DETERMINATION OF SOLUBLE PROTEIN CONTENTS FROM RVNRL

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ABSTRACT

This project was carried out to determine the soluble protein contents on RVNRL film vulcanisates, with respect to the RVNRL storage time, gamma irradiation dose absorbed by the latex and the effect of different leaching time and leaching conditions. These three factors are important in the hope to determine the best possible mean of minimizing the soluble protein contents in products made from RVNRL. Within the nine months storage period employed in the study, the results show that, the longer the storage period the less the soluble protein extracted from the film samples. Gamma irradiation dose absorbed by the samples, between 5.3 kGy to 25.2 kGy seems to influence the soluble protein contents of the RVNRL films vulcanisates. The higher the dose the more was the soluble protein extracted from the film samples. At an absorbed dose of 5.3 kGy and 25.2 kGy, the soluble contents were 0.198 mg/ml and 0.247 mg/ml respectively. At a fixed leaching temperature, the soluble proteins increases with leaching time and at a fixed leaching time, the soluble proteins increases with leaching temperature. The highest extractable protein contents was determined at a leaching time of 10 minutes and leaching temperature of 90°C. The protein analysis were done by using Modified Lowry Method.

INTRODUCTION

Over the last eight years or so, a number of papers have reported cases of contact urticaria (Kopman, A., Hannuksela, M., 1983), anaphylactic shock and related responses on exposure to dipped latex products (Turjanmaa, K. Reunala, T. Tuimala and Karkkainen, 1984). These reported cases were known as Type I response for allergic reaction to latex products due to the pesence of soluble protein.

Several methods were used to reduce the extractable protein contents of dipped latex products were reported (Faridah Yusof and Yeang, H.Y., 1992). These include the introduction of low protein, deproteinised latex to the latex based dipped product industries.

In this study, the potential of RVNRL (Radiation Vulcanised Natural Rubber Latex) is being exploited. This paper described the results of the determination of soluble protein contents from RVNRL film vulcanised with respect to the effect of different storage periods on RVNRL, the γ-irradiation dose absorbed by the latex and the effect of different leaching treatments, leaching time and leaching temperature.

MATERIALS AND METHODS

Materials

Latex used in these studies was of a high ammonia type. Proteins analysis were determined according to Modified Lowry Method. The standard used was Lowry Micro D.C. Protein Assay supplied by Bio-Rad Laboratories USA. The concentration of protein were determined at 570 nm by using UV-Spectrophotometer. The latter was calibrated using Didymium and Holmium Filter Type II supplied by Pye Unicam Ltd. Cambridge, England.

Methods

The studies were carried out on RVNRL film vulcanisates prepared by coagulant dipping method. The first set of studies were to see the effects of the storage periods of between 0 to 9 months on the soluble protein contents of RVNRL film vulcanisates and unirradiated latex films.

The second set of studies were to determined the effect of irradiation dose of between about 5 kGy to 25 kGy on the extractable proteins from their film vulcanisates. In this study, a set of film vulcanisates were initially leached in distilled water at 60°C for a period of 5 minutes, and another set of sample were leached under the similar conditions for 10 minutes. Leached samples were dried in an air convection oven at 70°C until transparent, before they were subjected to protein extraction by using phosphate buffer saline as the solvent. The amount of soluble proteins were determine from the solvent and the results are presented in Table 2. Their graphical representations are given in Figure 2. The studies were repeated by changing the leaching times to 5, 10, 15 and 30 minutes, but using RVNRL prepared at an irradiation dose of 12 kGy. The results are given in Table 3.

The third set of studies were to determine the effect of leaching temperature, 30°C, 60°C and 90°C on the soluble protein contents of RVNRL film vulcanisates. The leaching time used was 5 and 10 minutes. The results are given in Table 4 and their graphical representation are given in Figure 3.

In the three sets of studies, the samples which were initially leached by the conditions described above were dried in an air convection oven before they were finally leached in phosphate buffer saline for 3 hours. The final protein contents of the samples were analysed from the phosphate buffer solvent.

RESULTS AND DISCUSSIONS

Table 1 : Effects of The Storage Periods Within 9 Months on The Soluble Proteins

		Soluble Protein Contents (mg/ml)									
Time (Months)		0	1	2	3	4	5	6	7	8	9
Latex	**L1**	0.38	0.31	0.30	0.29	0.25	0.24	0.10	0.07	0.05	0.03
	L2	0.04	0.07	0.08	0.09	0.11	0.16	0.15	0.13	0.11	0.10
RVNRL	**L1**	0.30	0.38	0.40	0.30	0.22	0.18	0.13	0.08	0.08	0.05
	L2	0.03	0.04	0.04	0.05	0.04	0.03	0.02	0.01	0.009	0.007

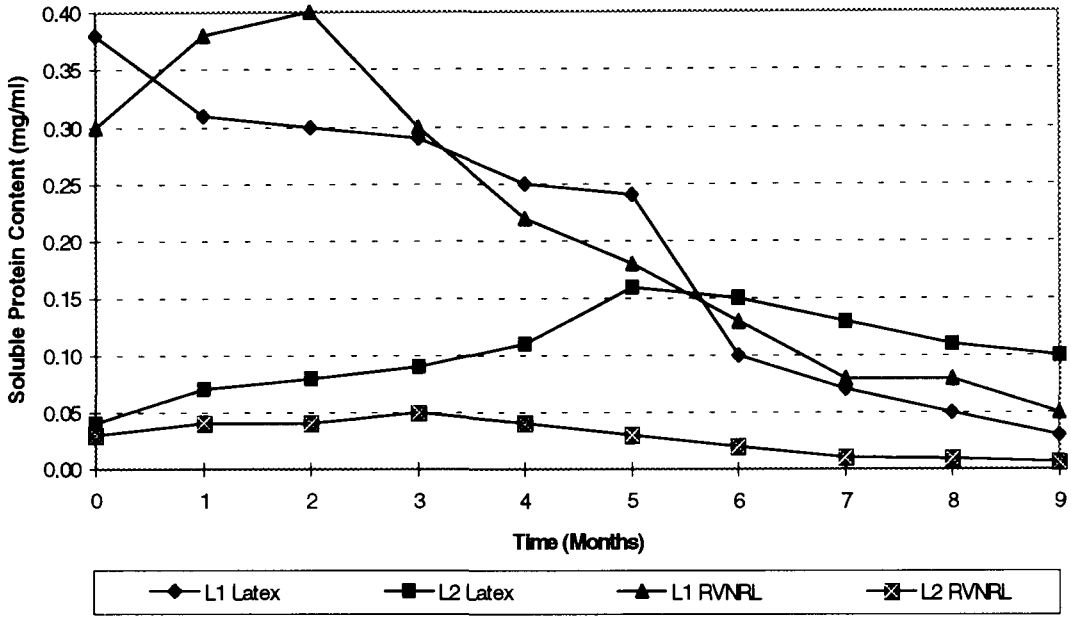

Figure 1: Soluble Protein Content Within 9 Months Storage Period

RESULTS AND DISCUSSIONS

From Table 1, in general the soluble protein from RVNRL film vulcanisates and unirradiated latex films were found to increase with storage times. The reason was not known for certain. However, there is a possibility that the long polypeptides chain which were initially broken down during the latex irradiation stage finally reform with storage times.

The soluble protein obtained from the samples leached in distilled water at 60°C for 3 minutes shown that the soluble protein contents from unirradiated latex decreases with storage periods. Under similar treatment, the soluble protein contents determined on RVNRL film vulcanisates were found to increase with storage time of up to 3 months before started to decrease again. See the results given in Table 1 as L1. Further extractions of proteins from the samples using phosphate buffer saline as solvent, it was found that the soluble proteins from unirradiated latex increases with storage periods of up to 5 months before started to decrease again, and RVNRL has shown the increased in soluble protein contents up to 3 months storage periods before started to decrease again. These results are given in Table 1 as L2.

Table 2 : The Effects of Irradiating The Latex To Different Doses on The Amount of Soluble Protein Contents

Dose, kGy	Soluble protein contents (mg/ml)	
	5 minutes leached	10 minutes leached
5.3	0.022	0.016
6.0	0.029	0.021
9.3	0.043	0.034
12.0	0.053	0.047
13.0	0.058	0.053
15.0	0.064	0.057
19.0	0.075	0.062
25.2	0.078	0.072

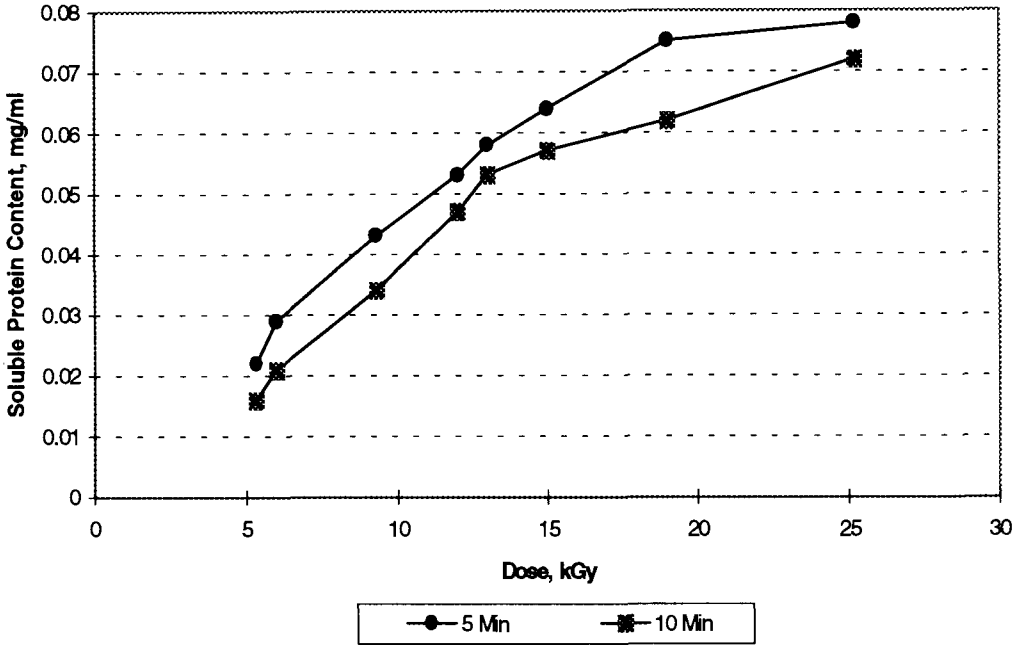

Figure 2: Soluble Protein Contents with the Effect of Irradiating The Latex To Different Doses.

Table 3: Effects of Leaching Times on The Soluble Protein Contents of a Latex Irradiated at 12.0 kGy

Leaching time, (mins)	Soluble protein contents (mg/ml)
5	0.053
10	0.047
15	0.035
30	0.025

From Table 2 and Figure 2, the results show that within the irradiation dose employed in the studies, the soluble protein contents increased as the irradiation dose increased. Similar trend of results were shown by both sets of samples leached for 5 and 10 minutes. However, the set of sample initially leached for 10 minutes have the lower extractable proteins.

As shown in Table 3, the extractable protein from a sample reduced as the initial leaching time increased. At a fixed leaching time, the soluble protein contents increased with irradiation dose. The increase in irradiation dose probably increase the breaking of polypeptides chain making it more soluble and easy to leach. Therefore, increased in soluble protein contents were observed.

Table 4 : Effect of Leaching Temperature and Time on The Amount of Soluble Protein Contents

Leaching time (min.)	Leaching temp. (°C)	Soluble protein contents (mg/ml)
5	30	0.061
	60	0.048
	90	0.028
10	30	0.051
	60	0.037
	90	0.021

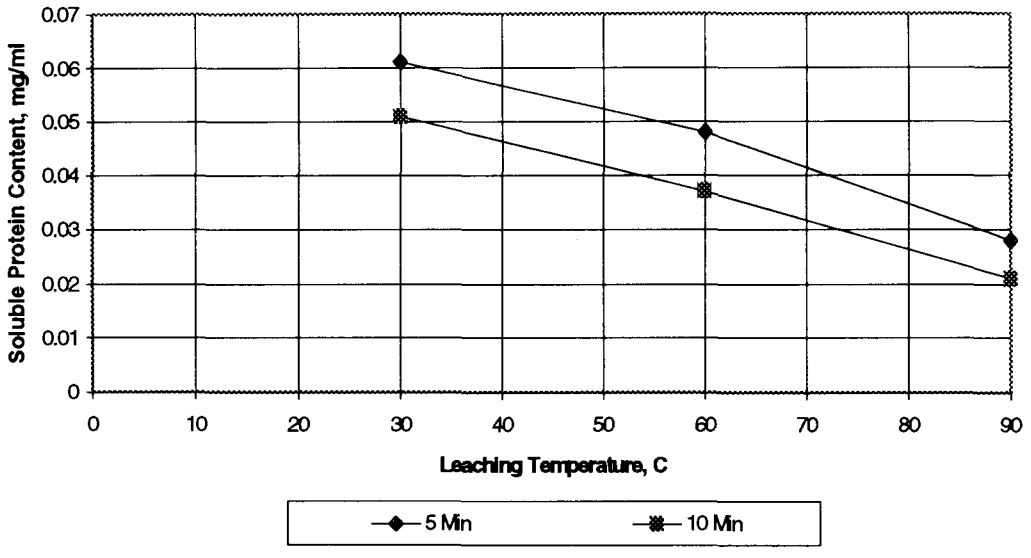

Figure 3: Soluble Protein Contents with the Effect of Different Leaching Temperature and Time.

As shown in Table 4 and Figure 3, at a fixed leaching time, 5 minutes the amount of soluble proteins extracted from the samples were influenced by the leaching temperature. The higher the leaching temperature, the lower was the remaining protein contents in the samples. These were exhibited by the decrease in the soluble protein determined on the phosphate buffer saline used.

Similar results were observed for the sample leached for 10 minutes. From these results, leaching temperature is an important parameter in reducing soluble protein level from products made from RVNRL. The function of the temperature is to activate the molecule chain of the polyisoprene molecules and hence open up the opportunity for the remaining protein to be leached out from the samples.

CONCLUSIONS

From the results of this studies carried out so far, the following conclusions are made :

1. Soluble protein contents of RVNRL film vulcanisates are influenced by many factors including the storage periods of the RVNRL, dose imployed in the latex irradiation stage as well as the leaching parameters.

2. RVNRL seems to exhibit high value of soluble protein contents. Proper leaching will help to reduce the proteins in any products made from it.

3. RVNRL is a potential material for latex based industry at this point in time.

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