Effect of Deproteinized Methods on the Proteins and Properties of Natural Rubber Latex during Storage

Kanjanee Nawamawat,[1] *Jitladda T. Sakdapipanich,**[1,2] *Chee C. Ho*[3]

Summary: Three different methods of deproteinization, i.e. saponification, surfactant washing and enzymatic treatment were employed to unravel the effect of deproteinized on the properties of natural rubber (NR) latex. The cleavage of proteins in NR latex was found to proceed with concomitant formation of low molecular weight polypeptides. This results in a lowering in gel formation of the enzyme-treated latex, indicating modification of the remaining proteins at the rubber chain-end. Washing NR latex with surfactant would efficiently reduce and remove proteins from NR latex particles through denaturation and transferring them to the serum phase. The relatively stable gel formed during storage of surfactant-washed NR latex is an indication of the absence of branch formation of proteins at the rubber molecule terminal. Saponification by strong alkali would hydrolyze the proteins and phospholipids adsorbed on the latex particle surface. The reason of the significantly higher gel formed in saponified NR latex is still not clear. The present study shows that deproteinization treatments result in modification of the proteins at the surface of NR latex particles and also those freely-suspended in the serum. The cleavage or the denaturation of the rubber proteins during purification by washing has a profound effect on the properties of the deproteinized NR latex upon storage, in particular the thermal oxidative aging properties of the rubber obtained.

Keywords: deproteinization; gel formation; natural rubber latex particle; proteins

Introduction

Natural rubber (NR) field latex (*Hevea brasiliensis*) contains about 30–40% rubber dispersed as rubber latex particles in water with some minor non-rubber constituents such as proteins, lipids, carbohydrates and sugars, metal ions, etc.[1] Structural studies of NR showed that the rubber molecule is composed of one ω-terminal, two *trans*-1, 4 isoprene units, a long chain of *cis*-1, 4 isoprene repeating units ending in an α-terminal.[2,3] Some of the proteins or phospholipids are thought to co-exist at the terminal ends of the rubber molecules[4,5] and present as a charged layer covering the rubber latex particle,[6] thereby stabilizing the latex particles against aggregation.

Storage hardening as manifested by an increase in the Mooney viscosity of the rubber, in prolonged storage of solid NR was found to be caused by the formation of branch-points and gel through reaction of functional terminal groups at the end of the NR molecule with proteins and phospholipids.[7] Besides, it has also been reported that the gel content in NR increases during the storage of NR latex concentrate in the presence of ammonia.[8] These findings suggest that the formation of branching and gel in latex are caused by the association of proteins and phospholipids at the ω- and α-terminals of the rubber molecules.

[1] Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Mahidol University, Bangkok 10400, Thailand
E-mail: scjtp@mahidol.ac.th
[2] Institute of Biomolecular Science, Mahidol University, Salaya campus, Nakornpathom, 73170, Thailand
[3] Faculty of Science, Engineering and Technology, University of Tunku Abdul Rahman, Jalan University, Bandar Barat, 31900 Kampar, Perak, Malaysia

Copyright © 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

The branch-points formed by both proteins and phospholipids can be broken-up by further transesterification of DPNR with sodium methoxide in toluene solution to form linear rubber molecules.[9] However, it is not clear whether the transesterification reaction results in ester linkage to give linear rubber molecules or in structural change of both terminal groups giving both branched molecules and linear molecules. The outstanding properties of NR have been traced to the presence of some non-rubber components[10] in the latex. The potential protein allergy risk in using NR products for some sensitized users has created a demand for deproteinized NR latex obtained through different protein-removal techniques.[11–18]

However, there is no direct evidence linking the deproteinization process to the breaking up of the branch-points in NR molecules. Thus, there is a need to study the effect of deproteinization on branching and also gel formation, in relation to physical properties of NR. In the present work, a series of experiments were conducted to elucidate the influence of deproteinization treatment on the proteins of NR latex and on physical properties of the rubber obtained via changes in branching and gel decomposition. The formation of gel in deproteinized NR latex on long storage period together with the physical properties change was also investigated.

Experimental Part

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 and then preserved by the addition of ammonia to 0.6% (v/v) in latex.

Washed NR (WSNR) Latex

FL-latex was first diluted to 15% (w/w) dry rubber content (DRC) with distilled water, and sodium dodecyl sulphate (SDS) was then added to achieve 1.0% (w/v) level, 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 an addition of SDS as mentioned above to further wash the rubber particles. This washing procedure was repeated 3 times and then the recovered cream was re-dispersed in distilled water to make WSNR latex at 30% DRC.

Enzymatic Deproteinized NR (DPNR) Latex

The DPNR latex was prepared by treating FL-latex with 0.04% (w/v) of a 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 purified by centrifugation at 19,000 rpm (43,300 g) for 40 min. The collected cream fraction was diluted with distilled water to make DPNR of 30% (w/w) drc.

Saponified NR (SPNR) Latex

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

Storage of NR Latex

Each of the treated latex was stored for 6 weeks at ambient temperature in air-tight containers 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 a calibration curve constructed from a solution mixture in toluene of synthetic *cis*-1,4 polyisoprene and various amounts of methyl stearate and used as an index of lipid content.[17] The Fourier transform infrared spectrum

Copyright © 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

(FTIR) was recorded with a JASCO-460 FTIR spectrometer. The gel content was measured by dissolving NR in anhydrous toluene in the dark for 1 week. The gel fraction was collected by centrifugation at 8,000 rpm (18,200 g) for 30 min. The gel content was calculated based on the weight ratio of gel fraction against the original rubber weight.

The composition of proteins in NR was investigated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The protein fractions was extracted from NR latex by precipitation using cold-acetone and kept at 4 °C for 12 h, followed by centrifugation at 13,000 rpm (29,500 g) at 4 °C for 15 min. The concentration of extracted proteins was measured by UV-VIS spectroscopy at 595 nm using a calibration curve of bovine serum albumin (BSA) as a standard protein. The extracted proteins were adjusted to a concentration of 8 μg/ml by lysis buffer [i.e., 50 ml of the aqueous solution composed of 24.02 g of urea (60.06 g/mol), 2.0 g of Triton® X-100, and 0.035 ml of 2-mercaptoethanol]. The SDS-PAGE analysis was done according to the procedure outlined by Hasma *et al.*,[19] 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, $[\eta]$, of the rubber samples was measured with a single bulb Ubbelohde viscometer (SCHOTT CT52) at 30 ± 0.01 °C in analytical grade toluene. The $[\eta]$ value was calculated using Equation (1).[20] 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}, $[\eta]$, C, and k' represent the specific viscosity, intrinsic viscosity, concentration expressed as g/dl, and Huggins' k' constant, respectively.

Rubber film were obtained by casting the latex on a glass plate and the stress-strain behavior of the film was measured by an INSTRON Model 5569 with the cross-head speed of 500 mm/min and load cell of 100 N. Plasticity retention index (PRI) of NR was determined by a Wallace Plastimeter, according to ASTM D3194-99 procedure. A constant compressive force of 10 ± 0.1 kg was used to press on the rubber pellet to control the thickness to within the range of *ca.* 3.1–3.5 mm, for 15 ± 0.2 sec. The thickness of the pressed specimen was recorded as the plasticity value. Three pellets from each rubber sample were measured and the median plasticity value was taken as P_o. The PRI value was estimated by the percentage ratio between the Wallace plasticity number after oxidative aging at 140 °C for 30 min (P_{30}) and the P_o as given in Equation (2).

$$PRI = [P_{30}/P_o] \times 100 \quad (2)$$

Results and Discussion

Non-rubber Components and Physical Properties of Deproteinized NR

The amount of protein in NR can be estimated in terms of the nitrogen content of the rubber. The efficiency of protein reduction by deproteinization is shown in Table 1. Nitrogen content of the rubber was markedly reduced by all the deproteinization methods indicating that proteins in NR are displaceable and removable. Washing with surfactant gives the best results whereas enzymatic and saponification methods show about the same efficiency. Ester content is indicative of the presence of phospholipid in NR. DPNR and WSNR show relatively higher ester content compared to FNR. This can be explained by the fact that DPNR and WSNR were prepared by centrifugation process which removed small-size rubber particles that do not have phospholipids linked at the terminal ends.[21] The slightly lower ester content in SPNR may be due to the removal of the

Copyright © 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

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

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

phospholipids through hydrolysis with NaOH and forming the sodium salts of the long chain fatty acids, the products of the hydrolysis.

Gel content is generally used to indicate the existence of branched or network structures in NR. Therefore, the amount of gel content is directly related to the contents of nitrogen and lipids in NR. The significant decrease in gel content after enzymatic treatment and surfactant washing respectively reflects the diminishing effect of nitrogen content on gel formation in NR (see Table 2). Since the gel content of SPNR is only decreased slightly, it may be inferred that gel formation for this rubber is not dependent on nitrogen (protein) content but by some other factors. The Huggins' k' constant, which is known as a qualitative index indicating the degree of long-chain branching, was measured to complement the results on gel formation. For a given polymer, the k' value is nearly independent of molecular weight and molecular weight distribution but increases in proportion to the extent of branching in a polymer chain.[22,23] A k' value of smaller than 0.45 is attributed to linear polymer.[22] Therefore, the very small difference in k' values of FNR, DPNR, and WSNR implies that the rubber molecules of FNR even after enzymatic treatment are still mostly linear. The presence of gel originally in FNR could be attributed to the formation of physical bonds or loose chemical bonds, which do not affect the degree of long-chain branching. In the case of SPNR, the k' value was higher than the rest indicating a higher degree of branching. The branch-point created in SPNR could be from the assist of degraded proteins fragments at the w-terminal, involving with some other factors, for instance the formation via ionic bond of NR molecules and the products of the hydrolysis.

Table 2.
Gel content, the branching factor, k' of FNR, DPNR, WSNR and SPNR samples.

Sample	Gel content (%w/w)	k'
FNR	10.8	0.37
DPNR	2.9	0.41
WSNR	3.6	0.37
SPNR	7.8	0.58

Using SDS-PAGE, the proteins obtained from NR using the three different deproteinization methods could be separated into several protein bands based on molecular weight difference. The major bands at 14.5 and 24 kDa are known to be derived from proteins covering the surface of the rubber latex particle.[23] It has been reported that the proteins in NR were broken down to low molecular weight protein fragments after saponification, i.e. lower than 6.5 kDa.[14] These degraded protein fragments may be trapped in NR matrix during coagulation. Therefore, it is necessary to include additional soaking process to eliminate this possible error. The proteins remained in DPNR latex obtained by enzymatic treatment were characterized and found to be made up of a very broad band with molecular weight lower than 6.5 kDa (Figure 1). Regarding oligopeptides are defined to consist of two to twelve amino acids,[25] the extracted proteineous substances from the serum of DPNR were proposed to be oligopeptides or low molecular weight protein fragments. Our result reveals that cleavage of proteins linked to or associated with the ω-terminal of the rubber molecules occurred during enzymatic treatment. On the other hand, the SDS-PAGE results on WSNR clearly showed that proteins at the surface of the rubber latex particles were removed to the serum phase by means of successive washing without suffering any change in molecular weight (Figure 2). Other higher molecular weight proteins, which are soluble proteins already present in NR, were found to have been transferred to the serum phase as well. Moreover, it has been reported

Copyright © 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

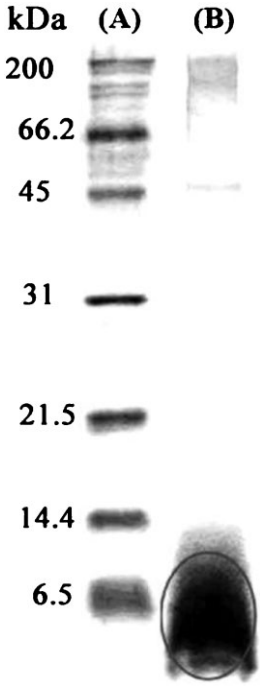

Figure 1.
SDS-PAGE of (A) standard protein bands of NR latex and (B) extracted proteins from serum phase of the enzymatic deproteinized latex.

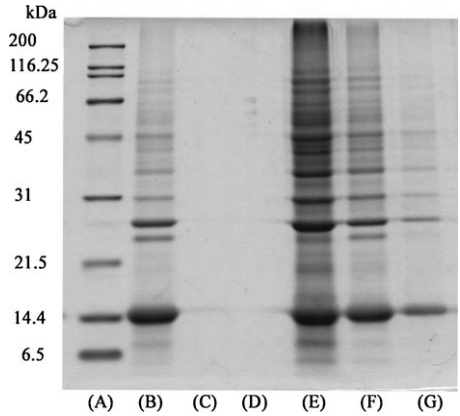

Figure 2.
SDS–PAGE of proteins from the rubber and serum fractions of surfactant washed NR latex from each centrifugation step. Lane (A): standard protein marker. Lanes (B), (C) and (D): extracted proteins from the rubber fraction of washed NR latex particles centrifuged 1, 2 and 3 times, respectively. Lanes (E), (F) and (G): extracted proteins from the serum phase of surfactant washed NR latex particles centrifuged 1, 2 and 3 times respectively.

that the low molecular weight fraction of rubber obtained from washed rubber showed ^{1}H-NMR and ^{13}C-NMR signals corresponded to the dimethylallyl group as initiating end.[26] Hence, it may be concluded that surfactant washing of latex would remove proteins in latex particles by displacement, of which possibly accompanied by denaturation, rather than degradation.

Based on the above results, the following mechanisms of deproteinization by enzymatic treatment and surfactant washing are proposed (Figure 3). Some of the oligopeptides and low molecular weight proteins fragments after enzyme treatment may remain in the serum phase of the DPNR latex or associate with the ω-terminal of the NR molecules of the latex particles. Besides, during surfactant washing, the proteins on the NR latex particles are dislodged from the particle surface (possibly denatured at the same time) and solubilized by SDS and went into the serum phase during the three centrifugation steps. On the other hand, the overall reactions of saponification are more complicated than it appears since alkali could react with both the proteins and lipids and other non-rubbers in NR latex.

The physical properties of the rubber treated by the three different deproteinization methods are compared in Figure 4. FNR showed the highest green strength which is attributed to the presence of non-rubbers in the latex, especially proteins and lipids. This is manifested in the phenomenon known as strain-induced crystallization[26] which refers to the rapidly increase in stress beyond 300–400% strain. The green strength of DPNR was found to be comparable to that for FNR, whereas that for WSNR was below these two. SPNR showed the lowest green strength with very little strain-induced crystallization. Since sodium hydroxide hydrolyzed both the proteins and phospholipids in the latex, it deprived SPNR of the proteins and lipids that can contribute to strain-induced crystallization. Hence a lower green strength of SPNR is expected.

Copyright © 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

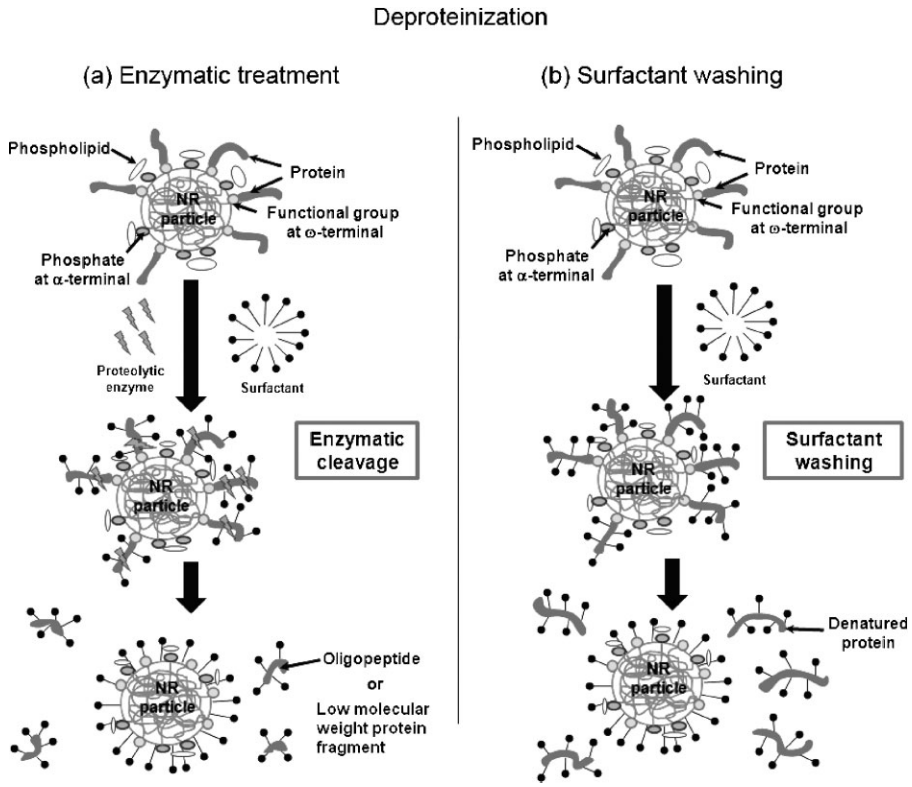

Figure 3.
A schematic representation of the proposed deproteinization mechanism by (a) enzymatic treatment and (b) surfactant washing or physical treatment.

Storage of Deproteinized NR Latex

FNR and deproteinized NR latices were stored at ambient temperature for various durations in order to investigate the role of residual proteins in deproteinized NR latex. It is clear that a change in gel content occurred in the first two weeks of storage (Figure 5). SPNR showed the highest rate of gel formation and also the highest gel content. The gel content of SPNR was reduced by only 30% after treatment with a polar solvent. It is well known that gel

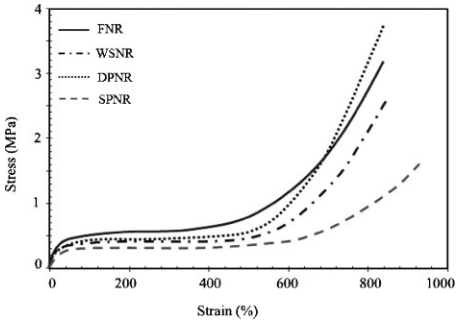

Figure 4.
Green strength of FNR after different deproteinization treatments.

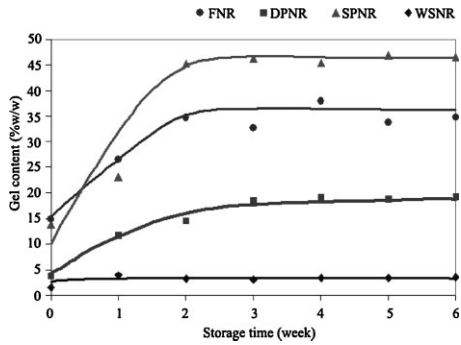

Figure 5.
Gel content of FNR and deproteinized NR after different treatment methods during storage in latex form.

Copyright © 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

formed via hydrogen bonding could be destroyed by polar solvent.[27] Thus it is concluded that the very high gel content formed upon storage of SPNR is not due to branch-point formation alone by the residual proteins in SPNR. Some other factors, possibly be ionic linkage, are likely to be involved. On the other hand, DPNR showed much lower gel content (about half) compared to that of FNR. It is assumed that the gel network is created by both proteins and phospholipids at the ω- and α-terminals of the rubber chain during storage.[18] Since most of the proteins were already removed, this result suggests that the gel formation process in DPNR could not be promoted at the same magnitude as that for FNR. In addition, the presence of gel fractions in DPNR is ought to be derived from some of the oligopeptides and low molecular weight protein fragments remaining in DPNR latex can associate with the ω-terminal to form branching point in DPNR. It is noticed that there is hardly any gel formed in WSNR during the storage period due likely to a lack of proteins. It is deduced that the gel formation in NR latex during storage was influenced by the residual proteinous substances remaining in the latex.

The green strength of NR is known to be dependent mainly on naturally-occurring network such as branching and gel through strain-induced crystallization.[28] The presence of long chain fatty acid in FNR is also known to promote strain-induce crystallization.[26] Figure 6 shows the relationship between the green strength and storage period for the rubber from the various deproteinized NR latices. The green strength of FNR is the highest among all the rubbers and increased rapidly within one week of storage and then leveled off. This correlates well with the high gel content of FNR implying an increase in branching network with storage. The green strength of rubber from DPNR latex also increased with storage but slightly smaller in values compared with FNR. There was no significant change in the green strength of rubber from WSNR latex during storage in agreement with the constant gel content observed during storage (Figure 5). The above results on DPNR and WSNR clearly show that the presence of proteins is important in the formation of branching and gel network in NR latex during storage. The relatively low green strength of SPNR throughout storage suggests that the saponification reaction reduced the proteins and lipids contents of the latex and hence diminished the strain-induced crystallization of the rubber.

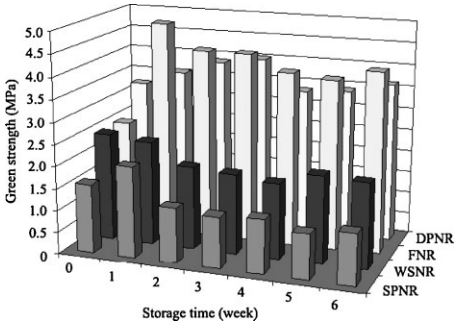

Figure 6.
Green strength of FNR and deproteinized NR after different treatment methods during storage.

Thus it is clear that gel formation during storage of FNR and the deproteinized latices depends on the presence of both proteins and phospholipids, which can link with the rubber molecules at the ω- and α-terminals, respectively. However, the reduction of proteins by different deproteinization techniques would affect the formation of gel in these latices and hence the physical properties of the NR obtained. A schematic representation of gel formation via association of proteins at the ω-terminal of rubber molecules during storage is given in Figure 7. The treatment of latex with enzyme would result in the cleavage of proteins. During storage, the remaining oligopeptides or protein fragments may associate and form a network. On the other hand, surfactant washing could effectively wash out almost all the proteins present, thus very little association at the ω-terminal could be expected. Therefore, the observed gel fractions in WSNR were most likely to have occurred mainly by linking of lipids at the α-terminal. On the other hand, the degraded fragments of proteins in the SPNR latex following

Copyright © 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

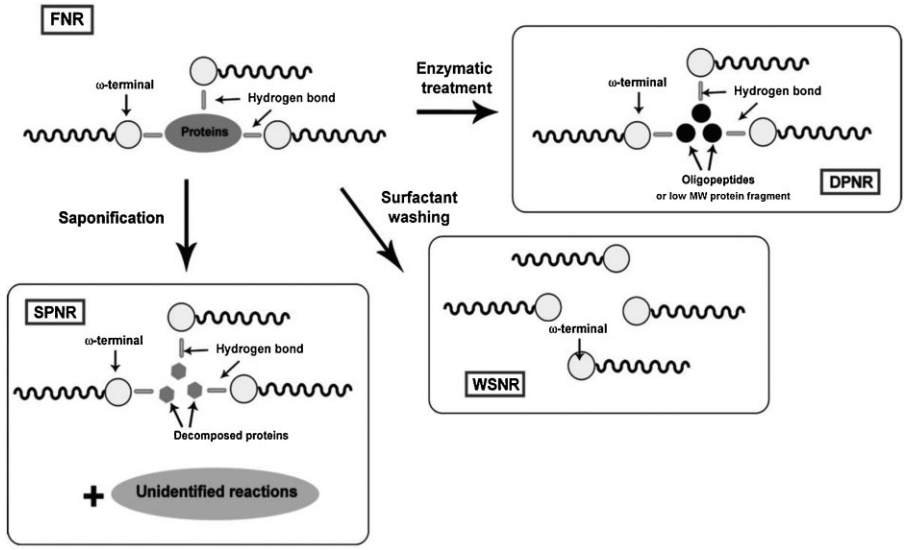

Figure 7.
The schematic representation of branching formation attributed to proteins of FNR and deproteinized NR.

hydrolysis during saponification could associate at the ω-terminal, resulting in the higher gel content during storage of the SPNR latex. To what extent hydrolysis reaction of saponification with lipids and minor constituents in NR are contributing towards gel formation is not clear.

From the above results, the presence of remnant oligopeptides or small protein fragments in DPNR and the near-complete removal of proteins by surfactant washing can be assessed indirectly by measuring the resistance to thermal oxidative aging represented as plasticity retention index (PRI). The measurement of softness by load-pressing is performed after preheating a rubber sample at 140 °C for 30 min. The more a rubber sample is softened by pressing, the more that rubber sample is susceptible to degradation by thermal oxidative aging. FNR showed the highest PRI value compared to the others, meaning that FNR has the highest resistance to thermal oxidative aging (Figure 8). This is due to the known fact that FNR contained a large amount of natural antioxidants in the form of amino acids present in NR.[29] The PRI values for DPNR and SPNR are comparable, but at slightly less than one-half that of FNR. This is clearly due to the loss of beneficial proteins and amino acids from the rubber, but whatever remaining can still bestow some resistance to thermal oxidative aging. The WSNR sample already appeared like a sticky glutinous material during the preheating procedure and therefore could not be subjected to load press. This indicates that WSNR has extremely low resistance to thermal oxidative degradation, in agreement with the notion of near absence of naturally occurring antioxidants such as amino acids and proteins in the NR after surfactant

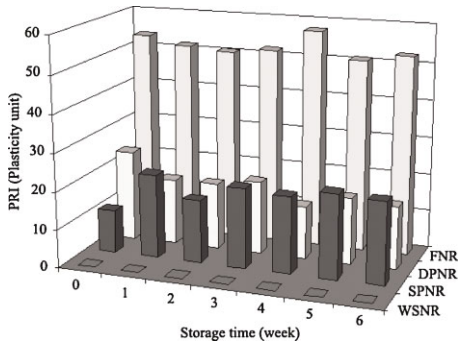

Figure 8.
Change in Plasticity retention index (PRI) of rubber from FNR and deproteinized NR after storage.

Copyright © 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

washing with centrifugation. This finding also corroborated the fact that DPNR still contains residual proteins such as oligopeptide and protein fragments.

Conclusions

Enzymatic treatment of NR latex results in the cleavage of proteins in DPNR latex. The residual proteineous substances in DPNR latex are responsible for gel formation during storage. The successive washing by surfactant leads to a WSNR that does not link proteins at the ω-chain ends of the rubber molecules. Consequently gel cannot be formed in WSNR during storage in the absence of proteins. The gel formed in SPNR latex is due to the residual degraded proteins but other unidentified reaction could also be involved. The green strength of deproteinized NR is depended on the presence of gel network and hence is related to the protein level in the latex. The higher PRI value of DPNR compared to WSNR supports the assumption that DPNR still contains naturally occurring antioxidant derived from the residual proteins.

Acknowledgements: The authors would like to express their gratitude to the Thailand Research Fund (TRF) through the RSA 5280009 and the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education. We also would like to thank the Thai Rubber Latex Co. Ltd. for the supply of fresh natural rubber latex.

[1] P. W. Allen, G. F. Bloomfield, *Chemistry and Physics of Rubber Like Substances*, L. Bateman, Ed., Maclaren and Sons, London **1963**, p. 51.
[2] Y. Tanaka, *Rubber Chem. Technol.* **2001**, *74*(3), 355.
[3] Y. Tanaka, *Prog. Polym. Sci.* **1989**, *14*(3), 339.
[4] D. Mekkriengkrai, J. T. Sakdapipanich, Y. Tanaka, *Rubber Chem. Technol.* **2006**, *79*(2), 366–379.
[5] L. Tarachiwin, J. Sakdapipanich, K. Ute, T. Kitayama, T. Bamba, E. Fukusaki, A. Kobayashi, Y. Tanaka, *Biomacromolecules*, **2005**, *6*(4), 1858.
[6] W. G. Wren, *Rubber Chem. Technol.* **1942**, *15*(1), 107.
[7] J. Yunyongwattanakorn, Y. Tanaka, S. Kawahara, W. Klinklai, J. Sakdapipanich, *Rubber Chem. Technol.* **2003**, *76*(5), 1228.
[8] S. Kawahara, Y. Isono, J. T. Sakdapipanich, Y. Tanaka, *Rubber Chem. Technol.* **2002**, *75*(4), 739.
[9] J. Tangpakdee, Y. Tanaka, *J. nat. Rubb. Res.* **1997**, *12*(2), 112.
[10] A. H. Eng, S. Ejiri, S. Kawahara, Y. Tanaka, *J. Appl. Polym. Sci., Appl. Polym. Symp.* **1994**, *53*, 5.
[11] B. L. Archer, *Proc. Int. Rubb. Conf., Kuala Lumpur.* **1975**, *2*, 532.
[12] W. S. E. Fernando, P. A. J. Yapa, P. P. Jayasinghe, *Kautsch. Gummi. Kunstst.* **1985**, *38*, 1010.
[13] S. Varghese, Y. Katsumura, K. Makuuchi, F. Yoshii, *Rubber Chem. Technol.* **2000**, *73*(1), 80.
[14] J. Yunyongwattanakorn, J. Sakdapipanich, Y. Tanaka, V. Wongsasutthikul, *Rubber Chem. Technol.* **2008**, *81*, 121.
[15] S. Kawahara, W. Klinklai, H. Kuroda, Y. Isono, *Polym Adv. Technol.* **2004**, *15*(4), 181.
[16] W. Klinklai, T. Saito, S. Kawahara, K. Tashiro, Y. Suzuki, J. T. Sakdapipanich, Y. Isono, *J. Appl. Polym. Sci.* **2004**, *93*(2), 555.
[17] D. Mekkriengkrai, J. T. Sakdapipanich, Y. Tanaka, *Rubber Chem. Technol.* **2006**, *79*(2), 366.
[18] K. Nawamawat, D. Mekkriengkrai, J. T. Sakdapipanich, Y. Tanaka, *Kautsch. Gummi. Kunstst.* **2008**, *66*, 518.
[19] H. Hasma, *J. nat. Rubb. Res.* **1992**, *7*(2), 102.
[20] J. T. Sakdapipanich, K. Nawamawat, S. Kawahara, *Rubber Chem. Tech.* **2002**, *75*(2), 179.
[21] L. Tarachiwin, J. T. Sakdapipanich, Y. Tanaka, *Rubber Chem. Technol.* **2003**, *76*(5), 1185.
[22] M. R. Ambler, R. D. Mate, J. R. Purdon, *J. Polym. Sci., Polym. Chem. Ed.* **1974**, *12*(8), 1771.
[23] G. M. Bristow, *J. Polym. Sci.* **1962**, *62*(174), S168.
[24] E. T. Creighton, *Proteins: Structure and molecular properties*, 2nd Edition, W. H. Freeman and Company, New York **1993**, 1.
[25] D. Mekkriengkrai, Biosynthesis study of natural polyisoprenes. Initiation step of rubber formation. Polymer Science and Technology [Ph.D thesis]. Bangkok, Thailand: Mahidol University; **2005**.
[26] T. Kawazura, S. Kawahara, Y. Isono, *J. Appl. Polym. Sci.* **2005**, *98*(2), 613.
[27] J. Tangpakdee, Y. Tanaka, *Rubber Chem. Technol.* **1997**, *70*(5), 707.
[28] S. Toki, I. Sics, S. Ran, L. Liu, B. S. Hsiao, *Rubber Chem. Technol.* **2004**, *77*(2), 317.
[29] R. F. A. Altman, *Rubber Chem. Technol.* **1948**, *21*(3), 752.

Copyright © 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim