THE ROLE OF PROTEINS ON THE THERMAL OXIDATIVE AGING OF RADIATION VULCANIZED NATURAL RUBBER LATEX

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Abstract

The effect of Hevea latex proteins on the aging properties of radiation vulcanized natural rubber latex (RVNRL) was investigated. Unpurified RVNRL films exhibited better aging properties than the purified RVNRL films. A sharp decrease in tensile strength was observed after aging when RVNRL films were leached in 1% NH_4OH. However, when these films were soaked in ethanol prior to leaching, the aging properties approximated those of the unleached samples. Kjeldahl and FT-IR analyses of the leached and unleached RVNRL films indicated a higher protein content for both the unleached and ethanol-soaked films than for leached films.

Electrophoretic analysis of the proteins present in the NH_3 extracts of leached RVNRL films showed a high concentration of hevein. This protein was not found in the NH_3 extracts of ethanol soaked films.

Hevein was shown to improve the aging properties of RVNRL.

INTRODUCTION

Radiation treatment for the vulcanization of natural rubber latex (RVNRL) is a potential alternative to the conventional sulphur vulcanization process. RVNRL utilizes gamma radiation to catalyze the crosslinking of rubber molecules. Various modifications to improve the process and quality of RVNRL products have been developed. Previous studies dealt with the development of sensitizers to enhance crosslinking resulting to a reduction of the required radiation dose, improvement of the properties of the products with leaching, and the use of antioxidants to improve the aging properties of natural rubber latex (NRL). However, certain physical properties of RVNRL are still inferior to those of sulfur-vulcanized rubber. These properties are important factors to consider in the manufacture of dipped rubber goods.

Natural rubber (NR) which contains unsaturated bonds in its chain undergoes thermal oxidation. NR vulcanizates that are conventionally cured with sulphur are not easily affected by this oxidative aging. Sulphur crosslinks are oxidized to sulphoxides, then to the sulphenic acids which are powerful antioxidants[1]. Radiation vulcanized natural rubber latex (RVNRL), on the other hand, does not have this auto-retarding property. The resistance of RVNRL to oxidation reaction depends on the natural antioxidants present in natural rubber latex. Among these are the tocotrienols, betaines, phenols, phospholipids and amino acids[2,3,4,5]. The role of proteins in the oxidative aging of NR is still quite unclear. The work of Morimoto indicated that proteins play an important role in the heat resistance of NR compound[4]. Hasma, on the other hand found that the Hevea proteins do not protect NR significantly against thermal oxidative aging[3]. This paper presents data to clarify the role of NRL proteins particularly hevein, in the oxidative aging of RVNRL.

MATERIALS AND METHODS

Sri-Lankan latex with the following properties was used for this study. It is a high stability (HS), low ammonia latex with TMTD-ZnO (LATZ) with a total solid content (TSC) of 60% and viscosity of 17.8 cP.

Extraction and Isolation of Proteins

Natural rubber latex (NRL) was centrifuged at 16,000 rpm for 2 hours at room temperature. A puncture was made on the coagulated rubber portion at the top of the centrifuge tube to run off the serum portion. The serum fraction was placed again in centrifuge tubes and frozen at -25°C. Recentrifugation was done for 2 hours while the serum was still in its frozen state. The bottom clear serum fraction was removed slowly with a pasteur pipette. The pH of the serum was adjusted to pH 6.0 with acetic acid and centrifuged to remove all the precipitated fraction. A portion of this serum was set aside for the isolation of hevein. Ammonium sulphate [$(NH_4)_2SO_4$] was added to the clear solution to 100% saturation. The precipitated proteins were redissolved in water and reprecipiated again with $(NH_4)_2SO_4$. α-globulin was isolated from the extracted proteins using Archer's procedure.[6]

The clarified serum was treated with $(NH_4)_2SO_4$ to 65% saturation. The resulting mixture was stored overnight at 4°C. The precipitate was discarded, and hevein was precipitated from the supernatant by saturation (100%) with $(NH_4)_2SO_4$ [7] This precipitate was dissolved in water. Desalting was done using the Amicon diaflo ultrafiltration membrane (YM2).

Preparation and Analysis of RVNRL Films

NRL was diluted to 50% TSC with NH_4OH and mixed with 5 phr n-butyl acrylate as sensitizer. NRL was irradiated at an absorbed dose of 15 kGy at room temperature using a ^{60}Co source at a dose rate of 1 kGy/hr. These were cast into films, and air dried. One set of films was leached in 1% NH_4OH overnight. The films were air dried then oven dried at 70°C for 3 hours to cure. Another set was soaked in ethanol overnight prior to leaching in NH_4OH.

Proteins were isolated from the NH_4OH extracts by saturation with $(NH_4)_2SO_4$ and analyzed by sodium dodecyl sulphate - polyacrylamide gel elctrophoresis (12.5%) (SDS-PAGE).

Nitrogen content was measured using the Kjeldahl analysis. FT-IR analyses of the films were carried out using the JEOL (JIR-100D) analyzer.

Determination of the Effect of NRL Proteins on the Aging Properties of RVNRL Films

NRL was purified by centrifuging the latex at 9,500 rpm for 1 hr. The rubber phase was separated from the serum and was redispersed in 5% sodium dodecyl sulphate (SDS) for 24 hrs. This was recentrifuged, rewashed with SDS followed by H_20 before it was finally redispersed in 1% NH_4OH.[8] Concentration of the latex was readjusted to 50%.

Extracted proteins were added to purified RVNRL and cast into films. The aging properties of the films were determined by placing these in a gear oven at 70°C for 168 hrs. Tensile strength measurements were taken every 24 hrs.

RESULTS AND DISCUSSION

The aging properties of unpurified RVNRL films was compared to purified RVNRL films. Fig. 1 indicates the aging properties of purified RVNRL films as inferior to that of the unpurified films. Tensile strength retention had gone down to 78% at the end of aging test as compared to the unpurified films which still show a tensile strength retention of 90%. Kjeldahl analysis indicated a decrease in nitrogen content from 0.29% (unpurified) to 0.06%. FT-IR spectra of both films (Fig. 2 & 3) showed differences in absorbance peaks at wavenumbers 3,280 cm^{-1} and 1,540 cm^{-1}. These peaks are indicative of the protein amide bonds. The absorbance at 3,280 cm^{-1} was higher for the unpurified film than the purified one. The unpurified RVNRL has an absorbance peak at 1540 cm^{-1} which was not present in the purified film.

Differences in the aging pattern of both the unpurified and purified RVNRL film may be associated with their protein content as seen in their FT-IR and Kjeldahl analyses. The removal of the proteins during the purification process of NRL resulted in the deterioration of the aging properties of the RVNRL films.

To confirm the effect of proteins on the aging property of RVNRL, increasing volumes of NRL serum (increasing % Nitrogen) were added to purified RVNRL and their aging properties determined. Fig. 4 indicates that the serum added films were very resistant to aging. Percent tensile strength retention at 0.31%-0.73% Nitrogen were still almost 100% at the end of the aging test. The aging properties of purified RVNRL also improved with the addition of NRL proteins at lower concentration (0.19%) but not quite as much as those films at higher nitrogen concentration. Similarly, extracted proteins from NRL serum which were added to purified RVNRL, also gave the same response (Fig. 5). Percent tensile strength retention decreased only very slightly ranging from 92-99% at the end of aging test. These values are higher than the control (78%).

The aging properties of the leached and unleached films were investigated. Fig. 6 shows two different aging trends for the leached and unleached films. The films which were leached in 1% NH_4OH had a very sharp degradation curve. Percent tensile strength retention was reduced to 0% after 96 hours of aging. The unleached RVNRL films on the other hand, still had a tensile strength retention of 74% at the end of aging test. When the films were soaked in ethanol prior to leaching, the aging properties approximated those of the unleached films.

Results of FT-IR and Kjeldahl analyses give strong indications that the observed differences in the aging pattern of the leached and unleached RVNRL films may be associated with their protein content. Kjeldahl analyses of the RVNRL films showed a decrease in protein content from 1.82% to 1.06% after leaching. Ethanol-soaked films had protein content of 1.56% which is close to that of the unleached films. Soaking the films in ethanol before leaching somehow reduces the loss of protein by leaching.

FT-IR data are in consonance with those from the Kjeldahl analyses. The FT-IR spectra of the leached and unleached RVNRL films showed differences in absorbance peak heights at wavenumbers 3,303 cm^{-1} and 1,585 cm^{-1} (Fig. 7) which are characteristic of the protein absorption bonds. Films soaked in ethanol prior to leaching have slightly less absorption at 3,303 cm^{-1} than the unleached films. On the other hand, the absorption peak at the same frequency is hardly discernible in leached films. Likewise, the absorption peak at 1585 cm^{-1} is higher for both the unleached and ethanol soaked films. It may be deduced from the data that proteins in the RVNRL films are extracted during the process of leaching such that the resulting leached films have poor aging properties. Soaking the films in ethanol could have precipitated most of the proteins rendering them insoluble in aqueous NH_3. Thus, the proteins are retained in the film and the resulting films have aging properties comparable with those of the unleached films.

To confirm this effect, proteins from the NH_3 extracts of both the leached and ethanol-soaked films were isolated and added to purified RVNRL. As shown in Fig. 8, these proteins substantially improved the aging properties of purified RVNRL. The effect of proteins isolated from the NH_3 extracts of leached films is the same as that from the total NH_3 extract of the leached RVNRL films. Proteins isolated from the NH_3 extracts of ethanol-soaked films also improved the aging properties of purified RVNRL but to a much lesser degree than the former.

Proteins from the NH_3 extracts of both leached and ethanol soaked films were analyzed by SDS-PAGE (12.5%). The protein hevein with a molecular weight of 13.5 Kda was found in the NH_3 extract of the leached film. This protein was not present in the NH_3 extract of ethanol soaked films (Fig. 9). Hevein is soluble in aqueous solutions , thus this protein is easily leached out in 1% NH_4OH. The removal of this specific protein from RVNRL may have been responsible for the poor aging property in leached RVNRL films. In as much as hevein is retained in the film when this is soaked in ethanol prior to leaching, these films exhibited good aging properties comparable with those of the unleached RVNRL films. Hevein may have a natural antioxidant property that makes the unleached RVNRL films more resistant to oxidative aging.

While it is ascertained that the NRL proteins could inhibit the thermal oxidative aging of RVNRL, it is not known whether all the NRL proteins would have the same effectivity in improving the aging properties of RVNRL. Two major proteins in NRL, α-globulin and hevein were studied for this purpose.

The addition of α-globulin to purified RVNRL had improved the aging properties of RVNRL as seen in Fig. 10. Likewise, a deceleration in the aging process of purified RVNRL occured when hevein was added to it (Fig. 11). Its effectivity in controlling the aging process is greater than the other NRL proteins. Tensile strength deceleration is lesser in purified RVNRL films added with hevein than those which were added with proteins not containing the hevein. Comparing the antioxidant properties of all the proteins studied (Fig. 12), hevein was shown to be the most effective. α-globulin and the NRL proteins without the heveins can also protect the RVNRL films against oxidative degradation but not as much as the heveins. This result is further confirmed when a minimum amount of hevein (0.05%) was added to purified RVNRL, and the % tensile strength retention was increased by 26% (Fig. 13).

The antioxidant property of hevein maybe attributed to its amino acid composition. Hevein is rich in cystine containing 16-19% of this amino acid[7,9]. Cystine could easily be reduced to cysteine. The -SH is a free radical scavenger which could prevent the formation of chain initiating radicals that catalyzes the oxidation reaction steps.

The effect of the amino acids cystine, cysteine and methionine on the aging properties of RVNRL were investigated. Fig.14 indicates that among the three, methionine exhibited a very good antioxidant property. Percent retention after aging is 26% as compared to the purified RVNRL (control) which has been reduced to an almost zero % retention. Cystine and cysteine on the other hand have very poor aging properties. Their aging curves are only slightly higher than that of the control. Cystine, cysteine and methionine are all S-containing amino acids that can be reduced to an -SH and which could act as good antioxidants. The data however indicated that it is only methionine that has this property. Chemical structures of these amino acids would show that cysteine and cystine have polar ends; -SH,-NH_2,-COOH for cysteine and two -NH_2 and -COOH for cystine. Methionine on the other hand has a polar end -COOH and -NH_2 on one end and a non-polar -CH_3 on the other end. This non-polar end could easily penetrate and interact with the rubber molecule, thus becoming a more effective inhibitor against its oxidative aging.

CONCLUSION

The results of this study show that Hevea proteins exert an inhibitory effect on the oxidative aging of RVNRL. Loss of proteins by leaching results in faster degradation of the RVNRL films. One of the proteins that is leached out by NH_4OH is hevein. Addition of hevein to purified RVNRL improved the aging properties of the RVNRL film. Thus, hevein serves as a natural antioxidant that inhibits the oxidative aging of RVNRL.

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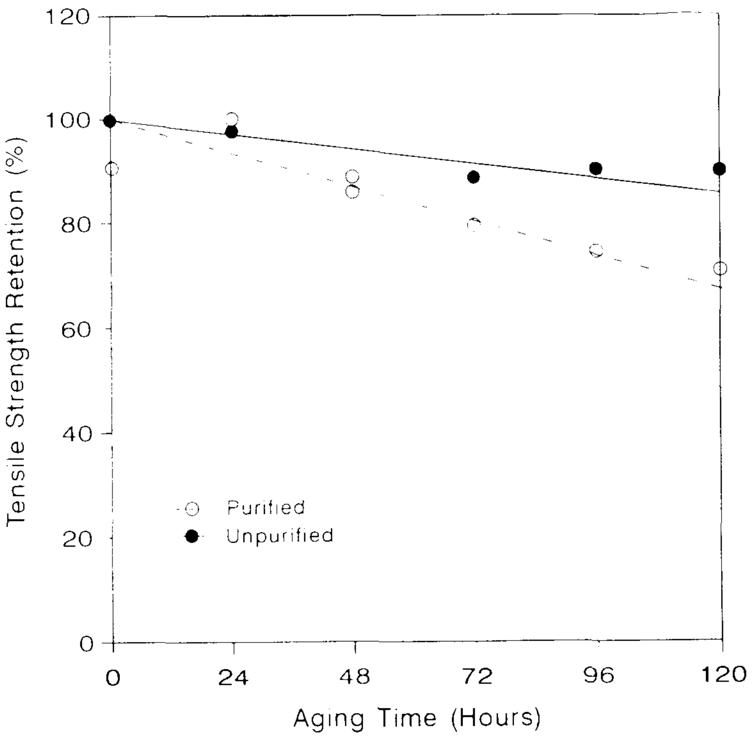

Figure 1: The Tensile Strength Retention of Purified and Unpurified RVNRL

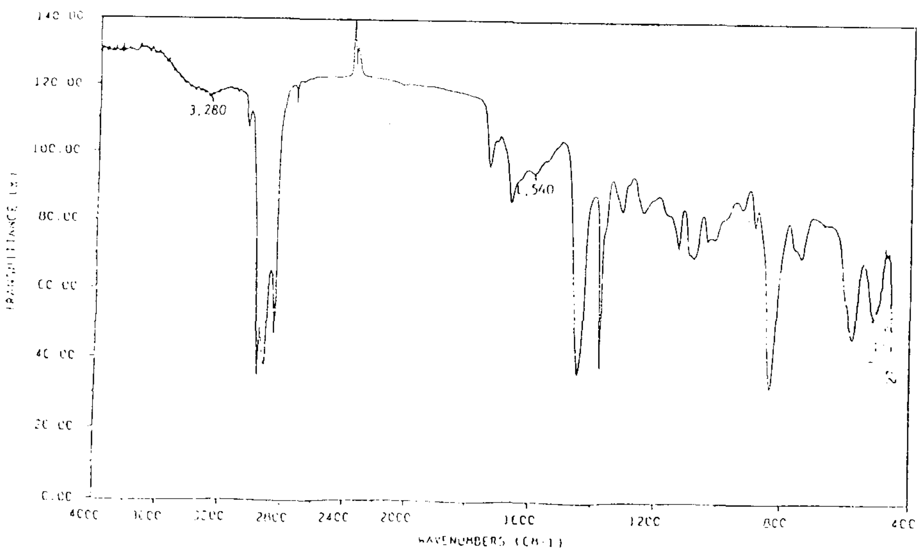

Figure 2: FT-IR Analysis of the Unpurified Film

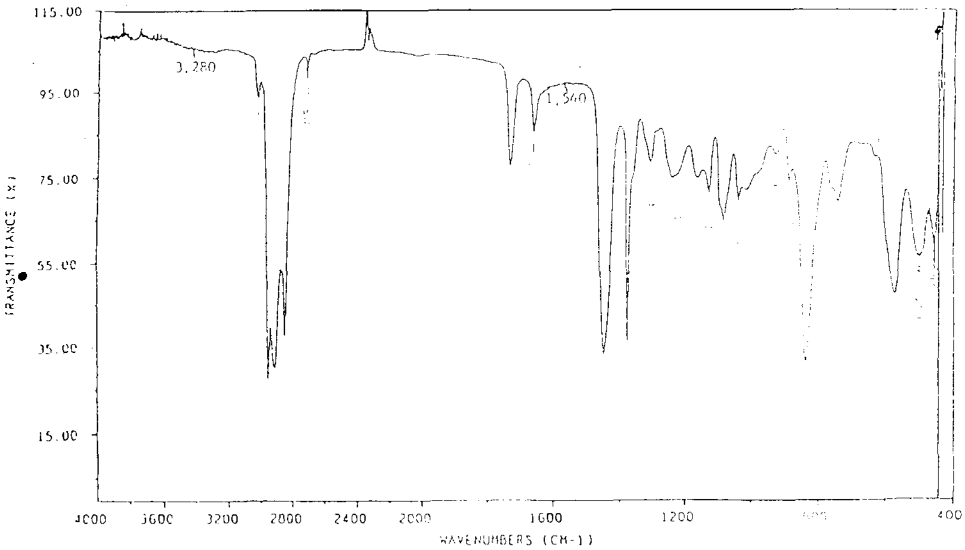

Figure 3: FT-IR Analysis of the Purified Film

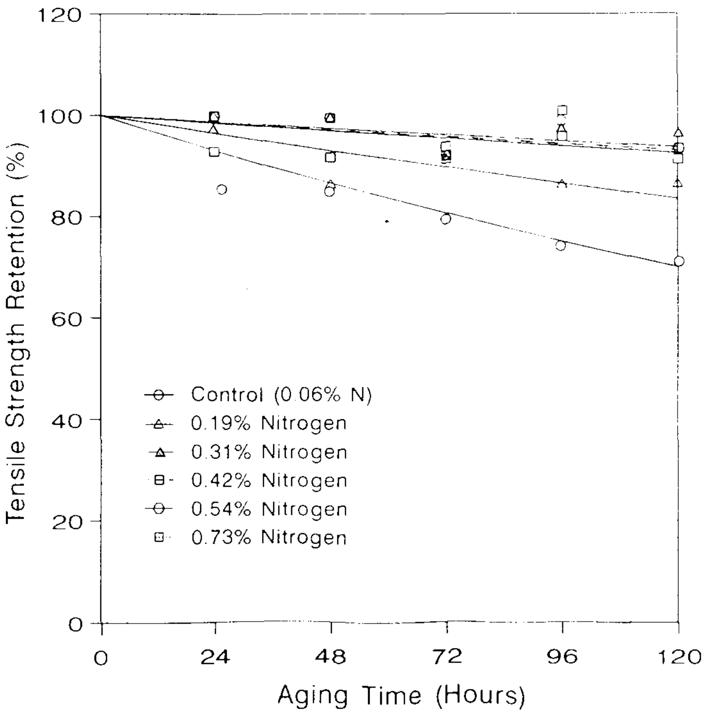

Figure 4: The Effect of Serum on the Tensile Strength of RVNPL

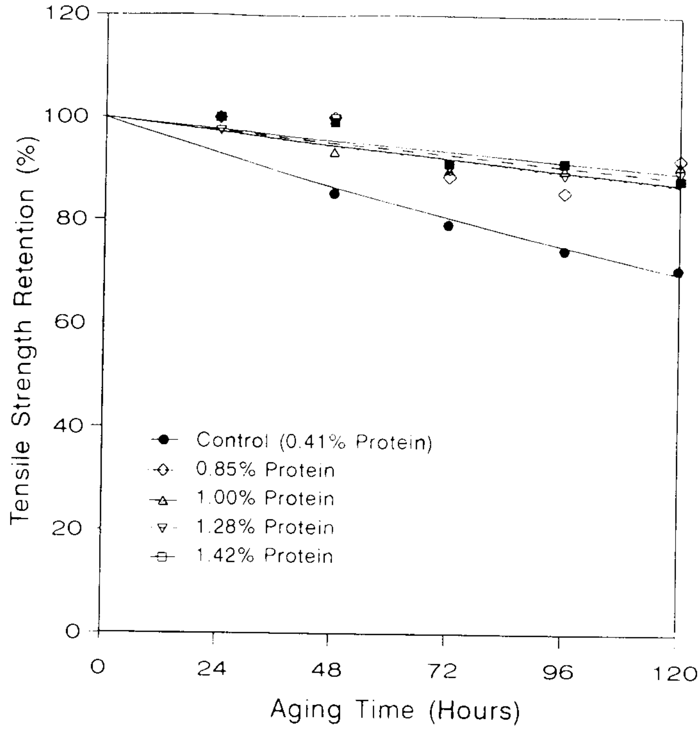

Figure 5: The Effect of Proteins on the Tensile Strength Retention of RVNRL

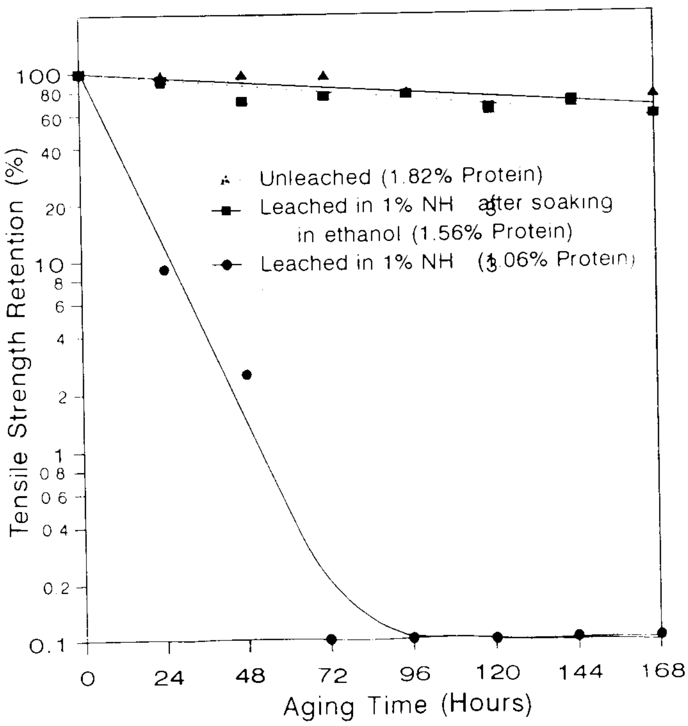

Figure 6: The Effect of Leaching in 1% NH_3 on the Tensile Strength Retention of RVNRL

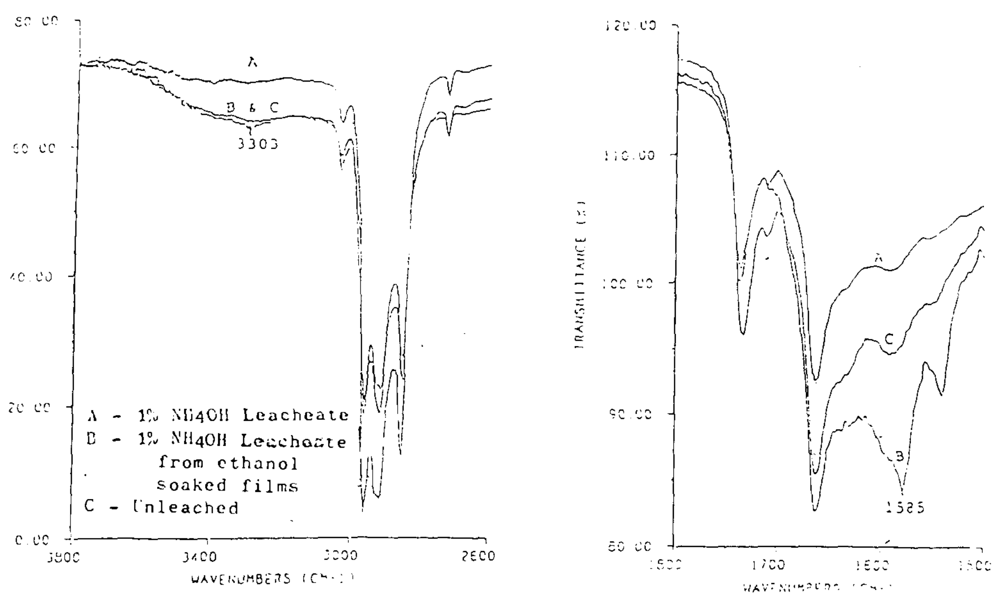

Figure 7: FT-IR Analysis of the Leached and Unleached RVNRL Film

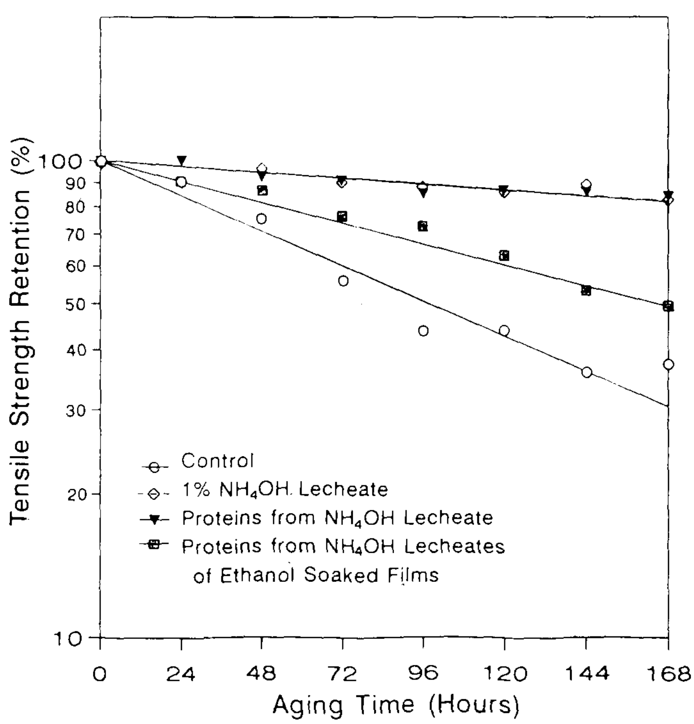

Figure 8: The Effect of Protein from NH_4OH leacheates on the Tensile Strength Retention of RVNRL

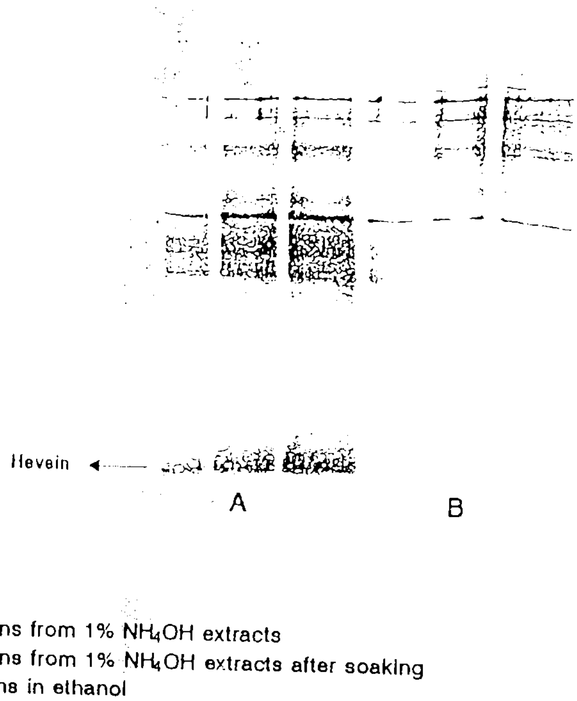

Figure 9: SDS-PAGE (12.5%) Electrophoresis of the Protein from the NH_4OH Extracts (At Different Concentrations)

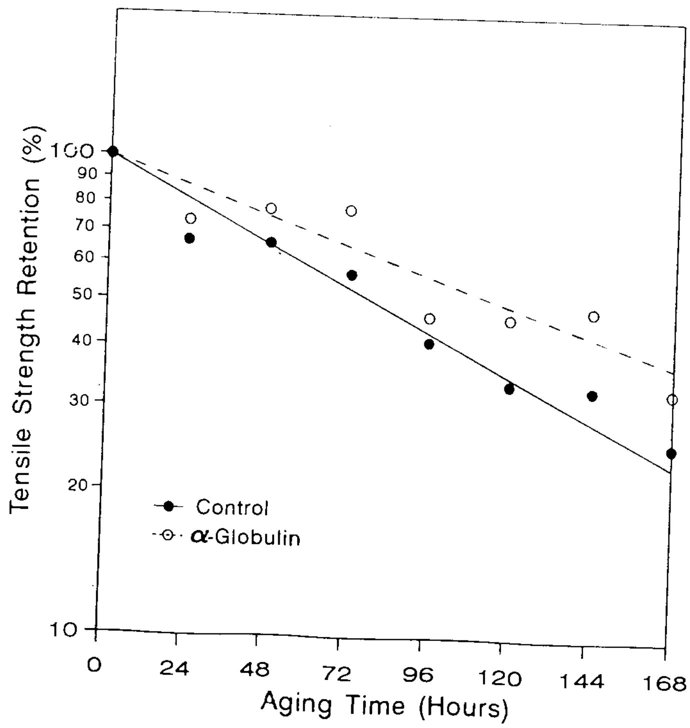

Figure 10: The Effect of Alpha Globulin on the Tensile Strength Retention of RVNRL

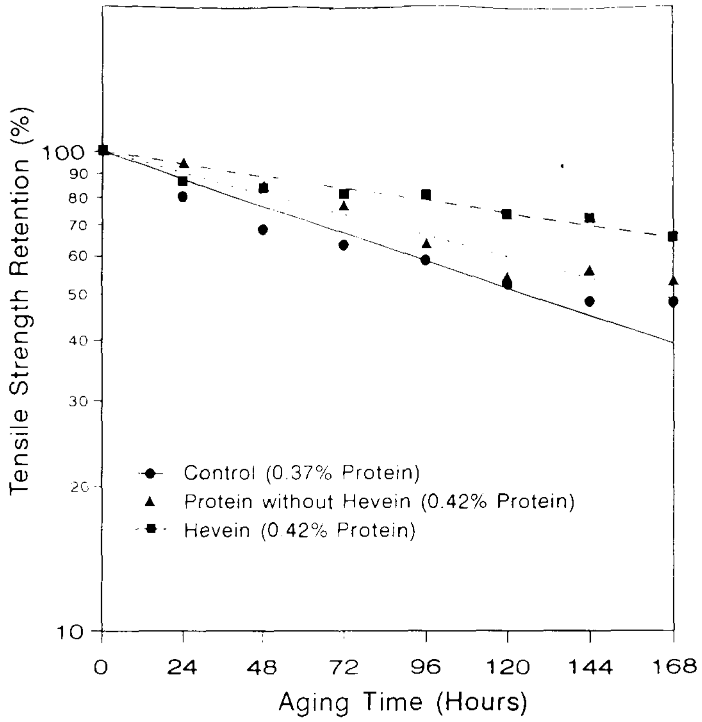

Figure 11: The Effect of the Havein on the Tensile Strength Retention of RVNRL

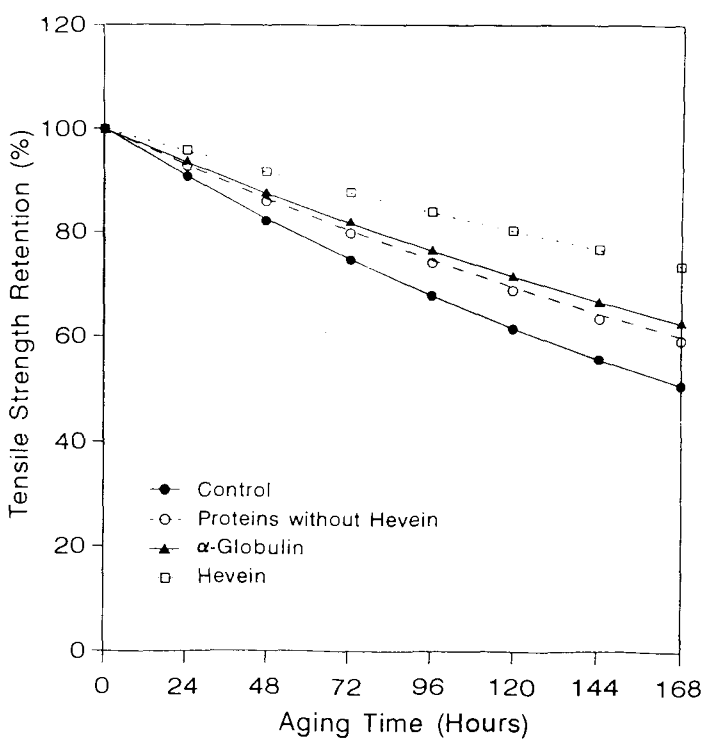

Figure 12: Comparison of the Effect of the Different Protein on the Ageing Properties of Purified RVNRL

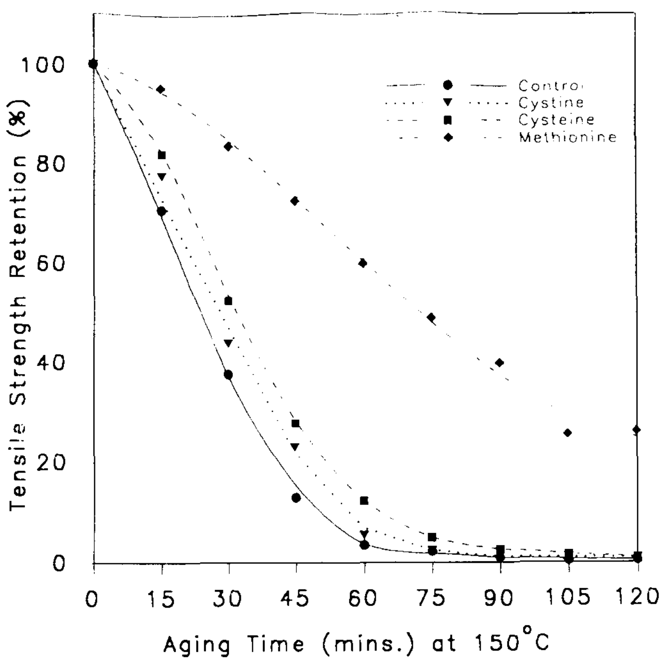

Figure 13: The Effect of Haaavein on the Tensile Strength Retention of RVNRL

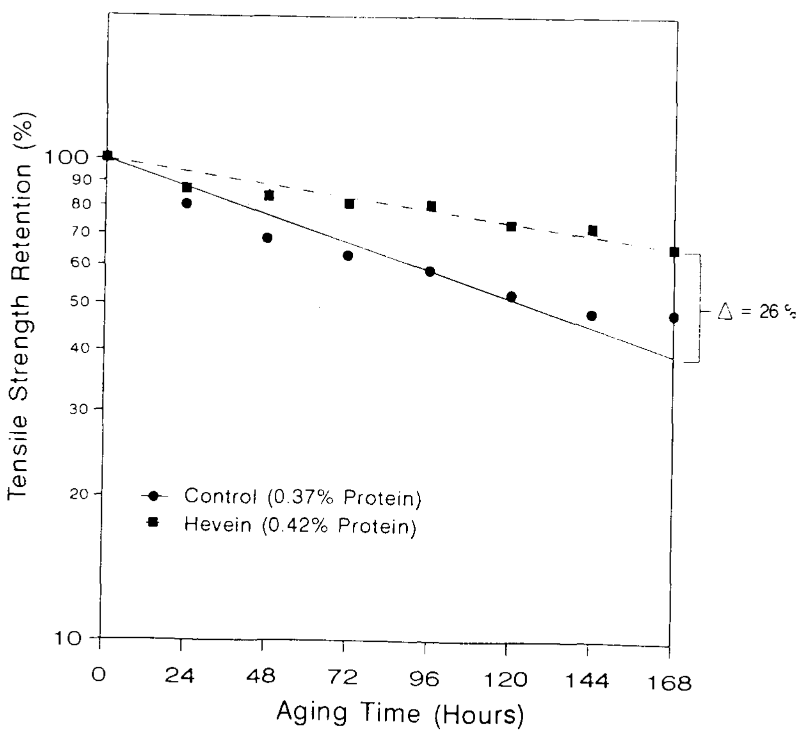

Figure 14: The Effect of Amino Acids on the Tensile Strength Retention of RVNRL