Vidyodaya J. of Sci. Vol 7. pp. 93 - 100

ENHANCEMENT OF DEPROTEINIZATION OF *HEVEA* RUBBER BY MATURATION OF PAPAIN TREATED *HEVEA* LATEX

P.A.J.Yapa
Department of Botany, University of Sri Jayewardenepure
Nugegoda
& W.S.E Fernando*
Rubber Research Institute, Agalawatte

Received on. 02. 10. 97
Accepted on. 22. 12. 97

Abstract

Possibility of improving the quality low protein rubber obtained by papain treatment of *Hevea* latex was investigated. A new method for the production of high quality low protein rubber from field latex was developed. It incorporates a new step of maturation into papain treatment presently used . Typically, the resulting rubber had a nitrogen content less than 0.10%, the reduction being in the region of 70-80% compared to 50-60% reduction in proteins by conventional papain treatment. Under ideal conditions the ash content was around 0.10%. A reduction in acetone extract value was also observed and it varied from 15-60%. Preliminary studies showed that technological and dynamic properties of resulting low protein rubber, are also satisfactory.

Key words: latex, papain, deproteinization, maturation

1. Introduction

Enzyme deprotenization of *Hevea* latex has drawn considerable amount attention in recent years as removal of proteins from *Hevea* rubber is now widely accepted to improve its technological and dynamic properties, particularly the heat build up (see Yapa & Yapa 1984 for a review). Of the various enzymes investigated by several workers papain treatment of field latex has been reported to reduce the protein content by about 30 - 50% (Yapa & Balasingham, 1974) whilst slightly higher reductions have been obtained by treatment of ammoniated latex with superase, an enzyme of bacterial origin (Chang et al, 1977).

However, it has been reported that removal of proteins is not the sole factor that contributes to improvement in quality of low protein rubber (Yapa & Yapa, 1984). For instance, Papain treated rubber with only 30-50% reduction in protein content has been reported to have better heat build up properties than that of rubbers with a much lower protein content (Loganathan, 1980). The superior quality of papain treated rubber has been attributed to microbial activity during treatment/coagulation period which is usually 24h. An extension of this period beyond 24h has been described as maturation (Yapa & Yapa, 1984).

* Present address: Dipped Products Ltd, Homagama

The objective of the present study was to explore the possibilities of using maturation of papain treated latex to further enhance the improvement in quality of low protein rubber obtained by papain treatment particularly with regard to low heat build up and low affinity for water.

2. Experimental Procedure

Materials: All laboratory and factory scale trials were carried out at the Dartonfield factory of the Rubber Research Institute at Agalawatte. The latex used was multiclonal but mainly PB 86. Commercial papain (white, Grade 1) was used in all trials.

Addition of papain: Fresh field latex was diluted with water appropriately as indicated in results, prior to addition of papain at 0.05% w/v on latex. Papain was dissolved in a small volume of water prior to addition. Nonidet T, a non-ionic detergent was also used in some trials at 0.1% w/v on latex and when used, it was added prior to the addition of the enzyme. Enzyme treatment brought about both coagulation and digestion of proteins. (Fig 1)

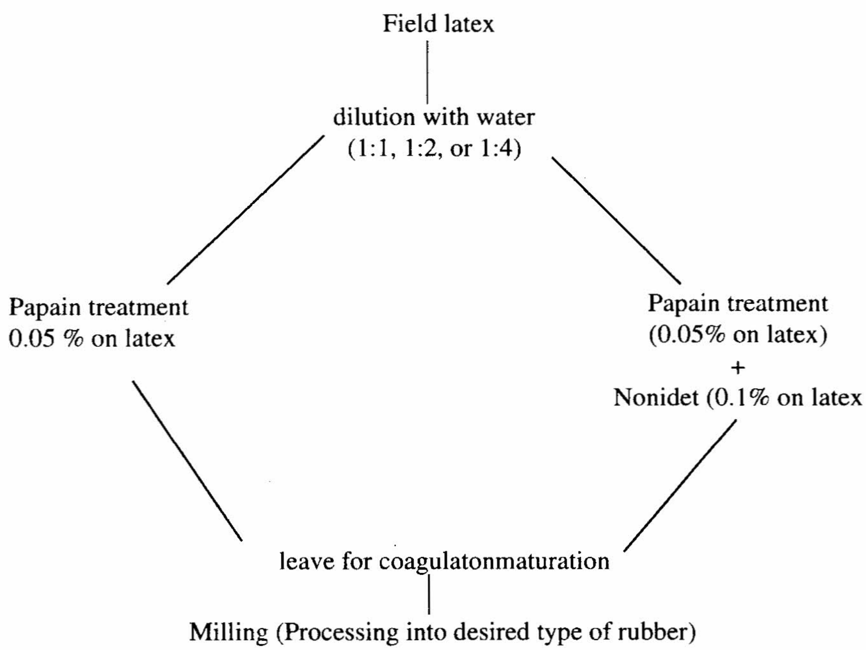

Fig 1 Deproteinization process investigated for the manufacture of low protein natural rubber from *Hevea* latex

Maturation: Instead of milling the samples after 24h of papain treatment as traditionally practiced, they were allowed to stand for a further period (eg 48h and 72h), which is described as maturation (Sri Lanka Patent 9574). Samples, after maturation, were milled into thin laces, dried, homogenized and tested for various properties.

Analysis: Nitrogen content of dry rubber was determined by the micro Kjeldahl method (Tong,1992). Wallace Plasticity and PRI were determined using a Wallace Plastimeter (Tong, 1992). Ash content of dry rubber was determined by the method of Tong (1992).

3. Results

Nitrogen content: The effect of dilution on nitrogen content of papain treated *Hevea* latex without maturation (ie conventional method) is given in Table 1. It can be seen that nitrogen content decreases with dilution and papain treatment upto 1:4 dilution after which no noticeable effect was observed.

The effect of dilution and maturaion on the degree of deproteinization is given in Table II. The protein content was found to reduce, on dilution of latex, a 72% reduction in the protein level was observed with 1:2 dilution compared to 58% with 1:1 dilution . The addition of Nonidet improved the degree of deproteinization by 12% in 1:1 dilution and by 6% in 1:2 dilution (Tabel I) . An overall reduction in protein content of 78% was observed with papain + Nonidet at 1:2 dilution compared to 70% with 1:1 dilution.

Table I Effect of dilution on nitrogen and ash contents

	Treatment/dilution	Nitrogen% w/w	Ash% w/w
1.	Acid (control)	0.38 ±0.017	0.10 ±0.013
2.	Papain 1:2	0.16 ±0.008 (P = 0.000)	0.17±0.009 (P = 0.000)
3.	Papain 1:4	0.15±0.008 (P=0.000)	0.15 ±0.008 (p=0.0001)
4	Papain 1:6	0.15 ±0.016 (P=0.0004)	0.15 ±0.009 (P=0.0001)

P. values - compared to control; n = 4

Table II Effect of dilution and maturation on deproteinization of *Hevea* latex

Treatment	Dilution 1:1 Nitrogen %	Dilution 1:1 % reduction in N compared to acid coagulation	Dilution 1:2 Nitrogen %	Dilution 1:2 % reduction in N compared to acid coagulation
1. Acid -control	0.34 ±0.007		0.35 ±0.01	
2 Papain (conventional method)	0.15 ±0.01	54	0.11 ±0.009	68
3 Papain (maturation)	0.14 ±0.008 (p=0.20)	58	0.098 ±0.006 (P=0.11)	72
4 Papain + Nonidet (maturation)	0.098 ±0.009 (P=0.007)	70	0.08 ±0.01 (P=0.03)	78

P values - compared to conventional papain method; n = 3

Maturation of latex after papain treatment also improved the degree of deproteinization at both dilutions by 4%.which was further improved by the addition of Nonidet, improvement over conventional treatment being 16% at 1:1 dilution and 10% at 1:2 dilution which had an overall reduction of 78%.

Wallace Plasticity (P) and Plasticity Retention Index (PRI)

The effect of dilution of latex on Plasticity and PRI is given in Table III. Papain treatment increased the Plasticity but it decreased when latex was diluted (ie 50 --- > 46). The Plasticity was further reduced by the addition of Nonidet and dilution. The PRI which gives a measure of oxidative resistance of NR, was found to be satisfactory despite dilution and maturation of latex. An occassional decrease in PRI was observed during these investigations and the reason for this is not known although it is suspected to be either due to nature of latex or the use of anticoagulants etc.

Table II Effect of dilution and maturation on deproteinization of *Hevea* latex

Treatment	Dilution 1:1		Dilution 1:2	
	Nitrogen %	% reduction in N compared to acid coagulation	Nitrogen %	% reduction in N compared to acid coagulation
1. Acid -control	0.34 ±0.007		0.35 ±0.01	
2 Papain (conventional method)	0.15 ±0.01	54	0.11 ±0.009	68
3 Papain (maturation)	0.14 ±0.008 (p=0.20)	58	0.098 ±0.006 (P=0.11)	72
4 Papain + Nonidet (maturation)	0.098 ±0.009 (P=0.007)	70	0.08 ±0.01 (P=0.03)	78

P values - compared to conventional papain method; n = 3

Maturation of latex after papain treatment also improved the degree of deproteinization at both dilutions by 4%,which was further improved by the addition of Nonidet, improvement over conventional treatment being 16% at 1:1 dilution and 10% at 1:2 dilution which had an overall reduction of 78%.

Wallace Plasticity (P) and Plasticity Retention Index (PRI)

The effect of dilution of latex on Plasticity and PRI is given in Table III. Papain treatment increased the Plasticity but it decreased when latex was diluted (ie 50 --- > 46). The Plasticity was further reduced by the addition of Nonidet and dilution. The PRI which gives a measure of oxidative resistance of NR, was found to be satisfactory despite dilution and maturation of latex. An occassional decrease in PRI was observed during these investigations and the reason for this is not known although it is suspected to be either due to nature of latex or the use of anticoagulants etc.

Table III Effect of dilution of latex on Po & PRI

Dilution	P_o	PRI
Acid (control), 1:1	44 ± 8	82 ± 6
Papain (1:2)	50 ± 13 (P=0.45)	70 ± 10 (P=0.024)
Papain (1:4)	46 ± 8 (P=0.69)	73 ± 11 (P=0.041)
Papain + Nonidet (1:2)	48 ± 11 (P=0.57)	78 ± 15 (P=0.63)
Papain + Nonidet (1:4)	44 ± 7 (P=1.0)	70 ± 8 (P=0.055)

P values - compared to control; n=6

Table IV Effect of dilution & maturation on ash content

	Treatment	Ash content %	% increase in reduction over conventional enzyme treatment
1	Acid, control	0.15 ± 0.06	
2	Papain, 1:2 dil. conventional	0.19 ±0.08	
3	Papain, 1:2 dil. new method	0.12 ±0.05 (P=0.29)	36.8
4	Papain, 1:4 dil. conventional	0.13 ±0.06	31.6
5	Papain, 1:4 dil. new method	0.10 ±0.02 (P = 0.48)	47.4

P values - new method vs conventional method, n = 4

Ash content: The effect of dilution on ash content of LPNR prepared by the new method is given in Table IV. At 1:2 dilution, an ash content of 0.12% and at 1:4 dilution an ash content of 0.10% was obtained by the new method. These ash levels were observed in our laboratory trials. However, in some of the large scale factory trials, very low ash contents such as 0.097% were observed. Unlike the nitrogen content low ash contents were inconsistent and the reason(s) for such variabilty is not known. However in general the ash contents were found to consideraby reduced compared to conventional papain treatment without a maturation step.

Other raw rubber properties : The typical raw rubber properties of LPNR prepared by the new method are given in Table VI. Both nitrogen and ash contents were well within the proposed revised specifications for deproteinized natural rubber (DPNR) Table V. The PRI was well above the proposed specification of 60 (minimum). Mooney viscosity was found to be higher than the proposed specification of 45 - 55. Although there is no specification for colour of the final rubber, the colour of all test samples prepared and found to be generally light coloured.

Table V Typical properties of low protein rubber prepared by the new method

Property	Acid control	Papain treatment (conventional)	Papain treatment (new method)	Specifications for DPNR
Ash % wt	0.15	0.19	0.12	No sample >
Nitrogen % wt	0.34	0.13	0.098	0.15 on test No sample > 0.15 on test
Volatile matter % wt	0.45	0.45	0.38	No sample > 0.5
P	40	42	40	-
PRI	88	90	87	60 (min)
Mooney viscosity	69	71	71	45 -55
Colour	1.5	2.0	2.0	-

Chang et al, (1977)

4. Discussion

Enzymolysis of *Hevea* latex is now widely known to improve the technological and dynamic properties of NR whilst the reduction in ash content which accompanies protein reduction, improves the moisture absorption properties of natural rubber (Yapa et al 1980).The maturation of the coagulam prior to processing has been reported to bring about a further improvement in these properties (Fernando, et al, 1984). The maturation process employed by Fernando et al (1984) did not result in a rubber with significantly low nitrogen and ash contents, which were 0.12% and 0.22 % respectively in papain treated rubber. It was felt that the quality of low nitrogen rubber can be further improved by optimising the maturation process and this was the objective of the present study.

In this study, incorporation of a maturation step and additional dilution to conventional papain treatment led to very low nitrogen and ash levels, the typical values of nitrogen and ash contents were 0.098% and 0.12% respectively. A nitrogen content of less than 0.10% can be obtained consistently by the maturation method.

However, the ash content was found to vary from batch to batch under almost similar conditions. Further refinement of the process is necessary in order to keep the ash content at low levels, consistently. Addition of sodium sulphite in the field as an anticoagulant is one factor that requires attention in this respect.

The PRI of papain treated rubber by maturation process, was comparable to that of normal acid coagulated rubber. The PRI was found to be extremely good and it was always well above the proposed specification of 60 (minimum), even when it showed a tendency to vary. This is in contrast to the low PRI values observed in our previously reported study on maturation (Fernando et al, 1984). The colour of the final rubber, although not so of vital importance in low protein rubber, was comparable to that of papain treated rubber prepared by the conventional enzyme treatment process. Although we have not made a full investigation on technological and dynamic properties of low nitrogen NR prepared by the new method, the indications from our preliminary studies are that they would be satisfactory. Another advantage of low nitrogen rubber is its resistance to mould growth which has been repeatedly observed in our laboratory studies.

Reducing the nitrogen and ash contents of natural rubber without affecting its inherent qualities, has been the objective of many of the studies in this field in the last few years including the present study. It has been reported that removal of protein is not the sole factor that contribute towards the improvement in quality of low nitrogen rubber (Yapa & Yapa, 1984). This view is based on the fact that heat build up properties of cup lump rubber is better than that of other conventional grades of rubber, although the protein content is not all that low. This improvement in heat build up properties in cup lump rubber has been attributed to a process of natural maturation in the field where microorganisms are also thought to play an important role in changing the nature of non-rubber constituents that goes with the rubber phase. The findings of the present study supports the contention on beneficial role of microorganisms in improving the technological and dynamic properties of NR when conditions were made suitable for their activity. As far as DPNR is concerned it opens up a convenient way to improve the technological and dynamic properties further without any added cost for manufacture.

5. Acknowledgements

Authors wish to thank Mr W.W.D.Jayasinghe, Mrs S.I.Yapa, Mr M.D.C Seneviratne of Biochemistry Department of RRISL for technical assistance.

6. References

Chang, W-P, Lau.C.H & Nambia, J. (1977) Deproteinized natural rubber from field latex. Proc. of the RRIM Planters Conf.(1977), 295-306

Fernando, W.S.E, Yapa, P.A.J, Medagama D.D. & Silva, D de (1984) Technological properties of DPNR Proc. of the Internat.Rubb.Conf.,Colombo (1984)

Loganathan, K.S. (1980) Private communication

Tong, N.Y. (1992) RRIM Test methods for Standard Malaysian Rubbers SMR Bulletin No 7, 1992

Yapa, P.A.J. & Balasingham, C.G. (1974) The proteolytic action of papain on proteins in *Hevea* latex RRISL Quart. J. **51**, 1974, 1-12

Yapa, P.A.J. Nadarajah, M & Loganathan, K.S. (1980) Use of papain treatment of NR latex to produce superior quality rubbers Plastics & Rubber: Materials & Applications Feb, (1980), 32-36

Yapa, P.A.J, & Yapa, S.I. (1984) Recent developments in the manufacture of deproteinized natural rubber: Proc. of the Internat. Rubb. Conf., Colombo, 1984.