รายงานวิจัยฉบับสมบูรณ์

โครงการ การศึกษาที่มาของสมบัติที่โดดเด่นของยางธรรมชาติ

(Elucidating the origin of outstanding properties of natural rubber)

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สัญญาเลขที่ **RMU 4980046**

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แขวงทุ่งพญาไท กรุงเทพ ฯ 10400

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัยและสำนักงาน
คณะกรรมการการอุดมศึกษา
(ความเห็นในรายงานนี้เป็นของผู้วิจัย สกว.ไม่จำเป็นต้องเห็นด้วยเสมอไป)

Abstract

Natural rubber (NR) from *Hevea brasiliensis* is the most practically representative *cis*-1,4-polyisoprene accounting for more than 99% of the world's natural source of rubber. This is due to the high productivity of the plant and excellent properties of NR, comparable to synthetic polyisoprene. Even though, the synthetic polyisoprene polymerized with Ziegler-Natta catalyst consists of the isoprene units in the *cis*-1,4 configuration higher than 98%, its property is inferior than NR. Why does NR show the outstanding properties? Besides a small amount of other isoprene configurations, non-rubber components have been believed to bring about properties characteristic of NR. However, the information was not complete, so it is necessary to do more deep research on this field to investigate the origin of outstanding properties of NR, which is the main objective of this work.

Variations in green strength of natural rubber (NR) by the film preparation method were studied to elucidate the origin of the stress-strain behavior characteristics of NR, in connection with the structure of branch-points in NR. The change of stress-strain behavior by the casting method and heat treatment is a phenomenon characteristic of NR, but no difference was observed in the case of synthetic polyisoprene (IR). It was found that proteins and phospholipids are associated with the formation of the network structure in NR. This network structure is responsible for high tensile strength and crystallizability under the deformation of NR. The removal of proteins and phospholipids resulted in the decomposition of network structure, leading to a significant decrease in the green strength and the crystallizability under deformation. An increase in the network chain density produced on increase in tensile stress and strain-induced crystallization during the deformation of NR. The stress-strain behavior of un-vulcanized NR strongly depends on the measuring condition, i.e. temperature and stretching speed. The stretching speed dependency of NR on the stress-strain behavior and the strain-induced crystallization of un-vulcanized and vulcanized state were different. This may be due to the effect of entanglements.

The structure of branch-points in NR was also analyzed by physical decomposition of branch-points using surfactant washing by high-speed centrifugation of NR latex. The diminution of nitrogen content is consistent with the disintegration of gel fraction. This suggests that the branching formation at □-terminal derives from proteins *via* supramolecular bond. Based on the result of the evaluation of the performance of deproteinized reaction by SDS-PAGE, enzymatic deproteinization was proposed to cleave the proteins so that the retained proteins would be oligopeptides or short-chain proteins. Surfactant washing with high-speed centrifugation denatured the proteins without degradation, whereas saponification reaction decomposed both proteins and fatty acids, although NaOH treatment of latex was not identified. Gel formation in the storage deproteinized NR latex could not be as fully accomplished as in the normal NR due to the lack of proteins leading to the deterioration in physical properties. Accelerated storage hardening test disclosed that both residual proteins and fatty acids play a role in the formation of gel fractions including the change of physical properties in the deproteinized NR.

บทคัดย่อ

ยางธรรมชาติจากฮีเวียบรสซิลเลียนซิส เป็น1,4 พอลิไอโซปรีนที่ใช้กันมากกว่า 99% ของยางธรรมชาติที่มีอยู่ในโลก อันเนื่องมาจากทั้งผลผลิตที่มากและสมบัติทางกายภาพที่ดีกว่ายางพอลิไอโซปรีนสังเคราะห์ ถึงแม้ว่ายางพอลิไอโซปรีนสังเคราะห์ที่สังเคราะห์โดยการใช้ซิกเกอร์-แนตตาคะตะลิตส์จะมีหน่วยไอโซปรีนมากกว่า 98% ก็ตาม แต่ก็มีสมบัติที่ด้อยกว่ายางฮีเวีย ทำไมยางธรรมชาติจึงมีสมบัติที่ดี? นั่นคือนอกจากเรื่องของโครงสร้างไอโซปรีนแล้วเชื่อว่ายังมีผลมาจากส่วนที่ไม่ใช่ยางที่ทำให้ยางฮีเวียมีสมบัติเฉพาะตัว แต่ก็ไม่มีข้อมูลที่อธิบายตรงนี้ ดังนั้นวัตถุประสงค์หลักของงานนี้จึงเป็นการศึกษาในระดับลึกเพื่อให้เข้าใจว่าทำไมยางธรรมชาติจึงมีสมบัติที่เด่น

จากศึกษาความปรวนแปรของสมบัติความแข็งแรงของฟิล์มยางธรรมชาติที่เตรียมด้วยวิธีการต่างๆ เพื่ออธิบายถึงสาเหตุของลักษณะความเค้นต่อความเครียดที่เป็นเอกลักษณ์เฉพาะตัวของยางธรรมชาติ ซึ่งสมบัตินี้เกี่ยวข้องกับโครงสร้างกิ่งก้านของสายโซ่ยาง การเปลี่ยนแปลงลักษณะความเค้นต่อความเครียด เนื่องจากวิธีการเตรียมแผ่นฟิล์มและการให้ความร้อนเป็นลักษณะเฉพาะตัวของยางธรรมชาติซึ่งไม่พบในกรณียางสังเคราะห์ ผลการทดลองพบว่าโครงสร้างร่างแหที่เกิดขึ้นเองตามธรรมชาติโดยการประกอบของโปรตีนและฟอสโฟลิปิด เป็นสาเหตุสำคัญส่งผลให้ยางธรรมชาติมีความแข็งแรงสูง และมีความสามารถในการเกิดผลึกขณะเปลี่ยนแปลงรูปร่างได้ดี การกำจัดโปรตีนและฟอสโฟลิปิดออกจากยางธรรมชาติ มีผลทำให้ความแข็งแรงและความสามารถในการเกิดผลึกขณะเปลี่ยนแปลงรูปร่างลดลงอย่างมาก ความหนาแน่นของโครงสร้างร่างแหที่เพิ่มขึ้นส่งผลทำให้ความแข็งแรงและความสามารถในการเกิดผลึกขณะเปลี่ยนแปลงรูปร่างเพิ่มขึ้น จากการทดลองยังพบว่าลักษณะความเค้นต่อความเครียดของยางธรรมชาติที่ไม่ได้ผ่านการวัลคาไนซ์ขึ้นกับเงื่อนไขของการทดลอง เช่น อุณหภูมิและความเร็วในการดึงตัวอย่าง ซึ่งความเร็วในการดึงตัวอย่างมีผลต่อลักษณะความเค้นต่อความเครียด และความ สามารถในการเกิดผลึกขณะเปลี่ยนแปลงรูปร่างของยางธรรมชาติที่ไม่ได้ผ่านการวัลคาไนซ์อย่างมาก โดยยางธรรมชาติแสดงพฤติกรรมที่แตกต่างกันก่อนและหลังการวัลคาไนซ์ ซึ่งสามารถอธิบายได้จากอิทธิพลของการพันกันของสายโซ่โมเลกุล

จากการวิเคราะห์โครงสร้างกิ่งก้านของยางธรรมชาติด้วย โดยทำการศึกษาโดยการสลายด้วยกระบวนการทางกายภาพ ด้วยการใช้สารลดความตึงผิวร่วมกับการปั่นล้างที่ความเร็วรอบสูง จากผลงานวิจัยพบว่าการลดลงของโปรตีนนั้นมีความสอดคล้องกับการแตกสลายเจลที่สนับสนุนว่าโปรตีนมีส่วนร่วมในการควบคุมการเกิดโครงสร้างกิ่งก้านที่ปลายด้านโอเมก้าโดยเกิดผ่านพันธะไฮโดรเจน จากผลการศึกษาความสามารถของกระบวนการดีโพรทีไนซ์โดยการวิเคราะห์ SDS-PAGE การทำดีโพรทีไนซ์ด้วยเอนไซม์สามารถตัดโปรตีนให้เหลือเป็นโอลิโกเปปไทด์ หรือเปปไทด์สั้นๆ ส่วนการปั่นล้างด้วยสารลดแรงตึงผิวจะไปทำให้ลักษณะธรรมชาติของโปรตีนเปลี่ยนไป โดยไม่เกิดการตัดโปรตีน ในขณะที่การทำปฏิกิริยาสะพอนิฟิเคชั่นนั้นคาดว่าทำปฏิกิริยากับทั้งโปรตีนและลิปิดในยางธรรมชาติ อย่างไรก็ตามการเกิดปฏิกิริยาเคมียังไม่ทราบแน่นอน จากผลการเก็บน้ำยางดีโพรทีไนซ์เป็นเวลานานพบว่า ปริมาณเจลไม่สามารถเกิดได้เท่ากับกรณีน้ำยางสดปรกติ เนื่องมาจากการขาดโปรตีน ส่งผลให้สมบัติทางกายภาพของยางแย่ลง นอกจากนี้ผลของการศึกษาการแข็งขึ้นของยางดีโพรทีไนซ์ภายใต้สภาวะเร่งพบว่าโปรตีนที่เหลืออยู่รวมไปถึงกรดไขมันมีบทบาทต่อการเกิดเจลและการเปลี่ยนแปลงของสมบัติทางกายภาพ

Project Code : RMU 4980046

Project Title : การศึกษาที่มาของสมบัติที่โดดเด่นของยางธรรมชาติ

(Elucidating the origin of outstanding properties of natural rubber)

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แขวงทุ่งพญาไท กรุงเทพ ฯ 10400

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Project Period : 3 years

Details :

PART 1 EFFECT OF NON-RUBBER PROTEINS AND FATTY ACIDS ON PHYSICAL PROPERTIES OF NATURAL RUBBER

PART 2 ORIGIN OF STRESS-STRAIN BEHAVIOR CHARACTERISTIC OF NATURAL RUBBER

PART 3 STRAIN-INDUCED CRYSTALLIZATION OF NATURAL RUBBER: EFFECT OF PROTEINS AND PHOSPHOLIPIDS

PART 4 ROLE OF PHOSPHOLIPIDS AND PROTEINS ON GEL FORMATION, STORAGE HARDENING AND CHANGE IN PHYSICAL PROPERTIES OF NATURAL RUBBER DURING ACCELERATED STORAGE

PART 5. STRUCTURE OF BRANCH-POINTS IN NATURAL RUBBER – REGULATION OF PROTEINS ON GEL FORMATION IN DEPROTEINIZED NATURAL RUBBER–

PART 1 EFFECT OF NON-RUBBER PROTEINS AND FATTY ACIDS ON PHYSICAL PROPERTIES OF NATURAL RUBBER

The outstanding green strength and strain-induced crystallization of un-vulcanized natural rubber (NR) were investigated by tensile test and synchrotron wide-angle X-ray diffraction (WAXD) measurement with respect to naturally occurring network. NR was purified by deproteinization, acetone-extraction and transesterification to remove proteins, free fatty acids and linked fatty acids, respectively, which resulted, in turn, in decomposition of the naturally occurring network. The green strength of NR was similar to that of deproteinized NR (DPNR), whereas it decreased after acetone extraction followed by transesterification. The transesterified DPNR did not show an abrupt increase in stress *versus* strain and its green strength was comparable to that of synthetic *cis*-1,4,-polyisoprene. The abrupt increase in stress *vesus* strain was associated with the strain-induced crystallization, detected by WAXD measurement for NR and DPNR. The effect of strain-induced crystallization was prooved with soluble and gel fractions of NR to be due to the naturally occurring network. The effect of fatty acids on green strength and strain-induced crystallization was also investigated in the present study.

Keywords: Green strength; Strain-induced crystallization; Naturally occurring network

Introduction

It is widely recognized that natural rubber (NR) from *Hevea brasiliensis*, shows several excellent properties such as high green strength[1,2] and tack[1] in the un-vulcanized state and high tensile strength[3] and crack growth resistance[4,5] and minimal heat build up in the vulcanized state. The outstanding properties of NR may be concerned with the fact that NR contains 6% of non-rubber components and characteristic structure, different from synthetic *cis*-1,4-polyisoprene (IR). As reported previously[6], a linear rubber chain in NR is proposed to consist of the initiating terminal, i.e., ω-terminal, two *trans*-1,4 isoprene units, about 5,000 *cis*-1,4 isoprene units, and the chain end group, i.e., α-terminal, linking up with phospholipids, containing fatty acid residues, aligned in that order. The α-terminal is presumed to consist of phospholipids, which play a role in branching formation of the rubber molecules.

In the previous work [10-12], we investigated the effect of non-rubber components such as proteins and fatty acids on the green strength of NR. We found that the green strength of NR didn't change after the removal of proteins, but the strain at break increased[10]. This may be concerned with the decomposition of physical linkage, formed with proteins at ω-terminal of NR, after deproteinization. The green strength of NR decreased to a low level comparable to that of IR, after the decomposition of fatty acids through transesterification[11,12]. This is attributed to the transesterification of deproteinized NR (DPNR), which resulted in the decomposition of branching points and the formation of linear rubber chains. Thus, the difference in the green strength may be concerned with the non rubber components, which are associated with the network structure of NR.

In the present study, we applied deproteinization and saponification process for removing proteins and fatty acids, respectively. Using these procedures, the effect of proteins and fatty acids in NR on physical properties and mechanical properties were investigated. These rubbers will be subjected to analyze for green properties such as Mooney and green strength and vulcanizate properties such as processing properties, tensile properties and dynamic properties. Furthermore, these rubbers were blend with styrene butadiene rubber (SBR) and investigated the processing properties, tensile properties and dynamic properties.

EXPERIMENTAL

1. *Preparation of samples for testing*

Fresh natural rubber latex (FL) will be used in this study as a starting material for preparing Fresh NR (FNR), Deproteinized NR (DPNR) and Saponified NR (SAP). FL was coagulated by the addition of flocculent and formic acid, followed by washing with water and dried at 50^{o}C. To remove proteins in NR, the deproteinization was carried out by incubating the fresh natural rubber latex with 0.04 w/v% proteolytic enzyme and 1 w/v% sodium dodecyl sulfate (SDS) for 12 h at 37^{o}C followed by coagulation with flocculent and formic acid, followed by washing with water and dried at 50^{o}C.

SAP-H was prepared by saponification of FL-latex with 0.2% w/v Triton X and 1.5% w/v NaOH for 3 h at 70^{o}C. Then, the resulting latex was coagulated by the addition of flocculent and formic acid, followed by washing with water. The obtained rubber was soaked in 1% w/v BHT suspended in water containing 0.2%SDS. The samples were dried at 50^{o}C.

SAP-L was prepared by soaking SAP-H, after washing it with water, in 2% w/v NaOH for 24 h at room temperature. After washing the sample with water, it was soaked in 1% w/v BHT suspended in water containing 0.2%SDS. The samples were dried at 50^{o}C.

2. *Characterization of rubber samples*

A LECO FP-258 nitrogen analyzer was used for nitrogen analysis based on the combustion by oxygen gas. The rubber sample of ca. 25 g was accurately weighed and subjected to the nitrogen analysis. The combustion of rubber sample converts the nitrogen compound to nitrogen gas, which is detected as nitrogen content in percentage by weight.

The content of long-chain fatty acid ester was determined by FT-IR measurement. A calibration curve was obtained for a mixture of methyl stearate and synthetic *cis*-1,4 polyisoprene (Kuraprene IR10). The content of fatty acid ester group per weight of rubber was determined by the intensity ratio of peaks at 1739 cm^{-1} (C=O) to 1664 cm^{-1} (C=C).

Gel content was measured by solubility measurement in toluene solution. The rubber sample dissolved in distilled toluene at the concentration of 0.1%w/v was kept in dark without stirring for a week at room temperature. The gel fraction was separated from sol fraction by centrifugation at 10,000 rpm for 30 min. The precipitated gel fraction was recovered by coagulation with methanol. The gel content was calculated from the percentage between weight ratio of gel and original rubber.

Ash content was investigated by thermal combustion in Furnace. The rubber sample of ca. 0.1 g was accurately weighed in a porcelain crucible and subjected to a furnace at 550^{o}C for 3 h. The residual ash was obtained and weighted for calculating the ash content.

3. *Mixing process and vulcanization*

The rubber samples (CV60, FNR, DPNR, SAP-H, SAP-L) were mixed with rubber chemicals in an internal mixer at 40^{o}C for 7 min using the rotor speed of 40 rpm and the fill factor 0.7. The obtained rubber compounds were passed through a two-roll mill and kept in the dark before being proceeded further for vulcanization.

The recipe of the compounds is as follows:

Rubber	100 phr
Sulpher	2 phr
Stearic acid	3 phr
ZnO	5 phr
MBT	1 phr
Antioxidant (6PPD)	2 phr

For vulcanization, the rubber compound was cured at 155^{o}C by compression molding. Cure time was varied according to the optimum cure time of each sample measured by Oscillating Disk Rheometer (ODR).

4. *Blend of rubber sample with styrene butadiene rubber (SBR)*

The rubber samples were blended with SBR and mixed with rubber chemicals in an internal mixer at 40^{o}C for 7 min using the rotor speed of 40 rpm and the fill factor 0.7. The obtained rubber compounds were passed through a two-roll mill and kept in the dark before being proceeded further for vulcanization.

Blend curing of rubber sample with SBR was carried out using the following tier recipe as follow:

Tire Recipe (for carcass**)**

NR	50 phr
SBR	70 phr
CB N660	43 phr
Aromatic oil	8 phr
ZnO	4 phr
Stearic acid	1.5 phr
TMQ	1.5 phr
MBT	0.5 phr
TMTD	1 phr
Sulfur	2.5 phr

For vulcanization, the rubber compound was cured at 155^{o}C by compression molding. Cure time was varied according to the optimum cure time of each sample measured by Oscillating Disk Rheometer (ODR).

5. *Determination of physical properties of rubber in un-vulcanized and vulcanized state*

In un-vulcanized state, rubber sheet for tensile measurement was prepared by casting of rubber latex on glass plate. The cast film was dried in an oven at 50^{o}C until get constant weight. The dried sheet was cut into dumbbell shape according to ASTM D 412-87. Testing was carried out by tensile testing machine (Instron model 4301) at the crosshead speed of 500 mm/min. Load cell of this experiment was 100 N.

In vulcanized state, tensile measurement was carried out by tensile testing machine (Instron model 4301) at the crosshead speed of 500 mm/min. Load cell of this experiment was 1 kN.

Mooney viscosity of rubber sample was determined by Mooney Torque Rheometer. Vulcanizing properties such as scorch time and cure time were measured by Oscillating Disk Rheometer (ODR). Dynamic properties and heat build up were investigated by Flexometer (Wallace De Mattia). Abrasion of sample was determing by DIN Abrasion Tester.

RESULTS AND DISCUSSION

1. Basic and Green properties of rubber samples

Table 1 Characterization properties of rubber samples

Sample	Nitrogen content (%w/w)	Fatty acid ester content (mmol/kg-rubber)	Gel content (%w/w)	Ash content (%w/w)
FNR	0.364	28.14	9.4	0.22
DPNR	0.021	28.17	1.8	0.20
SAPH	0.110	24.65	1.8	0.21
SAPL	0.094	20.68	3.8	0.21

It is clear that nitrogen content of DPNR, SAPH and SAPL was lower than that of FNR. This indicates that deproteinization and saponification can decompose proteins in NR. DPNR showed 0.021%w/w of N content while SAPH showed 0.110%w/w. After soaking with NaOH, the nitrogen content of SAPH further decreased to 0.094%w/w. Fatty acid ester content of DPNR was similar to that of FNR while SAPH showed lower fatty acid ester content. Fatty acid ester content of SAPH was 24.65 mmol/kg-rubber and further decreased to 20.68 mmol/kg-rubber after soaking in NaOH. It can be seen that deproteinization cannot remove or decompose the fatty acid in NR but saponification can do and more effective after soaking with NaOH. Figure 1 shows schematic representation of removing proteins and fatty acids by saponification and soaking process. The treatment with strong alkali can decompose proteins and some solubilize lipids covering the rubber particle. Therefore, saponification will be good procedure for getting purified solid NR. The gel content of rubber decreased after deproteinization and saponification process due to the

decomposition of proteins through the process. The ash content of all rubbers was about 0.2%w/w.

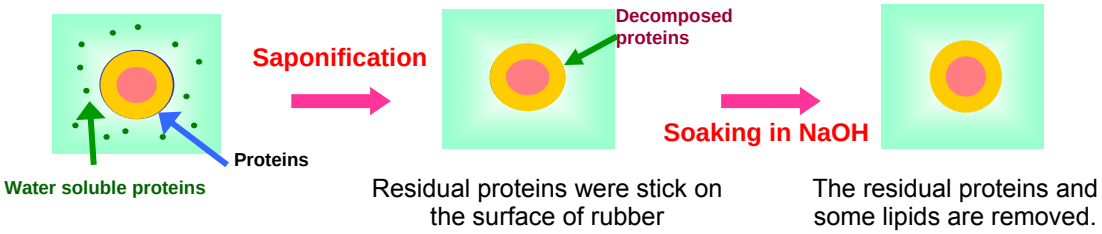

Figure 1 Schematic representation of removing proteins and fatty acids by saponification and soaking process

Table 2 shows Mooney viscosity and tensile properties of un-vulcanized rubbers. It can be observed that the mooney viscosity of saponified rubber and DPNR were less than that of the control rubber. This was caused by the reduction of gel content and non-rubber components, i.e. proteins and fatty acids, after deproteinization and saponification. Through both processes, not only rubber purity was improved but also processibity was better, due to low Mooney viscosity of rubber. Tensile strength of un-vulcanized rubber, i.e. green strength, was shown in Table 2. Green strength of FNR was 5.51 MPa while that of DPNR, SAPH and SAPL were 3.22, 1.97 and 1.86 MPa, respectively. It was clear that the green strength of rubber decreased after the removal of proteins and fatty acids by deproteinization and saponification. This suggests that proteins and fatty acids contributed to superior green strength of natural rubber. Modulus at 100%, 300% and 500% strain also showed the same trend as green strength. Strain at break of rubber increased after deproteinization and saponification. Stress-strain curves of FNR, DPNR, SAPH and SAPL were shown in Figure 2.

Table 2 Mooney viscosity and green strength of rubber in un-vulcanized state

Properties	FNR	DPNR	SAPH	SAPL
ML 1+4	125.35	88.07	72.64	87.95
Green strength (MPa)	5.51	3.22	1.97	1.86
Strain at break (%)	993	1020	1120	1150
100M (MPa)	0.39	0.38	0.31	0.32
300M (MPa)	0.40	0.42	0.34	0.36
500M (MPa)	0.50	0.44	0.34	0.35

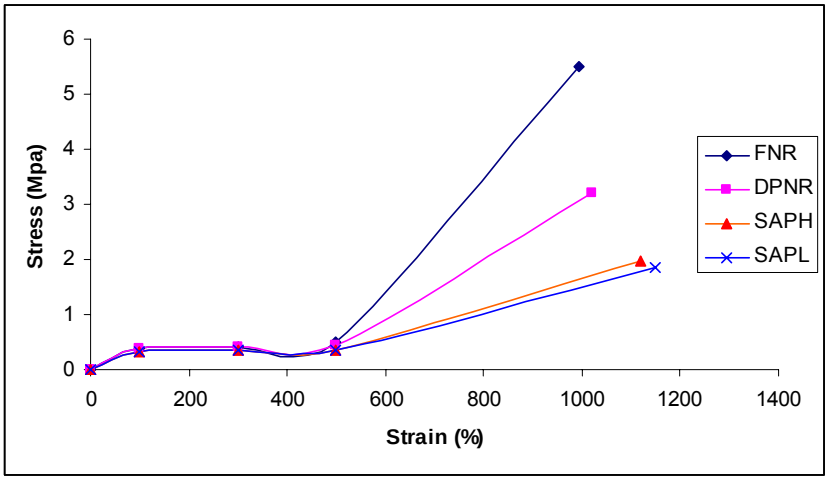

Figure 2 Stress-strain curves of un-vulcanized rubber samples

2. Tensile strength of Vulcanized rubbers

Table 3 Properties of vulcanized rubbers

Properties	CV60	FNR	DPNR	SAPH	SAPL
Scorch time (min)	2.55	1.43	4.15	1.42	1.46
Cure time (min)	7.38	6.19	11.23	5.52	4.36
Tensile strength (MPa)	16.76	13.79	10.83*	20.59	17.46
Strain at break (%)	723	674	745*	732	758
100M (MPa)	0.54	0.55	0.42	0.58	0.52
300M (MPa)	1.23	1.31	0.83	1.28	1.11
500M (MPa)	3.23	3.91	1.62	3.13	2.29

* Unbreakable

Scorch time of SAPH and SAPL was about 1.4 min, similar to that of FNR while DPNR showed the highest scorch time of about 4.15 min. Cure time of FNR was 6.19 min while SAPH and SAPL showed shorter cure time. DPNR showed the highest cure time of about 11.23. It can be observed that DPNR, which was removed proteins but still remained high fatty acid ester content, showed the longest cure time and scorch time. There are two possibilities for describing this phenomenon. First, it may be due to the removal of proteins, since proteins may accelerate the vulcanization. Second, SDS used in preparation procedure of DPNR, may disrupt the vulcanization. On contrary, saponified rubber, i.e. SAPH and SAPL, which was removed both proteins and fatty acids, showed the shortest cure time. This may be due to two reason; first, the residual base in saponified rubber may accelerate the vulcanization and second, the effect of fatty acid ester groups, which was removed after saponification.

Tensile properties and stress-strain curves of vulcanized rubbers were shown in Table 3 and Figure 3, respectively. Tensile strength of vulcanized saponified rubber was higher than that of FNR and CV60. Tensile strength of DPNR cannot be measured because the limitation of machine is about 750% strain. By the way, it was clear that the removal of protein and fatty acids didn't significantly affect physical properties in vulcanized state. This is due to the physical properties of vulcanized rubber mainly affects by s-s crosslink.

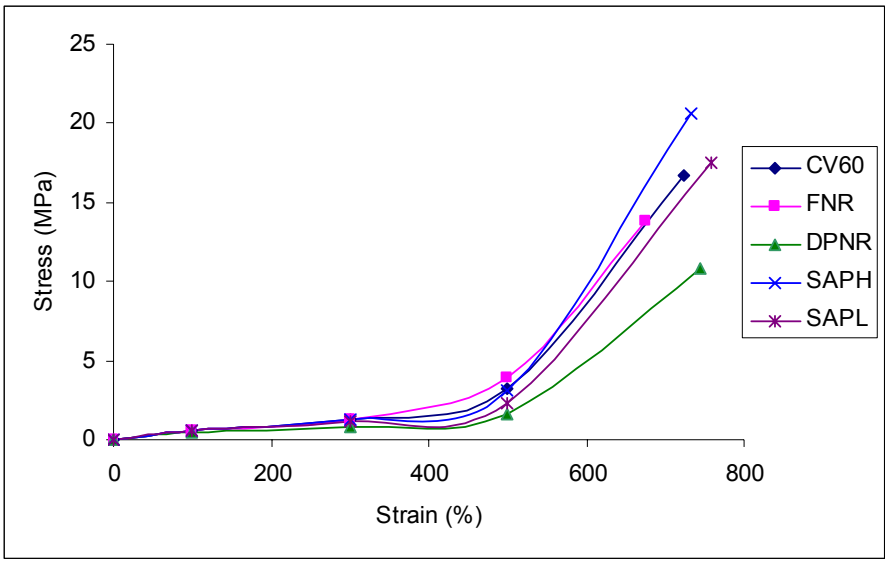

Figure 3 Stress-strain curves of cured rubbers

3. Dynamic properties of vulcanized rubber

Table 4 Dynamic properties of vulcanized rubber

Properties	CV60	FNR	DPNR	SAPL
Storage modulus E' (MPa)	1.262	1.505	1.157	1.694
Loss of modulus E" (MPa)	0.043	0.041	0.069	0.034
Tan δ	0.037	0.028	0.061	0.021
Heat build up (^{o}C)	12.5	9	20	12.5
Dynamic compression set (%)	100	89	178	51

Dynamic properties of vulcanized rubber were shown in Table 4. Storage modulus, loss modulus and tan δ of SAPL were almost the same as those of FNR. Whereas DPNR showed lower storage modulus, higher loss modulus and tan δ. This suggests that an elasticity of DPNR was lower than that of FNR and SAPL. Furthermore, heat build up of DPNR was very high, compared with other samples. Dynamic compression set of DPNR was also very high. In contrast, SAPL showed the lowest compression set. This indicates that SAPL showed the highest elasticity.

4. Tensile strength of rubber blend with SBR

Table 5 Properties of rubber blend with SBR

Properties	CV60	FNR	SAPH	SAPL
Scorch time	1.54	1.50	1.45	1.40
Cure time	3.43	3.42	3.30	3.13
Tensile strength (MPa)	10.89	12.59	11.49	11.87
Strain at break (%)	365	338	323	331
100M (Mpa)	1.99	2.58	2.38	2.41
300M (Mpa)	8.26	9.86	8.42	10.41

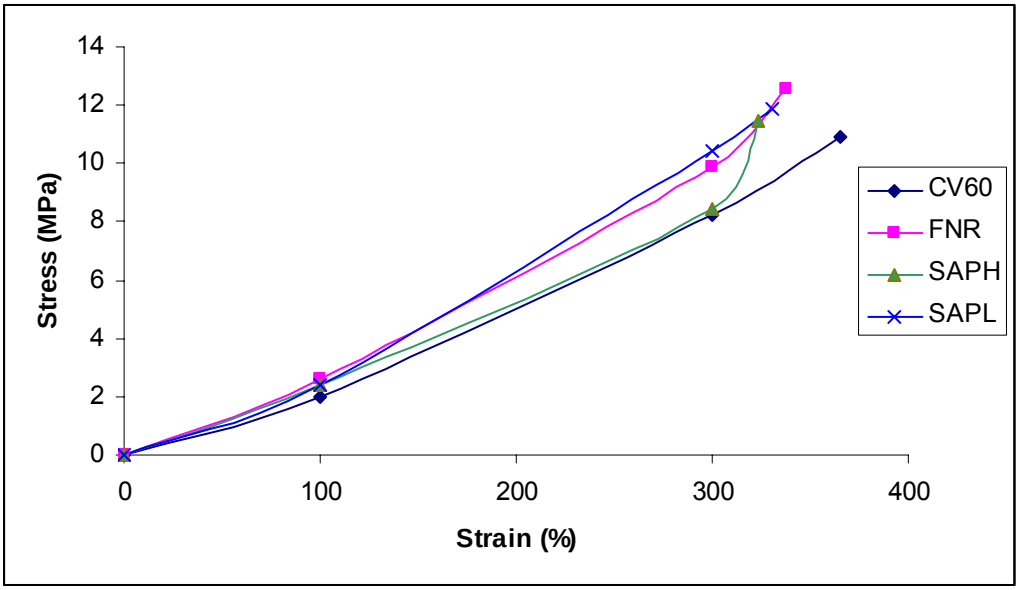

Figure 4 Stress-strain curve of rubber blend with SBR

Rubber sample was blend with SBR according to the recipe of tire for carcass. Physical and mechanical properties of rubber blend were investigated in order to concerning the possibility in application of purified rubber. Table 5 shows scorch time, cure time and tensile properties of rubber blend. Scorch time and cure time of saponified rubber blend was similar to that of FNR blend and CV60 blend. The tensile strength was nearly same in all cases. It can be explained by the high ratio of SBR in rubber blend (SBR 70 phr per NR 50 phr). Thus, scorch time, cure time and tensile properties of rubber blend mainly affected by SBR's properties.

4. Dynamic properties and abrasion resistance of rubber blend

Table 6 Dynamic properties and abrasion resistance of rubber blend

Properties	CV60	FNR	SAPH	SAPL
Storage modulus E' (MPa)	5.512	5.172	5.080	5.069
Loss of modulus E" (MPa)	0.153	0.162	0.152	0.153
Tan δ	0.034	0.031	0.030	0.030
Heat build up (^{o}C)	9.5	8.0	9.5	9.0
Dynamic compression set (%)	7.6	2.7	0.8	0
Abrasion (cm^3)	0.107	0.100	0.098	0.106

Dynamic properties and abrasion resistance of rubber blend were shown in Table 6. Storage modulus, loss modulus, Tan δ, heat build up and abrasion were not different in all cases. The difference was observed only on dynamic compression set of rubber blend. Dynamic compression set of saponified rubber blend (SAPH and SAPL) was very low, comparing with in the case of FNR and CV60. This elucidates that SAPH and SAPL have high elasticity. From above results, it can be seen that the properties of vulcanized saponified rubber is not different from control natural rubber excepting for the higher purity, the lower Mooney viscosity and the lower compression set.

CONCLUSION

Through deproteinization and saponification process, proteins in NR were decomposed, resulting in the decrease of gel content and Mooney viscosity. Fatty acids in NR were removed by saponification and soaking process in NaOH solution. DPNR and saponified NR showed a clear decrease in Mooney viscosity and small green strength. This demonstrates that the removal of non-rubber components, i.e. proteins and fatty acids, brings about good processability of NR. The removal of proteins in NR through deproteinization resulted in high scorch time and cure time while the removal of proteins and fatty acids through saponification contributed to low cure time. Not only the removal of proteins and fatty acids but the residual base in saponified rubber was also expected to responsible for the low cure time of saponified rubber. Vulcanized DPNR showed low tensile properties, low storage modulus, high heat build up and high dynamic compression set. In contrast, vulcanized saponified rubber showed high tensile properties, high storage modulus, low heat build up and low dynamic compression set. This clearly indicates that the purification of NR by saponification resulted in good physical and dynamic properties of cured rubber. Based on these findings it can be concluded that saponification of NR provides good processability, easily vulcanization and high elasticity of vulcanized rubber.

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PART 2 ORIGIN OF STRESS-STRAIN BEHAVIOR CHARACTERISTIC OF NATURAL RUBBER

The variation of the green strength of natural rubber (NR) by the preparation method of the film was studied to elucidate the origin of the stress-strain behavior characteristics of NR, in connection with the structure of branch-points in NR. The rubber film prepared by casting from NR latex showed the highest modulus and green strength compared to those by casting from toluene solution and by hot-press of dry rubber. The modulus and green strength of the NR latex casting film decreased after hot-press treatment of the film. On the other hand, no difference was observed in the case of synthetic *cis*-1,4-polyisoprene rubber (IR) by the preparation method of film and heat treatment. This stress-strain behavior characteristic of NR is ascribed to the change in branch-points of the NR film by the casting method, which may result in the difference of entanglement and crystallizability on stretching.

Keywords: Green strength, Stress-strain behavior, Natural rubber

INTRODUCTION

The green strength, the resistance to deformation of an elastomer and fracture before vulcanization, is an important property of elastomers such as processability and tackiness. Elastomers having an appropriate green strength are desirable in tire manufacture for tire production so that it will not creep and hence distort excessively before molding or tear during the expansion that occurs upon molding or in the second stage for a radial tire. The most meaningful measure for the characteristics of the green strength is obtained by examining the stress-strain curves at a given rate of strain. The shape of stress-strain curve is an important criterion for determining the green property. The green strength of elastomers has been commonly attributed to long-chain branching[1,2], interaction between polar groups,[1] the presence of gel[1,3], chain entanglement[2,4] and crystallization on stretching[3]. The difference in these factors has been responsible for the difference in green strength for various elastomers.

Natural rubber from *Hevea brasiliensis* (NR) is known to show very high green strength compared with synthetic rubbers, especially synthetic *cis*-1,4 polyisoprene (IR). This difference of green strength has been attributed to the crystallizability derived from the regularity of isoprene units in the *cis*-1,4 configuration and non-rubber constituents presented in NR. The former is presumed to be a reason why NR shows very high green strength compared with IR, while lower green strength of Guayule rubber from *Parthenium argentatum* is believed to be due to the absence of non-rubber components characteristic of NR. One of the characteristics of NR can be seen in the crystallization at low temperature. The rate of crystallization of NR at -25^{o}C was almost the same before and after deproteinization, while it decreased significantly after removing free fatty acids by acetone-extraction[5]. The rate was partly recovered after removing the linked fatty acids in the acetone-extracted sample by transesterification, which can be ascribed to the improvement of rubber purity[6]. The rate of crystallization of acetone-extracted NR was accelerated by adding 1% (w/w) methyl linoleate[7]. However, the crystallization of transesterified DPNR was suppressed by the addition of methyl linoleate as in the case of IR[8] due to

the lack of fatty acids linked to rubber molecules[7,9]. These findings demonstrated that both of the outstanding green strength and high crystallizability of NR are presumed to be derived from the linked fatty acids, which promote crystallization on stretching as well as at low temperature. A similar tendency of NR and IR was observed for crystallization on stretching. In the case of crystallization of cured rubber on stretching, it was reported that NR started crystallization at the strain of 270%, while that of cured IR was 330% by dilatometric measurement [10].

NR recovered from skim latex, i.e., the rubber from small rubber particles, shows low green strength comparable to that of IR[11]. The rubber from small rubber particles was found to be a linear polymer having no fatty acid ester group linked or associated with NR chain[12]. These findings suggest that the presence of long-chain branching in the ordinary NR is the origin of high green strength characteristic of NR. Here, it is necessary to take into consideration that the most of branch-points in ordinary NR are formed by both functional terminal groups via hydrogen bonding and/or association of phospholipid groups at the chain end, i.e., α–terminal[13,14]. Proteins in NR are considered to originate branch-points by hydrogen bonding[15]. As a proof, the gel content in ordinary NR decreases by enzymatic deproteinization of latex. However, it is clear that proteins have no direct effect on the green strength, since deproteinized NR (DPNR) shows almost the same green strength as that of NR before deproteinization[16]. Consequently, it seems reasonable to assume that the phospholipid groups in NR, which are presumed to linked at the α–terminal[13,14], act an important role to develop the green strength.

In an attempt to gain greater understanding on the origin of green properties characteristic of NR, we have conducted a series of experiments on the relationship between the structure of NR and green strength. In the previous work, we have carried out the measurement of green strength only for rubber film obtained by solution casting method[11,17]. However, we disclosed that the green strength of NR depends on the method of preparing the test sheet, i.e., casting from latex, rubber solution or hot-press of dry rubber. In general, the preparation of film by hot-press of raw rubber is usually applied for preparing the test sheet, as it can be seen in ASTM D6746-02. This standard method for preparing test sheet indicates that the sample shall be pressed at 100^{o}C for 5 min. However, in fact, this condition is not suitable for all rubbers since the viscosity and elasticity of rubber depend on the chemical structure and molecular weight. Furthermore, no information has been given on the relationship between the condition of hot press and the green strength. Therefore, in this paper, an attempt was made to show the variation of green strength by changing the preparation method of testing film in connection with the structure of branch-points of NR comparing with that of IR. Here, the rubber was prepared in three ways: casting form rubber latex, casting from rubber solution in toluene and hot-press of dry rubber. The effect of temperature and time of hot press treatment for the test film was also investigated in order to analyze the effect of presumed branch-points on the stress-strain behavior.

EXPERIMENTAL

Preparation of NR films for the measurement of stress-strain curve was carried out in three ways: (A) casting from rubber latex of 30% dry rubber content (DRC) and drying in an oven at 50^{o}C for 24 h, (B) casting from rubber solution

(~3%w/v) in toluene including 1 phr BHT, keeping at room temperature in the dark to evaporate the solvent and eliminating the residual toluene by an vacuum oven at 40^{o}C for 12 h, and (C) hot-press of solid NR. Here, the rubber film from hot-press method was made in two ways. (C-1) The rubber film obtained by latex casting was pressed in a mold, which was sandwiched with two slide polyester films, and pressed at 100-160^{o}C for 10-40 min. After heating, the mold was cooled down immediately to room temperature under pressure by cooling system for 15 min. The resulting rubber film was subjected to the measurement of stress-strain curve. (C-2) The rubber gum sandwiched with two slide polyester films was pressed in a mold and at 100-160^{o}C for 10-40 min. After pressing, the mold was cooled down immediately to room temperature under pressure for 15 min using the cooling system. In order to eliminate the residual molding strains, all the test sheets were stored at room temperature for 5 days before use.

Rubber samples

KRATON IR-309 (KRATON Polymers LLC) latex provided by SRI R & D Ltd, and NR latex provided by Thai Rubber Latex Co, Thailand were used as IR and NR latices, respectively. NR in solution was prepared by dissolving the NR film, obtained by casting the latex, in toluene solution. NR gum was prepared by coagulation of NR-latex with 5%w/v formic acid followed by washing and drying at 50 ^{o}C for 24 h.

MEASUREMENT

The test pieces for green strength measurement were stamped out using a type C dumbbell die according to ASTM D 412-87. Measurement was carried out by an Instron Model 5569 at room temperature. The testing crosshead speed of 500 mm/min was applied with load cell of 100 N. The thickness of sample was 0.5-1.5 mm. The measurement was repeated 3-4 times for each sample.

The molecular weight of NR samples was determined by size exclusion chromatography (JASCO-Borwin) using two columns in series, packed with polystyrene-divinylbenzene copolymer gel having the exclusion limits of 2.0×10^{7} and $4 \times 10^{5.}$ The rubber solution was prepared by dissolving rubber into THF (LabScan, HPLC grade) at the concentration of 0.05% w/v and filtered through a Millipore prefilter and 0.45 μm membrane filter (Alltech). THF was used as an eluent with a flow rate of 0.5 ml/min at 35 $\pm$ 0.01°C, monitoring with refractive index as a detector. Standard *cis*-1,4 polyisoprenes (Polymer Standard Service GmbH, Germany) were used for the calibration of molecular weight.

RESULTS AND DISCUSSION

Figure 1 shows the stress-strain curve of NR films prepared by latex casting and solution casting methods. Three to four test pieces of sample were measured and plotted separately. It can be seen that the toluene solution casting film, represented by solid lines, showed the stress-strain behavior lower than that of the latex casting film, represented by doted lines, while the green strength, the stress at break, of both samples was almost the same of about 6.5 MPa. This observed value is in the level of green strength reported before [6,11,17,20]. The

stress of latex casting film increased significantly at about 300% strain, while the stress of solution casting film increased significantly at about 400% strain. As it was reported before[18], the increase in the stress is attributed to the crystallization of rubber on straining. Thus, the slower onset of crystallization in the solution casting film compared with that of the latex casting film is attributed to the lower stress-strain behavior. It is reasonable to assume that the onset of crystallization on stretching is related to the number of branch-points per chain and chain entanglement. This assumption suggests that the branch-points and/or chain entanglement of the NR film prepared by latex casting are higher than that of NR film prepared by solution casting.

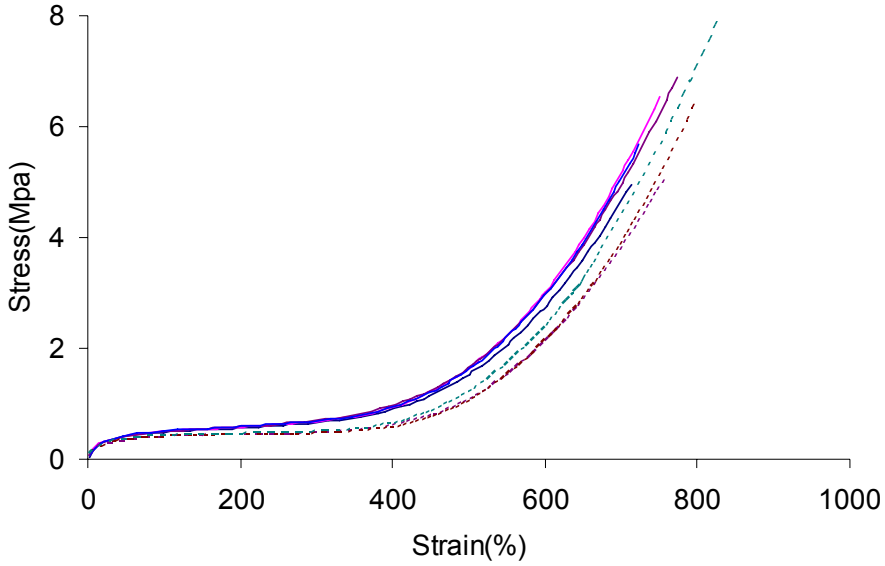

Figure 1 Stress strain curves of NR films obtained by latex casting (solid lines) and by toluene solution casting (doted lines)

In **Figure 2**, the stress-strain behavior of NR film prepared by hot-press method was compared with that of NR film by latex casting and solution casting methods, which was represented as the mean of each curve in **Figure 1.** Here, the hot-pressed film from latex casting and rubber gum were prepared by pressing rubber in the mold and sandwiched with two slides at 100^{o}C for 15 min under high pressure. In the case of NR press-gum, the green strength was very low and strain at break was also very low compared with that of hot-pressed film from latex casting. This can be explained by the high elasticity of NR gum resulting in the difficulty to prepare a uniform film with smooth surface, although this method has been usually used to prepare the film for tensile testing. It is remarkable that the hot-press latex film showed very low green strength compared with that of before hot-press treatment. This indicates that the stress-strain curve changes dramatically after hot-press treatment of NR latex film. Based on the assumption that the branch-points and entanglement affect the

stress-strain behavior, the hot-press treatment of NR latex film is presumed to bring about the structural change of rubber chains by treatment at high temperature and high pressure.

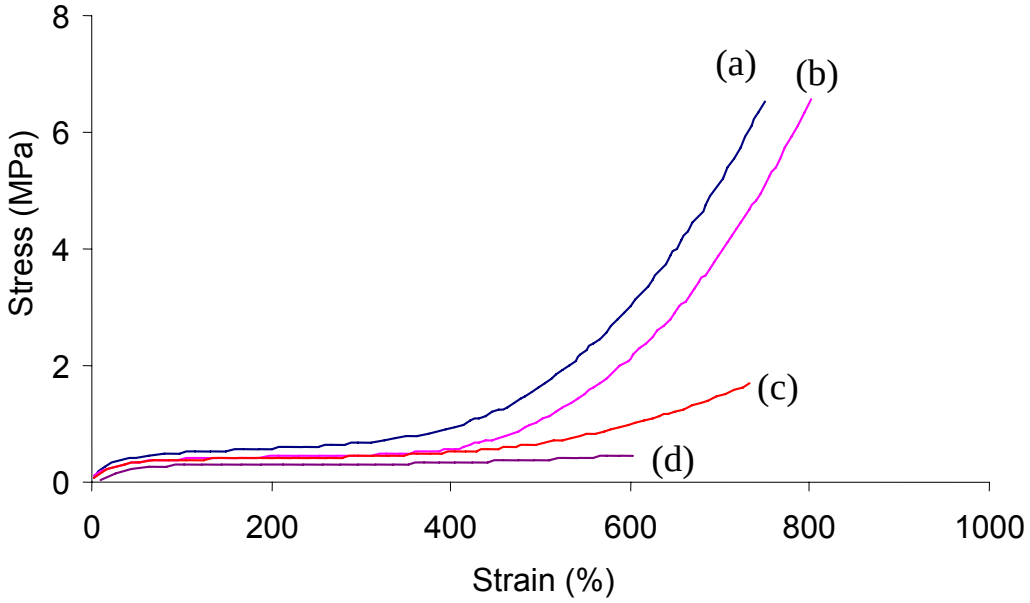

Figure 2 Stress-strain curves of NR sheet prepared by: (a) Latex casting, (b) Toluene solution casting, (c) Hot-press of latex casting film at 100°C for 15 min and (d) Hot-press of rubber gum at 100°C for 15 min

Oxidative degradations of rubber film after solution casting can be negligible by adding 1 phr BHT as an antioxidant. It was confirmed by measurement of the molecular weight by GPC of NR samples before and after casting. The molecular weight of the rubber samples in various casting methods showed almost the same value as shown in **Table 1**. This indicates that the lower stress-strain curve of NR film from toluene solution casting was not caused by the degradation of rubbers. The possibility of degradation of NR under high temperature and pressure during the hot-press treatment is also neglected, because the samples from latex casting film showed almost the same molecular weight before and after hot-press treatment.

Table 1 Molecular weight of rubber samples obtained from latex casting, toluene solution casting and hot-press at 100°C for 15 min

Sample	Casting method	M_w (x10^6)	M_n (x10^5)	Polydispersity
NR	Latex form	1.49	1.47	10.1
NR	Solution form	1.42	1.27	11.2
NR	Hot-press	1.53	1.43	10.7

The effect of temperature and time for hot-press treatment was also investigated to check the influence on the green strength and strain at break. NR latex cast film was used for this experiment, which is comparable to NR gum, in order to eliminate the effect of inhomogeneiity of film. To neglect the degradation of rubber under high temperature during hot-press treatment, an antioxidant, Wingstay-L, was added in NR latex before casting in latex form and before coagulation of NR gum. **Tables 2** and **3** show green strength and strain at break of NR latex casting film after hot-press treatment at various conditions. The green strength and strain at break of the NR latex casting film and NR gum showed no significant change after increasing the temperature and time of hot-press treatment. This means the decrease of green strength after hot-press is not related with temperature between 100 and 160^{o}C and time of hot-press for 10 to 40 min. The addition of antioxidant did not assist to increase the green strength of NR. This result confirms that the decrease of green strength was not caused by the degradation of rubber during hot-press treatment. The absence of change in green strength of both cases clearly indicates that the very low stress-strain behavior of the hot-press film is derived from the decrease of crystallizability and/or entanglement, which are caused by decrease in the branch-points.

Table 2 Green strength and strain at break of NR latex casting film after hot-press treatment at various temperatures and times

Temperature (^{o}C)	Time (min)	NR latex casting film			
		Without antioxidant		With antioxidant	
		Green strength (MPa)	Strain at break (%)	Green strength (MPa)	Strain at break (%)
100	10	1.36 ± 0.32	594 ± 64	1.08 ± 0.10	651 ± 46
100	20	1.50 ± 0.32	623 ± 48	1.07 ± 0.09	666 ± 41
100	30	1.11 ± 0.38	617 ± 75	0.89 ± 0.13	633 ± 37
100	40	1.41 ± 0.26	690 ± 51	1.08 ± 0.15	695 ± 27
120	10	1.57 ± 0.95	674 ± 22	1.02 ± 0.26	586 ± 61
140	10	1.15 ± 0.21	596 ± 68	0.85 ± 0.29	653 ± 62
160	10	0.71 ± 0.07	567 ± 67	0.48 ± 0.07	574 ± 55

Table 3 Green strength and strain at break of NR gum after hot-press treatment at various temperatures and times

Temperature (^{o}C)	Time (min)	NR gum			
		Without antioxidant		With antioxidant	
		Green strength (MPa)	Strain at break (%)	Green strength (MPa)	Strain at break (%)
100	10	0.56 ± 0.10	590 ± 78	0.63 ± 0.07	616 ± 60
100	20	0.61 ± 0.06	636 ± 31	0.55 ± 0.04	625 ± 64
100	30	0.57 ± 0.03	565 ± 53	0.50 ± 0.04	568 ± 49
100	40	0.54 ± 0.07	615 ± 39	0.51 ± 0.10	612 ± 94
120	10	0.49 ± 0.03	652 ± 56	0.44 ± 0.08	647 ± 78
140	10	0.35 ± 0.03	686 ± 42	0.35 ± 0.03	593 ± 65
160	10	0.31 ± 0.06	491 ± 117	0.28 ± 0.03	436 ± 115

This assumption can be confirmed by measurement of the stress-strain behavior for IR films prepared in a similar way as NR. As shown in **Figure 3**, the casting film from IR latex before and after hot-press treatment showed very low stress-strain behavior, which was almost the same as that observed for the solution casting film. This clearly indicates that the change of stress-strain behavior of NR by casting method and heat treatment is a phenomenon characteristic of NR. By considering the difference in structure, NR contains 100% *cis*-1,4 isoprene unit, while KRATON IR-309 was estimated to contains about 92% *cis*-1,4 isoprene unit.

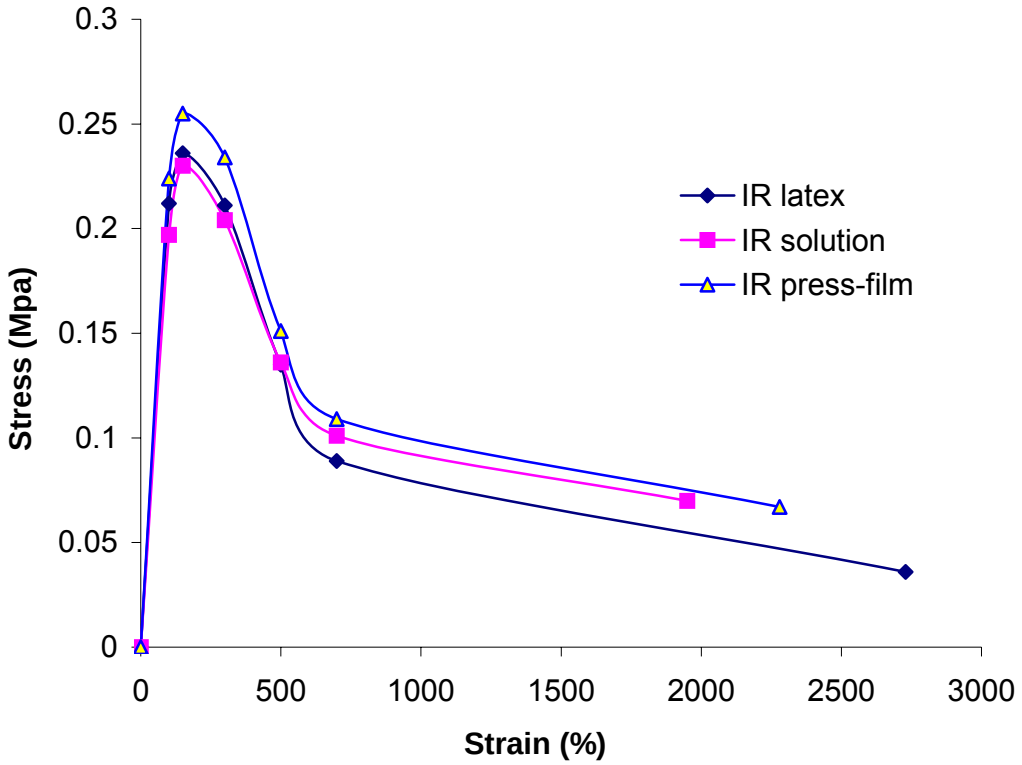

Figure 3 Stress-strain curves of IR film prepared by latex casting, toluene solution casting and hot-press of latex casting film at 100°C for 15 min

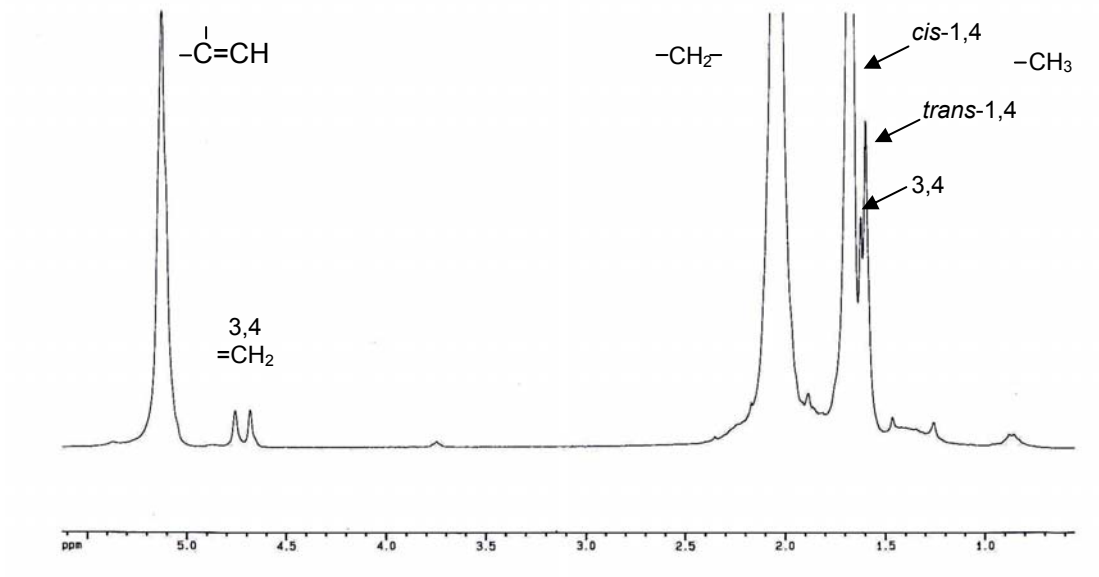

Figure 4 ^{1}H-NMR of synthetic polyisoprene, KRATON IR 309

As shown in **Figure 4**, ^{1}H-NMR spectra of KRATON IR-309 exhibited the small signals resonated at 1.60, 1.62 and 4.75 ppm, which are assignable to –CH_3 of *trans*-1,4 unit, –CH_3 of 3,4 unit and =CH_2 of 3,4 unit, respectively. The irregularity in structure of KRATON IR-309 will contribute to lower the crystallizability compared with NR. Accordingly, no difference in the stress-strain behavior observed in the latex casting film of IR is attributed to the absence of crystallizability and low entanglement due to no long-chain branching, considering the polymerization with anionic initiator for KRATON IR-309. This assumption is supported by very low stress-strain behavior of transesterified DPNR, comparable to that of IR[17]. Transesterified DPNR is confirmed to be a linear NR decomposed almost all the branch-points by treatment with protease followed by treatment with sodium methoxide to decompose the ester linkages, which results in the decomposition of branch-points composed of lipids and phospholipids.

We have disclosed that NR is composed of long-chain branched molecules, the branch-points of which are composed of both terminal groups of rubber chain[15]. It has been reported that the □–terminal group of rubber chain in fresh NR latex consists of mono- and di-phosphate groups linked with phospholipids.[13,14] The association or micelle formation of phospholipids was presumed to form long-chain branching as illustrated in **Figure 5.** Here, the branch-points are postulated to be derived from the micelle formation of phospholipids attached to the α–terminal group of rubber chain. It is well known that phospholipids form a micelle in aqueous media. Rubber particle in latex was reported to be covered by a bi-layer composed of lipids at the inside and proteins at the outside[19]. The polar terminal groups in rubber molecules are assumed to be on the surface of rubber particles in latex as illustrated in **Figure 6**. The α–terminal groups may associate together on the surface of rubber particles to form branch-points. The branched structure of rubber molecules in latex can be held even after casting and drying to form a film. On the other hand, the branch-points composed of phospholipids can form a reversed micelle in toluene solution, which is expected to less effective to form branch-points by considering the concentration of rubber in toluene solution. It is reasonable to consider that the rubber film obtained by casting from toluene solution show lower stress-strain curve due to the reduction of long-chain branching. The high temperature and high pressure in hot-press method will result in the conformational change of long-chain fatty acid chains in phospholipids leading to the low effective to form the long-chain branch-points or associated phospholipids. This will result in the decrease of number of long-chain branch-points. Moreover, in this experiment the rubber after hot-press was cooled immediately under pressure. So, the conformational change of long-chain fatty acid was fixed and cannot be rearranged to recover the original branch-points. This behavior resulted in the decrease in green strength of rubber film from latex casting after hot-press.

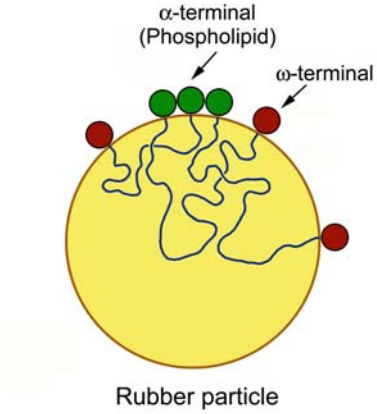

Figure 5 Presumed structure of branch-points of rubber chain in latex

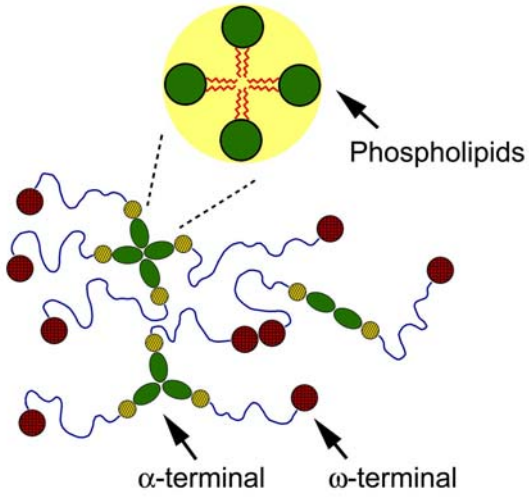

Figure 6 Presumed location of both terminal groups in rubber particle

CONCLUSION

The latex casting film of NR showed the highest stress-strain behavior comparing with that by solution casting and by hot-press, whereas no difference was observed by preparation method of testing film in synthetic IR. It is remarkable that hot-press treatment of the latex film brings about very low green strength. It was confirmed that oxidative degradation of rubber was not participated in the change of stress-strain curve by the addition of antioxidant and analysis of molecular weight. No significant effect of temperature was observed after hot-press at 100-160^{o}C for 10-40 min on the green strength of NR. This indicates that a mild heat treatment results in the decrease of stress-strain behavior. These findings support the assumption that branch-points composed of mainly phospholipids decrease by heat treatment of latex casting film and by casting

from toluene solution, which will result in the decrease of entanglement of rubber chains and crystallizability on stretching and contribute to lower the stress-strain behavior.

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PART 3 STRAIN-INDUCED CRYSTALLIZATION OF NATURAL RUBBER: EFFECT OF PROTEINS AND PHOSPHOLIPIDS

Strain-induced crystallization of natural rubber (NR) during uni-axial deformation was studied in real time by synchrotron wide angle X-ray diffraction (WAXD) technique. Strain-induced crystallization of NR was investigated together with the effect of proteins and phospholipids on stress-strain behavior. Proteins and phospholipids in NR were decomposed by deproteinization and lipase treatment, respectively. The naturally occurring network, formed by proteins and phospholipids, was found to be responsible for high stress-strain behavior and crystallizability of un-vulcanized NR on stretching. The effect of naturally occurring network in vulcanized natural rubber was also investigated. It was found that the naturally occurring network plays a role even in vulcanized rubber.

Keywords: Strain-induced crystallization; Natural rubber, Naturally occurring network, X-ray

INTRODUCTION

Natural rubber (NR) from *Hevea brasiliensis* is the most practically representative of cis-1,4-polyisoprene accounting for more than 99% of the world's natural source of rubber. This is due to the high productivity of the plant and excellent properties such as high tensile and tear strength, good crack growth resistance and minimal heat build up, comparable to synthetic polyisoprene (IR). Even though IR consists of the isoprene units in the cis-1,4 configuration higher than 98%, which is polymerized with a Ti/Al catalyst , its mechanical properties are poorer than NR. Besides a small amount of other isoprene configurations, the differences in mechanical properties between NR and IR have been presumed to be related with 6%w/w of non-rubber components, essentially proteins and phospholipids, which contribute to the formation of network structure.

As reported previously [1], a linear rubber chain in NR is proposed to consist of *ω*-terminal, two *trans*-1,4 isoprene units, about 1,000-3,000 *cis*-1,4 isoprene units, and *α*-terminal. The *α*-terminal is composed of mono- or di-phosphate group linked with phospholipids [2,3], which play a role in branching formation of the rubber molecules [4]. On the other hand, the *ω*-terminal of rubber molecule in NR was postulated to be a modified dimethylallyl group linked with a functional group, which is associated with proteins to form crosslinks through intermolecular hydrogen bonding formation [5]. The proposed structure of a linear rubber chain and naturally occurring network in NR are shown in Figure 1. Here, the non-rubber components composed of mostly proteins and phospholipids are presumed to be major constituents to form branch-points by interaction with rubber chains at both chain-ends. These components with polar functional groups are immiscible with rubber chains and expected to form a network structure.

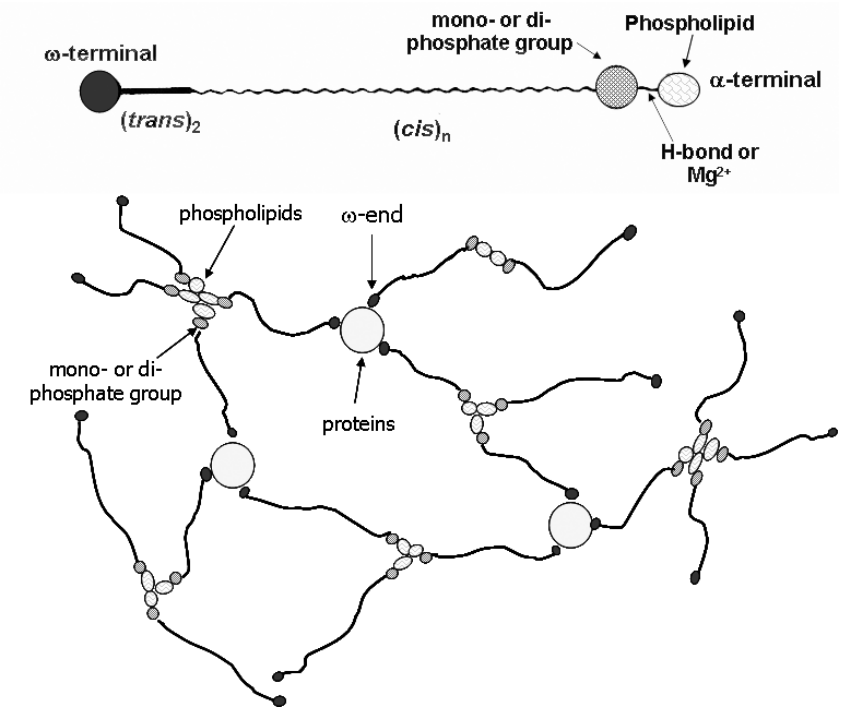

Figure 1 Proposed structure of linear rubber chain and naturally occurring network in NR

The effect of proteins and phospholipids on the tensile strength of un-vulcanized NR was investigated in the previous work [6-8]. The removal of proteins in NR showed no change in the tensile strength, whereas it increased strain at break [6]. The decomposition of branching points through transesterification decreases the tensile strength of NR to very low level comparable to that of IR [7,8], since the transesterification process decomposes the branching points containing ester linkages of phospholipids and phosphate group, thus render NR into the linear rubber chains. Although the effect of proteins and phospholipids on tensile strength of NR has been studied, little information has been provided on the relation with strain-induced crystallization.

Since strain-induced crystallization has been believed to cause the outstanding tensile strength and good crack growth resistance of NR, many attempts have been performed to investigate the strain-induced crystallization of NR in vulcanized [9-24] and un-vulcanized state [25-33]. Molecular orientation and strain-induced crystallization of vulcanized NR have been studied by the use of many techniques such as ^{2}H NMR [9,10], infra-red (IR) absorption [11], birefringence [12,13] and wide angle X-ray diffraction (WAXD) [14-24] techniques. However, the change in molecular orientation of un-vulcanized NR has not been clarified yet. Therefore, the purpose of this work is to demonstrate the change in molecular orientation (orientated amorphous and crystalline parts) during deformation of un-vulcanized NR in relation with the effect of proteins and phospholipids in the rubber. Furthermore, the effect of both components on molecular orientation during deformation of vulcanized NR was also investigated.

EXPERIMENTAL

Sample preparation

NR latex used in this study was from regularly tapped *Hevea* tree of RRIM 600 clone, provided by Thai Rubber Latex Co, Thailand. NR latex was cast on plate and dried in an oven at 50^{o}C for 24 h.

Deproteinized NR (DPNR) was prepared by incubation of NR latex with 0.04 %w/v proteolytic enzyme (KAO KP-3939) and 1 %w/v Triton-X for 12 h at 37^{o}C followed by centrifugation twice.

Lipase-treated DPNR (L-DPNR) was prepared in order to remove neutral lipids and polar lipids, including phospholipids and glycolipids in NR [2]. The DPNR-latex (10% dry rubber content) was mixed with lipase from microorganism (*Candida rugosa,* Sigma) at 37ºC with pH 7.2 and incubated at 37°C from 48 hr at the lipase: DPNR concentration ratio of 5:1. The rubber fraction was recovered by centrifugation at 13,000 rpm for 30 min. Then, the cream fraction was re-dispersed in 0.5% w/v Triton X solution and treated with 0.1%w/v proteolytic enzyme for 12 h at 37^{o}C in order to eliminate the residual lipase. The cream fraction was recovered again by centrifugation at 13,000 rpm for 30 min.

L-DPNR latex was used as a starting material for the re-addition of proteins and phospholipids. Proteins were extracted from fresh NR latex by washing with surfactant. Fresh NR latex was dispersed with 1% Triton-X, followed by centrifugation at 19,000 rpm for 30 min to separate serum from rubber fraction. The serum fraction was added with cold acetone and kept it at 4^{o}C for 12 h in order to precipitate proteins in the serum. The precipitated proteins was collected and re-dispersed in 0.5% Triton-X solution. Then, it was added into L-DPNR latex in the same ratio of original latex. Phosphatidyl choline (1 phr) was used in this study as a representative of phospholipids in NR.

Transesterified NR (TENR) was prepared by the reaction of NR with freshly prepared sodium methoxide in toluene solution at room temperature for 3 h, followed by precipitation with excess amount of methanol [7].

NR, DPNR and L-DPNR was mixed with 2 phr dicumyl peroxide (DCP) in 4%w/v chloroform solution. Then, the rubber solution was cast and dried at room temperature to be 1 mm thickness film. The received film was vulcanized at 150^{o}C for 30 min.

Characterization

The nitrogen content was analyzed by a Leco Nitrogen Analyzer (model FP 528) with the sensitivity of 0.001%. The quantity of long chain fatty acid ester group was determined by FTIR measurement based upon the calibration curve prepared by using a mixture of methyl stearate and synthetic *cis*-1,4-polyisoprene, Kuraprene IR10. The gel content was determined by toluene solubility measurement.

Network chain density (υ) of vulcanized samples was estimated according to followed equation based on the classical theory of rubber elasticity [34].

$$\sigma = \upsilon kT(\alpha - \alpha^{-2})$$

Where σ is the force per unit area, υ is the number of network chain in unit volume, k is the Boltzmann constant, T is the absolute temperature, and α is the elongation ratio. A plot of σ versus $(\alpha - \alpha^{-2})$ gives a straight line and the value of

υ is calculated from the initial slope. The crosslink density of vulcanized rubbers was shown in Table 1.

Table 1 Crosslink density of peroxide vulcanized rubber

Vulcanized rubber	Crosslink density x 10^4 (mol/cm^3)
V-NR	0.97
V-DPNR	0.80
V-LDPNR	0.66

Tensile and synchrotron WAXD measurement

Strain-induced crystallization was observed by Synchrotron X-ray measurement at the X27C beam line in the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory (BNL). The wavelength used for measurement was 0.1371 nm. The WAXD patterns were recorded by a MAR-CCD X-ray detector. Exposure time for each image was 30 sec. The tensile machine for X-ray measurement was a tabletop stretching machine from the Instron Company, which was modified specifically for X-ray study in a symmetric deformation mode. This apparatus was allowed the X-ray to illuminate the same sample position during straining. The sample was stretched at the speed of 10 mm/min. The original sample length was 30mm. The strain is calculated by (l-lo)/lo where l is the length of the sample during stretching and lo is the original length. The stress was calculated by F/Ao, where F is a measured force and Ao is the original cress section of the sample. The experiment was carried out at 25^{o}C.

The WAXD pattern was regarded to the contribution of isotropic, orientated amorphous and crystalline components. The following process was used in this study to evaluate isotropic, oriented amorphous and crystalline part of each WAXD pattern. That is, after subtraction of background scattering and normalization, the isotropic amorphous component was subtracted referring to the WAXD pattern of un-stretched sample. The rest that contains the oriented amorphous and crystalline components was integrated for all angles. Then, the peak fitting program was used to decompose into the orientated amorphous and crystalline components. The calculation of % anisotropic, % orientated amorphous and % crystalline part is described as follows:

$$\% \text{ Crystalline part} = \frac{\text{Total area of crystalline peaks x 100}}{\text{Total area of all components before subtraction}}$$

$$\% \text{ Orientated amorphous part} = \frac{\text{Total area of orientation peaks x 100}}{\text{Total area of all components before subtraction}}$$

$$\% \text{ Anisotropic part} = \% \text{ Crystalline part} + \% \text{ Orientated amorphous part}$$

RESULTS AND DISCUSSION

It is well known that un-vulcanized NR shows upturn in stress during stretching but un-vulcanized IR does not, since un-vulcanized NR has naturally occurring network and un-vulcanized IR does not have any network. Proteins and phospholipids contribute to the formation of naturally occurring network in un-vulcanized NR. Thus, the role of proteins and phospholipids was investigated in the present study by decomposition of these components in NR by deproteinization and lipase treatment, respectively. As shown in Table 1, the nitrogen content of NR, represent the amount of proteins, decreased to almost 0% after deproteinization. The effect of lipase treatment for DPNR was confirmed by the analysis of long-chain fatty acid ester content decreasing from 25.6 to 16.2 mmol/kg rubber. This indicates that some neutral lipids and polar lipids, including phospholipids, were removed. It should be note that the efficiency of lipase may be not enough to decompose all of lipids. On the other hand, transesterification decompose and remove almost proteins and lipids. Since proteins and phospholipids are associated with the formation of naturally occurring network in raw NR, the decomposition of proteins and/or phospholipids is expected to contribute the deconstruction of network structure as evaluated by the decrease of gel content.

Table 2 Characterization of un-vulcanized rubbers

Sample	Nitrogen content (%w/w)	Ester content (mmol/kg-rubber)	Gel content (%w/w)
NR	0.75	25.6	23.9
DPNR	0.01	25.8	5.15
L-DPNR	0.01	16.2	0
TENR	0.02	0	0

Stress-strain curves of un-vulcanized NR, DPNR, L-DPNR and TENR are shown in Figure 2. The stress of NR increased significantly at the strain of about 3.0, while the stress of DPNR and L-DPNR increased significantly at strain of about 4.0 and 6.0, respectively. The decomposition of proteins and phospholipids may reduce the network density and increase the strain that stress increase. It is clear that TENR cannot be extended more than strain of 4.0 and did not show the increase in stress during stretching. This is ascribed to the absence of network structure in TENR. NR showed higher stress than DPNR and L-DPNR for all range of deformation. The removal of proteins and phospholipids may destroy the naturally occurring network, resulting in the decrease of tensile properties. During retraction, NR, DPNR and L-DPNR recovered and showed clear hysteresis with permanent sets 130%, 150% and 170%, respectively. The decomposition of proteins and phospholipids may not only decrease their modulus but also increase their permanent set because of the destruction of network points.

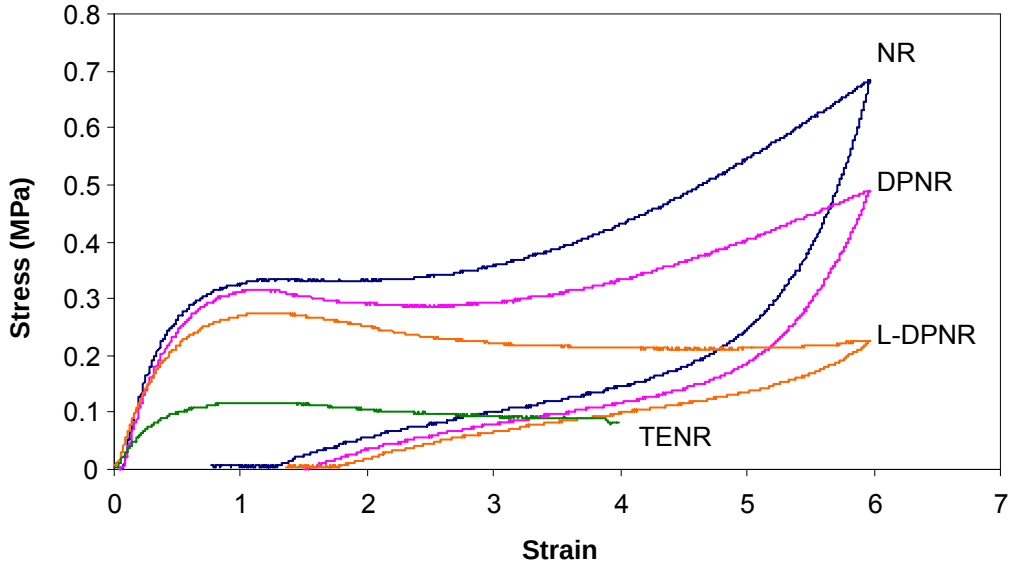

Figure 2 Stress-strain of raw NR, DPNR, L-DPNR and TENR during stretching and retraction

The WAXD patterns collected at strain of 6.0 for NR, DPNR, L-DPNR samples and at strain of 4.0 for TENR sample are shown in Figure 3. The stretched NR, DPNR and L-DPNR showed 120, 200 and 201 crystalline reflections with an isotropic scattering halo. However, the stretched TENR showed only an isotropic scattering halo from random amorphous chains. The peculiar characteristic of TENR is ascribed to the decomposition of naturally occurring network through transesterification.

The changes in the anisotropic part during extension and retraction of NR, DPNR and L-DPNR are shown in Figure 4. The anisotropic part consists of orientated amorphous and crystalline part. The anisotropic part was detected at strain of about 2.0 for NR and DPNR, while at about 3.0 for L-DPNR. All samples showed the increase of anisotropic part during stretching. It is interesting to note that the majority of chains (>90%) remain in the un-orientated amorphous state even at a high strain. The increase in anisotropic part of NR was faster than that of DPNR and L-DPNR. At a given strain, NR also showed higher anisotropic part than DPNR and L-DPNR did. This indicates that proteins and phospholipids, existing in NR, play a role on the molecular orientation during deformation.

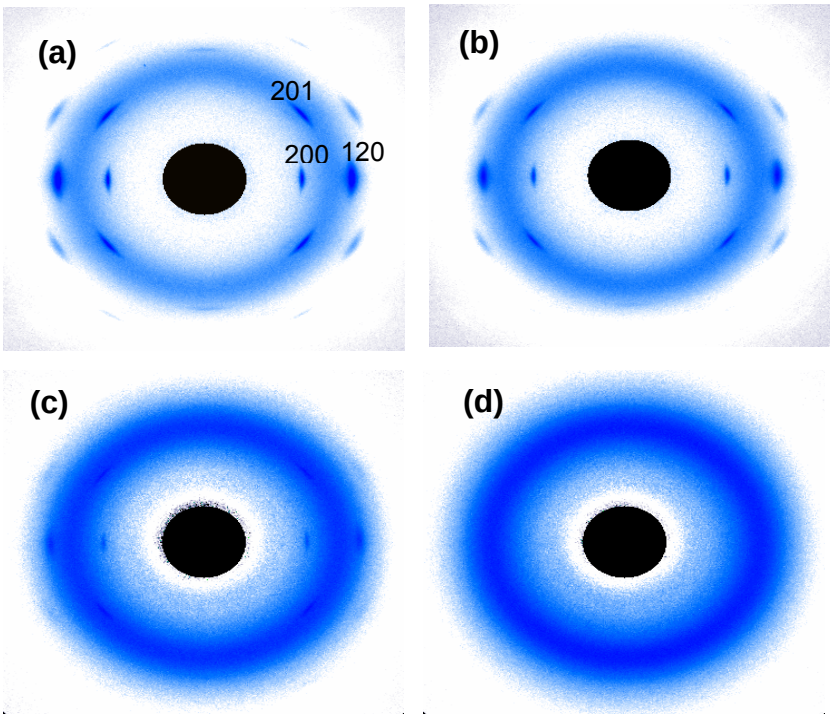

Figure 3 WAXD patterns of (a) NR, (b) DPNR and (c) L-DPNR collected at strain of 6 and (d) TENR collected at strain of 3.8

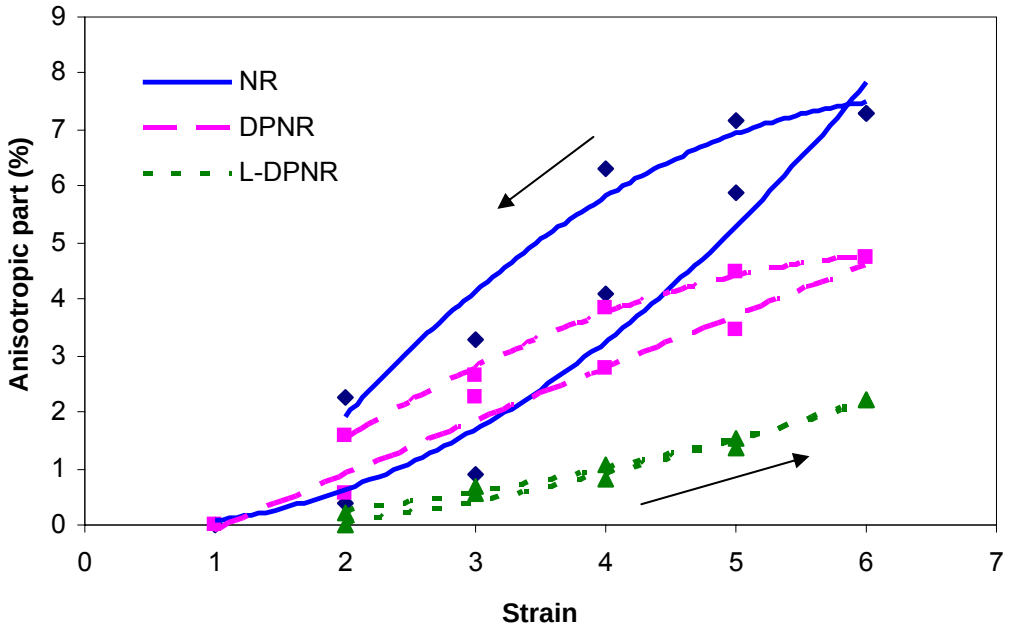

Figure 4 Change in anisotropic part of NR, DPNR and L-DPNR during extension and retraction

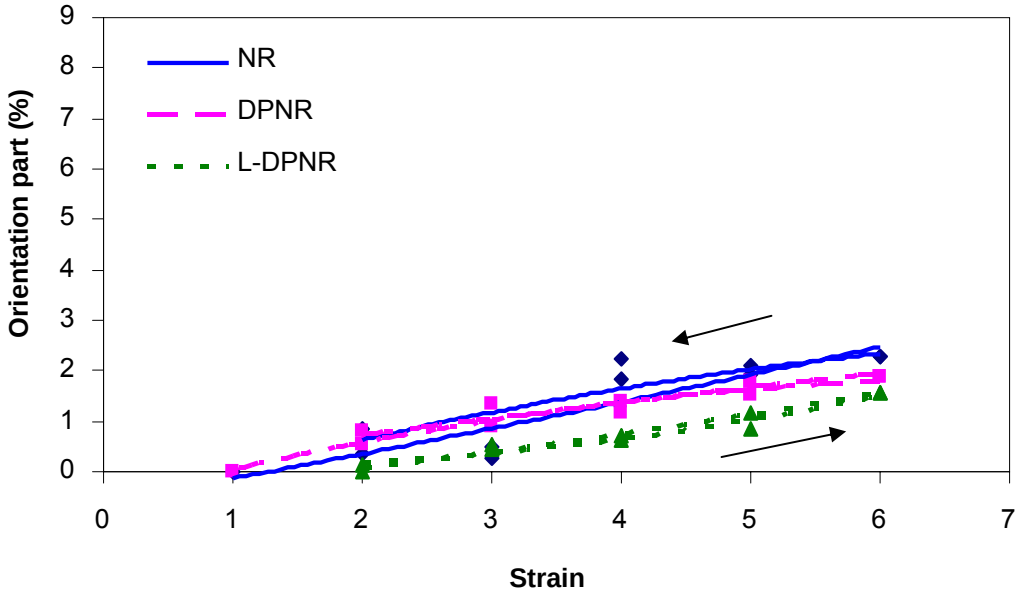

Figure 5 Change in orientated amorphous part of NR, DPNR and L-DPNR during extension and retraction

Figure 5 shows the changes in orientated amorphous part in NR, DPNR and L-DPNR during extension and retraction. The orientated amorphous part was observed at strain of about 2.0 for NR and DPNR, while it was about 3.0 for L-DPNR. The fraction of orientated amorphous molecules in un-vulcanized rubber is lower than 5%. This value is almost similar to that of vulcanized NR, which was reported before [18,19].

The changes in the crystalline part of rubber during extension and retraction are shown in Figure 6. The crystalline part appeared at strain of about 3.0 for NR and DPNR but at strain of about 4.0 for L-DPNR. The fraction of crystalline part in un-vulcanized rubber was much lower than that in vulcanized rubber. The maximum fraction of crystalline part of vulcanized NR is about 20% [18,19], while in un-vulcanized NR, it is only about 5%. At a given strain, the fraction of strain-induced crystal was suppressed after the removal of proteins through deproteinization. Furthermore, the removal of proteins and phospholipids by deproteinization and lipase treatment resulted in very low strain-induced crystal as well as slower rate of crystallization. This finding suggests that proteins and phospholipids is an important factor for high tensile strength and the crystallizability under uni-axial deformation of un-vulcanized NR.

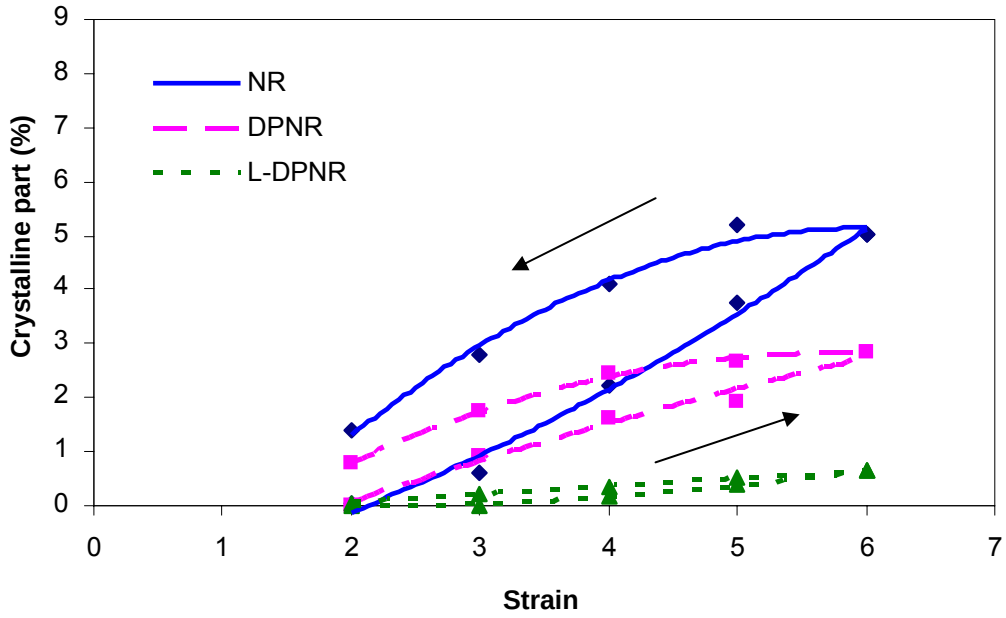

Figure 6 Change in crystalline part of NR, DPNR and L-DPNR during extension and retraction

To clarify the effect of proteins and phospholipids, the re-addition of proteins and phospholipids into L-DPNR was carried out. Proteins extracted from fresh NR latex by washing with surfactant were added into L-DPNR latex in the same ratio of original latex. Phosphatidyl choline (1 phr) was used in this study as a representative of phospholipids in NR. The stress-strain curves of L-DPNR after the addition of extracted proteins and/or phosphatidyl choline are shown in Figure 7. The addition of extracted proteins and/or phosphatidyl choline into L-DPNR did not improve the tensile properties. Furthermore, the addition of proteins resulted in the reduction of its tensile strength. This suggests that high tensile strength of NR is not directly caused by proteins and/or phospholipids but it relates with the characteristic structure of NR, which is originated from the bonding of proteins and phospholipids with the terminal unit of rubber chains. However, it should be considered that the simple addition of extracted proteins and phosphatidyl choline may be not efficient way to re-construct the naturally occurring network in NR.

The WAXD patterns of L-DPNR after the addition of extracted proteins and/or phosphatidyl choline at break point are shown in Figure 8. The crystalline reflection in WAXD pattern of L-DPNR, added with proteins and/or phosphatidyl choline showed lower intensity than that in the original L-DPNR. This implies that the addition of extracted proteins and/or phosphatidyl choline decrease the crystallizability of rubber chains.

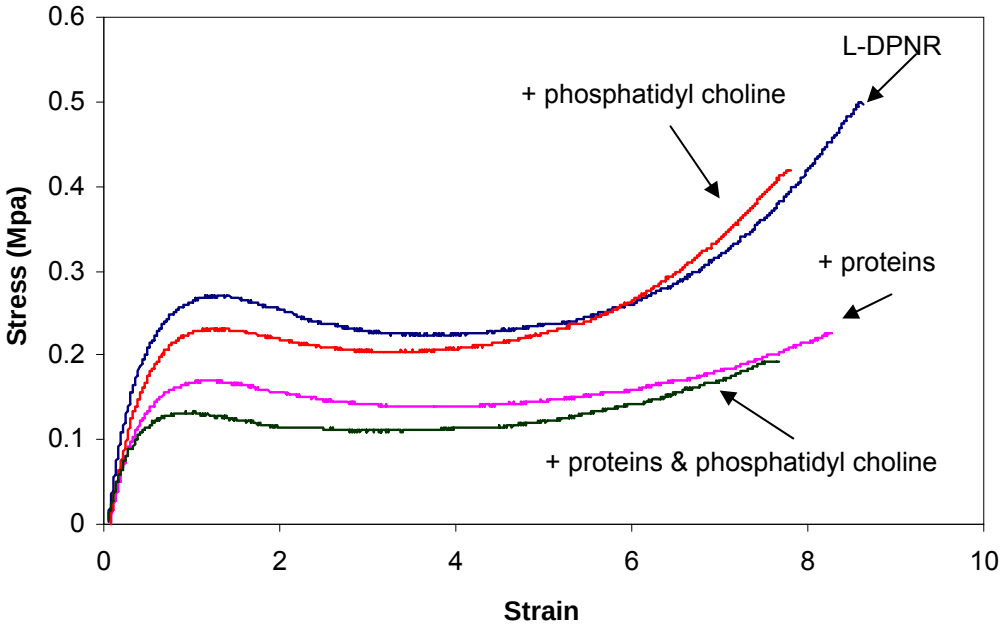

Figure 7 Stress-strain curves of L-DPNR adding with extracted proteins and/or phosphatidyl choline

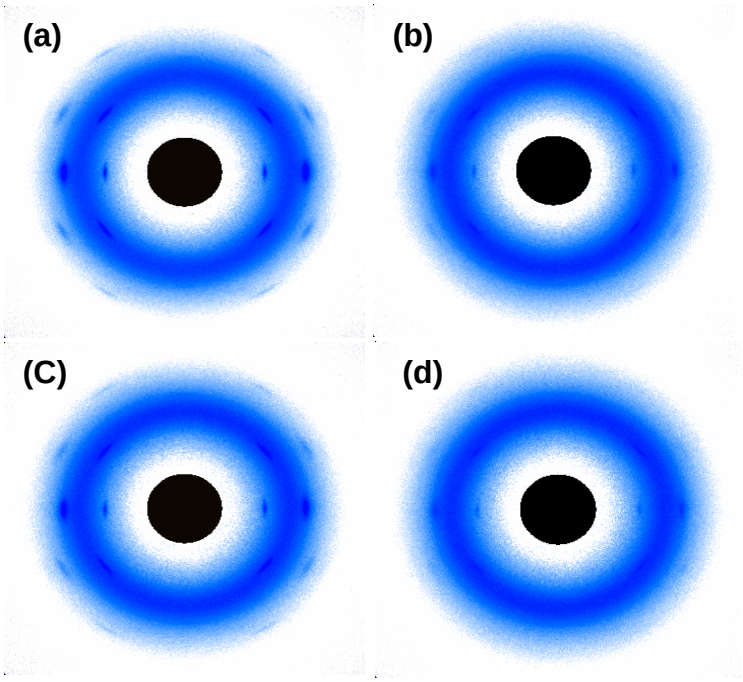

Figure 8 WAXD patterns of (a) L-DPNR, (b) L-DPNR adding extracted proteins, (c) L-DPNR adding phosphatidyl choline and (d) L-DPNR adding extracted proteins and phosphatidyl choline collected at break point

The changes of crystalline part of L-DPNR after the addition of proteins and/or phosphatidyl choline are shown in Figure 9. The strain-induced crystallite of L-DPNR appeared at strain of 4.0, whereas L-DPNR added with proteins and/or phosphatidyl choline showed at strain of 5.0. The mass fraction of crystalline part and the rate of crystallization of L-DPNR decreased after the

addition of extracted proteins and/or phosphatidyl choline. This indicates that the addition of extracted proteins and/or phosphatidyl choline suppressed the crystallization of rubber on stretching. Considering the decrease in crystallizability of NR after the removal of proteins and phospholipids through deproteinization and lipase treatment, proteins and phospholipids should be an accelerating factor for strain-induced crystallization. However, the re-addition of extracted proteins and/or phosphatidyl choline into L-DPNR showed no improvement of stain-induced crystallization. This suggests that proteins and phospholipids themselves do not assist the strain-induced crystallization of NR directly but the formation of naturally occurring network with proteins and phospholipids at terminal unit of rubber chains induces crystallization.

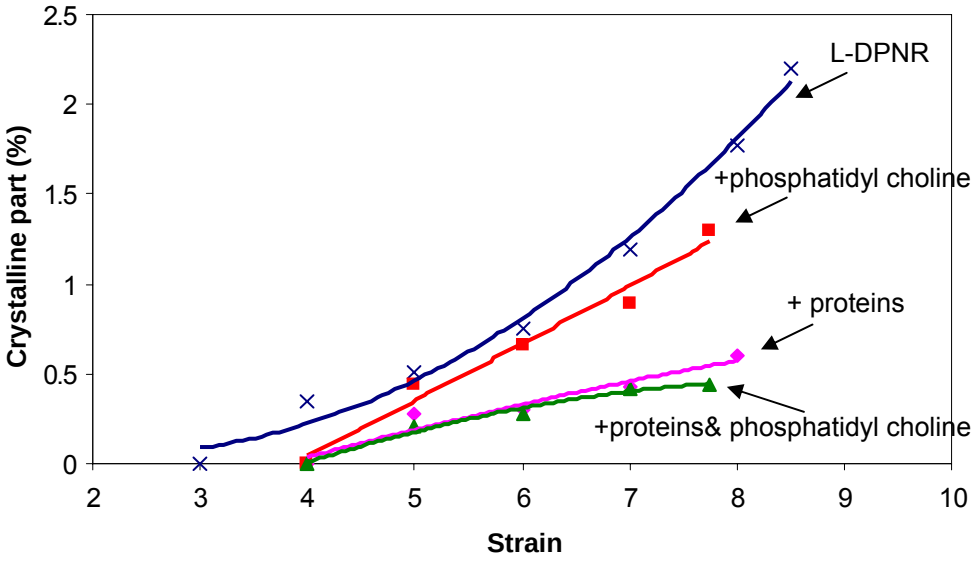

Figure 9 Change in crystalline part of L-DPNR adding with extracted proteins and/or phosphatidyl choline

We also made an attempt to clarify the effect of proteins and phospholipids on strain-induced crystallization of rubbers in vulcanized state. To maintain the naturally occurring network, formed by proteins and phospholipids in raw rubber, we avoided the usage of internal mixer and two-roll mill. Thus, we applied peroxide vulcanization in chloroform solution. Using this process, the crosslink density of rubber is very low as shown in Table 1.

The stress-strain curves of vulcanized NR, DPNR and L-DPNR are shown in Figure 10. Vulcanized NR showed higher stress than that of vulcanized DPNR and vulcanized L-DPNR at all strain. This indicates that the naturally occurring network, formed by proteins and phospholipids, assists to increase the tensile strength of vulcanized rubbers. During retraction, all vulcanized rubbers recovered and showed hysteresis with permanent sets 100%.

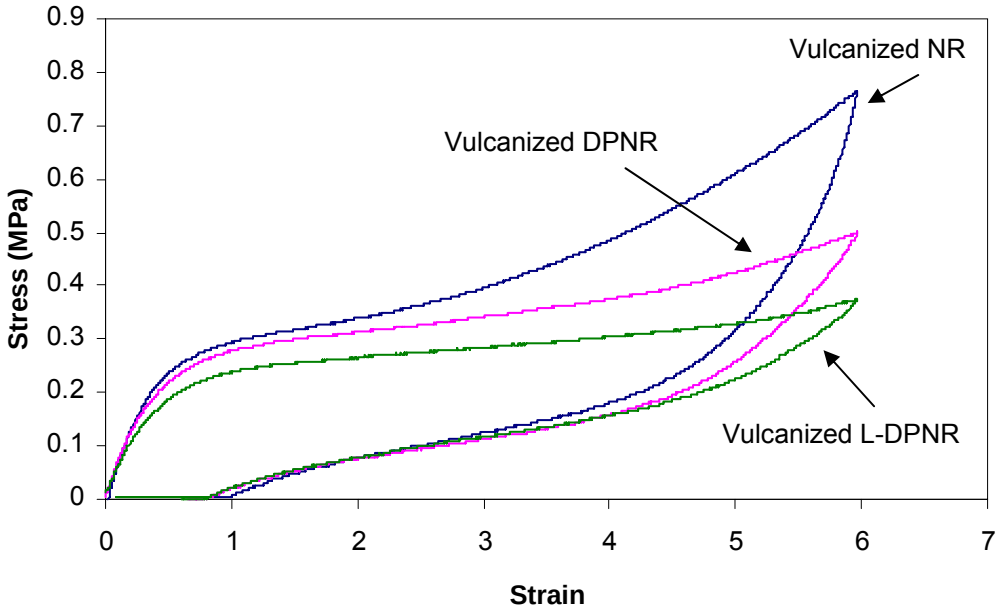

Figure 10 Stress-strain curves of vulcanized NR, DPNR and L-DPNR during extension and retraction

The WAXD patterns of vulcanized NR, vulcanized DPNR and vulcanized L-DPNR samples, collected at strain of 6.0 are shown in Figure 11. All stretched vulcanized rubbers showed 120, 200 and 201 crystalline reflections with an isotropic scattering halo, the same pattern of un-vulcanized rubbers. It is observed that the intensity of crystalline reflection of all samples was almost the same. This may be due to peroxide crosslinking network predominantly affects the strain-induced crystallization of vulcanized rubber.

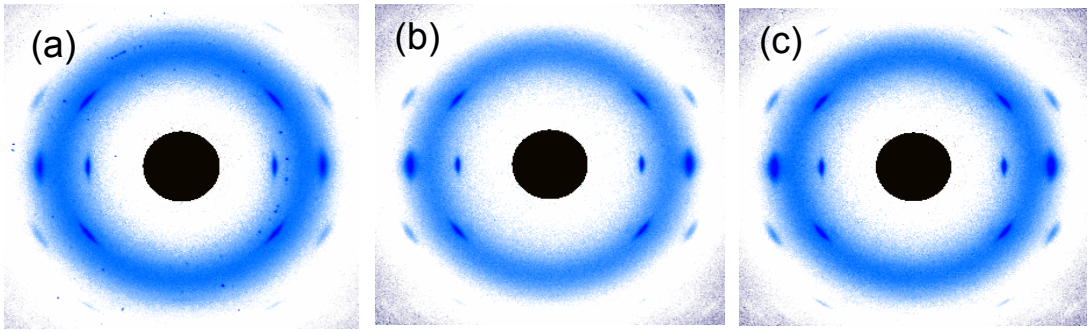

Figure 11 WAXD patterns of (a) vulcanized NR, (b) vulcanized DPNR and (c) vulcanized L-DPNR at strain of 6

The changes in the anisotropic part of vulcanized rubbers during extension and retraction are shown in Figure 12. The anisotropic part was detected at strain of about 2.0 and increased during stretching for all samples. The anisotropic part of vulcanized NR was a little bit higher than that of vulcanized DPNR and vulcanized L-DPNR. However, the maximum anisotropic part of all vulcanized rubbers was almost same.

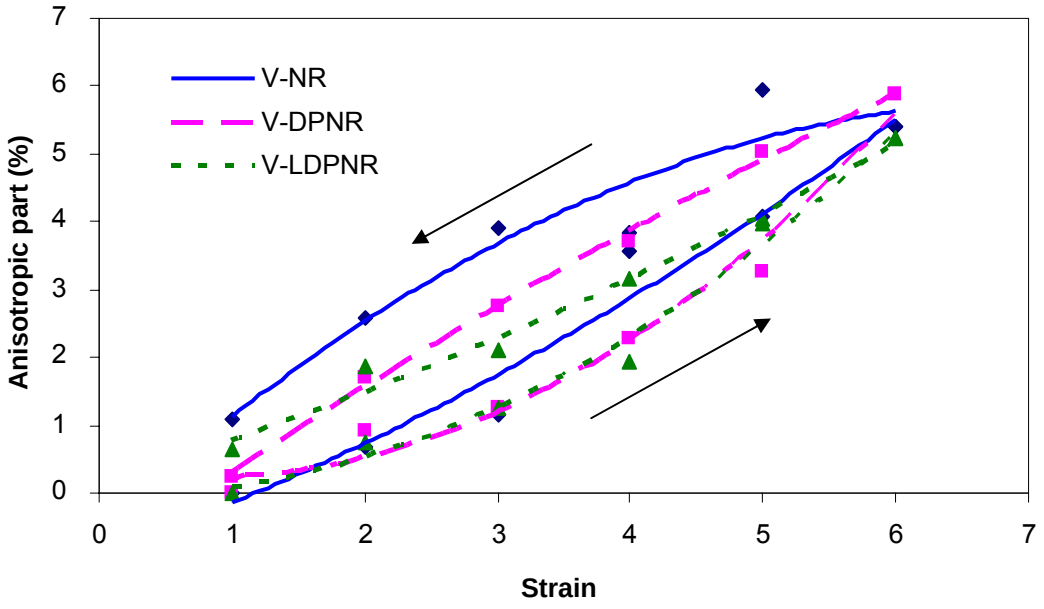

Figure 12 Change in anisotropic part of vulcanized NR, vulcanized DPNR and vulcanized L-DPNR during extension and retraction

Figure 13 shows the change in orientated amorphous part in vulcanized rubbers during extension and retraction. The orientated amorphous part of all vulcanized rubbers was observed at strain of about 2.0 and the maximum was lower than 3%. The changes in the crystalline part of rubbers during extension and retraction are shown in Figure 14. The strain-induced crystallization appeared at strain of about 3.0 for vulcanized NR and vulcanized DPNR, while at strain of about 4.0 for vulcanized L-DPNR. It is clear that the strain at onset of crystallization of NR, DPNR and L-DPNR was unaltered after vulcanization. L-DPNR showed slower appearance of strain-induced crystallite than NR and DPNR in un-vulcanized and vulcanized state. At a given strain, the fraction of crystalline part of vulcanized NR was a little bit higher even through the maximum fraction of crystalline part of all vulcanized samples was almost the same. This implies that naturally occurring network, formed by proteins and phospholipids, plays a role on strain-induced crystallization even in vulcanized state.

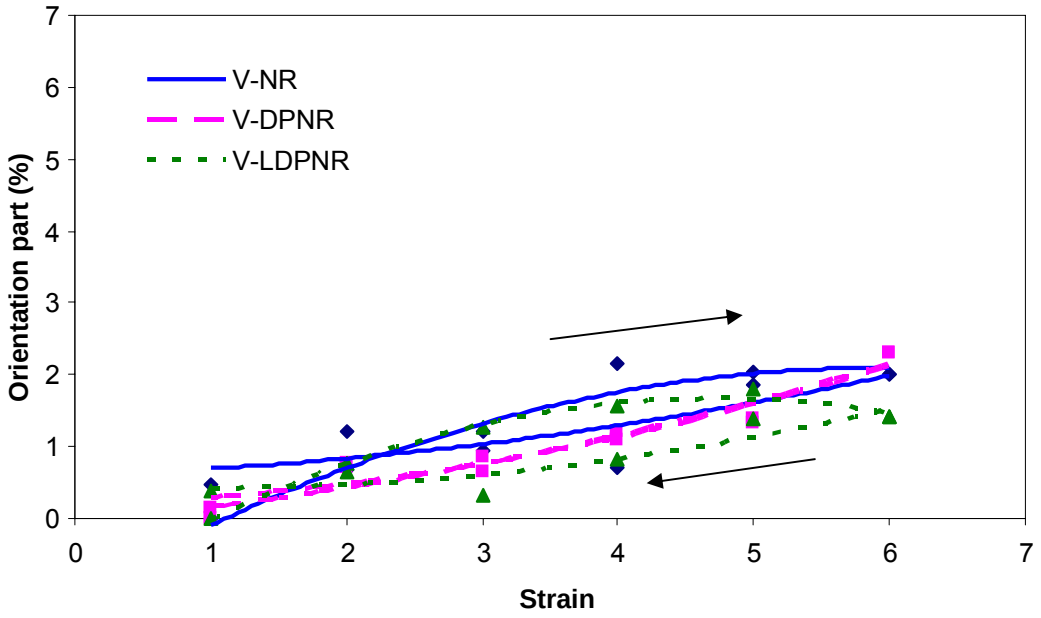

Figure 13 Change in orientated amorphous part of vulcanized NR, vulcanized DPNR and vulcanized L-DPNR during extension and retraction

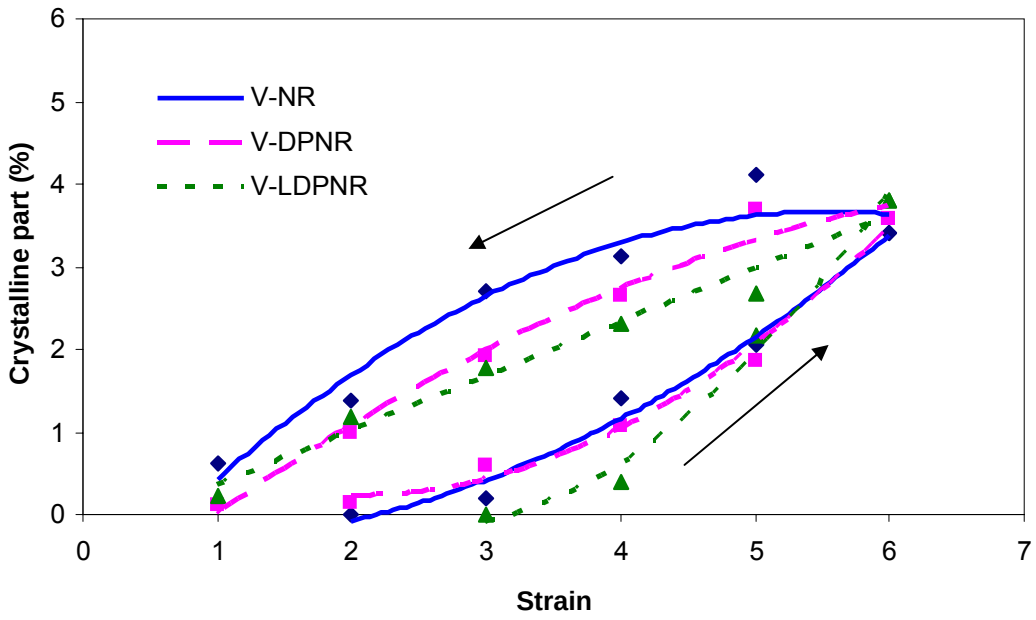

Figure 14 Change in crystalline part of vulcanized NR, vulcanized DPNR and vulcanized L-DPNR during extension and retraction

CONCLUSION

The naturally occurring network, formed by proteins and phospholipids, is responsible for high tensile strength and crystallizability under deformation of un-vulcanized NR. The removal of proteins and phospholipids by deproteinization and lipase treatment, respectively, resulted in the decrease of stress-strain behavior and crystallizability under deformation. The addition of extracted

proteins and/or phosphatidyl choline into L-DPNR was not effective to improve the stress-strain behavior and crystallizability on stretching since the superior properties are not directly caused by proteins and phospholipids. The naturally occurring network, formed by proteins and phospholipids, is presumed to contribute to the stress-strain behavior and the strain-induced crystallization under deformation of NR even in vulcanized state.

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PART 4 ROLE OF PHOSPHOLIPIDS AND PROTEINS ON GEL FORMATION, STORAGE HARDENING AND CHANGE IN PHYSICAL PROPERTIES OF NATURAL RUBBER DURING ACCELERATED STORAGE

The effect of non-rubber components i.e. proteins and long-chain fatty acid ester group in phospholipids on gel formation, storage hardening and change in physical properties of natural rubber (NR) during accelerated storage were investigated. Proteins and phospholipids in NR were decomposed by deproteinization and transesterification, respectively. Centrifuged natural rubber (CNR) and deproteinized natural rubber (DPNR) showed significant increase in Wallace plasticity value, Mooney viscosity and tensile strength during storage while transesterified natural rubber (TENR) and transesterified deproteinized natural rubber (TE-DPNR) showed very low and almost constant values during storage. This suggests that proteins are not responsible for storage hardening but phospholipids instead play an important role on storage hardening. The formation of crosslinking in storage NR was further studied and discussed in the present work. The interaction between long-chain fatty acid ester groups of phospholipids at chain ends of NR molecules was proposed to be the possible mechanism for the formation of crosslinking during accelerated storage.

INTRODUCTION

Solid natural rubber (NR) consists of about 94% rubber hydrocarbon and 6% non-rubber components such as proteins, lipids, sugars, etc [1]. These non-rubber components have been considered to play important roles for bringing about outstanding properties of NR [2]. Proteins in NR have been believed to be an essential component controlling the properties of NR, especially cured properties [3]. Lipids in NR have been also regarded to play an important role to

govern the physical properties of NR as well as proteins [4]. Lipids in NR are composed of neutral lipids, glycolipids and phospholipids. The study on structural characterization [5-7] and purification [8-9] of NR indicates the presence of associated proteins and phospholipids at the initiating and terminating ends of rubber molecules, respectively. Both non-rubber constituents, i.e. proteins and phospholipids are presumed to form two types of branch-points [8-11]. The first is presumed to originate from the intermolecular interaction of proteins that can be decomposed by deproteinization [9]. Another is reported to form through the association of phospholipids [5,6] that can be decomposed by transesterification [10]. The removal of proteins and phospholipids through deproteinization and transesterification, respectively results in the decomposition of branch-points, which are expected to involve the change in physical properties of NR.

Prolonged storage of NR is known to progress the hardening, which is referred to as storage hardening. Storage hardening phenomenon in NR has long been recognized to be a factor affecting the processing properties such as Mooney viscosity and Wallace plasticity during storage. The change in the properties of NR during storage was presumed to be due to the cumulative effect of crosslinking and chain-scission [12]. It has been postulate that the hardening proceeds through the reaction between the rubber chain and so-called abnormal groups assumed to exist on rubber molecule such as epoxide [13-15], aldehyde [16-20], and lactone [21]. However, the recent work reveals that the active functional groups in the long chain fatty acid of phospholipids at the terminating ends of rubber molecules are the cause of the storage hardening in NR [22]. Even through the extensive works have been done on the storage hardening, the actual mechanism of the storage hardening is still under discussion.

The present work is an attempt to investigate the role of proteins and fatty acid ester groups in phospholipids on storage hardening and change in physical properties, i.e.; Mooney viscosity and tensile properties during accelerated storage condition. The formation of gel and chemical change in NR during storage were also discussed to establish the possible mechanism for storage hardening of NR.

EXPERIMENTAL

Sample preparation

Fresh natural rubber (FNR) was prepared by casting freshly tapped NR latex into thin film on a glass plate and drying in an oven at 50^{o}C for 2 days.

Centrifuged natural rubber (CNR) was prepared by centrifugation of fresh natural rubber latex at 13,000 rpm for 30 min. The cream fraction was dispersed in distilled water, followed by cast into thin film on a glass plate and dry in an oven at 50^{o}C for 2 days.

Deproteinized NR (DPNR) was prepared by deproteinization with urea [23]. FL-latex was stabilized with 1%w/v SDS. The rubber latex was incubated with 0.1%w/v urea at room temperature for 3 h, followed by centrifugation at 13,000 rpm for 30 min. The cream fraction was re-dispersed with 1%w/v SDS and re-centrifuged for 30 min. The received DPNR was cast into thin film and dried in the same condition.

Transesterified NR (TENR) and transesterified DPNR (TE-DPNR) were prepared by the reaction with freshly prepared $NaOCH_3$ under nitrogen

atmosphere in the dark at room temperature for 3 h, followed by precipitation with methanol. Purification of the resulting rubber was carried out by precipitation from toluene solution into methanol.

Accelerated storage hardening test

The accelerated storage hardening test was carried out on the rubber samples in preheated (to 60^{o}C, 30 min) desiccator. Then, phosphorus pentaoxide (P_2O_5) was placed in preheated desiccator. The desiccator was seal and placed in an oven at 60^{o}C. The accelerated rubbers were collected at 0h, 6h, 12h, 24h and 48h.

Characterization

Nitrogen content of the sample was analyzed by a Leco Nitrogen Analyzer (model FP 528) with the sensitivity of 0.001%. Calculation of nitrogen content was performed by built-in software. The quantity of long chain fatty acid ester group was determined by FTIR measurement based upon calibration by a mixture of methyl stearate and synthetic *cis*-1,4-polyisoprene, Kuraprene IR10. The content of fatty acid ester group per weight of rubber was determined by the intensity ratio of peaks at 1739 cm^{-1} (C=O) to 1664 cm^{-1} (C=C) [24].

Gel content was determined in dried toluene to give a concentration of 0.1% w/v and kept in dark without stirring for a week at room temperature. The insoluble fraction was separated from sol fraction by centrifugation. The gel content was determined as the weigh percentage of the gel fraction against the total sample weight.

The rubber sample of ca. 0.1 g was accurately weighed in a porcelain crucible and pyrolzed in a furnace at 550oC for 2.5 h. The ash content was calculated from percentage of weight ratio between ash product and original rubber used.

Physical and Mechanicl properties

Wallace plasticity value was determined by Wallace Plastimeter, according to ASTM D3194-99. A constant compressive force of 10 ± 0.1 kgf for 15 sec was pressed on the rubber pellet for 15 ± 0.2 sec. The thickness of specimen at the end of this period was taken as the plasticity measurement. The median of three pieces was taken as the Wallace plasticity value.

Measurement of Mooney viscosity (MS1+4) was carried out by using a TECHPRO Mooney Viscometer. The temperature of testing was 100 ± 1^{o}C and the small rotor size was used to measure viscosity at the strain rate of about 2 sec^{-1}. The rubber sample was preheated at 100^{o}C for 1 min with continuous shear for 4 min followed by measuring the decay of torque when the rotor was stopped.

Tensile strength measurement was carried out by an Instron Model 5569 at room temperature. The testing crosshead speed of 500 mm/min was applied with load cell of 100 N. The test pieces were stamped out using a type C dumbbell die according to ASTM D412-87. The thickness of sample was 0.5-1.5 mm. The measurement was repeated 3-4 times for each sample.

RESULTS AND DISCUSSION

The roles of non-rubber components were investigated by composition alternations through centrifugation, deproteinization and transesterification. Table1 shows nitrogen content, fatty acid ester content, ash content and gel content of the rubber samples. The nitrogen content of NR from fresh latex decreased from 0.58% w/w to 0.22% w/w after centrifugation, to 0.04%w/w after deproteinization and further decrease to 0.001% w/w after transesterification. The reduction of nitrogen content indicates the removal of proteins through those processes. The ester content of rubber after transesterification decreased remarkably, to about zero for both TENR and TE-DPNR. The ash content of FNR decreased after centrifugation, and deproteinization. The gel content of FNR decreased from 18.2% to 17.0 after centrifugation and to zero after deproteinization and transesterification. The decrement of gel content suggests that branch points can be decomposed by deproteinization and transesterification. We have confirmed that transesterification could remove all ester linkages, believed to be included in the formation of branch-points [8,10]. The rubber after transesterification i.e. TENR and DP-TENR was proved to be composed of linear rubber molecules [25].

Table 1 Nitrogen content, ester content, ash content and gel content of rubber samples

Sample	N-content (%w/w)	Ester content (mmol/kg)	Ash content (%w/w)	Gel content (% w/w)
FNR	0.58	10.02	1.15	18.2
CNR	0.22	13.08	0.79	17.0
DPNR	0.04	16.22	0.39	~ 0
TENR	0.36	~0	1.15	~ 0
TE-DPNR	0.001	~0	1.17	~ 0

Stress-strain curves of FNR, CNR, DPNR, TENR and TE-DPNR are shown in Figure 1. Stress at 100%, 300%, 500% and at break (tensile strength) of FNR decreased after deproteinization and transesterification. FNR, CNR and DPNR showed the significant increase in stress during stretching at strain of about 4. On the contrary, TENR and TE-DPNR showed very low tensile strength and didn't show any increase in stress during stretching. This behavior can be explained by linear rubber molecules of TENR and TE-DPNR, which was obtained by the decomposition of branch-points through transesterification.

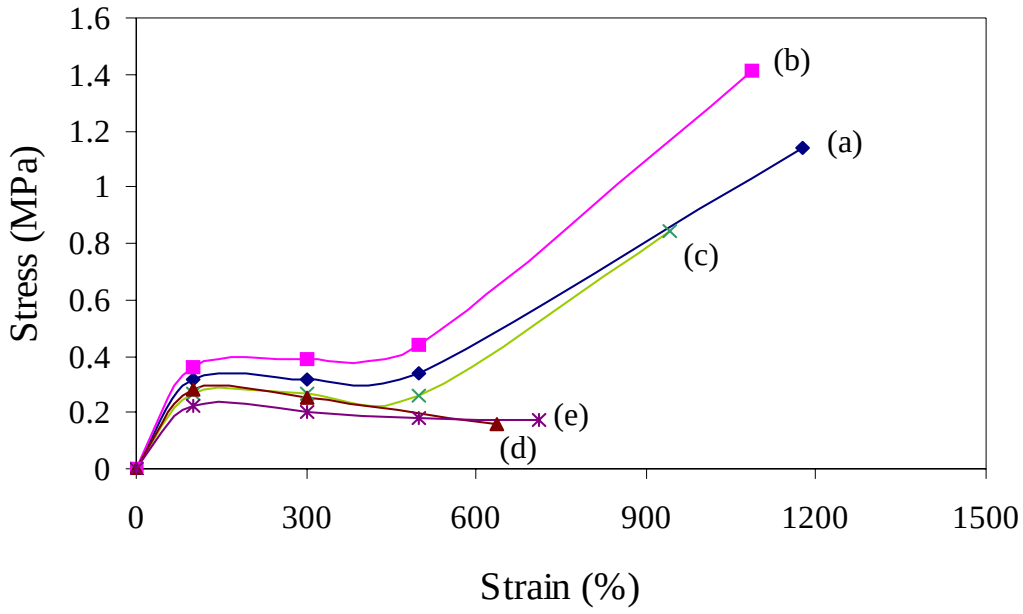

Figure 1 Stress-strain curves of FNR (a), CNR (b), DPNR (c), TENR (d) and TE-DPNR (e).

Change in gel content of rubber samples after accelerated storage over P_2O_5 at 60°C for various times are shown in Figure 2. The gel content of FNR and CNR increased from about 20% w/w to 48% w/w while that of DPNR increased from 0% w/w to 38% w/w after storage for 48 hours. It is clear that the gel formation occurred even without proteins as shown by the increase in gel content of DPNR after storage. This indicates that proteins are not the major factor for the formation of gel after storage under accelerated condition. On the other hand, the gel content of TENR and TE-DPNR showed little change (less than 1% w/w) after storage over P_2O_5 for 48 hours. This indicates that the gel formation cannot proceed in NR without the long-chain fatty acid ester group, which can be decomposed by transesterification. In other words, the long-chain fatty acid ester group is an important factor for gel formation in NR.

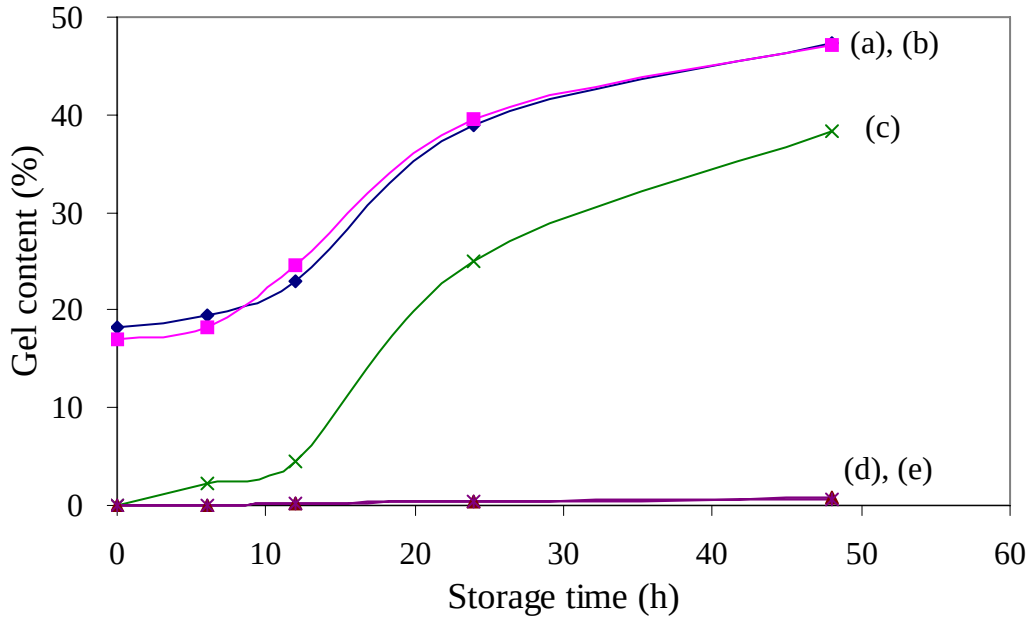

Figure 2 Change in gel contents of FNR (a), CNR (b), DPNR (c), TENR (d) and TE-DPNR (e) after accelerated storage over P_2O_5 at 60°C for various times

The change in Wallace plasticity value was used as representative data to indicate the storage hardening in the rubber. Change in Wallace plasticity values of rubber samples after accelerated storage over P_2O_5 at 60°C are shown in Figure 3. The plasticity value of FNR and CNR slowly increased about 10 units after storage for 48 h while that of DPNR abruptly increase about 30 units after storage for 48h. At the initial stage of storage, DPNR showed lower plasticity value than FNR and CNR. This indicates that the removal of proteins through deproteinization resulted in the reduction in the plasticity value, which can be explained by the decomposition of network structure after deproteinization. However, the plasticity value of DPNR after storage for 48 h was almost the same as that of FNR and CNR. This implies that the formation of network structure during storage under accelerated condition is not predominantly caused by proteins. It is clear that the plasticity values of TENR and TE-DPNR didn't show any significant change in the plasticity value after accelerated storage. They showed very low and almost constant plasticity value. This finding suggests that fatty acid ester groups in phospholipids play an important role in the storage hardening of NR.

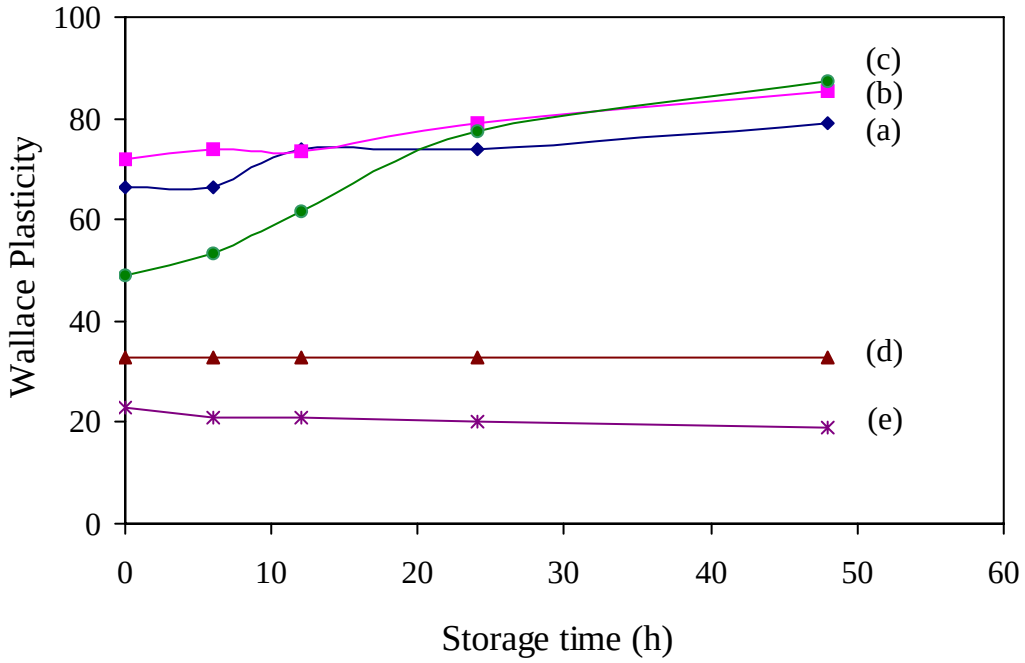

Figure 3 Change in Wallace plasticity values of FNR (a), CNR (b), DPNR (c), TENR (d) and TE-DPNR (e) after accelerated storage over P_2O_5 at 60°C for various times

Change in Mooney viscosities (MS1+4) of rubber samples after accelerated storage over P_2O_5 at 60°C is shown in Figure 4. At starting point, Mooney viscosity of FNR decreased from 60 units to 50 units after deproteinization and further decreased to about 40 units after transesterification. The decrement in Mooney viscosity value of FNR after deproteinization and transesterification might be due to the decomposition of branch-points through the removal of proteins and phospholipids, respectively. After accelerated storage test, FNR showed no significant change in Mooney viscosity while CNR show the increase in Mooney viscosity step by step. The Mooney viscosity of DPNR rapidly increased after storage under P_2O_5 at 60°C for 24 h. In contrast, the Mooney viscosity of TENR and TEDP were very low and showed no significant change after accelerated storage. These results are consistent with the change in gel content and Wallace plasticity values shown in Figure 5 and 3, respectively. The constant Mooney viscosity of TENR and TE-DPNR confirms that the progress hardening cannot proceed in NR after the decomposition of fatty acid ester groups in phospholipids by transesterification.

Tensile strength and elongation at break of rubber samples before and after accelerated storage were investigated as shown in Figure 5 and 6, respectively. Tensile strength or stress at break of FNR, CNR and DPNR increased with increasing storage time while that of TENR and TE-DPNR was almost constant. The elongation at break of FNR, CNR and DPNR showed no clear tendency with storage time while that of TENR and TE-DPNR was almost the same. It is well known that the strength of rubber depends on the amount of network so the increase in tensile strength during accelerated storage is the

supporting evidence for the formation of network structure in rubber. Accordingly, TENR and TE-DPNR has no formation of network during storage.

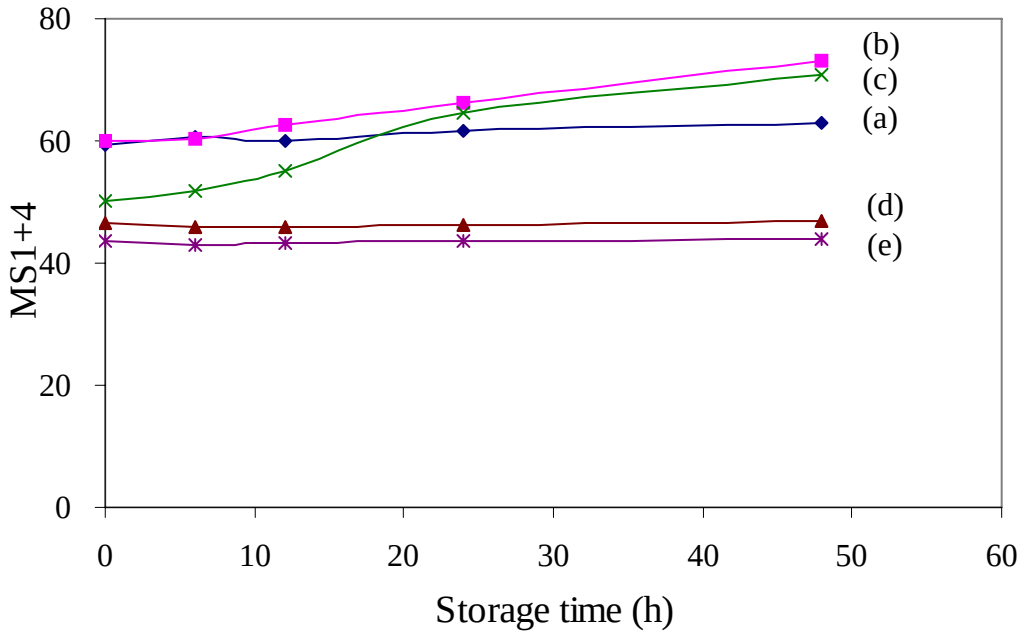

Figure 4 Change in Mooney viscosities of FNR (a), CNR (b), DPNR (c), TENR (d) and TE-DPNR (e) after accelerated storage over P_2O_5 at 60°C for various times

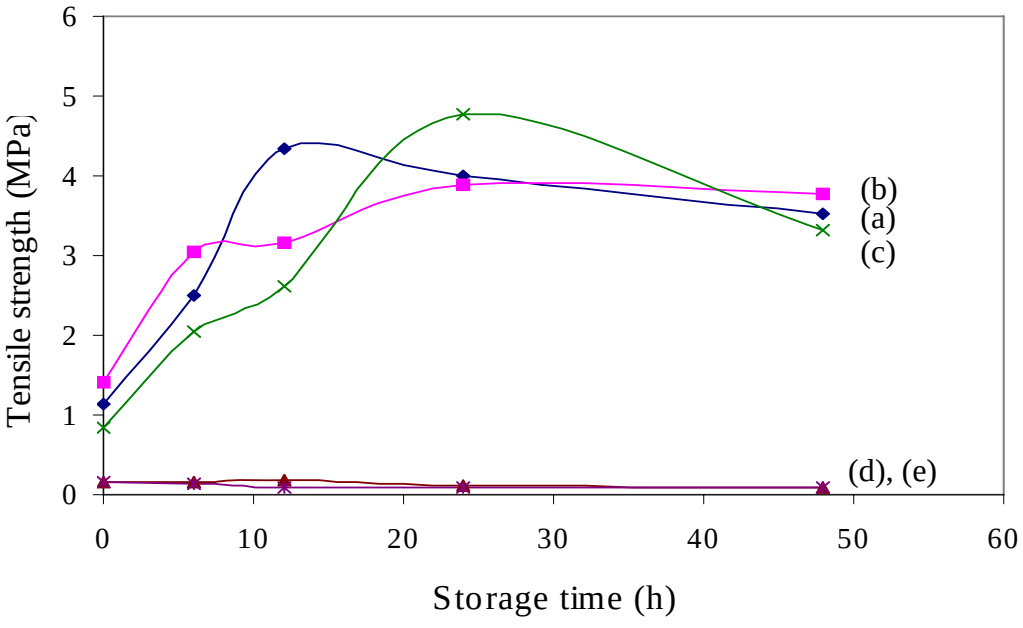

Figure 5 Change in tensile strength of FNR (a), CNR (b), DPNR (c), TENR (d) and TE-DPNR (e) after accelerated storage over P_2O_5 at 60°C for various times

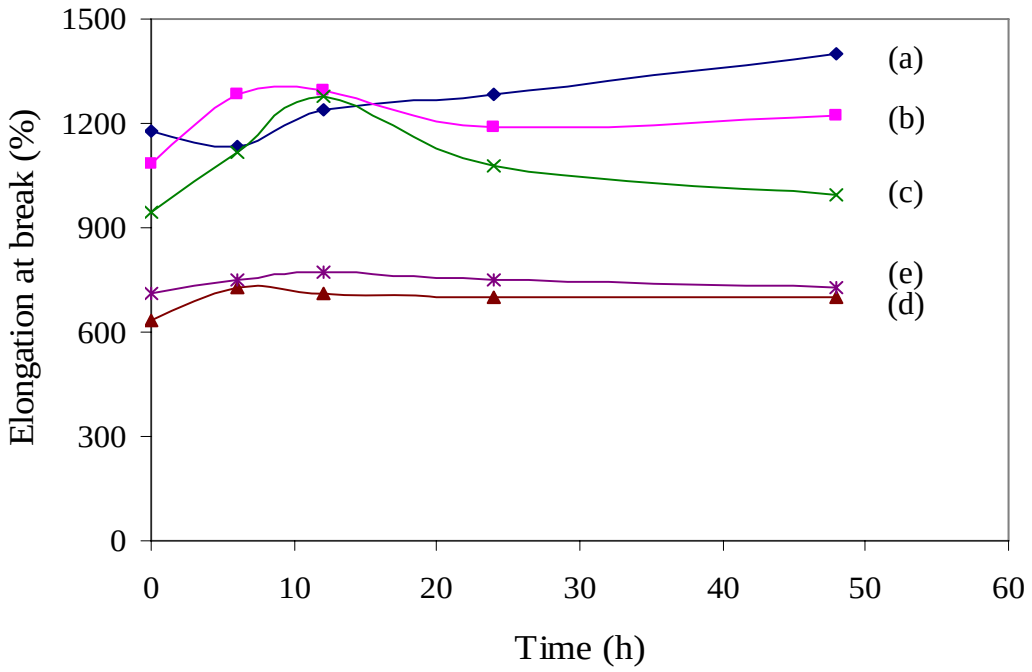

Figure 6 Change in strain at break of FNR (a), CNR (b), DPNR (c), TENR (d) and TE-DPNR (e) after accelerated storage over P_2O_5 at 60°C for various times

Table 2 Gel content of storage FNR, CNR and DPNR before and after treating with transesterification

Sample	Storage time (h)	Gel content (%w/w)	
		Before TE*	After TE*
FNR	0	18.2	~0
	48	47.2	~0
CNR	0	17.0	~0
	48	47.0	~0
DPNR	0	~0	~0
	48	38.3	~0

TE* : Transesterification

The formation of network structure in rubber sample after accelerated storage over P_2O_5 was further investigated by treating with transesterification and FTIR measurement. If the gel fraction of rubber, causing the storage hardening, was generated by the linkages of long-chain fatty acid ester groups, it should be completely decomposed after treating with transesterification. The results showed that the gel fraction can be decomposed by transesterification for all rubber samples, as shown in Table 2. The gel content of storage rubbers decreased to almost zero after treating with transesterification. This result reveals that the formation of gel during accelerated storage is originated by the interaction of long-chain fatty acid ester groups in phospholipids of NR.

FTIR spectra of CNR, DPNR and TE-DPNR are shown in Figure 7. CNR showed the band at 1738 cm^{-1} and 1710 cm^{-1}, corresponding to the characteristic band of fatty acid ester and aldehyde groups, respectively. It is clear that the band at 1710 cm^{-1} increased with increasing storage time. On contrary, DPNR showed the characteristic band of fatty acid ester group without the band of aldehyde group at 1710 cm^{-1} and TE-DPNR was not observed for both characteristic bands. The disappearance of band at 1710 cm^{-1} after deproteinization and transesterification indicates that these aldehyde groups are not linked with the rubber main-chain and they might be existed in the degraded product of non-rubber components like unsaturated fatty acids, which can be removed by these processes.

In the past, the formation of crosslinking during storage hardening was proposed to be originated by a bimolecular aldol condensation between aldehyde groups in NR chain [16,19,20]. According to this assumption, the crosslinking points are formed by covalent carbon-carbon bond. It is remarkable that this type of bonding cannot be decomposed by transesterification. However, the present study clearly showed that the gel fraction of storage NR can be decomposed by transesterification. Furthermore, FTIR spectra of storage DPNR clearly showed the absence of aldehyde groups even the progressive hardening was occurred. Therefore, it may be reasonable to disagree with this hypothesis. Another assumption was proposed that some chemical reaction between proteins (amino acids) and the abnormal groups such as epoxide [14] and aldehyde [18] groups in rubber chain cause the storage hardening. These hypotheses are also denied by considering the occurrence in gel formation and storage hardening of DPNR as mentioned previously. Base on the present work, it can be deduced that proteins are not the major factor but long chain fatty acid ester groups in phospholipids are responsible for the formation of gel and storage hardening during accelerated storage.

CONCLUSION

The storage hardening phenomenon and increase in physical properties i.e. Mooney viscosity and tensile strength were detected in FNR, CNR and DPNR during storage while no progressive hardening and constant physical properties were observed for TENR and TE-DPNR during storage. This suggests that proteins are not important for storage hardening but fatty acid ester groups in phospholipids are responsible for this phenomenon. The gel fraction of storage rubbers was found to be decomposed by tranesterification, suggesting the presence of fatty acid ester linkages at the crosslink-points of rubber molecules. Therefore, it is reasonable to presume that the interaction of fatty acid ester group in phospholipids at chain ends of rubber molecules is responsible for the formation of crosslinking during storage.

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PART 5 STRUCTURE OF BRANCH-POINTS IN NATURAL RUBBER – REGULATION OF PROTEINS ON GEL FORMATION IN DEPROTEINIZED NATURAL RUBBER–

The various kinds of treatment to diminish the proteins content in natural rubber (NR), i.e., enzymatic deproteinization, saponification, and surfactant washing, were employed to investigate the role of proteins decomposition in NR latex. The cleavage of proteins and retaining of oligopeptides and short peptide proteins by means of enzyme treatments was revealed. The washing with surfactant was believed to denature and separate proteins from rubber particle to water phase without decomposition. The reaction of strong alkali in saponification was still unclear but expected to react both of proteins and fatty acids in NR latex. The physical properties of deproteinized NR such as green strength and Mooney viscosity were suppressed compared to original NR owing to the lack of proteins, which regulated the branching and gel formation. Gel formation during storage was still observable in the case of enzyme treated NR (DPNR) although the rate was slower than that of original NR. The steeply boosted of gel content in saponified NR (SPNR) was expected to be not only the residual proteins but also the unidentified side reactions. Without proteins associated at ω-terminal, gel and branching in WSNR latex could not be perfectly formed even for long period of storage. The formation of gel and branching in deproteinized NR latex during storage was conclusive to depend on the association of decomposed proteins at ω-terminal.

Keyword: Hevea brasiliensis, proteins decomposition, deproteinization, gel formation

INTRODUCTION

Natural rubber (NR) latex contains colloidal rubber particles suspended in water with 30-40% of rubber content and non-rubber constituents such as proteins, lipids, sugar, and *etc* [1]. Structural studies of NR disclosed that the rubber molecule is composed of □-terminal, two *trans*-1, 4 isoprene units, a long sequence of *cis*-1, 4 isoprene units, and □-terminal. [2,3]. The □-terminal was expected to be a modified dimethylallyl group linked or associated with proteins by hydrogen bond, although the structure of functional group has still been unidentified [4]. On the other hand, the □-terminal was found to consist of mono- and di-phosphates groups linked up with phospholipids [5]. The proteins and phospholipids are reported to be the double layer surrounded the rubber particles, in which protein layer on the surface acted as a stabilizer [6]. The outstanding properties of NR have been presumed to be derived from the high *cis*-1, 4 content and the presence of some non-rubber components [7]. It has been reported that freshly tapped NR latex contains *ca.* 1% proteins. In concentrated latex, after centrifugation, proteins are distributed in the cream, serum and bottom fractions *ca.* 27.2%, 47.5% and 25.3%, respectively [8,9]. However, some proteins exuding from NR product were found to give rise to a so-called Type I rubber allergy, which can result in life-threatening anaphylactic shock [10]. Consequently, removal of proteins in NR is strongly required. In the past decades, many efforts have been made to produce deproteinized NR latex using different protein-removing treatments such as the treatment of latex with a proteolytic enzyme [11,12], radiation treatment of latex with □-ray [13], treatment of rubber

products with aqueous solution of strong alkali [14], treatment of latex with sodium hydroxide [14], and treatment of enzymatic treated latex with urea [15,16]. Moreover, we have recently reported that proteins on the surface of rubber particle can be removed by successive washing with centrifugation in the presence of surfactant [17,18]. In the course of washing with centrifugation, the reduction of nitrogen content accompanied with the decrease of gel content, showing a role of proteins on branching and gel formation in NR through hydrogen bond [18].

It is well-known that prolonged storage of solid NR causes the increase of Mooney viscosity, which is termed storage hardening. The phenomenon was ascribed to the formation of branch-points and gel by the reaction with functional terminal groups at the chain-end of NR with proteins and phospholipids [19]. It is also reported that the gel content in NR increases during the storage of concentrated latex in the presence of ammonia [20]. It is remarkable that the increase of gel fraction is observed even in the case of enzymatic deproteinized NR (DPNR) latex, although the rate of gel formation is slower than that of ordinary NR latex. These findings suggest that the formation of branching and gel in latex are derived from the association of proteins and phospholipids at the □-and □-terminals, by considering the presumed structure of rubber molecule. It was reported that branch-points formed by both proteins and phospholipids can be decomposed by further transesterifying DPNR with sodium methoxide in toluene solution to form linear rubber molecules [21]. Nevertheless, it is not clear whether the transesterification treatment is toward to the ester linkages giving the formation of linear rubber molecules or the effect of structural change of both terminal groups for both branched molecules and linear molecules. Furthermore, no direct evidence was reported about the decomposition of branch-points in NR in connection with the removal of proteins alone. Thus, it is noteworthy to evaluate the effect of deproteinization on branching and also gel formation, which relating to physical properties of deproteinized NR, more in details.

EXPERIMENTAL

Fresh Natural Rubber (FNR) latex

Fresh field latex (FL-latex), provided by Thai Rubber Latex Co. Ltd, was obtained by tapping from regularly tapped mature *Hevea* rubber trees of clone RRIM 600 and collected in an ice-cooled cup. FL-latex was filtered with muslin cloth to remove impurities, preserved by the addition of ammonia to make 0.6% (v/v) in latex.

Washed NR (WSNR) latex

FL-latex was diluted to 15% (w/v) dry rubber content (DRC) with distilled water and added sodium dodecyl sulphate (SDS) to make 1% (w/v), followed by centrifugation at 19,000 rpm (43,300 g) for 60 min. The recovered cream fraction was re-dispersed in distilled water followed by the addition of SDS as mentioned above to wash the rubber particles. This washing procedure was repeated for 3 times, and then the recovered cream was re-dispersed in distilled water to make WSNR latex with 30% DRC.

Enzymatic Deproteinized NR (DPNR) latex

The DPNR latex was prepared by treating FL-latex with 0.04% (w/v) proteolytic enzyme (KP-3939, KaO Co.) in the presence of 1% (v/v) of

polyethylene glycol *p*-isooctylphenyl ether (Triton® X-100) by incubation at 37°C for 12 h with stirring. The resulting latex was centrifuged twice at 19,000 rpm (43,300 g) for 40 min each. The collected cream fraction was diluted with distilled water to make 30% DRC.

Saponified NR (SPNR) latex

FL-latex was diluted with distilled water to make 15% (w/v) DRC latex in the presence of 0.5% (v/v) Triton® X-100 and saponified with 2% (w/v) NaOH at 70°C for 3 h, followed by neutralization with 2% (v/v) formic acid.

Storage of NR latex

Each of prepared latex was storage for 6 weeks at ambient temperature with regularly shaking every day.

Characterization of non-rubber components and physical properties analysis

The nitrogen content in NR was analyzed by a LECO-FP258 Nitrogen Analyzer. The content of long-chain fatty acid ester was calculated based on the calibration curve from a mixture of synthetic *cis*-1,4 polyisoprene and various amounts of methyl stearate and used as an index of lipid content.[17] The measurements of Fourier transform infrared spectroscopy (FTIR) were carried out with a JASCO-460 FTIR spectrometer. Gel content was evaluated by dissolved NR in anhydrous toluene in the dark for 1 week. Gel was collected by centrifugation at 8,000 rpm (18200 *g*) for 30 min. The gel content was calculated based on the weight ratio of gel fraction against the original rubber weight.

The effect of each deproteinization treatment to proteins in NR was investigated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Each of the protein fractions was extracted from each NR latex specimen by precipitation using cold-acetone and kept at 4°C for 12 h, followed by centrifugation at 13,000 rpm (29500 *g*) at 4°C for 15 min. The concentration of extracted protein was measured by UV-VIS spectroscopy at 595 nm using calibration curve from standard protein bovine serum albumin (BSA). The extracted proteins was then adjusted the concentration to 8 □g/ml by lysis buffer, i.e., 50 ml of the aqueous solution composed of 24.024 g of urea (60.06 g/mol), 2 g of Triton® X-100, and 0.035 ml of 2-mercaptoethanol. The SDS-PAGE analysis was preceded according to the procedure outlined by Hasma *et al* [9], with 4% stacking gels and 15% separating gels at a constant current of 50 mA for 1 h. The molecular weight reference marker was run simultaneously in all experiments. The SDS-PAGE gels were subsequently stained with Coomassie Blue R-250 staining system for 20 min and de-stained several times in de-staining solution.

Intrinsic viscosity, [η], of rubber sample was measured with a single bulb Ubbelohde viscometer (SCHOTT CT52) at 30±0.01°C in analytical grade toluene. The intrinsic viscosity [η] was calculated using Equation (1).[22] The Huggins' *k'* constant was calculated from the slope of the plot of Equation (1).

$$\eta_{sp}/C = [\eta] + k'[\eta]^2 C \quad (1)$$

Where, η_{sp}, [η], C, and *k'* represent the specific viscosity, intrinsic viscosity, concentration expressed as g/dl, and Huggins' *k'* constant, respectively.

The stress-strain behavior of rubber film obtained by casting of latex on a glass plate was measured by an INSTRON Model 5569, with the crosshead speed of 500 mm/min and load cell of 100 N. Mooney viscosity (ML1+4) was analyzed using a TECHPRO Mooney Viscometer at 100±1°C with a strain rate of 1 sec^{-1}. Mooney viscosity was determined as the torque at 4 min. Mooney viscosity was determined as the torque at 4 min. A measurement of decay torque, when the rotor stopped for 30 sec was defined as MR_{30}.

RESULTS AND DISCUSSION

Non-rubber components of deproteinized NR

In this study, DPNR and WSNR were processed through the centrifugation, while that of SPNR was directly subjected to acid coagulation. By considering that some parts of proteins in NR can be removed not only by the treatments but include the method to collect the rubber such as centrifugation or acid coagulation. Thus, the control experiment of DPNR and WSNR was done by directly coagulate the latex with acid after reaction was finished without centrifugation, whereas SPNR was subjected to centrifuge twice for collecting solid rubber as control.

Table 1 Nitrogen content and ester content in FNR, DPNR, WSNR, and SPNR.

Sample	Nitrogen content (%w/w)	Ester content (mmol/kg rubber)
FNR	0.65	14.0
DPNR	0.03 (0.14)*	24.3 (20.1)*
WSNR	0.02 (0.31)*	25.1 (21.9)*
SPNR	0.12 (0.03)**	12.3 (21.3)**

*Values were obtained from directly coagulated rubber latex without centrifugation.

**Values were obtained from subjected SPNR latex to centrifugation twice then coagulation with acid.

Table 1 shows the nitrogen and long-chain fatty acid content in purified NR samples, i.e., DPNR, SPNR and WSNR. It is clear that the enzymatic deproteinization and surfactant washing methods reduced the nitrogen content significantly to a level of 0.02-0.03% (w/w) after centrifugation of latex, whereas the ester content remarkable increased. This is due to the loss of impurities and some of small rubber particles in the course of centrifugation, because it has been reported that there are no lipids linked to NR molecules from small rubber particles [23]. WSNR and DPNR without centrifugation showed higher nitrogen content than that from normal DPNR and WSNR with centrifugation. This indicates that the decomposed proteins by enzymatic treatment in DPNR latex and denatured proteins by SDS in WSNR can be removed from the rubber fraction to the serum by centrifugation, while coagulation with formic acid remains a part of these residual proteinous compounds. SPNR coagulated after centrifugation showed very low nitrogen content, whereas higher nitrogen content

was observed when SPNR latex was directly coagulated without centrifugation. This also ascribed to be due to the adsorption of decomposed proteins in acid coagulated SPNR coagulum. As a proof, it was found that the nitrogen content in SPNR decreased to 0.02% (w/w) after soaking the coagulum from SPNR latex with aqueous NaOH solution [14]. Regarding to the usual SPNR procedure is performed without centrifugation, thus some decomposed proteins might still exist in SPNR. In the case of ester content in SPNR, a slight reduction of the ester content was observed. This may be owing to the reaction of NaOH was not specific to protein but could react with lipids. High ester content in SPNR after centrifugation is relevant to the effect of the loss of small rubber particles and impurities as mentioned before.

Branching and gel in deproteinized NR

Table 2 shows the gel content of FNR, DPNR, WSNR and SPNR together with [η] and *k*' values. FNR contained 10% (w/w) gel fraction, which was drastically reduced to 2.9% (w/w) and 3.6% (w/w) by enzymatic treatment and surfactant washing, respectively. Whereas, SPNR showed insignificantly change in the gel content. By considering the diminishing of nitrogen content as shown in **Table 1**, it can be assumed that the loss of nitrogenous components in NR could significantly decrease the gel content. The *k'* values of samples indicate that the *k'* of FNR retained about 0.3-0.4 whether after enzymatic deproteinized or surfactant washing. This implies that this kind of residual long-chain branching is not particularly derived from proteins, but mainly generated by the association of phospholipids at the α-terminal or ionic linkage derived by magnesium. In the case of SPNR, the higher of *k'* value compared to the others was noticeable, which indicates the higher degree in branching.

Table 2 Gel content, branching factor, *k'*, and intrinsic viscosity, [η], of FNR, DPNR, WSNR, and SPNR samples.

Sample	Gel content (%w/w)	*k'*	[η]
FNR	10.8	0.37	4.86
DPNR	2.9	0.41	3.84
WSNR	3.6	0.37	3.82
SPNR	7.8	0.58	2.96

It can be presumed that the residual decomposed proteins in SPNR latex can probably assist to create the branch-point at ω-terminal of NR based on the analysis of nitrogen content. Theoretically, the viscosity of branched polymer is uniformly lower than that of a linear polymer of the same molecular weight, because the size of polymer coil of branched molecules is smaller than linear molecules [24]. The intrinsic viscosity, [η], showed the relevant results with the *k'* value, i.e., SPNR showed lower [η] than the others indicating the presence of more branch-points in SPNR.

Physical properties of deproteinized NR

The green strength of FNR before and after deproteinization was analyzed and depicted in **Figure 1**. FNR showed high green strength, which has been proposed to be owing to the persistence of non-rubber components conducting the phenomenon known as strain-induce crystallization [25], which refer to the rapidly increasing in stress after 300-400% strain. The green strength of DPNR was found to be in the same level as FNR, whereas WSNR showed a clear decrease of the green strength. SPNR showed the lowest green strength with a little occurrence of strain-induce crystallization. This supposed to be due to the unspecific reaction of saponification, which is able to react with lipids including fatty acids in NR. Based on the assumption that the presence of free fatty acids in FNR can promote the strain-induce crystallization [26], so the reduction of lipids in SPNR may lead to the suppression of strain-induce crystallization and lower strength in SPNR.

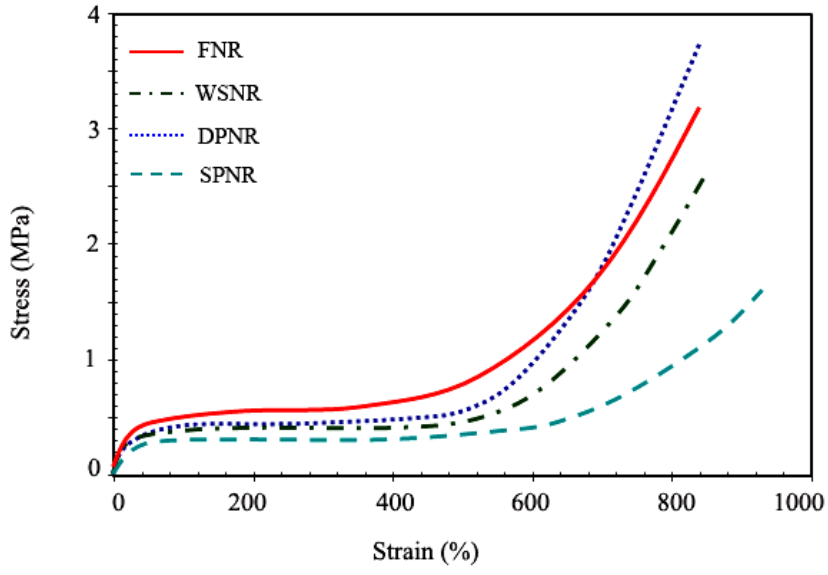


Figure 1 Green strength of FNR before and after deproteinization with different methods.

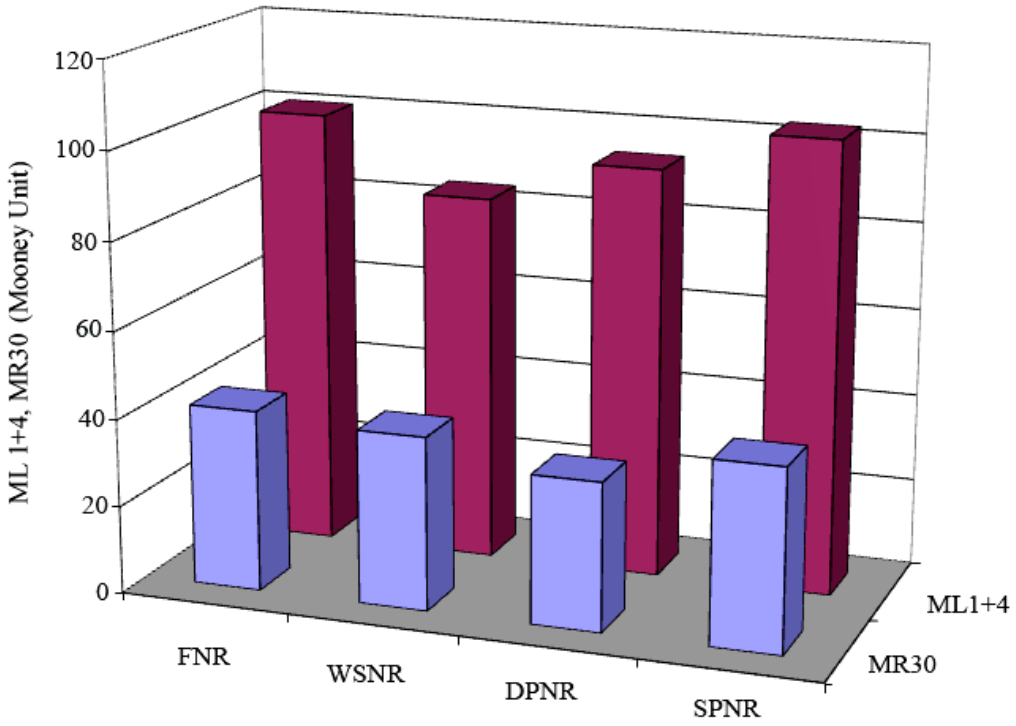


Figure 2 Mooney viscosity (ML1+4) and Mooney relaxation (MR_{30}) of FNR after different deproteinization treatments.

Figure 2 shows the Mooney viscosity of each sample. FNR showed higher Mooney viscosity than that of DPNR and WSNR. It is clear that the reduction of some parts of proteins in NR leads to the decreasing of Mooney viscosity. DPNR showed a slightly higher Mooney viscosity compared to WSNR, which was in accordance with the green strength. Nevertheless, SPNR showed insignificantly change of Mooney viscosity. By considering the *k'* results together, these suggest that SPNR has higher branch structure, although the exact reaction was still unknown. The MR_{30} value of each sample, even before or after treatments, showed insignificant difference. These findings mean that the relaxation properties, which related to the elastic behavior of NR, were not strongly affected by the removal of some parts of branch-points derived from proteins.

From the divergence of physical properties between DPNR and WSNR, the different performance between enzymatic reaction and physical treatment of surfactant washing was then proposed. **Figure 3** illustrates SDS-PAGE of extracted proteins from serum phase of DPNR latex, the protein band with molecular weight lower than 6.5 kDa was clearly noticeable. Theoretically, oligopeptide consists of two to twelve amino acids [27], therefore the extracted protein compounds from the serum of DPNR were proposed to be oligopeptides or low molecular weight short-chain proteins. This result reveals that the cleavage of proteins in which linked or associated to the □-terminal of NR molecules was taken place in the course of enzymatic treatment.

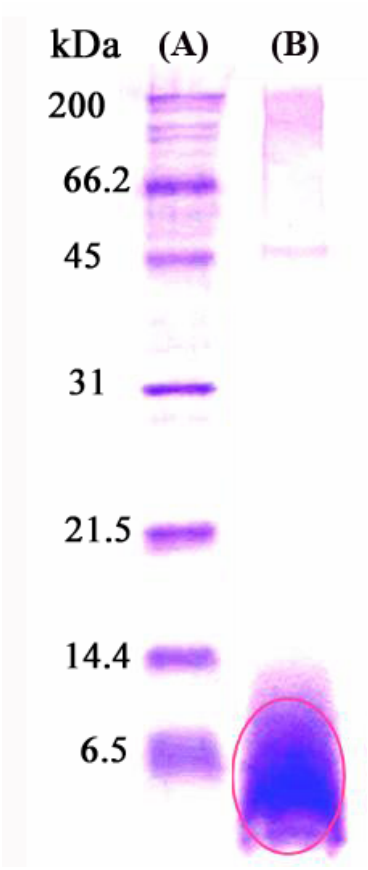


Figure 3 SDS-PAGE of (A) standard protein bands and (B) extracted proteins from serum phase of DPNR latex.

On the other hand, the SDS-PAGE results of WSNR clearly showed that proteins on the surface of rubber particles were removed to the serum fraction by means of successive washing without changing the molecular weight. Other higher molecular weight proteins, which consist in NR as soluble proteins, were found to be removed to the serum fraction as well. Moreover, it has been reported that the low molecular weight rubber fraction obtained from washed rubber showed ^{1}H-NMR and ^{13}C-NMR signals corresponded to the dimethylallyl group at the initiating end [28]. Hence, it can be concluded that the surfactant washing treatment would successively remove proteins by denaturing proteins to lose their native shapes without degradation. Based on the above results, the scheme showing the proposed mechanism of deproteinization by enzymatic treatment and surfactant washing was illustrated in **Figure 4**. Here, the oligopeptides and short-chain proteins, remaining in the serum fraction of DPNR latex, might be again associated to the ω-terminal of NR molecules, and be a driving force to form branch-point in DPNR, whereas denatured proteins in WSNR latex ought to be covered by SDS micelle and vanished to serum by means of centrifugation. This is probably being the reason why DPNR showed higher green strength and Mooney viscosity rather than WSNR.

Nevertheless, the molecular weight of proteins in serum phase of SPNR has been reported to be lower than 6.5 kDa [14]. This demonstrates that saponification caused degradation of proteins into small fragments. However, the reaction of saponification towards to NR was still suspicious owing to the high reactivity of alkali, which would react with both proteins and lipids, including fatty

acids. Thus, the physical properties of SPNR were depended not only on the effect of proteins removal from saponification treatment but also the residual of non-rubber components in which expected to be mainly the residual fatty acids content.

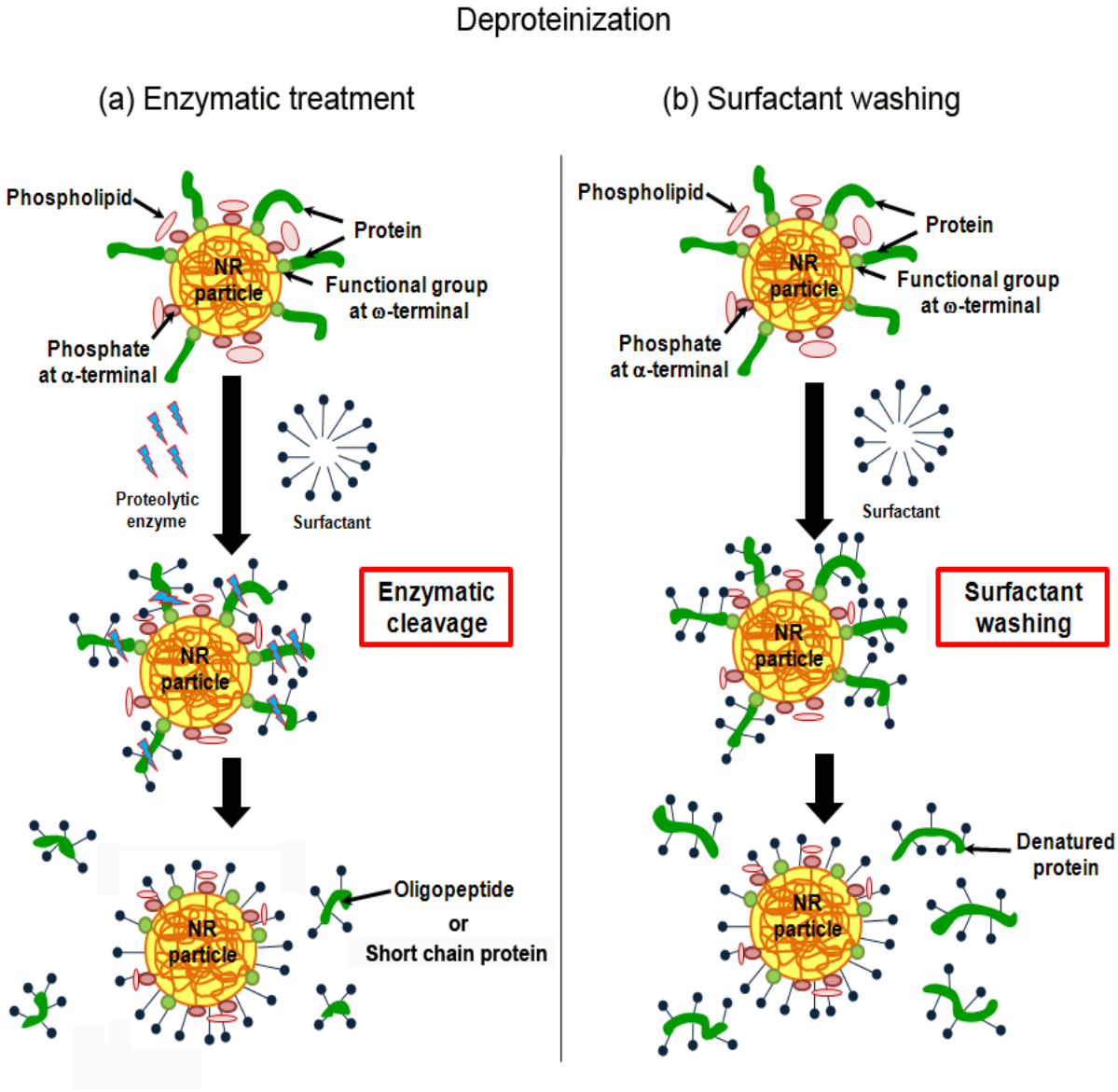


Figure 4 The presumed schematic of deproteinization mechanism of (a) enzymatic treatment and (b) surfactant washing or physical treatment.

Change of basic characteristics during storage of deproteinized NR latex

The physical properties of NR are reported to change during long time storage period [19,20]. The comparison of change in physical properties between NR and deproteinized NR will provide information on the effect of storage. **Figure 5** shows the change of gel content during storage of deproteinized NR latex samples. It is clear that the apparent change occurred in the first couple weeks. SPNR showed the highest rate of gel formation, which was higher than that from FNR. This kind of gel fraction in SPNR was thus expected to be not due to the formation of branch-point by the residual proteins in SPNR only. To proof this

hypothesis, the polar solvent addition was applied, which could decompose gel fraction derived from hydrogen bond [29]. It has been found that the gel content of SPNR was reduced only 30% after polar solvent treatment. Consequently, the gel fraction in SPNR is presumed to be caused by some unusual reactions, which have not been clarified yet.

DPNR showed a slower rate of increasing in the gel content compared to FNR owing to a part of proteins was already removed. It is assumed that the natural crosslink network would be generated by the participation of both proteins and phospholipids at the initiating □-terminal and terminating □-terminal of rubber chain ends during storage [18]. This result suggests that the gel formation in DPNR was not accomplished as effective as FNR case. However, this result disclosed that the gel formation in DPNR still proceeds even after deproteinization by enzyme treatment. This confirmed the previous assumption that some parts of oligopeptides and short chain proteins might remain in DPNR latex, and can associated to the □-terminal to form branching point in DPNR. Nevertheless, it is noteworthy to notice that gel content of WSNR was almost constant through the storage period owing to the lack of proteins. From these findings, it can be deduced that the gel formation in NR latex during storage was regulated by the residual amounts of proteinous compounds in latex.

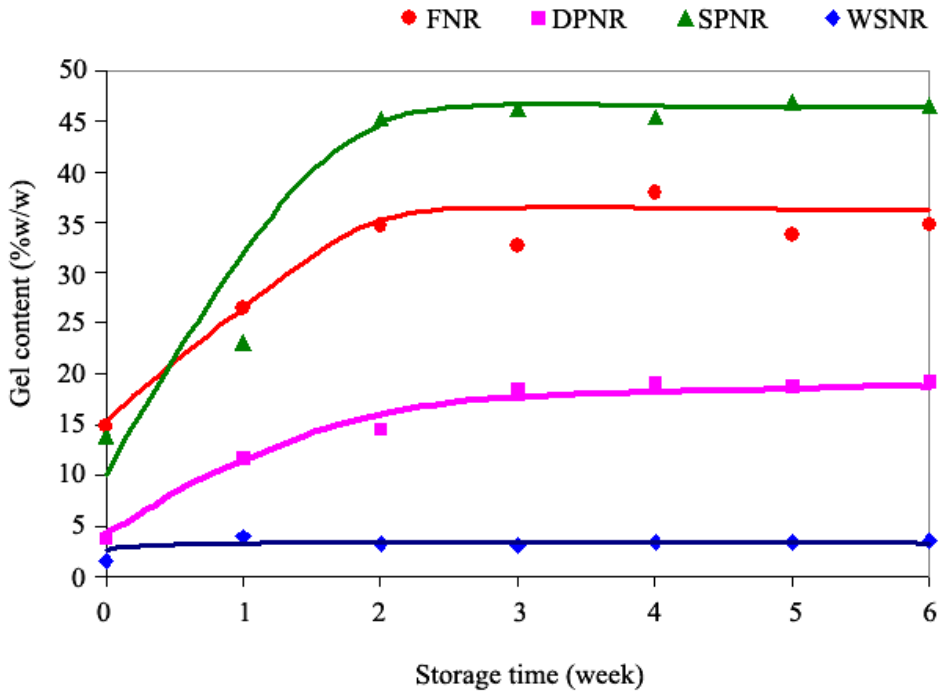
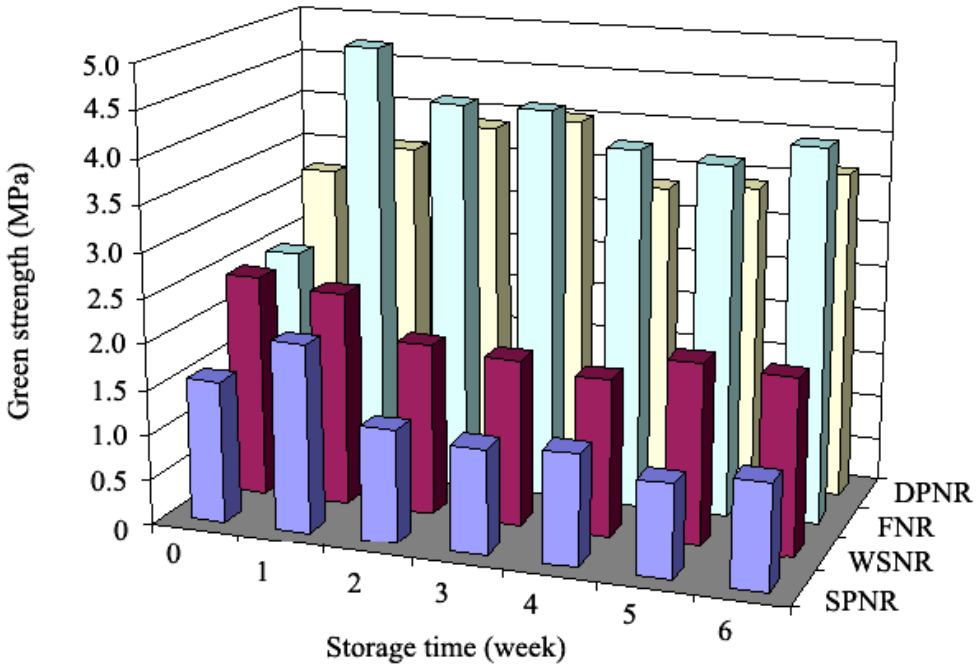


Figure 5 Increase of gel content of FNR and deproteinized NR with different methods during storage in latex form.

As described previously, the green strength of NR has been proposed so far to be mainly controlled by the naturally occurring network such as branching and gel fraction through the strain-induce crystallization [25]. In addition, the presence of free fatty acids in FNR was also believed to promote the strain-induce crystallization as well [26]. **Figure 6** shows the relationship between the green strength and storage period for the rubber from deproteinized NR latices.

After storage for 1 week, the green strength of FNR rapidly increased and then leveled off. This is relevant with the gel content results implying that the increase of branching network as storage time increased. The storage DPNR latex also showed a slight increase in the green strength of DPNR, while that of WSNR latex showed no significant change for WSNR in the green strength through the storage period, which is in accordance with the constant gel content. The suppression of gel formation and green strength of DPNR and WSNR is supporting evidence assuring that proteins is either the important parameter



controlling the formation of branching and gel in NR latex during storage. The low green strength of SPNR throughout the long storage period suggests that the lack of strain-induce crystallization by saponification reaction leads to the inferior in green properties even the gel content markedly increase.

Figure 6 Green strength of FNR and protein removed NR with various treatments during storage period.

Figure 7 illustrates the change of Mooney viscosity of deproteinized NR from various treatments during storage. FNR showed the increase of Mooney viscosity as increasing the storage period up to 2 weeks and leveled off due to the increase of gel fraction after storage. This network would restrict the movement of the rotor leading to higher Mooney viscosity than that of the initial FNR. The raised Mooney viscosity during storage was noticeable in DPNR, whereas WSNR showed a slight change. As shown schematically shown before (*cf.* **Figure 4)**, it can be explained that, as for DPNR, both of the association of oligopeptides or short chain proteins with the□-terminal and phospholipids at the□-terminal could generate branching point, which obviously represented as the elevated of gel content. On the other hands, WSNR latex was presumed to contain none of proteins to link at the □-terminal, thus the incomplete gel formation during storage of WSNR was observable leading to the insignificant

change of green strength and Mooney viscosity. In the case of SPNR, it is interesting to note that Mooney viscosity markedly increased as storage time increased up to 2 weeks, and then leveled off. This can be ascribed that SPNR contained residual decomposed proteins, which can also generate branching and gel networks. Nonetheless, the reactivity of alkali in saponification is high in which the certain reaction with NR has not been known, therefore, at the present, the crosslinking reaction was suspected to cause the high Mooney viscosity.

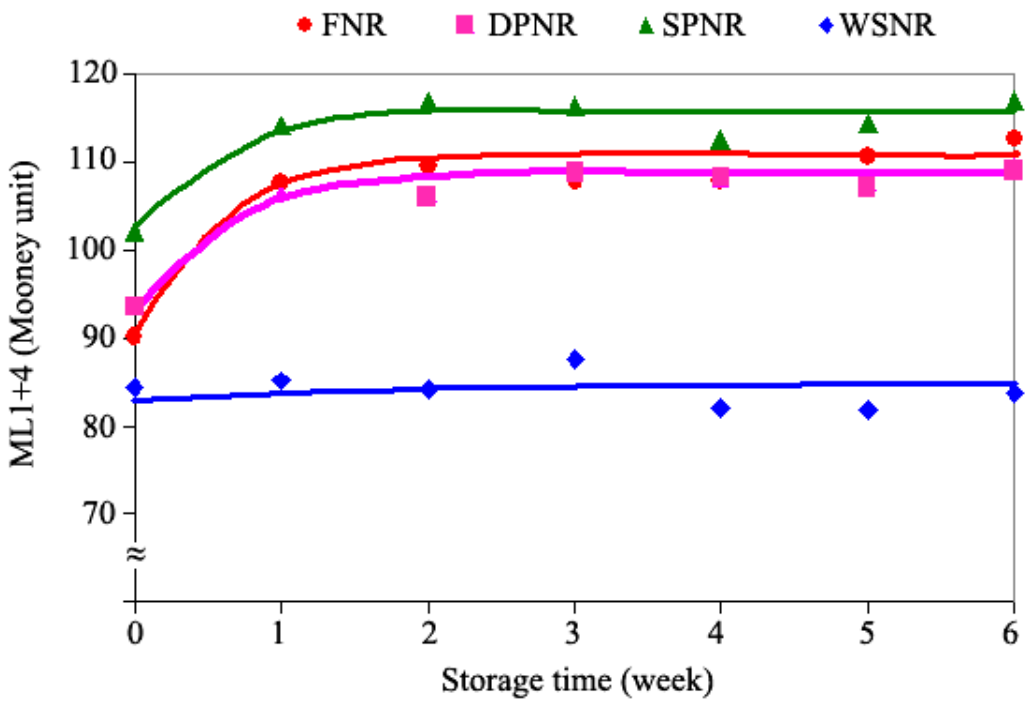


Figure 7 Mooney viscosity change during storage of FNR and deproteinized NR.

Based on the above findings, it is revealed that the gel formation during storage of FNR and deproteinized latices intensively depends on the presence of both proteins and phospholipids at the ω- and α-terminals, respectively. However, the diminution of proteins by different techniques would affect to the formation of gel in deproteinized latex towards to physical properties of the obtained NR. **Figure 8** introduced the schematic representation of branching formation derived by proteins at the ω-terminal in FNR and deproteinized NR during storage. The treatment with enzymatic deproteinization would result in the cleavage of proteins. During storage, the oligopeptides might associate and form branching network. The surfactant washing treatment can totally wash out the proteins at ω-terminal, thus the branching at the ω-terminal cannot be formed. Hence, the gel fraction in WSNR was expected to be predominantly created by phospholipids at the α-terminal. Saponification of latex was reported to decompose proteins [14]. The decomposed proteins, which believed to include in the SPNR latex, would associate at the ω-terminal giving the higher gel content during storage the SPNR latex.

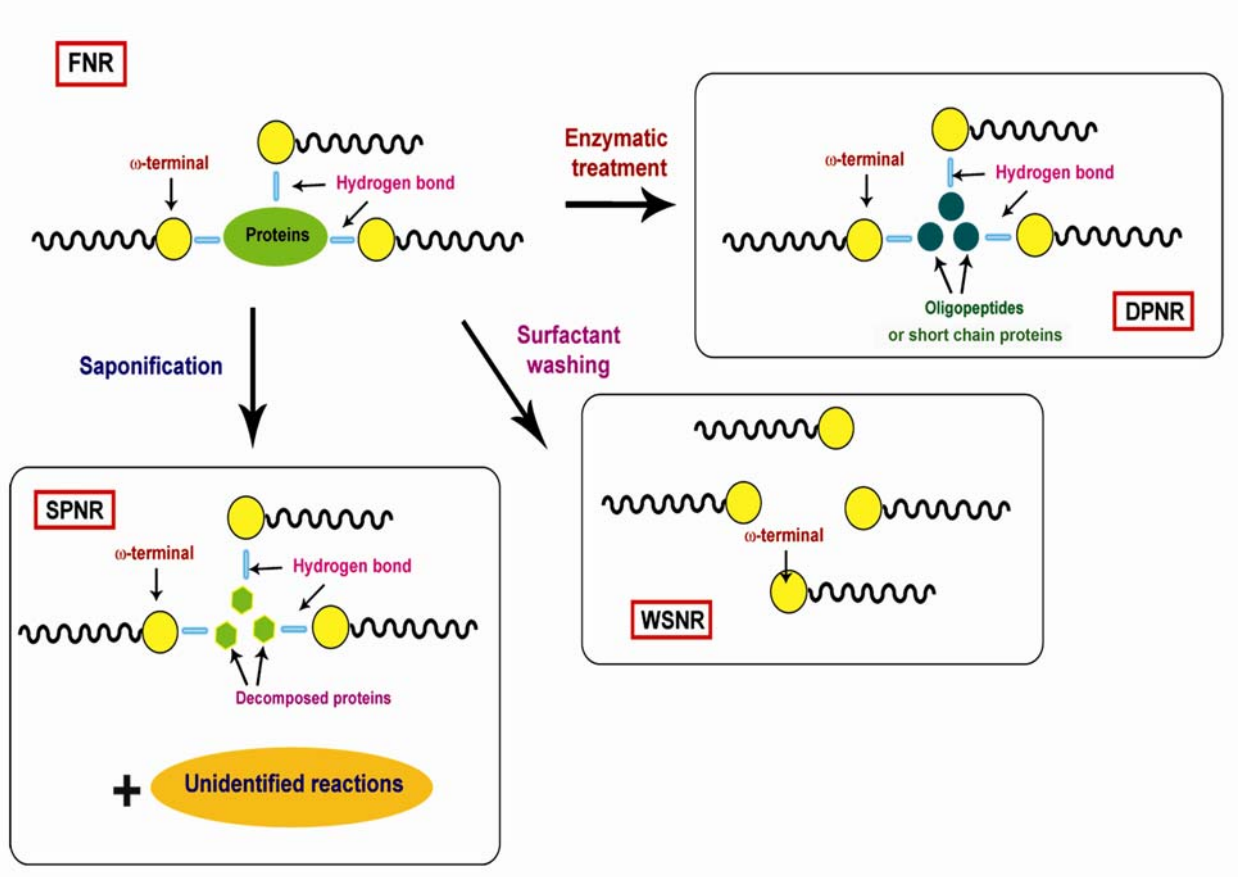


Figure 8 The schematic representation of branching formation derived from proteins of FNR and deproteinized NR.

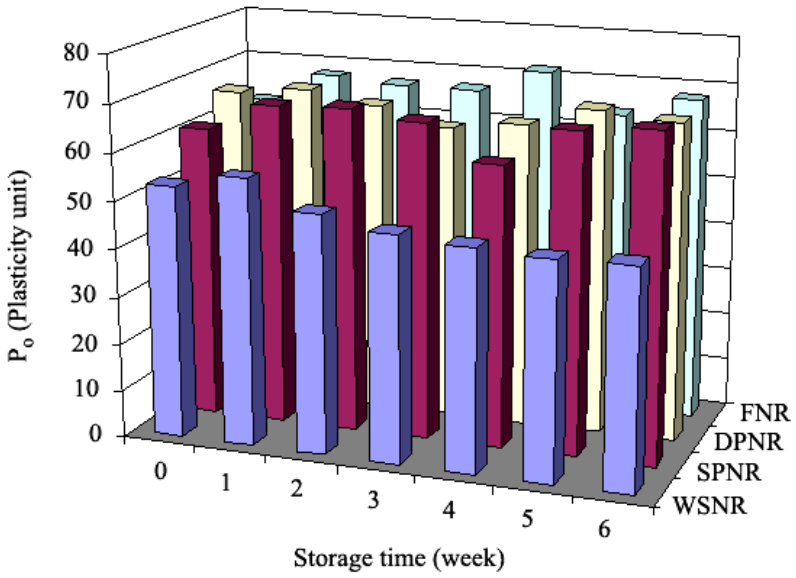


Figure 9 Plasticity index (P_o) of rubber from FNR and treated FNR with different treatments during storage.

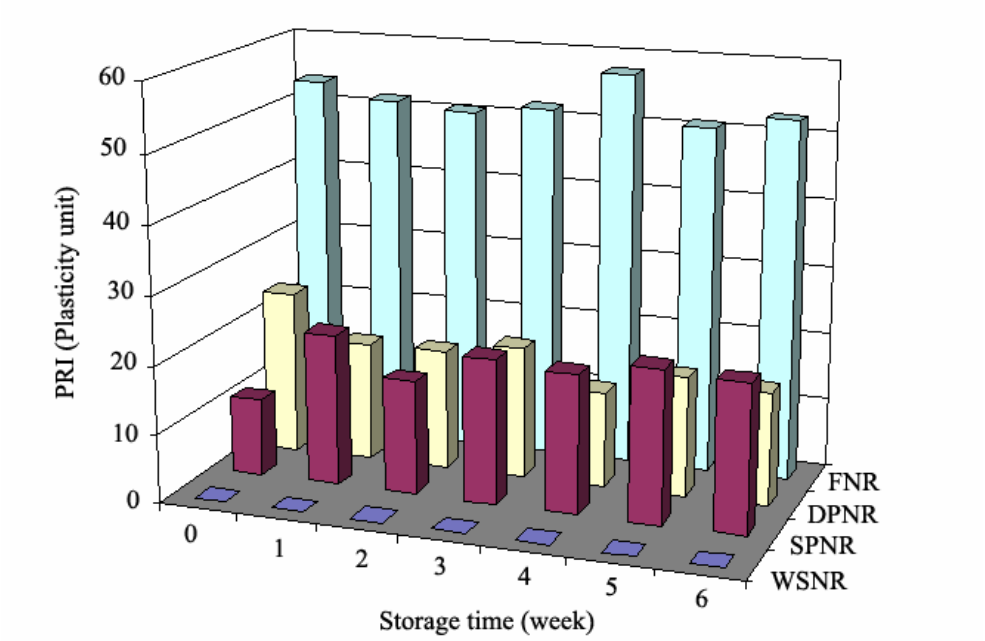


Figure 10 Change of Plasticity retention index (PRI) of rubber from FNR and treated FNR with different treatments during storage.

Plasticity measurement is normally used for indicating stiffness of raw NR. The resistance to oxidative aging is usually represented as plasticity retention index (PRI), which the measurement is performed after preheating at 140°C for 30 min. The changes of plasticity index (P_o) and PRI during storage of different treated FNR were illustrated in **Figures 9** and **10**, respectively. FNR, DPNR, and SPNR, showed almost equivalent P_o values with no change even after storage. However, WSNR showed slightly lower P_o value compared to the others, but no change along the storage period. This suggests that the stiffness of rubber from FNR and deproteinized NR latex was not directly depended on the storage time. As illustrated in **Figure 10**, FNR showed the highest PRI value compared to the others, which means that FNR could mostly resist to oxidative aging. This is due to the fact that FNR contained a large amount of natural antioxidants, which were believed to be amino acids and choline components presenting in NR [30]. DPNR and SPNR showed the comparable PRI values, which was lower by one-half of that from FNR due to the loss of proteins. The extremely low resistance to oxidative degradation was found in the case of WSNR. It was obvious that WSNR samples were sticky like a glutinous after preheated, which could not be subjected to measure. This is because of the lack of naturally occurring antioxidants after successively washing with centrifugation. In other words, based on the results of DPNR, this can be the supporting evidence showing that DPNR still persists of residual proteins such as oligopeptide and short-chain proteins.

CONCLUSION

The gel content of DPNR and WSNR decreased significantly in relevant with the decomposition of branch-points by means of proteins removal. The green strength of FNR, DPNR and WSNR was depended not only on the amount of gel but also the presence fatty acid esters. The decrease of long-chain fatty acid ester content by saponification resulted in the suppression of strain-induce crystallization in SPNR giving lower green strength than the others. The

performance of enzyme treatment was disclosed to be the cleavage of proteins in DPNR latex. The residual proteinous compounds in DPNR latex are proposed to be a driving force to form gel fractions during storage. The successive washing by surfactant led to the achievement of WSNR containing no protein linked at the □-chain ends. The gel fraction in WSNR was not changed during the storage period, indicating that the formation of gel in WSNR latex is consequently incompletely created without proteins. The gel formation in SPNR latex is proposed to be not only from the residual decomposed proteins but also from the unidentified reaction accompanied with the NaOH treatment, which is still suspicious. The green strength and Mooney viscosity of DPNR was relatively higher than WSNR, and gradually increased as storage time increased. The higher PRI value of DPNR compared to WSNR supports the assumption that DPNR still consists of naturally occurring antioxidant derived from the residual proteins.

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Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ (ระบุชื่อผู้แต่ง ชื่อเรื่อง ชื่อวารสาร ปี เล่มที่ เลขที่ และหน้า) พร้อมแจ้งสถานะของการตีพิมพ์ เช่น submitted, accepted, in press, published

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