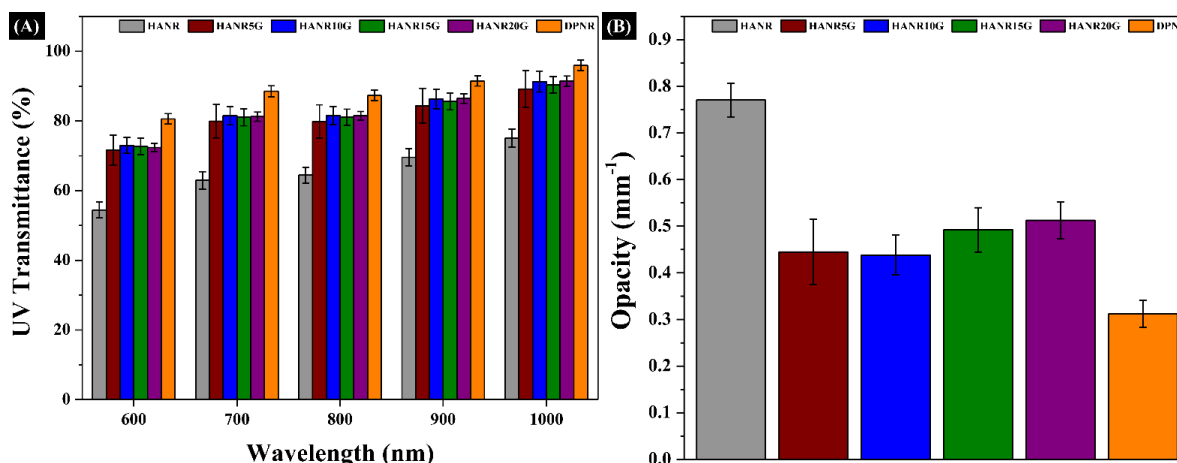
It is necessary for the film application that the material has the ability to absorb ultraviolet (UV) light. This value determines not only how long a food product may be stored in a film suited for food packaging but also how quickly the film will degrade when used for general purposes. If the percentage of light that is absorbed is higher, then the light transmittance will be lower. It is possible that the film will control oxidation degradation more effectively if it has a higher UV absorption [11,17]. In our research, UV absorption was determined in terms of the percentage of light that was transmitted (light transmittance (%)), beginning at 600 nm. This number represents the midpoint of the visible wavelength range for human vision. It is used as well in the process of determining the opacity of the film, which is then followed by Eq. (1). In our investigation, the light transmittance of the latex films at wavelengths below 600 nm could not be determined from those of the rubber films.

Table 2 and Figure 4 both show the optical properties of the material, including its light transmittance and opacity levels respectively. The higher the transparency of a film material is indicated by lower opacity values, which are also sometimes referred to as transparency values. Based on these results, it was determined that a lower in the amount of glucose added to the latex film resulted to an improvement in the film's opacity. The light transmittance of the film remained the same even after greater amounts of glucose were added at 10 phr. This indicates that the addition of 10 phr of glucose is all that is sufficient to improve the optical characteristics of the film caused by the leaching out of water-soluble non-rubber particles. However, the light transmittance and also the opacity value of the rubber film with adding glucose as HANR10G are still lower than those values from DPNR.

**Table 2**

The percent of light transmittance at various wavelength and opacity value measured at 600 nm of the rubber films

Films	Light transmittance (%) at various wavelengths (nm)					Opacity (mm <sup>-1</sup> )
	600	700	800	900	1000	
HANR	54.5 ± 2.3	62.9 ± 2.5	64.5 ± 2.3	69.6 ± 2.5	75.1 ± 2.6	0.77 ± 0.04
HANR5G	71.6 ± 4.3	79.9 ± 4.8	79.9 ± 4.8	84.4 ± 5.0	89.2 ± 5.2	0.44 ± 0.07
HANR10G	73.0 ± 2.3	81.5 ± 2.6	81.6 ± 2.6	86.3 ± 2.8	91.3 ± 3.0	0.44 ± 0.04
HANR15G	72.7 ± 2.3	81.1 ± 2.4	81.1 ± 2.3	85.6 ± 2.4	90.4 ± 2.4	0.49 ± 0.05
HANR20G	72.4 ± 1.2	81.2 ± 1.3	81.5 ± 1.2	86.4 ± 1.4	91.5 ± 1.4	0.51 ± 0.04
DPNR	80.6 ± 1.5	88.5 ± 1.5	87.3 ± 1.5	91.5 ± 1.5	95.9 ± 1.5	0.31 ± 0.03



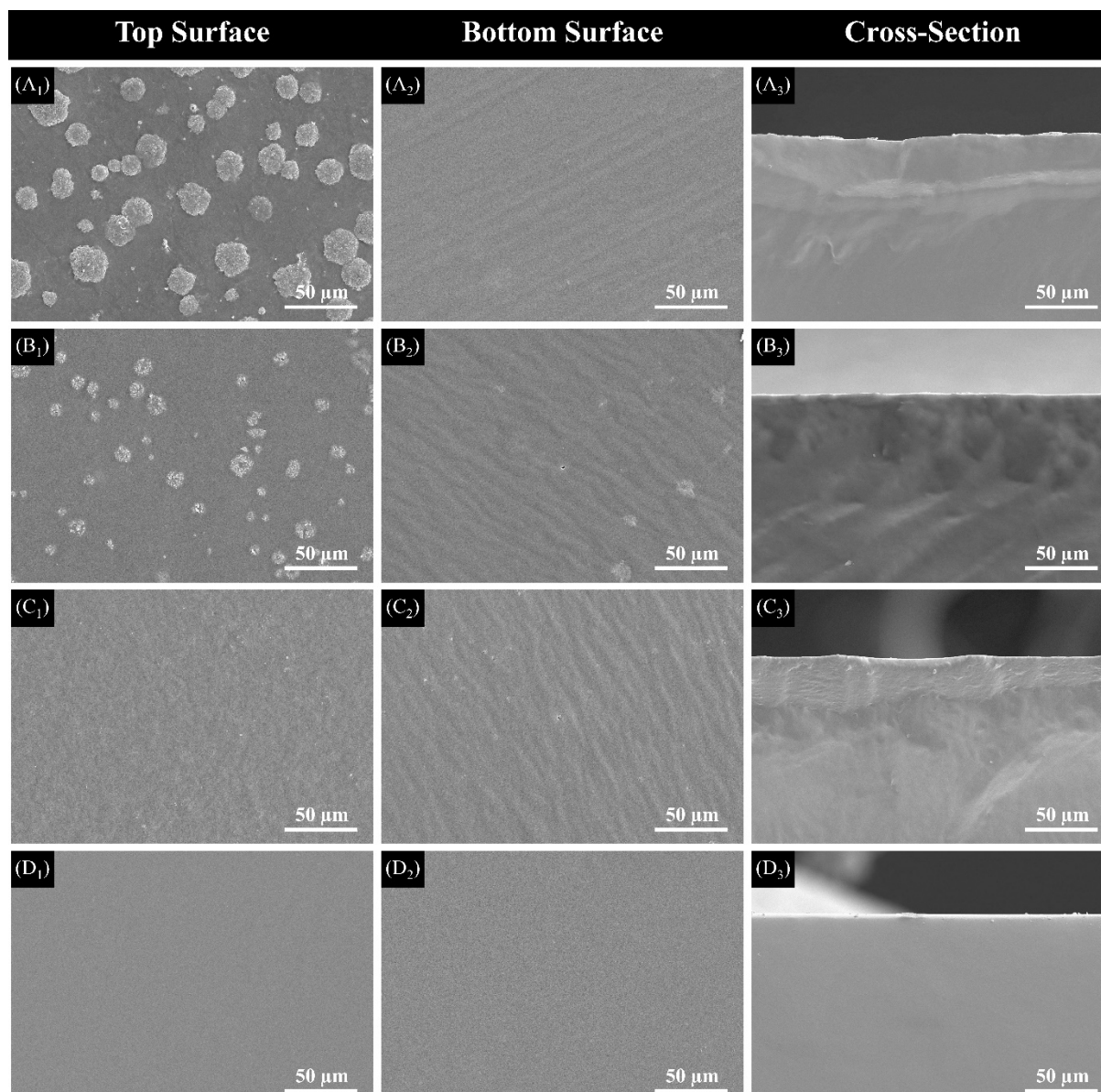
**Fig. 4.** The optical properties of rubber films: (A) Light transmittance (%) at various wavelengths from 600 to 1000 nm and (B) opacity value

### 3.2 Morphological Studies of Rubber Films

The morphological images that were observed by SEM at the top surface, the bottom surface, and the cross-section of the films are shown in Figure 5. An aggregation of rubber particles could be seen on the top surface of the HANR (A<sub>1</sub>) and HANR10G (B<sub>1</sub>) films in the SEM images of that surface area. It was found through the mechanism of film production that proteins and phospholipids had a role in the aggregation of rubber particles [18]. This occurrence can only be seen on the top of the film because it is the side that was exposed to the air while the film was drying, whereas the bottom of the film (all of the middle images) is facing to the mould. When higher amounts of glucose were added

to the rubber film, the amount of protein was decreased, which resulted in a reduction in the coagulation of the rubber (Figure C<sub>1</sub>). As a result of this, the HANR20G film turns into a very dense and completely smooth film. The dense layer that was lacking of holes was seen in the cross-section of all of the films that had glucose added to film (A<sub>3</sub> through C<sub>3</sub>). Although the fact that glucose has been suggested to be a pore forming for a rubber latex film with a content of 30% [18], this research found that it had no effect on the higher rubber solid content of 60%. It can be achieved to see a smooth and dense film on the DPNR (D<sub>1</sub> to D<sub>3</sub>). This indicates that the DPNR technique has the ability to remove the majority of protein as well as other water-soluble non-rubber particles from the rubber film.

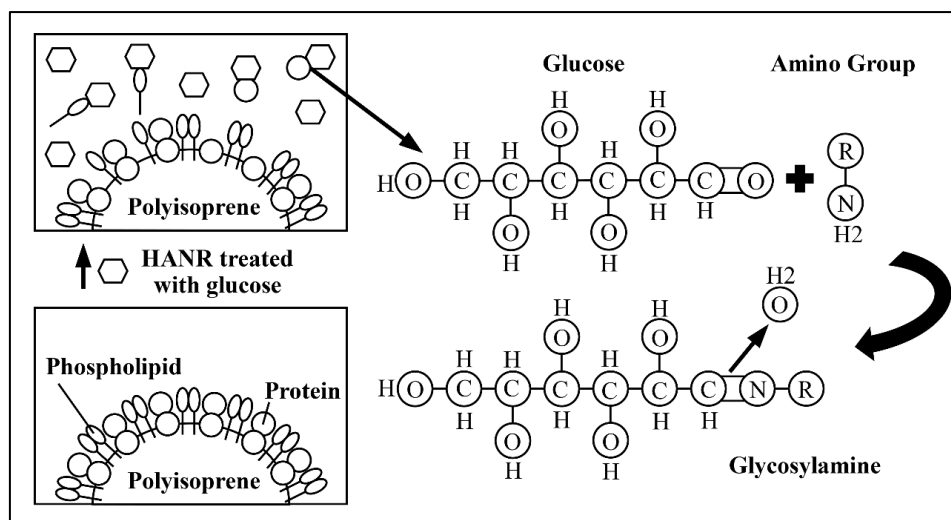




**Fig. 5.** SEM image (magnification:  $\times 500$ ) at top surface (left row images), bottom surface (middle row images) and cross section (right row images) of latex films of: (A) HANR, (B) HANR10G, (C) HANR20G, and (D) DPNR

### 3.3 Nitrogen Content Results of Rubber Films

Since glucose was known to have the ability to bind with protein through the Maillard reaction [19] is the main reason for our use of glucose as a protein remover in the research that we are working. The Maillard reaction is a chemical reaction that takes place between an amino acid from a protein and a carbonyl group from a reducing sugar, as represented in the schematic model as shown in Figure 6. This is a kind of chemical reaction that is used for browning food in a procedure that does not need enzymes in food process. This process is possibly used in natural rubber due to the fact that the rubber particles are covered with phospholipids and proteins [20,21]. As a result of this reaction, it was reasoned that by increasing the amount of glucose present in the rubber film, the aggregation between glucose and protein-phospholipid would be reduced or absent from the morphological studies as mentioned earlier.



**Fig. 6.** Schematic model of Maillard reaction between glucose and protein binding in rubber

In order to determine the amount of protein contained within the rubber film, the TNA technique was applied to analyse the nitrogen content. This is because proteins consist of an atom of nitrogen with an amino group inside their chemical structure. When it relates to protein content, a higher nitrogen concentration indicates a greater amount. The nitrogen content of the dry rubber (HANR-B) was around 0.347% when protein-removing chemicals were not used, and the rubber was not immersed in water. A content of the nitrogen, particularly the type that is soluble protein inside the latex solution, has been leaching out of the film when it was subjected to water (about 7%). However, when glucose was added to the latex of the rubber, the amount of nitrogen that was contained inside the film of the rubber decreased even further, as can be shown in Table 3 and also in Figure 7(a). Due to the Maillard reaction, glucose interacts with the lipids and proteins in rubber, and it is simultaneously washed out during the step in which the rubber is immersed in water.

**Table 3**

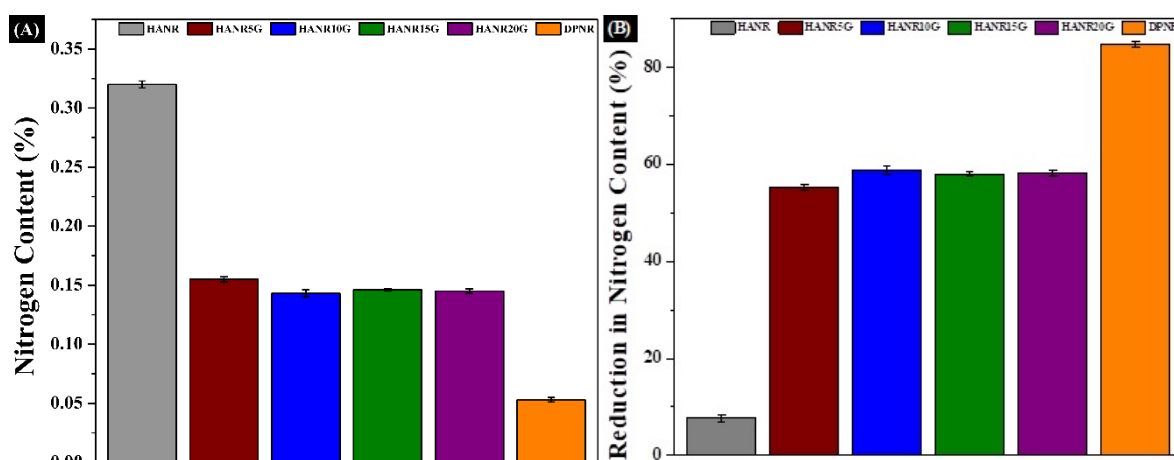
Nitrogen content of rubber films determined by TNA

Films	Nitrogen Content (%)	Reduction in Nitrogen Content (%)
*HANR-B	0.347 ± 0.005	-
HANR	0.320 ± 0.003	7.69 ± 0.59
HANR5G	0.155 ± 0.002	55.24 ± 0.49
HANR10G	0.143 ± 0.003	58.69 ± 0.68
HANR15G	0.146 ± 0.001	58.02 ± 0.27
HANR20G	0.145 ± 0.002	58.21 ± 0.47
DPNR	0.053 ± 0.002	84.73 ± 0.47

\*HANR-B: the prepared rubber film without water immersion

As shown in Table 3 and also in Figure 7 (b), the decreasing of nitrogen content from rubber using glucose can lower the amount of protein contained within the rubber by more than 58% at 10 phr of glucose or higher. Although the effectiveness of deproteinization using glucose is lower than that of the DPNR process (85%), using glucose as an alternative for removing protein from rubber is a simple process that only requires one step, compared to the DPNR method, which requires three steps for deproteinization requiring the use of a variety of chemicals. In addition, the rubber films produced by reducing proteins with glucose have a tendency to produce films with film qualities that are similar to those produced by the DPNR technique, although the fact that these films have lower values than

those produced by DPNR. In addition, the reduction of protein and lipids had an important effect on the discoloration of the rubber film [22]. From this result, the finished film products were lighter in colour with a higher percentage of light transmission, as shown in film opacity study results.

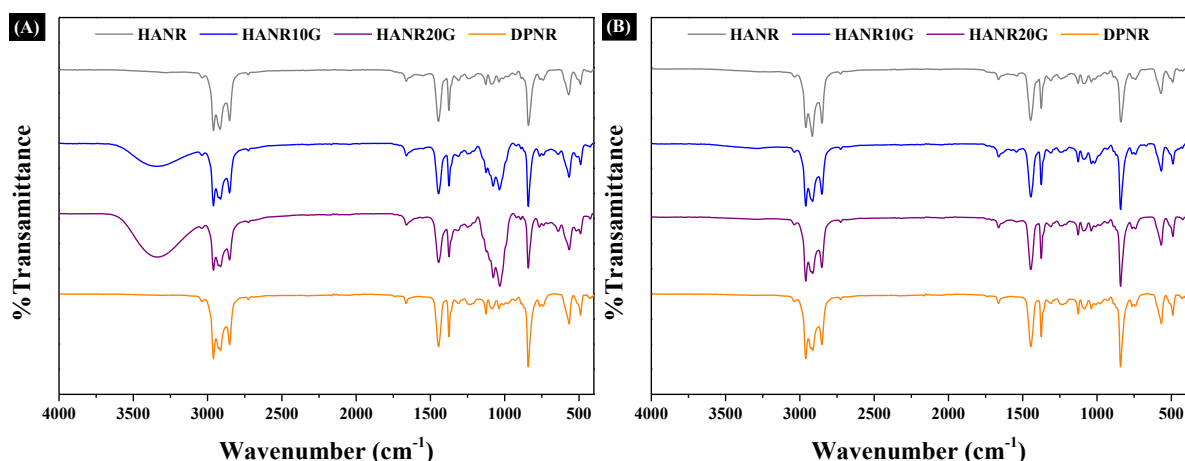


**Fig. 7.** (A) Nitrogen content determined from TNA and (B) Reduction in nitrogen content of rubber films

### 3.4 Chemical Structure Characterization by FTIR

The FTIR technique was applied in order to determine the chemical structure of the sample by analysing the peaks that formed by all of the functional groups of the material. The infrared (IR) spectra of selected rubber films, including HANR, HANR10G, HANR20G, and DPNR, are shown in Figure 8 before (A) and after (B) water immersion, which was performed to remove glucose and all other water-soluble non-rubber compounds that were incorporated inside the rubber. Even though some soluble material had been leached out during water immersion, the amount was small in comparison to the total rubber content. From this result, the majority of the spectrum peaks of HANR and DPNR at before and after water immersion were very similar. These peaks are representative of the characteristic peaks that are found in *cis*-1,4-polyisoprene, which is the chemical term for rubber. The main characteristic peaks from polyisoprene functional groups were shown at  $3035\text{ cm}^{-1}$  (=CH stretching),  $2960\text{ cm}^{-1}$  (C–H stretching of  $\text{CH}_3$ ),  $2915\text{ cm}^{-1}$  (C–H stretching of  $\text{CH}_2$ ),  $2850\text{ cm}^{-1}$  (C–H stretching of  $\text{CH}_2$  and  $\text{CH}_3$ ),  $1660\text{ cm}^{-1}$  (C=C stretching),  $1445\text{ cm}^{-1}$  (C–H bending of  $\text{CH}_2$ ),  $1375\text{ cm}^{-1}$  (C–H bending of  $\text{CH}_3$ ),  $1125\text{ cm}^{-1}$  (C–H bending), and  $840\text{ cm}^{-1}$  (C=CH wagging) [23,24]. In respect to fact, proteins and phospholipids each have their own functional groups that are different from those of polyisoprene; however, these groups could not be distinguished from polyisoprene in IR spectra due to the lower quantity of chemicals present and the FTIR technique that was applied to identify characteristics at the film's surface; it did not pass through the film body.

Before and after being immersed in water, the IR spectra of HANR10G and HANR20G films were very different from each other. Before being immersed in water, the IR peak intensity was detected to be at  $3340\text{ cm}^{-1}$  (–OH and N–H stretching), which is caused by the interaction of glucose and natural rubber proteins via hydrogen bonds, and at  $1075\text{ cm}^{-1}$  (C–O–C stretching) of the anhydroglucose ring of the glucose molecule [5,25]. After being immersed in water, the films lost their intensity at both peaks. It was demonstrated from IR results that all of the added glucose was leaching out, in addition to taking out protein and other water-soluble chemicals by incorporating as described in the Maillard reaction. According to these results, the final IR spectra of all of the rubber films that were obtained after being submerged in water were very similar.



**Fig. 8.** FTIR spectra of rubber films of HANR, HANR10G, HANR20G, and DPNR: (A) before water immersion and (B) after water immersion

### 3.5 Water Absorption of Rubber Films

The results of the percentage of water absorption by rubber films are presented in Table 4 and also shown in Figure 9. When an additional amount of glucose was added, the rubber film exhibited less of a capacity to absorb water. The HANR film had a greater water absorption percentage (12.95%) than the HANR5G, HANR10G, HANR15G, and HANR20G films, which had lower water absorption percentages of 8.90, 3.56, 3.50, and 2.62% respectively. Natural rubber latex generally has a limited water absorption capacity due to its hydrocarbon structure [26]. However, HANR had the highest water absorption percentage out of all the film samples. This may be due to the fact that the majority of hydrophilic molecules, such as proteins and other water molecules, remained inside the rubber film after the water immersion, as can be observed from the nitrogen content results of the HANR in Table 3 and also from the SEM image (Figure 5 (A1)). Because of this, the remaining compounds have the potential to form bindings with the molecules of water, which could result in increased water absorption. As a result of the addition of glucose to the rubber latex, the rubber films had a decreased water absorption capacity from the protein, and other water-soluble molecules were bound and leached out with the glucose. As was mentioned before, the DPNR had the lowest water absorption, which can be explained to its minimized protein level. In addition to the film characteristics in terms of water absorption, the results of this study can be used as an indirect method to measure protein content in a simple way. Based on the HANR's water absorption results in this study, it seems that HANR10G is the most effective at extracting protein from rubber films by using glucose as a protein remover. The results did not show more protein removal at increased glucose added to the rubber film, which led to slightly higher water absorption as shown in the results.

**Table 4**

The water absorption results of rubber films

Films	Water Absorption Value (%)
HANR	12.95 ± 2.81
HANR5G	8.90 ± 0.81
HANR10G	3.56 ± 0.76
HANR15G	3.50 ± 0.43
HANR20G	2.62 ± 0.57
DPNR	1.44 ± 0.26

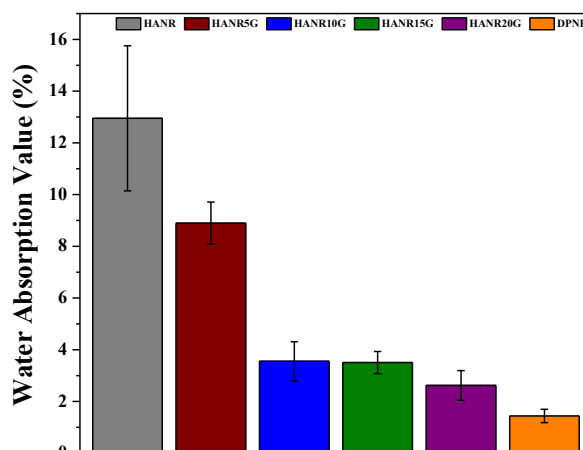


Fig. 9. Water absorption values of rubber films

### 3.6 Tensile Properties of Tubber Films

In Table 5, the results of tensile testing on rubber films are summarized in terms of ultimate tensile strength (UTS), elongation at break (EB), 100% modulus (100% E) and 500% modulus (500% E). The results from the study of comparing the UTS and EB values were also presented in plot form, which can be seen in Figure 10 (A) and Figure 10 (B), respectively. All of these results were examined using the green rubber films, no additional chemical components and unvulcanization, were used during the testing process. Although the rubber film with 5 phr glucose addition had somewhat higher tensile values than the rest of the film samples, the tensile results showed that all films had similar results within practically the same range. This indicates that the tensile characteristics of the rubber film are unaffected by the addition of glucose in order to remove the protein from the rubber film, and that the deproteinized rubber film retains its original rubber elasticity as HANR. When force is applied to un-vulcanized rubber, the molecules have the potential to stretch out of their coiled shape, which results in the rubber becoming loose and more easily break. This is different from the process of cross-linking achieved during vulcanization, which leads to greater improvements in tensile strength [27]. The 100% E and 500% E, or tensile stress at 100% and 500% elongation, respectively as defined by ASTM D412, of all sample were determined. The highest 100% E and 500% E value were presented by HANR and DPNR, while the lowest value was presented by HANR5G. All samples, however, remained within the same range for both values when the standard deviation value was taken into consideration. Additionally, our research showed better tensile strength and 100% modulus than the previous research conducted by Zhao group, which examined the mechanical characteristics of unvulcanised natural rubber through coagulation with various sugars, including glucose, almost two to three times [28].

**Table 5**

Tensile properties in term of ultimate tensile strength, elongation at break, 100% and 500% modulus of rubber films

Films	Ultimate Tensile Strength (MPa)	Elongation at Break (%)	100% Modulus (MPa)	500% Modulus (MPa)
HANR	1.56 ± 0.24	769.2 ± 34.0	0.35 ± 0.02	0.61 ± 0.06
HANR5G	2.01 ± 0.12	844.5 ± 38.3	0.32 ± 0.03	0.50 ± 0.05
HANR10G	1.43 ± 0.31	737.5 ± 19.7	0.34 ± 0.05	0.61 ± 0.05
HANR15G	1.47 ± 0.37	786.7 ± 63.7	0.33 ± 0.05	0.50 ± 0.08
HANR20G	1.29 ± 0.06	729.5 ± 23.1	0.34 ± 0.03	0.57 ± 0.07
DPNR	1.62 ± 0.05	804.1 ± 23.6	0.35 ± 0.08	0.61 ± 0.12

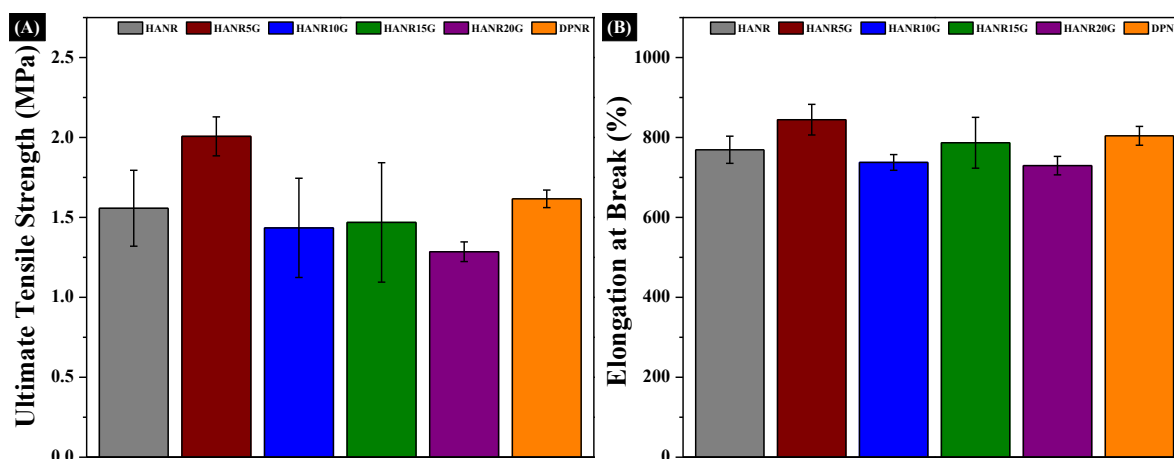


Fig. 10. (A) Ultimate Tensile Strength and (B) Elongation at Break of rubber films

#### 4. Conclusions

This study presents an application of glucose as a substance for the removal of proteins from rubber films. The formation of hydrogen bonds between glucose and protein inside the rubber can result to their simultaneous leaching, hence causing a reduction in the protein content. The Maillard reaction, originally used to explain food processing, has been newly employed in this study to explore rubber films. While the protein removal achieved through the addition of glucose was comparatively lower than the protein removal achieved through using of chemicals and high-speed centrifugation on latex (or DPNR), the application of glucose as a protein remover represents an easy method. The production process can be simplified into a single step by only using environmentally friendly chemicals such as glucose and water. The experimental findings indicated that using a glucose concentration of 10 phr yielded good results with respect to optical characteristics, water absorption properties, and mechanical properties. While the results obtained from this approach are generally lower compared to DPNR, they exhibit a tendency to be in close proximity. The finding of this study has the potential to contribute to the development of protein-free rubber films, particularly those that have undergone vulcanization, for potential future applications on broader basis.

#### Acknowledgement

This research was funded by a grant from the National Research Council of Thailand (NRCT) (Grant number 125345). The authors are grateful to the Graduate School and the Department of Mining and Materials Engineering and the Department of Chemical Engineering, Faculty of Engineering, Prince of Songkla University for the provision of facilities and equipment. The authors would like to thank Office of Scientific Instrument and Testing (OSIT), Prince of Songkla University for instrumental characterization support.

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