**Effect of Protein Crosslinking by Maillard Reaction on Natural Rubber Properties**

**Introduction**
Natural rubber (NR) is an industrially significant polymer because of its various applications and products covering the fields of medicine, laboratory, chemistry, technology, and engineering. Fresh NR latex tapped from the *Hevea Brasiliensis* tree is composed of about 25-35% rubber fraction, 5% non-rubber components, with water accounting for the remaining [1,2]. The non-rubber components, such as proteins and phospholipids, are not only distributed in the serum fraction, but also found on the rubber particle surface. The protein-phospholipid layers covering the rubber particle surface are especially interesting due to their ability to enhance the colloidal stability of NR latex [3-5]. Major components of the adsorbed proteins are most likely α-globulin and hevein [6-7]. The in-depth structural analysis revealed that rubber molecules in NR are composed of long-chain branched structure [8]. Proteins in NR were presumed to form branch-points via hydrogen bonding between the rubber chains. It is well-known that proteins in NR play an important role in controlling NR properties. Unpurified NR presents shorter cure time than purified NR because there are some phospholipids and proteins acting as natural accelerators for curing reaction [9]. The accelerated degradation of saponified skim rubber due to the decrease of nitrogenous substances acting as antioxidants after saponification was also reported [10]. Proteins in NR markedly affected the vulcanization characteristics, physical properties and mechanical properties of NR [11]. Maillard reaction is a type of non-enzymatic browning which involves the reaction of carbonyl groups and the amines of proteins. This reaction plays an important role in food processing which results in the formation of aroma, taste and color in foods [12-14]. The chemistry underlying the Maillard reaction is very complex because a wide range of reaction products are formed [15]. The improved thermal stability of modified soy protein concentrate resin could be attributed to the crosslinks formed by glutaraldehyde with soy protein concentrate [16-17]. It is reported that the addition of nutrient-rich banana skin powder into NR latex could induce the Millard reaction, a proten crosslinking, leading to the reduction of allergenic protein and the improvement of mechanical properties of the NR products [18]. The formation of brownish compounds by Maillard reaction occurred in NR containing glucose after vulcanization process due to the presence of N-cyclohexylbenzothiazole-2-sulfenamide (CBS), a nitrogenous accelerator [19]. Recently, Maillard crosslinking of proteins in fresh NR latex had the remarkable effect on bulk NR properties, i.e., solvent resistance, hardness, resistance to oxidation, rheological behavior and resistance to stretching out [20]. In this paper, proteins in fresh NR latex were cross-linked by the Maillard reaction prior to coagulation. The obtained rubber was then compounded and properties of the rubber compound such as cure characteristics, mechanical properties and thermal resistance were investigated.

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Industrial grade and used as received. Tetrasulfenamide (TBBS), tetramethyl thiosulfane (TMTD) and sulfur were in-
sulfenamide (TBBS), tetramethyl thiourea, N-tert-butyl-2-benzothiazole.
3.46%, respectively. Other compounding
to ISO 2004, were 30.73%, 27.27% and
38 KGK
of fresh NR latex, determined according
tent (DRC) and non-rubber content (NRC)
tal solid content (TSC), dry rubber con-
tation in Rayong province, Thailand. To-
NR latex was obtained from rubber plan-
ized water and dried at 70˚C for 24 h.
subsequently sheeted on a two-roll mill
drochloric acid. The NR coagulum was
glass plate and then put in an oven at
ASTM: E573-01. The latex was cast on the
were used. Fresh NR latex was obtained from rubber planta-
total solid content (TSC), dry rubber con-
total solid content (TSC) and non-rubber content (NRC)
of fresh NR latex, determined according to
ISO 2004, were 30.73%, 27.27% and
3.46%, respectively. Other compounding
chemicals such as zinc oxide (ZnO), stea-
ric acid, N-tetra-butyl-2-benzothiazole
sulfenamide (TBBS), tetramethyl thiu-
amine and then evaporated of water was com-
plete. NR film was put on the sample
holder. The measurement conditions we-
were set as follows; crystal: ZnSe, angle: 45
degrees, light source: middle range infra-
red (4000-650 cm-1), resolution: 4 cm-1,
detector: TGS. Changes in peak area at
wavenumber 1,480 - 1,575 cm-1, referred
to amide band (C-N stretching and N-H
bending), were recorded.

**Experimental**

**Materials**

Formaldehyde (37% solution) and glutar-
aldehyde (25% solution) were purchased from J.T Baker and Sigma-Aldrich, respec-
tively. Their original pH was adjusted to
be a neutral pH by using potassium carbo-
nate (K2CO3) before being used. Fresh NR latex was obtained from rubber planta-
tion in Rayong province, Thailand. To-
total solid content (TSC), dry rubber con-
tent (DRC) and non-rubber content (NRC)
of fresh NR latex, determined according to
ISO 2004, were 30.73%, 27.27% and
3.46%, respectively. Other compounding
chemicals such as zinc oxide (ZnO), stea-
ric acid, N-tetra-butyl-2-benzothiazole
sulfenamide (TBBS), tetramethyl thiu-
ram disulfide (TMTD) and sulfur were in-
dustrial grade and used as received.

**Sample preparation and characterization**

Formaldehyde (HCHO) and glutaralde-
yde (C5H8O2) were added to fresh NR latex
in amounts of 0, 50, 100 and 200 mmol/
kg of latex. The latex mixture was conti-
nuously stirred for 1 h at room tempera-
ture and then coagulated with 5 wt% hy-
drochloric acid. The NR coagulum was subsequently sheeted on a two-roll mill
(Labtech LRM150), washed with deio-
nized water and dried at 70°C for 24 h.
Nitrogen content of the dried NR was
measured in accordance with SMR: Bulle-
tin No. 7-1992. Attenuated total reflec-
tance infrared (ATR-IR) spectroscopy
was confirmed by ATR-IR spectroscopy. The most
distinctive spectral features for proteins
were the strong amide I and amide II
bands. Amide I band (1,600-1,690 cm-1) is
referred to C=O stretching and amide II
bending, was calculated as shown in Equation 1;

\[
\text{Swelling ratio} = \frac{\text{Ws} - \text{Wd}}{\text{Wd}} \times 100\%
\]

Where Wd and Ws are specimen weights before and after immersion, respectively. Determination of hardness was carried out using a Wallace Shore A durometer according to ISO 7619 Part 1. Tensile properties were determined using a uni-
versal testing machine (Instron 3366 se-
ries) following ISO 37 (die type 1). The
ternal resistance of the rubber vulcanizates
was determined by ageing the speci-
mens in an air-circulated oven at 70°C for
24 h prior to the measurement of hard-
ness and tensile properties. The thermal
resistance is represented in terms of the
relative properties, i.e., the ratio of the
properties after ageing to those before
ageing.

**Results and discussion**

**Characterization of the prepared samples**

Crosslinking of proteins in NR was con-
firmed by ATR-IR spectroscopy. The most
distinctive spectral features for proteins
are the strong amide I and amide II
bands. Amide I band (1,600-1,690 cm-1) is
referred to C=O stretching and amide II
band (1,480-1,575 cm-1) is corresponded
to C-N stretching and N-H bending [21],

\[
\text{Swelling ratio} = \frac{\text{Ws} - \text{Wd}}{\text{Wd}} \times 100\%
\]
Figure 1 represents the ATR-IR spectra of the NR film after treating fresh NR latex with various contents of Maillard reagents. Changes in peak area of amide II band at wavenumbers 1,548 cm⁻¹ in the ATR-IR spectra of the NR films were observed after treating NR latex with HCHO and C₅H₈O₂. Table 1 summarizes the data obtained from the spectra. Clearly, peak area of amide II band decreased with increasing HCHO and C₅H₈O₂ concentrations. It was explained that the aldehyde groups of HCHO and C₅H₈O₂ might combine with amine groups of proteins, forming a methylene bridge (–CH₂–) crosslink in the case of HCOH and a quaternary pyridinium crosslink or a more complicated crosslink type with numerous terminal formyl groups via Michael addition to double bonds in the case of C₅H₈O₂ [22-24]. Mechanisms of protein crosslinking by HCHO and C₅H₈O₂ as shown in Scheme 1.

Properties of the rubber compounds
The effect of Maillard crosslinking of proteins in NR on Mooney viscosity of the rubber compounds is presented in Table 3. It was found that Mooney viscosity values of the rubber compounds prepared from untreated and treated NR were not significantly different indicating that the Maillard reaction had no significant effect on viscosity of the rubber compounds. Table 3 also presents the influence of Maillard crosslinking of proteins in NR on cure characteristics such as scorch time and optimum cure time of the compounds at 150°C. Very interestingly, scorch time and optimum cure time of the compounds prepared from untreated and treated NR are presented in Table 3.

Figure 2: Swelling ratio of the vulcanizates.

![Swelling ratio of the vulcanizates.](image)

Figure 3: Effect of crosslinking of proteins in NR on thermal ageing resistance of the vulcanizates.
NR treated with C₅H₈O₂ were significantly lower than those of the compounds prepared from NR treated with HCHO and untreated NR, respectively. As expected, scorch time and optimum cure time of the compounds appeared to decrease continuously with increasing HCHO and C₅H₈O₂ concentrations. This is simply attributed to the increased protein content in NR (see Table 2) because it is widely known that proteins in NR could accelerate sulfur vulcanization [11, 25].

Mechanical properties of rubber vulcanizates

Table 4 shows tensile strength, 100% modulus and hardness of the vulcanizates. Obviously, tensile strength of the vulcanizates was found to increase with increasing HCHO and C₅H₈O₂ concentrations. The results clearly show that crosslinking of proteins in NR could lead to the significant improvement of tensile strength. It has previously been reported that proteins are congregated and dispersed in NR matrix in a similar manner as nano-filler [26]. With cross-linking via Maillard reaction, the nano-sized harden proteins might be able to act as nano-filler leading to a considerable increase of tensile strength. At a given concentration of the Maillard reagents, tensile strength of the vulcanizate prepared from NR treated with C₅H₈O₂ was significantly higher than that of the vulcanizate prepared from NR treated with HCHO. Explanation is given by the greater reactivity of C₅H₈O₂ towards the Maillard reaction resulting in a greater protein content in NR, as evidenced by the increased nitrogen content. Hardness and 100% modulus of the vulcanizates prepared from HCHO-treated NR were comparable with those of the vulcanizate prepared from untreated NR. However, by treating with C₅H₈O₂, 100% modulus and hardness of the vulcanizates were slightly enhanced. As modulus and hardness of the rubber vulcanizate are closely related to cross-link density, the results in Table 4 therefore indicated the significant increase of cross-link density when proteins in NR were cross-linked by C₅H₈O₂. This is further confirmed by the swelling test results as shown in Figure 2. It was found that the swelling ratio was noticeably reduced when the NR was treated by C₅H₈O₂. The higher cross-linked protein content might be the possible explanation for such finding.
Thermal properties of rubber vulcanizates

The effect of Maillard crosslinking of proteins in NR on thermal resistance of the vulcanizates represented in terms of the relative properties is shown in Figure 3. It could be seen that the values of relative hardness and relative 100% modulus of all vulcanizates were greater than 1 indicating the post curing effect after thermal ageing. However, the relative hardness and relative 100% modulus of the vulcanizates were not extensively changed after treating with either HCHO or C$_5$H$_8$O$_2$ implying that crosslinking of proteins in NR had a little effect on relative hardness and relative 100% modulus of the vulcanizates. Interestingly, the relative tensile strength and elongation at break of the vulcanizates sharply increased when NR was treated with HCHO and C$_5$H$_8$O$_2$. The improvement of thermal resistance was more obvious in NR treated with C$_5$H$_8$O$_2$. As it is widely accepted that proteins in NR could act as natural antidegradants for rubber [10, 27-28] and the improved thermal stability of modified soy protein concentrate resin can be attributed to the crosslinks formed by glutaraldehyde with soy protein concentrate [16-17], explanation for the improvement of thermal resistance is therefore given by the increased protein content through the Maillard reaction.

Conclusions

ATR-IR techniques confirmed the protein crosslinking in NR. Changes in peak area of amide II band (C-N stretching and N-H bending) at wavenumbers 1,548 cm$^{-1}$ in the ATR-IR spectra of the NR films were noticed after treating NR latex with HCHO and C$_5$H$_8$O$_2$. The higher amount of proteins in treated NR could result in acceleration of sulfur vulcanization. Tensile strength of the vulcanizates prepared from treated NR was obviously greater than that of the vulcanizates prepared from untreated NR. However, the increases of 100% modulus and hardness were only pronounced when NR was treated by C$_5$H$_8$O$_2$. The Maillard crosslinking of proteins in NR could also significantly improve the thermal resistance of the vulcanizates.

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References