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Preparation and Properties of Low Protein NR Latex Using Stabilised Liquid Papain

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An attempt was made to produce low protein natural rubber (NR) latex suitable for making surgical gloves with low levels of extractable protein content in a single centrifuging process. Stabilised liquid papain from papaya plant (Carica papaya) at two dosages viz.*, 0.25 p.h.r. and 0.5 p.h.r. was used for latex deproteinization. A process was standardised for the deproteinization of NR latex by conducting laboratory, pilot plant and plant trials. Processing and technological properties of these low protein latices were assessed. Surgical gloves were prepared in an automatic dipping plant under different conditions of leaching. The extractable protein content and physical properties of the gloves were determined. The results showed that low protein latex prepared using 0.25 p.h.r. of liquid papain produced gloves of extractable protein (EP) content less than 50 µg/dm^2. Antigenic protein content obtained for the samples were also less than 10 µg/dm^2. Continuous production of surgical gloves from this latex by on line wet gel and post-cure leaching was found to give low protein gloves with good physical properties. The effect of slurry dip on protein content could be controlled more effectively using this latex as the amount of EP content in the original latex was low and hence the amount of proteins leaching into the slurry would also be less.*

Storage behaviour of this latex and its vulcanizates was studied in comparison to normal centrifuged latex (cenex). Changes in raw latex properties such as volatile fatty acids, mechanical stability time, potassium hydroxide number and zinc oxide viscosity were comparable for both the latices. But the change in EP content for low protein latex vulcanizates was from 68 to 88 µg/dm^2 where as for the cenex, the increase was from 150 to 368 µg/dm^2. Physical properties of latex film vulcanizates were more or less unaffected by storage.

Key words: deproteinization; extractable protein; gloves; leaching; natural rubber latex; papain; storage behaviour; cenex

Natural rubber (NR) latex protein allergy of Type 1 (immediate) hypersensitivity has emerged as a serious health related issue since the late eighties[1–3]. Several techniques are being adopted to reduce the allergenic potential of NR latex gloves through the production of low protein and powder free gloves. Use of low protein latex produced either by mechanical or chemical means is common for reducing protein content of latex

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based products. The mechanical process involves multiple centrifugation or membrane filtration of diluted latex. The chemical treatment involves a combination of digestion with a proteolytic enzyme or displacement of adsorbed proteins using a surfactant and the subsequent purification of the treated latex by centrifugation or creaming. Even though the latex products industry has been offered both commercial and experimental low protein latices, the commercial uptake is low.

Papain is a proteolytic enzyme preparation derived from papaya plants (*Carica papaya*). It has been used for the production of deproteinized natural rubber (dry form)[4]. In this work, an attempt is made to produce low protein latex using this enzyme preparation in a single centrifuging process and to evaluate its suitability for the production of surgical gloves in an automatic dipping plant. Storage behaviour of this latex and the vulcanizates prepared from it are also studied in comparison to normal centrifuged latex (cenex) for a period of six months.

MATERIALS AND METHODS

The study was conducted in three phases. In the first phase, laboratory trials on deproteinization of NR latex using stabilised liquid papain were carried out. In the second phase, low protein latex was prepared in a pilot plant scale using Alfa Laval centrifuge and its processing and technological properties were determined. In the third phase, low protein latex was prepared in a commercial centrifuging unit and evaluated for the production of surgical gloves in an automatic dipping plant.

Preparation of Low Protein Latex

Preserved field latex containing one percent ammonia (PFL), collected from the Rubber Research Institute of India Experiment Station was desludged and used as the starting material. Stabilised Liquid Papain (LP) supplied by M/s. Senthil Papain and Food Products (P) Ltd. Coimbatore, India was used for deproteinization. A typical composition of Liquid Papain is given in *Table 1*. All other chemicals used were of laboratory reagent grade.

Laboratory trials showed that treatment of NR latex with 0.25 p.h.r. (parts per hundred rubber) of papain as 5% aqueous solution had reduced the stability of the latex. This may be due to the acidic nature and mineral content present in the sample as shown in *Table 1*. It was seen that stability of latex was maintained when liquid papain was initially treated with 1 p.h.r. potassium oleate and then used for deproteinization. Enzyme digestion was done at two concentrations of papain *viz* 0.25 p.h.r. and 0.50 p.h.r.. PFL was mixed with the enzyme, kept at room temperature for 48 h, treated with ammonium laurate (0.01% w/v on latex), stored for a further 24 h and diluted to 25% w/w dry rubber content (DRC). It was then concentrated by centrifuging at 6000 rcf in a laboratory centrifuge. Dry films were prepared from the latex concentrate. The percentage of nitrogen (N) and extractable protein (EP) content of the films were determined by *ASTM D 3533-90*[5] and *ASTM D 5712-99*[6] respectively. Two samples of centrifuged latex were prepared without any enzyme treatment, but with and without dilution of PFL to 25% w/w DRC before centrifuging.

Pilot plant trials on deproteinization using the Alfa Laval centrifuge machine were carried out to assess the raw latex and technological properties. Plant scale trials were conducted to evaluate its suitability for the production of surgical gloves. Sample code, batch size, N and EP content of all the control and experimental latices are given in *Table 2*.

TABLE 1. TYPICAL COMPOSITION OF LIQUID PAPAIN

Parameter	Liquid papain
Activity, TU/mg	208.15
Total plate count, CFU/gm, (FDA)	20.00
Moisture content, %	1.66
Mineral content, %	2.16
pH of aqueous solution	5.40
Pathogens	Free
Yeast and mould count, CFU/gm	<10
Lead, arsenic and heavy metals	Below detectable limit
Aflatoxin & pesticides	Absent

TABLE 2. TRIALS ON DEPROTEINIZATION OF PRESERVED FIELD LATEX

Type	Sample code	Dosage of papain, p.h.r.	Nitrogen content of dry film, % w/w	EP content of dry film, $\mu g/dm^2$
Laboratory trials	1	0.25	0.16	135
	2	0.50	0.13	142
	3	Nil	0.26	330
	[a]4	Nil	0.22	260
Pilot plant, Batch size–200 L PFL	5	0.25	0.09	60
	6	0.50	0.08	62
	7	Nil	0.18	137
	[a]8	Nil	0.16	120
Plant trial-Batch size–6000 L PFL	9	0.25	0.09	58
	10	0.50	0.08	61
	11	Nil	0.18	130
Plant trial-Batch size–12000 L PFL	12	0.25	0.09	55

[a]PFL diluted to 25% w/w DRC

Pilot Plant Trials – Raw Latex and Technological Properties

Properties of latex concentrate (Samples 5, 6 and 7) are given in *Table 3*. Dry rubber content (DRC), total solids content (TSC), alkalinity (NH_3), mechanical stability time (MST), volatile fatty acid (VFA) number, potassium hydroxide (KOH) number, coagulum content and viscosity were determined as per *ASTM D 1076-02*[5]. Ash content of the dry film was determined as per *ASTM D 1278-91a*[5] . The zinc oxide viscosity (ZOV), zinc oxide stability time (ZST) and zinc oxide heat stability time (ZHST) were determined as reported earlier[7].

Latex compounds were prepared and diluted to 50% w/v total solids (TS). Films were prepared from the latex compound by coagulant dipping using 10% w/v calcium chloride solution as coagulant. These were then dried at 70ºC and vulcanized at 100ºC for 1 h in air.

TABLE 3. PROPERTIES OF LATEX CONCENTRATE

Parameter	Sample code 5	6	7
Dry rubber content, % w/w	60.22	60.01	60.56
Total solids content, % w/w	61.16	60.97	62.27
Non-rubber solids, % w/w	0.94	0.96	1.71
Alkalinity as ammonia, % w/w	0.69	0.72	0.78
Mechanical stability time, s	>1000	>1000	>1000
Volatile fatty acid number	0.01	0.01	0.01
KOH number	0.44	0.45	0.48
Coagulum content, % w/w	0.01	0.01	0.01
Nitrogen content of dry film, % w/w	0.09	0.08	0.18
EP content of dry film, μg/dm^2	60	62	137
Ash content of dry film, % w/w	0.07	0.08	0.23
Brookfield viscosity, Spindle No.2, 60 r.p.m., mPa s	60	64	110
Zinc oxide viscosity (5 min after adding ZnO), mPa s	64	68	115
Zinc oxide viscosity (60 min after adding ZnO), mPa s	72	79	130
Zinc oxide stability time, s	111	98	142
Zinc oxide heat stability time, s	150	135	230

TABLE 4. PHYSICAL PROPERTIES OF LATEX FILM VULCANIZATES[a]

Parameter	Sample code 5	6	7
		Before ageing	
500 % modulus, MPa	1.44	1.48	1.55
Tensile strength, MPa	25.00	24.21	25.10
Elongation at break, %	1305	1338	1300
		Percentage retention after ageing at 100° C for 22 h	
500 % modulus	94	92	95
Tensile strength	92	90	94
Elongation at break	102	102	106

[a](Parts by weight) Natural rubber–100, Potassium hydroxide–0.2, Sulphur–2.0, Zinc dibutyl dithiocarbamate–1.0 and Zinc oxide–0.6

Physical properties of the films were determined as per *ASTM D 412-98a*[5], using a 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 were determined. The results are given in *Table 4*.

Plant Scale Production and Evaluation of Surgical Gloves

Surgical gloves were prepared from the papain treated latices in two phases, in the first phase using latex 9, 10 and 11 and in the second phase using latex 12 in a commercial glove manufacturing unit, M/s. Kanam Latex Industries, Nagercoil, India.

During production, the gloves were subjected to different wet gel and post-cure leaching processes as shown:

(a) Without leach
(b) One wet gel leach (42ºC)
(c) One wet gel leach (42ºC) and one post-cure leach (50ºC)
(d) One wet gel leach (42ºC) and two post-cure leaches (50ºC and 68ºC)
(e) One wet gel leach (42ºC) and three post-cure leaches (50ºC, 68ºC and 87ºC)

At each of the above leaching conditions, gloves from Samples 9, 10 and 11 were collected after running the plant for 30 min, while gloves from Sample 12 were collected after running the plant for 8 h. Glove samples were tested for protein content and antigenic protein content of Sample 9 as per *ASTM D 6499-03*[6]. Tests were carried out at Donald Guthrie Foundation for Education and Research, USA. The results are given in *Table 5*. Physical properties of the gloves from Sample 9 were tested as per *ASTM D-3577-01a*[6] and the results are given in *Table 6*. The inspection report obtained from the factory during the production is represented in *Table 7*.

Storage Behaviour of Latices

Low protein latex (Sample 5) and the cenex (Sample 7) prepared in the pilot plant were stored for a period of 6 months. The properties of latices and their vulcanizates were tested periodically and the results are given in *Tables 8* and *9* respectively.

RESULTS AND DISCUSSION

Properties of Low Protein Latex

Results in *Table 2* show that dilution of PFL to 25% w/w DRC before centrifuging has reduced the nitrogen content in both laboratory and pilot plant trials. However, the values were not within the tentative limits specified for low protein latex (nitrogen content 0.10% w/w max.)[8]. A comparison of the results for Samples 1 and 2 with 3 showed that both concentrations of liquid papain (0.25 p.h.r. and 0.5 p.h.r.) were capable of reducing the nitrogen content of NR latex under laboratory

TABLE 5. EXTRACTABLE PROTEIN CONTENT AND ANTIGENIC PROTEIN CONTENT OF SURGICAL GLOVES

Treatment details	Extractable protein content, µg/dm²				Antigenic protein content, µg/dm²
Sample code	9	10	11	12	9
Without leach	100	110	298	43	5.7
One wet gel leach	87	95	268	37	7.1
One wet gel leach and one post-cure leach	50	65	208	32	5.2
One wet gel leach and two post-cure leaches	45	52	173	30	–
One wet gel leach and three post-cure leaches	43	48	146	22	–

TABLE 6. PHYSICAL PROPERTIES OF GLOVES FROM SAMPLE 9

Treatment	500% modulus		Tensile strength		Elongation at break	
	Before ageing GPa	% retention after ageing	Before ageing MPa	% retention after ageing	Before ageing %	% retention after ageing
Without leach	1.69	115	27.10	88	1086	89
One wet gel leach	1.76	117	28.12	90	1071	90
One wet gel leach and one post-cure leach	1.80	117	29.62	91	1062	92
One wet gel leach and two post-cure leaches	1.85	119	29.70	91	1059	92
One wet gel leach and three post-cure leaches	2.02	118	30.12	92	1043	94

TABLE 7. 100% INSPECTION REPORT OF SURGICAL GLOVES

Day of Production	Particulars	High quality	B grade	Pinhole	Damage	Plant damage
1^{st} day	Right	63660	938	1918	575	184
	%	94.60	1.40	2.90	0.90	0.30
	Left	64793	723	1579	259	319
	%	95.70	1.10	2.30	0.40	0.50
2^{nd} day	Right	62522	392	1891	136	201
	%	95.98	0.60	2.90	0.20	0.30
	Left	64119	495	490	113	109
	%	98.10	0.76	0.80	0.20	0.16

conditions. The nitrogen and EP content of low protein latex Samples 5 and 6 were lower than Sample 7 confirming that enzyme treatment of PFL with liquid papain was effective in the pilot plant scale.

In the plant trial where low protein latex was used for line production of gloves, 60% centrifuged latex was used as the control (Sample 11). A comparison of EP content of low protein latices 9 and 10 showed that EP content of the sample with 0.25 p.h.r. of papain was less than that for the sample with 0.5 p.h.r. of papain. Hence a dosage of 0.25 p.h.r. papain was selected for large scale production (Sample 12) and its results were found to be conforming to the results of Sample 9.

Properties of latex concentrate are given in *Table 3*. The NRS content and ash content of papain treated samples were lower than those of the control sample. This showed that the latex was purified by this treatment through the removal of non-rubbers. The low ash content may be due to the removal of mineral matter by soap treatment and dilution during the process. This also showed that no additional mineral matter has been deposited in the latex by the papain treatment, but the latex has become more pure. Other latex properties such as dry rubber content (DRC), total solids content (TSC), alkalinity (NH_3), mechanical stability time (MST), volatile fatty acid (VFA) number, potassium hydroxide (KOH) number and coagulum content were within the limits specified by the Bureau of Indian Standards *(IS 5430-1981)*[9]. Initial Brookfield viscosity of low protein latices was lower than the control. Zinc oxide viscosity (ZOV) of the samples increased with time and the increase was less for low protein latices compared to the control. This may be due to the presence of potassium oleate in the low protein latices. Zinc oxide stability time (ZST) and zinc oxide heat stability time (ZHST) of deproteinzed latices were lower compared to the control. The increased sensitivity of deproteinized latex towards zinc oxide may probably be a consequence of increased aqueous ionic content due to enzymatic degradation of proteins to amino acids and polypeptides[10]. Another factor is that the latex may be partially destabilised by the enzymatic degradation and perhaps by the partial desorption of surface bound proteinaceous stabilizers. Lepetit[11] and Rajammal and co-workers[12] had reported similar results for natural rubber latex, when digested with proteolytic enzyme preparations, trypsin and anilozyme respectively.

Physical properties of dipped latex film vulcanizates before and after ageing are given in *Table 4*. The tensile properties and ageing resistance of the low protein latices were comparable to the control (Sample 7).

Plant Trials – Properties of Surgical Gloves

The EP content of the surgical gloves from latices 9, 10, 11 and 12 are given in *Table 5*. Even without giving any leaching, the values were 100 μg/dm^2, 110 μg/dm^2 and 43 μg/dm^2 for Samples 9, 10 and 12 respectively while the value for the control Sample 11 was 298 μg/dm^2. It was observed that the EP content of the gloves from Samples 9, 10 and 12 could be brought down to 87 μg/dm^2, 95 μg/dm^2 and 37 μg/dm^2 respectively with one wet gel leach and for Sample 11 it was 268 μg/dm^2. During leaching, hydrophilic materials were removed from latex dipped products by dissolving in water[13]. This resulted in the improvement in physical properties such as tensile strength and film clarity, prevention of surface blooms and reduction in water absorption.

The incorporation of post-cure leaching had reduced the EP content. The EP values of Samples 9, 10 and 12 were reduced to 50 μg/dm^2, 65 μg/dm^2 and 32 μg/dm^2 respectively after one post-cure leach while for Sample 11 it was 208 μg/dm^2. The EP content of the gloves was further reduced during successive post-cure leaching. It was reported that there was a considerable reduction in the EP content with one post-cure leach. This may be due to the fact that a substantial amount of water-soluble proteins was generated upon drying and vulcanization of dipped products causing those proteins to be drawn towards the surface away from the bulk during this stage, which was removed during post-cure leaching[14–16].

A comparison of the EP content of low protein latices 9 and 10 showed that the EP content of the sample with 0.25 p.h.r. papain was less than that for the sample with 0.5 p.h.r. papain. Hence, a dosage of 0.25 p.h.r. papain may be sufficient for enzyme treatment of latex. The results showed that the use of this low protein latex will help in reducing or even eliminating the post-cure leaching for obtaining gloves with an EP content of less than 50 μg/dm^2.

Cornstarch powder is used in the manufacture of gloves to facilitate easy removal from the mould as well as to prevent the gloves from sticking together[17,18]. It is reported that while a substantial amount of EP in gloves can be effectively removed when the slurry dip is performed after drying, there is a danger of protein build-up with time in the slurry during continuous production of gloves. A serious concern about cornstarch powder is that it acts as a carrier of airborne allergens and causes respiratory allergic reactions because natural rubber latex allergens have been shown to bind to cornstarch[19].

The EP content of low protein gloves is found to be less than the control sample before and after giving the leaching treatments. It is expected that these low values could be maintained through out the process, as the amount of proteins leaching into the slurry and binding to cornstarch will also be less.

While comparing Samples 9 and 12, the EP content was lower for gloves from Sample 12. Even though the treatment details were same for both the samples, the initial PFL and batch sizes were different. The good manufacturing practices in the glove factory during continuous production might have contributed to the reduction in the protein content. Lower values of protein content for Sample 12 (43 $\mu g/dm^2$ – 22 $\mu g/dm^2$) showed that the papain treatment was also effective in large scale production.

Antigenic protein content of surgical gloves from Sample 9 is also included in *Table 5*. The values obtained for the samples were within the limit specified by *ASTM D 3577-01a*[6], which was 10 $\mu g/dm^2$.

TABLE 8. EFFECT OF STORAGE ON PROPERTIES OF LOW PROTEIN LATEX AND CENEX

Properties	Sample code	Period of storage (months) 1	2	3	4	5	6
Dry rubber content, % w/w	5	60.22	60.22	60.20	60.18	60.15	60.10
	7	60.56	60.55	60.52	60.49	60.45	60.42
Non rubber solids, % w/w	5	0.94	0.95	0.99	1.03	1.08	1.13
	7	1.71	1.72	1.74	1.78	1.80	1.88
Volatile fatty acid number	5	0.010	0.011	0.013	0.016	0.018	0.022
	7	0.010	0.012	0.014	0.017	0.020	0.023
KOH number	5	0.44	0.44	0.46	0.49	0.53	0.57
	7	0.48	0.49	0.50	0.52	0.54	0.57
Mechanical stability time, s	5	>1000	>1000	>1000	>1000	>1000	>1000
	7	>1000	>1000	>1000	985	975	963
Brookfield viscosity, mPa s	5	60	60	62	63	65	67
	7	110	111	113	114	116	118
Zinc oxide viscosity (5min. after adding ZnO) mPa s	5	64	64	66	68	69	74
	7	115	115	117	118	120	123
Zinc oxide viscosity (60min. after adding ZnO) mPa s	5	72	73	74	74	76	80
	7	130	131	132	132	133	136
Zinc oxide stability time, s	5	111	105	101	94	83	70
	7	142	140	136	133	130	122
Zinc oxide heat stability time, s	5	150	140	132	120	113	100
	7	230	225	223	209	200	196

Note: Sample 5 = low protein latex; Sample 7 = cenex

Effects of leaching on tensile properties of the gloves prepared with 0.25 p.h.r. of liquid papain (Sample 9) before and after ageing at 100ºC for 22 h are given in *Table 6*. The tensile properties and ageing resistance of the gloves were within the specified limits.

The results of 100% inspection report given in *Table 7* show that 95% to 98% of the gloves produced were of high quality. B grade gloves range from 0.6% to 1.4%, pinhole gloves from 0.8% to 2.9%, damage from 0.2% to 0.9% and plant damage from 0.16% to 0.5%.

Storage Behaviour of Latices

The effect of storage on properties of low protein latex and cenex are given in *Table 8*. Changes in properties observed on DRC, NRS, VFA number and KOH number during storage were comparable for both the latices. The MST values of low protein latex were greater than 1000 s during the period under study showing good mechanical stability characteristics. Brookfield viscosity increased on storage for both the latices. Zinc oxide viscosity of the low protein latex increased during storage and the increase was comparable to cenex. ZST and ZHST of low protein latex decreased during storage and the decrease was more for the low protein latex. Results obtained for the nitrogen content of the coagulated film and the EP content of the vulcanizate is represented in *Figures 1* and *2* respectively. Nitrogen content almost remained the same for both the latices whereas EP content increased during storage. The increase in EP content for low protein latex was from 68 µg/dm^2 to 88 µg/dm^2, whereas for the cenex the increase was from 150 µg/dm^2 to 368 µg/dm^2. The results of low protein latex showed that not much water-soluble protein was generated during storage of the latex and

TABLE 9. EFFECT OF STORAGE ON PHYSICAL PROPERTIES OF LATEX FILM VULCANISATE

Properties	Sample code	Period of storage (months) 1	2	3	4	5	6
		Before ageing					
500% modulus, MPa	5	1.44	1.40	1.36	1.23	1.16	1.03
	7	1.55	1.50	1.38	1.30	1.24	1.13
Tensile strength, MPa	5	25.00	24.95	24.86	24.46	23.76	23.00
	7	25.20	25.10	24.92	24.81	23.98	23.35
Elongation at break, %	5	1305	1296	1278	1243	1211	1185
	7	1300	1295	1280	1276	1260	1240
		Percentage retention after ageing at 100ºC for 22 h					
500% modulus	5	87	79	76	67	65	52
	7	89	82	81	77	75	67
Tensile strength	5	92	90	87	85	85	80
	7	91	91	90	89	88	85
Elongation at break	5	101	100	100	102	103	103
	7	106	105	104	102	101	100

Note: Sample 5 = low protein latex; Sample 7 = cenex

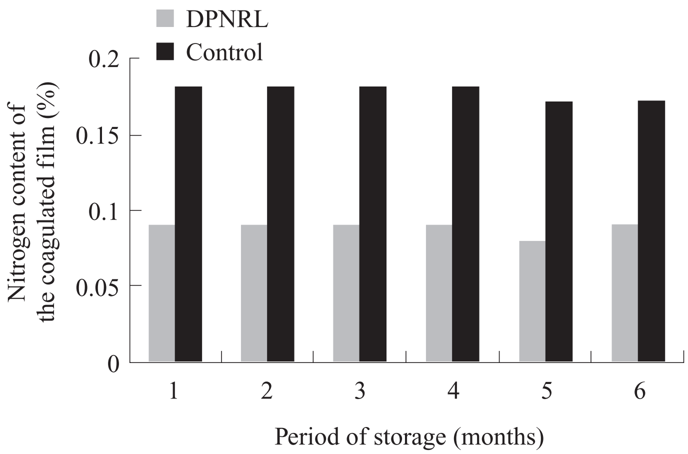

Figure 1. Effect of storage of latex on nitrogen content.

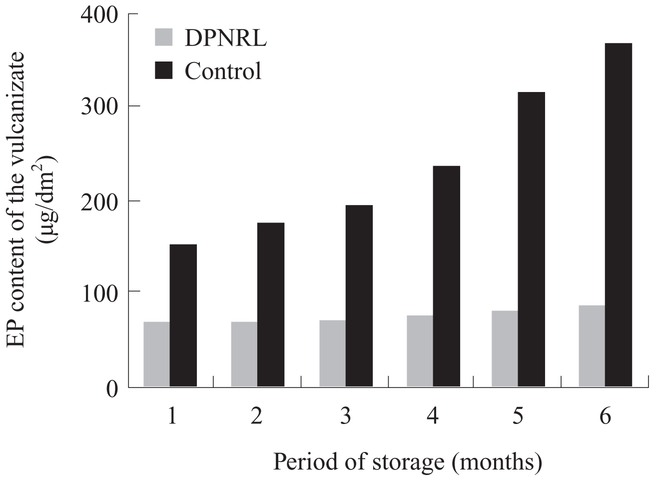

Figure 2. Effect of storage of latex on EP content.

preparation of latex film vulcanizates. This may be due to the fact that during digestion of field latex with papain, most of the proteins may have undergone decomposition and are removed during centrifuging. The residual proteins may be the non-removable types that are strongly bound to or within the rubber particles[10]. Unlike the cenex, proteins will not be hydrolysed by ammonia during storage. Effect of storage on physical properties of latex film vulcanizates before and after ageing is given in *Table 9*. The results showed that tensile properties and ageing resistance of low protein latex were comparable to the cenex and were more or less unaffected by storage.

CONCLUSIONS

Low protein latex was prepared in a single centrifuging process using food grade stabilised liquid papain. The optimum conditions for the deproteinization process were identified and were used for plant scale production of surgical gloves in an automatic dipping plant. Low protein latex produced using 0.25 p.h.r. stabilised liquid papain was found to produce gloves with an EP content of less than 50 µg/dm^2 with one wet gel and one post-cure leach. Plant trials showed that the control of proteins by online wet gel and post-cure leaching was more effective with this low protein latex. Antigenic protein content estimated for selected treatments was within the limit specified by ASTM. The effect of slurry dip on protein content could be controlled more effectively as the amount of proteins leaching into the slurry and binding to cornstarch would be less. The changes in raw latex properties such as VFA, MST, KOH number and zinc oxide viscosity observed during storage were comparable for both the latices. The change in extractable protein content for low protein latex vulcanizate was from 68 µg/dm^2 to 88 µg/dm^2 whereas for the cenex, the increase was from 150 µg/dm^2 to 368 µg/dm^2. Physical properties of latex film vulcanizates were more or less unaffected by storage.

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REFERENCES

1. LEYNADIER, F. AND DRY, J. (1991) Allergy to Latex. *Clinical Reviews in Allergy*, **9**, 317–377.

2. TURJANMAA, K. (1994) Contact Urticaria from Latex Gloves, *Protective Gloves for Occupational Use*, *(Eds. Gunh, Mellstrom, Jan, E. Wahberg, Howard, I. Maibach)*, Chapter **17**, 241–254. Boca, Raton, Florida: CRC Press.

3. WOODS, J.A., LAMBERT, S., PLATTS-MILLS, T.A.E., DRAKE, D.B. AND EDLICH, R.F. (1997) Natural Rubber Latex Allergy: Spectrum, Diagnostic Approach and Therapy, *J. Emergency Medicine*, **15(1)**, 71–85.

4. YAPA, P.A.J. (1977) Enzyme Deproteinization of *Hevea* Latex 1. Preparation and Properties of DPNR and Viscosity Stabilized DPNR, *J. Rubb. Res. Inst. Sri Lanka*, **54**, 508–519.

5. ANNUAL BOOK OF ASTM STANDARDS (2005) Section 9, Rubber, Vol. 09.01.

6. ANNUAL BOOK OF ASTM STANDARDS (2005) Section 9, Rubber, Vol. 09.02.

7. GEORGE, K.M., RAJAMMAL, G., PEETHAMBARAN, N.R. AND MATHEW, N.M. (2001) Enzymatic Deproteinization of Natural Rubber Latex. *Indian J. nat. Rubb. Res.*, **14(1)**, 7–13.

8. MA'ZAM, M.S., KOK, N.P., HASMA, H., MOK, K.L., ASRUL, M., LAI, P.F. AND SAADIAH, S. (2002) Low Protein Natural

Rubber Latices. *2nd Inter. Rubb. Glove Conf. and Exhibition 2002, 10–12 September 2002, Kuala Lumpur*.

9. INDIAN STANDARD (1981) Specification for Ammonia Preserved Concentrated Natural Rubber Latex.

10. BLACKLEY, D.C. (1997) Natural Latices. *Polymer Latices: Science and Technology*, 2nd Ed., Vol.2. London: Chapman and Hall.

11. LEPETIT, F. (1947) Natural, Thermal and Biochemical Degradation of Ammoniated *Hevea* Latex to Give Heat Sensitivity. *Transactions of the Institution of the Rubber Industry*, **23**, 104.

12. RAJAMMAL, G., GEORGE, K.M., MATHEW, S., PEETHAMBARAN, N.R. AND MATHEW, N.M. (2004) Stability and Vulcanization Characteristics of Enzyme Deproteinized Natural Rubber Latex, *J. nat. Rubb. Res.,* **17(2)**, 95–102.

13. LATEX DIPPING – LEACHING TREATMENTS, (1977) Technical Information Sheet, L16, MRPRA.

14. DALRYMPLE, S.J. AND AUDLEY, B.G. (1992) Allergenic Proteins in Dipped Products: Factors Influencing Extractable Protein Levels, *Rubb. Dev.,* **45(2/3)**, 51.

15. YEANG, H.Y., SUNDERASAN, E., BAKRI, A.R.S., GHAZLY, H. AND HAMZAH, S. (1993) Studies on the Behaviour and Characterisation of Latex Glove Proteins. *Proc. Int. Rubb. Conf., Kuala Lumpur, June 1993*.

16. BODYCOAT, I. (1993) Manufacture of Hypoallergenic Rubber Products. *Proc. Int. Conf. on Latex Protein Allergy, 1993, Amsterdam, the Netherlands*, 43–45.

17. HASMA, H. AND WAN ROSLINAH (2005) Binding Propensity of Modified Corn Starch and Oat Starch for NR Latex Proteins and Ways to Minimise the Interaction. *J. Rubb. Res*., **8(2)**, 79–89.

18. TOMAZIC, V.J., SHAMPAINE, E.L., LAMANNA, A., WILHROW, T.R., ADKINSON, N.F. AND HAMILTON, R.G. (1994), Cornstarch Powder on Latex Products as an Allergen Carrier, *J. Allergy Clin. Immunol*., **93**, 751–758.

19. SEGGEV, J.S., MAWHINNEY, T.P., YUNGINGER, J.W. AND BRAUN, S.R. (1990) Anaphylaxis due to Cornstarch Surgical Glove Powder. *Ann. Allergy*, **65**, 152–155.