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EXTRACTABLE PROTEIN CONTENT OF RADIATION VULCANIZED NATURAL RUBBER LATEX FILMS

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Abstract

The effects of processing conditions on extractable protein content of coagulant dipped radiation vulcanized natural rubber latex films have been investigated. Drying of wet-gel of radiation vulcanized latex films even at a relatively low temperature of 70°C resulted in increases of extractable protein content of the films. The extractable protein content is dependent upon both the temperature and time of drying of wet-gel deposit. Wet-gel leaching of film alone is not adequate to reduce the extractable protein content of films to low levels. Combination of wet-gel leaching, post-leaching, a dip in corn starch slurry, followed by drying at a low temperature of 70°C reduces the extractable protein content of films to very low levels.

INTRODUCTION

Type 1 or immediate hypersensitivity reactions to natural rubber (NR) latex was first reported in Germany in 1927[1]. The next published case appeared in 1979[2]. Between 1989 - 1992, the US Food and Drug Administration reported a number of cases of immediate hypersensitivity reactions associated with latex allergy[3]. These immediate hypersensitive reactions have been attributed to proteinous allergens present in NR latex. These incidents have led to strenuous efforts in the rubber industry to reduce the levels of proteins in NR latex products.

Great amount of work has been carried out by the Rubber Research Institute of Malaysia (RRIM) on reduction of protein content of sulphur vulcanized NR latex products[4-12]. The work by Esah et. al.[8] showed that natural rubber latex films containing extractable protein content of less than 0.1 mg/g as determined by the RRIM Modified Lowry Microassay gave low degree of allergenic reactions in persons showing latex hypersensitivity. So far, there is no report published on extractable protein content of films or products made from radiation vulcanized NR latex (RVNRL). This paper discusses the effects of processing conditions on extractable protein content of RVNRL films.

EXPERIMENTAL

Two batches of HA latices, designated as A and B, were used to prepare RVNRL. The RVNRL was prepared by using n-butyl acrylate (5 p.h.r.) as a sensitizer and irradiating the latex mixture with 12 kGy of gamma rays. Irganox 1520 (1 p.h.r.) antioxidant was added into the RVNRLs. The total solids of the latex compounds were adjusted to 45% by adding appropriate amount of distilled water.

Coagulant dipped films were prepared using 10% calcium nitrate in industrial methylated spirits (IMS) and 30% cyclohexylamine acetate in IMS. The cyclohexylamine acetate was prepared by mixing cyclohexylamine, acetic acid and IMS. The following formulation was used to prepare a 30% cyclohexylamine acetate solution :

	parts by weight
Cyclohexylamine	18.6
Acetic acid	11.4
IMS	70.0

A glass plate was used as a former. The former was immersed in the coagulant and then placed in an oven at 70°C to partially dry the coagulant. It was then immersed in the latex compound for 10 seconds. The wet-gel was allowed to consolidate at room temperature for a few minutes, then leached in distilled water at 50°C. The water contained in 2.5 liter beaker was changed after dipping three sets of 2-3 samples. The latex film was dried at 70 - 100°C. Some of the dried samples were given post-leaching treatment at 50°C in distilled water and slurry dip before final drying at 70°C for 40 minutes. The slurry was prepared by mixing corn starch, Vulcastab LW (cetyl oleyl alcohol-ethylene oxide condensate) as a wetting agent and water. The following formulation was used to prepare a 10% corn starch slurry :

	parts by weight
Corn starch	10.0
Vulcastab LW	0.1
Water	89.9

A sulphur prevulcanized latex was also prepared using HA latex (B). Comparison of extractable protein content of RVNRL (B) films and those of sulphur prevulcanized latex (B) films will be made. The following formulation was used to prepare a sulphur prevulcanized natural rubber latex :

	parts by weight
HA latex (B)	10.0
Potassium laurate	0.25
Potassium hydroxide	0.25
Sulphur	1.0
Zinc diethyldithiocarbamate	0.4
Zinc oxide	0.2

The compound was heated at 60°C for 6 hours. Irganox 1520 (1 p.h.r.) was added in the prevulcanized latex. Coagulant dipped films were prepared using the procedure as described in the preceding paragraph.

The soluble protein content of film samples was analyzed using the RRIM Modified Lowry Microassay with pre-precipitation of protein using trichloroacetic and phosphotungstic acids and the results calibrated against bovine serum albumin[13].

RESULTS AND DISCUSSION

All the data shown in the table of results are those for dipped films using calcium nitrate as the coagulant or otherwise stated. The thickness of films prepared using RVNRL and sulphur prevulcanized NR latex is in the range 0.2 - 0.3 mm.

EFFECT OF LEACHING CONDITIONS AND DRYING TEMPERATURES UPON EXTRACTABLE PROTEIN CONTENT OF RVNRL (A) FILMS

RVNRL films, cast or coagulant dipped, that were dried at room temperature showed very low soluble protein content of 0.06 - 0.12 mg/g despite that the films were unleached (Table 1). Data on extractable protein content of unleached sulphur prevulcanized HA latex (A) films that are dried at room temperature are not available. For comparison, it was shown earlier[4] that an unleached sulphur prevulcanized latex cast film that was dried at room temperature also showed a low extractable protein content of 0.30 mg/g. In this case, the prevulcanized latex was prepared using 1.0 p.h.r. sulphur, 1.0 p.h.r. zinc diethyldithiocarbamate and 0.25 p.h.r. zinc oxide and the latex compound was prevulcanized at 70°C for 2 hours.

Table 1: Extractable protein content of unleached radiation vulcanized NR latex (A) films dried at room temperature

Type of film	Protein content (mg/g)
Cast	0.059
Coagulant dipped, calcium nitrate	0.118
Coagulant dipped, cyclohexylamine acetate	0.061

The extractable protein content of leached RVNRL films that were dried at 70°C - 100°C (Table 2) was found to be significantly higher in comparison to those of unleached RVNRL films that were dried at room temperature (Table 1). In the present investigation, it took about 90 minutes, 60 minutes and 30 minutes to dry wet-gel of RVNRL at 70°C, 80°C and 100°C respectively. The extractable protein content appeared to be affected by a combination of both the drying temperature and drying time. Drying of leached wet-gel of RVNRL at 70°C for 90 minutes tended to produce higher extractable protein content than drying the leached wet-gel at 100°C for 30 minutes. However, the effect of drying temperature and drying time of leached wet-gel of RVNRL became less pronounced with increasing wet-gel leaching time.

Table 2: Effect of leaching and drying conditions on extractable protein content of radiation vulcanized NR latex (A) films

Leaching and drying conditions							
Wet-gel leaching at 50°C (min.)	Drying at 70°C (min.)	Drying at 80°C (min.)	Drying at 100°C (min.)	Post-leaching at 50°C (min.)	Slurry dip (s)	Post-drying at 70°C (min.)	Protein content (mg/g)
1	90	-	-	-	-	-	2.200
3	90	-	-	-	-	-	1.957
5	90	-	-	-	-	-	1.490
1	-	60	-	-	-	-	1.852
3	-	60	-	-	-	-	1.719
5	-	60	-	-	-	-	1.526
1	-	-	30	-	-	-	1.714
3	-	-	30	-	-	-	1.551
5	-	-	30	-	-	-	1.385
-	-	-	30	1	-	40	1.326
-	-	-	30	2	-	40	1.137
-	-	-	30	0.8	10	40	1.319
-	-	-	30	1.8	10	40	1.080
1	-	-	30	0.8	10	40	0.870
2	-	-	30	0.8	10	40	0.495
1	-	-	30	1.8	10	40	0.547
2	-	-	30	1.8	10	40	0.413

Wet-gel leaching alone appeared to be not so effective in bringing down extractable protein content of films to low values. Thus for instance, the sample that had been wet-gel leached for 5 minutes at 50°C and dried at 100°C showed a relatively high extractable protein content of about 1.4 mg/g. A lower extractable protein content of about 1.1 mg/g was obtained when sample dried at 100°C underwent post-leaching alone for 2 minutes at 50°C followed by drying at 70°C. The importance of post-leaching of latex films in the reduction of extractable protein content of latex vulcanizates, in particular prevulcanized latex films has been demonstrated earlier[6,9]. The effect of corn starch slurry dip in the reduction of extractable protein content for samples that did not undergo wet-gel leaching process appeared to be insignificant.

Combination of wet-gel leaching, post-leaching, slurry dip and then followed by drying at a relatively mild temperature of 70°C produced a much more significant reduction of extractable proteins compared to the other leaching procedures described in the preceding paragraph. For instance, the sample that had been wet-gel leached for 2 minutes at 50°C, then dried at 100°C, post-leached for 1.8 minutes at 50°C, dipped into corn starch slurry for 10 seconds and then dried at 70°C showed extractable protein content of about 0.4 mg/g.

FURTHER INVESTIGATION ON FACTORS AFFECTING EXTRACTABLE PROTEIN CONTENT OF FILMS PREPARED FROM RVNRL (B) AND DRIED AT 100°C

The results in Table 2 show that drying of wet-gels of RVNRL at 100°C for 30 minutes tended to produce lower extractable protein content of films than drying the wet-gels at 80°C for 60 minutes or at 70°C for 90 minutes. Furthermore drying of film at a high temperature of say 100°C would increase productivity. A more detailed study on extractable protein content of films dried at 100°C using RVNRL (B) was carried out.

Effect of leaching conditions on extractable protein content of RVNRL (B) films

Table 3 shows that prolonged wet-gel leaching of film for 10 minutes at 50°C reduced the extractable protein content of RVNRL (B) film from a value of more than 2.5 mg/g to a value of 0.85 mg/g. Combination of wet-gel leaching and post-leaching reduce further the extractable protein content. However when the wet-gel leaching time was kept constant at 2 minutes, increasing the post-leaching time from 1.5 minutes to 3 minutes did not bring about further significant reduction of extractable proteins.

Table 3: Effect of leaching and drying conditions on extractable protein content of radiation vulcanized NR latex (B) films

Leaching and drying conditions					
Wet-gel aching at 50°C (min.)	Drying at 100°C(min.)	Post-leaching at 50°C (min.)	Slurry dip (s)	Post-drying at 70°C(min.)	Protein content(mg/g)
3	30	-	-	-	2.457
5	30	-	-	-	1.980
10	30	-	-	-	0.854
2	30	1	-	40	0.870
2	30	1.5	-	40	0.475
2	30	2	-	40	0.446
2	30	3	-	40	0.401
1	30	0.75	10	40	0.113
2	30	0.75	10	40	0.100
3	30	0.75	10	40	0.103
4	30	0.75	10	40	0.071

Combination of wet-gel leaching, post-leaching and slurry dip resulted in reduction of extractable protein content to very low values of about 0.1 mg/g. However when the post-leaching time was kept constant at 0.75 minutes, increasing the wet-gel leaching time from 1 minute to 4 minutes did not bring about further significant reduction of extractable proteins.

Comparison of extractable protein content of RVNRL (A) films and RVNRL (B) films

Comparison of results in Table 2 and Table 3 for films that underwent wet-gel leaching alone and dried at 100°C seem to suggest that RVNRL (B) has a higher extractable proteins than RVNRL (A). The RVNRL (B) films that underwent wet-gel leaching for 3 minutes at 50°C and then dried for 30 minutes at 100°C showed extractable protein content of about 2.5 mg/g. The corresponding RVNRL (A) film showed extractable protein content of about 1.5 mg/g.

Although RVNRL (B) seemed to have higher extractable proteins than RVNRL (A), however the proteins in RVNRL (B) films tended to be more easily removed than the proteins in RVNRL (A) films. This observation applies for both type of leaching procedures used i.e. wet-gel leaching or wet-gel leaching plus post-leaching of films.

Effect of cyclohexylamine acetate on extractable protein content of RVNRL (B) films

Comparison of the results shown in Table 3 and Table 4 indicate that the type of coagulant used to make dipped products also affect extractable protein content. The results clearly show that dipped films prepared using cyclohexylamine acetate produced substantially higher extractable protein content than those prepared using calcium nitrate. For instance, RVNRL (B) film that was prepared using cyclohexylamine acetate, wet-gel leached for 2 minutes at 50°C, post-leached for 1.5 minutes at 50°C and then dried for 40 minutes at 70°C showed extractable protein content of about 1.7 mg/g. The corresponding film prepared using calcium nitrate showed extractable protein content of about 0.5 mg/g.

Table 4: Effect of using cyclohexylamine acetate as a coagulant on extractable protein content of radiation vulcanized NR latex (B) films

Leaching and drying conditions				
Wet-gel leaching at 50°C (min.)	Drying at 100°C (min.)	Post-leaching at 50°C (min.)	Post-drying at 70°C (min.)	Protein content (mg/g)
2	30	1	40	1.946
2	30	1.5	40	1.667
2	30	2	40	1.413
2	30	3	40	0.644

Comparison between extractable protein content of RVNRL (B) and sulphur prevulcanized latex (B) films

RVNRL (B) films that were wet-gel leached for 3 - 5 minutes and then dried at 100°C for 30 minutes showed higher extractable protein content (Table 4) than the corresponding sulphur prevulcanized latex (B) films (Table 5). However when the wet-gel leaching time was increased to 10 minutes or the films were given wet-gel leaching as well as post-leaching, the difference in the extractable protein content of RVNRL (B) films and sulphur prevulcanized latex (B) films became insignificant.

Table 5: Effect of leaching and drying conditions on extractable protein content of sulphur prevulcanized latex (B) films

Leaching and drying conditions					
Wet-gel leaching at 50°C (min.)	Drying at 100°C (min.)	Post-leaching at 50°C (min.)	Slurry dip (s)	Post-drying at 70°C (min.)	Protein content (mg/g)
1	30	-	-	-	1.403
3	30	-	-	-	1.251
5	30	-	-	-	1.144
10	30	-	-	-	0.822
2	30	0.5	-	40	0.485
2	30	1	-	40	0.464
2	30	1.5	-	40	0.419
2	30	2	-	40	0.215
2	30	3	-	40	0.161
2	30	1	10	40	0.233
2	30	2	10	40	0.213
2	30	3	10	40	0.198
2	30	4	10	40	0.108

It is noteworthy that the extractable protein content of sulphur prevulcanized latex films is also dependent upon the type and the levels of compounding ingredients used[7, 9].

CONCLUSION

Wet-gel leaching of RVNRL films alone could not reduce the extractable protein content of these films to a low value of say 0.1 mg/g if the films are dried at temperature range of 70°C - 100°C. When the films are dried at a relatively high temperature of 100°C, a leaching procedure comprising wet-gel leaching, post-leaching, a slurry dip followed by final drying at 70°C could produce films having low extractable protein content. The extractable protein content of RVNRL films tends to be also affected by the source of latex used. With adequate leaching of films, the extractable protein content of RVNRL films tends to be rather similar to that of sulphur prevulcanized latex films. The use of cyclohexylamine acetate as a coagulant for dipping resulted in significantly higher extractable protein content of RVNRL films compared to when using calcium nitrate as a coagulant.

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