

CHAPTER IV

DISCUSSION

In accordance with the discoveries of previous workers neutral lipids in Hevea brasiliensis consist of carotenoid pigments. But here found that carotenoid pigments are not existed only in neutral lipids eluted with hexane-diethyl ether (95:5 and 75:25, v/v). The pigments can also be found in more polar fraction eluted with diethyl ether alone. The pigment in hexane-diethyl ether (95:5) resembles β -carotene plus closely related isomer which is not distinguishable chromatographically from β -carotene. It is wellknown that carotenes are very susceptible to air oxidation and isomerization in solution due to various factors such as photo isomerization, thermal isomerization and isomerization by catalysts including organic acids. Nadarajah and Karunaratne (1971) have suggested that Frey-Wyssling globules of the yellow fraction consist mostly of fat and fat-like substances and account for most of the acetone extract residue. Fatty acids can bring about isomerization of the pigment.

The long process of experiment had also changed the pigment, so that it differed slightly from naturally occurring β -carotene. Beadle and Zscheile (1942) have reported that β -carotene can be transformed by heat into pseudo α -carotene (neo- β -carotene), one of the more stable isomers of β -carotene, which has absorption maxima at wavelength very close to that of α -carotene (λ_{max} at 422, 446, 474 nm). In case of carotenoid pigment in the second fraction (hexane-diethyl ether, 75:25)

and the third fraction (diethyl ether, separation of these pigments on TLC developed with hexane-diethyl ether-acetic acid (90:10:1) indicated that there are several kinds of derivative existed in the latex, and they are more polar than β -carotene standard. From their absorption spectra, one pigment should be β -carotene-5,8-epoxide which has λ_{max} at 409, 428 and 450 nm. These results suggest that the pigments in both the hexane diethyl ether (75:25) fraction and the diethyl ether fraction should be an oxygenated carotenoid or hydroxylated carotenoid. The results of this work using spectrophotometry and TLC can not identify the exact structure of these pigments. However, these pigments existed in any derivatives forms have the yellow color resulting in the discoloration of rubber.

Tocotrienols existed in rubber latex are both in free and esterified forms but only free form has an effect on the discoloration because it has free OH-group which can be oxidized by oxygen. Comparing with free α -tocopherol standard, the absorption spectra and TLC of each fraction show that free tocotrienols exist only in the hexane-diethyl ether (75:25) fraction. However, the method used in this work can not distinguish the iso-forms of free tocotrienols (α -, β -, γ - and δ -form).

Among the verifying indicators; tocotrienols, carotenoids and polyphenols, which have been expected to have an effect on discoloration of rubber, only total carotenoids content has been found to vary with clone, PB 5/51 has the highest amount ($\approx 190\mu\text{g}$) and RRIM 600 ($\approx 60\mu\text{g}$), and the degree of discoloration is the same in raw rubber. The total amount of tocotrienols and polyphenols are not significantly different among 3 rubber clones tested, ranging in 0.07-0.08 g and 0.11-0.13 g respectively. Anyhow the method of extraction can not remove absolutely

all these factors from the latex, because the rubber coagulation occur on contact with the solvent (Hasma, 1984). The mild extraction procedure is used by intention to prevent any undesirable changes taking place in the lipids during extraction.

In case of polyphenol, Nadarajah and Karunaratne (1971) have reported that the phenolic content of the latex samples can increase or decrease during storage at room temperature (about 28°C) or in the refrigerator. An increase in phenolic content is probably caused by oxidation of amino acids to aminophenols by the enzyme peroxidase.

In case of PPO activity, there are significant difference among 3 fractions of latex. Serum fraction shows the highest activity, the latter is lutoids and rubber fraction. In contrast, Low and Wiemken (1984) reported dominant proportion of PPO with the lutoid fraction by using Ficoll gradient. However there are no significant difference in the total PPO activity per gram dry weight among these 3 rubber clones.

The presence of PPO in larger proportion in the serum fraction might be stimulated by the tapping frequency used in Rayong province, where tappings are continuous for 3 days stop on the 4th day, and start again. In Malaysia and Indonesia rubber plantations are larger, and tapping is on alternate day (one day tapping/one day stop), so that physiological difference, such as enzyme distribution, and activity stimulation can be different. Analogous example is Ethephon stimulation of RRIM 600 trees has been reported to reduce the PPO activity markedly and caused shifting in lutoid density (Low and Wiemken, 1984).

In considering the relationship between the color index of rubber and the amount of these verifying indicators, the results indicate that only the total amount of carotenoids has correlation with the color index of control untreated rubber, i.e. the rubber clone which has the low amount of carotenoids will give the low value of color index. The amount of other indicators are not significantly different among 3 clones tested.

In the experimental design to see the effect of removing total lipids and polyphenols, in comparison with addition of each verifying factor individually, the intention of this study is to extrapolate from 3 rubber clones tested to the extreme conditions of minute amounts on one end and very large amounts for the 4 verifying factors selected on the other extreme. By using the same set of latex specimens, the cause and the mechanism of discoloration have been followed up in complete process starting from fresh field latex to rubber vulcanizate of processing the rubber. Greater loss occurs on diluting the latex to 15% DRC to prepare sheet rubbers. The air dried sheet rubbers contains less tocotrienols about 45% reduction than the normal acid coagulated Hevea crumbs.

Addition of carotenoid shows about 28-45% higher degree of color index than control rubber. This result indicates that carotenoids have outstanding effect on the discoloration of raw rubber. In order to reduce this effect, the bleaching agents have been used. Alkali metal salt of tolyl mercaptan is now used in the crepe industry as the bleaching agent. Tillekeratne et al. (1984) have indicated that the bleaching effect of thiols on carotenoids in the latex is due to free radical process because this bleaching effect is not observed in

complete darkness. During the bleaching action, carotene molecule is excited to a high energy state by absorbing visible light and then there is transfer of energy to a thiol molecule, thus exciting it. The excited thiol molecule then breaks up into RS^* (thiol radical) and H^* free radicals, and the RS^* free radical ultimately reacts with the unsaturated structure of the carotene molecule thereby disrupting the conjugation of double bonds and thus making it colorless.

Like carotenoids, polyphenols have much effect on the discoloration of raw rubber. The color index of polyphenol-added rubber are higher than that of control rubber and also that of carotenoid-added rubber. During processing of latex to air dried rubber, PPO the polyphenols oxidizing enzyme must be destroyed by heat, but the discoloration of polyphenol added rubber still continue. Rinderknecht and Jurd (1958) have shown that polyphenols can combine with amino acid at room temperature to give a yellow color, and Hess (1958) has shown that oxidized polyphenolic compounds react with glycine to give purple products. Burton et al. (1963) has proposed that oxidized polyphenols may de-amine amino acids with the formation of keto acids, which lose carbon dioxide to form aldehydes. Such aldehydes would also react with NH_2 groups, and undergo condensation with substance likely to be present in naturally occurring material, which has not been fully explored, to give intermediates of high browning potential. The brown color of dry rubber is also probably due to these similar reaction. However, these discoloration can be inhibited by sodium metabisulfite. Burton et al. (1963) have shown that sulfite can limit the reaction between carbonyl and amino group to give discolored product and hence retard non-enzymatic browning.

In case of PPO, latex was first added with 0.08 M Tris-HCl buffer containing 1.0 M Sucrose (TS buffer), followed by addition of PPO. The results in Table 3.4 indicate that TS buffer has an effect on the discoloration of raw rubber, probably due to sucrose existed in the large amount in TS buffer. Sugar can react with amino compounds in the latex to give a color substance (Burton, 1963). The color index of PPO-added rubbers are not different from that of buffer-added rubbers. But it could not be concluded that PPO has no effect on discoloration because the effect of TS buffer could cover the effect of PPO, thus disguising the effect of PPO. In order to reduce this enzymatic discoloration, sodium metabisulfite the inhibitor of PPO is used. Sodium metabisulfite is a strong reducing agent and also inhibit non-enzymatic discoloration by removing oxygen from reaction sites or by reducing highly reactive orthoquinone to polyphenols (Nadarajah and Kannaratne, 1971). Besides sodium metabisulfite, oxalic acid or a mixture of oxalic acid and formic acid (3:1) can be used as an inhibitor and as the coagulant because oxalic acid is also a reducing agent. During acid coagulation (6-8 h) by formic acid only, the pH of latex is lowered in weak acid, thus making the pH suitable for PPO activity. The better method for rubber coagulation should be steam coagulation because this method takes only 3 min and can destroy the PPO completely by heat and does not change the pH of the latex. When comparing the color index of polyphenol-added rubber and PPO-added rubber, the results show that polyphenol has more drastic effect on discoloration than PPO. This result indicates that non-enzymatic discoloration is predominant over enzymatic discoloration.

When raw rubber has been mixed with vulcanizing ingredients, the compound rubber obtained exhibits higher degree of color than raw

rubber. The effect of some indicators on discoloration of compound rubber were different from the effect on raw rubber. Carotenoids and Tris buffer containing sucrose which affected on the discoloration of raw rubber does not have an effect on compound rubber. Only the effect of increasing polyphenols can continue by increasing the dark color of compound rubber and vulcanized rubber. TS buffer containing 1.0 M Sucrose has an effect on the color of vulcanizate, but less than polyphenols, which indicates that processing of rubber in each step can increase discoloration. Acid-coagulated rubber was heated at 60°C until dried. The time used in this step was usually over 12 h. This heating is adequate to promote the reaction of sugar/amino acid and polyphenol/amino acid, and to burn the sucrose to singe. These reactions result in increasing color index of polyphenol-added rubber and TS buffer. Vulcanizing ingredients mixed with raw rubber can also react with some substance retained in the rubber such as proteins and those factors added in each treatment, causing the discoloration of rubber. Some vulcanizing ingredients per se have color such as sulfur, Shellflex and MBT. However, comparison of color between raw rubber and compound rubber are based on visual inspection, and compound rubbers are more opaque than raw rubber, so that the degree of discoloration are subjective. However, the effect of vulcanizing ingredients on discoloration are strong enough to cover the effect of depleted lipids and polyphenols, or increasing carotenoids and TS buffer observed in raw rubbers. In processing of compound rubber to vulcanized state, the rubber must be heated at 150°C, 3-4 min depending on their cure time derived from cure characteristic curve. The color index of vulcanizates are therefore increased with the same reasons as in that of raw rubber.

Besides discoloration, the effect of these verifying indicators

on Mooney viscosity has been demonstrated. Reduction of total lipids and polyphenols in the latex result in reduction of the Mooney viscosity. This finding is not similar to the previous work (Yip and Subramaniam,) which indicated that lipids can act as plasticizers, a high content of acetone extract rubber which contain considerable quantities of lipids could induce lower values of Mooney viscosity. The reason why the lipid-removed rubber has lower viscosity than control rubber is probably because some lipids such as fatty acid which are cross-linked with the rubber chain have been removed, so that the rubber chains can move or flow easily. However, the lipid substances that affect the viscosity of rubber are not tocotrienols, carotenoids and polyphenols because addition of these substances do not complement Mooney viscosity. In case of adding TS buffer and PPO, Tris-sucrose buffer reduced the viscosity whereas PPO has no effect on the viscosity. The Mooney viscosity reduction caused by Tris-sucrose buffer is probably due to both amino group and sucrose, because the reaction between sugars and amino containing substance at high temperature can hinder or compete with cross-linking between rubber particles resulting high flow ability of rubber. In addition the Mooney viscosity of rubber has been shown to be decreased when proteins are removed (Chang et al., 1977). Mooney viscosity has been known as rubber clonal characteristic, it depends on the molecular weight of the polyisoprene polymers, molecular weight distribution and branching of the polymer chains including linkages to some cross linking non-rubber molecules (Yip and Subramaniam, 1984). Nair (1970) and Subramaniam (1975) have indicated that rubber with high molecular weight show high viscosity and vice versa.

The overall cure characteristics are not affected by variation of total lipids, tocotrienols, carotenoids, and polyphenols used in this

research probably because the compounding formulation used consist of 4 accelerators and 2 activators. Addition of TS buffer results in reduction of cure time. This reduction may be due to Tris-(hydroxy methyl)-amino methane which have an amino group, and acting as vulcanizing agent. In case of PPO, addition of this indicator reduces the cure rate of rubber. Bloomfield (1973) have suggested that proteins can interfere with the homogenization of vulcanizing ingredients, thus reduction in cure rate of NR.

The effect of each verifying indicator on physical properties : 300% modulus, elongation at break, tensile strength, tear strength, hardness and specific gravity tested, the results show that extracted rubber and rubber enriched with tocotrienols, carotenoids, polyphenols and PPO exhibit reduction of tensile strength and tear strength. These finding could be artefacts caused by mole surface or by heterogeneous mixing of compound rubbers, because addition of each indicator cannot increase these properties. Like the results of this research tensile strength and tear strength of vulcanizate prepared from the rubber in the same laboratory by another worker show the lower value than it was expected, but these physical properties of vulcanizate prepared from commercial TTR 5L and crepe rubber, and pressed with the same mold exhibit the higher value. These finding suggests that there are other factors affected on this physical properties such as storage time of raw rubber before mixing with vulcanizing ingredients.

From overall results, it can be concluded that polyphenols are the most important indicator affected on discoloration of rubber because polyphenol-added rubber show the higher degree of color index than other treatment in every step of processing. In the processing of raw rubber

to compound and vulcanized rubber PPO has been destroyed by heat, but the discoloration can still occur. This result indicate that non-enzymatic discoloration has much prolonged effect than enzymatic discoloration. Polyphenol can react with amino acid to give the color product. However we could not remove the polyphenols out of the rubber, because it can make the disadvantages effect to the other properties such as antioxidation. In producing the light color rubber, we should remove the proteins instead of the polyphenols because aminophenols formation would be limited. Parallel research in the same laboratory show that deproteinized rubber has lower color index than control rubber (Wonnop Visessanguan, 1992).

However, the results of this research have been derived from merely 2-4 samples which could vary due to climatic condition, seasonal variation, tapping system and rubber processing, thus probably making wide variation of the mean. This problem is common for biological materials collected under seasonal variation etc. and could not be eliminated in a short period of research of 2-3 years.