



Improving deproteinized skim natural rubber latex with a further leaching process

Bhumin Than-ardna, Hiroshi Tamura and Tetsuya Furuike*

Faculty of Chemistry, Materials and Bioengineering, Kansai University, Osaka 564-8680, Japan

Received 31 October 2018

Revised 7 January 2019

Accepted 18 January 2019

Abstract

In this study, a process for the removal of proteins from skim natural rubber latex (SNRL) was developed. This process involves a one-step incubation with urea in the presence of a surfactant and an organic solvent, followed by leaching. After 1 h of incubation in 0.1 wt% urea, 1 wt% sodium dodecyl sulfate (SDS) and 1% ethanol, the extractable protein (EP) content was lower than that of a deproteinized SNRL (D-SNRL) prepared via a single step. To further reduce the protein content, leaching with SDS coupled with 1–3 centrifugation cycles was performed. The EP content of D-SNRL with an additional leaching step and 3 centrifugation cycles under the optimum conditions was found to be 0.179 mg/g. Additionally, the nitrogen content, as determined by the Kjeldahl method, confirmed that the protein content was decreased to 0.053%, which was lower than those of the SNRL and D-SNRL samples processed in a single step. Fourier transform infrared spectra confirmed that the protein contents of these samples decreased with an increasing number of leaching steps. Thermogravimetric analysis showed quite similar thermal stability for the product of each deproteinization process. The tensile strength and elongation at breakage of the rubber film decreased after protein removal. Scanning electron microscopy revealed that all rubber films had quite smooth surfaces. This deproteinization method with an additional leaching step allowed for highly efficient protein reduction of up to 96%. This method is expected to improve the quality of skim latex products for several biomedical applications.

Keywords: Skim latex, Natural rubber, Deproteinization, Proteins, Rubber film

1. Introduction

Natural rubber latex (NRL) is obtained from *Hevea brasiliensis* trees. After freshly tapped latex is extracted from *Hevea* trees, fresh natural rubber latex (FNRL) has a consistency similar to that of milk. Then, NH_3 is added to stabilize the NRL system [1] and inhibit bacterial activity [2]. FNRL is a colloidal system of *cis*-1,4 polyisoprene suspended in a mixture containing 36 wt% rubber or dry rubber content (DRC), 2 wt% protein, 1.5 wt% carbohydrate, and 1.3 wt% lipid. This mixture is typically altered to increase the rubber fraction to 60 wt% DRC in the concentrated NRL [3]. Skim natural rubber latex (SNRL) is obtained from a centrifugation process as a by-product with 4–6 wt% DRC. NRL is mainly used in dipping processes for the production of medical gloves, condoms, and other NRL products. In contrast, SNRL is processed into skim natural rubber (SNR) products such as skim crepe and skim blocks or is discarded as waste. Since increasing amounts of SNRL are being produced as an industrial by-product, characterization methods for SNRL and SNR were investigated. These materials contain a higher amount of proteins than does NRL, and the SNR particles are smaller and can be more easily converted into a smooth film product [2, 4–6]. Moreover, skim rubber exhibits good adhesion and has been studied for use as a urea encapsulant in controlled

release applications [7].

According to a novel rubber particle model, the particle surfaces are surrounded by a mixed layer of proteins and phospholipids, which provide colloidal stability [8]. More than 250 types of proteins can be found in latex, each with different functional groups. Of these 30–60 are believed to cause allergic reactions in humans [1]. Thus, irradiation [9–10], saponification [11], and enzymatic methods, as well as surfactant and urea-based methods [3, 12–18], have been developed for deproteinization of NRL.

An irradiation method has been demonstrated to effectively and easily remove proteins from rubber [9–10]. Protein reduction in FNRL has also been achieved using a saponification method [11]. Furthermore, surfactants such as sodium dodecyl sulfate (SDS), Triton X-100, and rheodol are used to remove proteins because they affect protein conformation and reduce the amount of protein in NRL. Enzymatic treatments the most effective methods of removing proteins because the proteins in NRL decompose at their amide groups in the presence of lytic enzymes [3, 12–15]. However, enzymatic treatment uses proteins, which might cause allergies in some users. Deproteinization of NRL using urea in the presence of a surfactant can reduce protein content from approximately 0.38 to 0.005 wt% nitrogen, with an allergenic protein concentration of approximately 1.0 $\mu\text{g/mL}$. Urea unfolds the proteins and the

*Corresponding author. Tel.: +81-6-6368-1121

Email address: furuike@kansai-u.ac.jp

doi: 10.14456/easr.2019.8

surfactant facilitates further protein denaturation [12, 16-17]. Adding polar organic solvents, such as acetone, ethanol, or 2-propanol to the surfactant also increases protein removal. Protein-free NRL can be obtained using urea in the presence of SDS and organic solvents [18].

A few studies employed deproteinization processes to reduce allergenic proteins in SNRL, as it has a higher protein content than NRL [19]. Additionally, surfactants such as SDS, Triton X-100, and polyethylene glycol (PEG) have been used for protein extraction from SNRL [3, 11, 13, 20]. Protein extraction using PEG was shown to be effective in SNRL treatments. Furthermore, papain was used in an enzymatic deproteinization of SNRL, reducing the nitrogen content from 1.18 to 0.56 wt%. Several methods have been used to remove proteins from skim latex, but so far none were efficient. Thus, this study aimed to develop a process to remove proteins from SNRL in the latex phase by combining a urea treatment in the presence of a surfactant and the use of organic solvents and a leaching treatment, which has not been reported yet. This novel deproteinization method could be used to obtain low-protein SNRL products for a variety of biomedical applications and other value-added skim rubber products.

2. Materials and methods

2.1 Materials

The SNRL used in this study was supplied by the Thai Eastern Group Co., Ltd., Chonburi, Thailand and it contained 3.87% DRC. Urea, sodium dodecyl sulfate (SDS), ethanol,

methanol, and acetone were purchased from Wako Pure Chemical Industries, Ltd.

2.2 Preparation of D-SNRL

In the experimental procedure, SNRL was incubated with urea and several organic solvents (acetone, methanol, and ethanol) containing 1 wt% SDS for 1, 2, 6, 12, or 24 h. Subsequently, the suspension was centrifuged at 10,000 rpm for 30 min to obtain a cream fraction. 20 mL of the cream fraction was re-dispersed in 40 mL of distilled water and then dried at 60 °C. The coagulum was dissolved in toluene, and the resulting solution was cast into a film with dimensions of 1 cm × 1 cm × ~0.15 mm. A leaching procedure was performed on the abovementioned cream fraction after it was re-dispersed in 40 mL of 0.5–1 wt% SDS and subjected to 1-3 cycles of centrifugation. The material was then re-dispersed in water and cast into a film. A schematic representation of the experimental procedure for preparing DSNR is shown in Figure 1.

2.3 Protein content determination by a modified Lowry method

A calibration curve of standard protein (ovalbumin) was prepared. Phosphate-buffered saline (PBS) was prepared by dissolving 3 g of PBS in 1 L of distilled water. Then, 0.1 g of ovalbumin was added to 50 mL of PBS to form a 2000 ppm ovalbumin solution. Subsequently, the ovalbumin solution was diluted to final concentrations of 2, 4, 6, 8, and 10 ppm, which was confirmed by measuring its absorbance at 750 nm using a spectrophotometer.

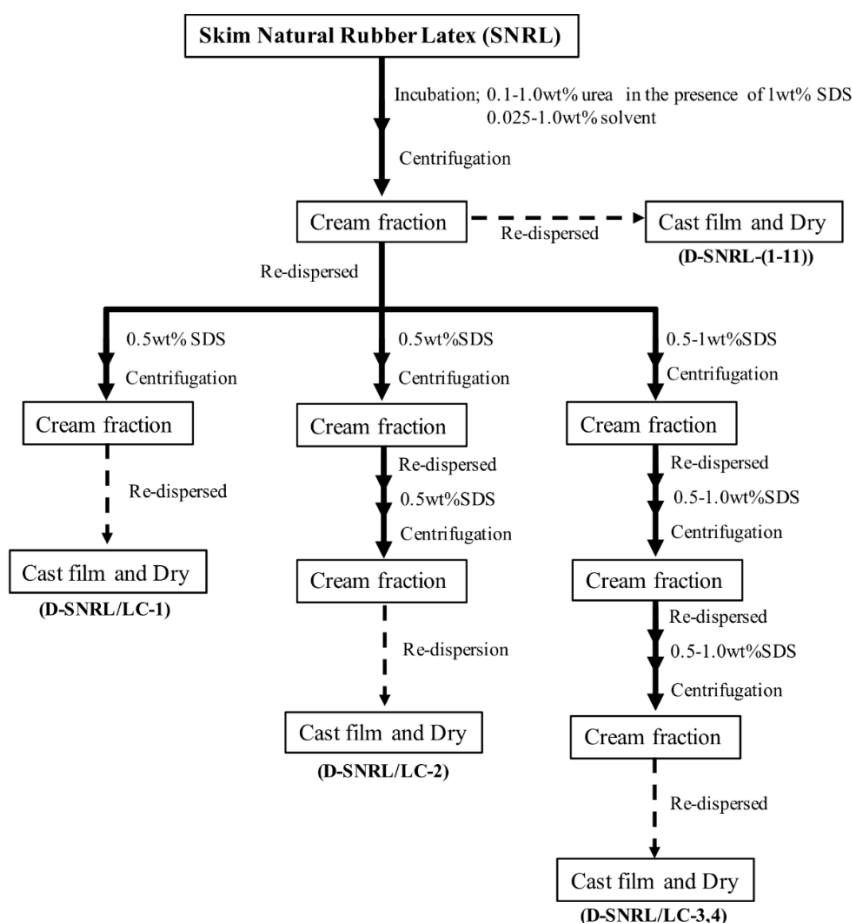


Figure 1 Schematic representation of the experimental procedure

The protein content of the rubber film was determined by a modified Lowry method according to ASTM D5712. A small piece of rubber film was extracted with 2 mL of PBS and shaken at 120 rpm for 120 min. After that, the small film was removed and 1 mL of the extract solution was centrifuged at 3600 rpm for 15 min. Aqueous reagent A (tartrate solution) was prepared by combining 0.22 g of sodium carbonate, 0.44 g of sodium hydroxide, and 0.18 g of sodium tartrate in 100 mL of diluted water. Reagent B (copper sulfate solution) was prepared by adding 7 g of copper sulfate to 100 mL of diluted water. Reagent C (alkaline copper tartrate solution) was then prepared by mixing 1 mL of reagent B and 150 mL of reagent A. Subsequently, 0.1 mL of sodium deoxycholate (DOC) was added to the solution, mixed thoroughly, and left standing for 10 min. Then, 0.2 mL of a freshly prepared solution of trichloroacetic acid (TCA) and phosphotungstic acid (PTA) in a 1:1 ratio was added, thoroughly mixed, and allowed to stand for 30 min for protein precipitation. This was followed by centrifugation at 6000 rpm for 15 min. After this step, 0.6 mL of 0.2 M NaOH was added to clarify the solution and 0.625 mL of reagent C was added and left to stand for 15 min. Reagent D (0.075 mL, Folin reagent diluted 50%, was then added, mixed vigorously and allowed to stand for 30 min. Finally, the absorbance of this extract solution was measured at 750 nm within 1 h. Ovalbumin was used as a standard protein and the amount of protein was reported as protein remaining (mg/g), as defined in Eq. (1):

$$\text{Extractable protein (EP)}[\text{mg/g}] = \frac{C \times V \times F}{W}, \quad (1)$$

where C is the protein concentration of extract ($\mu\text{g/mL}$), V is the volume of extraction buffer (mL), F is the dilution factor, and W is the weight of the specimen (g). The reported values of extractable protein are reported as the average of six specimens.

2.4 Nitrogen content

The nitrogen contents in the SNRL and D-SNRL samples were measured according to a standard test method for rubber nitrogen content (D3544). Briefly, the rubber sample was mixed with anhydrous potassium sulfate (K_2SO_4) and copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), then digested with concentrated H_2SO_4 and heated until the color turned clear green. The digested solution was distilled, and the distillate was immediately titrated with 0.02 M NaOH using methyl red–methylene blue as an indicator. The nitrogen content was calculated using Eq. (2):

$$\text{Nitrogen content (\%)} = \frac{(V_2 - V_1)M \times 0.0140}{w} \times 100, \quad (2)$$

where V_1 is volume of NaOH solution required for complete titration of the receiving flask contents (mL), V_2 is volume of NaOH solution required for titration of the blank (mL), M is the molarity of the NaOH solution, w is the mass of sample used (g), and 0.0140 is the millimol mass of nitrogen.

2.5 Fourier transfer infrared spectroscopy

The functional groups of the thin rubber sample films were characterized by Fourier transform infrared spectroscopy (FTIR) measurements using a JASCO FT/IR–4200 spectrophotometer (Japan). The results were obtained

at a resolution of 4 cm^{-1} and scanned in the range of $400\text{--}4000 \text{ cm}^{-1}$.

2.6 Thermogravimetric analysis

A thermogravimetric/differential thermal analyzer (TGA, EXSTAR TG/DTG 6200, SII, Japan) was used to analyze the thermal properties of the rubber samples. First, 8 mg of each samples was heated from $25\text{--}600^\circ\text{C}$ at a rate of 20°C/min under a nitrogen atmosphere. The thermogravimetric analysis (TGA) and differential thermogravimetric analysis (DTG) curves of the samples were then plotted.

2.7 Tensile strength

The mechanical properties of the obtained samples were determined using a universal testing machine (STA-1150, A&D company, Ltd., Japan) equipped with a 50 N load cell at a constant speed of 10 mm/min under ambient conditions. The sample was cut to a length of 2 cm and a width of 0.5 cm. It was then mounted in tensile grips before analysis for an average of more than 10 replicates. The tensile strength and elongation at its break point were determined from the stress-strain curves obtained from the force-distance data.

2.8 Scanning electron microscopy

SEM images were acquired (JSM6700, JEOL, Japan). They were used to analyze the morphology of the obtained samples at an accelerating voltage of 5 kV with $2000\times$ of magnification.

3. Results and discussion

3.1 Deproteinization of SNRL

SNRL with 3.89% DRC was incubated with 0.1 wt% urea and 0.025 wt% acetone used as denaturing agents in the presence of 1 wt% SDS as a stabilizer to prevent latex coagulation at room temperature. This was done with stirring for 1, 2, 6, 12, or 24 h. The EP contents as determined by the Lowry method are shown in Table 1. The EP contents varied widely due to the presence of various monolayers of mixed proteins and phospholipids on the rubber surfaces [5] and the wide range rubber particle sizes [2]. These factors significantly affected deproteinization through their interactions with proteins and phospholipids [21]. An incubation time of 1 h resulted in a minimal EP content, where the protein content was reduced from 4.963 to 3.250 mg/g (per weight of rubber film). At a longer incubation time of 2 h, the EP content increased. This was likely due to aggregation of particles during protein removal resulting from a reduction of repulsive forces from the negatively charged proteins.

Table 1 Extractable protein content of D-SNRL incubated for various times

Sample	Incubation time (h)	Extractable protein (mg/g)
SNRL	-	4.963 ± 1.706
D-SNRL-T1	1	3.250 ± 0.355
D-SNRL-T2	2	4.536 ± 0.427
D-SNRL-T6	6	4.185 ± 0.235
D-SNRL-T12	12	4.505 ± 0.561
D-SNRL-T24	24	3.535 ± 1.454

Table 2 EP content of D-SNRL under various process parameters

Sample	Urea (%)	Organic solvent	Leaching process with SDS (%)			Extractable protein (mg/g)	P value
			1	2	3		
D-SNRL-1	0.1	0.025% acetone	–	–	–	3.250 ± 0.355	1.70 × 10 ⁻⁵
D-SNRL-2	0.1	0.1% acetone	–	–	–	2.490 ± 0.471	2.47 × 10 ⁻⁵
D-SNRL-3	0.1	1.0% acetone	–	–	–	2.070 ± 1.435	2.40 × 10 ⁻⁶
D-SNRL-4	0.1	0.025% methanol	–	–	–	2.366 ± 1.146	1.22 × 10 ⁻⁶
D-SNRL-5	0.1	0.1% methanol	–	–	–	1.854 ± 0.321	6.12 × 10 ⁻¹¹
D-SNRL-6	0.1	1.0% methanol	–	–	–	1.674 ± 0.852	9.49 × 10 ⁻¹²
D-SNRL-7	0.1	0.025% ethanol	–	–	–	2.381 ± 0.472	6.13 × 10 ⁻⁸
D-SNRL-8	0.1	0.1% ethanol	–	–	–	1.972 ± 0.065	1.12 × 10 ⁻¹⁰
D-SNRL-9	0.1	1.0% ethanol	–	–	–	1.601 ± 0.530	2.80 × 10 ⁻¹²
D-SNRL-10	0.5	1.0% ethanol	–	–	–	1.682 ± 0.186	7.33 × 10 ⁻¹¹
D-SNRL-11	1.0	1.0% ethanol	–	–	–	1.627 ± 0.360	1.79 × 10 ⁻¹¹
D-SNRL/LC-1	0.1	1.0% ethanol	0.5	–	–	1.540 ± 0.315	8.78 × 10 ⁻¹⁰
D-SNRL/LC-2	0.1	1.0% ethanol	0.5	0.5	–	0.587 ± 0.209	9.17 × 10 ⁻¹⁴
D-SNRL/LC-3	0.1	1.0% ethanol	0.5	0.5	0.5	0.205 ± 0.196	8.54 × 10 ⁻¹⁶
D-SNRL/LC-4	0.1	1.0% ethanol	1.0	1.0	1.0	0.179 ± 0.132	8.66 × 10 ⁻¹⁵

Thus, prolonged incubation time may affect the coagulation of rubber particles, leading to lower protein denaturation on rubber particles. However, when the incubation time was increased from 2 to 6 h, the EP content decreased, implying that the stability of the colloidal systems was variable and depended on the removal of proteins. During the first 12 h of incubation time, the EP content continued to increase, whereas the EP content decreased with increasing incubation time from 12 to 24 h. This may imply that the interplay between the colloidal stability of the system and the denaturation process was disturbed. Consequently, the denaturation system may require time to adjust to a new colloidal stability equilibrium before urea and organic solvent denaturants are added and these additives are known to be effective denaturants [19]. Therefore, nonspecific trends of the EP content with increasing incubation time can be expected. This confirmed the heterogeneity of the protein removal trends from natural rubber, as similar protein denaturation from SNRL has been reported using ethanol and ethylene glycol as organic solvents [19-21]. Therefore, the optimal incubation time should be chosen to minimize the EP content. However, to obtain a sufficiently low EP content in skim latex, the urea concentration and levels of other organic solvents was tuned to investigate deproteinization and further leaching in the latex phase.

Table 2 shows the effect of the various organic solvents and urea content on the removal of proteins from natural rubber. All reported values are the average of six replicates with p-values <0.05, indicating that the results were significantly different between the D-SNRL and SNRL samples. The effect of organic solvents, such as acetone, methanol, and ethanol, were determined for protein removal. The D-SNRL-1–9 samples represented the effect of organic solvents such as acetone, methanol, and ethanol, where the concentration of solvents varied from 0.0025 to 1 wt%. It can be seen that the EP contents of the D-SNRL-1–9 samples decreased when the organic solvents were increased from 0.025 to 1.0 wt% for each solvent tested. Methanol and ethanol more significantly affected protein removal than acetone. However, ethanol seems to be more efficient at removing protein, which may indicate that its small dielectric constant resulted in lower solubility of the proteins, inducing precipitation [22-23]. Due to the restrictions for various

biomedical applications, ethanol is considered to be the most suitable organic solvent of those tested. The EP content was reduced to 1.601 mg/g (67.74% reduction), implying that increased solvent concentration could dissolve and induce phospholipid precipitation in an aqueous medium [23], facilitating protein removal from the rubber particles whereas, in a previous report, one-step incubation resulted in maximum protein reductions of only 52% [19]. The EP contents were nearly identical regardless of the urea concentration. However, it is likely that most proteins on the rubber particle surface were attached via physical interactions and urea can denature protein adhering to natural rubber [12]. The proteins covering the rubber particle surfaces could be removed upon unfolding, which would affect protein activity and reduce interactions with rubber particle surfaces, allowing the proteins to be easily removed by the extraction medium [12, 24].

A further leaching process with SDS and 1–3 centrifugation cycles showed that the EP content of D-SNRL/LC-1, D-SNRL/LC-2, and D-SNRL/LC-3 decreased to 1.54, 0.587, and 0.205 mg/g, respectively. Increasing the SDS concentration with three centrifugation cycles dramatically decreased the EP content to 0.179 mg/g. SDS is an anionic surfactant with a high efficiency [25], and could extract proteins from the latex, effectively lowering the protein content. Thus, it can be concluded that SDS acts as a stabilizer in the latex that can easily solubilize and remove the proteins on rubber particles by leaching and centrifugation [26]. It can also be concluded that most of the proteins in the SNRL are attached to the particles via weak physical interactions [12, 16]. Compared to some of the reported methods to remove protein in skim latex using PEG as a surfactant, the EP content in the serum extracted from the treatment was as high as 40 mg/g of rubber [20]. The removal of proteins from the latex phase, and protein extraction from skim rubber were studied. The extracted protein content in the skim rubber film was up to 11 mg/g of rubber [11].

3.2 Nitrogen content

To further investigate the amount of protein in the rubber samples, the nitrogen content was determined for the

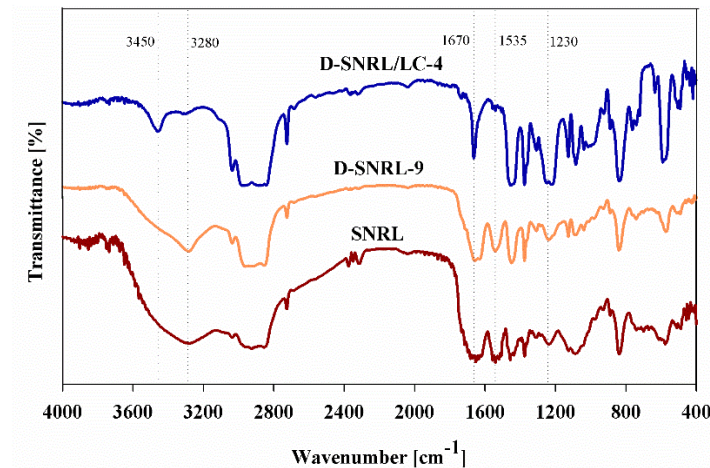


Figure 2 FTIR spectra of the SNRL, D-SNRL-9, and D-SNRL/LC-4 samples

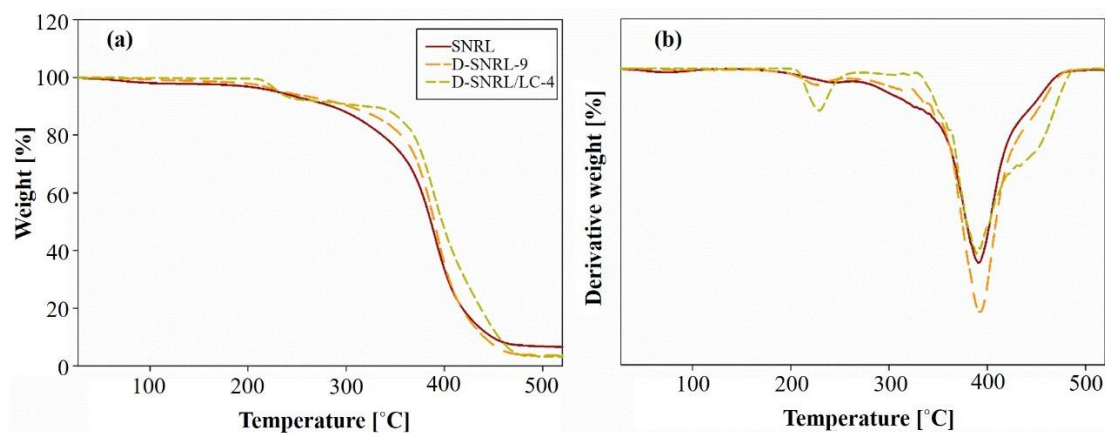


Figure 3 TGA (a) and DTG (b) curves of the SNRL, D-SNRL-9, and D-SNRL/LC-4 samples

products of each process. Table 3 shows the results of the nitrogen content analysis (as a rough approximation of the quantity of amino groups), which is directly related to the protein content of the rubber latex. The nitrogen content of the product obtained from the one-step incubation decreased from 2.118% to 1.036% and that of the leaching process product was further decreased to 0.056%. Comparing the EP and nitrogen contents as a measure of total protein content, the nitrogen content of D-SNRL-9 and D-SNRL/LC-4 was consistent with the EP value shown in Table 2. This suggested that the deproteinization with a further leaching process enhanced removal of proteins from skim latex.

Table 3 Total nitrogen content of the products of each process

Sample	Nitrogen content (wt %)	Protein reduction (%)
SNRL	2.118 ± 0.098	–
D-SNRL-9	1.036 ± 0.112	51.08
D-SNRL/LC-4	0.056 ± 0.028	97.35

3.3 Fourier transform infrared spectroscopy (FTIR)

To confirm the results above, FTIR spectra of the products were recorded, as shown in Figure 2. The spectra of SNRL, D-SNRL-9, and D-SNRL/LC-4 were prepared. After deproteinization, the D-SNRL-9 spectra show a

disappearance of the bands corresponding to the amino acids or dipeptides in natural rubber (N–H stretching) at approximately 3280 cm^{-1} [10, 18, 27–28]. The peak at 3280 cm^{-1} in the D-SNRL/LC-4 sample also decreased and almost disappeared. The peaks at approximately $1510\text{--}1580$ and $1600\text{--}1700 \text{ cm}^{-1}$ corresponding to amine and amide II (N–H bending), respectively, showed sharp peaks after each step of the treatment [2, 15]. Moreover, the sample showed a small peak at 3450 cm^{-1} , which corresponds to urea [23]. The peak at 1230 cm^{-1} arising from S=O stretching can be attributed to SDS, implying that the SDS attached to the skim rubber film [24]. These FTIR results confirmed that the protein content decreased after the leaching process.

3.4 Thermal gravity analysis (TGA)

TGA and DTG curves of the SNRL, D-SNRL-9, and D-SNRL/LC-4 samples are shown in Figures 3(a) and 3(b), respectively. The decomposition of all samples showed similar thermal behavior. The SNRL and D-SNRL samples, after protein removal, were thermally degraded at $25\text{--}500^\circ\text{C}$. In the range of $25\text{--}200^\circ\text{C}$, all the samples initially exhibited a small mass loss, which can be attributed to the evaporation of water and ammonia residues. For the D-SNRL samples, a small degradation peak at approximately 225°C likely arose from residual SDS [20]. The thermal degradation of natural rubber showed decomposition peaks between 350°C and 460°C , corresponding to dipentene, isoprene, and

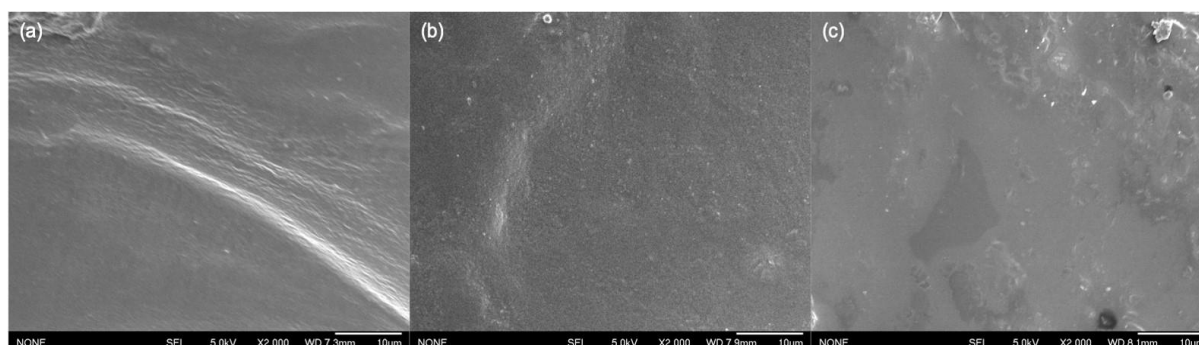


Figure 4 SEM images of (a) SNRL, (b) D-SNRL-9, and (c) D-SNRL/LC-4 samples

p-menthene [29-30]. Moreover, SNRL showed a degradation peak at 391.5 °C, whereas two decomposition temperatures were observed for D-SNRL, 392.2 and 443.9 °C for D-SNRL-9 as well as 392.9 and 448.1 °C for D-SNRL/LC-4. At temperatures up to 460 °C, degradation of nonvolatile residues occurred [31]. Additionally, major degradation peaks of the SNRL and D-SNRL samples were not significantly altered by further protein removal, implying that the thermal stability of the D-SNRL sample was not affected [32-34].

3.5 Tensile strength

Table 4 shows the tensile strength and elongation at breakage for the SNRL, D-SNRL-9, and D-SNRL/LC-4 films. The tensile strength did not dramatically decrease after deproteinization of D-SNRL-9 and D-SNRL/LC-4, but the SNRL tensile strength was slightly higher than that of the deproteinized samples. This was probably due to the lower crystallization of rubber. Proteins and phospholipids linked to fatty acid ester groups contribute to the strength of rubber films. Moreover, from the elongation at breakage of these samples, it can also be seen that deproteinization decreased the elongation at breakage due to a reduction in the rigidity of the rubber film, indicating that removing the protein affected the mechanical properties of rubber films. These results are similar to those reported by the Chaikumpollert group [18].

Table 4 Tensile strength and elongation at breakage

Sample	Tensile strength (MPa)	Elongation at break (%)
SNRL	0.481 ± 0.307	498.795 ± 149.442
D-SNRL-9	0.454 ± 0.231	400.486 ± 110.488
D-SNRL/LC-4	0.435 ± 0.250	372.150 ± 186.784

3.6 Scanning electron microscopy (SEM)

SEM images for the SNRL, D-SNRL-9, and D-SNRL/LC-4 samples are shown in Figure 4. In these SEM images, all the rubber films showed a nearly smooth surface texture. The SNRL film image was somewhat ambiguous, indicating that the natural rubber particles in the latex stage were distributed in a mixed layer of protein and phospholipid complexes. Thus, a matrix-layer may occur after coagulation of the latex containing non-rubber components. After the deproteinization process, the D-SNRL-9 and D-SNRL/LC-4 films showed considerably smoother surfaces with the presence of some agglomerated particles, which indicates that non-rubber components were distributed throughout the

film. From these results, it was concluded that the surfaces of SNRL rubber films obtained after the removing proteins films were quite similar.

4. Conclusions

Deproteinization of SNRL was successfully performed by incubation with urea in the presence of a surfactant (SDS) together with the addition of ethanol followed by a leaching processes. This combination of reagents successfully denatured proteins in the SNRL samples. The incubation time of SNRL did not affect the EP content in a meaningful way due to the variable colloidal stability of the latex during deproteinization. One-step incubation under optimum conditions resulted in a 67.74% protein content reduction. This was followed by a subsequent leaching process with SDS and centrifugation. The SDS concentration and the number of centrifugation cycles significantly affected the protein content. This occurred since SDS facilitated protein unfolding and detachment from the rubber particles. Therefore, SDS acted as a stabilizer that assisted in protein leaching from skim latex. Centrifugation with 1% SDS (3 cycles) resulted in a significantly reduced EP content compared to the D-SNRL samples. This yielded an effective protein reduction of 96.39%. Determination of the nitrogen content of the test samples confirmed that the protein content decreased after subsequent leaching processes, yielding an overall protein reduction of 97.35%. These results were further confirmed by the FTIR spectra of the samples. They showed a near absence of protein related peaks. Moreover, the thermal stability of the SNRL and D-SNRL samples was similar, suggesting that the protein content of SNRL was significantly reduced. Deproteinization of the rubber films resulted in a reduction of the tensile strength and elongation at breakage compared to those of skim natural rubber. SEM images demonstrated that the texture of the surface of the rubber films after protein removal were quite smooth and were similar to that of the SNRL film. These results are superior to those previously reported for SNRL deproteinization methods. This novel deproteinization approach for skim latex can be applied in the production of low-protein products and value-added skim rubber.

5. Acknowledgements

This work was financially supported by a Private University Research Branding Project, MEXT, 2016–2020 and in part by the Kansai University Outlay Support for Establishing Research Centers, 2016–2017. ‘Development and application of biocompatible polymer materials having sol-gel transition’.

6. References

- [1] Huber MA, Terezhalmay GT. Adverse reactions to latex products: preventive and therapeutic strategies. *J Contemp Dent Pract.* 2006;7:97-106.
- [2] Rippel MM, Lee LT, Leite CA, Galembeck F. Skim and cream natural rubber particles: colloidal properties, coalescence and film formation. *J Colloid Interface Sci.* 2003;268:330-40.
- [3] Perrella FW, Gaspari AA. Natural rubber latex protein reduction with an emphasis on enzyme treatment. *Methods.* 2002;27:77-86.
- [4] Tangboriboonrat P, Tanunchai, T, Tiyaipiboonchaiya C. Creaming skim natural rubber latex for encapsulation of urea fertiliser. *Plast Rubber Compos.* 1999;28:357-62.
- [5] Blackley DC. High polymer latices: their science and technology. London: Maclaren; 1966.
- [6] Gazeley KF, Gorton ADT, Pendle TD. Latex concentrates: properties and composition. In: Roberts AD, editor. *Natural rubber science and technology.* Oxford: Oxford University Press; 1988. p.62-98.
- [7] Sakdapipanich JT, Rojruthai P. Molecular structure of natural rubber and its characteristics based on recent evidence. In: Sammour R, editor. *Biotechnology-Molecular Studies and Novel Applications for Improved Quality of Human Life.* Croatia: InTech; 2012. p. 213-38.
- [8] Nawamawat K, Sakdapipanich JT, Ho CC, Ma Y, Song J, Vancso JG. Surface nanostructure of Hevea brasiliensis natural rubber latex particles. *Colloids Surf A Physicochem Eng Asp.* 2011;390:157-66.
- [9] Parra DF, Martins CFP, Collantes CH, Lugao AB. Extractable proteins from field radiationvulcanized natural rubber latex. *Nucl Instrum Methods Phys Res Sect B.* 2005;236:508-12.
- [10] Sakdapipanich J, Kalah R, Nimpaiaboon A, Ho CC. Influence of mixed layer of proteins and phospholipids on the unique film formation behavior of Hevea natural rubber latex. *Colloids Surf A Physicochem Eng Asp.* 2015;466:100-6.
- [11] Kalapat N, Watthanachote L, Nipithakul T. Extraction and characterization of proteins from skim rubber. *Kasetsart J (Nat. Sci.).* 2009;43:319-25.
- [12] Kawahara S, Klinklai W, Kuroda H, Isono Y. Removal of proteins from natural rubber with urea. *Polym Adv Techno.* 2004;15:181-4.
- [13] George KM, Alex R, Joseph S, Thomas KT. Characterization of enzyme-deproteinized skim rubber. *J Appl Polym Sci.* 2009;114:3319-24.
- [14] Eng AH, Tanaka Y, Gan SN. FTIR studies on amino groups in purified Hevea rubber. *J Nat Rubber Res.* 1992;7:152-5.
- [15] Watthanaphanit A, Rujiravanit R. Sericin-bound-deproteinated natural rubber film containing chitin whiskers as elasto-gel dressing. *Int J Biol Macromol.* 2017;101:417-26.
- [16] Klinklai W, Saito T, Kawahara S, Tashiro K, Suzuki Y, Sakdapipanich JT, et al. Hyperdeproteinized natural rubber prepared with urea. *J Appl Polym Sci.* 2004;93:555-9.
- [17] Yamamoto Y, Nghia PT, Klinklai W, Saito T, Kawahara S. Removal of proteins from natural rubber with urea and its application to continuous processes. *J Appl Polym Sci.* 2008;107:2329-32.
- [18] Chaikumpollert O, Yamamoto Y, Suchiva K, Kawahara S. Protein-free natural rubber. *Colloid Polym Sci.* 2012;290:331-8.
- [19] Azmi M, Argo KA, Suriani AB, Azira AA, Masanobu S, Paul B, et al. Preparation of multiwall carbon nanotubes (MWCNTs) stabilised by highly branched hydrocarbon surfactants and dispersed in natural rubber latex nanocomposites. *Colloid Polym Sci.* 2014;292:3013-23.
- [20] Danwanichakul P, Than-ardna, B. Permeation of salicylic acid through skim natural rubber films. *Ind Crops Prod.* 2018;122:166-73.
- [21] Danwanichakul D, Rattanaphan O, Srisatjang J, Danwanichakul P. Extraction of protein from skim natural rubber latex using PEG as a surfactant via low speed centrifugation and continuous flow. *J Appl Polym Sci.* 2014;131(4):1-9.
- [22] Mattos C, Ringe D. Proteins in organic solvents. *Curr Opin Struct Biol.* 2001;11:761-4.
- [23] Chaikumpollert O, Yamamoto Y, Suchiva K, Nghia PT, Kawahara S. Preparation and characterization of protein-free natural rubber. *Polym Adv Techno.* 2012;23:825-8.
- [24] Danwanichakul P, Yooyanyong A, Danwanichakul D. The effect of remaining SDS on protein release in later leaching steps of deproteinized natural rubber. *KKU Eng J.* 2016;43(1):41-6.
- [25] Manroshan S, Mustafa A, Mok KL, Kawahara S, Amir-Hashim MY, Booten K. Comparison between sodium dodecyl sulfate and polyfructose surfactant systems in urea deproteinisation of natural rubber latex. *J Rubber Res.* 2009;12:1-11.
- [26] Nawamawat K, Sakdapipanich JT, Ho CC. Effect of deproteinized methods on the proteins and properties of natural rubber latex during storage. *Macromol Symp.* 2010;288:95-103.
- [27] Pukkate N, Kitai T, Yamamoto Y, Kawazura T, Sakdapipanich JT, Kawahara S. Nano-matrix structure formed by graft-copolymerization of styrene onto natural rubber. *Eur Polym J.* 2007;43:3208-14.
- [28] Prasertkittikul S, Chisti Y, Hansupalak N. Deproteinization of natural rubber using protease immobilized on epichlorohydrin cross-linked chitosan beads. *Ind Eng Chem Res.* 2013;52:11723-31.
- [29] Mathew AP, Packirisamy S, Thomas S. Studies on the thermal stability of natural rubber/polystyrene interpenetrating polymer networks: thermogravimetric analysis. *Polym Degrad Stab.* 2001;72:423-39.
- [30] Jayadevan J, Unnikrishnan G. Novel membranes from physico-chemically modified deproteinized natural rubber latex: development, characterisation and drug permeation. *New J Chem.* 2018;42:14179-87.
- [31] Asaletha R, Kumaran MG, Thomas S. Thermal behaviour of natural rubber/polystyrene blends: thermogravimetric and differential scanning calorimetric analysis. *Polym Degrad Stab.* 1998;61:431-9.
- [32] De Oliveira LCS, De Arruda EJ, Favaro SP, Da Costa RB, Gonçalves PS, Job AE. Evaluation of thermal behavior of latex membranes from genetically

- improved rubber tree (*Hevea brasiliensis*). *Thermochimica acta*. 2006;445:27-31.
- [33] Rao V, Johns J. Thermal behavior of chitosan/natural rubber latex blends TG and DSC analysis. *J Therm Anal Calorim*. 2008;92:801-6.
- [34] Jayadevan J, Alex R, Gopalakrishnapanicker U. Deproteinised natural rubber latex grafted poly (dimethylaminoethyl methacrylate)–poly (vinyl alcohol) blend membranes: synthesis, properties and application. *Int J Biol Macromol*. 2018;107:1821-34.