

## SOME ASPECTS OF PHENOLIC DISCOLOURATION IN NATURAL RUBBER\*

BY

P. A. J. YAPA  
(Biochemist, RRISL)

### SUMMARY

*Quantitative and qualitative studies were made on the phenolic compounds in the latex of two clones : PB 86 and RRIC 7. Five phenolics were detected in RRIC 7 and three in PB 86 by paper chromatography. In both clones two of these compounds were found to be predominant : one of them was a phenolic, identified as tyrosine and the other is probably an indole compound. A slight increase in the phenolic content was observed after Ethrel stimulation. Clonal variation of thiol content in latex was also studied. Addition of thiols to latex improved the colour and the oxidative resistance of rubber.*

### INTRODUCTION

Certain types of natural rubber such as pale crepe, are graded visually on the basis of the lightness in colour of the dry rubber. Darkening which usually occurs during both the processing stage and storage of rubber has therefore been a problem of considerable concern. Two types of darkening have been reported in biological systems ; enzymatic and nonenzymatic. Oxidation of phenolic compounds in latex by polyphenol oxidase is of the first type and is generally considered responsible for discolouration of most of the clonal latices. Nonenzymatic browning in natural products has been related to processes such as sugar-amino acid condensation (Reynolds, 1963 ; Hashiba, 1976) and ascorbic acid decomposition (Huelin, Clegg & Morton, 1965). There have been few studies of this type on *Hevea* latex.

Discolouration of crepe rubber is normally inhibited or prevented by the use of chemicals e.g. oxalic acid to retard enzymatic discolouration (Nadarajah & Karunaratne, 1964) and sulphites for nonenzymic browning (Burton *et al.*, 1963). In addition, thiols, such as glutathione and cysteine, are known to react with oxidised phenolic compounds to form colourless products. The nature of phenolic compounds in latex and their role in discolouration are not yet fully understood. The present investigation was started to get information on this subject, with special reference to the role of thiols in inhibiting the darkening of rubber.

### MATERIALS AND METHODS

Latex samples collected from Dartonfield Group were used in all experiments. Preliminary investigations on the determination of thiol content in F-serum was carried out by the colourimetric method of Liddell & Saville (1959). Clonal variation of thiol content was studied by the nitroprusside method (Chinhard & Hellerman, 1954). Absorbances were measured on a Perkin-Elmer spectrophotometer model 4802.

Phenolic compounds were extracted by the method described by Nadarajah & Karunaratne (1963) and they were separated by two dimensional paper chromatography on Whatman No. 1 paper ; chromatograms were first run in 2% CH<sub>3</sub>COOH followed by n-Butanol-acetic acid-water (63 : 27 : 10). The compounds were located by spraying with the ferric chloride-ferricyanide reagent, p-dimethylaminobenzaldehyde,  $\alpha$ -nitroso- $\beta$ -naphthol and ninhydrin (Hathway, 1969).

The following procedure was adopted in studying the effect of addition of thiol compounds on the colour and Plasticity Retention Index (PRI). About 200 ml of field latex diluted (1 : 1) with water was used in each experiment. Thiol compounds

\* Paper presented at the 32nd Annual Sessions of the Sri Lanka Association for the Advancement of Science (SLAAS) on 9th December, 1976. Ed.

(glutathione or cysteine hydrochloride, obtained from BDH, U.K.) was added at a concentration of 0.2% on the original volume of latex. Coagulation was effected with 2% HCOOH.

Quantitative determination of the content of phenolic compounds was carried out by the colourimetric method of Swain & Hills (1959). Absorbances were measured on a Beckmann Spectrophotometer at 750 m $\mu$ .

## RESULTS

### Clone RRIC 7

Five phenolic compounds were detected in this clone with FeCl<sub>3</sub> - ferricyanide reagent (Fig. 1); spots 5 and 6 were detected only in the fresh extracts. Spots 1, 2, 3, 4 and 5 were all ninhydrin-positive. Spots 1 & 2 gave positive reactions with FeCl<sub>3</sub> - Ferricyanide reagent as well as ninhydrin. Spot 1 was identified as tyrosine. Chromatographic behaviour of spot 2 showed a similarity to that of tryptophan. Both tryptophan and spot 2 gave a pink colour with p-dimethylaminobenzaldehyde which is a specific reagent for indoles and hydroxyindoles; they also gave yellow spots with  $\alpha$  - nitroso  $\beta$  - naphthol reagent. These facts indicate that it is an indole. Compared with the intensity of spots 1 and 2, spots 3, 4, 5 and 6 appeared to be present in trace amounts and no real attempt was made to identify them. However, spot 3 was identified tentatively as dihydroxyphenylalanine (DOPA).

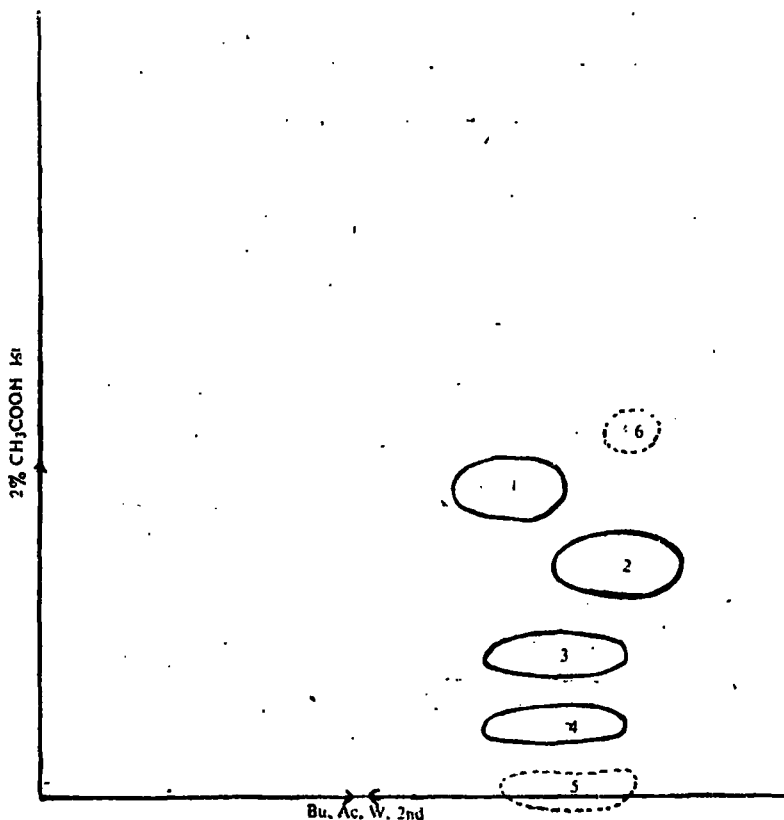


Fig. 1 — Phenolics in extractive from RRIC 7. Chromatogram run first in 2% CH<sub>3</sub>COOH followed by n-Butanol-acetic acid-water (63 : 10 : 27 v/v) solvent system. Detected with ferric chloride-ferricyanide reagent spots, 1 tyrosine 2 tryptophan (?), - non phenolic 3 dihydroxyphenyl alanine (?) 4, 5 & 6 unknown.

When phenolic extracts were analysed for amino acids, a bright reddish pink spot, in addition to the amino acid spots, was obtained in the usual 'histidine' area. This spot remained unfaded for several months at room temperature, whilst all other ninhydrin-positive spots had disappeared. Although it did not show a colour reaction with  $\text{FeCl}_3$  - ferricyanide reagent, a whitish or sometimes a pale blue area in a blue background was noticed. It is suspected that this is the histidine betaine (ergothionine) which has been detected in RRIC 7 by several workers (Tan & Audley, 1968 ; Nadarajah *et al.*, 1972).

#### Clone PB 86

Three phenolic compounds were detected with  $\text{FeCl}_3$  - ferricyanide reagent, in this clone (Fig. 2). All the spots (1, 2, 3 & 4) were ninhydrin positive. The behaviour of spot 1 was as in clone RRIC 7. It was identified as tyrosine. The intensity of spots obtained under similar conditions with  $\text{FeCl}_3$  - ferricyanide reagent was low in PB 86 as compared with RRIC 7, indicative of a higher content of phenolic compounds in RRIC 7 than in PB 86. These results are in accordance with those of Karunaratne & Nadarajah (1964).

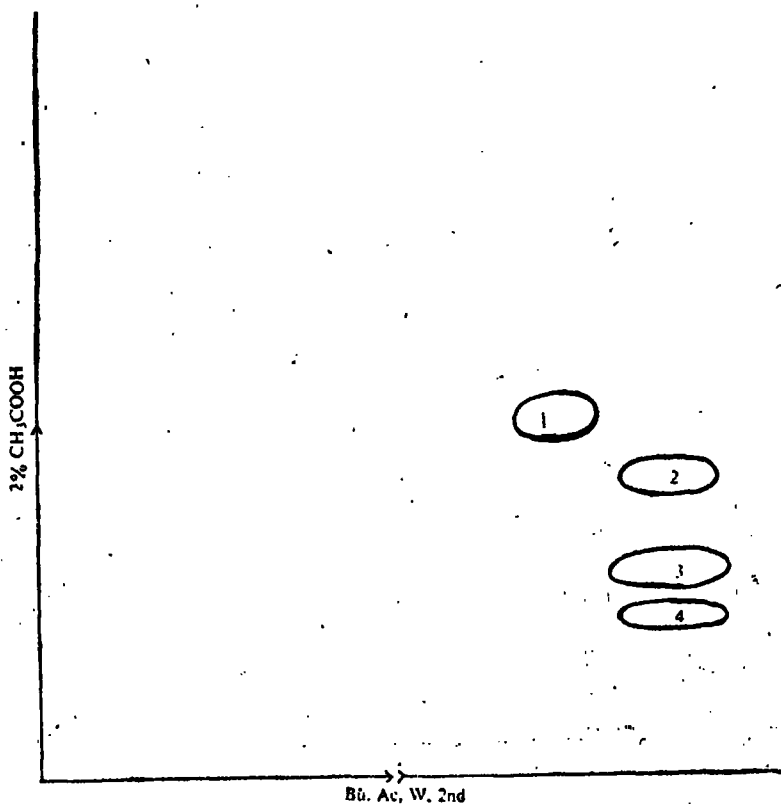


Fig. 2 — Phenolics in latex of clone PB 86 chromatogram run first in 2%  $\text{CH}_3\text{COOH}$  followed by n-Butanolacetic acid-water (63 : 10 : 27 v/v) solvent system. Sprayed with ferric chloride-ferricyanide reagent spots 1 tyrosine 2 tryptophan (?) -non phenolic 3 Dihydroxyphenylalanine (?), 4 unknown.

Results of amino acid analysis of the two clones are again complementary to the above findings (Table 1). It can be seen that the tyrosine content in RRIC 7 is higher than that of PB 86.

TABLE 1  
A QUANTITATIVE COMPARISON OF FREE AMINO ACIDS IN *Hevea* LATEX

	Altmann (1940)*	Whitby & Greenberg* (1941)	Drake (1951)*	Soe* (1960)	Kasinathan <i>et al.</i> (1971)		Present Study**	
					PB 86	RRIC 7	PB 86	RRIC 7
Glycine	Y	—	Y	Y	T	—	×	×
$\alpha$ Alanine	Y	—	Y	Y	T	7	×××××	×××××
Valine	Y	Y	—	T	5	T	×	×
Leucine	Y	Y	Y	Y	T	T	×	×
Isoleucine	Y	Y	Y	Y	T	T	×	×
Phenylalanine	Y	Y	—	Y	1	1	×	×
<hr/>								
Tyrosine	Y	Y	—	Y	T	1	×	×××
Tryptophan	Y	—	—	Y	T	3	×	×××
Cysteine	Y	—	—	Y	T	T	×	×
<hr/>								
Aspartic	—	Y	Y	Y	1	T	×××	×
Glutamic	Y	—	Y	Y	4	T	×××××	×××××
Arginine	—	Y	—	T	—	—	×××	×
Serine	—	—	Y	T	2	1	×	×××
Threonine	—	—	—	T	1	—	×	×
Lysine	Y	Y	—	Y	—	T	—	—

-5

\* as quoted by Blakely (1966)    \*\* (One × is approx. equivalent to  $0.4 \times 10^{-5}$  g)

The colour, on visual judgement, and the PRI of the dry rubber of both PB 86 and RRIC 7 are summarized in Table 2.

TABLE 2  
EFFECT OF ADDITION OF GLUTATHIONE AND CYSTEINE ON PRI & COLOUR

Treatment	RRIC 7			PB 86		
	PRI	% increase in PRI	Colour	PRI	% increase in PRI	Colour
1. Control — acid	67.3	—	slight discolouration	77.5	—	no discolouration
2. 0.2% glutathione	76.9	14.2	no discolouration	87.8	13.3	no discolouration
3. 0.2% cysteine	73.1	8.6	no discolouration	90.5	16.8	no discolouration

### Effect of Ethrel stimulation on phenolic content

During the course of this study it was brought to my notice by an estate that its rubber (pale crepe) from Ethrel stimulated areas was slightly discoloured. Latex from stimulated and unstimulated (control trees) was analysed for phenolic content for 15 weeks, and the results are shown in Fig. 3. It appears on the present evidence that the latex from stimulated trees has a slightly higher phenolic content than that from control trees. The discolouration of stimulated latex was confirmed by the Lovibond Colour Test (Table 3). However, further studies have to be carried out to confirm that Ethrel stimulation affects the content of phenolic compounds in latex.

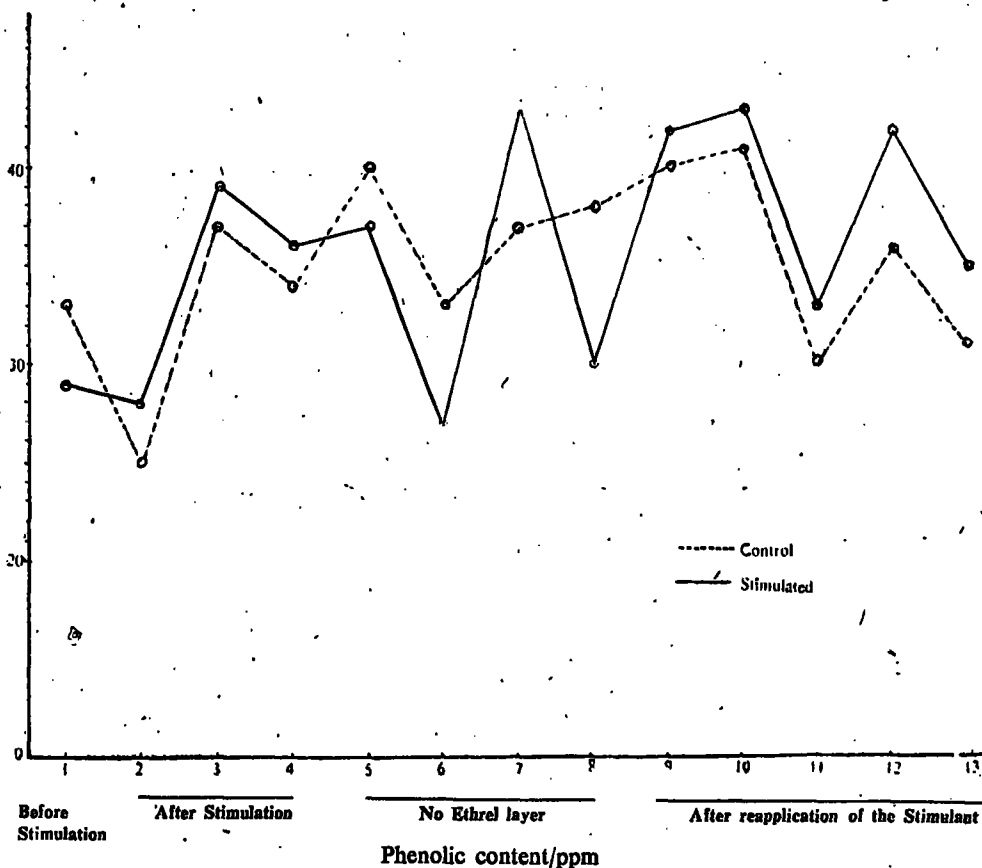


Fig. 3 — Effect of Ethrel stimulation in phenolic content in NR latex.

TABLE 3

EFFECT OF ETHREL STIMULATION ON LOVIBOND COLOUR INDEX OF RUBBER

Sample	Lovibond colour index	
	unstimulated	stimulated
1	5.0	7.0
2	7.0	8.0
3	5.5	6.0
4	5.0	5.0
5	4.5	5.0

TABLE 4

THIOL CONTENT OF F-SERUM OF SOME CLONES

Clone	Thiol/ppm.
RRIC 7	70
Nab 12	99
PB 86	119
RRIC 45	142

## DISCUSSION

The darkening of rubber is a clonal characteristic and the two clones investigated in this study are well known for their contrasting discolouration patterns; PB 86 gives a rubber with hardly any noticeable discolouration, whilst RRIC 7 normally yields dark coloured rubbers. Of the different types of mechanisms responsible for discolouration observed in biological systems, phenolic oxidation seems to be the main cause in NR latex. The most common substances associated with enzymatic browning are the cinnamic acids, especially caffeic and chlorogenic acids, tyrosine which is oxidized to a melanin type pigment and the catechins and leucoanthocyanidins (Martin & Ruberte, 1976). Tyrosine has been detected in NR latex by a number of researchers and at high concentrations it has often been associated with discolouration of the final rubber. Nadarajah & Karunaratne (1964) have shown that the phenolic content of RRIC 7 latex is four times that of PB 86, the results of this study broadly confirm their observations. Further, this study shows that the latex of clone RRIC 7 contains a greater number of phenolic compounds than that of clone PB 86.

Amongst the several phenolic compounds identified, the possible presence of DOPA is of some importance in that it shows a probable pathway for the formation of dark pigments. DOPA can undergo oxidation, the products of which can then undergo polymerization to form pigments of the melanin type which are usually brown to black in colour (Contarow & Schepartz, 1967).

Phenolic compounds have been reported to react, after enzymatic oxidation with thiols such as cysteine and glutathione, to form colourless products (Mason, 1955). If this is so, a clone with a high phenolic content can still yield a colourless or lightly coloured rubber, provided that it contains sufficient thiols. It also reveals a means of controlling the discolouration. The results of this study throw some light on this. PB 86 was found to have a higher thiol content than RRIC 7. One would have assumed on these results that a part or complete inhibition of discolouration of RRIC 7 can be achieved by supplying it with sufficient thiols. This was found to be true with cysteine, which when added to latex of RRIC 7 gave a relatively colourless rubber. It appears in general, that thiols play some role in discolouration. In addition, the oxidative resistance which is expressed as PRI, is also improved considerably on the addition of thiols. It has been reported by Sivabalasundaram & Nadarajah (1966), that cysteine has an adverse effect on the PRI. On the contrary a noticeable improvement in PRI, on addition of both glutathione and cysteine (as cysteine HCl) to latex, was observed repeatedly in the present investigation. The presence of thiols in high concentration in latex therefore, serves two purposes *viz.* improvement in colour and oxidative resistance of rubber.

The products of condensation between sugars and amino acids (Amadori compounds) are known to play a role in discolouration in certain types of systems (Reynolds, 1963; Hashiba, 1976). However, little or no attention has been paid to the possible involvement of these Amadori compounds in the darkening processes in NR. Tryptophan was detected in significantly large quantities in the bottom fraction and it has been detected in *Hevea* latex by several others as well. (Drake, 1951; Soe, 1960; Kasinathan *et al.*, 1971) Fructose-tryptophan is amongst Amadori compounds, the oxygen dependent browning of which is one of the most important factors contributing to the browning of Soya sauce during storage (Hashiba, 1976). The other Amadori compounds composed of aromatic or heterocyclic amino acids are fructose-tyrosine, fructose-phenylalanine, fructose-histidine. The participation of Amadori compounds tryptophan-fructose as well as several others in darkening of rubber cannot be completely ruled out, and needs further study. Hashiba (1976) has also reported that the oxygen-dependent browning of Amadori compounds composed of aromatic or heterocyclic compounds was synergistically accelerated by the presence of  $Fe^{2+}$  and  $Mn^{2+}$ . This probably adds to the generally accepted importance of using iron-free water in the manufacture of certain grades of rubber such as pale crepe.

The increase in phenolic content in latex of stimulated trees, as observed in this study, has not been reported previously. It is known that colour development of several varieties of apples *e.g.* McIntosh, Early Italian, is greatly enhanced by Ethrel (Proebsting & Mills, 1969; Edgerton & Blanpied, 1970). However they also report that internal browning is reduced by the treatment. Whether the reduction in internal browning has any association with enhancement of colour is not known. The recent studies (Archer *et al.*, 1973; Audley *et al.*, 1975) on the effect of Ethrel and the fate of Ethrel itself have elucidated this. These studies show not only that Ethaphon or Ethrel can be translocated to various tissues but also that these tissues can metabolise the compound (Audley *et al.*, 1975). It is possible that some of these substances formed have some physiological activity and the increase in phenolic content may represent the outcome of just one of them.

#### ACKNOWLEDGEMENTS

I wish to thank Dr. O. S. Peries, Director and Mr. M. Nadarajah, Head, Rubber Chemistry Department for valuable suggestions on the manuscript. Author also wishes to thank Messrs S. W. Karunaratne, C. G. Balasingham and P. P. Jayasinghe for initiation of the experiment, on Ethrel stimulation at Culloden Estate. Thanks are also due to Miss R. Ponnampalam for carrying out some of the work and to Messrs M. D. C. Seneviratne and L. J. Wickremasinghe for technical assistance.

#### REFERENCES

- ARCHER, B. L., AUDLEY, B. G. AND MANU, N. P. (1973). Decomposition of 2-Chloroethyl phosphonic acid in stems and leaves of *Hevea brasiliensis*. *Phytochemistry* **12**, 1535.
- AUDLEY, B. G. AND TAN, C. H. (1968). The uptake of ergothioneine from the soil into the latex of *Hevea brasiliensis*. *Phytochemistry* **7**, 1999-2000.
- AUDLEY, B. G. ARCHER, B. L. AND CARRUSTHERS, I. B. (1975). Translocation and metabolism of Ethephon and related compounds in *Hevea brasiliensis*. *Int. Rubb. Conf. 1975, Kuala Lumpur* (Preprint).

- BLACKLEY, D. C. (1966). *High Polymer Latices*, Vol. 1, London : Maclaren & Sons Ltd.
- BURTON, H. S., MCSWEENEY, D. J. AND PANDHI, P. N. (1963). Non-enzymatic browning : Browning of phenol and its inhibition by sulphur dioxide *Nature, Lond.* **199**, 559.
- CANTAROW, A. AND SCHEPARTZ, B. (1967). *Biochemistry* p. 597 - 602, Philadelphia : W. B. Saunders Company.
- CHINARD, F. P. AND HELLERMAN, L. (1954) : Determination of sulfhydryl groups in certain biological substances. *Methods in Biochemical Analysis*, Vol. 1 (Glick, D., ed.) p. 1 - 27, New York : Interscience Publishers Inc.
- CLEGG, K. M. AND MORTON, A. D. (1965). Carbonyl compounds and the non-enzymic browning of lemon juice. *J. Sci. Fd. Agric.* **16**, 191 - 198.
- EDGERTON, L. J. AND BLANPIED, G. D. (1970). Interaction of Succinic acid dimethyl hydroxide, 2-chloroethylphosphoric acid and auxins 2,2 — on maturity, quality and abscission of apples. *J. Amer. Soc. Hort. Sci.* **95**, 664 - 666.
- HASHIBA, H. (1976). Participation of Amadori Rearrangement products and carbonyl compounds in Oxygen-dependent browning of Soya Sauce. *J. Agric. Food Chem.* **24**, 70 - 73.
- HATHEWAY, D. E. (1969). Plant phenols and tanins. In *Chromatographic and Electrophoretic techniques*, vol. 1, Chromatography (Smith, J. ed.) p. 390 - 437, London : W. Heinemann Medical Books Ltd.
- HUELIN, F. E. (1953). Studies on the anaerobic decomposition of ascorbic acid. *Fd. Res.* **18**, 633 - 639.
- KASINATHAN, S., NADARAJAH, M., AND TIRIMANNA, A. S. L. (1971). A study of the free amino acids in the latex of NR. *RRIC Bulletin* **6**, 1971, 29 - 34.
- LIDDELL, H. F. AND SAVILLE, B. (1959). Colourimetric determination of cysteine. *Analyst* **84**, 188 - 190.
- MARTIN, F. W. AND RUBERTE, R. (1976). The polyphenol of *Dioscorea alata* (Yam) tubers associated with oxidative browning. *J. Agric. Food Chem.* **24**, 67 - 70.
- MASON, H. S. (1955). Reactions between quinones and proteins, *Nature, Lond.* **175**, 771.
- MCMULLEN, A. I. (1960). Thiols of low molecular weight in *Hevea brasiliensis* latex. *Biochem. Biophys. Acta.* **41**, 152 - 154.
- NADARAJAH, M. AND KARUNARATNE, S. W. (1964). Some observations on the non-rubber constituents of *Hevea latex* Part II, Discolouration of latex and of rubber. *Q. Jl. Rubb. Res. Inst. Ceylon* **40**, 1 - 12.
- NADARAJAH, M., TIRIMANNE, A. S. L., COOMARASAMY, A. AND KASINATHAN, S. (1971). Some naturally occurring antioxidants in *Hevea brasiliensis* latex. *Q. Jl. Rubb. Res. Inst. Ceylon.* **48**, 202 - 211.

- PROBSTING, E. L. AND MILLS, H. H. (1969). Effect of 2-chloroethane phosphonic acid and its interaction with gibberellic acid on quality of "Early Italian" prunes. *J. Amer. Soc. Hort. Sci.* **94**, 443 - 446.
- REYNOLDS, T. M. (1963). Chemistry of non-enzymic browning. The reaction between aldoses and amines. *Adv. in Fd. Res.* **12**, 1 - 52.
- SIVABALASUNDERAM, J. AND NADARAJAH, M. (1966). Factors affecting the plasticity retention index of natural rubber. *Q. Jl. Rubb. Res. Inst. Ceylon* **42**, 13 - 28.
- SWAIN, T. AND HILLIS, W. E. (1959). The phenolic constituents of *Prunns domestica*. The quantitative analysis of phenolic constituents, *J. Sci. Fd. Agric.* **10**, 63 - 68.
- TAN, C. H. AND AUDEY, B. G. (1968). Ergothioneine and hercynine in *Hevea brasiliensis* latex. *Phytochemistry* **7**, 109 - 118.