REDUCTION OF PROTEIN CONTENT IN SKIM RUBBER VIA BOTH EXTRACTIONS IN SKIM LATEX AND FROM RUBBER FILMS

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Abstract

Low-protein skim natural rubber latex suitable for manufacturing of special products for those who are allergic to proteins. Produced by centrifugation using water soluble polymer and surfactant. In this work, 6%wt/v polyethylene glycol solution was added into skim natural rubber latex and the mixture was centrifuged under various conditions, i.e. 1000, 2000, 3000 rpm and 5, 15, 20 min, to extract proteins from rubber particles in suspension before coagulation process. The skim natural rubber films were prepared from coagulated rubber and then was extracted with different extracting medium including water, 2%wt/v SDS, 2%wt/v TritonX-100 and 2%wt/v NaOH solution. The rubber film was centrifuged in the extracting medium at various conditions. The results of protein extractions from rubber particles and from the rubber film were found to be consistent. The extractable protein content at a low centrifugal speed increased with increasing centrifugal time. At a higher speed, increasing centrifugal time may not increase the extractable protein content. It was also found that 2%wt/v SDS solution could extract protein better than 2%wt/v NaOH, 2%wt/v TritonX-100 and distilled water, respectively.

Keywords: skim natural rubber latex, extractable protein, polyethylene glycol (PEG), sodium dodecyl sulfate (SDS)

Introduction

Natural rubber latex is obtained from rubber trees (*Hevea brazilenses*) grown in Malaysia, Indonesia and Thailand. It looks similar to milk but it consists mainly of cis-1, 4 polyisoprene. Field rubber latex containing 30-33%wt rubber is centrifuged to yield a latex with a rubber concentration of 60%wt and 4-6%wt skim natural rubber latex. Small factories throw skim rubber away as a waste, whereas large manufacturers recover rubber from skim natural rubber latex by using highly concentrate sulfuric acid. Natural rubber latex contains proteins, fats, carbohydrates, and inorganic substances such as potassium, magnesium, zinc, copper and iron. There are more than 250 kinds of proteins in the latex, with a total amount between 1-1.8 %wt depending on the source of latex. Each kind of proteins has different functional groups and 30-60 kinds are believed to cause allergic reactions to human (Huber et al. 2006).
Nowadays, natural rubber latex can be manufactured into a variety of products such as gloves, condoms, balloons, and medical products (Rogero et al. 2003), which are important in daily lives. Some people are allergic to proteins in products made of natural rubber latex due to the combination of the chemicals and proteins in latex gloves when they are exposed to

human skin (Honeycutt et al. 2006). Allergic reactions are mostly shown in the form of rashes and skin infections. Eliminating or reducing allergy problems of proteins in natural rubber latex can be done by using chemical method, physical means, or both as in centrifugation to precipitate proteins (Honeycutt et al. 2007). In addition, using enzyme to degrade proteins, using polymer such as polyethylene glycol (PEG) to extract protein in concentrate rubber latex (Abhilash et al. 2009), using surfactant, washing with water (leaching) or chemical solution to remove residual proteins in the latex and using ^{60}Co gamma source to reduce the water-soluble protein content in the final product were reported (Parra et al. 2005).
Using non-ionic surfactants to treat natural rubber latex can reduce allergens more than 95%. This method is a comparatively better method and it will not affect the mechanical properties to a greater extent (Ichikawa et al. 1993; Schloman et al. 2002). The surfactants which are commonly used are Triton X-100, sodium dodecyl sulfate (SDS) and phosphate buffered saline (PBS). Using Triton X-100 solution together with washing in 4 to 5 steps will reduce allergens up to 98-99% (Schloman et al. 2006) and SDS solution can extract protein better than PBS solution (Kalapat et al. 2009). At the end, reducing protein content from skim natural rubber latex is one method to increase value of the final special product. Therefore, in this study, protein extraction of skim natural latex in liquid phase by using a polymer solution and then protein extraction of skim natural rubber film with non-ionic surfactant to remove residual proteins in the latex film was investigated.

Methodology

Materials

Skim natural rubber latex was obtained from Rubber Estate Organization (total solid content of 7.67%, dry rubber contents of 4.229%, initial protein content in serum of 0.66 mg/ml, total protein content on the surface of the rubber chip of 11.17 mg/g rubber and pH of 9.03. Polyethylene glycol 6000 (PEG-6000) and Bovine serum albumin (BSA) were purchased from Sigma-Aldrich Co.LLC. (Germany). Acetic acid was purchased from Merck. Sodium dodecyl sulfate (SDS) was purchased from Ajax Finechem Pty Lty. Triton X-100 and Toluene were purchased from Panreac Quimica S.L.U. Dye reagent concentrate was purchased from Bio-Rad.

Methods

Protein extraction from skim natural rubber by a polymer solution (PEG6000)
30 ml of 6%wt/v PEG6000 solution were added to 30 ml of skim rubber latex. This was followed by centrifugation at a speed of 1000, 2000 or 3000 rpm and for 5, 15 or 30 min and the mixture was stirred by an overhead stirrer at 150 rpm for 12 min. After that the rubber was coagulated with acetic acid solution and then it was dried in an oven at 100 °C for a day. The shown result is the average of three samples.

Preparation of latex films
The latex films were cast from the solution of coagulated rubber in toluene and the solution was poured into a glass plate. Subsequently, the cast film was allowed to dry at room temperature and then removed from the plate and cut to a size of 0.5 cm x 0.5 cm with thickness of 0.033±0.02 mm of before testing.

Protein extraction from rubber films with different extracting medium
The dried films of all samples were divided and washed with extracting medium. One set of films were extracted in 2%wt/v SDS solution, using centrifugation under various conditions of 1000, 2000, 3000 rpm and 5, 15, 30 min. Other sets of films were extracted in distilled water, 2%wt/v Triton X-100 solution and 2%wt/v NaOH solution, using centrifugation at a speed of 1000 rpm for 5 min. Extracting medium was measured for protein content by Bradford method using Bovine serum albumin (BSA) as the standard protein.

Results

Extraction of protein from skim rubber in latex by using polymer solution (PEG6000).

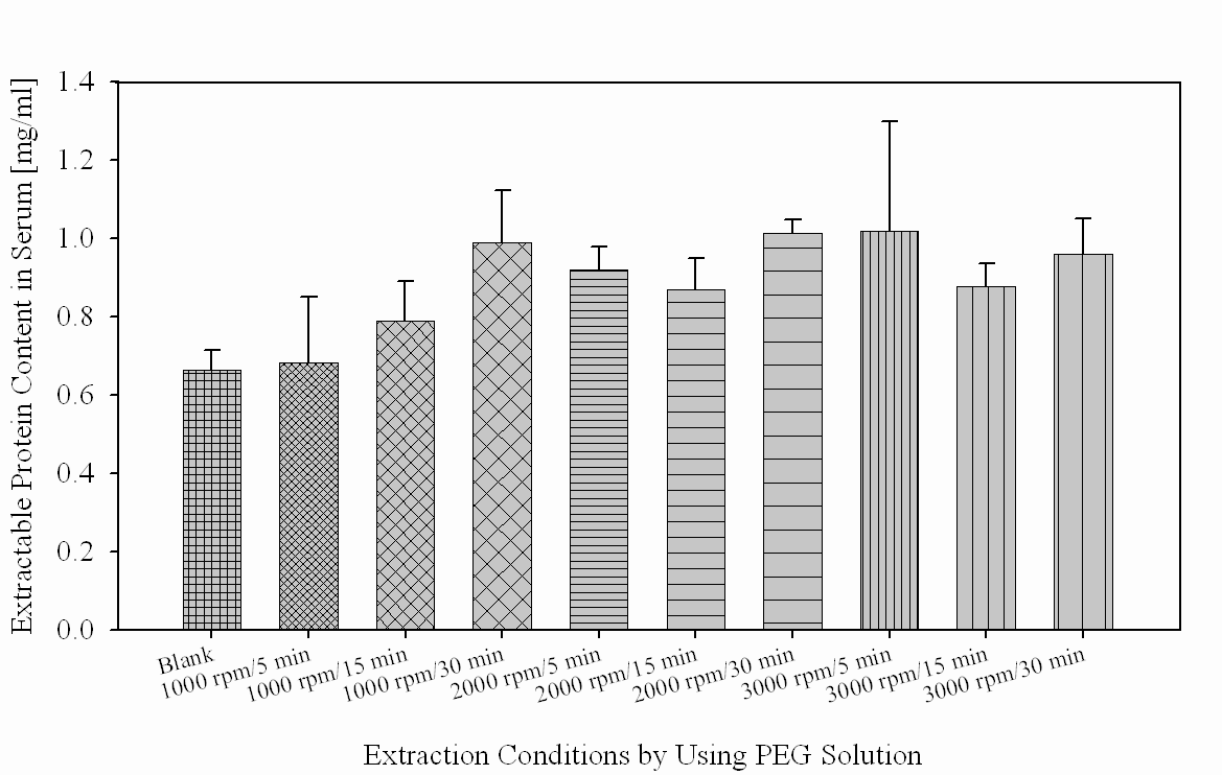

Figure 1 Extractable protein content in serum

Figure 1 shows the extractable protein content in serum after the rubber latex was extracted with the PEG6000 solution using various conditions of centrifugation. It is observed that the extractable protein content of the blank sample, which was extracted by distilled water, is the least, showing that mixing PEG6000 solution in skim natural rubber latex can extract proteins out of rubber particle surface, there by increasing extractable protein content in serum. In addition the extractable protein content in serum when using the speed of 1000 rpm increases with increasing time of centrifugation from 5 to 30 min because at a lower speed, when increasing time of centrifugation more number of PEG6000 molecules diffuse and reach the surface of rubber particles so they can interact with protein molecules and remove them from rubber particles to the serum. However, the extractable protein content in serum, when centrifugation speeds are 2000 and 3000 rpm, shows no definite trend and independent on time. This is because at a higher speed, PEG6000 molecules will move faster to reach rubber particles, when the time of centrifugation is increased, protein molecules are probably removed from polymer and stick to skim natural rubber latex again so that increasing time did not increase the extractable protein content at a high speed of centrifugation.

The % increase of the extractable protein content (EP) in serum can be calculated by using Equation (1). This equation shows the percentage of the increase of extractable protein content in serum in mg/(ml serum · g rubber) compared with the content in serum of unextracted rubber latex. It was found that the extraction at a centrifugal speed of 3000 rpm for 5 min could yield the increase of extractable protein content in skim rubber latex of 53.76%.

$$\% \text{ increase of EP} = \frac{\text{EP of extracted rubber - EP of unextracted rubber}}{\text{EP of unextracted rubber}} x\,100 \qquad (1)$$

Extraction of protein from rubber films by using different extracting medium.

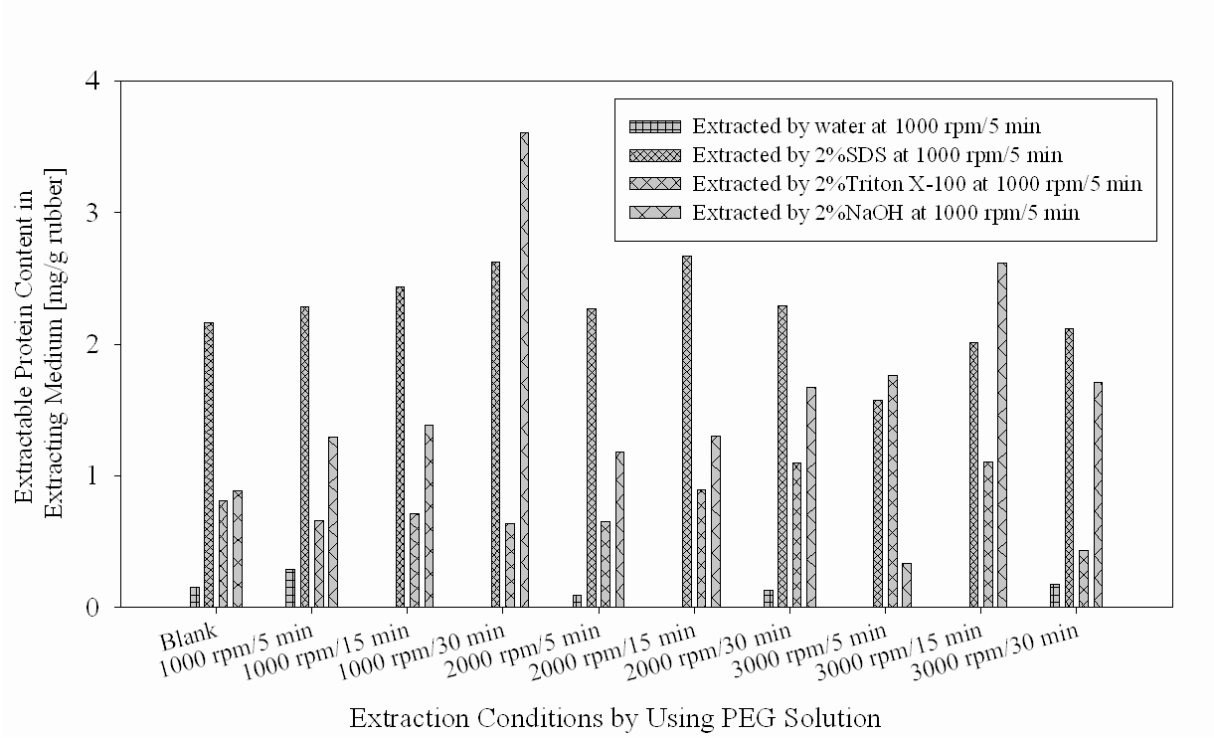

Figure 2 Extractable protein content in different extracting medium

Figure 2 shows the result of extractable protein content in distillated water, 2 types of surfactant which are 2%wt/v SDS solution and 2%wt/v TritonX-100 solution, and 2%wt/v NaOH solution, using the same condition of centrifugation speed at 1000 rpm for 5 min. The result shows that when extracted with 2%wt/v SDS solution, the extractable protein content was the highest because SDS is a surfactant consisting of two parts: a structure that is less appealing to the solvent (hydrophobic group) and another group that is most attracted to the solvent (hydrophilic group). When a surfactant was dissolved in the solvent, the hydrophobic group in the molecular structure undergoes structural changes in the solvent and increase the Gibbs free energy in the system so that SDS will gather at the surface of the film and more protein amount could be extracted. TritonX-100 is also a surfactant but less polar than SDS, thereby, having less attractive interaction with the proteins. Even though NaOH solution could extract proteins better than SDS in some samples, the final rubber product is likely to be degraded by the basic condition. Comparing all these, SDS showed the best performance in extracting protein from rubber film.

The effect of speed of centrifugation and time to extraction of protein from rubber films on extractable protein content in 2% wt/v SDS solution.

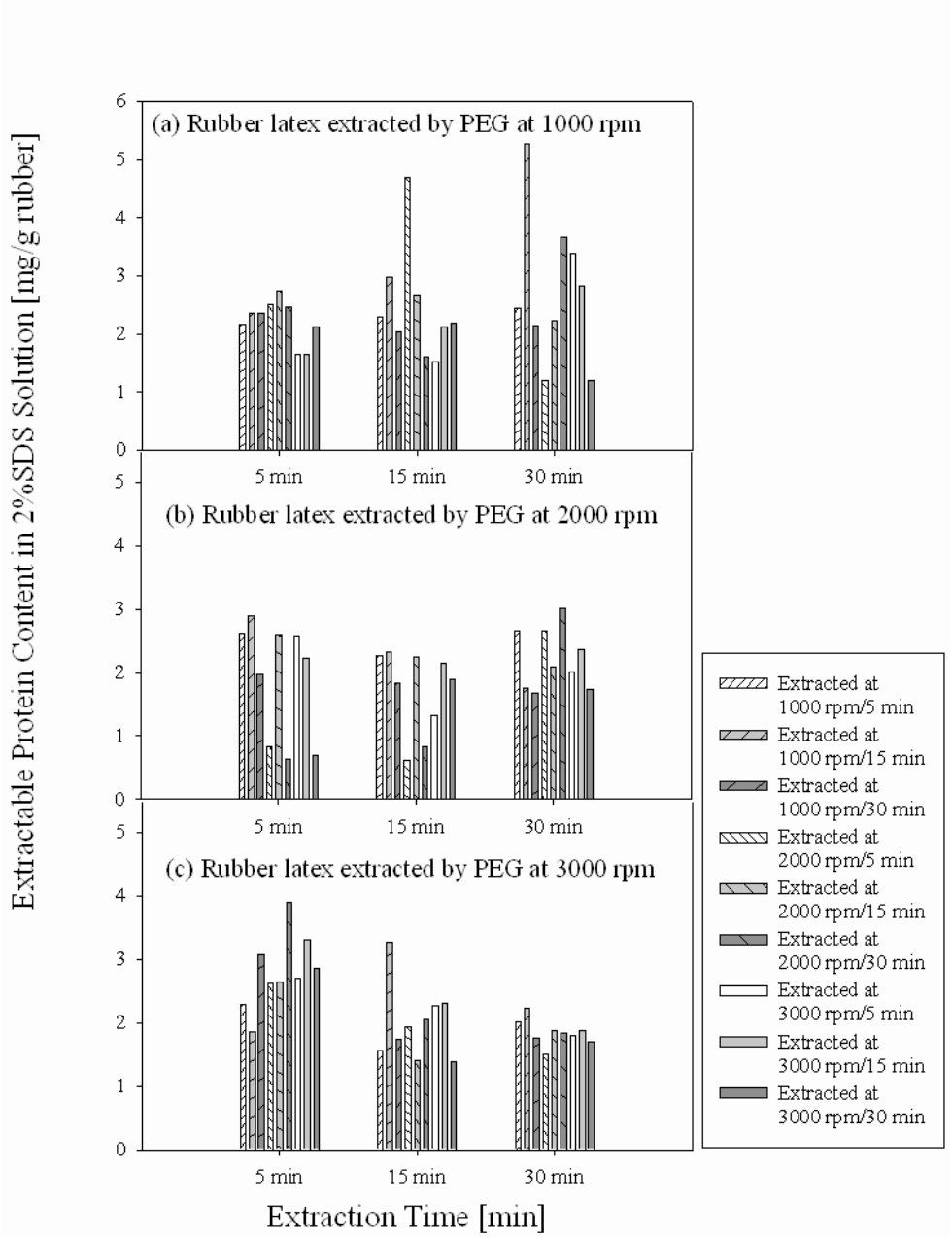

Figure 3 Extractable protein content in SDS solution

The extractable protein content in extracting medium is defined here.

$$\text{EP in extracting medium} = \frac{\text{Protein content in extracting medium (mg)}}{\text{Weight of rubber film (g)}} \qquad (2)$$

It could be observed in the previous section that extraction with 2%wt/v SDS solution yielded the highest extractable protein content. Therefore, 2%wt/v SDS solution was chosen as the extracting medium for the rubber film and the experiment were done by varying the speeds of centrifugation to be 1000, 2000 and 3000 rpm and the centrifugal time to be 5, 15 and 30

min. It is observed that when considering the result at each speed of centrifugation, no clear trend is seen as shown in Figure 3 so we decided to average the result of all eight conditions and found that the average of extractable protein contents from rubber films showed similar trend to the extractable protein content in serum as previously shown in Figure 1. Therefore, it is of interest to conclude that at a low speed of 1000 rpm, the extractable protein content in 2%wt/v SDS solution increased with increasing time of centrifugation and at high speed of 2000 and 3000 rpm extractable protein contents in 2%wt/v SDS solution were independent on time. However, at 3000 rpm and 5 minute more proteins can be extracted.

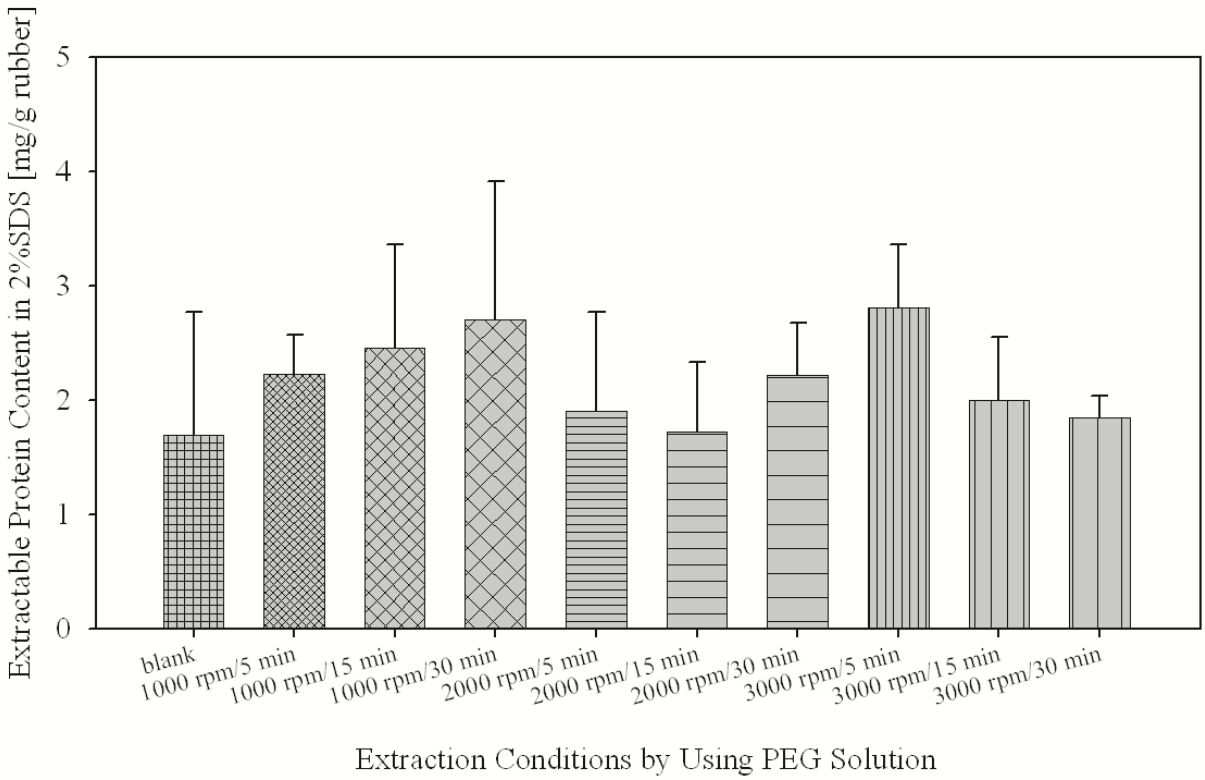

Figure 4 The average extractable protein content in 2%SDS solution

Considering Figures 1 and 4, the trend of extractable protein content in serum is the same as that of extractable protein content in 2%wt/v SDS solution. This may be concluded that using 6%wt/v PEG6000 to extract protein from rubber in latex not only allows more protein to be dissolved in to serum, it also allows the proteins to be dissolved in surfactant (2%wt/v SDS solution) more efficiently. This is a probably due to the fact that PEG is already attached to the coagulation rubber before casting a film, when the film is extracted with the 2%wt/v SDS solution, the polymer molecule could help facilitate the extraction in extracting medium again.

Discussion and Conclusion

Both protein extractions in skim latex phase and from rubber film were studied. It was found that the extractable protein content at a low speed increased with increasing centrifugal time from 5 to 30 min while the extractable protein content at a high speed did not depend on time of centrifugation. However extraction in skim latex phase at centrifugal speed of 3000 rpm for 5 min can yield an increase in protein content of 53.76%.

The comparison of extraction with different extracting medium showed that 2%wt/v SDS solution can extract proteins better than 2%wt/v NaOH solution, 2%wt/v TritonX-100 solution and distilled water, respectively. The results of the extractable protein content in different extracting mediums were varied probably because of the attachment of the polymer inside of rubber matrix from the previous stage of extraction. This should be investigated in more detail in the future.

Acknowledgements

The financial support from National Research Council of Thailand (NRCT) for the fiscal year of 2012 is highly acknowledged.

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