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# SOME NATURALLY OCCURRING ANTIOXIDANTS IN *HEVEA BRASILIENSIS* LATEX

BY

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## INTRODUCTION

The natural rubber (NR) producing countries with the exception of India and Brazil produce NR for export and Ceylon exports over 95% of her production of NR. Because of the present marketing arrangements for NR, there can be a considerable time lag between the production of NR and its consumption. This can result in the bulk oxidative ageing of raw rubber. An important specification for NR (Bateman & Sekhar, 1966) is the plasticity retention index (PRI) which is a measure of the resistance of raw rubber to oxidative degradation. It has been recommended that producer limits of PRI be at least ten units higher than consumer limits, to give allowance to deterioration during storage (Anon 1970).

Natural antioxidants are known to be present in NR latex. These antioxidants are so powerful that they have been able to protect NR from deterioration during the coagulation and drying processes that there has been no necessity to add stabilizers in the preparation of raw NR as in the case of synthetic rubbers (SRs). Thus, though styrene-butadiene rubber (SBR) is about half to one-third less susceptible to oxygen absorption than NR, stabilizers have to be added during its preparation before the flocculation and drying stages. The subject of natural antioxidants of raw NR has become more important, because of the increasing use to which preserved NR field latex is being used, and because of higher temperatures used in drying new process rubbers. In the manufacture of new process rubbers, higher temperatures such as 100°C are used for drying whilst in the past the temperature of drying seldom exceeded 65°C. NR latex is being used in road construction (Fernando & Nadarajah, 1969) and also could be used in unvulcanized carpet backing (Mursalo & Hannam, 1970). Raw NR is used in a consumer product as sole crepe.

NR field latex has antioxidants some of which are predominantly water-soluble and some of which are predominantly rubber-soluble. Thus when the latex is converted to total solids, all the antioxidants are retained, whilst, if it is converted by coagulation into rubber, most of the non-water-soluble antioxidants are retained in the rubber. Hence whilst the PRI of total solids from clone RRIM 501 was 136, the PRI of crepe made from the same latex was 105 (Sivabalasunderam & Nadarajah, 1966). The antioxidants present in NR latex are mainly phospholipids, amino acids, phenols, tocotrienols and betaines.

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**Tocotrienols in NR**

Dunphy *et al.* (1965) have shown that γ, δ and α tocotrienols in the free and esterified form are present in surprisingly large amounts at about 0·09 g per 100 g of 60% DRC high ammonia centrifuged NR latex. These tocotrienols are soluble in rubber and would be expected to be present predominantly in the rubber phase at about 0·15% on the NR. Davies & Kam (1968) have found the tocopherol and tocotrienol class of compounds in the acetone extracts of uncured and cured NR. The relative amounts present appeared to be independent of the type of accelerator or antioxidant system used.

The method used by us for the extraction and isolation of the tocopherols and tocotrienols from field latex stabilized with ammonia was by the method of Folch *et al.* (1951) which was used by Dunphy *et al.* (1965). The method used was as follows :—

Field latex containing about 30%—40% rubber and stabilized with ammonia was used. The latex was extracted by the method of Folch *et al.* (1951); 20 ml of latex was homogenised for ten minutes in a Waring blender (half speed) with 150 ml of a chloroform/methanol, 2 : 1 mixture. The extract was filtered from the solid rubber using a filter paper and was washed in the following manner. A beaker of 250 ml capacity was placed inside a larger beaker of 2 : 1 capacity. The larger beaker was filled with distilled water upto 9/10 of the volume. Then the extract was slowly introduced into the smaller beaker using a burette. The large beaker was covered with a clock glass and left overnight. The next day about 3/5 of the original volume of the extract remained at the bottom of the small beaker. At the interphase (chloroform/water) there was white 'fluff' consisting of phospholipids. The water was removed by suction and the lower chloroform layer was taken with fluff. A portion of it was dried with anhydrous sodium sulphate. To the other portion methanol was added until the extract became clear. This portion was also dried using anhydrous sodium sulphate.

The method used by us for the extraction and isolation of the tocopherols and tocotrienols from total solids or from coagulated rubber was by acetone extraction, as used by Davies & Kam (1968). The method used was as follows :—

A 1 g sample ± 0·01 g was extracted for 12 hours with acetone, under reflux; the acetone was then distilled off and the residue was dissolved in 0·5 ml of carbon tetrachloride, and 0·015 ml was spotted on the chromatograms.

Thin-layer chromatographic techniques were used in the separation of the tocopherols and the tocotrienols. The chromatographic plate was impregnated with kiesel G (silica gel G) which serves as the solid phase and heated to 110°C. The chromoplate was developed with the solvent chloroform in the first direction and with a mixture of 20% di-isopropyl ether and light petroleum ether (b.p. 60°—80°C) in the second direction (Whittle & Pennock, 1967). The spray reagents used were Emmerie Engel reagent (dipyridyl-ferric chloride), ceric sulphate or diazotised orthoanisidine. The ratio of the amounts of tocotrienols present were estimated visually, by running known amounts of the compounds and comparing the intensity of the spots.

*Clonal variation of tocotrienol content in field latex:* The results of the amount of free tocotrienols in field latex from several clones by visual estimation are given in Table 1. α tocopherol was present in all these clones in 'trace' amounts, and the esterified form was removed during the extraction process and was therefore not present.

TABLE 1

VISUAL ESTIMATION OF TOCOTRIENOLS IN LATEX OF VARIOUS CLONES

| Tocotrienol | Clone | | | | | | |
|---|---|---|---|---|---|---|---|
| | RRIM 513 | RRIC 45 | PB 86 | RRIC 52 | RRIC 88 | RRIC 7 | Nab 15 |
| δ - tocotrienol | 2 | 1 | 1 | 1 | 1 | 1 | 1 |
| γ - tocotrienol | 10 | 7 | 7 | 6 | 6 | 6 | 5 |
| α - tocotrienol | 3 | 3 | 8 | 8 | 5 | 2 | 2 |
| α - tocopherol | T | T | T | T | T | T | T |
| PRI of total solids | 133 | 120 | 120 | 113 | 105 | 102 | 88 |
| PRI of coagulated rubber | 100 | 96 | 86 | 78 | 85 | 81 | 75 |
| DRC | 37·0 | 34·7 | 34·9 | 39·6 | 36·6 | 34·9 | 40·0 |

In the visual estimation, intensity of 1 is approximately equivalent to $2 \cdot 0 \times 10^{-6}$g.

The ratio of the tocotrienols in centrifuged latex found by Dunphy *et al.* (1965) for γ : α : δ was about 20 : 10 : 1 but the ratio obtained by us shows a clonal variation. γ tocotrienol is also the principal tocotrienol found by us in most of the clones tested, the exceptions being RRIC 52 and PB 86. The next important tocotrienol found by us was α tocotrienol. According to Lea & Ward (1959), who investigated the relative antioxidant activities of the known tocopherols, activity decreases in the order monomethyltocol, dimethyltocol and trimethyltocol and in the order 8 methyltocopherol, 7 methyltocopherol and 5 methyltocopherol. We would expect the trienols to have the same order of antioxidant activity as the tocopherols. α tocotrienol is trimethyltrienol, γ tocotrienol is 7, 8 dimethyltocotrienol and δ tocotrienol is 8 methyltocol respectively. Hence the descending order of antioxidant activity would be expected to be δ, γ and α tocotrienols respectively. Table 1 shows that RRIM 513 which has the highest amount of δ and γ tocotrienols also has the highest PRI, whilst Nab 15 which has the lowest amount of these trienols has the lowest PRI in the coagulated rubber.

*Effect of centrifuging on tocotrienol content:* The results by visual estimation of the amount of the tocotrienols in the acetone extract of total solids and coagulated rubber from centrifuged, skim and field latex from clone PB 86 by running known amounts of the compounds are given in Table 2. It is seen that skim latex contains more tocotrienols and centrifuged latex less tocotrienols than field latex. The PRI values obtained are also given in Table 2.

TABLE 2

VISUAL ESTIMATION OF TOCOTRIENOLS IN CENTRIFUGED, SKIM AND FIELD LATEX FROM CLONE PB 86

| Sample | Tocotrienol content | | | | PRI |
|---|---|---|---|---|---|
| | δ | γ | α | Esters | |
| Field latex (total solids) | 2 | 12 | 10 | 4 | 120 |
| Field latex (rubber) | 2 | 10 | 11 | 6 | 86 |
| Centrifuged latex (total solids) | 2 | 9 | 9 | 4 | 97 |
| Centrifuged latex (rubber) | 2 | 5 | 5 | 4 | 43 |
| Skim latex (total solids) | 3 | 14 | 12 | 8 | 106 |
| Skim latex (rubber) | 1 | 1 | 1 | 1 | 5 |

In the visual estimation, intensity of 1 is approximately equivalent to $2 \cdot 0 \times 10^{-6}$g.

*Effect of coagulation conditions on tocotrienol content :* The results obtained by visual estimation of the tocotrienols present in the acetone extract of the samples by running known amounts of the compounds are given in Table 3.

Different coagulation conditions, *i.e.* controlled acid coagulation, bacterial coagulation, autocoagulation and processes used in pale crepe manufacturing, such as fractionation into yellow and white fractions and the use of RPA No. 3 have no significant effect on the tocotrienol content of the rubber obtained and is the same as that present in latex T.S. Hence tocotrienols can be said to be rubber-soluble antioxidants. However the amount of ester present varies, being highest in auto-coagulation and lowest in the yellow fraction.

TABLE 3

VISUAL ESTIMATION OF TOCOTRIENOLS IN RUBBER FROM CLONE PB 86 OBTAINED BY DIFFERENT COAGULATION CONDITIONS

| Sample | Tocotrienol content | | | | PRI |
|---|---|---|---|---|---|
| | δ | γ | α | Esters | |
| Field latex (T.S.) | 2 | 12 | 10 | 4 | 120 |
| Rubber by autocoagulation | 2 | 12 | 11 | 9 | 79 |
| Rubber by acid coagulation | 2 | 12 | 10 | 3 | 78 |
| Rubber by bacterial coagulation | 2 | 10 | 9 | 5 | 80 |
| Yellow fraction | 2 | 11 | 10 | 3 | 107 |
| Yellow fraction RPA No. 3 | 2 | 11 | 10 | 3 | 103 |
| White fraction | 2 | 10 | 9 | 4 | 92 |
| White fraction RPA No. 3 | 3 | 11 | 11 | 8 | 88 |

In the visual estimation, intensity of 1 is approximately equivalent to $2 \cdot 0 \times 10^{-6}$g.

*Effect of alkali on tocotrienol content :* Alkali which would be expected to convert the tocotrienols into a salt appears to have an adverse effect on the antioxidant action of the tocotrienols. Table 4 shows that latex with 0·6% sodium hydroxide (T.S.) has a lower PRI than latex with 1·2% ammonia (T.S.) which has a lower PRI than latex (T.S.). The rubber prepared from alkali-treated latex has a still lower PRI as the sodium and ammonium salts of the tocotrienols would be expected to be leached out in the preparation of the rubber. The results of the amount of the tocotrienols by visual estimation in the acetone extract of the total solids and coagulated rubber from latex preserved with 1·2% ammonia and 0·6% sodium hydroxide from clone PB 86, by running known amounts of the compounds are given in Table 4. The values of PRI are also given in Table 4. According to Deuel (1951), the tocopherols are acted on by alkalies only slowly. The results in Table 4 are after only one week's treatment with alkali. Treatment for longer periods has been found by us to have a more adverse effect, especially on the coagulated rubber.

TABLE 4

VISUAL ESTIMATION OF TOCOTRIENOLS IN TOTAL SOLIDS AND IN RUBBER FROM ALKALI-PRESERVED LATEX FROM CLONE PB 86

| Sample | Tocotrienol content | | | | PRI |
|---|---|---|---|---|---|
| | δ | γ | α | Esters | |
| Latex (T.S.) | 2 | 12 | 10 | 4 | 120 |
| Latex with 1·2% ammonia (T.S.) | 2 | 12 | 10 | 4 | 95 |
| Latex with 1·2% ammonia (rubber) | 1 | 7 | 6 | 3 | 39 |
| Latex with 0·6% sodium hydroxide (T.S.) | 1 | 7 | 6 | 3 | 68 |
| Latex with 0·6% sodium hydroxide (rubber) | 1 | 5 | 4 | 3 | 52 |

In the visual estimation, intensity of 1 is approximately equivalent to $2 \cdot 0 \times 10^{-6}$g.

*Effect of oxidation on tocotrienol content of rubber from clone PB 86*

Samples of shell scrap, of panel scrap and of coagulated rubber from clone PB 86 were oxidised at 100°C for 1 hr and for 4 hr and at 140°C for 1 hr and for 4 hr. The acetone extracts of these samples were examined for tocotrienol content and the results by visual estimation, by running known amounts of the compounds, are given in Table 5. It will be seen that shell scrap and panel scrap are low in tocotrienol content and this may be explained by the fact that the tocopherols are reasonably resistant to destruction in visible light but are quite susceptible to alteration in ultra-violet light (Deuel, 1951).

TABLE 5

TOCOTRIENOLS IN CUP LUMP, PANEL SCRAP AND OXIDISED RUBBER FROM CLONE PB 86

| Sample | Tocotrienol content | | | | PRI |
|---|---|---|---|---|---|
| | δ | γ | α | Esters | |
| Cup lump | 1 | 2 | 3 | 5 | 71 |
| Panel scrap | T | 2 | 2 | 3 | 63 |
| Unheated rubber | 2 | 10 | 9 | 4 | 92 |
| Rubber heated for 1 hr at 100°C | 1 | 10 | 9 | 4 | — |
| Rubber heated for 4 hr at 100°C | 1 | 10 | 9 | 4 | — |
| Rubber heated for 1 hr at 140°C | 1 | 8 | 7 | 3 | 74 |
| Rubber heated for 2 hr at 140°C | 1 | 6 | 6 | 2 | 76 |

In the visual estimation, intensity of 1 is approximately equivalent to $2{\cdot}0 \times 10^{-6}$g.

The tocopherols are sensitive to oxidation in the presence of oxygen, but are quite stable when heated to 100°C or to 200°C in the absence of oxygen (Deuel, 1951). Heating at 100°C in air has a negligible effect, but heating at 140°C in air has some effect on the tocotrienols. Thus the tocotrienols of NR appear to be reasonably stable at 100°C which is the drying temperature of new process rubber.

**Betaines, amino compounds and phenolic compounds in NR**

Altman (1941) was the first worker to draw attention to the presence of betaines in NR latex and to mention its practical importance in rubber chemistry, since they may belong to the much discussed but as yet unidentified " natural age resistors in rubber". He identified trigonelline as being present in NR latex. Amino and phenolic compounds are known to be antioxidants in natural rubber. Tan & Audley (1968) have shown the presence of the three betaines, trigonelline, ergothioneine and hercynine in the readily sedimentable fraction (bottom fraction) of NR latex from mature trees. The results obtained by us on the presence of these betaines and other amino compounds in the bottom fraction of latex from clones RRIC 45, PB 86, RRIC 7 and Nab 15 are given in Table 6. The presence of these compounds is one of the factors which contribute to the high PRI of the yellow fraction (Table 3). The methods used by us for the extraction of phenols and betaines from the bottom fraction are as follows :

Ten ml of latex were collected directly from the tapping cut into 40 ml of 0·6 m tris-mannitol buffer and centrifuged at 14,000 rpm for 20 minutes to obtain the bottom fraction. The bottom fraction was treated with 3 ml of water, centrifuged and the supernatant solution of phenates was evaporated to dryness over a steam bath. This was acidified with a 0·5 ml of 0·1 m acetic acid and then 1 ml of methanol was added to it. The suspension was centrifuged and the supernatant was used for chromatographic analysis. Paper chromatography (descending technique) was used to detect phenols first with acetic acid : water (2% solution) and then butanol : acetic acid water, 6 : 1 : 2 as the solvents. The spraying reagents used were :-

(1) Folin denis reagent

(To 750 ml of water, added 100 g of sodium tungstate 20 g phosphomolybdic acid and 50 ml of othophorphoric (syrupy). Reflux for 2 hours, cooled and diluted to 1000 ml).

(2) Potassium iodate saturated aqueous solution.

(3) Diazotised para nitro aniline

5% p-nitro aniline in 2N, HCl, 5% NaNO and 20% sodium acetate were mixed in an ice bath in the ratio 1 : 10 : 30 and used for spraying immediately.

For betaines, the solvent used for paper chromatography was butanol : acetic acid : water (4 : 1 : 5) by volume and diazotised para nitro aniline used as the spraying reagent.

TABLE 6

VISUAL ESTIMATION OF BETAINES AND OTHER AMINO COMPOUNDS IN BOTTOM FRACTION OF VARIOUS CLONES

| Compounds | | Clone | | | |
|---|---|---|---|---|---|
| Betaines | Other amino compounds | RRIC 45 | PB 86 | RRIC 7 | Nab 15 |
| Hercynine | | 8 | 15 | 12 | 8 |
| Ergothioneine | | 8 | 6 | 8 | — |
| Trigonelline | | 1 | — | 4 | 2 |
| | Histidine | 20 | 30 | 15 | 18 |
| | Tyrosine | 1 | 1 | 3 | 1 |
| | Unknown spot | — | — | 12 | — |
| PRI of total solids | | 120 | 120 | 102 | 88 |

Nadarajah & Karunaratne (1964) have determined the phenolic content of the bottom fraction of various clones and shown that there is a clonal variation and that the latex of clone RRIC 7 had the highest phenolic content, being 49 ppm as tyrosine. These phenols since they are present in small amounts would be expected to be water-soluble. Tan & Audley (1968) have stated that tyrosine is the only water-soluble phenolic compound present in *Hevea brasiliensis* latex bottom fraction. The unknown spot from clone RRIC 7 may be a phenolic compound.

Morris & Sekhar (1959) have stated that amines are present in latex and are responsible for the discolouration of pale crepe during storage. The presence of excessive amounts of these amines in any latex can be judged by a simple polymerization experiment in which methyl methacrylate monomer containing peroxide is added to the stabilized latex. An increase in temperature of more than 5°C within 10 minutes of mixing indicates the presence of excessive amines. The temperature rise in fresh latex (3 hours after tapping) and in three-day old latex within 10 minutes and in 25—30 minutes (the maximum temperature noted) is given in Table 7. Nab 15 and RRIC 7 gave a rise in temperature of 5·3° and 5°C respectively, whilst PB 86 and RRIC 45 gave a rise in temperature of 3·7° and 1°C respectively. When the latex was kept for three days, RRIC 7 and Nab 15 gave a rise of only 3°C and 1°C respectively, whilst PB 86 and RRIC 45 gave a rise of 3·7° and 3·9°C respectively. It thus appears that in Nab 15 the amines are volatile or have become inactivated with time and that in RRIC 45 amines have been formed during storage. The lowering in temperature may also be due to formation of inhibitors such as phenols by atmospheric oxidation during storage.

TABLE 7

TEMPERATURE RISE IN °C IN FIELD LATEX AS A RESULT OF POLYMERIZATION

| Clone | Fresh field latex | | Preserved field latex | |
|---|---|---|---|---|
| | In 10 minutes | Max. temp. | In 10 minutes | Max. temp. |
| RRIC 45 | 1 | 11·3 | 3·9 | 13·1 |
| PB 86 | 3·7 | 15 | 3·7 | 13·1 |
| RRIC 7 | 5 | 15·6 | 3 | 14 |
| Nab 15 | 5·3 | 16·1 | 1 | 14·3 |

Boucher & Carlier (1964) have stated that the amino acids corresponding with the first members of the aliphatic series, unsubstitued or substituted by ethyl, phenyl or propyl groups are good antioxidants.

Ng Tet Soei (1960) has shown the presence of glycine, alanine, and phenyl alanine in NR latex. These amino acids would be expected to be important antioxidants present in the aqueous phase especially as they form complexes with prooxidants such as copper ions (Heinisch *et al.*, 1961). The extraction of the free amino acids was carried out according to the method of Altman (1941). In this method chemical or enzymic hydrolysis is reduced to a minimum. Ten ml of latex was allowed to flow, drop by drop, into 90 ml of absolute alcohol, which contained ·01 ml of toluene. The resulting mixture was heated to 60°C and agitated with a mechanical stirrer. A spongy rubber coagulum was formed and it was macerated in a mortar with 3 ml of absolute ethanol. The alcoholic extract was filtered through Whatman No. 1 paper and the filtrate evaporated to 2 ml ; 0·1 ml of this extract was used for chromatography. Two dimensional chromotography was carried out using n-butanol : acetic acid : water (12 : 3 : 5 v/v/v) as the developing solvent in the first direction and phenol:water:ammonia (500 : 125 : 0·5 v/v/v/) as the developing solvent in the second direction. These studies on the free amino acids of field latex from clones RRIC 45, RRIC 7, PB 86 and Nab 15 have shown (Table 8) the occurrence of the antioxidant amino acid alanine in appreciable concentration in all these clones. The other antioxidant amino acids, leucine and isoleucine were also observed in these clones.

Table 8 gives the amino acids and the amounts present as estimated visually in clones Nab 15, PB 86, RRIC 7 and RRIC 45 by running known amounts of the compounds. In the visual estimation intensity of 1 is approximately equivalent to $0{\cdot}4 \times 10^{-5}$g.

TABLE 8

VISUAL ESTIMATION OF THE FREE AMINO ACIDS IN FIELD LATEX

| Amino acids | Clone | | | |
|---|---|---|---|---|
| | NAB 15 | PB 86 | RRIC 7 | RRIC 45 |
| Cysteine | T | T | T | T |
| Aspartic acid | T | 1 | T | T |
| Glutamic acid | 3 | 4 | 7 | 6 |
| Serine | 1 | 2 | 1 | 1 |
| Glycine | — | T | — | T |
| Threonine | 1 | 1 | — | 1 |
| α-Alinine | 5 | 7 | 7 | 7 |
| Tyrosine | T | T | 1 | T |
| Tryptophan | 1 | T | 3 | 1 |
| Valine | T | 5 | T | T |
| Leucine and isoleucine | T | T | T | T |
| Lysine | — | — | T | T |
| Histidine | T | T | 1 | T |
| α-Phenylalanine | 2 | 1 | 1 | 1 |
| Glutamine | 1 | T | 4 | T |

Despite the presence in raw NR of naturally occurring antioxidants, it is well recognised that for most applications further antioxidant is necessary. The beneficial influence of the use of a rubber having a high value of PRI is detectable even in the presence of 2 pphr of a powerful antioxidant (NRPRA — NR Technical Information Sheet No. 107). The use of types of natural rubber containing high levels of naturally occurring antioxidants will give the best ageing resistance.

In conclusion it may be said that where field latex, or to some extent, concentrated latex made from it is used as such, then the antioxidants such as phenols, amino acids would play an important part in addition to the tocotrienols. However where rubber is made from field latex in the form of RSS, new process rubbers, pale crepe, estate brown crepe etc., then the tocotrienols would be expected to be very important antioxidants.

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