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Removal of Proteins and Its Effect on Molecular Structure and Properties of Natural Rubber

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Abstract

Removal of protein from natural rubber was carried out via deproteinization of natural rubber in the latex stage. Then, its effect on the molecular structure and properties of natural rubber was investigated. Urea, SDS, and acetone were used as denaturing agent, a surfactant, and a polar solvent in the deproteinization, respectively. Various deproteinized natural rubbers were obtained after one, two, and three times of centrifugation with and without acetone, namely DPNR1, DPNR2, DPNR3, DPNR1-A, DPNR2-A, and DPNR3-A. It was found that the total protein content significantly decreased as increasing the number of centrifugations; however, the total fatty acid contents showed a slight decrease. Structural characteristics analyzed by nuclear magnetic resonance spectroscopy indicated no changes in the chemical structure of natural rubber after deproteinization. However, the removal of proteins significantly enhanced the resolution of the NMR signals. Gel content and tensile properties of natural rubber showed a decrease in the removal of proteins, which was associated with the decrease in the number of the inherent branching points formed in natural rubber.

Keywords: deproteinized natural rubber, proteins, NMR signal, tensile property

1. Introduction

Natural rubber (NR) is well known to be a polymer possessing outstanding green properties. Recently, some findings suggest that the presence of non-rubber components was the origin of the extraordinary performances of natural rubber which completely distinguished from synthetic rubbers [1-3]. The non-rubber components are proposed to constitute the branching points at two terminal groups [4]. The decrease in the number of branch points at both terminal groups of rubber molecules is presumed to decrease the entanglements and subsequently decrease mechanical properties.

In previous work [5], the fundamental structure of natural rubber molecules and characteristics of long-chain branching were disclosed to consist of *ω*-terminal, two *trans*-1,4-isoprene units, about 5000 *cis*-1,4-isoprene and *α*-terminal as illustrated in Fig. 1. The *ω*-terminal was expected to be dimethylallyl groups modified with a functional group that links with proteins to form crosslinking via intermolecular hydrogen bonds. The *α*-terminal was presumed to consist of a phospholipid containing fatty acid ester groups from which the branching structure is formed.

The gel in natural rubber was considered as branched molecules, in which the branch points were formed at two terminal groups via proteins and phospholipids [6]. Therefore, the gel content is presumed to increase as increasing the number of branched points. The removal of non-rubber components decreases the gel content as well as reduces mechanical properties of natural rubber.

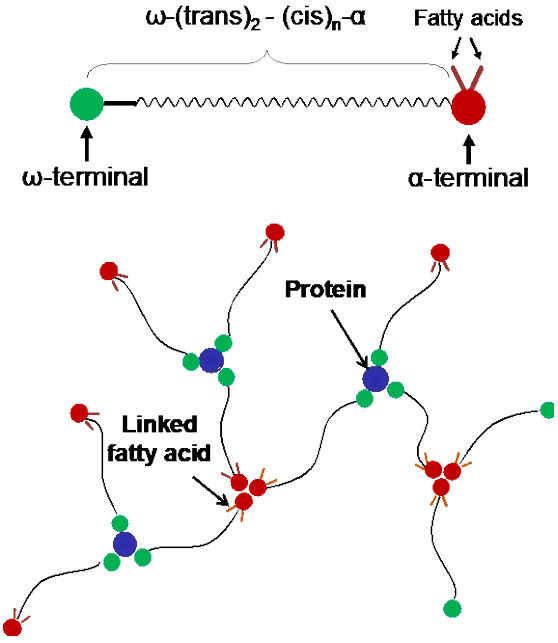

Fig. 1. Fundamental structure of natural rubber and branch points in natural rubber

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Purification of NR could be performed by deproteinization, acetone extraction, and transesterification. In deproteinization process, the protein was denatured and cleaned out of the NR latex by centrifugation. At the same time, some non-rubber components and small rubber molecules in the serum fraction are also removed during the deproteinization. So far, NR could be purified by acetone extraction to remove several acetone-dissolve components such as free fatty acid and other acetone-soluble compounds. The fatty acids that are linked to NR molecules, however, can be removed by transesterification using fresh CH_3ONa. The branch points and properties of NR were found to decrease after transesterification since the linked fatty acid ester are known to be responsible for strain-induced crystallization for NR. In previous works [7,8], proteins were removed by deproteinization using enzymatic reagents or urea denaturants. Usage of polar organic solvent, i.e., acetone, showed the increase in proteins removal efficiency. However, the effect of non-rubber components on mechanical properties during centrifugation and acetone extraction on was not elucidated yet.

In the present work, an attempt to elucidate the relationship between non-rubber components removal, structure, and properties of NR was carried out. Various DPNRs were prepared after one, two, and three times of centrifugations, as well as with and without acetone extraction. The composition of non-rubber components and the properties of natural rubber were determined and their relationship was discussed.

2. Experiment

2.1. Materials

High ammonia natural rubber latex (HANR, 60 wt% DRC) used in this work is purchased from Golden Hope (Malaysia). Sodium dodecyl sulfate (SDS, 99 wt%) was purchased from Chameleon Reagent (Japan). Urea (99.5 wt%) was bought from Nacalai-Tesque (Japan). Acetone was from Tokyo Chemical Industry Co, Ltd. All other chemicals were analytical grade.

2.2. Removal of Proteins from Natural Rubber

Removal of proteins from natural rubber was carried out by deproteinization with urea as a denaturing agent and sodium dodecyl sulfate (SDS) as a surfactant. Fig. 2 shows the procedure for the removal of proteins from natural rubber. HANR latex was diluted with SDS water solution to obtain HANR latex with 30 wt% DRC and SDS 1 wt%. After that, urea and subsequent acetone were gradually added to obtain the latex mixture with 0.1 wt% urea and 0.25 wt% acetone. The mixture was incubated for 1 hour before the first centrifugation. After that, the cream fraction was redispersed into SDS water solution to produce DPNR1-A latex. The DPNR1-A latex was then centrifuged the second time and obtained the cream fraction. This cream fraction was redispersed into SDS water solution to obtain DPNR2-A latex with SDS 1 wt% and acetone 0.25 wt%. The DPNR2-A was centrifuged the third time to get the cream fraction. The final cream fraction was redispersed with SDS water solution to get DPNR3-A latex. As-cast films of DPNR1-A, DPNR2-A, and DPNR3-A were prepared by casting the latexes onto a petri dish followed by drying. A similar deproteinization procedure was carried out without using acetone and three samples were obtained, that is, DPNR1, DPNR2, and DPNR3. Acetone extraction of these samples was carried out within 24 hours.

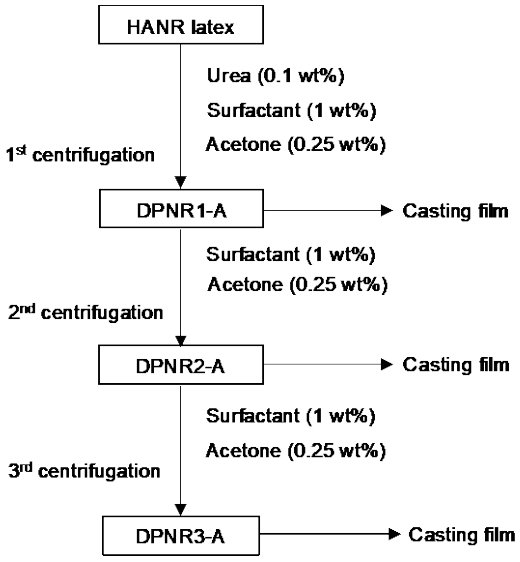

Fig. 2. Removal of proteins from natural rubber

2.3. Material Characterizations

FT-IR spectra of rubber samples were recorded using JASCO FT-IR 410 at 100 scans in the range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. Several drops of rubber solution in chloroform were placed onto KBr dish and let it dried naturally before measurement. Mixtures of synthetic polyisoprene and stearic acid were used to calibrate the total fatty acid content and linked fatty acid content using FTIR.

The gel content of the rubbers was determined by swelling rubber sample in dried toluene. About 0.4 g of each sample was swollen in 40 ml of toluene for one week in the dark. Then, the mixtures were centrifuged at 15°C for 30 mins to collect the gel fraction. The gel fraction was dried for 5 days. Gel content was calculated from the fraction of dried gel and the initial rubber sample.

Nitrogen content was determined by Kjeldahl method as published elsewhere [9]. A rubber sample of about 0.1 g was digested with concentrated acid H_2SO_4 and distilled with water vapor. The distillates were titrated with H_2SO_4 0.005 M to determine the amount of NH_3 produced.

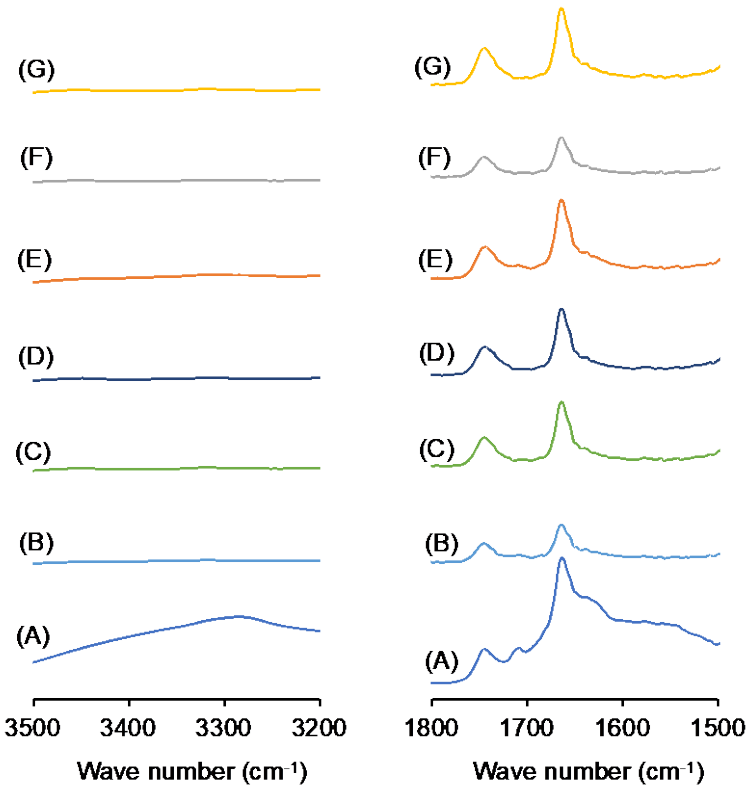

Fig. 3. Fourier transform infrared spectra for (A) HANR, (B) DPNR1 and (C) DPNR2, (D) DPNR3, (E) DPNR1-A, (F) DPNR2-A and (G) DPNR3-A

NMR measurements were performed with a JEOL ECA-400 FT-NMR spectrometer operating at 400 MHz for ^{1}H. NR samples were dissolved in C_6D_6. ^{1}H-NMR measurements were carried out at 50 °C at a pulse repetition time of 7 s for 1000 scans. The chemical shift was referred to as the proton of benzene at 7.16 ppm.

The tensile strength of the rubbers was measured according to JIS K6251 (Japan).

3. Results and Discussion

3.1. FT-IR Spectroscopy

Fig. 3 shows FT-IR spectra of HANR, DPNR1,2,3, and DPNR1,2,3-A in ranges of 3200-3500 cm^{-1} and 1500-1800 cm^{-1}. The absorption peaks at 3280 cm^{-1} and 3320 cm^{-1} were identified to the proteins, i.e., monopeptides and dipeptides, present in natural rubber as reported in the previous literature. The adsorption peak at 1739 cm^{-1} and 1704 cm^{-1} were assigned to the ester and fatty acid groups, respectively. After the first centrifugation, the broad peak at 3280 cm^{-1}, assigned to the proteins, disappeared and the small peak at 3320 cm^{-1}, assigned to monopeptides and dipeptides, appeared. After three times of centrifugation, the peak at 3280 cm^{-1} also disappeared. It suggested that the proteins were decomposed to mono- and dipeptides before removing out by centrigufation. On the other hand, the peak height of the absorption peak at 1739 cm^{-1} did not change much. Therefore, the ester content, which was calculated from the peak height at 1739 cm^{-1} to the peak height at 1664 cm^{-1}, was almost constant.

3.2. Non-Rubber Components

Table 1 shows the nitrogen content, total fatty acid ester content, and linked fatty acid ester content for various DPNRs. The nitrogen content, which represents the amount of protein in natural rubber, decreased as the number of centrifugations increased. That is, from 0.304 wt% for HANR to 0.130 wt% for DPNR1, 0.063 wt% for DPNR2, and 0.014 wt% for DPNR3. During protein removal with acetone, the nitrogen content of natural rubber is further decreased. Particularly, nitrogen content remarkably reduced from 0.304 wt% for HANR to 0.048 wt% for DPNR1-A, to 0.006 wt% for DPNR2-A, and to 0.000 wt% for DPNR3-A. The further decrease in nitrogen content demonstrated the better efficiency in proteins removal. In previous work [7], acetone was reported to affect the removal of proteins via the removal of fatty acids ester. However, in this work, the total fatty acid ester contents of HANR and DPNRs were slightly decreased during centrifugation.

Table 1. Non rubber composition and gel content of various DPNRs

Samples	Total nitrogen content (wt%)	Total fatty acid content (mmol/kg rubber)	Linked fatty acid content (mmol/kg rubber)
HANR	0.304	55.5	11.4
DPNR1	0.130	55.7	-
DPNR2	0.063	55.5	-
DPNR3	0.014	54.2	-
DPNR1-A	0.048	58.6	17.3
DPNR2-A	0.006	54.5	18.1
DPNR3-A	0.000	52.5	17.9

Table 2. Gel content of DPNRs before and after acetone extraction

Samples	Gel content (wt%)	
	before AE	after AE
HANR	39.8	37.5
DPNR1	38.0	35.9
DPNR2	35.8	30.6
DPNR3	33.7	29.5
DPNR1-A	39.4	35.8
DPNR2-A	38.6	30.3
DPNR3-A	30.5	25.3

Table 2 shows the gel content of various rubbers. It seems to decrease as increasing the number of centrifugation, and it further decreases after acetone extraction. The decrease in gel content and free fatty acids after proteins removal suggested that they may relate to formation of branch points in gel fraction of rubber samples. However, the presence of acetone during deproteinization doesn't affect much of the gel of the rubbers.

Fig. 4 plots the relationship between gel content and non-rubber components. It demonstrated that linked fatty acid increased from HANR to DPNR1-A during the first centrifugation. It may be due to removing serum fraction, which contains small rubber molecules and free fatty acids. Therefore, the concentration of fatty acid ester content increased. From the second centrifugation, the linked fatty acid was constant. This figure confirmed that the proteins play a role in decreasing the gel content of various deproteinized natural rubbers.

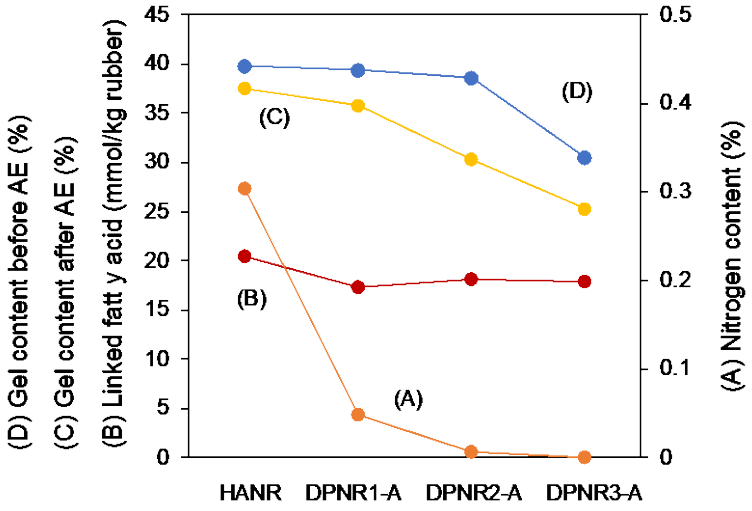

Fig. 4. Relationship between nitrogen content (A), linked fatty acids content (B) and gel content after AE (C), and gel content before AE (D) of HANR, DPNR1-A, DPNR2-A and DPNR3-

3.3. NMR Spectroscopy

Fig. 5 shows the ^{1}H-NMR spectra of HANR, DPNR1-A, DPNR2-A, and DPNR3-A after acetone extraction. The three major signals at 1.77, 2.2, and 5.2 ppm were assigned to methyl proton, -CH_3, methylene proton, -CH_2-, and methine proton, -CH of the *cis*-1,4-isoprene unit. The small signals that appeared at 0.9 ppm and 1.3-1.4 ppm were due to the presence of fatty acids. The signal from 3.8 to 4.3 ppm, that was belonged to signal of phospholid was shown in Fig. 6. It was noted that the phopholipid signals' resolution increases after deproteinization, from HANR to DPNR1-A, DPNR2-A, and DPNR3-A. The enhanced resolution and spitting patterns of these signals may be due to removing proteins that were closed to phospholipids. Besides, the removal of proteins may improve the solubility of the rubber in an organic solvent.

Fig. 7 presents the expanded spectra of HANR, DPNR1-A, DPNR2-A, and DPNR3-A from 1.3-2.4 ppm. It was observed that the small signals at 1.64-1.66 ppm were assigned to methyl protons of two *trans*-1,4-isoprene units. The ^{1}H-NMR spectra show the increase in the signal intensity, which was tabulated in Table 4. The signal intensities were found to increase as the number of centrifugation increased. It may reveal that the location of proteins was close to the ω-terminal of rubber molecules

Table 4: Intensity ratios of the signals at 1.64-1.66 ppm to the signal at 1.77 ppm

Samples	$I_{1.64\text{-}1.66\ ppm}$ (%)
HANR-AE	0.00017
DPNR1-A-AE	0.00007
DPNR2-A-AE	0.00007
DPNR3-A-AE	0.00043

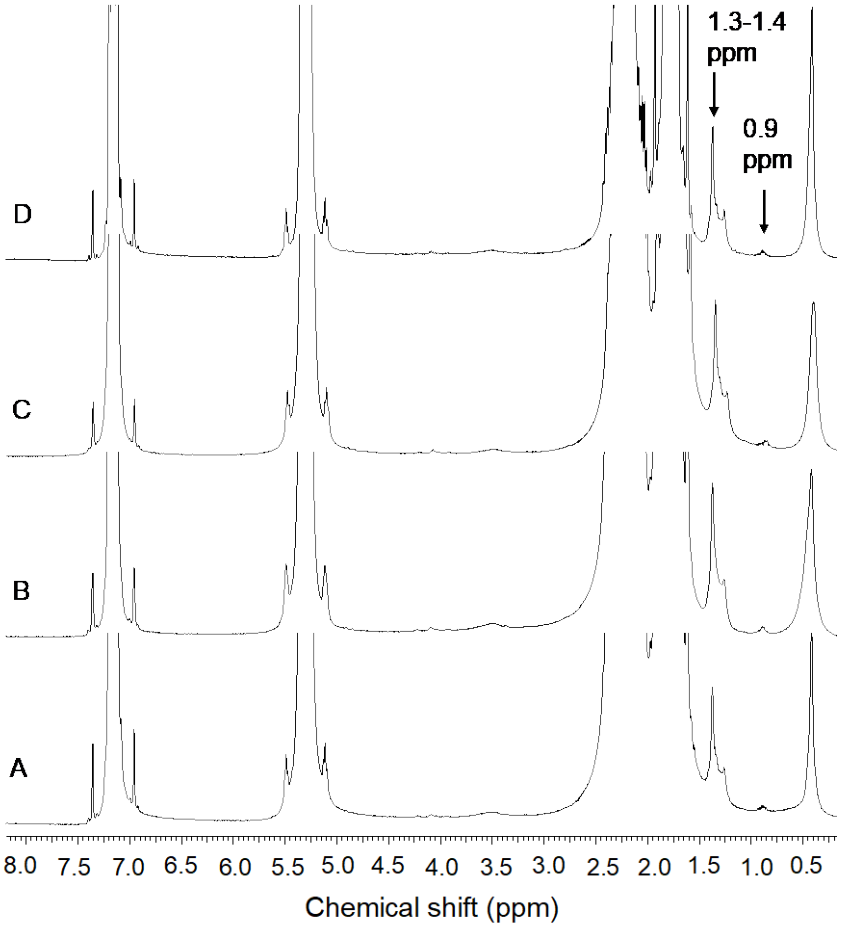

Fig. 5. ^{1}H-NMR spectra of (a) HANR, (b) DPNR1-A, (c) DPNR2-A and (d) DPNR3-A after AE

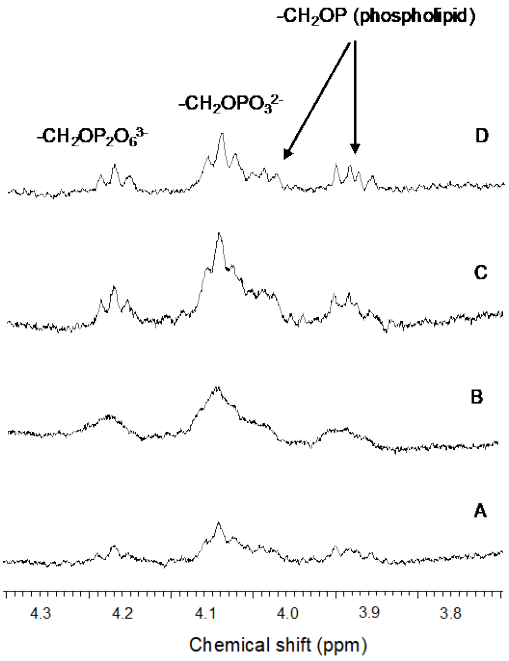

Fig. 6. Signals of phospholipid and phosphate groups of (A) HANR-AE, (B) DPNR1-A-AE, (C) DPNR2-A-AE and (D) DPNR3-A-AE

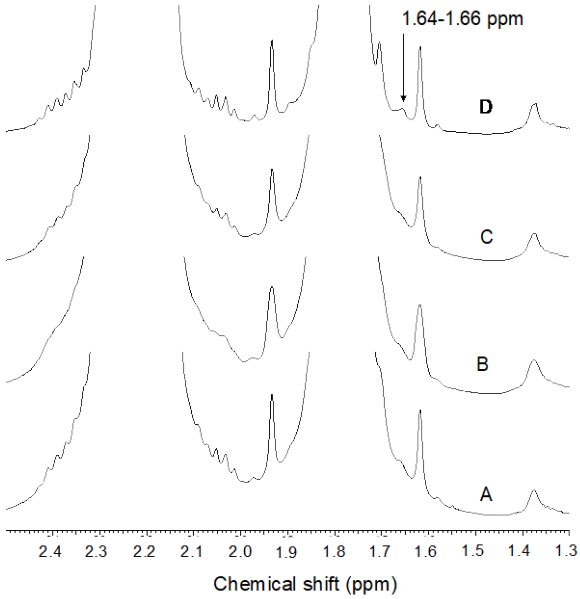

Fig. 7. Expanded ^{1}H-NMR spectra of (a) HANR, (b) DPNR1-A, (c) DPNR2-A and (d) DPNR3-A after AE

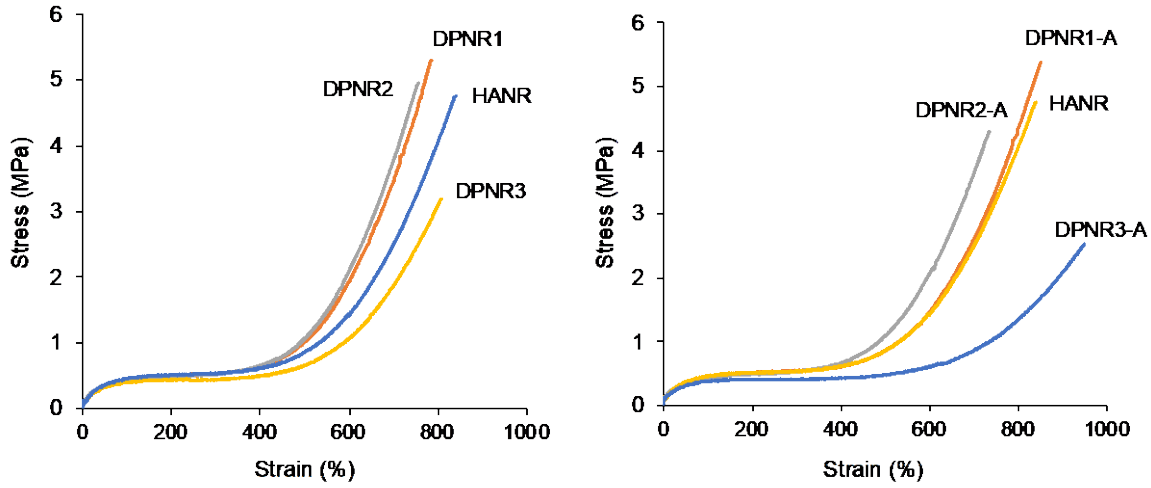

Fig 8. Stress-strain curves of DPNR1, DPNR2, DPNR3 and DPNR1-A, DPNR2-A, DPNR3-A

3.4. Mechanical Properties

Fig. 8 shows the stress-strain curves for various deproteinized natural rubbers prepared with and without acetone. It could be seen that the removal of proteins decreased stress at the break of the rubbers in both cases: use and don't use acetone. DPNR3 showed a significant decrease in stress at break compared to HANR. However, DPNR3-A was further decreased in stress at break compared to DPNR3. It suggested that natural rubber becomes softer after removing protein completely. This may relate to the role of proteins in accelarating the upturn of stress at break versus strain. Since proteins may act as the crosslinking points as well as reinforcing filler in NR as reported in the previous work [10].

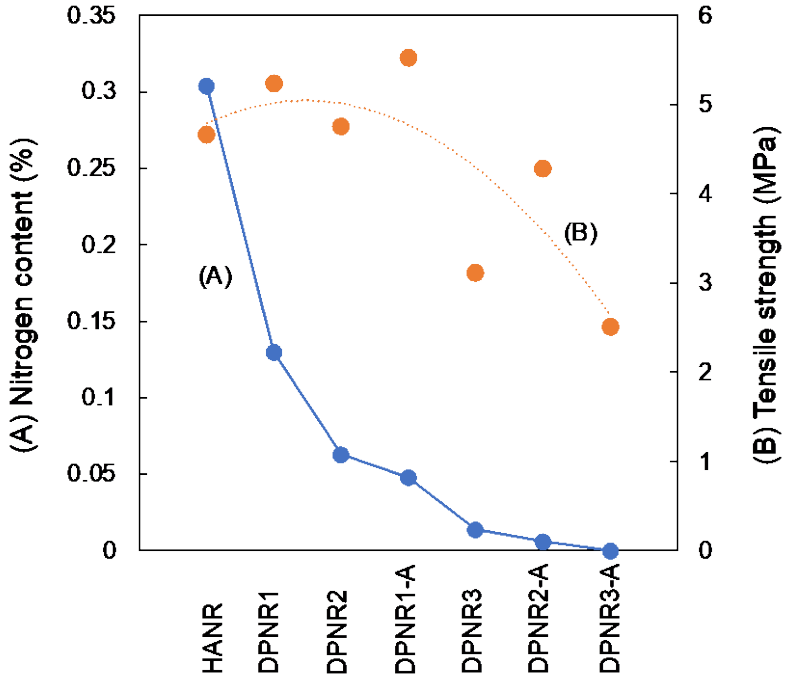

Fig. 9. Relationship between nitrogen content and mechanical properties of natural rubber

Fig. 9 shows the plots of tensile strength and nitrogen contents of the various DPNRs. As can be seen, when nitrogen content decreased from 0.048 wt% to 0.000 wt%, the tensile strength of the rubber seemed to decrease. This result demonstrated that nitrogen content and gel content play a role in natural rubber's mechanical properties. The role of proteins may be a natural rubber filler that contributes to the inherent outstanding properties of natural rubber.

4. Conclusion

Natural rubber was purified by deproteinization using urea, sodium dodecyl sulfate, and acetone. The nitrogen content of the rubbers decreased with the increasing number of centrifugations. The chemical structure of the rubber molecule main chain was not interfered with by removing protein; however, the resolution and intensities of the signal of fatty acid ester, phospholipid and *trans*-1,4-isoprene units increased. The removal of proteins also caused the reduction in gel content and tensile strength of natural rubber. The results in the study suggested that the proteins in rubber molecules may be associated with the ω-terminal and with phospholipids at the α-terminal as well.

Acknowledgments

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