Removal of Protein from Natural Rubber in Pilot Scale Toward Production of Low Protein Rubber Gloves

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Abstract

Removal of protein from natural rubber was carried out at pilot scale in latex stage to prepare low-protein natural rubber (LPNR) and the resulting product was subsequently subjected to rubber glove production. The LPNR was prepared by the novel procedure to remove proteins from natural rubber using urea as denaturing agent together with a polar solvent, i.e. ethanol in the presence of sodium dodecyl sulfate (SDS) as a surfactant. The condition for the removal of protein was investigated in term of number of centrifugation in large scale. The residual protein, which is assessed by the amount of nitrogen in the sample, was determined by Kjeldahl method. The results showed that in the presence of ethanol, the nitrogen content decreased from 0.38 wt.% to approximately 0.07 wt.% after two times of centrifugation. The rubber glove made from the LPNR latex contains the lowest nitrogen content among various commercial rubber gloves.

Keywords: removal of protein, pilot scale, latex stage, rubber glove, extractable protein

1. Introduction

The removal of proteins from natural rubber (NR) at pilot scale may attract attention to become an important process in rubber industry since it allows to prepare low protein natural rubber (LPNR) latex at bulk scale necessary for production of high quality rubber product, i.e. low-protein rubber glove. The removal of proteins from NR was performed through a continuous process to prepare deproteinized natural rubber (DPNR) rapidly and efficiently. The proteins existing on the surface of NR particles in the latex state may cause a latex allergy mediated by type I immunoglobulin E in sensitive individuals. The proteins are either chemically bonded or physically held by the rubber particles. The former is cleaved with a proteolytic enzyme such as alkaline protease and the latter is denatured with urea which may change the conformation of the proteins [1]. In previous work, the removal of proteins was primarily performed in the latex stage with a proteolytic enzyme that might decompose the proteins. In fact, it has already been reported that the total nitrogen content of NR was reduced to less than 0.02 wt.% after incubation with a proteolytic enzyme, which was about 1/20 of that of untreated NR [2]. However, deproteinization using that enzyme must be performed in a batch system due to the long incubation time and strict temperature control. Therefore, it is quite important to develop a novel technique to apply for continuous process.

The removal of protein from natural rubber using urea and polar solvent has been developed so far in the previous work [3]. It is clearly shown that protein is efficiently removed from natural rubber latex, in which nitrogen content is about 0.00 wt.%. Therefore, the combination of both urea and polar solvent is proved to be very useful technique to detach protein from natural rubber.

In the present work, we apply the concept of denaturing protein using urea and surfactant to remove protein from natural rubber at pilot scale. The resulting LPNR was used as a staring material for rubber glove production. The nitrogen content, which is proportional to the protein present in NR, were compared among various rubber gloves from different sources. The mechanical properties of corresponding rubber gloves also were measured to figure out whether the removal of proteins affect the performance of glove products or not.

2. Experimental

2.1 Materials

Natural rubber latex used in this work is high ammonia natural rubber provided from MERUFA joint-stock company. Sodium dodecyl sulfate (SDS,

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99%) was purchased from Kao chemicals company (Taiwan). Urea (99.5%) was obtained from Merck (Germany). The other chemicals were purchased from Sigma-Aldrich.

2.2 Purification of sample

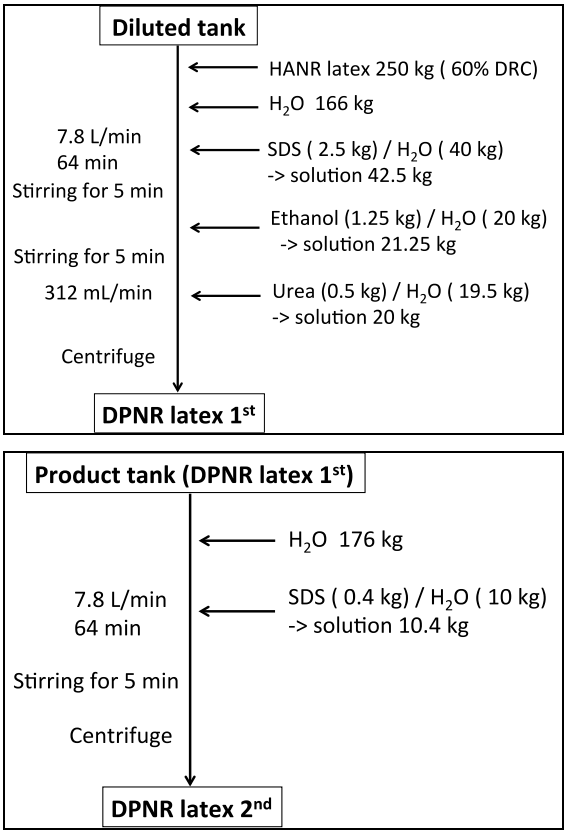

Fig. 1. Operation of Test plant

The removal of protein was carried out at pilot scale, which is shown in the Figure 1. The NR latex used in this study was Vietnam commercial high-ammonia natural rubber (HANR) latex. The latex was diluted with water and surfactant followed by adding polar solvent of 0.25 wt.%. Subsequently, the urea solution was added into the latex with the flow rate of 312 ml/min. The mixture, then, was subjected to the first continuous centrifugation. The resulting concentrated latex fraction was redispersed in a 0.2 wt.% SDS and washed for the second centrifugation. The latex was subjected to glove manufacturing process. The sample for nitrogen content determination was coagulated with methanol and completely dried at 50 °C for about one week.

2.3 Nitrogen content determination

Measurement of nitrogen content is performed according to Kjeldahl method described in the reference [4]. A 0.1 g dried rubber was digested with about 2.5 ml concentrated H_2SO_4 in the presence of 0.65 g catalyst mixture (K_2SO_4:$CuSO_4$:Se=15:2:1) until obtaining the clear green homogenous solution. The resulting solution was distilled and preserved in H_3BO_3 2 wt.% and the nitrogen content was determined by titration the distillated with 0.01N H_2SO_4 using methyl red as an indicator.

2.4. Extractable protein content measurement

Extractable protein content of rubber glove was determined by the modified Lowry method using DC protein assay. The rubbers films was extracted with phosphate buffer with a ratio of 1g-rubber/5 ml phosphate buffer. The extraction process was maintained under continuous stirring for about 6 hours at 40 °C. The extractable protein content in the extracted solution was determined by the Bio-Rad DC protein assay [5,6] using bovine gamma globulin (BGG) as a standard.

2.5 Tensile strength measurement

The tensile test was performed at room temperature using a Toyo Seiki Strograph VG10E, according to JIS K6251. Dumbbell-shaped rubbers No.7 were subjected to cut the sample and the thickness of the rubber glove, which was about 0.1-0.2 mm, was measured by thickness gage (dial type) (Mitutoyo, Japan). Crosshead speed used was 200 mm/min. The measurement was repeated three times for each sample.

3. Results and discussion

3.1 Removal of protein from natural rubber

The total nitrogen content of HANR (control sample) and DPNRs, which is proportional to the content of proteins present in the rubber, were shown in Table 1. The three different operations showed the similar results indicating that the consistency and stability of operation of the production line. It is shown that the nitrogen content of the rubber after the second centrifugation is almost reduced about half, which is about 0.06-0.08 wt.%.

Table 1. Nitrogen content of various DPNRs prepared at pilot scale

Operation	Product	Number of centrifugation	Nitrogen content (wt.%)
-	HANR	-	0.380
1	DPNR1	1	0.168
	DPNR2	2	0.082
2	DPNR1	1	0.153
	DPNR2	2	0.072
3	DPNR1	1	0.130
	DPNR2	2	0.063

3.2 FT-IR spectroscopy

Figure 2 shows FT-IR spectra for HANR, DPNR1 and DPNR2, ranging from 3200 to 3500 cm^{-1} and from 1500 to 1800 cm^{-1}. In the FT-IR spectra of HANR, six absorption peaks appeared at around 3280, 1730, 1710, 1660, 1624 and 1540 cm^{-1}. The peaks at 3280, 1624 and 1540 cm^{-1} were identified to stretching vibrations of N-H, amide I and amide II bonding, respectively, which can be attributed to the presence of proteins. The intensity of these signals were reduced from HANR to DPNR1 and DPNR2. The reduce in the signal intensity is confirmed by the decrease in the amount of proteins represented by the nitrogen content estimated by Kjeldahl method. The absorption peaks at 1730 cm^{-1} was assigned to stretching vibration of C=O of fatty acid ester group, whose intensity reflects amount of fatty acid present in NR samples.

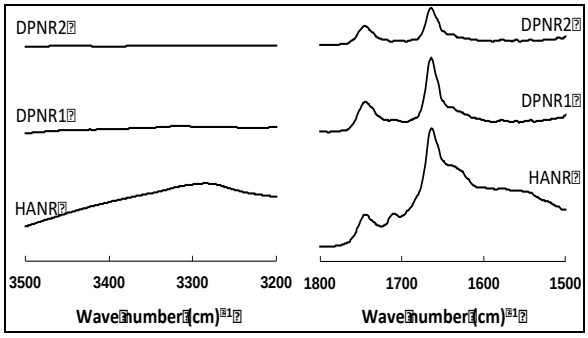

Fig. 2. FT-IR spectra of HANR, DPNR1 and DPNR2

The LPNR latexes were used as starting materials for glove manufacturing process. Although DPNR2 contains the lowest nitrogen content, considering the cost to produce this product, in this work, we select DPNR1 for glove manufacturing. In that process, the latex was mixed with a recipe of processing compounds include sulfur, zinc oxide, accelerators, pigments, stabilizers and antioxidant.

The protein content of the rubber glove was examined by determination of nitrogen content and the extractable protein content. Table 2 shows the nitrogen content and the extractable protein content of the rubber gloves made from LPNR latex (glove A) and rubber glove which is commercially available from Duy Hang company (glove B).

Table 2. Nitrogen content and extractable protein content of DPNR rubber gloves

Product	Nitrogen content (wt.%)	Extractable protein content (µg/g-rubber)	Made from
Glove A	0.175	83.75	DPNR1
Glove B	0.404	118.53	(*)

It could be seen that the nitrogen content and the extractable of protein content of glove A are quite smaller than those of glove B (commercial product *). In previous work [7], high level of extractable protein content was found to be associated with the positive allergic responses while very low extractable protein content shows very weak or no allergic problem. Therefore, in this case, rubber glove containing high nitrogen content or high extractable proteins may have high possibility to bring about the type I allergy to hypersensitive persons.

3.3 Tensile strength

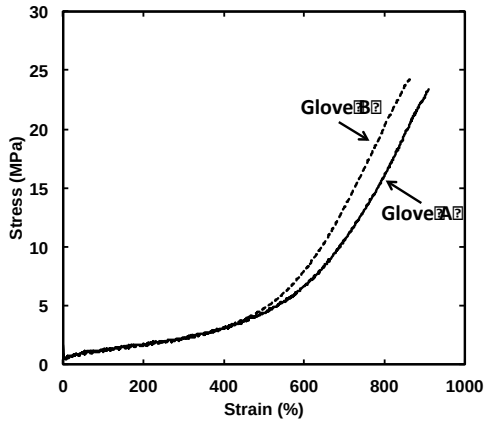

Fig. 3. Stress-strain curves of glove A and glove B

Figure 3 shows the stress-strain curves of glove A and glove B. Both products have similar mechanical properties that stress at break of about 24-25 MPa and strain at break of about 850-900%. This result implies that the mechanical properties of final product is insignificantly affected by the removal of proteins.

3.4 Nitrogen content of various rubber gloves

Table 3. Nitrogen content of various commercial rubber gloves

Product	Nitrogen content (wt.%)	Commercial products from
Glove C	0.2289	Nam Long company
Glove D	0.2069	Nam Cuong company
Glove E	0.1944	Khai Hoan company
Glove F	0.2945	Dunlop company, Japan
Glove G	0.1919	Dunlop company, Japan

Several commercially available rubber gloves from different companies were subjected to the nitrogen content determination for screening test. The results are listed in Table 3. It is found that all rubber

gloves (from glove C to glove G) contain higher nitrogen content than that of glove A and lower than that of glove B. It could be inferred that the amount of extractable protein of glove C to glove G may also higher than that of glove A and lower than that of glove B. It may be concluded that the low protein natural rubber latex plays an important role in preparation of latex-dipped products, such as medical glove or medical products to prevent the unexpected allergic symptoms for users, particularly who are atopic.

4. Conclusion

The removal of proteins from NR latex at pilot scale was made by incubation of the latex with urea, a polar solvent in the presence of SDS. The system was scaled up to work up to about 500 kg of rubber latex each time of running. The total nitrogen content and extractable protein content of DPNR and its rubber glove was quite low compared to those of various rubber gloves. This study strongly indicates that removal of proteins can be achieved in large scale and the low proteins NR latex may be sufficient to be a starting material for manufacturing of various high quality products such as low protein rubber glove.

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