SOME STUDIES ON CYCLIZATION OF BROMELAIN TREATED RUBBER

By

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SUMMARY

Pineapple juice (PAJ) treatment of *Hevea* latex produces a low protein rubber which can be cyclized easily by the p-toluene sulphonic acid method. The rate of cyclization was generally comparable to or better than that of papain treated rubber. Sodium bisulphite was found to activate the reaction whilst the action of Nonidet T and RPA 3 was somewhat inhibitory. PAJ treatment can be used in crepe, block and sheet rubber manufacture and the resulting rubbers can all be cyclized easily. Use of PAJ as a part coagulant with acid improves drying characteristics without having any adverse effects on cyclization.

INTRODUCTION

Manufacture of cyclized rubber from natural rubber (NR) has been studied by several workers (Nadarajah *et al* 1973). The commercial production of this important chemical derivative of NR was started in Sri Lanka in 1973. Proteins have been reported to inhibit the cyclization reaction (Jannsen, 1956), and one of the important requirements for cyclization of NR by the p-toluene sulphonic acid method, as reported by Fisher (1927), is the removal of proteins from the starting rubber. Nadarajah *et al* (1973), have in this connection reported successful cyclization of *Hevea* rubber of which the proteins had been partially removed by papain treatment of latex; Coomarasamy & Silva (1977) have further investigated this process with particular attention to properties of the cyclized product prepared by the p-toluene sulphonic acid method.

Papain treatment of latex with a few improvements such as the addition of RPA 3 and Nonidet has been used in Sri Lanka for the preparation of starting material to be used in the production of cyclized rubber. The high cost of enzyme treatment, the longer drying time and discolouration of the rubber, are the problems associated with papain coagulation. Recent studies in this laboratory have shown that pineapple juice (PAJ) treated rubber can be successfully cyclized by the p-toluene sulphonic acid method. Pineapple is known to contain a proteolytic enzyme named bromelain and some effects of the addition of pineapple juice to latex have been already reported by John (1969). No reduction in the protein content has been reported in his work, but in our studies, a low protein rubber that can be readily cyclized, was consistently obtained by PAJ-treatment. This was further investigated with particular attention being paid to the cyclization pattern of PAJ-treated rubbers and the results obtained are reported.

EXPERIMENTAL

All experiments were carried out with latex obtained from the Institute's Estate at Dartonfield. Pineapple (*Annas comosus*), juice was extracted by maceration of fresh fruits and it was added to latex at the concentrations given in Tables 1—6. Papain which was mainly used for comparative purposes, was added to latex at 0.05% (w/v) of the latex, as reported previously (Yapa, 1977). The dried rubbers were cyclized after incorporation of 10% p-toluene sulphonic acid and by initiation of the reaction with an IR lamp. The rate of cyclization was measured with circular test pieces of identical thickness.

RESULTS

Protein content:

Comparison of the degree of deproteinization obtained by bromelain, and various pineapple juice and papain treatments are given in Table 1. The highest reduction (64%) was obtained with purified bromelain, followed by pineapple juice and papain. Protein fraction obtained by $(NH_4)_2 SO_4$ precipitation of fresh pineapple juice was also used on latex without further purification and this too was found to be fairly effective. However, the degree of deproteinization obtained by PAJ treatment alone was sufficient to bring it down to level required for a complete cyclization (Table 1).

TABLE 1. PROTEIN CONTENT OF ENZYME TREATED RUBBERS AND THEIR CYCLIZATION PATTERNS

Treatment		*Protein Content %*	*% reduction compared to control*	*Cycliza-tion reaction*	*Reaction Time (Sec)*	*Reaction pattern*
1. HCOOH 2%(control)	...	2.45	—	incomplete	300	very slow
2. Papain 0.05%	...	1.23	49.8	complete	226	slow
3. Papain (fresh) 0.05%	...	1.05	57.1	complete	86	very fast
4. Papain purified 0.05%	...	1.05	57.1	—	—	—
5. Bromelain 0.01%	...	0.88	64.0	complete	117	fast
6. PAJ, (precipitated)	...	1.14	53.5	complete	127	normal
7. PAJ, 2% on latex	...	1.05	57.1	complete	147	normal
8. PAJ, 4% on latex	...	1.05	57.1	complete	105	fast

Cyclization pattern:

The fastest cyclization reaction was obtained with rubber treated with fresh papain whilst rubber treated with old papain gave a slow reaction with p-toluene sulphonic acid. The protein content of the latter too was high, being 1.23% compared to 1.14% or less in other, (Table 1). Increase of the concentrations of PAJ added, accelerated the rate of cyclization but had no noticeable effect on deproteinization.

The type of PAJ used, depending on the variety and maturity of fruits, was also found to have an effect on the rate of cyclization which in any event was complete and fast when used fresh. For instance, a satisfactory cyclization could be obtained normally with a concentration as low as 1% PAJ in latex. The cyclization pattern of PAJ treated RSS is given in Table 2, and it is seen that PAJ can be used as a part coagulant with acid without adversely affecting the cyclization reaction. The combined treatment (acid-PAJ) was used in order to improve the poor drying characteristics of rubber treated with PAJ alone. The colour and general appearance of sheets were also improved considerably by the combined treatment.

TABLE 2. CYCLIZATION PATTERNS OF PAJ-TREATED RSS

Treatment		*Initiation/ sec*	*Completion sec*	*Reaction pattern*
1. Acid (HCOOH)	....	40.0	300	Complete, very slow
2. Acid/Pineapple juice	....	41.6	140.6	Complete, normal
3. Pineapple juice	....	31.7	111.6	Complete, fast

Effect of incorporation of Nonidet T and RPA 3:

Nonidet T and RPA 3 were used with PAJ treatment and their effects on cyclization are given in Table 3. Addition of Nonidet and RPA 3, either individually or in combination, did not improve the rate of the cyclization reactions observed with PAJ alone. RPA 3 did not exhibit any noticeable change in the rate of cyclization, whilst the action of Nonidet was found to be inhibitory. The combination of Nonidet and RPA 3 together with PAJ was also found to retard the rate of cyclization, and addition of $NaHSO_3$ did not have any favourable effect on the rate of reaction. However, $NaHSO_3$ when used alone with PAJ, showed its usual catalytic action on the rate of reaction.

TABLE 3. EFFECT OF INCORPORATION OF RPA 3 AND NONIDET WITH PAJ TREATMENT, ON CYCLIZATION

Treatment	*Time(sec)taken for* *Initiation*	*Completion*	*Reaction pattern*
1. Acid control	48	300	incomplete
2. Papain	33	104	complete, fast
3. Pineapple juice (PAJ)	35	106	complete, fast
4. PAJ + RPA 3	38	106	complete, fast
5. PAJ + RPA 3 + Nonidet ...	49	125	complete, normal
6. PAJ + RPA 3 + Nonidet + $NaHSO_3$	48	127	complete, normal
7. PAJ + Nonidet	45	120	complete, normal
8. PAJ + Nonidet + $NaHSO_3$ ...	52	118	complete, normal
9. PAJ + RPA 3 + $NaHSO_3$ ...	53	122	complete, normal
10. PAJ + $NaHSO_3$	37	104	complete, fast

Effect of Na_2SO_3 and $NaHSO_3$:

The effect of Na_2SO_3 and $NaHSO_3$ which are used to prevent pre-coagulation and enzymatic discolouration, respectively, was also investigated, and the results are shown in Table 4. These sulphites were added to latex at concentrations recommended for estate practice, namely 0.05% & 0.15% respectively and no inhibitory action on cyclization was observed with PAJ treatment. We have often observed both sulphites exhibiting a catalytic effect on the cyclization reaction and the results indicated in Table 4 are typical of such a trial. A catalytic effect can be seen with both $NaHSO_3$ and Na_2SO_3. However, no such catalytic action was observed when they were used with papain treatment (Table 4).

TABLE 4. EFFECT OF Na_2SO_3 AND $NaSHO_3$ TREATMENTS ON CYCLIZATION OF PAJ & PAPAIN TREATED RUBBER

Treatment	*Time taken for/sec* *Initiation*	*Completion*	*Cyclization pattern*
1. Control - HCOOH	34	280	Complete-very slow
2. Papain	31	100	Normal
3. Pineapple juice	31	105	Normal
4. Na_2SO_3 + Papain	43	144	Slow
5. Na_2SO_3 + PAJ	22	97	Fast
6. $NaHSO_3$ + Papain	38	110	Normal
7. $NaHSO_3$ + PAJ	30	87	Very fast

Colour of dry rubber:

The colour characteristics of dry rubber prepared with papain and PAJ with other combinations, are given in Table 5: with PAJ, the final colour of the rubber was severely affected and was worse than that of papain treated rubber. However, PAJ in combination with $NaHSO_3$ showed a remarkable improvement in colour which was better than that of the papain-$NaHSO_3$ treatment. PAJ behaved similarly with the boric acid-ammonia system which is normally used in the preservation of latex in central processing factories such as the one at Mawanella.

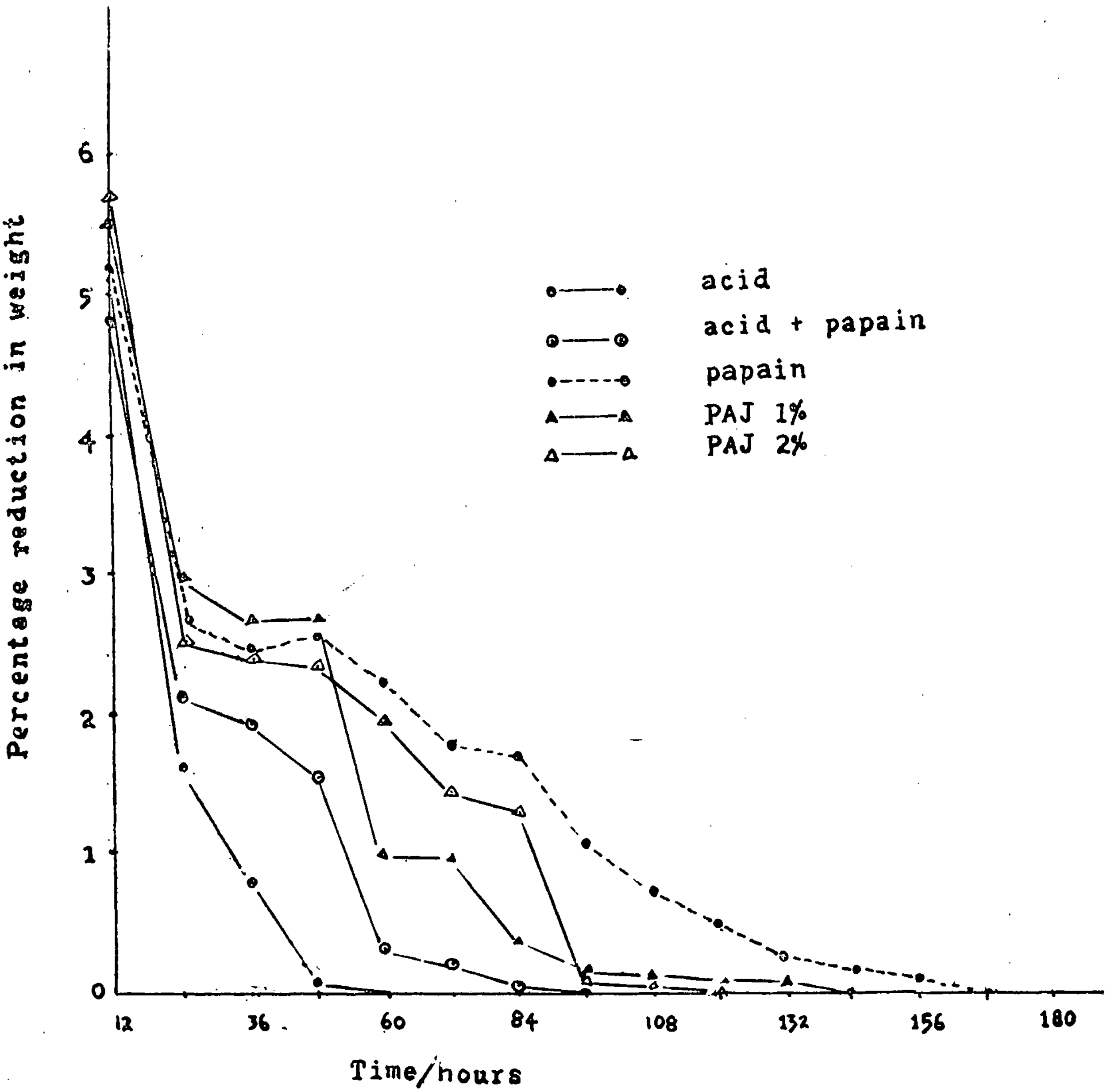

Fig. 1 Drying pattern of papain and PAJ treated rubbers

TABLE 5. LOVIBOND COLOUR OF PAPAIN & PAJ - TREATED RUBBERS

Treatment	Lovibond colour
1. HCOOH only	1.0
2. PAJ only	4.5
3. Papain only	3.5
4. $NaHSO_3$ + HCOOH	1.0
5. $NaHSO_3$ + PAJ	1.5
6. $NaHSO_3$ + Papain	2.0
7. Boric acid + NH_3 + HCOOH	1.0
8. Boric acid + NH_3 + PAJ	1.5
9. Boric acid + NH_3 + Papain	3.5

Drying characteristics:

The drying time of PAJ treated sheet rubber was investigated and the results are shown in Fig. 1. It is seen that drying time can be reduced considerably by using PAJ treatment as compared to papain treatment; drying time of PAJ treated rubber at 1% level, was 156 hours compared to over 170 hours when papain is used alone. Drying period can be reduced further by using a higher concentration of PAJ in latex, as seen in Fig. 1. In addition, it could also be improved by using PAJ in combination with acid, as a part coagulant.

DISCUSSION

The results of this study show that PAJ can be used on *Hevea* latex to obtain a rubber with a low protein content and also that this rubber can be used successfully in the production of cyclized rubber by the p-toluene sulphonic acid method. Pineapple juice was one of the several waste carbohydrate substrates used on latex by John (1969) for the purpose of assisted biological coagulation. Although pineapple juice is known to contain a proteolytic enzyme called bromelain, in John's studies there was apparently no proteolytic action as reflected in nitrogen content of PAJ treated rubber (Table 2, John, 1969). However, in this study, we have consistently observed a reduction in protein content with PAJ treatment of latex, irrespective of clonal variation The local variety of pineapple known as 'Murusi' was used in all our trials to obtain PAJ. The degree of deproteinization by PAJ was similar to that of papain treatment, the protein content usually being around 1.05%.

The fact that PAJ treated rubber cyclises readily and completely with p-toluene sulphonic acid, can be attributed to the removal of inhibitory proteins (Jannsen, 1956). Unless tree-fresh papain is used the rate of cyclization of PAJ treated rubber was generally faster than that of papain treated rubber, (Table 1). The PAJ used in these studies, was always from fresh fruits. The low pH of PAJ could contribute to the higher rate of cyclization, as it is well known that the cyclization reaction is catalysed by acids.

Sodium sulphite which is added to latex in the field as an anticoagulant, has been reported to have an inhibitory effect on cyclization (Nadarajah *et al*, 1973). A similar effect was observed in the present study too. However, no such inhibitory effect was observed when PAJ was used on latex to which Na_2SO_3 had been added; the cyclization pattern was either normal or faster depending on the clone, with Na_2SO_3 - PAJ combination (See Table 4). The addition of sodium bisulphite to prevent enzymatic discolouration also, did not have any adverse effect on cyclization when PAJ treatment was used. It was either normal or faster than usual, exerting some catalytic effect depending on the nature of the clone. The ability of PAJ treatment to be used on latex to which Na_2SO_3 or $NaHSO_3$ was added without affecting the cyclization reaction, is another advantage of the PAJ treatment.

The addition of sodium bisulphite was also found to improve the colour of rubber considerably, almost to the level of acid coagulated rubber. Papain on the other hand, did not make any noticeable impact on the colour, when used with $NaHSO_3$. PAJ-treatment also behaved satisfactorily with the Boric acid-NH_3 preservative system, giving a light coloured rubber. One of the disadvantages of cyclized rubber produced from papain treated rubber, is its dark colour and several attempts have been made without much success, to improve it by starting with a light coloured rubber (Coomarasamy *et al*, 1978). In this context, the improvement of colour of the initial raw material by combined treatment with $NaHSO_3$ - PAJ, is of great importance, in obtaining a light coloured cyclized derivative.

The peptiser, RPA 3, has been reported to activate cyclization of papain-treated rubber (Coomarasamy & Silva, 1977). It has also been used with the surfactant Nonidet P 40. However, in this study on PAJ-treated rubber, no activation was observed with RPA 3. Nonidet exhibited a slightly inhibitory effect; this surfactant has been used (Coomarasamy & Silva, 1977) in combination with RPA 3, to assist in the deproteinization process with papain but its actual individual effect on cyclization has not been reported.

ACKNOWLEDGEMENTS

The Authors wish to thank Dr. O. S. Peries, Director, RRISL, Dr. R. L. Wickramasinghe, Director, National Institute of Plantation Management and Mr. S. W. Karunaratne, Head, Rubber Chemistry Department for their valuable comments on the manuscript. We also thank Mr. M. D. C. Seneviratne for technical assistance.

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