

Characterization of Enzyme-Deproteinized Skim Rubber

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ABSTRACT: Skim natural rubber latex is a protein-rich byproduct obtained during the centrifugal concentration of natural rubber latex. Skim latex has a very low dry rubber content (4–8%), and the rubber particles are smaller in size. It has a higher proportion of nonrubber solids, which are mostly proteinaceous in nature. It is difficult to coagulate, and it takes more processing time. The proteins in skim latex can be decomposed by proteolytic enzymes. This article discusses the use of stabilized liquid papain from the papaya plant (*Carica papaya*) for deproteinization followed by creaming for quick and easy coagulation of skim latex. The technological properties and aging characteristics of the deproteinized skim rubber are compared with those of conventionally prepared skim rubber and block rubber. The deproteinized skim rubber showed enhanced quality parameters. Particle size analysis revealed that deproteinization and creaming of the skim latex did not markedly change the particle size. Gel permeation chromatography

showed a reduction in the quantity of fatty acids after deproteinization and creaming, which was reflected in the improvement of the aging characteristics in comparison with the control sample. Vulcanizates prepared with the deproteinized skim rubber had higher resilience, lower heat buildup, lower compression set, and good tensile strength and elongation at break in comparison with conventionally prepared skim rubber, and the properties were almost comparable to those of block rubber. The improvement in the mechanical properties and aging characteristics could be attributed to the reduction of the protein content, the partial removal of unsaturated fatty acids, and the removal of metal ions that were pro-oxidants during the deproteinization and creaming process. © 2009 Wiley Periodicals, Inc. *J Appl Polym Sci* 114: 3319–3324, 2009

Key words: deproteinization; gel permeation chromatography (GPC); mechanical properties; skim rubber; vulcanization

INTRODUCTION

Skim latex is a byproduct of natural rubber latex concentrate production. When field latex is concentrated by centrifugation, about 10–15% of the total rubber together with an enhanced proportion of the nonrubber constituents of the original latex remains in the serum phase with 5–8% dry rubber content; this is known as skim latex. It is mostly composed of very small rubber particles with a high specific surface area.^{1,2} The absorbed, soluble, and suspended components together make the nonrubber solid content in skim latex much higher than that in field latex. The rubber recovered from it, skim rubber, retains a considerable proportion of these nonrubber ingredients.

Natural rubber, when processed into block rubber or sheet rubber, contains rubber hydrocarbon, acetone-soluble fatty materials, and proteins with average concentrations of 95%, 3%, and 2%, respectively. Skim rubber, however, is only about 70–85% rubber hydrocarbon, 5–10% acetone-soluble fatty materials, and 10–20% proteins.^{3,4} Consequently, the process-

ability and vulcanizate properties of skim rubber are different from those of latex and field grades of natural rubber. Skim rubber has a bad odor and rapid curing behavior with wide variations in the mechanical and aging properties. The concentration of copper ions, which are pro-oxidants, is usually high in skim rubber,⁵ and they affect aging adversely. The dirt content is low, and at present, skim rubber is recovered from skim latex by direct coagulation with concentrated sulfuric acid.⁶ The resulting rubber is of little value because of the presence of many nonrubber components included during the course of coagulation.⁷ Furthermore, acid coagulation requires a large amount of acid to neutralize the high ammonia content in the skim latex, and the addition of concentrated sulfuric acid can result in some side reactions on the rubber molecule, such as degradation and cyclization.⁸ Another problem is that if the skim latex is stored for longer periods, the small rubber particles in the skim latex cannot be coagulated by acid coagulation.⁸ Autocoagulation of the skim latex is another method to recover the skim rubber from the skim latex⁹ (i.e., putrefactive coagulation), and it requires considerable storage space.

A possible solution appears to be the removal of the undesirable nonrubber constituents from the skim latex while the rubber is recovered. Morris¹⁰ developed a method to recover skim rubber by the

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addition of a pancreatic enzyme to skim latex before coagulation with sulfuric acid. Recently, another method was developed for recovering the small rubber particles in skim latex; it involves the concentration of skim latex by a membrane-separation procedure.¹¹ However, this method seems to be too expensive for the large-scale production of skim latex. Therefore, we have been seeking cost-effective alternatives such as the use of enzymes. Proteolytic enzymes will decompose the protein components in skim latex, and the decomposed proteins dissolved in the serum phase can be removed by creaming. Papain is a proteolytic enzyme derived from papaya plants (*Carica papaya*) useful for the deproteinization of natural rubber¹² and natural rubber latex.¹³ For the deproteinization of natural rubber latex using liquid papain, it was observed that the treatment of natural rubber latex with a 5% aqueous solution reduced the stability of the latex, possibly because of the acidic nature and mineral content present in the sample. However, the stability of the latex was maintained when liquid papain was initially treated with potassium oleate soap and then used for deproteinization. This article discusses the use of this enzyme for deproteinization along with quick and easy coagulation of skim latex. The processing and technological properties of this skim rubber are compared with those of conventionally prepared skim rubber and block rubber [Indian Standard Natural Rubber 10 (ISNR 10)].

EXPERIMENTAL

Materials

Skim latex collected from the Rubber Research Institute of India Experiment Station (Kerala, India) was used as the starting material. Stabilized liquid papain, supplied by Senthil Papain and Food Products, Ltd. (Coimbatore, India), was used for deproteinization. A typical composition of liquid papain is given in Table I. A 5% solution of the enzyme in water treated with potassium oleate to remove the excess mineral matter¹³ was used for the study. All other chemicals were laboratory reagent grade.

Preparation of deproteinized skim rubber (DPSR)

The skim latex used for the study contained a 5.41% (w/w) concentration of dry rubber and a 9.23% (w/w) concentration of total solids, and the obtained rubber had a 2.32% (w/w) concentration of nitrogen with an initial plasticity (P_0) of 39 and a plasticity retention index (PRI) of 13. The process variables studied for the deproteinization of skim latex included the concentration of the enzyme and the potassium oleate soap used for the treatment of pa-

TABLE I
Composition of Liquid Papain

| Parameter | Liquid papain |
|---------------------------------|------------------------|
| Activity (TU/mg) | 208.15 |
| Total plate count (CFU/g; FDA) | 20 |
| Mineral content (%) | 2.16 |
| pH of aqueous solution | 5.4 |
| Pathogens | Free |
| Yeast and mold count (CFU/gm) | <10 |
| Lead, arsenic, and heavy metals | Below detectable limit |
| Aflatoxin and pesticides | Absent |

FDA = Food and Drug Administration.

pain before its addition to the skim latex. Liquid papain at different concentrations (0.25 and 0.5 phr) in 5% aqueous solutions was initially treated with 0.5 or 1.0 phr potassium oleate. This was then mixed with the skim latex, kept at room temperature for 48 h, treated with potassium oleate (0.01% w/v on latex as a 10% solution), and stored for another 24 h. Sodium metabisulfite (0.05 phr as a 1% solution) was added to the latex to obtain a good color for the coagulum before creaming. It was then subjected to creaming with ammonium alginate (0.3% w/v on latex as a 3% solution). The cream was coagulated with 5% H_2SO_4 , and the coagulum was granulated and soaked for 24 h in an oxalic acid (1% w/v) solution. The crumbs were dried, and raw rubber properties such as the nitrogen content, P_0 , and PRI were determined per IS 4588:1986. Control samples of creamed latex were prepared without any enzyme treatment but with and without potassium oleate. DPSR, prepared by the standardized method, was compared with the conventionally produced skim rubber (control) and block rubber (ISNR 10) for raw rubber properties such as the nitrogen content, ash content, P_0 , PRI, accelerated storage hardening (ΔP_0), and Mooney viscosity [ML(1 + 4)100°C] according to IS 4588:1986.

The particle size distribution of the centrifuged latex (cenex) and its serum fraction (skim latex) before and after deproteinization followed by creaming was determined with a Malvern Zetasizer Nano series particle size analyzer (Worcestershire, UK) using a laser diffraction technique. The refractive index of the sample was taken to be 1.52. The fatty acids present in the skim rubber were determined from the acetone-extractable materials with a Waters 510 gel permeation chromatograph (USA) attached to a 410 diffraction refractometer. A Waters free fatty acid column was used for the study. The solvent was a 25/35/45 (v/v) tetrahydrofuran/water/acetonitrile mixture at a flow rate of 2 mL/min. Samples were injected at a temperature of 45°C. The column was standardized for the elution time with high-performance liquid chromatography grade fatty acids

TABLE II
Effect of the Papain Concentration on the Properties of Raw Rubber

| Papain concentration (phr) | Potassium oleate concentration (phr) | Nitrogen content (% w/w) | P_0 | PRI |
|----------------------------|--------------------------------------|--------------------------|-------|-----|
| 0.0 | 0.0 | 1.18 | 38 | 56 |
| 0.0 | 0.5 | 0.98 | 38 | 58 |
| 0.0 | 1.0 | 0.89 | 40 | 59 |
| 0.25 | 0.5 | 0.58 | 39 | 60 |
| 0.25 | 1.0 | 0.57 | 40 | 60 |
| 0.50 | 0.5 | 0.58 | 40 | 59 |
| 0.50 | 1.0 | 0.56 | 40 | 60 |

such as lauric, myristic, palmitic, oleic, linoleic, linolenic, and stearic acids.

To compare the rate of vulcanization and the technological properties, a gum rubber compound made according to the ACS 1 formulation (ASTM D 3184 1A) and filled compounds containing high-abrasion furnace black (HAF) were prepared with a laboratory model two-roll mixing mill. The cure characteristics of the samples were determined with a Tech Pro Rheotech MD+ (Winona, MN) at 150°C. Vulcanizates were molded with an electrically heated hydraulic press to their respective optimum cure. The properties of the vulcanizates were determined according to the relevant ASTM standards. Aging of the vulcanizates was carried out at 70°C for 7 days in an air oven as per ASTM standards.

RESULTS AND DISCUSSION

Standardization of the process using liquid papain

Skim rubber prepared by the conventional method (the control sample) had a nitrogen content of 2.32%. The results in Table II show that creaming of the skim latex reduced the nitrogen content to 1.18%. The addition of potassium oleate still reduced the nitrogen content, but the values were higher than those obtained for the papain-treated samples. A comparison of the results for papain-treated samples showed that both concentrations of liquid papain (0.25 and 0.5 phr) and potassium oleate (0.5 and 1.0 phr) were capable of reducing the nitrogen content of skim latex, and the values were almost the same. Hence, 0.25 phr papain and 0.5 phr potassium oleate were selected as the optimum dosages.

Soaps, being highly surface-active, are known to displace proteins from latex particles.¹⁴ In our previous study,¹³ we observed that hydrolysis by a combination of an enzyme and soap helped to further reduce the protein content in the latex, and a dosage of 0.01% soap on latex was found to be optimum for the papain treatment. Hence, a dosage of 0.01% potassium oleate as a 10% solution was added to the

TABLE III
Properties of Skim Rubber and DPSR in Comparison with ISNR 10

| Parameter | Control | DPSR | ISNR 10 |
|-----------------------------------|---------|------|---------|
| Nitrogen content (% w/w) | 2.30 | 0.62 | 0.35 |
| Ash content (% w/w) | 0.53 | 0.40 | 0.31 |
| P_0 | 39 | 38 | 51 |
| PRI | 17 | 56 | 59 |
| ΔP_0 | 1 | 3 | 15 |
| Mooney viscosity [ML(1 + 4)100°C] | 70 | 76 | 87 |

skim latex before creaming. Although the enzyme treatment and creaming reduced the nitrogen content significantly, it affected the PRI of the rubber adversely. It has been reported that normal skim latex contains more than 15 ppm copper ions on total solids, and an appreciable proportion of these copper ions are retained in the relatively small amount of coagulum obtained from the skim latex.¹⁰ It was found that the PRI could be improved by immersion of the coagulum in a 1% oxalic acid solution for 24 h, and the improvement observed for the enzyme-treated sample may have been due to the extraction of copper ions from the rubber.¹⁵ PRI values in our studies were also improved by an oxalic acid treatment.

Raw rubber properties

Table III shows that for the DPSR sample, the nitrogen and ash content values were reduced to about 70 and 25%, respectively, of those of the control sample, but the values were still higher than those for ISNR 10. The P_0 values for the three samples were within the specified limit. The low PRI value for the control sample was due to the absence of the oxalic acid treatment. The Mooney viscosity of natural rubber changes during storage as it undergoes hardening, which results in higher bulk viscosity

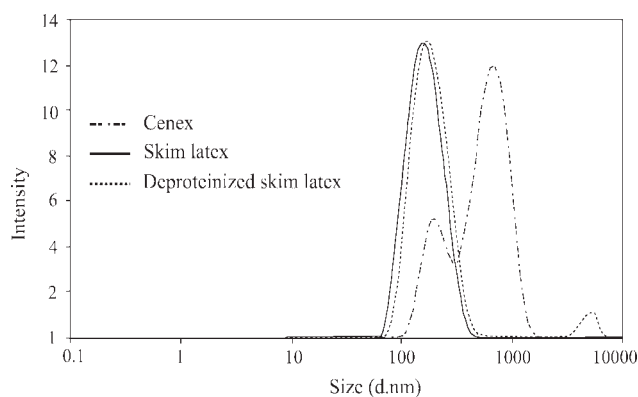


Figure 1 Particle size distribution of latices.

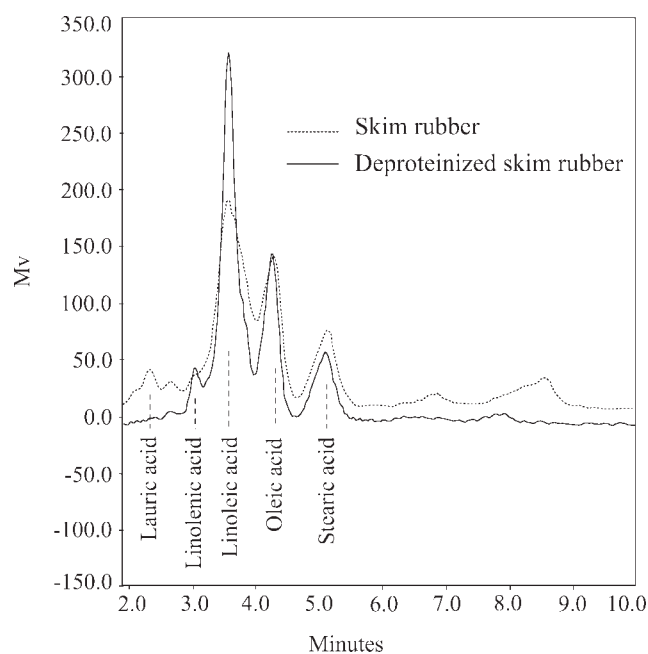


Figure 2 Chromatogram showing the fatty acids in skim rubbers.

when it is stored at the ambient temperature and humidity over a period of time. This hardening process is accelerated at elevated temperatures and under dry conditions. The formation of crosslinking and branching during accelerated storage has been presumed to be due to the chemical bonding between the active functional groups in the long-chain fatty acid of phospholipids at the terminating ends of rubber molecules.¹⁶ Small skim rubber particles do not have unsaturated fatty acids containing terminal end groups, and so skim rubber does not harden during storage.¹⁷ The ΔP_0 values for the control and DPSR samples also support these findings. The Mooney viscosities for the control and enzyme-treated samples were within the specified limit.

Particle size distribution

The particle size distributions for the cenex and its skim fraction and that after deproteinization and

TABLE IV
Formulations of the Compounds

| Ingredient | ACS 1 | HAF-filled |
|---------------------|-------|------------|
| Skim natural rubber | 100 | 100 |
| Zinc oxide | 6.0 | 5.0 |
| Stearic acid | 0.5 | 2.0 |
| HAF black (N300) | — | 40 |
| Naphthenic oil | — | 4 |
| Antioxidant TDQ | 0 | 1.0 |
| CBS | — | 0.6 |
| MBT | 0.5 | — |
| Sulfur | 3.5 | 2.5 |

TDQ = 2,2,4-trimethyl 1,2-dihydroquinoline; CBS = *N*-cyclohexyl-2-benzothiazole sulfenamide; MBT = 2-mercaptobenzothiazole.

creaming of the serum fraction are listed in Figure 1. Cenex showed a bimodal particle size distribution, whereas skim latex and deproteinized skim latex showed a unimodal particle size distribution. The shift in the particle size distribution curve after deproteinization was only marginal; therefore, it can be assumed that deproteinization and creaming of skim latex did not significantly change the particle size of the skim latex.

Fatty acid content

Fatty acids present in skim rubbers obtained by conventional and enzyme-treated methods are shown in Figure 2. A comparatively higher proportion of fatty acids was present in the control sample. The fatty acids included lauric, linolenic, linoleic, oleic, and stearic acids. The higher quantity of fatty acids retained in skim rubber adversely affected the aging characteristics.¹⁸ There was a reduction in the quantity of fatty acids after deproteinization and creaming. As explained later, the aging characteristics were improved after deproteinization and creaming because of the lower level of unsaturated fatty acids, which could act as pro-oxidants of rubber.

TABLE V
Cure Characteristics of ACS 1 and HAF-Filled Mixes

| Parameter | ACS 1 | | | HAF-filled | | |
|---|---------|------|---------|------------|------|---------|
| | Control | DPSR | ISNR 10 | Control | DPSR | ISNR 10 |
| Minimum torque (dN m) | 0.6 | 0.4 | 0.6 | 0.7 | 0.6 | 0.6 |
| Maximum torque (dN m) | 8.4 | 6.4 | 6.4 | 14.5 | 7.0 | 9.6 |
| Rheometric torque (dN m) | 7.8 | 6.0 | 5.8 | 13.8 | 6.4 | 9.0 |
| Scorch time at 150°C (min) ^a | 2.4 | 3.8 | 3.2 | 2.0 | 3.0 | 2.6 |
| Optimum cure time at 150°C (min) ^b | 10.0 | 16.0 | 11.0 | 8.0 | 13.0 | 9.0 |

^a Time for the torque to increase 2 units above the minimum value.

^b Time to achieve 90% of the cure.

TABLE VI
Mechanical Properties of ACS 1 and HAF-Filled Mixes

| Parameter | ACS 1 | | | HAF-filled | | |
|--------------------------------|---------|------|---------|------------|------|---------|
| | Control | DPSR | ISNR 10 | Control | DPSR | ISNR 10 |
| 100% modulus (MPa) | 1.2 | 0.9 | 0.8 | 3.9 | 2.7 | 2.3 |
| 200% modulus (MPa) | 1.9 | 1.4 | 1.2 | 7.8 | 6.0 | 6.2 |
| 300% modulus (MPa) | 2.9 | 1.9 | 1.7 | 13.8 | 12.0 | 12.6 |
| Tensile strength (MPa) | 15.6 | 20.5 | 19.8 | 20.1 | 25.0 | 25.9 |
| Elongation at break (%) | 663 | 720 | 756 | 425 | 520 | 538 |
| Tear strength (N/mm) | 33.5 | 37.5 | 37.0 | 74.5 | 78.3 | 81.0 |
| Shore A hardness | 40.0 | 36.0 | 34.0 | 64.0 | 60.0 | 58.0 |
| Resilience (%) | 61.7 | 65.2 | 68.0 | 61.0 | 65.0 | 66.0 |
| Heat buildup (°C) ^a | 8.0 | 6.0 | 6.0 | 25.0 | 21.0 | 18.0 |
| Compression set (%) | 28.8 | 24.8 | 25.0 | 38.9 | 34.0 | 35.0 |

^a Change in the temperature.

Cure characteristics

ACS 1 (American Chemical Society) and HAF-filled compounds were prepared per the formulations given in Table IV. The cure characteristics of both compounds are presented in Table V. The control sample had a higher rheometric torque for both ACS 1 and carbon black filled mixes than for DPSR and ISNR 10. The optimum cure time and scorch time were also lower for the control sample. High levels of fatty acids and proteins present in the control sample may have been responsible for this cure behavior. Nitrogenous compounds and fatty acids are known to take part in sulfur vulcanization.¹⁹ The comparatively slow curing for the enzyme-treated sample could be attributed to the lower protein content of the treated rubber. However, in the case of carbon black filled mixes, the effect of proteins was less conspicuous because there was activation of curing in the presence of carbon black. Proteins and fatty acids retained in the enzyme-treated skim rubber appeared to be sufficient to activate the vulcanization process.

Mechanical properties

The mechanical properties of the mixes are given in Table VI. The high modulus and hardness observed

for the control sample in the case of ACS 1 and HAF-filled mixes may be due to the high level of proteins in the sample. It has been reported that proteins can improve the modulus and hardness of a rubber vulcanizate because of the reinforcing characteristics of proteins.²⁰ The tensile strength, elongation at break, tear strength, resilience, heat buildup, and compression set were better for the enzyme-treated sample and were almost comparable to those of block rubber. The improvement in the mechanical properties may be attributed to the reduction of the protein content and partial removal of unsaturated fatty acids.

Aging characteristics

The aging resistance of the vulcanizates was assessed by the determination of the tensile properties before and after aging. Figures 3 and 4 show the aging resistance with respect to the modulus and tensile strength of the vulcanizates after aging at 70°C for 7 days. Retention of the modulus and strength for the enzyme-treated samples was better than that for the control sample and was comparable to that for the block rubber sample. The improvement in aging characteristics may be attributed to

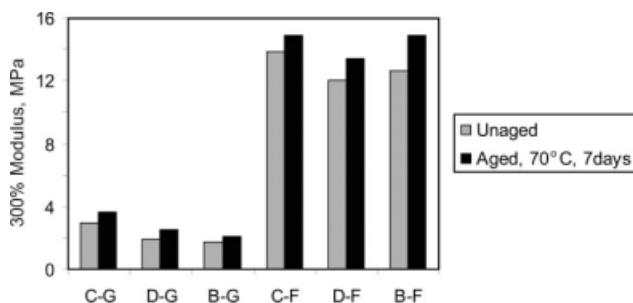


Figure 3 Effect of aging on 300% modulus. B-G = ISNR 10 gum; B-F = filled ISNR 10; C-G = control gum; C-F = filled control; D-G = DPSR gum; D-F = filled DPSR.

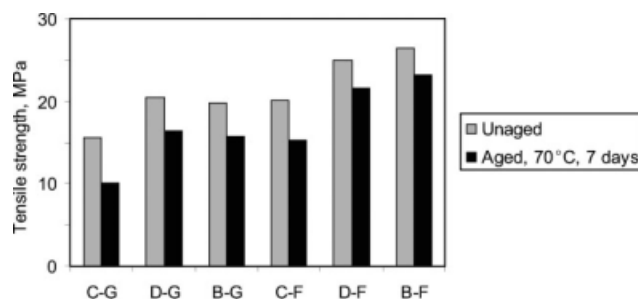


Figure 4 Effect of aging on tensile strength. B-G = ISNR 10 gum; B-F = filled ISNR 10; C-G = control gum; C-F = filled control; D-G = DPSR gum; D-F = filled DPSR.

the partial removal of unsaturated fatty acids and metal ions, which were pro-oxidants, during the deproteinization and creaming process.

CONCLUSIONS

Liquid papain was used for the deproteinization and easy coagulation of skim latex. Advantages noted for the deproteinization and creaming process over the conventional method were the production of odorless and high-quality rubber by easy coagulation and the recovery of around 15–20% more rubber. The size of the rubber particles remained almost unchanged after deproteinization by this technique. Because of the reduction of the protein content and fatty acids, the deproteinized rubber showed better mechanical properties and aging characteristics than normal skim rubber, and the properties achieved were almost comparable to those of conventional block rubber.

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