Indian Journal of Natural Rubber Research, **14**(1) : 7 - 13, 2001

ENZYMATIC DEPROTEINIZATION OF NATURAL RUBBER LATEX

K. Mariamma George, G. Rajammal, N.R. Peethambaran and N.M. Mathew

George, K.M., Rajammal, G., Peethambaran, N.R. and Mathew, N.M. (2001). Enzymatic deproteinization of natural rubber latex. *Indian Journal of Natural Rubber Research*, **14**(1) : 7 - 13.

An attempt was made to produce low protein natural rubber latex suitable for making dipped goods with low extractable protein content. A laboratory process was standardized for the deproteinization of natural rubber latex using a proteolytic enzyme. Using the standardized process deproteinized latex was prepared on a pilot plant scale and its processing and technological properties were assessed.

Key words : Deproteinization, Extractable protein, Leaching, Natural rubber latex, Protein allergy, Proteolytic enzyme, Vulcanization.

K. Mariamma George (for correspondence), G. Rajammal, N.R. Peethambaran, and N.M. Mathew, Rubber Research Institute of India, Kottayam – 686 009, India (E-mail : rrii@vsnl.com).

INTRODUCTION

The use of natural rubber (NR) latex products is facing serious challenges owing to allergic effects of some of the extractable proteins (EP) contained (Pendle, 1994). Several techniques are being adopted for manufacturing products with very low EP content. Use of low protein latex is one of the important requirements for this. NR latex contains 2 to 3 per cent proteins. During centrifuging, only a part of the soluble proteins are removed. The adsorbed proteins on the surface of the rubber particles cannot be removed by this process (Blackley, 1997). Several methods have been adopted for the removal of bound proteins from the surface of the rubber particles. Peethambaran and Thomas (1985) reported that enzymes can be used for this purpose.

Anilozyme-P is a proteolytic enzyme preparation useful for deproteinization of NR (Rajammal and Thomas, 1978). In this work, an attempt was made to produce low protein latex using this enzyme in a single centrifuging process and to evaluate its properties.

MATERIALS AND METHODS

The study was conducted in two phases. In the first phase, laboratory trials on deproteinization of latex was made and in the second phase, the processing and technological properties of the low protein latex prepared on a commercial centrifuge

using the method standardized in the laboratory trials, were evaluated.

Preparation of low protein latex

Preserved field latex (PFL) containing one per cent ammonia, collected from the RRII Experiment Station was used. The enzyme, Anilozyme-P, a neutral protease derived from a *Bacillus* sp. containing variable quantities of α–amylase (M/s. Anil Starch Products Ltd., Ahmedabad) was used for deproteinization. The active portion of this enzyme preparation is readily soluble in water. The insoluble fraction has no enzyme activity and can easily be removed by filtration. A 5 per cent solution of the enzyme in water was prepared at room temperature and the insoluble materials were removed by filtration. All the other chemicals used were of laboratory reagent grade.

The various process variables such as concentration of the enzyme preparation, effect of addition of different levels of surfactant and dilution of latex were studied as detailed below :

The PFL was treated with different concentrations of the enzyme *viz.*, 0, 0.5, 1 and 1.5 parts per hundred rubber (phr) and stored for 48 h at room temperature and then centrifuged at 6000 rpm in a laboratory centrifuge for 30 min. To study the effect of surfactants, PFL was mixed with 1 phr of the enzyme, kept for 48 h and treated with different concentrations of ammonium laurate (0 to 0.5% on latex) and centrifuged after 24 h. To ascertain the effect of dilution of latex, the PFL was treated with 1 phr enzyme preparation and 0.1 per cent ammonium laurate. The treated latex was diluted to different DRC levels (35, 30, 25 & 20) and then concentrated by centrifuging. Films were prepared from the latex concentrate by casting in shallow glass dishes followed by drying at 70°C overnight. The percentage of nitrogen (N) and extractable protein (EP) content of the films were determined by IS:3708 Part I (1966) and ASTM D 5712-95 respectively.

Experimental sample (LE-I) was prepared by single centrifuging of PFL treated according to the optimum conditions derived. A control sample (LC-I) was also prepared after diluting PFL to 25 per cent DRC without any enzyme treatment. These samples were again subjected to enzyme treatment and centrifuging using the laboratory centrifuge and coded as shown in Table 1.

Preparation of low protein latex for evaluation of processing and technological properties was undertaken using PFL collected from the Pilot Latex Processing Centre (PLPC), RRII Central Experiment Station, Chethackal. The latex was treated with 1 phr of the enzyme, stored for 48 h,

Table 1. **Processing and coding of samples**

Sample	Treatment and processing	Sample after processing
LC - 1	Centrifuging	LC - 2
	Enzyme treatment and centrifuging	LCE - 2
LE - 1	Diluting and centrifuging	LE - 2
	Enzyme treatment and centrifuging	LEE - 2
PFL	Single centrifuging (control)	A
	Double centrifuging (control)	B
PFL	Enzyme treatment and centrifuging	C
	Enzyme treatment and double centrifuging	D

Table 2. **Raw latex properties**

Parameter	Latex sample			
	A	B	C	D
Dry rubber content, %	61.21	62.74	61.73	63.01
Total solids content, %	62.07	62.99	62.82	63.28
Non-rubber solids, %	0.86	0.25	1.09	0.27
Alkalinity as ammonia, %	0.69	0.67	0.69	0.68
Mechanical stability time, sec	>1000	960	>1000	420
Volatile fatty acid number	0.02	0.01	0.02	0.01
KOH number	0.44	0.27	0.55	0.24
Copper content, ppm	0.31	0.21	Trace	Trace
Manganese content, ppm	Trace	Trace	Trace	Trace
pH	10.8	11.0	10.6	10.9
Ash content, %	0.28	0.15	0.33	0.16
Magnesium content, %	Trace	Trace	Trace	Trace
Acetone extract, %	2.54	2.22	2.59	2.45
Nitrogen content, %	0.16	0.10	0.10	0.09
EP content, mg/kg	120	40	45	35

treated with ammonium laurate (0.1% on latex), stored for further 24 h and diluted to 25 per cent DRC. It was then subjected to single and double centrifuging. Control samples were also prepared (Table 1). The samples were stored for a period of one month before testing.

Processing and technological properties

Raw latex properties were determined as per ASTM standards and are given in Table 2. Brookfield viscosity of the samples was determined as per BIS method. The zinc oxide viscosity (ZOV), zinc oxide stability time (ZST) and zinc oxide heat stability time (ZHST) were also determined. For the measurement of zinc oxide viscosity, the latex was treated with 1 phr of zinc oxide (as 40% dispersion) and viscosity of the compounded latex was measured after 5 and 60 min using Brookfield viscometer. For determining ZST, the latex was treated with 1 phr of zinc oxide (as 40% dispersion), kept for 1 h and the mechanical stability time of the latex compound was measured using an MST apparatus. For the measurement of ZHST, the latex was treated with 1 phr of zinc oxide (as 40% dispersion), kept for 1 h and the time for complete coagulation when

Table 3. **Viscosity characteristics**

Property	Latex sample			
	A	B	C	D
Brookfield viscosity, Spindle No.2, 60 rpm, mPa s	110	107	108	91
Zinc oxide viscosity (5 minutes after adding ZnO), mPa s	115	112	113	95
Zinc oxide viscosity (60 minutes after adding ZnO), mPa s	130	128	128	108
Zinc oxide stability time, s	154	102	89	96
Zinc oxide heat stability time, s	224	280	228	274

Table 4. **Formulation of latex compound**

Ingredient	Parts by weight (wet)
Centrifuged latex, 60%	167.0
Potassium hydroxide 10% solution	0.2
Sulphur 50% dispersion	2.0
Zinc dibutyldithocarbamate 50% dispersion	1.0
Zinc oxide 50% dispersion	0.6
Antioxidant BHT[a] 50% dispersion	2.0

[a]2, 6-di-t-butyl-p-cresol

heated at 90°C in a water bath was noted. Results are given in Table 3.

Latex compounds were prepared as per the formulation given in Table 4 and diluted to 50 per cent total solids (TS). Films wereprepared from the latex compound by coagulant dipping using 10 per cent calcium chloride solution as coagulant. These were then dried at 70°C and vulcanized for 1 h at 100°C in a hot air oven.

Tensile properties of the films were determined as per ASTM D-3188, using Universal Testing Machine (Instron, Model 4411). Films were subjected to ageing at 100°C for 22 h in a multicellular ageing oven and tensile properties determined. EP content of the films were determined before and after wet / dry film leaching (70°C for 2 min).

RESULTS AND DISCUSSION

Standardization of the process

The effect of varying concentrations of enzyme on the N and EP content of latex concentrate prepared from PFL is shown in Figure 1. It is seen that as the concentration of the enzyme increased, N and EP content of the latex films decreased and this effect almost stabilized when the concentration of the enzyme exceeded 1 phr. Hence, in this

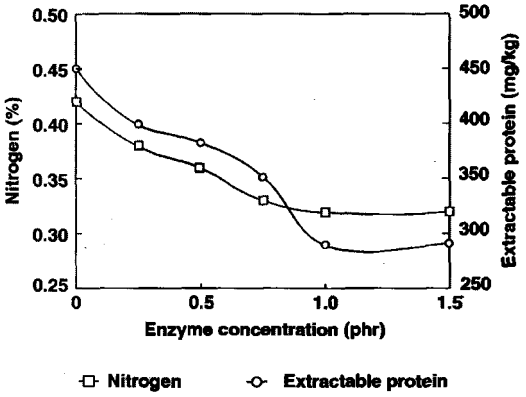

Fig. 1. Effect of enzyme concentration on nitrogen and extractable protein content

study, 1 phr of the enzyme was selected as the optimum dosage. Being a protease, this enzyme catalyses the hydrolysis of proteins which are bound to the latex particles, into water soluble polypeptides and amino acids, which get removed during the centrifuging process (Rajammal and Thomas, 1978).

The effect of varying concentrations of ammonium laurate on the N and EP content of latex concentrate prepared from enzyme treated PFL is plotted in Figure 2. As the concentration of ammonium laurate increased, the N and EP content of the latex films decreased. But a dosage of 0.10 per

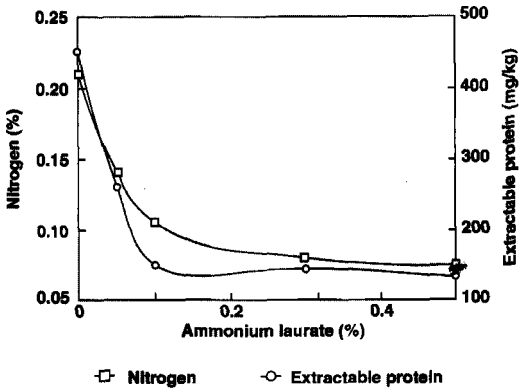

Fig. 2. Effect of ammonium laurate concentration on nitrogen and extractable protein content

cent on latex was found to be the optimum, as latex containing higher dosage of soap is unsuitable for dipped goods manufacture. Soaps being highly surface active are known to displace proteins from latex particles (Chin *et al.*, 1974). Hydrolysis by a combination of enzyme and soap helps to further reduce the protein content in the latex as is evident from the results.

The effect of dilution on deproteinization by enzyme and ammonium laurate treated PFL is shown in Figure 3. The results indicate that due to dilution the N and EP content decreased. Dilution of latex helps in the increased dissolution of the non-rubber constituents in latex and these are removed along with the serum during the centrifuging process. A dilution to 25 per cent DRC was selected as the optimum as further reduction in DRC adversely affected the efficiency of centrifuging.

The effect of double centrifuging and multiple enzyme treatment as determined from N and EP content measurements is given in Figure 4. Double centrifuging of PFL reduced the protein content in latex considerably (sample LC-2). This is because

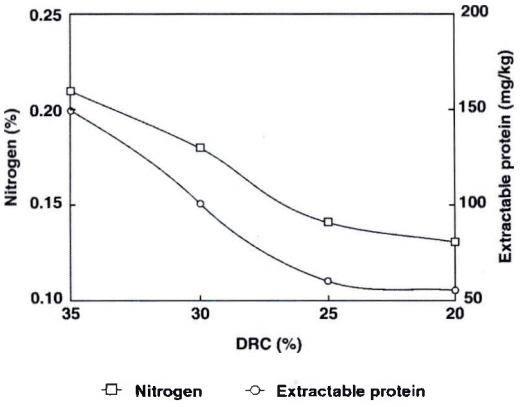

Fig. 3. Effect of dilution on nitrogen and extractable protein content

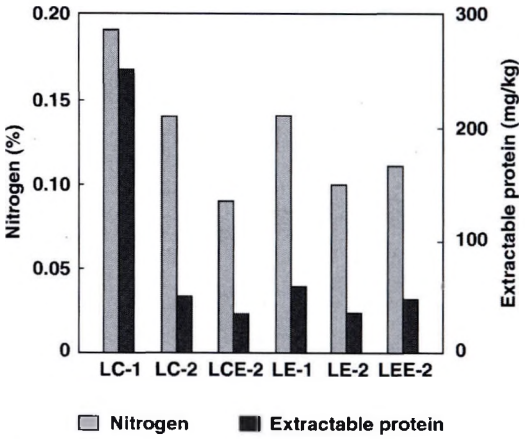

Fig. 4. Effect of centrifuging and enzyme treatment on nitrogen and extractable protein content

more non-rubbers are removed during the repeated centrifuging process. A combination of single enzyme treatment and double centrifuging helped to remove the protein content further (samples LCE-2 & LE-2). Multiple enzyme treatment and centrifuging (sample LEE-2) did not show any appreciable reduction in EP content.

Comparison of the results of samples LC-2 and LE-1 indicates that the effect of double centrifuging of PFL on reduction of EP content can be achieved by enzyme treatment of PFL with single centrifuging.

Properties of low protein latex

The N and EP content of low protein latex samples B and C processed in the plant trials were similar (Fig. 5) confirming that enzyme treatment of PFL with single centrifuging had the same effect as that of double centrifuging of PFL. Further centrifuging of sample C did not show any marked reduction in nitrogen content (sample D).

Latex properties such as dry rubber content (DRC), total solids content (TSC), non-rubber solids (NRS) content, alkalinity (NH_3), mechanical stability time (MST),

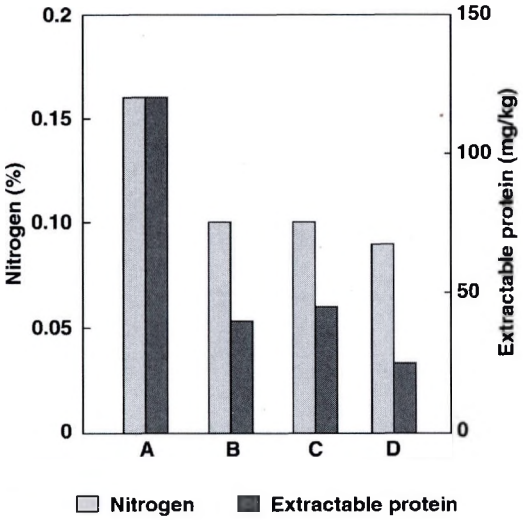

Fig. 5. Effect of latex properties on nitrogen and extractable protein content

volatile fatty acid (VFA) number, potassium hydroxide (KOH) number, copper (Cu) and manganese (Mn) (Table 2), were within the limits specified by the Bureau of Indian Standards (IS 5430-1981). The NRS content of samples B and D were lower. This may be due to the effect of double centrifuging. NRS of sample C was higher compared to sample A. This could be due to the presence of residual materials from the enzyme preparation. The higher ash content in sample C indicates inorganic residues of the enzyme in latex. Acetone extract of all the four samples were comparable.

The viscosity changes of samples before and after the addition of zinc oxide are shown in Table 3. Initial Brookfield viscosity of the samples were comparable. Zinc oxide viscosity (ZOV) of the samples increased with time and were comparable for all the samples. Zinc oxide stability time (ZST) of samples B, C and D were lower compared to sample A. This may be due to the lower levels of bound proteinaceous materials in the latices B, C and D. Proteinaceous materials bound to the surface of the rubber particles in NR latex contribute to the stability of the latex, with steric and hydration effects (Blackley, 1997). Zinc oxide heat stability time (ZHST) of all the four samples were comparable.

The initial tensile properties of all the latex film vulcanizates were comparable (Table 5). After ageing at 100^{0}C for 22 h, a sharp reduction in tensile properties was noted for samples B, C and D compared to A. This may be due to removal of more natural antioxidants from the latex during deproteinization / double centrifuging.

Table 5. **Physical properties of vulcanized films**

Sample	500% modulus (MPa)	Tensile strength (MPa)	Elongation at break (%)
Before ageing			
A	1.71	28.96	1086
B	1.63	23.80	1250
C	1.79	25.67	1024
D	1.65	22.85	1200
After ageing*			
A	1.73	20.27	1050
B	1.33	15.0	1210
C	1.72	16.7	1150
D	1.34	13.5	1163

*At 100^{0}C for 22 h

The effect of wet and dry film leaching on the EP content of latex film vulcanizates is shown in Fig. 6. It was seen that even without leaching, EP content of sample C was lower than leached films of sample A and were comparable to samples B and D. Wet and dry film leaching were capable of reducing the EP content in all the films, the effect being more pronounced with dry film leaching. During drying and vulcanization, migration of moisture in the latex film to the

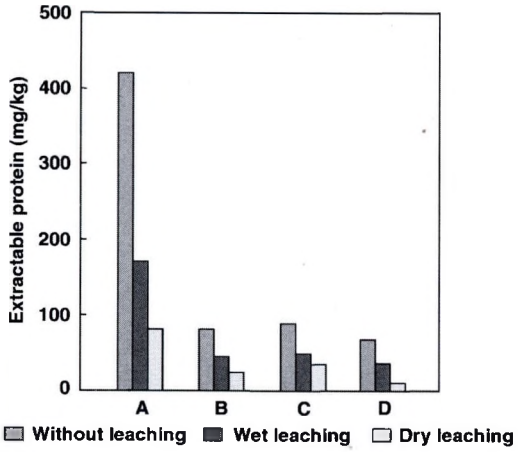

Fig. 6. Effect of leaching on extractable protein content

outer surface takes residual compounding ingredients and some of the proteins along with it, which is removed during dry film leaching (Bodycoat, 1993).

CONCLUSION

Low protein latex can be prepared in a single centrifuging process after treatment with the proteolytic enzyme. The optimum conditions for the deproteinization process have been identified. The extent of deproteinization is equivalent to that of double centrifuging of PFL. Multiple enzyme treatment followed by centrifuging of the latex has no significant effect on deproteinization. The properties of the raw latex and latex film vulcanizates were found to be satisfactory. EP of vulcanizates from the low protein latex was low, even before leaching.

ACKNOWLEDGEMENT

The facilities and support rendered by the PLPC of the RRII Central Experiment Station, Chethackal are gratefully acknowledged.

REFERENCES

Blackley, D.C. (1997). Polymer latices : Science and technology. Ed. 2. Chapman and Hall, London, pp. 76-113.

Chin, P.S., Chang, W.P., Lau, C.M. and Pong, K.S. (1974). Deproteinized natural rubber (DPNR). *Proceedings of the Rubber Research Institute of Malaysia, Planters' Conference*, 1974, Kuala Lumpur, Malaysia, pp. 252-262.

Bodycoat, I. (1993). Manufacture of hypoallergenic rubber products. *Proceedings of International Conference on Latex Protein Allergy*, 1993, Amsterdam, The Netherlands, pp. 43-45.

Peethambaran, N.R. and Thomas, E.V. (1985). Preparation of electricians' gloves from natural rubber latex. *Journal of Polymer Materials*, **2** : 58-63.

Pendle, T.D. (1994). Challenges for NR latex products in medical and food related applications. MRPRA Publication, UK, pp. 3-17.

Rajammal, G. and Thomas, E.V. (1978). Preparation and properties of deproteinized natural rubber produced using indigenous enzymic formulations. *Rubber Board Bulletin*, **15**(3&4) : 45-49.