

CHAPTER 3

RESULTS

3.1 Characterization of carotenoid pigments in fresh latex

Carotenoids are the wellknown pigment in rubber latex, which reside in the lipid fraction. To study the effect of carotenoids on rubber discoloration, the total lipids fraction of fresh latex (clone PB 5/51) extracted by chloroform-methanol (2:1) were fractionated on a silica gel column eluted with hexane-diethyl ether (95:5), (75:25) and (0:100) respectively. Every fraction contains yellow pigments which are characterized by its absorption spectra in the wavelength range 350-500 nm in hexane (Figure 3.1). Comparing with β -carotene standard, the first fraction shows nearly identical λ_{max} at 425, 450 and 475 nm, while the second and the third fraction show very similar λ_{max} at 405, 425 and 450 nm. Separation of these three fraction on TLC developed with hexane-diethyl ether-acetic acid (90:10:1) (Figure 3.1) suggests that the first fraction contains the pigment which is slightly more polar than β -carotene standard. It may be the mixture of α - and β -carotene. The second and the third fraction contain pigments which are more polar than β -carotene standard. Both the second and third fraction pigments may be the polar carotenoid derivatives such as ketocarotenoids, hydroxylated carotenoids or epoxy-carotenoids.

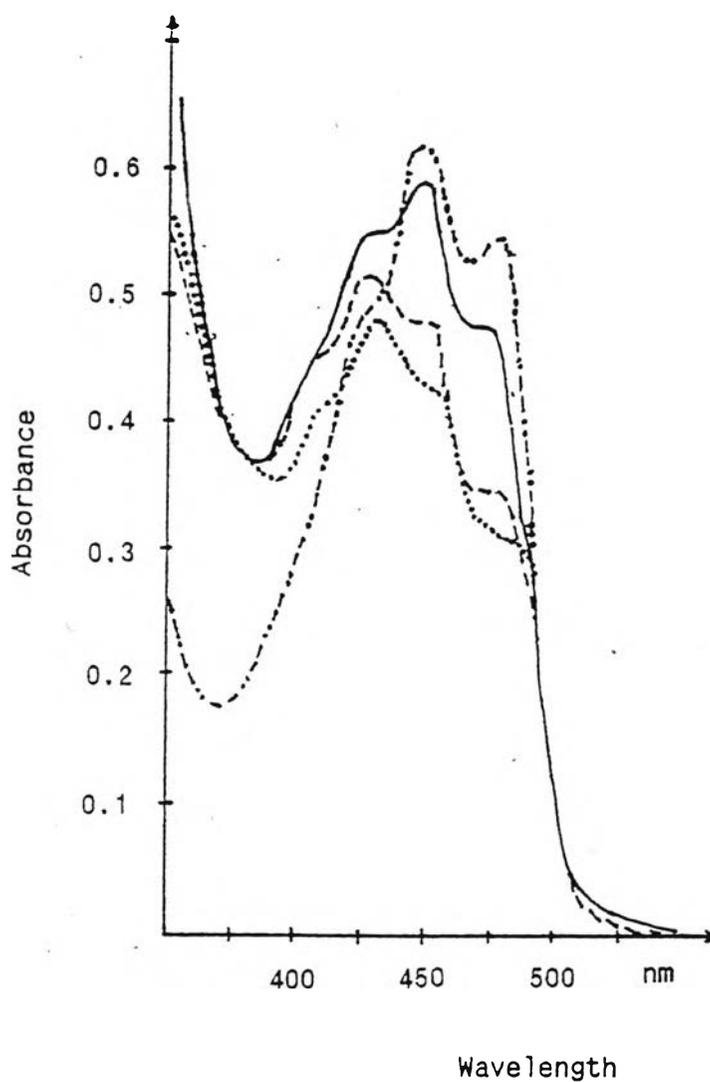


Figure 3.1 Absorption spectra of pigments eluted from silica gel column of latex lipids in hexane solution.

The absorption spectra of the 3 separated fractions in the following order are :

- 1) Hexane:diethyl ether (95:5), (—)
- 2) Hexane:diethyl ether (75:25), (---)
- 3) Diethyl ether, (.....)
- 4) β -carotene standard in hexane, (-·-·-·-)

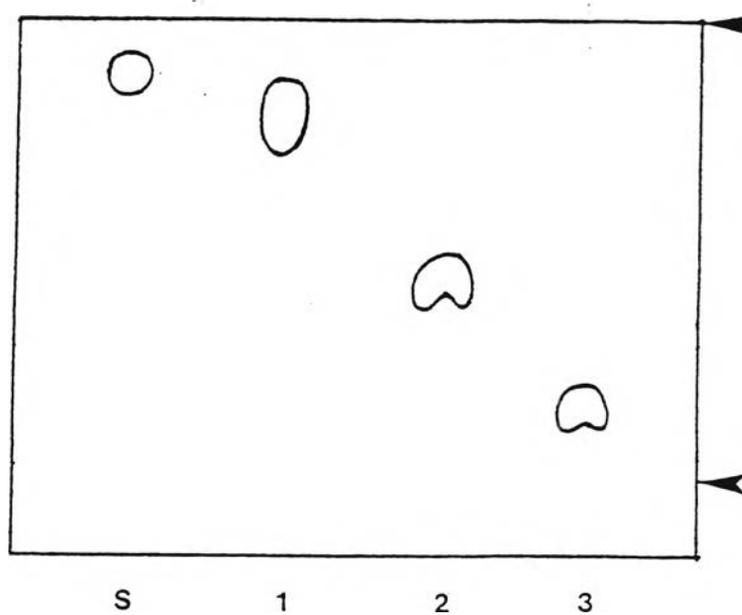


Figure 3.2 TLC profile of carotenoid pigments eluted from silica gel column.

The chromatographic plate was developed in hexane-diethyl ether-acetic acid (90:10:1)

Lane S, β -carotene standard

Lane 1, hexane-diethyl ether (95:5)

Lane 2, hexane-diethyl ether (75:25)

Lane 3, diethyl ether

3.2 Characterization of tocotrienols in fresh latex

Free tocotrienols, an alcohol lipid, exist in rubber latex as natural antioxidant. To study the effect of free tocotrienols as retarder of rubber discoloration which is caused by enzymatic reaction, tocotrienols were extracted out of the total lipids, and analyzed separated fractions from silica gel column by comparing with α -tocopherol standard having structure closed to tocotrienols, only the second fraction shows nearly identical λ_{max} at 298 nm, while the first and the third fraction show nearly λ_{max} at 285 and 290 nm respectively (Figure 3.3). Separation of these 3 fractions on TLC developed with hexane:diethyl ether:acetic acid (90:10:1) and visualized under ultraviolet light confirms that only the second fraction contains free tocotrienols because the spot of the second fraction has the same R_f as α -tocopherol standard (Figure 3.4). However, there are several forms of free tocotrienols, α -, β -, γ - and δ -form, which can not be distinguished by absorption spectroscopy and one dimensional TLC separation.

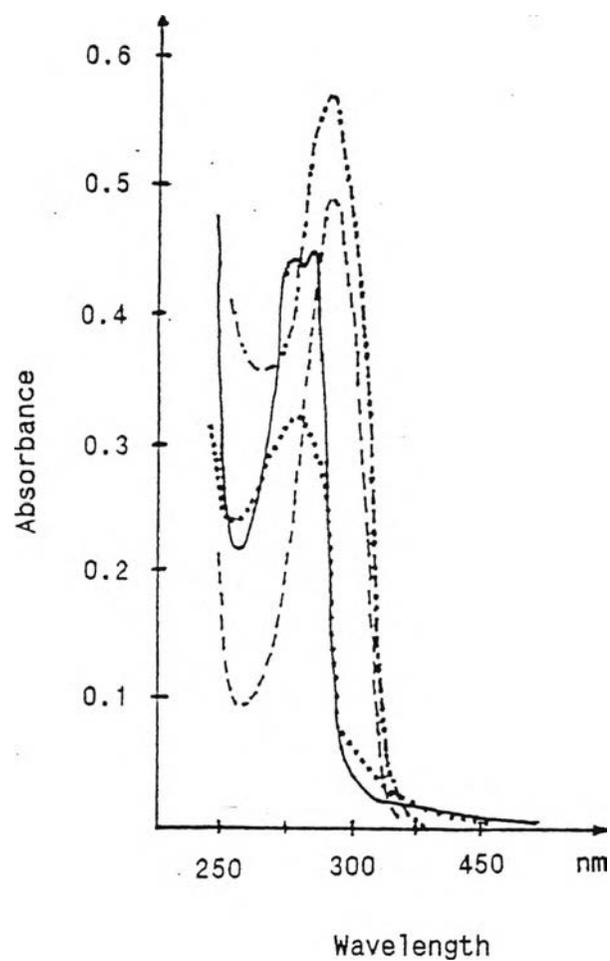


Figure 3.3 Ultraviolet absorption spectra of tocotrienol fractions eluted from silica gel column of latex lipid in ethanol.

The absorption spectra of the three separated fraction in the following order are :

- 1) Hexane:diethyl ether (95:5), (—)
- 2) Hexane:diethyl ether (75:25), (---)
- 3) Diethyl ether, (.....)
- 4) α -Tocopherol standard in ethanol, (·-·-·-·)

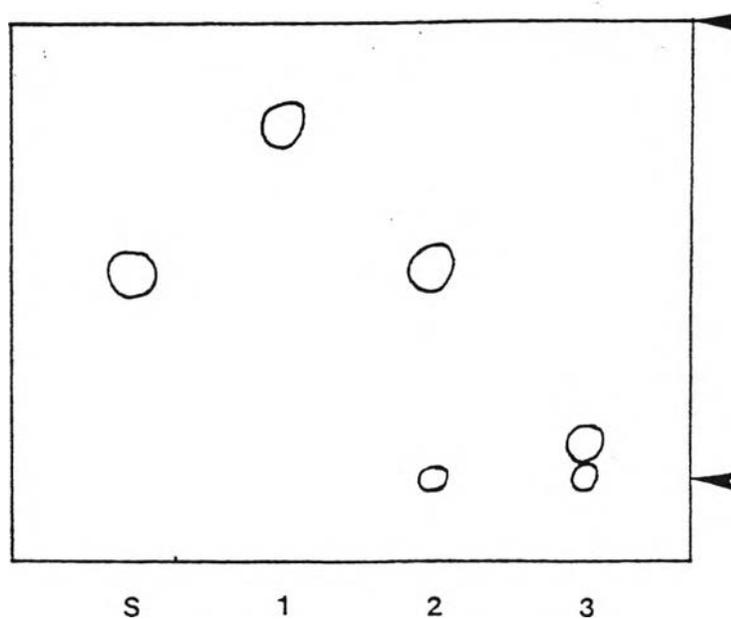


Figure 3.4 TLC profile of tocotrienols eluted from silica gel column.

The chromatographic plate was developed in hexane-diethyl ether-acetic acid (90:10:1)

Lane S, α -tocopherol standard

Lane 1, hexane:diethyl ether (95:5)

Lane 2, hexane:diethyl ether (75:25)

Lane 3, diethyl ether

3.3 Comparison of tocotrienols, carotenoids and polyphenols contents among rubber clones

The amount of these varying indicator : tocotrienol, carotenoid and polyphenol in fresh latex have been determined in 3 rubber clones : RRIM 600, GT 1 and PB 5/51 by spectrophotometric method using the absorption coefficients of γ -tocotrienol, β -carotene and the standard graph of tyrosine to estimate the total amount of tocotrienols, carotenoids and polyphenols respectively.

Table 3.1 shows that PB 5/51 contains the highest amount of total lipids content while RRIM 600 has the lowest lipids. However, there is no significant difference at 98% confidence because the variation among 4 samples yielding high standard deviation. For tocotrienols content, there is no significant difference among 3 rubber clones (0.07-0.08 g/100g dry wt. rubber). Only the carotenoid content that significant difference has been clear; PB 5/51 shows the highest amount of total carotenoids content (190 μ g). In case of polyphenols content, there is no significant difference in all 3 rubber clones (0.11-0.13 g/100g dry wt. rubber).

These results suggest that carotenoids may be an indicator which affect on the color of rubber although existing in a very small quantity (0.003-0.007 % total lipid).

Table 3.1 Comparison of total lipids, tocotrienols, carotenoids and polyphenols contents in the latex collected from different rubber clones

Composition (in 100g dry wt. rubber)	Rubber clone		
	RRIM 600	GT 1	PB 5/51
Total lipid (g)	1.87±0.40	2.21±1.08	2.82±1.40
Tocotrienols (g)	0.08±0.02	0.07±0.02	0.07±0.01
(% Total lipid)	(4.3)	(3.2)	(3.2)
Carotenoids (µg)	59.65±6.52 ^a	119.33±46.74 ^b	189.51±54.59 ^c
(% Total lipid)	(0.003)	(0.005)	(0.007)
Polyphenols (g)	0.12±0.02	0.11±0.04	0.13±0.05

Value presented are means of 4 latex specimen ± standard deviation
a, b, c, is an significant difference at 95% confidence analyzed by t-test

3.4 Distribution of polyphenol oxidase activity

The distribution of PPO activity in various fractions of latex has been investigated after fractionation of latex into rubber, serum and luteoid fractions in the presence of stabilizing buffer. Table 3.2 shows that serum fraction contains the highest PPO activity, which is significantly higher than luteoid fraction and rubber fraction in every latex specimen of all three rubber clones collected. Although, there is no significant difference in the total PPO activity among these three rubber clones, there is a trend that RRIM 600 contains lower amount of total PPO activity.

3.5 The correlation between color index and these verifying indicators

In order to emphasize the effect of each verifying indicator on color index, fresh latex has been divided into 5 portions, the first portion is for control untreated rubber, the second portion has been extracted with chloroform - methanol (2:1), and into the other 3 portions, each verifying indicator has been added at the amount of "mean + 2 S.D." to estimate the effect of each verifying indicator as per cent difference in color index ($\pm \% \Delta$), where plus sign (+) indicates darker color and minus sign (-) indicates lighter color.

Table 3.2 Distribution of polyphenol oxidase activity in various fractions of fresh field latex

Latex clone	PPO activity in various fractions ($\times 10^3$ unit/100g dry weight of rubber)			Total ($\times 10^3$ unit/100g dry weight of rubber)
	Rubber	Serum	Lutoid	
RRIM 600	4.0 \pm 8.0 ^a	118.4 \pm 54.8 ^b	18.4 \pm 13.9 ^c	140.8 \pm 65.8
GT 1	7.0 \pm 8.2 ^a	184.0 \pm 54.9 ^b	38.3 \pm 15.0 ^c	229.4 \pm 71.9
PB 5/51	9.6 \pm 8.2 ^a	161.7 \pm 105.0 ^b	103.1 \pm 133.0 ^b	267.7 \pm 182.0

Values presented are mean of 4 latex specimens \pm standard deviation. Significant difference of PPO activity between fractions within each reube clone are marked by different letter (a, b, c) analysed by t-test at 95% confidence

Table 3.3 and Figure 3.5 show that among control specimens of 3 rubber clones collected, GT 1, has a trend to give the highest color index, followed by PB 5/51 and RRIM 600 respectively. But only the color index of RRIM 600 is significantly lower than that of GT 1 and PB 5/51 at 95% confidence. Removal of total lipids including polyphenols resulted in the reduction of color index. In RRIM 600, the color index decreased from 7.1 to 4.4, which is not significant difference because the original color is white. In GT 1 and PB 5/51, the color index decreased significantly from 10.8 to 4.2 (-60%) and 8.8 to 4.8 (-44%) respectively. In case of tocotrienols, the addition of this indicator sometimes has a trend to reduce the color index and sometimes has a trend to increase the color index. However it is not significant difference at 95% confidence. Addition of carotenoids increases the color index of GT 1 (+45%) and PB 5/51 (+40%) significantly, but not significant in RRIM 600 which has the least amount of carotenoid among 3 rubber clones. Addition of polyphenol increased the color index most significantly in every clone. The color index of RRIM 600 and PB 5/51 increased up to 89% and 97%.

The result suggests that the color index of raw rubber is a clonal characteristic depending on non-rubber constituents existed in the latex especially for carotenoid pigments and very likely polyphenols. Polyphenols and carotenoid pigments have a remarkable effect on the color of raw rubber although some rubber specimens values of carotenoids have been deviated from this conclusion, probably resulting from variation of rubber latex collected at different time. Tocotrienols which is firstly expected to retard the enzymatic discoloration has no effect on the color of raw rubber.

In case of PPO, fresh latex was first stabilized with 0.08 M Tris-HCl buffer pH 7.0 containing 1.0 M sucrose (TS buffer), followed by addition of PPO. Table 3.4 shows that addition of buffer alone resulted in a significant increase in color index comparing with its control. However, addition of PPO (Mean + 2 S.D. of each clone) does not increase the color index significantly comparing with the latex plus TS buffer.

This result suggests that TS buffer has much effect on the discoloration of raw rubber but PPO does not.

Table 3.3 Comparison of color index and % difference in color index of dried rubber before and after extraction of total lipids including polyphenols, or addition of verifying indicators

Rubber clone	Color index of rubber samples (\pm % Difference from control)				
	Control (No treatment)	Extracted -Lipid+polyphenol	+Tqcotrienol ($x \pm 2$ S.D.)	+Carotenoid ($X \pm 2$ S.D.)	+Polyphenol ($X \pm 2$ S.D.)
RRIM 600	4.5	4.0 (-11)	5.0 (+11)	7.0 (+55)	7.5 (+ 57)
	6.0	4.0 (-33)	6.5 (+ 8)	8.0 (+33)	14.0 (+133)
	9.0	4.5 (-50)	6.0 (-33)	10.0 (+11)	16.0 (+ 78)
	9.0	5.0 (-44)	7.5 (-28)	10.0 (+11)	16.0 (+ 78)
	Mean \pm S.D.	7.1 \pm 2.2	4.4 \pm 0.5 (-34)	6.3 \pm 1.0 (-10)	8.8 \pm 1.5 (+28)
GT 1	12.0	3.0 (-75)	12.0 (0)	16.0 (+ 33)	16.0 (+ 33)
	7.0	3.0 (-51)	13.0 (+86)	15.0 (+114)	12.0 (+ 71)
	12.0	6.0 (-50)	14.0 (+17)	14.0 (+ 17)	16.0 (+ 33)
	12.0	5.0 (-58)	12.0 (0)	14.0 (+ 17)	16.0 (+ 33)
	Mean \pm S.D.	10.8 \pm 2.5	4.3 \pm 1.5 (-60)	12.8 \pm 1.0 (+25)	14.8 \pm 1.0 (+ 45)
PB 5/51	7.0	3.5 (-50)	12.0 (+71)	12.0 (+ 71)	16.0 (+129)
	6.0	4.5 (-25)	8.5 (+48)	10.0 (+ 68)	16.0 (+166)
	12.0	6.0 (-50)	10.0 (-17)	12.0 (0)	16.0 (+ 33)
	10.0	5.0 (-50)	10.0 (0)	12.0 (+ 20)	16.0 (+ 60)
	Mean \pm S.D.	8.8 \pm 2.8	4.8 \pm 1.0 (-44)	10.1 \pm 1.4 (+24)	11.5 \pm 1.0 (+ 40)

Table 3.4 Comparison of color index of dried rubber before and after addition of PPO



Rubber clone	Color index (+% difference)		
	Before adding	After adding	
	- TS buffer	+ TS buffer	+ TS buffer and PPO
RRIM 600	4.5	14.0 (+211)	16.0 (+256)
	6.0	11.0 (+ 83)	12.0 (+100)
	9.0	14.0 (+ 56)	14.0 (+ 56)
	9.0	14.0 (+ 56)	14.0 (+ 56)
	Mean	7.1±2.3	13.3±1.5 (+101)
GT 1	12.0	14.0 (+ 17)	14.0 (+ 17)
	7.0	14.0 (+100)	14.0 (+100)
	12.0	14.0 (+ 17)	16.0 (+ 33)
	12.0	14.0 (+ 17)	16.0 (+133)
	Mean	10.8±2.5	14.0±0 (+ 35)
PB 5/51	7.0	12.0 (+ 71)	16.0 (+129)
	6.0	14.0 (+133)	16.0 (+167)
	12.0	14.0 (+ 17)	16.0 (+ 33)
	10.0	14.0 (+ 40)	16.0 (+ 60)
	Mean	8.8±2.8	13.5±1.0 (+ 65)

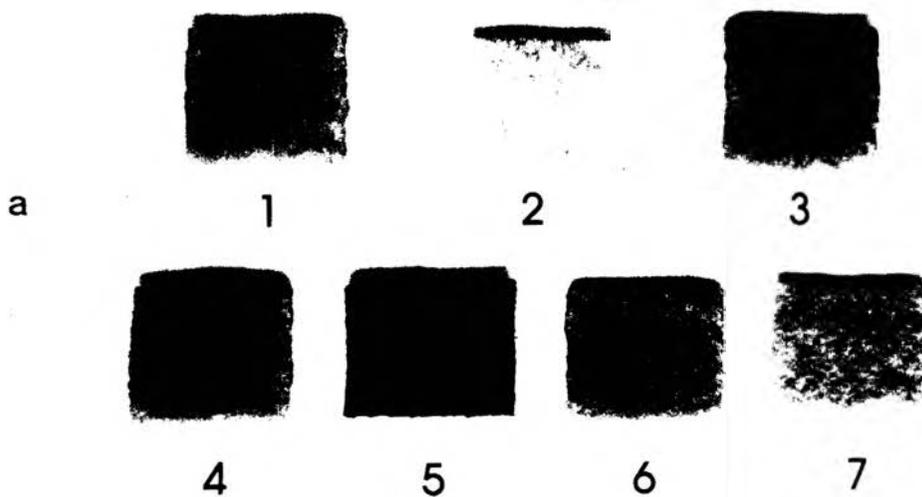
Figure 3.5 Comparison of color of control raw rubber with lipids depleted rubber and enrichment of various discolorating factors

Raw rubber produced by acid coagulation from latex with and without treatments are compared among 3 rubber clones :

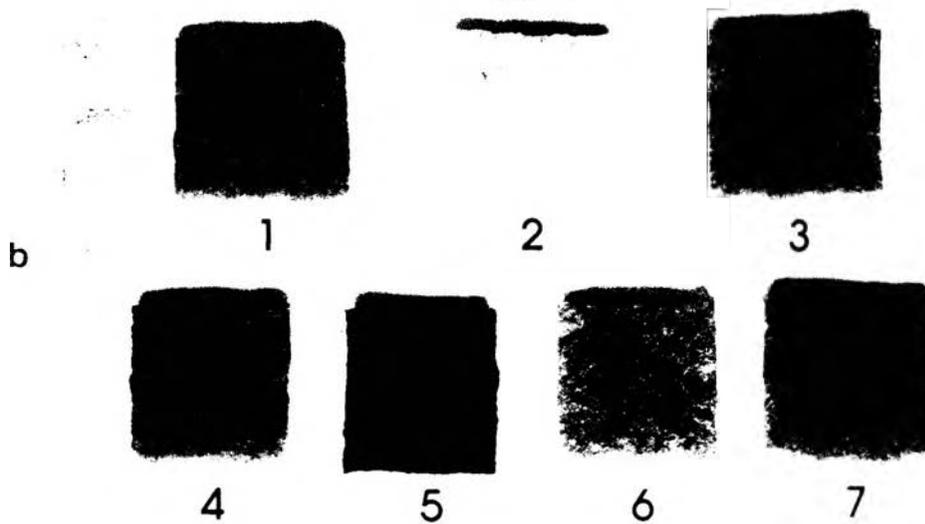
a) RRIM 600, b) GT 1 and c) PB 5/51

- where
- | | |
|------------------------|-----------------------------|
| 1. control | 2. - lipids and polyphenols |
| 3. + tocotrienols | 4. + carotenoids |
| 5. + polyphenols | 6. + TS buffer |
| 7. + Ts buffer and PPO | |

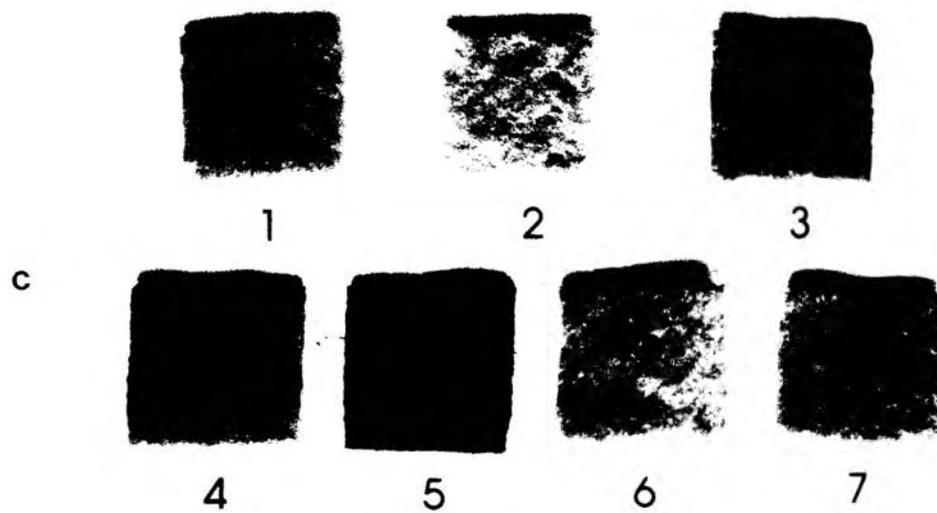
RRIM 600



GT 1



PB 5/51



3.6 Effect of total lipids, tocotrienols, carotenoids, polyphenols and polyphenol oxidase on discoloration of compound and vulcanized rubber

When these raw rubbers prepared under various treatments have been mixed by similar compounding formulation, and compound again for relative changes in color of rubber compared, Figure 3.6 shows that removal of total lipids and polyphenols is nearly similar to that of control rubber. Addition of tocotrienols and carotenoids have no effect on the color of compound rubber while addition of polyphenols increased the color of compound rubber most significantly in all 3 rubber clones. Addition of TS buffer alone, and TS buffer with PPO did not increased the color of compound rubber. This result implies that increasing polyphenol content should react with chemicals in the compounding mixture and have remarkable effect on the color of compound rubber.

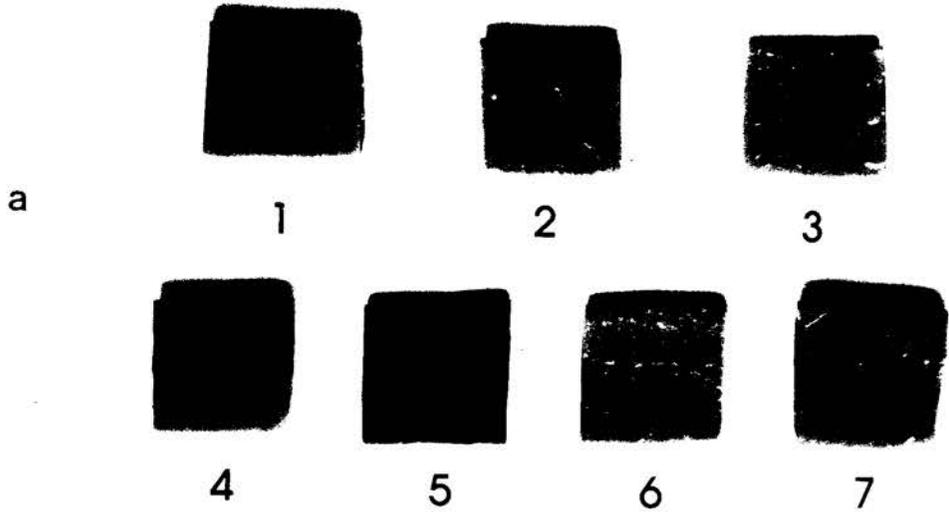
Figure 3.6 Comparison of color of compound rubbers

Raw rubber produced under various condition (200 g) was mixed according to the compounding formulation (2.16) and compared among 3 rubber clones :

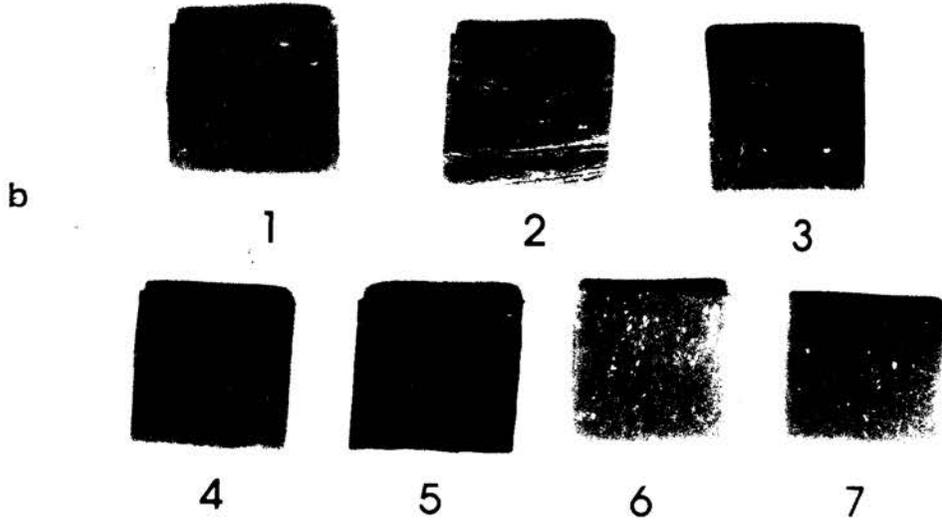
a) RRIM 600, b) GT 1 and c) PB 5/51

- where
- | | |
|------------------------|-----------------------------|
| 1. control | 2. - lipids and polyphenols |
| 3. + tocotrienols | 4. + carotenoids |
| 5. + polyphenols | 6. + TS buffer |
| 7. + Ts buffer and PPO | |

RRIM 600



GT 1



PB 5/51

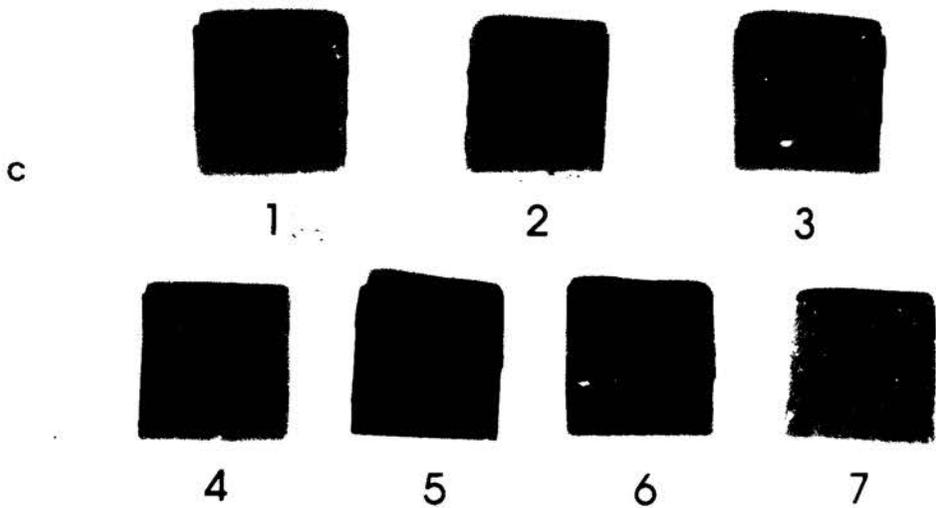
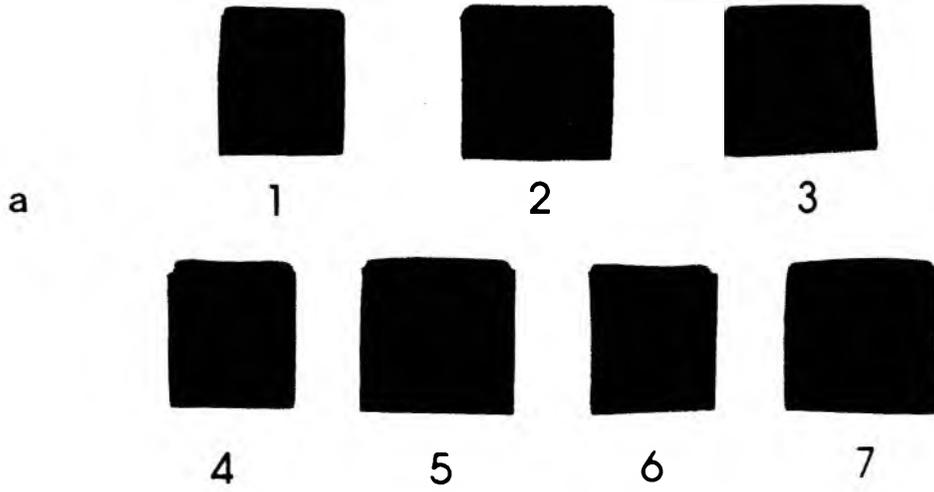


Figure 3.7 Comparison of color of vulcanized rubbers depleted rubber

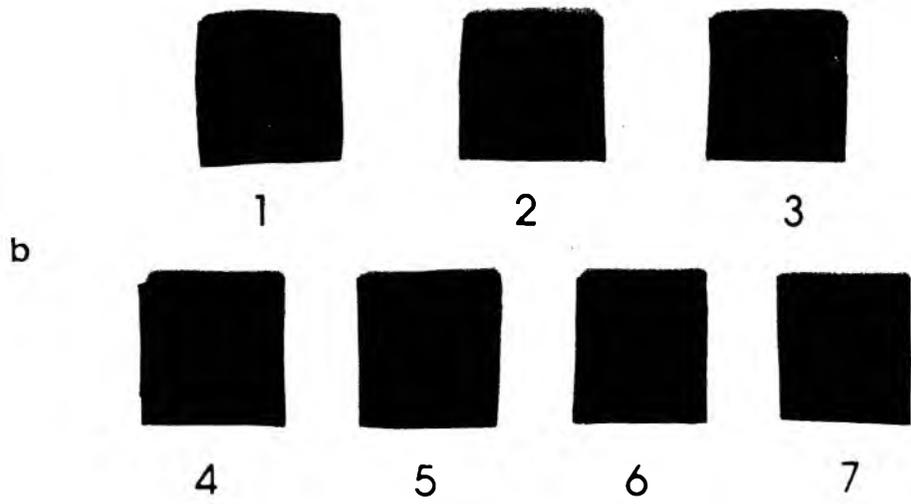
Compound rubber produced from raw rubber which produced under various condition was vulcanized to its optimum cure state, and compared among 3 rubber clones :

- a) RRIM 600, b) GT 1 and c) PB 5/51
- where
- | | |
|------------------------|-----------------------------|
| 1. control | 2. - lipids and polyphenols |
| 3. + tocotrienols | 4. + carotenoids |
| 5. + polyphenols | 6. + TS buffer |
| 7. + Ts buffer and PPO | |

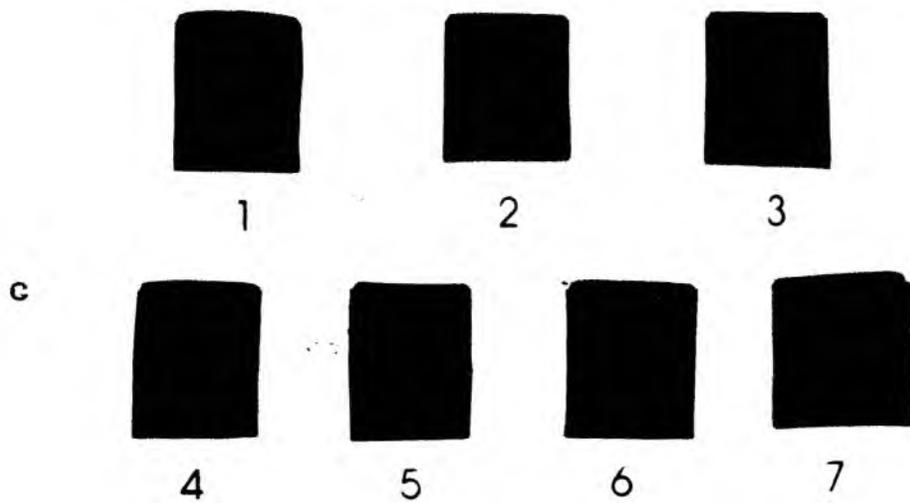
RRIM 600



GT 1



PB 5/51



Vulcanization of compound rubbers give rise to vulcanizates of higher degree in color than the corresponding compound rubber, as shown in Figure 3.6. Removal of total lipids including polyphenols does not have any effect on the color of vulcanizate comparing with untreated vulcanizate. Addition of tocotrienols and carotenoids also do not have any effect on the color of vulcanizate, but addition of polyphenols increase the color most significantly in all 3 rubber clones. By adding TS buffer alone, the vulcanizate show darker color than control vulcanizate, but less than polyphenol-added vulcanizate. However, addition of PPO does not increased the color of vulcanizate comparing with vulcanizate prepared from latex plus TS buffer. This result suggests that polyphenols and TS buffer have a profounding effect on the color of vulcanized rubber, but depletion of all the lipids and polyphenols did not help much in improving the color of vulcanizate. This experiment is designed to extrapolate how these varyfying indicators affect the color of raw rubber, compound rubber and finally vulcanized rubbers to understand not only the cause but also the mechanism of discoloration. Addition of polyphenol has the most remarkable effect on the color of raw rubber in which moderate temperature (50-60°C) has been used for drying, and the intensity of black color has increased with high temperature ($\approx 150^\circ\text{C}$) in the vulcanization. TS buffer has a profounding effect only on the color of vulcanizate or enhanced by high temperature. Removal of these varyfying indicators except proteins by chloroform-methanol extraction did not improve much the color of compound and vulcanized rubber.

3.7 The effect of decreasing total lipids including polyphenols, and increasing tocotrienols, carotenoids, polyphenols and polyphenol oxidase on Mooney viscosity of raw rubber

Besides discoloration, the effect of these verifying indicators on Mooney viscosity has been investigated as shown in Table 3.5 and 3.6 as % difference in Mooney viscosity number (± 4 ML (1+4) 100°C) from control untreated raw rubber.

It is clearly demonstrated that removal of total lipids which also include polyphenols leads to reduction in the Mooney viscosity (about 10-30%) in all 3 rubber clones. Addition of tocotrienols, carotenoids and polyphenols sometimes reduced the viscosity and sometimes increase the viscosity to a less extent ($\pm 1-14\%$). However, the Mooney viscosity has been affected significantly by the addition of 0.08 M Tris-HCl buffer containing 1.0 M sucrose. About 10-30% reduction in the Mooney viscosity has been observed, where merely 1.0 M sucrose 0.08 M Tris-buffer has been added, and adding both PPO and buffer have result in more or less no difference from buffer alone.

Since Mooney viscosity is the physical property of rubber particles per se, these results indicate that some non-rubber constituents extracted by chloroform: methanol (2:1, v/v) have an effect on the Mooney viscosity. However, these are not tocotrienols, carotenoids, polyphenol and PPO. The reduction of Mooney viscosity affected by TS buffer may be due to sucrose existed in the buffer.

Table 3.5 Mooney viscosity of dired rubber after various treatments and per cent difference in viscosity number compared with control untreated rubber

Rubber clone	Mooney viscosity of raw rubber, ML(1+4)100°C (+%Difference)				
	Control	-Total lipids and polyphenols	+Tocotrienols	+Carotenoids	+Polyphenols
RRIM 600	51.9	42.9 (-17)	54.2 (+4)	56.8 (+9)	58.9 (+14)
	70.1	56.6 (-19)	69.7 (-1)	67.9 (-3)	71.5 (+ 2)
GT 1	79.9	72.8 (- 9)	80.9 (+1)	83.6 (+5)	80.6 (+ 1)
	80.7	60.0 (-26)	78.1 (-3)	79.4 (-2)	73.0 (-10)
PB 5/51	87.6	61.0 (-30)	86.9 (-1)	88.4 (+1)	88.5 (+ 1)
	90.4	77.5 (-14)	94.2 (+4)	91.2 (+1)	89.2 (- 1)

Table 3.6 Mooney viscosity of dried rubber before and after addition of PPO

Rubber clone	Mooney viscosity of raw rubber (ML(1+4)100°C) (+%Difference)				
	Before treatment - TS buffer	After treatment			
		+ TS buffer	+ TS buffer and PPO		
RRIM 600	51.9	37.5	(-28)	40.1	(-21)
	70.1	55.2	(-21)	58.5	(-15)
GT 1	79.9	64.3	(-20)	63.3	(-21)
	80.7	67.0	(-17)	58.5	(-28)
PB 5/51	87.6	79.4	(- 9)	78.2	(-11)
	90.4	81.8	(- 9)	80.6	(-11)

3.8 The effect of decreasing total lipids including polyphenols, and increasing tocotrienols, carotenoids, polyphenols and PPO on cure characteristics of raw rubber

Other physical property investigated are cure characteristics which indicate the essential data such as scorch time, cure time and rate of cure used in converting compound rubber to vulcanizate. In order to emphasize the effect of each verifying indicator on cure characteristics, air dried rubber added with each verifying indicator, extracted rubber and control untreated rubber were mixed with the vulcanizing ingredients, left for 24 h and then curing behaviors of the compound rubbers are determined.

Cure characteristics of control untreated rubber and of treated rubber are shown in Figure 3.8-3.12. The minimum torque (ML) and the maximum torque (MH) of control rubber in all clones tested range from 14.0-16.6 and 3.4-3.8 min. In case of the cure rate PB 5/51 shows the highest value at 0.56 in lb./min, followed by RRIM 600 (0.53) and GT 1 (0.45) respectively. Removal of total lipids including polyphenols has no drastic effect on the ML of all clones tested. The reduction sometimes decrease the MH and the T_s , and sometimes increased the MH and the T_s comparing with their controls. In case of T90 and cure rate ($T_{90}-T_s$), lipid-depleted rubbers show a trend to increase these values, however these differences are very small. Addition of tocotrienol has less effect on the ML, T_s , $T_{90}-T_s$, but has a trend to decrease the MH of all 3 rubber clones. The effect of increasing carotenoids and polyphenols on cure characteristics is uncertain in all 3 rubber clones and most likely not significant. In case of PPO, rubber added with TS buffer shows a small decrease in cure time (T_{90}) about minus 11-23%

comparing with its control. Addition of PPO tends to reduce the M_f and cure rate ($T_{30}-T_5$) of all clones tested while has less effect on the other characters.

These results suggest that decreasing of total lipids which included polyphenols and increasing tocotrienols, carotenoids and polyphenols at mean + 2 S.D. values of each clone have no drastic effect on cure characteristics of all 3 rubber clones though in some case, these treatments slightly affect the cure characteristics. In case of adding TS buffer alone, the cure time of all 3 rubber clones become shorter than that of their control, where addition of PPO plus TS buffer result in no further change of the cure time but small reduction in cure rate.

Figure 3.8 Effect of decreasing total lipids including polyphenols on cure characteristics

(□ Control ; ▣ - Total lipids plus polyphenol)

a) Minimum torque (ML) and maximum torque (MH)

b) Scorch time (T_s) and cure time (T_{90})

c) Cure rate ($T_{90}-T_s$)

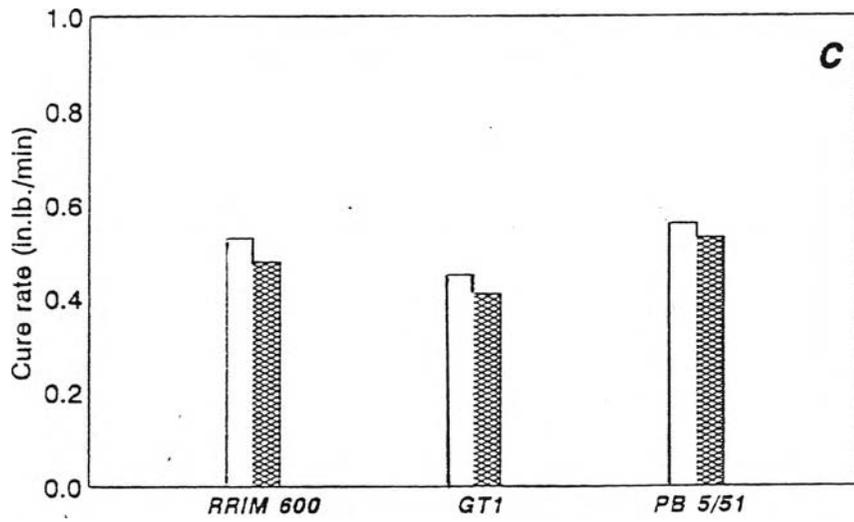
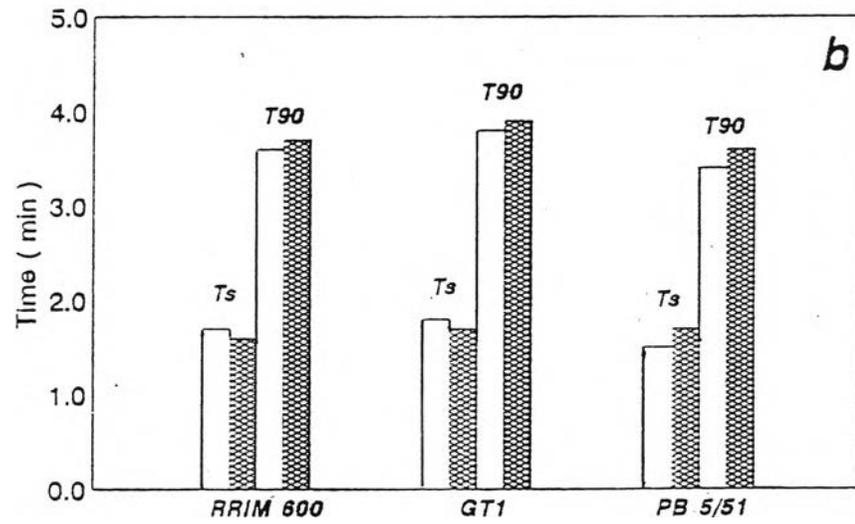
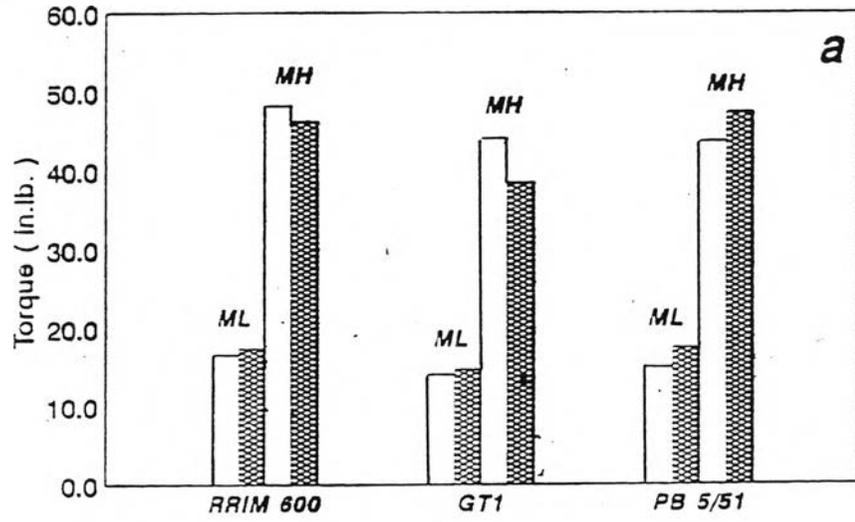


Figure 3.9 Effect of increasing tocotrienols on cure characteristics

(□ Control ; ⊠ + Tocotrienols)

- a) Minimum torque (ML) and maximum torque (MH)
- b) Scorch time (T_s) and cure time (T_{90})
- c) Cure rate ($T_{90}-T_s$)

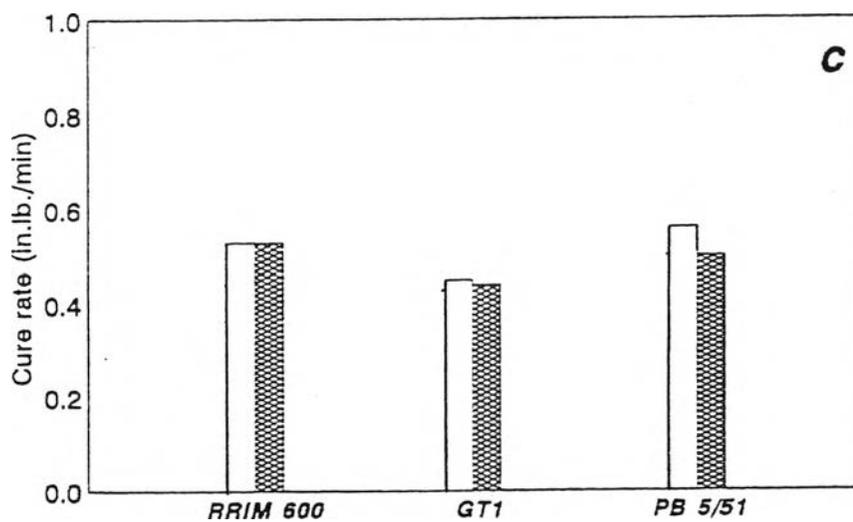
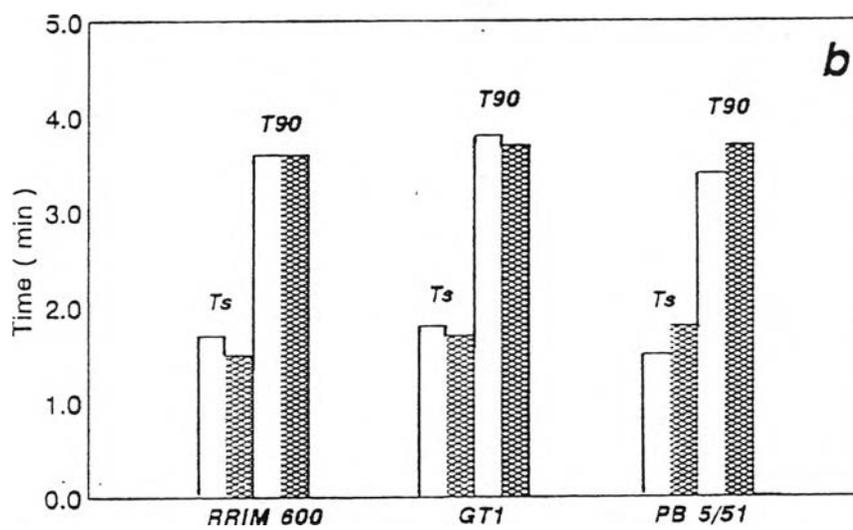
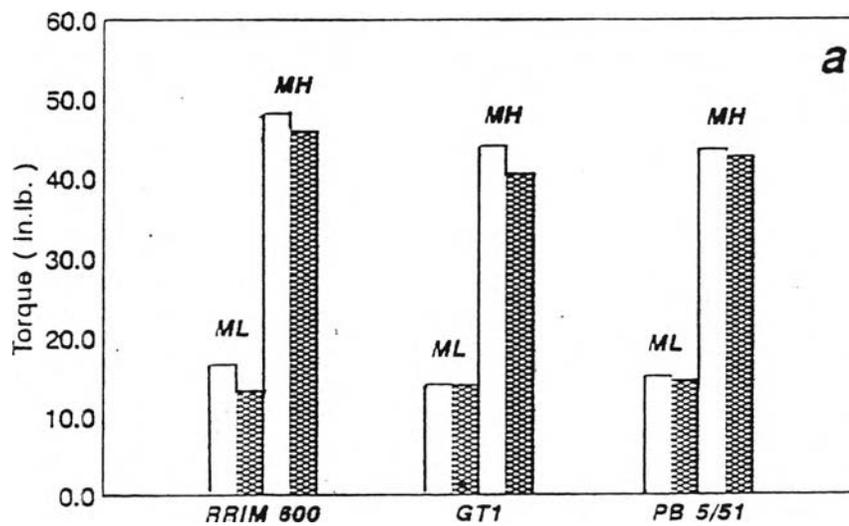


Figure 3.10 Effect of increasing carotenoids on cure characteristics

(□ Control ; ⊠ + Carotenoids)

- a) Minimum torque (ML) and maximum torque (MH)
- b) Scorch time (T_s) and cure time (T_{90})
- c) Cure rate ($T_{90}-T_s$)

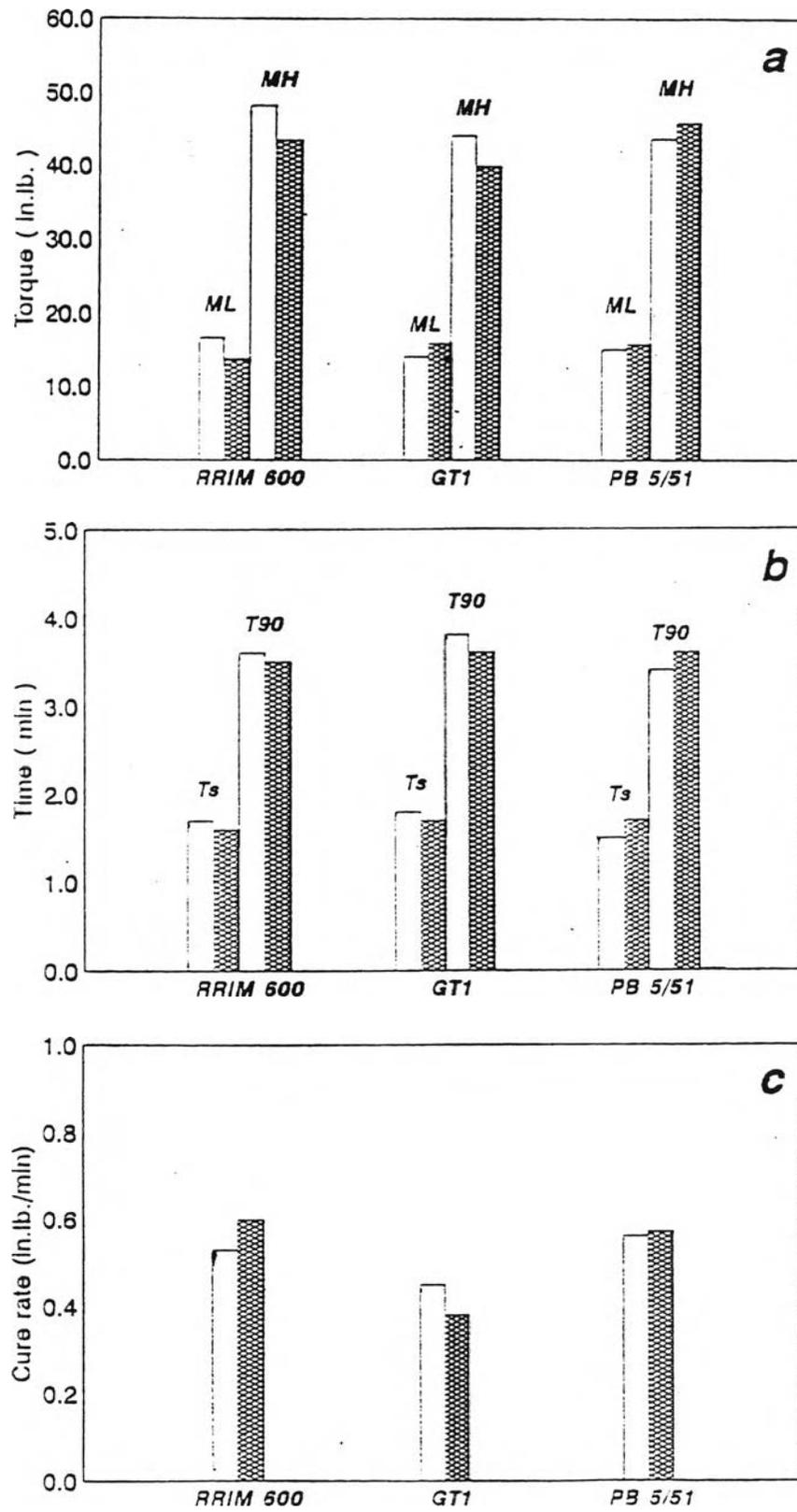


Figure 3.11 Effect of increasing polyphenols on cure characteristics

(□ Control ; ▣ + Polyphenols)

a) Minimum torque (ML) and maximum torque (MH)

b) Scorch time (T_s) and cure time (T_{30})

c) Cure rate ($T_{30}-T_s$)

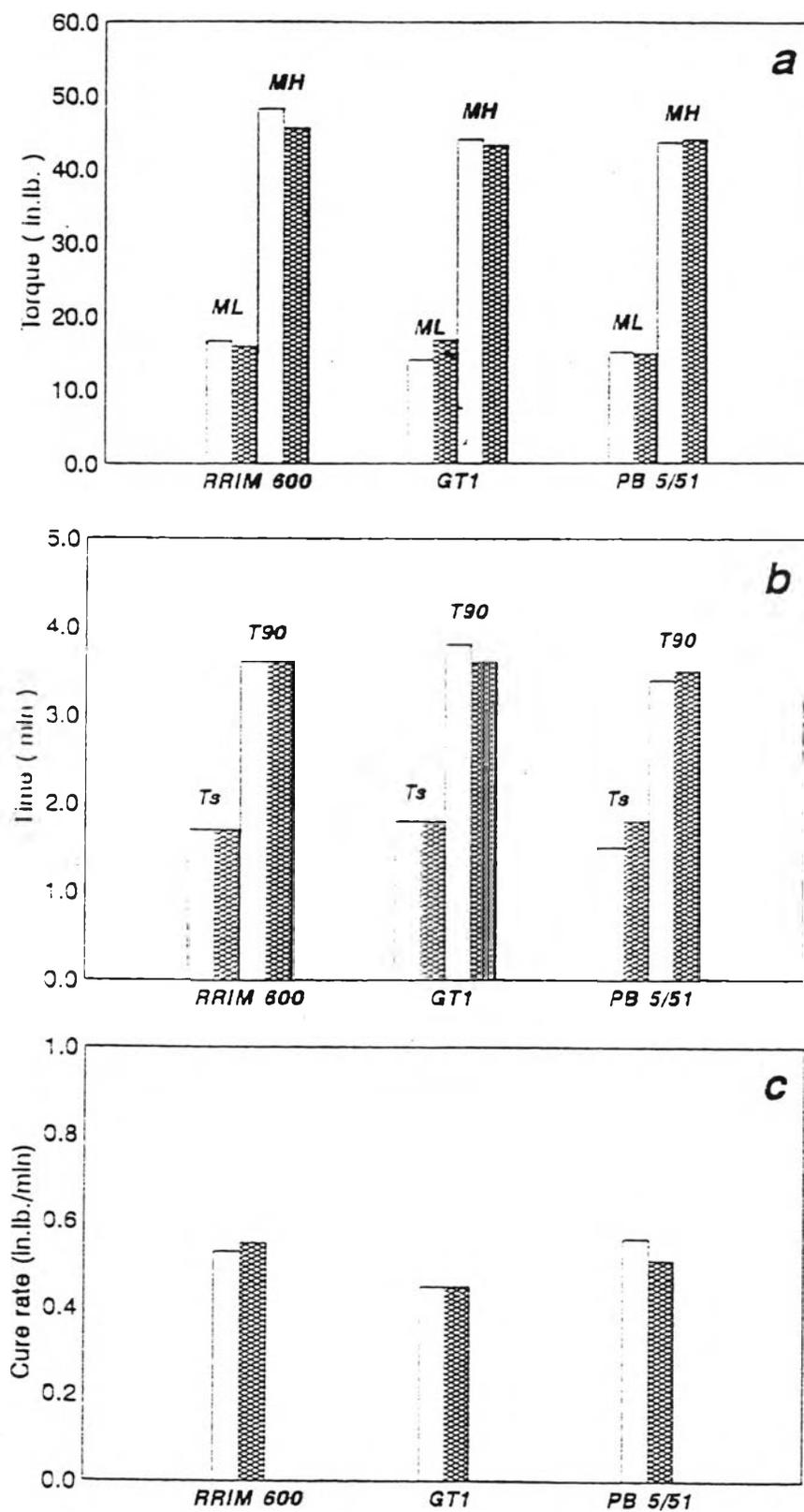


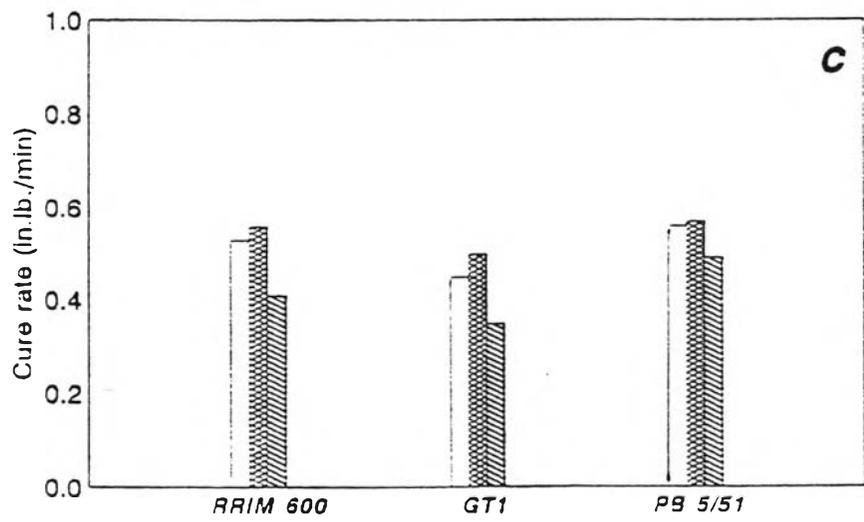
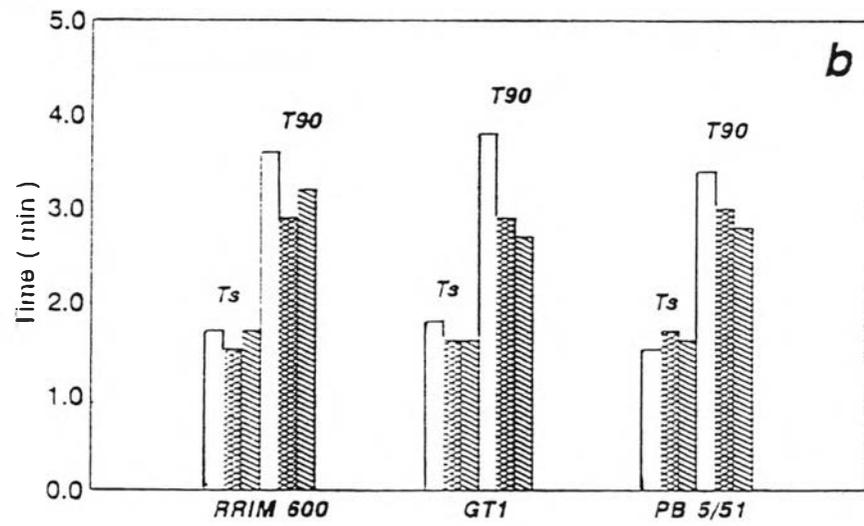
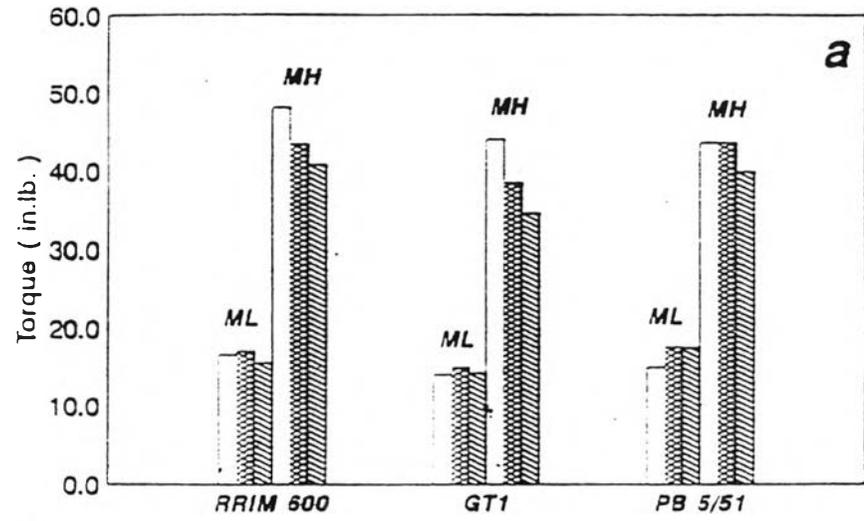
Figure 3.12 Effect of addition of TS buffer and polyphenols oxidase on cure characteristics

(□ Control ; ▣ + TS buffer; ▤ + TS buffer and PPO)

a) Minimum torque (ML) and maximum torque (MH)

b) Scorch time (T_s) and cure time (T_{90})

c) Cure rate ($T_{90}-T_s$)



3.9 The effect of decreasing total lipids including polyphenols and increasing tocotrienols, carotenoids, polyphenol and PPO on physical properties of vulcanized rubber

In order to understand the effect of each verifying indicator on the physical properties of rubber vulcanizates which usually are the final products in rubber industry, 200 g of raw rubber from each treatment is mixed with vulcanizing ingredients to be compound, and cured to its optimum state as analyzed previously before preparing the test piece.

Physical properties measured are 300% modulus, elongation at break, tensile strength, tear strength, hardness and specific gravity. Removal of total lipids including polyphenols did not change the 300% modulus (16.7–20.4 kg/cm²), hardness (55–56 shore A) and specific gravity (1.13–1.14) of vulcanizate. The elongation at break, tear strength and tensile strength decreased with total lipid plus polyphenols extraction. RRIM 600 and PB 5/51 are most affected from extraction, and the high tear strength and tensile strength decreased to the same level as GT 1. Addition of tocotrienols which consist about 4% of total lipids and play important role as natural antioxidant cannot improve any physical properties of the vulcanizate, but decrease the tear strength and tensile strength of the vulcanizate, especially RRIM 600. Addition of carotenoids which consist only 0.003% of total lipids results in reduction of tensile strength (17–25%) and tear strength (15–50% at the same % reduction as in addition of tocotrienols, and reduces elongation at break in vulcanizate clone RRIM 600 and GT 1 but not PB 5/51. Addition of polyphenol reduces most significantly tensile strength (18–36%) and tear strength (10–33%), but has less effect on the other properties. In case of adding TS buffer, it decreases tear

strength only in vulcanizate clone PB 5/51 but not RRIM 600 and GT 1. Addition of this buffer did not have an effect on the other properties. However, addition of PPO has an effect on tensile strength and tear strength which have been decreased significantly ranging from 28-38% and 26-63% respectively, while it has no effect on 300% modulus, elongation at break, hardness and specific gravity.

These results suggest that decreasing total lipids plus polyphenols and increasing of tocotrienols, carotenoids, polyphenols and PPO individually can disturb the tensile strength and tear strength of the vulcanizates. Of all 3 rubber clones tested GT 1 is less sensitive to these treatments where RRIM 600 is the most sensitive clone when subjected to variation of these verifying indicators followed by PB 5/51.

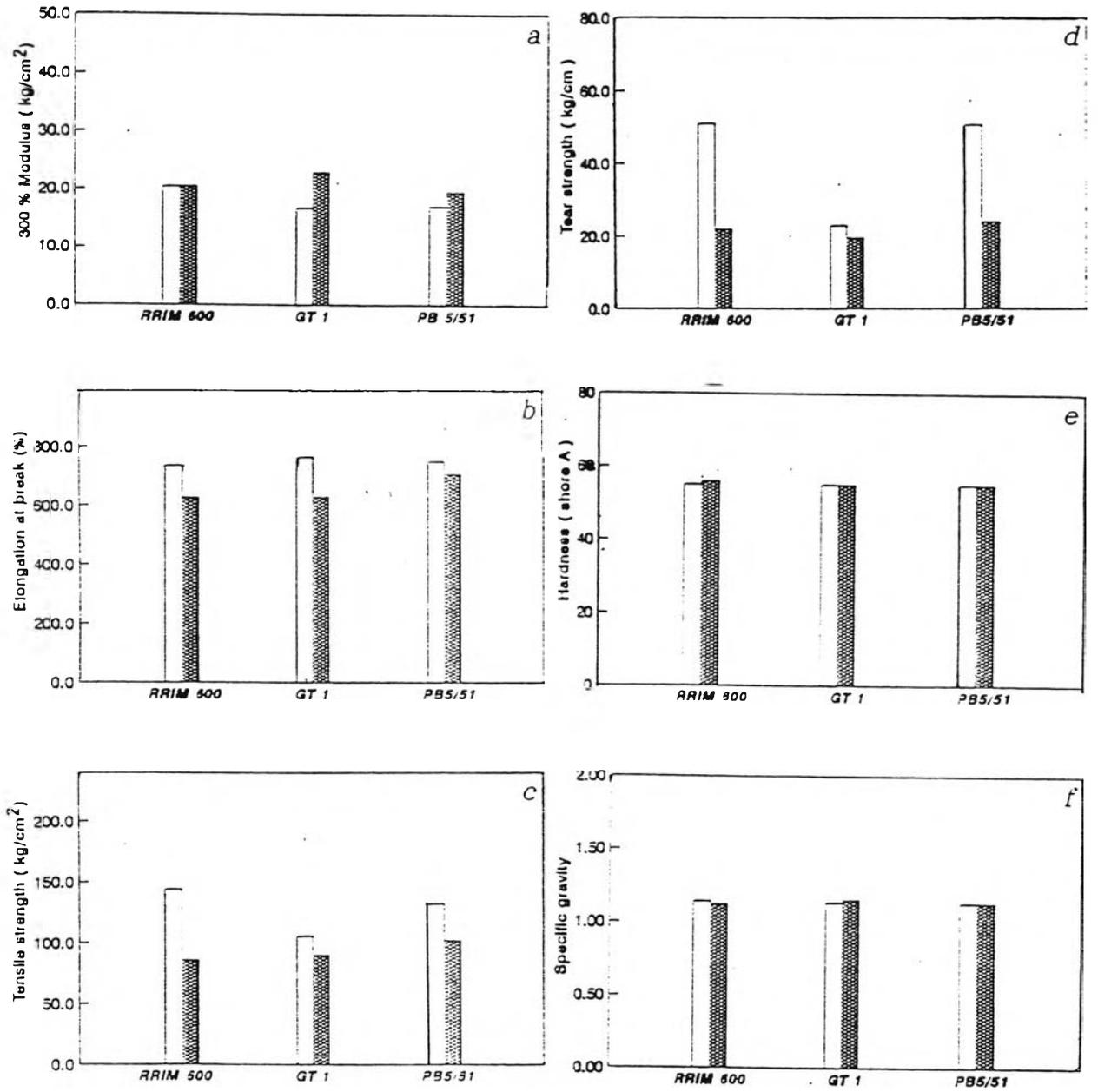


Figure 3.13 Effect of decreasing total lipids including polyphenols on some physical properties of vulcanized rubber

(□ Control ; ▨ Total lipids and polyphenol depleted)

a) 300% Modulus

d) Tear strength

b) Elongation at break

e) Hardness

c) Tensile strength

f) Specific gravity

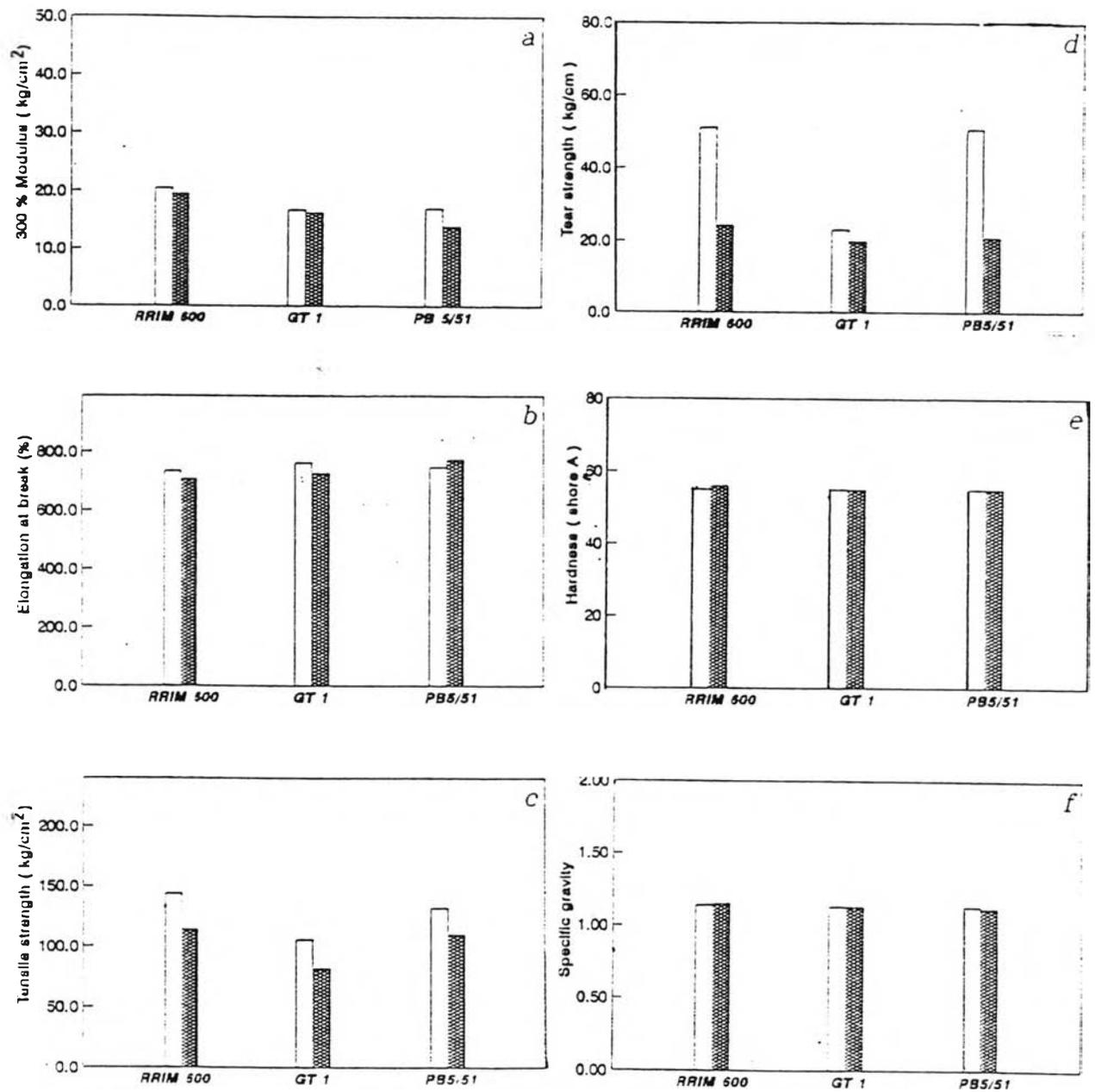


Figure 3.14 Effect of increasing tocotrienols on some physical properties of vulcanized rubber

(□ Control ; ▨ + Tocotrienols)

a) 300% Modulus

b) Elongation at break

c) Tensile strength

d) Tear strength

e) Hardness

f) Specific gravity

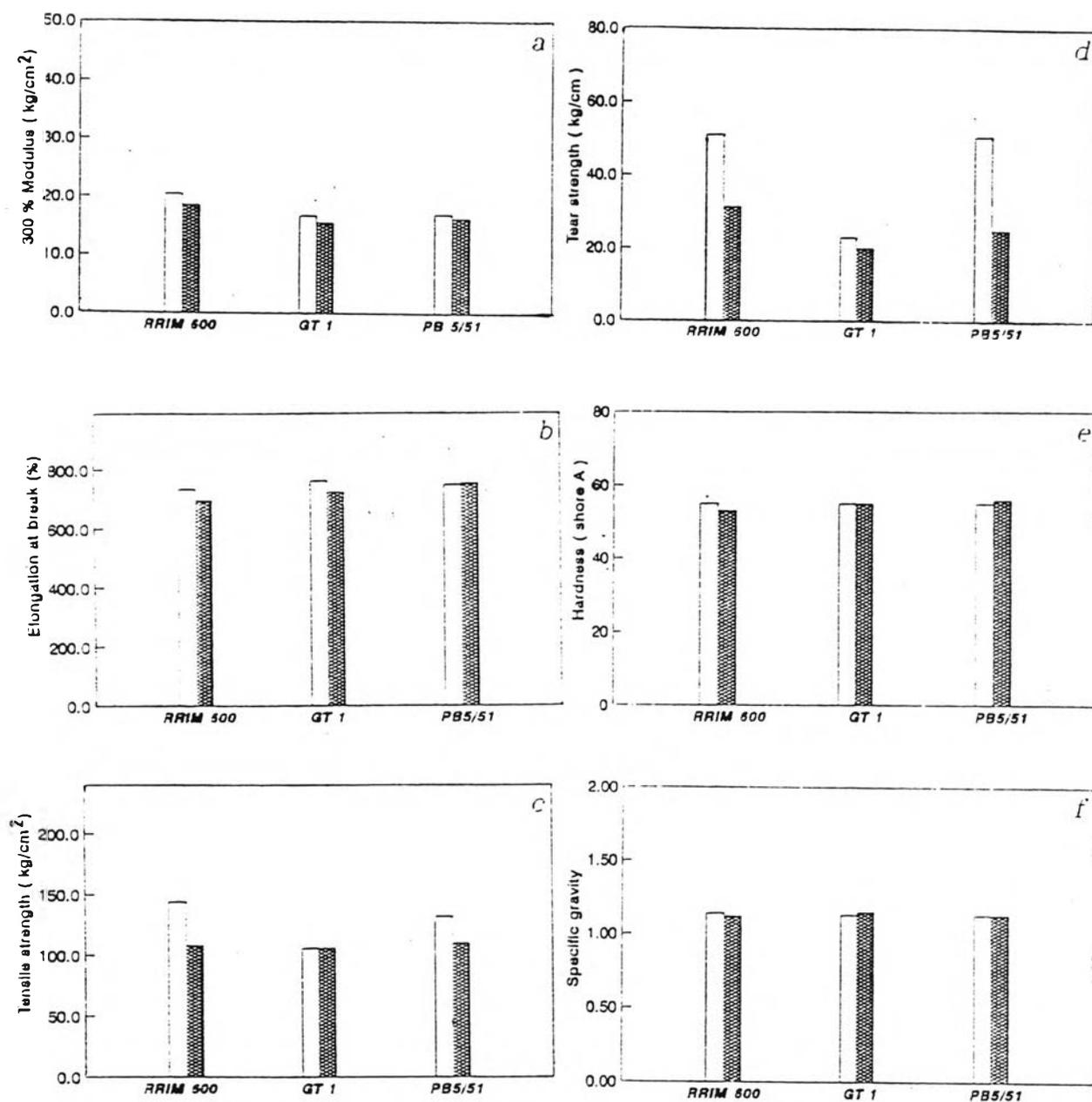


Figure 3.15 Effect of increasing carotenoids on some physical properties of vulcanized rubber

(□ Control ; ▨ + Carotenoids)

a) 300% Modulus

b) Elongation at break

c) Tensile strength

d) Tear strength

e) Hardness

f) Specific gravity

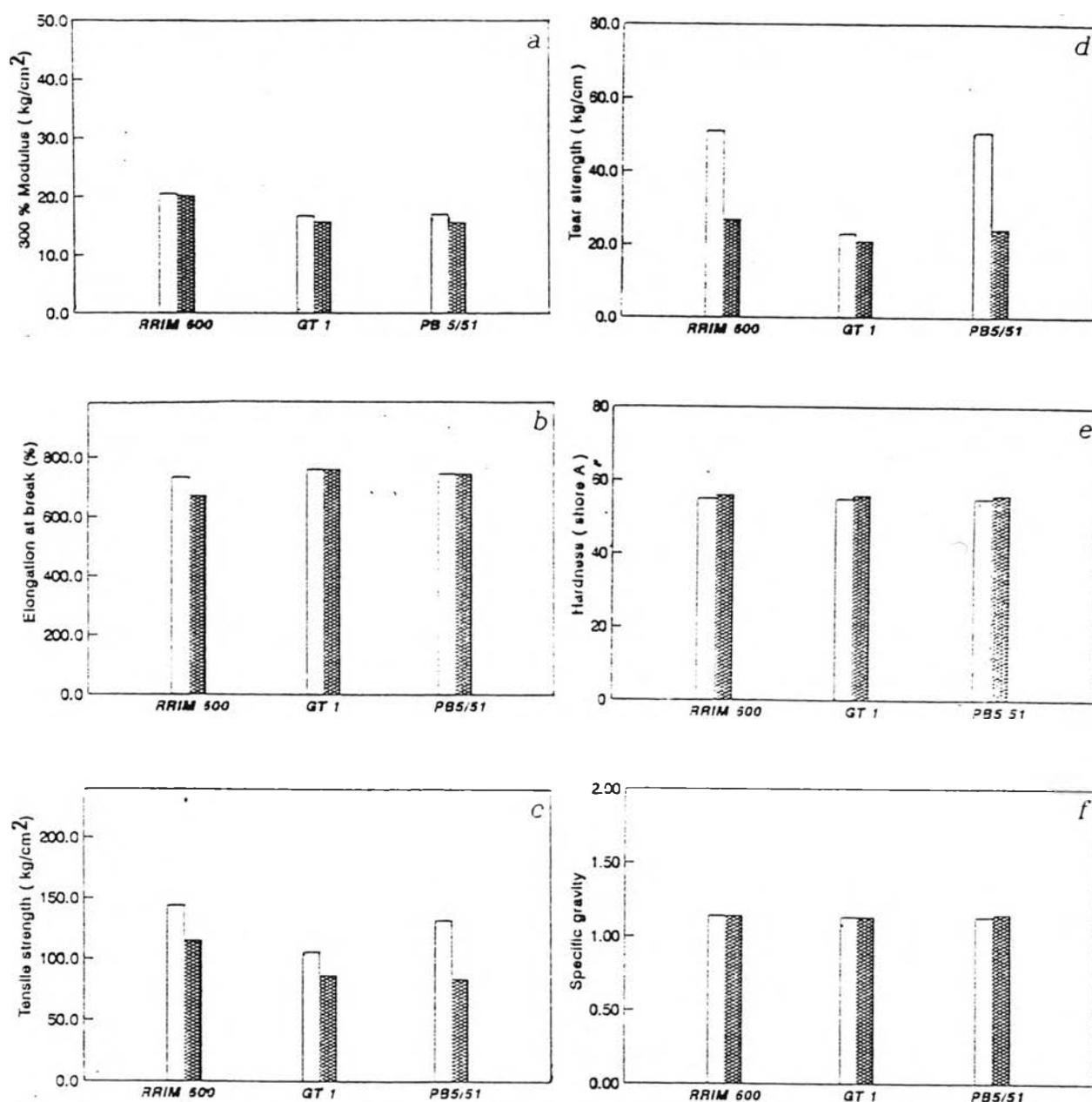


Figure 3.16 Effect of increasing polyphenol on some physical properties of vulcanized rubber

(□ Control ; ▨ + Polyphenols)

a) 300% Modulus

b) Elongation at break

c) Tensile strength

d) Tear strength

e) Hardness

f) Specific gravity

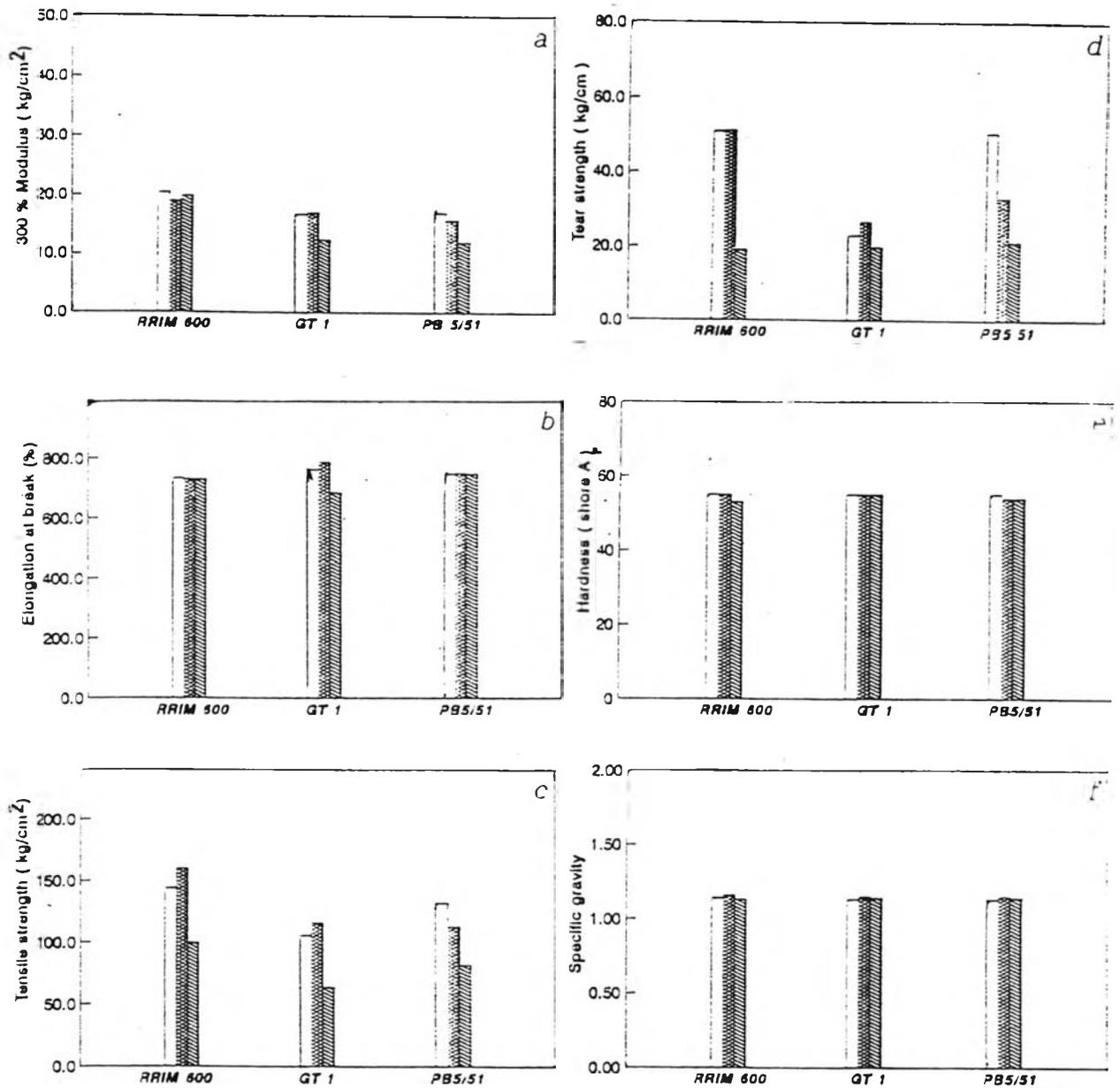


Figure 3.17 Effect addition of TS buffer and polyphenol oxidase on some physical properties of vulcanized rubber

(□ Control ; ▨ + TS buffer; ▩ + TS buffer and PPO)

a) 300% Modulus

d) Tear strength

b) Elongation at break

e) Hardness

c) Tensile strength

f) Specific gravity