CHAPTER III

RESULTS

3.1 Optimum condition for enzyme activity with casein substrate

Papain and Alcalase T are proteolytic enzymes chosen for latex deproteinization. Preliminary study using casein as substrate indicates that the specific activity of Alcalase is 300.3 CDU/mg in standard assay condition of Te Whaiti's method (1978), pH 7.6 of 0.05 M Tris-HCl, 45°C for 20 min,and Papain is 410.0 CDU/mg at specified standard condition at pH 6.0 of phosphate-cysteine EDTA buffer, 40°C for 60 min. Alcalase shows the highest specific activity in the pH range 9.4-10.0, at temperature range 55-60°C (Figure 3.1 a, b), whereas Papain shows the highest specific activity in lower pH range 6.5-7.0 and similar optimum temperature 50-60°C (Figure 3.2 a, b).

3.2 Optimum condition for deproteinization of concentrated latex

Concentrated latex is firstly used because it is convenient to obtain the material all year round and some serum proteins had been already removed during centrifugation, so that the initial total nitrogen is about 0.19-0.22 g%. Since the pH of concentrated latex (0.7 % NH_3) is about 10, the first step is to remove ammonia by evaporation until the pH drop to the desired pH range and diluted to 25% DRC. The effect of pH on

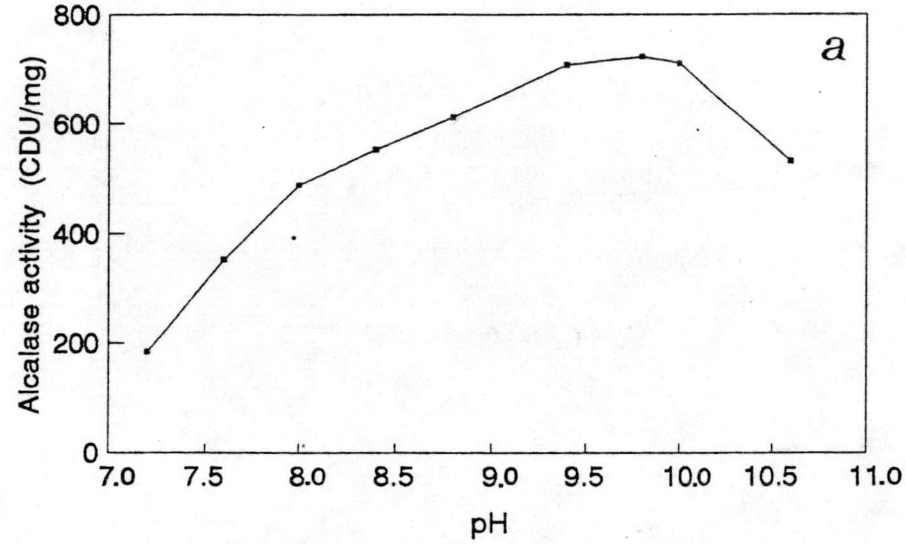
Figure 3.1 Optimum condition for Alcalase activity

a) The effect of pH on Alcalase activity

Alcalase activity was determined with casein substrate at various pH ; 7.2-9.0 in 0.05 M Tris-buffer,and 8.6-10.0 in 0.025 M Glycine buffer at 55°C for 20 minutes.

b) The effect of temperature on Alcalase activity

Alcalase activity was determined with casein substrate in 0.05 M Tris-HCl buffer pH 7.6 at various temperatures from 25-70°C for 20 minutes.


a
Alcalase activity (CDU/mg)
800
600
400
200
0
7.0
7.5
8.0
8.5
9.0
9.5
10.0
10.5
11.0
pH

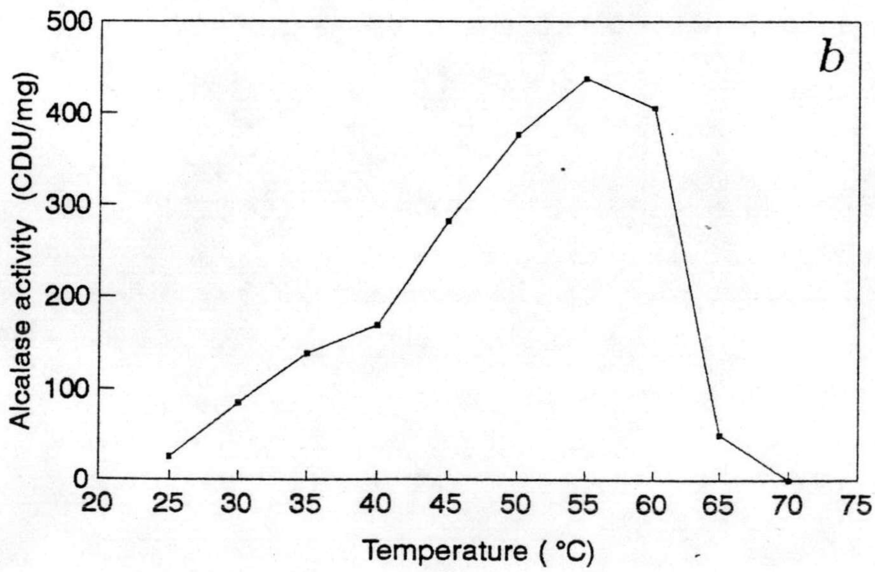

b
Alcalase activity (CDU/mg)
500
400
300
200
100
0
20
25
30
35
40
45
50
55
60
65
70
75
Temperature (°C)

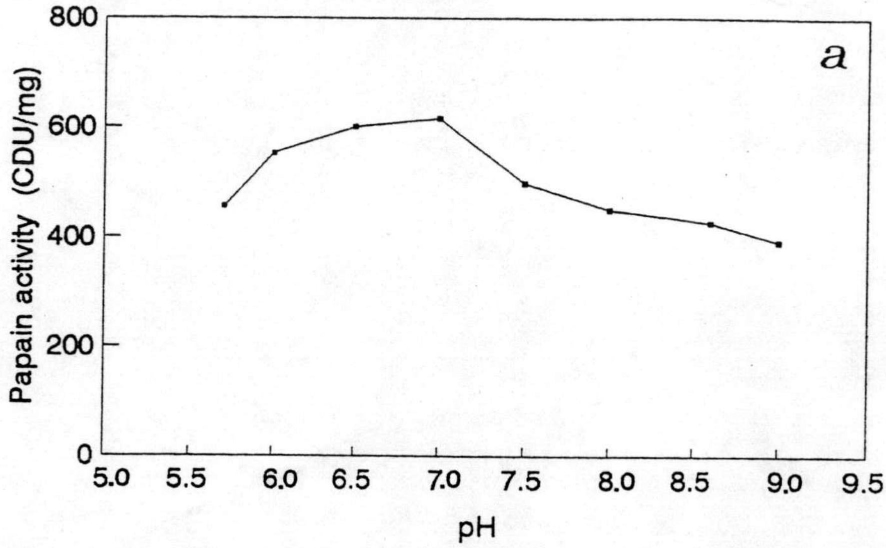
Figure 3.2 Optimum condition for Papain activity

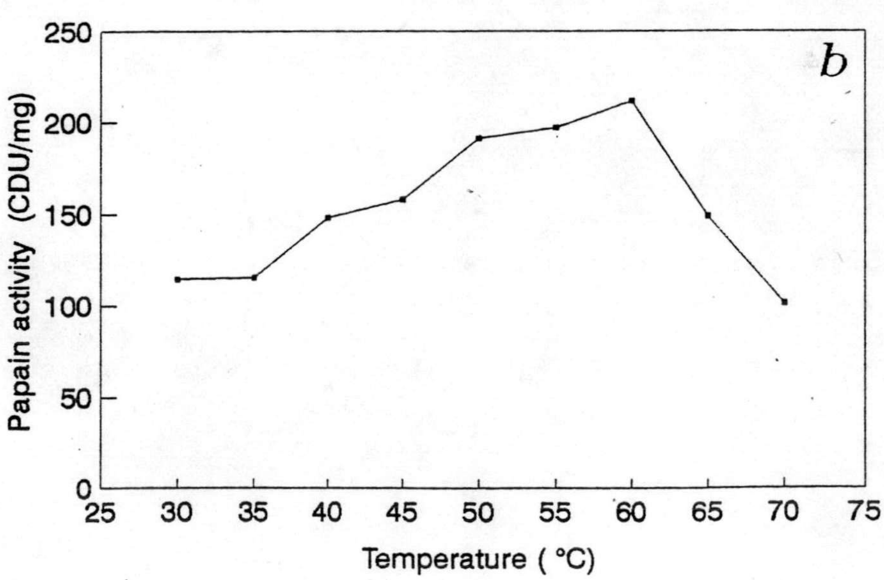
a) The effect of pH on Papain activity

Papain activity was determined with casein substrate at various pH ; 5.7-8.0 in 0.05 M phosphate buffer, and 8.6-9.0 in 0.05 M Glycine buffer at 45°C for 20 minutes.

b) The effect of temperature on Papain activity

Papain activity was determined by incubating with casein substrate in 0.05 M phosphate buffer pH 6.0 at various temperatures from 30-70°C for 20 minutes.


a
Papain activity (CDU/mg)
800
600
400
200
0
5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5
pH


b
Papain activity (CDU/mg)
250
200
150
100
50
0
25 30 35 40 45 50 55 60 65 70 75
Temperature (°C)

latex deproteinization by Alcalase has been monitored by measuring per cent total nitrogen (g%) retained in the resulted raw rubber, in which pH 8-9 has been found to yield the lowest nitrogen content (0.060 g%) after 6 h. (Figure 3.3 a). The optimum temperature is between 40-60°C (Figure 3.3 b) and the Alcalase concentration is 0.3 p.h.r. (Figure 3.3 c) respectively.

Deproteinization of concentrated latex 60% by Papain has been investigated at pH ranging from 8-11 as shown in Figure 3.4. The lowest per cent nitrogen content 0.15 g% obtained with pH range 8-9 is significantly higher than deproteinization with Alcalase (0.060 g%). Besides at pH below 8, coagulation of rubber has been observed, so that no further investigation has been performed with Papain on concentrated latex 60%.

To evaluate the deproteinization of concentrated latex 60% by Alcalase at optimal condition which are pH 8-9,Alcalase 0.3 p.h.r.,at 40-50 °C for 10 h (Figure 3.5), the critical step yielding reduction in %N, and retaining of the good processibility properties of solid rubber , another two parameters are measured,"Initial Plasticity" (Po) and "Plasticity Retention Index" (PRI). After ammonia evaporation at increasing temperature (50-70°C). Table 3.1 suggests that different temperature of evaporation has no effect on raw rubber processibility properties.

Figure 3.3 Optimum condition for depreteinization of concentrated latex 60%

The 25% DRC latex was prepared from concentrated latex 60% and then treated with Alcalase at verifying condition. The digested latex was withdrawn at time intervals and coagulated with 2% composite mixture of acid. Total nitrogen of dry coagulum was determined.

a) The effect of pH on latex deproteinization

Treatment of latex at various pH was performed at fixed Alcalase concentration of 0.3 p.h.r. at 50°C.

—•— pH 8-9, —+— pH 9-10, —✳— pH 10-11

b) The effect of temperature on latex deproteinization

The latex was prepared in the optimal pH range and then treated with Alcalase 0.3 p.h.r. at various temperatures.

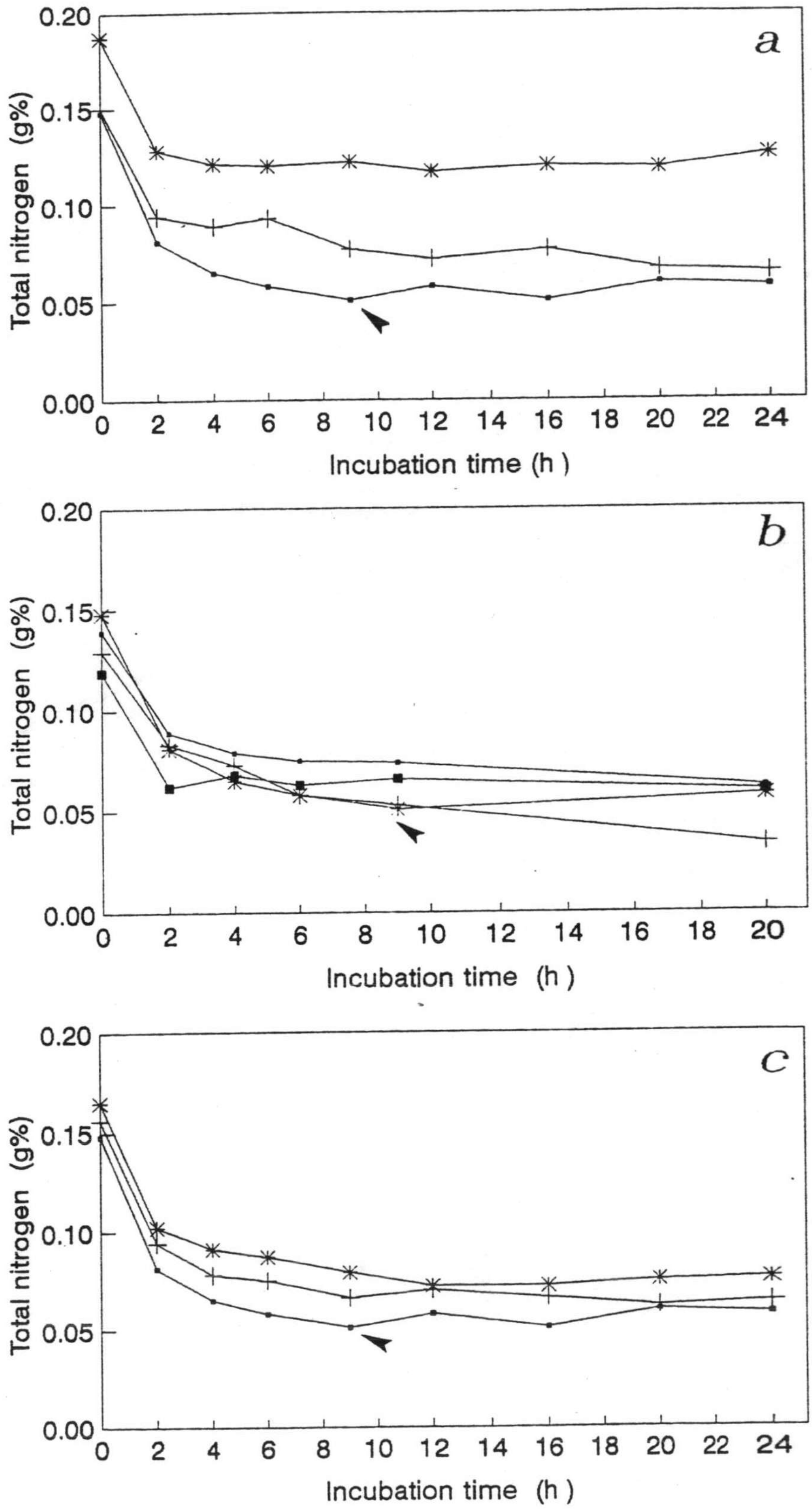
—•— at 30°C, —+— at 40°C

—✳— at 50°C, —■— at 60°C

c) The effect of Alcalase concentration on latex deproteinization

The latex was prepared in the optimal pH 8-9 and then treated with Alcalase at various concentrations at 50°C

—■— 0.3 p.h.r., —+— 0.2 p.h.r., —✳— 0.1 p.h.r.


a
Total nitrogen (g%)
0.20
0.15
0.10
0.05
0.00
0 2 4 6 8 10 12 14 16 18 20 22 24
Incubation time (h)
b
Total nitrogen (g%)
0.20
0.15
0.10
0.05
0.00
0 2 4 6 8 10 12 14 16 18 20
Incubation time (h)
c
Total nitrogen (g%)
0.20
0.15
0.10
0.05
0.00
0 2 4 6 8 10 12 14 16 18 20 22 24
Incubation time (h)

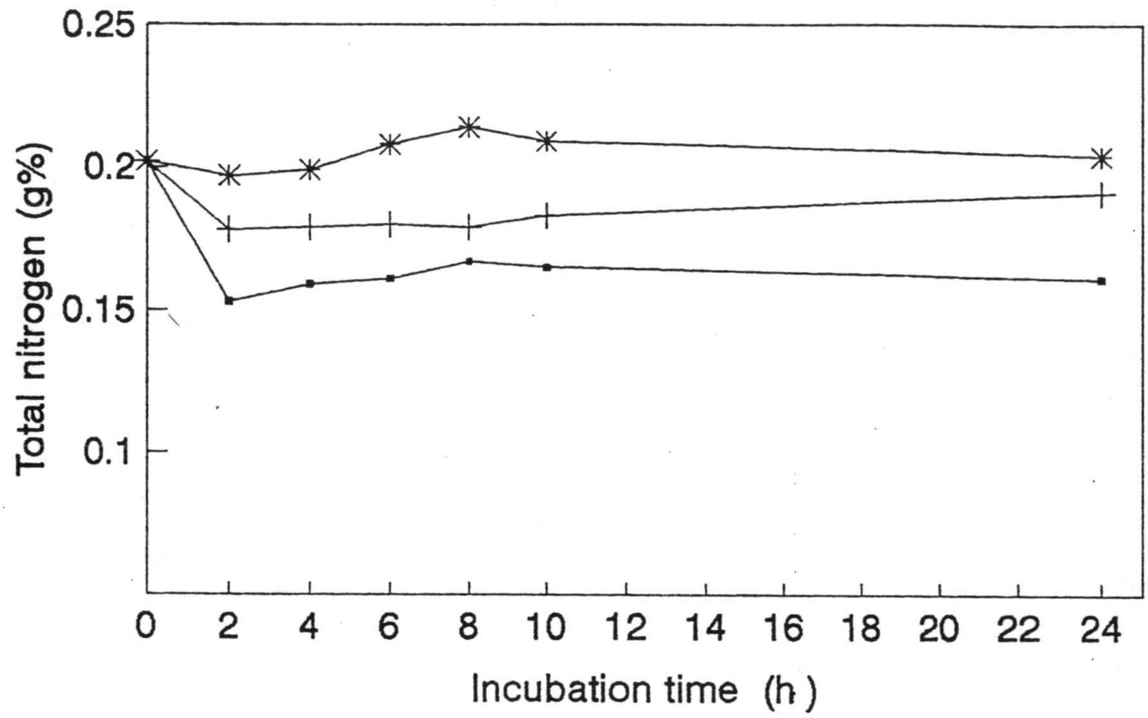


Figure 3.4 Effect of pH on concentrated latex deproteinization by Papain

Concentrated latex 60% was prepared as 25% DRC latex and then treated with Papain 0.3 p.h.r. at various pH at 50°C in a shaking waterbath. The nitrogen content of rubber was determined by semi-Kjeldahl method at time intervals.

—▪— pH 8-9, —+— pH 9-10, —✳— pH 10-11

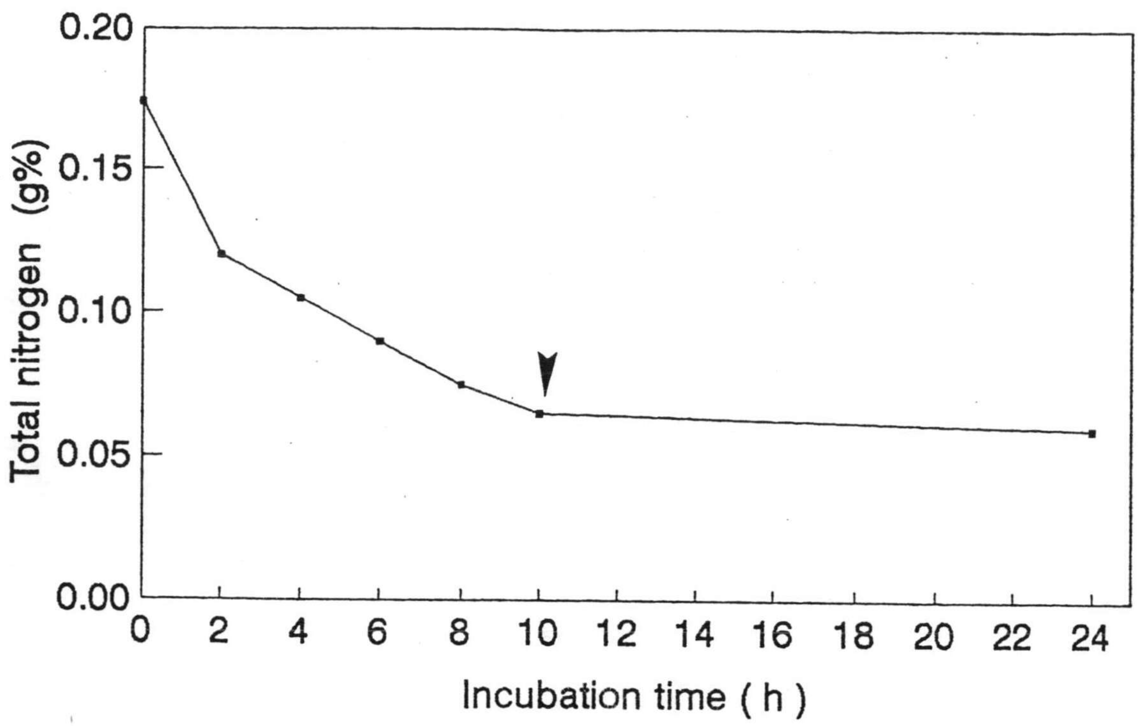


Figure 3.5 Optimal condition for concentrated latex deproteinization by Alcalase

Concentrated latex 60% was diluted to 25 % DRC and deproteinized by Alcalase 0.3 p.h.r. at pH 8-9, 40-50°C. The minimum nitrogen content of 0.06 g% was obtained after 10 h digestion.

Table 3.1 Effect of ammonia evaporation at various temperatures on raw rubber properties.

Concentrated latex 60% was evaporated at various temperatures in a shaking waterbath and withdrawn at time intervals to measure pH and prepare total solid film. The properties of thin film were analyzed.

Property	Control	At 50 °C	At 60 °C	At 70 °C
Total nitrogen (g%)	0.276	0.282	0.273	0.270
Initial plasticity (P_o)	73.3	71.8	72.0	73.3
Plasticity Retention Index (PRI)	76.1	77.6	76.2	76.1
Time (min)	-	120	60	40
Final pH	10.3	9.0	8.98	9.03

After Alcalase digestion, latex proteins have been hydrolyzed into small oligopeptides and soluble amino acids, which are readily soluble in water and easily removed from rubber particles by acid coagulation, therefore the dilution volume with water suitable for the process has been studied. Table 3.2 shows that the dilution ratio of latex : water = 1:5 is necessary to obtain the minimum %N of raw rubber. Coagulation of rubber by formic acid or composite mixture of sulfuric acid and phosphoric acid 1:1 resulted in the same amount of g%N reduction, but very low Po and zero PRI were obtained (Table 3.2) in experiment 1 and 2. These unacceptable values have been corrected to an approved level (minimum Po = 30, and PRI not less than 60) in experiment 3 and 4 by Thiourea treatment. Table 3.3 summarizes the change in raw rubber properties of raw rubber coagulated stepwise during the process. Significant reduction in %N occurs after dilution of digested latex (step F), and thiourea-treatment is necessary to obtain the specified Po and PRI.

The total process time required for deproteinization of concentrated latex 60% with Alcalase is about 35 h starting from ammonia evaporation at 60°C until the pH decrease to 8-9 and diluted to 25% DRC with adding 0.15 p.h.r. hydroxylamine hydrochloride and 0.05 p.h.r. sodium metabisulfite. Alcalase is then added to 0.3 p.h.r. and incubated with shaking at 40-50°C for 10 h. The latex is then diluted with 5 volumes of water and coagulated with 2% solution of 1:1 composite mixture of sulfuric and phosphoric acid. The coagulum is then washed and sheeted through a two-roll mill. The coagulum should be dipped in 0.2% thiourea solution before drying at 60°C for 24 h in an air-circulating oven.

Table 3.2 Effect of dilution and coagulant on raw rubber properties.

After enzyme treatment, the digested latex was diluted with various ratio of water and coagulated with either 2% formic acid or 2% composite mixture. The rubber properties of dry coagulum were determined. Exp. 1,2 represent for non-thiourea treatment;Exp. 3,4 for thiourea treatment

Exp.	Dilution ratio	2% Formic acid			2% Composite mixture		
		P_o	PRI	g%N	P_o	PRI	g%N
1	1:0	36.0	0	0.084	23.3	0	0.088
	1:2	25.3	0	0.063	20.2	0	0.078
	1:5	23.8	0	0.050	19.6	0	0.059
	1:10	19.4	0	0.052	21.3	0	0.060
2	1:0	43.1	0	0.085	34.3	0	0.094
	1:2	36.1	0	0.052	37.0	0	0.054
	1:5	36.3	0	0.055	35.8	0	0.054
	1:10	38.6	0	0.051	36.1	0	0.067
3	1:2	58.3	66.4	0.092	51.5	73.8	0.086
	1:5	59.9	62.4	0.082	58.3	64.2	0.074
	1:10	61.4	60.6	0.083	52.2	45.2	0.072
4	1:2	57.7	70.7	0.095	56.4	70.0	0.088
	1:5	58.5	66.7	0.076	56.4	67.7	0.085
	1:10	57.5	66.2	0.078	58.8	63.9	0.086

Table 3.3 Tracing the raw rubber properties along steps in DPNR production scheme.

Following each step in DPNR production scheme (Figure 2.1), the raw rubber prepared from dry coagulum as thin film were determined for Po, PRI,and total nitrogen (g%)

Treatment	Batch #1		
	P_o	PRI	g%N
A: Concentrated latex 60%	72.1	81.6	0.370
B: Ammonia evaporation at 60 ^oC ,1 h	76.4	77.5	0.366
C: Dilution to 25 %DRC with mixing additives	62.5	77.9	0.380
D: Alcalase addition at zero time	58.3	79.4	0.383
E: After incubation at 45 ^oC, 10 h	56.2	70.3	0.325
F: Dilution to 5% DRC Coagulation			
- Thiourea treatment	56.1	1.8	0.062
+ Thiourea treatment	61.4	73.0	0.069
Dried at 60 ^oC, 24 h			

3.3 Optimum condition for deproteinization of field latex

Fresh field latex differs from concentrated latex in its lower DRC (25-40%) but higher nitrogen content (0.4-0.7%). Normally variation of %DRC from 20-40% were reported, depending on several factors such as rubber clone, age of rubber tree, day of tapping, temperature, humidity etc., so that the %DRC of each lot must be determined and adjusted to 25% DRC before use. The advantage of using field latex as starting material is that the pH of field latex is in the range of 6-6.5 which is easier to adjust the pH of latex to the optimal pH of Papain or Alcalase. The disadvantage of using field latex is that the nitrogen content of starting material is higher than concentrated latex 60%. Besides there are so many soluble organic compounds in the serum and lutoid fractions which might interfere with the enzyme activity, so that higher concentration of enzyme could be required.

By using field latex, clone RRIM 600 as model, the effect of pH on deproteinization by Alcalase is monitored by determination of % Total nitrogen at various pH ranges (8-11), and it is shown in Figure 3.6 a that the highest % nitrogen reduction is obtained at pH 8-9. The optimal temperature is 60°C (Figure 3.6 b) and the optimal concentration of Alcalase is 0.4 p.h.r. (Figure 3.6 c) where the maximum 68% nitrogen reduction has been observed after 6 h.

In case of Papain, the optimum pH for deproteinization is between 7-8 (Figure 3.7 a) and optimal temperature is between 40-60°C (Figure 3.7 b) and suitable Papain concentration is 0.3 p.h.r. in order to reach 67% nitrogen reduction within 2 h (Table 3.7 c). Although there are variation in the initial nitrogen content in each lot of fresh field latex and result in different amount of per cent nitrogen after enzyme

Figure 3.6 Optimal condition for deproteinization of fresh field latex

Fresh field latex was prepared to 25% DRC latex and then treated with Alcalase at varyfying conditions, pH, temperature and concentration. The latex was withdrawn at time intervals and coagulated with steam and determined for total nitrogen retaining in the rubber. Nitrogen reduction was calculated, comparing with the started total nitrogen of non-treated rubber.

a) The effect of pH or latex deproteinization

—•— pH 8-9, —+— pH 9-10, —✳— pH 10-11

b) The effect of temperature on latex deproteinization

—+— at 40°C, —✳— at 50°C, —■— at 60°C

c) The effect of Alcalase concentration on latex deproteinization

—•— 0.2 p.h.r., —+— 0.3 p.h.r., —✳— 0.4 p.h.r.

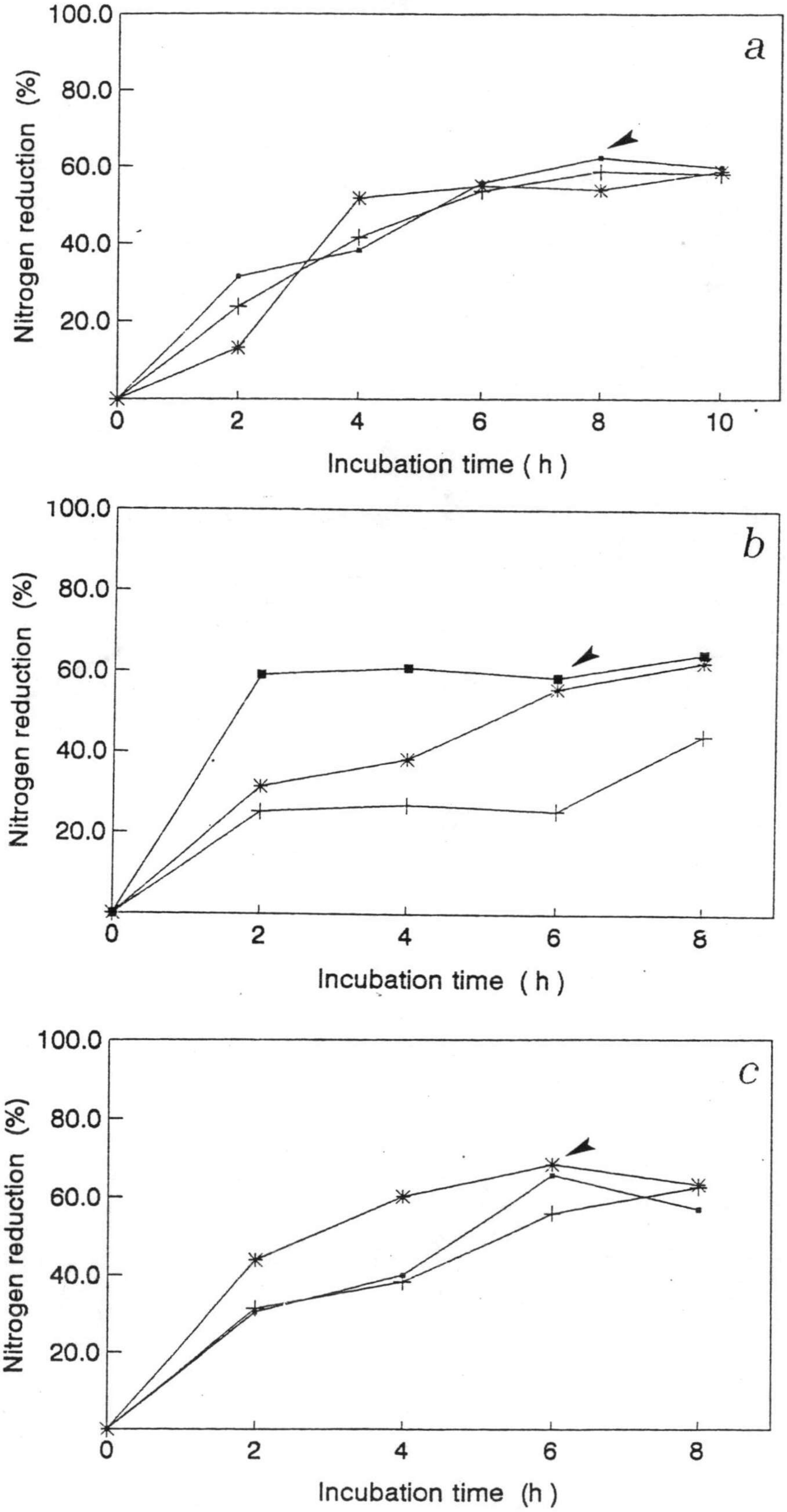

a
Nitrogen reduction (%)
100.0
80.0
60.0
40.0
20.0
0
2
4
6
8
10
Incubation time (h)
b
Nitrogen reduction (%)
100.0
80.0
60.0
40.0
20.0
0
2
4
6
8
Incubation time (h)
c
Nitrogen reduction (%)
100.0
80.0
60.0
40.0
20.0
0
2
4
6
8
Incubation time (h)

Figure 3.7 Optimization of fresh field latex deproteinization by Papain

a) The effect of pH on latex deproteinization

—•— pH 7-8, —+— pH 8-9, —*— pH 9-10

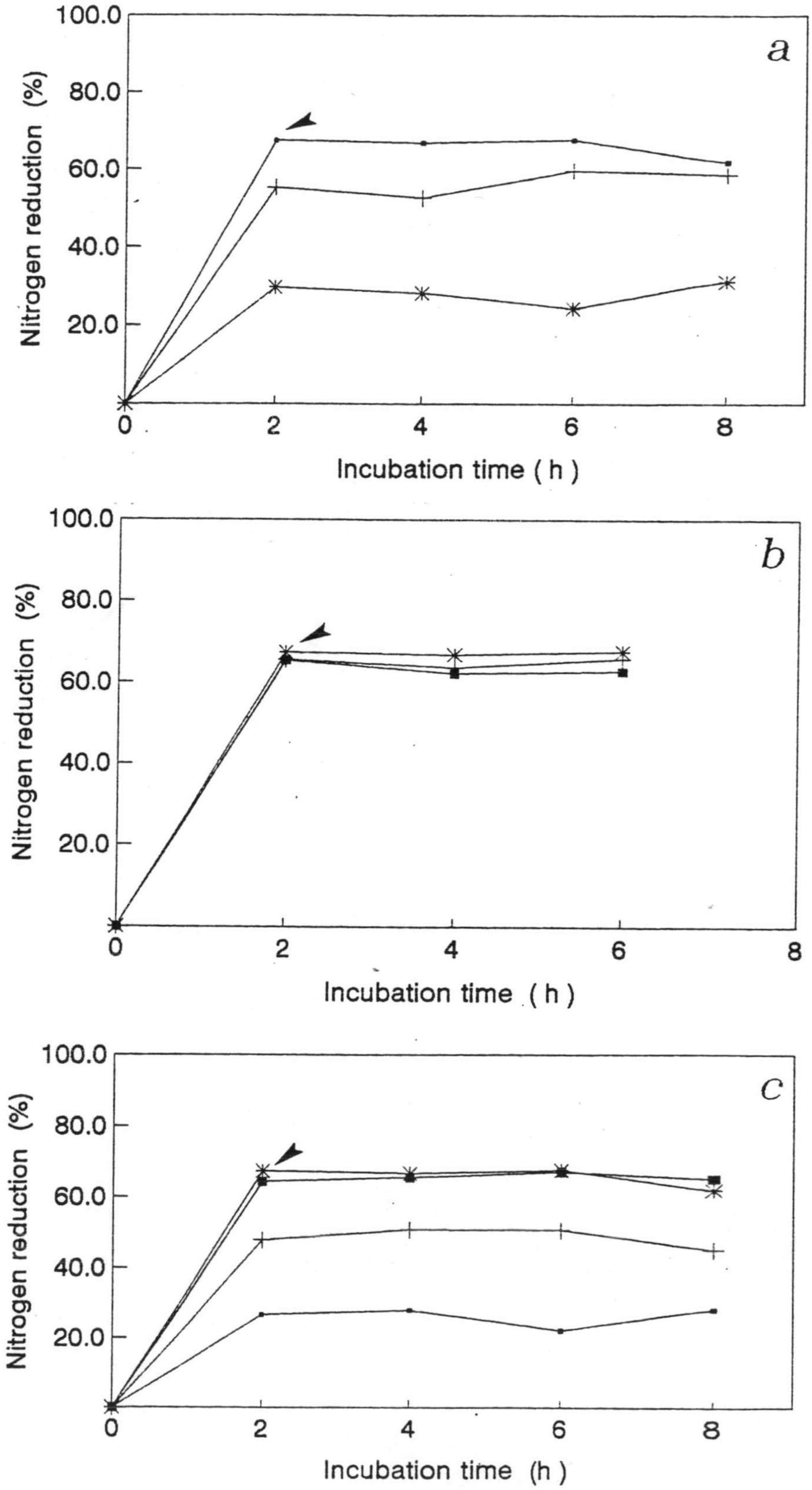
b) The effect of temperature on latex deproteinization

—+— at 40°C, —*— at 50°C, —■— at 60°C

c) The effect of Papain concentration on latex deproteinization

—•— 0.1 p.h.r., —+— 0.2 p.h.r.

—*— 0.3 p.h.r., —■— 0.4 p.h.r.


a
Nitrogen reduction (%)
100.0
80.0
60.0
40.0
20.0
0
2
4
6
8
Incubation time (h)
b
Nitrogen reduction (%)
100.0
80.0
60.0
40.0
20.0
0
2
4
6
8
Incubation time (h)
c
Nitrogen reduction (%)
100.0
80.0
60.0
40.0
20.0
0
2
4
6
8
Incubation time (h)

treatment, but the per cent of nitrogen reduced from initial concentration are the same among 3 lots.

Deproteinization by Papain shows more advantageous than Alcalase, because the lower concentration of Papain (0.3 p.h.r.) is required, and only 2 h of digestion can reduce nitrogen content to the minimum or the same per cent reduction of 70%. However the amount of nitrogen retained in the resulted rubber (0.13-0.16 g%) is still higher than the accepted value (0.09-0.12 g%) for DPNR of the RRIM standard.

3.4 Addition of nonionic detergent as proteins solubilizer and latex stabilizer

In order to increase % nitrogen reduction, nonionic detergent, Triton X-100 has been considered for its capacity to solubilize proteins and also stabilize the colloidal state of rubber particles. The criteria used for selecting optimal concentration of Triton X-100 are; prolonged coagulation during enzyme treatment, but complete coagulation under steam coagulation, and maximum %N reduction. Table 3.4 shows that 0.9 p.h.r. of Triton X-100 can stabilize latex during enzymolysis about 8-10 h after enzyme addition and the final % nitrogen reduction of 76% can be obtained after steam coagulation.

Table 3.4 Optimal concentration of Triton X-100 for latex deproteinization by Alcalase and Papain.

The 25% DRC latex,added with various concentrations of Triton X-100, was incubated with enzyme at the optimal condition in fixed duration of time. The digested latex was coagulated with steam and dry coagulum was determined for nitrogen content.

Triton X-100 (p.h.r.)	Observation		% N reduction	
	During enzymolysis	After steam coagulation	+Papain	+Alcalase
0	Cloting	Complete coagulation	69.8	66.4
0.6	Cloting	Complete coagulation	60.6	72.8
0.7	Cloting	Complete coagulation	75.7	66.2
0.8	Cloting	Complete coagulation	76.9	71.4
0.9	No Cloting	Complete coagulation	75.5	75.8
1.0	No Cloting	No coagulation	ND*	ND*

ND* : Non detectable because the latex was not coagulated by steam.

By adding 0.9 p.h.r. Triton X-100, raw rubber produced from deproteinization by Papain and Alcalase at the optimal condition require at least 2 h and 6 h for reaction time respectively. (Figure 3.8)

The optimal condition and processing of DPNR production from fresh field latex can be concluded as follow; The latex, preserved with 0.4 p.h.r. NH_3 and 0.9 p.h.r. Triton X-100 at the rubber plantation, is adjusted to optimal pH range 7-8 for Papain and 8-9 for Alcalase deproteinization, 0.15 p.h.r. of hydroxylamine hydrochloride and 0.05 p.h.r. of sodium metabisulfite are then added to stabilize viscosity and control the color of rubber. Only clear solution of Alcalase 0.4 p.h.r. or Papain 0.3 p.h.r. is then added and incubated for 6 h or 2 h with shaking at 50-60°C.(Figure 3.8) After that, latex is coagulated with steam by autoclaving 3 minutes at 121°C under pressure 15 lb/inch^2. The coagulum then milled into crepe and washed with water and dried at 60°C.

3.5 Quality of deproteinized raw rubber

The properties of raw rubber material indicate the quality of rubber in term of per cent non-rubber impurities (total nitrogen content, dirt, ash, volatile matter and color index) and the processibility of rubber (Po, PRI and Mooney viscosity). Three lots of deproteinized rubber, produced from concentrated latex (containing 0.19-0.22 g% nitrogen) by Alcalase contain less non-rubber contaminants, particularly total nitrogen which is lowered to 0.05-0.06 g%, ash 0.02-0.04 g% and color index 2-3.5. While those parameters indicating good processibility are still accepted by RRIM specification (Table 3.5). Moreover the resulted DPNR posses the viscosity-stabilized characteristic which is defined as the difference of the initial plasticity (ΔP) of rubber before

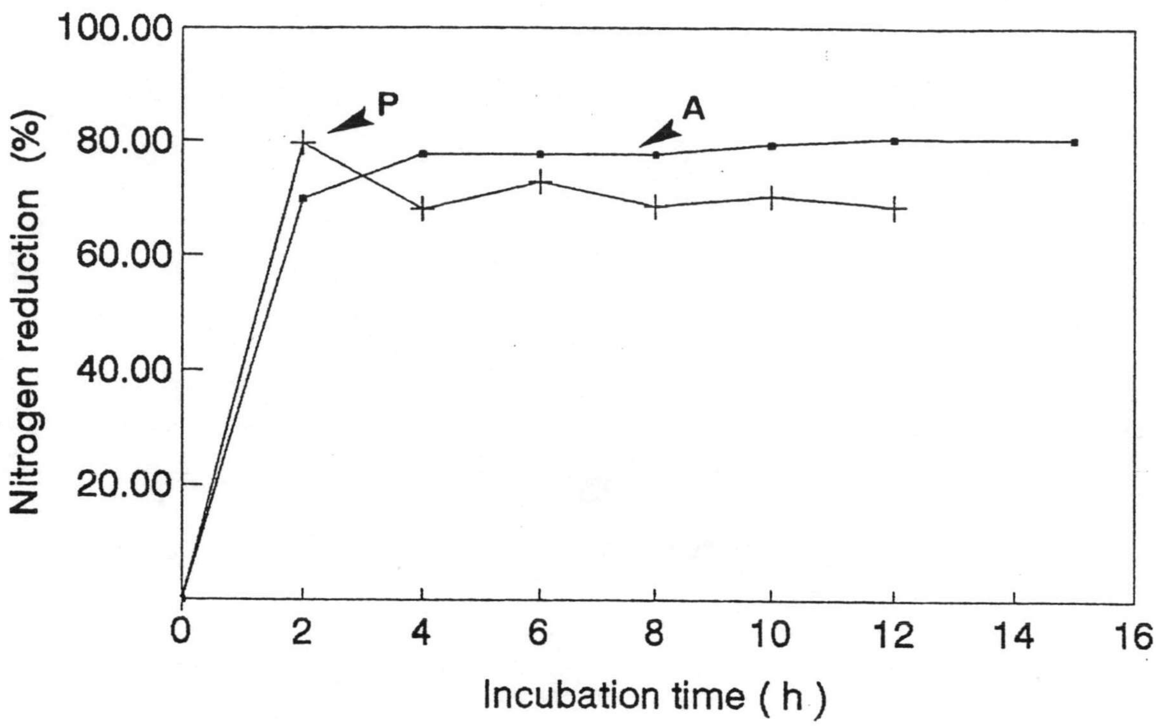


Figure 3.8 Optimal incubation time for deproteinization of field latex by Alcalase and Papain

The latex was adjusted to 25 %DRC at pH 7-8 for Papain and 8-9 for Alcalase ,steam coagulated ,dried ,and determined for the nitrogen reduction at time intervals.

—•— Alcalase treatment (A), 0.4 p.h.r. Optimal time 6 h

—+— Papain treatment (P) 0.3 p.h.r. Optimal time 2 h and after ageing over P_2O_5 at 60°C for 24 h. The color index of the DPNR is significantly lower than the control. (Figure 3.9 a)

Table 3.5 Raw rubber properties of deproteinized rubber, produced from concentrated latex 60%

Property	Control	DPNR	DPNR of RRIM specification
Nitrogen content(g%)	0.19-0.22	0.05-0.06	0.066
Dirt (g%)	0.006-0.010	0.006	0.006
Ash (g%)	0.05	0.02-0.04	0.052
Volatile matter (g%)	0.10-0.13	0.07-0.10	0.28
Initial plasticity (P_O)	58-60	54-57	39
Plasticity Retention Index (PRI)	75-76	65-77	66
ΔP (P_H-P_O)	16-17	2-8	not more than 9
Mooney viscosity (ML1+4 ,100 °C)	83-87	84-88	-
Color index	3.5-4.0	2.0-3.5	-

* As quoted by Chang et al.,1977

In case of deproteinized rubber produced from fresh field latex, Table 3.6 summarizes the raw rubber properties of DPNR, produced from 3 clones of rubber, PB 5/51, GT 1 and RRIM 600 by Papain and Alcalase in comparison with control untreated raw rubber of identical clone. After enzyme treatment, Non-rubber impurities have been decreased so that the retained amount of nitrogen, volatile matter and dirt are within the acceptable limit of RRIM specification except ash content. The initial plasticity and plasticity retention index also decreased after enzyme treatment particularly Alcalase treatment which greatly affected on PRI of deproteinized rubber from rubber clone PB 5/51 and GT 1. Hence the resulted PRI were below the proposed specification (PRI>60) and needed improvement with thiourea. While DPNR produced from RRIM 600 satisfied the PRI requirement.

The viscosity-stabilized characteristic (ΔP) shows that deproteinized rubber, produced from fresh latex of 3 clones,either by Alcalase or Papain can pass the specified value ($\Delta P<9$).

The Mooney viscosity of deproteinized rubber is always less than its control (Table 3.6 and Figure 3.10) especially the lower-viscosity clone RRIM 600 (10 units) ,although clone GT 1 and PB 5/51 which are the higher viscosity clones show smaller decrease in viscosity about 3-5 units. Figure 3.11 shows the correlation between the net Mooney viscosity reduction (ΔMV) and total nitrogen reduction (ΔN) in the range of 0.3-0.55 g%N (r^2 = 0.4) and indicates that the reduction of nitrogen content (ΔN) less than 0.35 g% does not affect the Mooney viscosity of the rubber and if % total nitrogen reduction (ΔN) is more than 0.55 g%, the maximum Mooney reduction is between 10-15 units from the started value.

Table 3.6 Raw rubber properties of deproteinized rubber from fresh field latex clone : PB5/51, GT 1 and RRIM 600

Property	PB 5/51			GT 1			RRIM 600		
	Control	+Alcalase	+Papain	Control	+Alcalase	+Papain	Control	+Alcalase	+Papain
N-content (g%)	0.465±0.014	0.096±0.023	0.094±0.010	0.572±0.025	0.106±0.019	0.113±0.020	0.738±0.107	0.198±0.039	0.166±0.023
Dirt (g%)	0.004±0.001	0.002±0.001	0.005±0.002	0.004±0.003	0.004±0.002	0.004±0.002	0.004±0.001	0.004±0.002	0.003±0.002
Ash (g%)	0.436±0.148	0.384±0.204	0.312±0.066	0.419±0.088	0.226±0.042	0.259±0.054	0.286±0.012	0.428±0.201	0.320±0.084
Volatile matter (g%)	0.408±0.060	0.257±0.103	0.249±0.033	0.441±0.097	0.222±0.049	0.246±0.021	0.527±0.010	0.457±0.084	0.420±0.047
P_o	62.5±1.5	57.5±0.80	56.4±2.1	48.4±1.4	40.4±0.6	39.5±0.5	34.0±6.4	26.2±5.4	25.4±6.0
PRI	89.9±3.8	46.1±4.9	74.4±3.7	91.7±3.6	57.5±5.6	69.1±8.0	95.6±6.9	80.2±4.5	85.7±7.0
$\triangle P$ (P_H-P_o)	23-29	1-3	3-6	34-38	1-5	3-7	24-56	5-6	3-5
Mooney viscosity (ML1+4 ,100 ^oC)	89.7±2.1	86.4±2.5	85.4±2.9	73.5±1.4	66.3±0.8	66.9±1.6	56.3±4.9	44.5±5.9	45.2±5.6
Color index	4.5±0.5	4.0±1.0	4.0±0.5	5.0±1.0	12.0	7.0±1.0	5.0±1.0	3.0±1.0	5.0±1.0

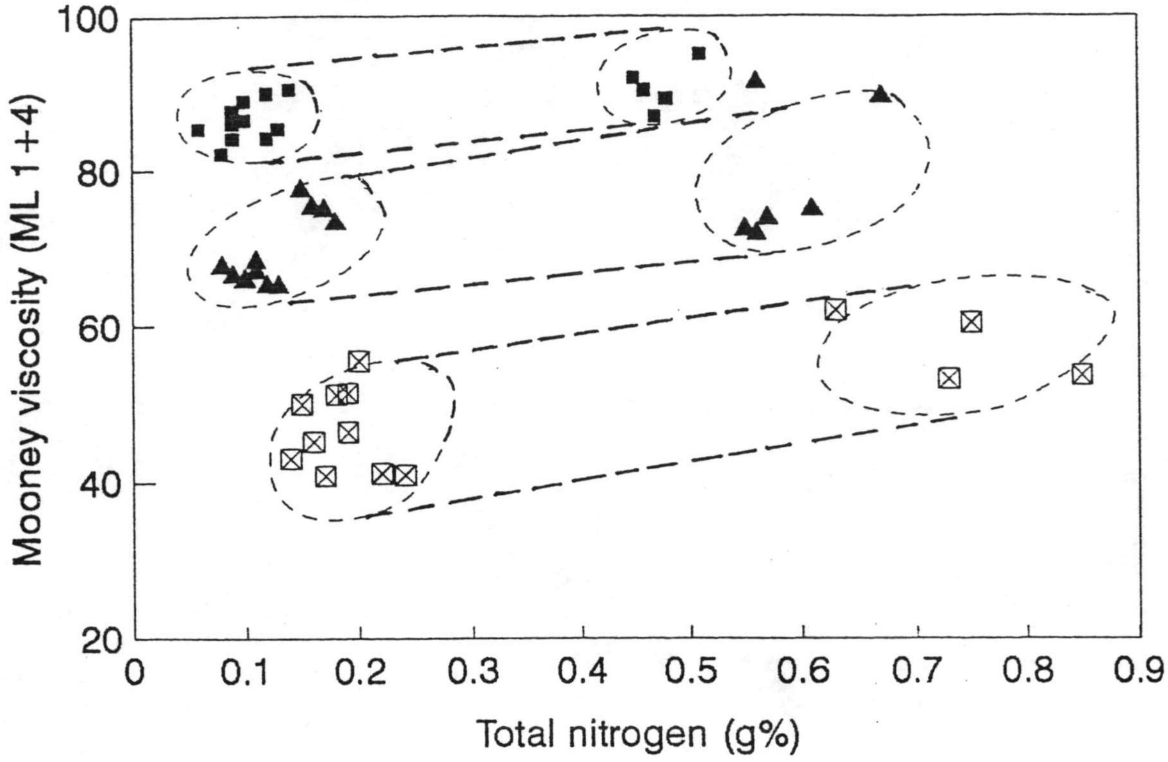


Figure 3.10 Correlation between total nitrogen and the Mooney viscosity of 3 rubber clones

Total nitrogen of rubber specimens from each clone before and after deproteinization were plotted with their corresponding Mooney viscosity , Clone PB 5/51 (■) contains the lowest amount of total nitrogen followed by GT 1 (▲) and RRIM 600 (⊠), but the Mooney viscosity of clone PB 5/51 is higher than GT 1 and RRIM 600.

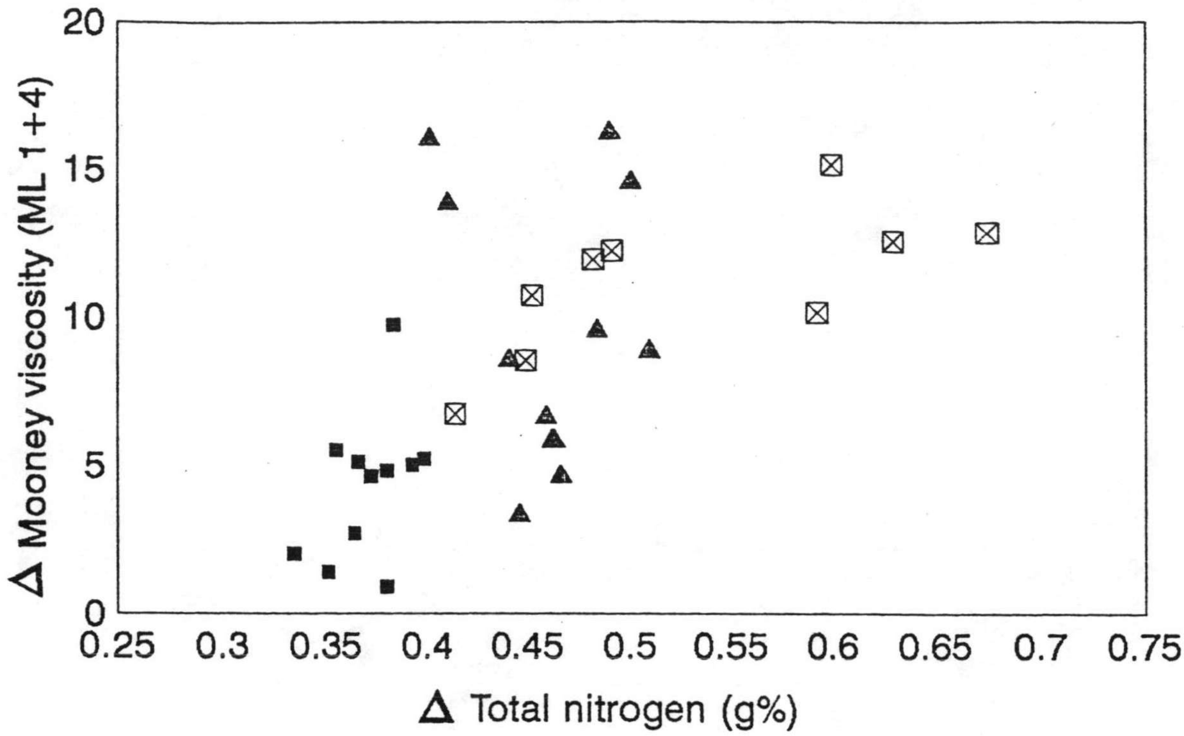


Figure 3.11 Correlation between total nitrogen reduction and the Mooney viscosity reduction

Δ Total nitrogen and Δ Mooney viscosity stand for the difference between its value of dry rubber before and after enzyme treatment.

PB 5/51 (■), GT 1 (▲), RRIM 600 (⊠)

Most of produced DPNR show the color index lower than 6, the acceptable value for light-color rubber, except GT 1, only the Alcalase-treated rubber shows dark color. Clonal of rubber latex is the main influence on color index of the raw rubber (Figure 3.9 b, c,and d). The color index of rubber processed from fresh latex clone, RRIM 600, recommended as white grade rubber is significantly lower than rubber processed from fresh latex clone : PB 5/51 and GT 1. Comparing with non-enzyme treated rubber, the color of enzyme-treated rubber are lighter about 1-2 index differences.

In conclusion, when using both retained total nitrogen and color index as criteria, the obtained DPNR from treatment of concentrated latex 60% with Alcalase give the lower non-rubber impurities and better processibility than treatment of fresh field latex. While PB 5/51 seems to be the most suitable clone for DPNR production by Papain and/or Alcalase with added thiourea treatment.

3.6 Processibility of deproteinized rubber

Processibility of the rubber in the factory is usually performed by mixing the rubber with the chemical ingredients to obtain the compound rubber or "green stock" and followed by curing or vulcanization. The compounding formula used in this research is a normal formulation for making the outsole of sport shoe, where the high modulus, high resilience, high durability rubber and lighter color are required.

Since in this process, the compound rubber should flow throughout a complicate mold during the short and precise time duration while the appearance and physical properties of vulcanizate should be uniform. A

Figure 3.9 Color comparison among raw DPNR specimens and non deproteinized rubber from various sources

a) From concentrated latex 60%

1. Non-treated rubber
2. Alcalase-treated rubber

b) From fresh latex clone PB 5/51

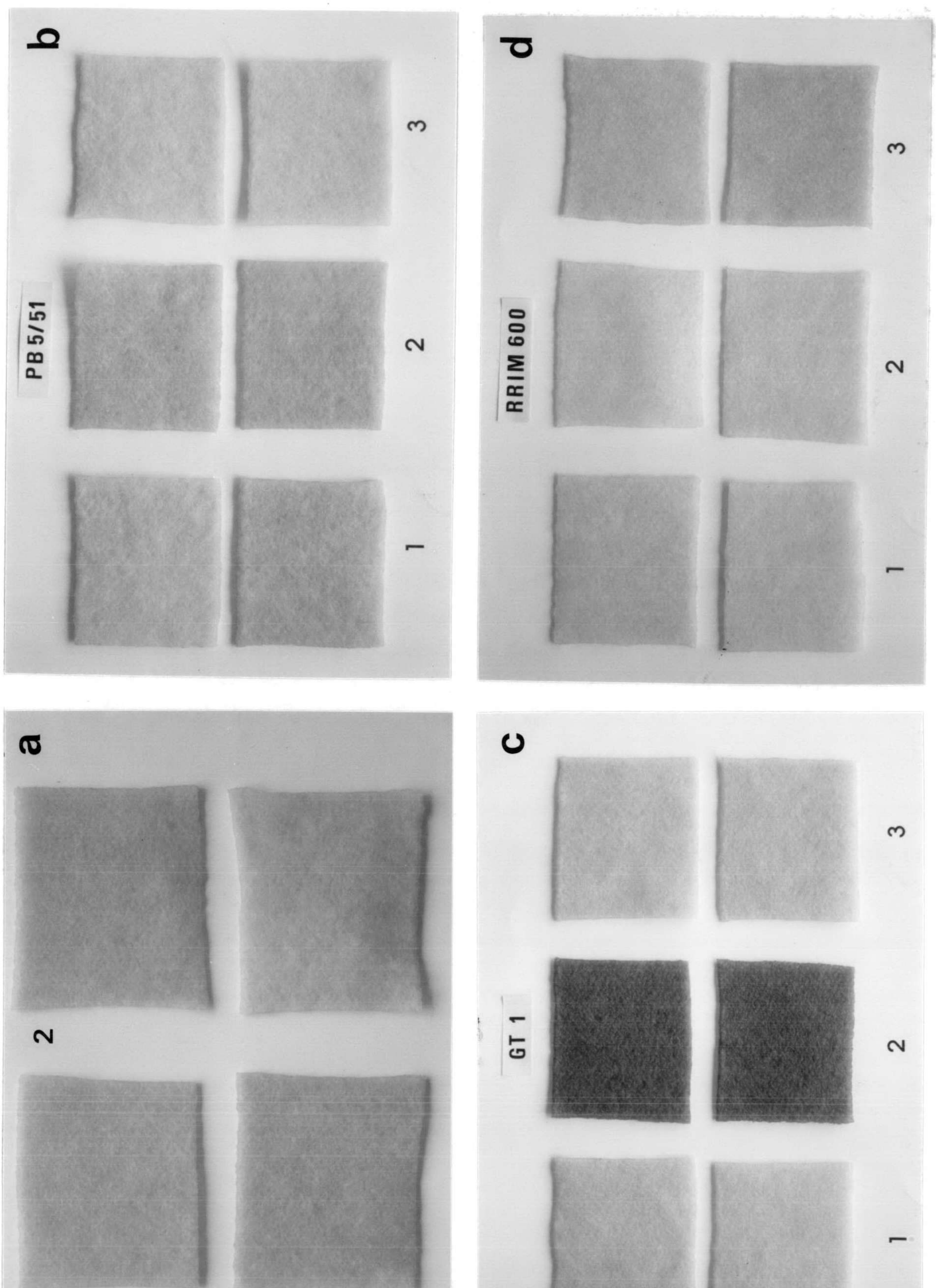
1. Non-treated rubber
2. Alcalase-treated rubber
3. Papain-treated rubber

c) From fresh latex clone GT 1

1. Non-treated rubber
2. Alcalase-treated rubber
3. Papain-treated rubber

d) From fresh latex clone RRIM 600

1. non-treated rubber
2. Alcalase-treated rubber
3. Papain-treated rubber


a
1
2
b
PB 5/51
1
2
3
c
GT 1
1
2
3
d
RRIM 600
1
2
3

typical cure characteristic curve of the compound formulation is shown in Figure 3.12.

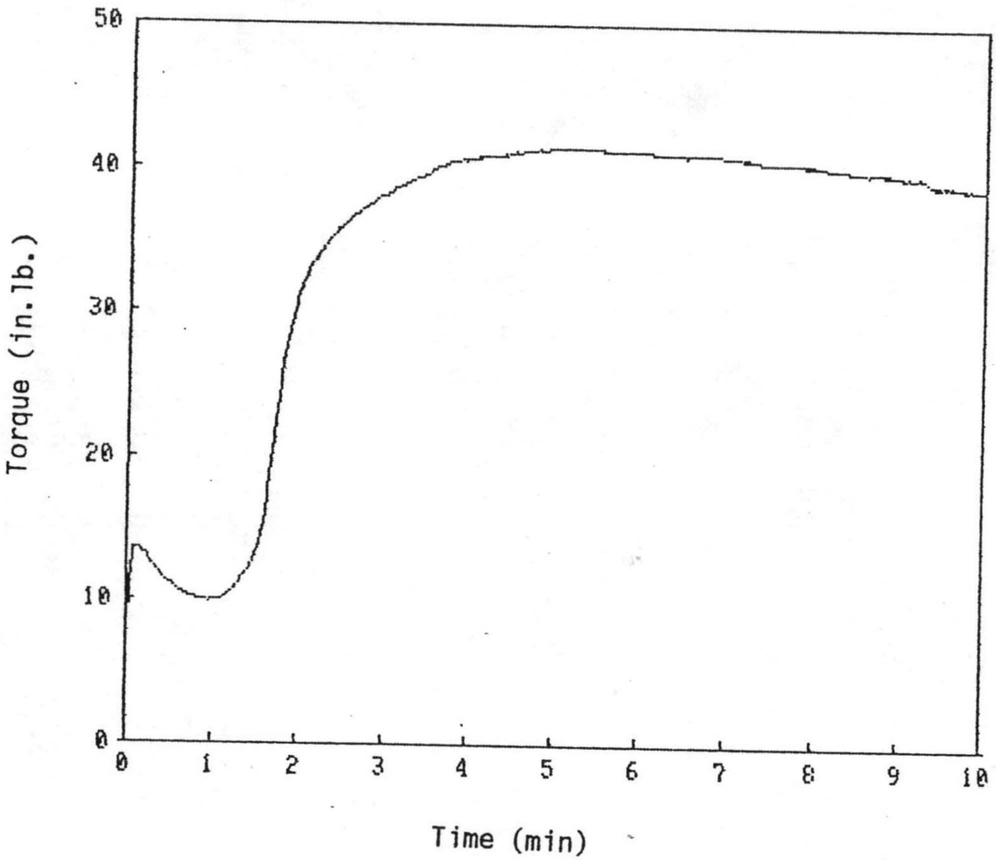


Figure 3.12 Rheograph of a typical sample of compounded rubber and DPNR

Cure characteristic of the compound rubber was run for 10 min at 155°C with disc oscillating at 3° arc, in a Rheometer model 100S (Monsanto)

The difference between the cure characteristics of DPNR and the control is evident in cure rate. The cure rate of deproteinized rubbers seem to increase in all 3 clones, and also deproteinized rubber made from concentrated latex 60% (Fig 3.13 d), where other cure characteristics are similar. The scattered plot between total nitrogen of rubber and cure rate indicate 3 different groups of rubber,high ,moderate and low cure rate in corresponding to an increase in total nitrogen (g%) of the rubber (Figure 3.14). From correlation analysis, Total nitrogen broadly correlates with cure rate at % N lower than 0.12 g%, the specified maximum value of DPNR at RRIM station, while the other 2 groups with total nitrogen of 0.15-0.4 g% and exceed 0.4 g% show no correlation between cure rate and nitrogen content.

It is noted that deproteinization by Alcalase and Papain show no significant difference on the cure behaviors of raw rubber produced from fresh latex clones : PB 5/51 and GT 1 but in clone RRIM 600, the marked difference in cure parameters have been observed. Alcalase treatment has retarded the rate of cure by extending in scorch and cure time which normally are in range 1.3-1.7 min and 2.8-3.0 to 2 min and 3.8 min respectively (Figure 3.13 a ,and b). There is no significant difference between torque rise (MH-ML) of high total nitrogen and low total nitrogen rubber. (Figure 3.15)

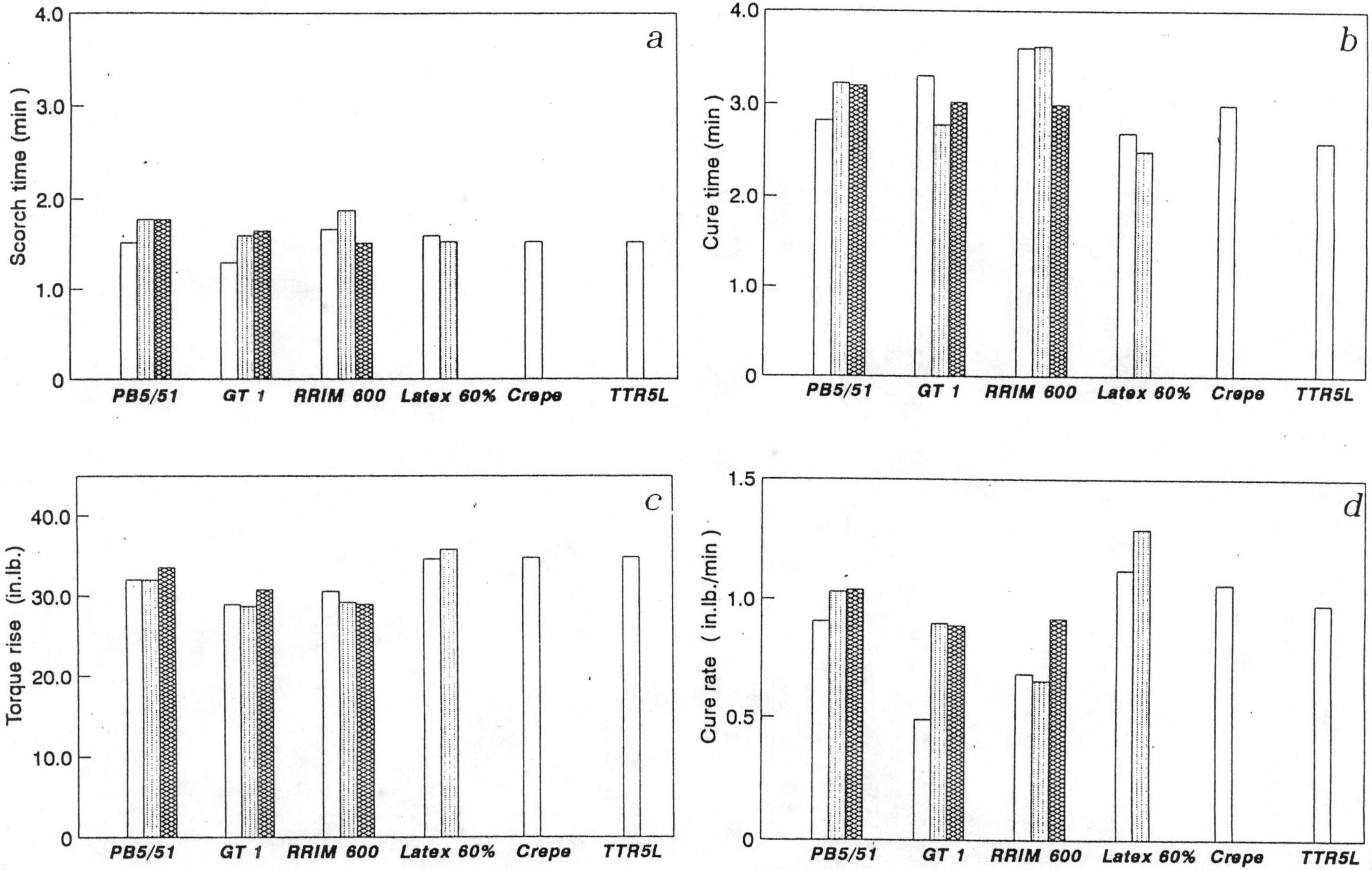
It can be concluded that the deproteinization of rubber increases the rate of cure when the reduction of nitrogen contents are lower than 0.12 g% for this compound formulation.

Figure 3.13 Rheological properties of DPNR

Control untreated rubber ☐ , Papain-treated rubber ▩ ,and Alcalase-treated rubber ▤ were mixed and cured under the same condition and measured for

a) Scorch time b) Cure time

c) Torque rise (MH-ML) d) Cure rate



Scorch time (min)
4.0
3.0
2.0
1.0
0
a
PB5/51
GT 1
RRIM 600
Latex 60%
Crepe
TTR5L
Cure time (min)
4.0
3.0
2.0
1.0
0
b
PB5/51
GT 1
RRIM 600
Latex 60%
Crepe
TTR5L
Torque rise (in.lb.)
40.0
30.0
20.0
10.0
0
c
PB5/51
GT 1
RRIM 600
Latex 60%
Crepe
TTR5L
Cure rate (in.lb./min)
1.5
1.0
0.5
0
d
PB5/51
GT 1
RRIM 600
Latex 60%
Crepe
TTR5L

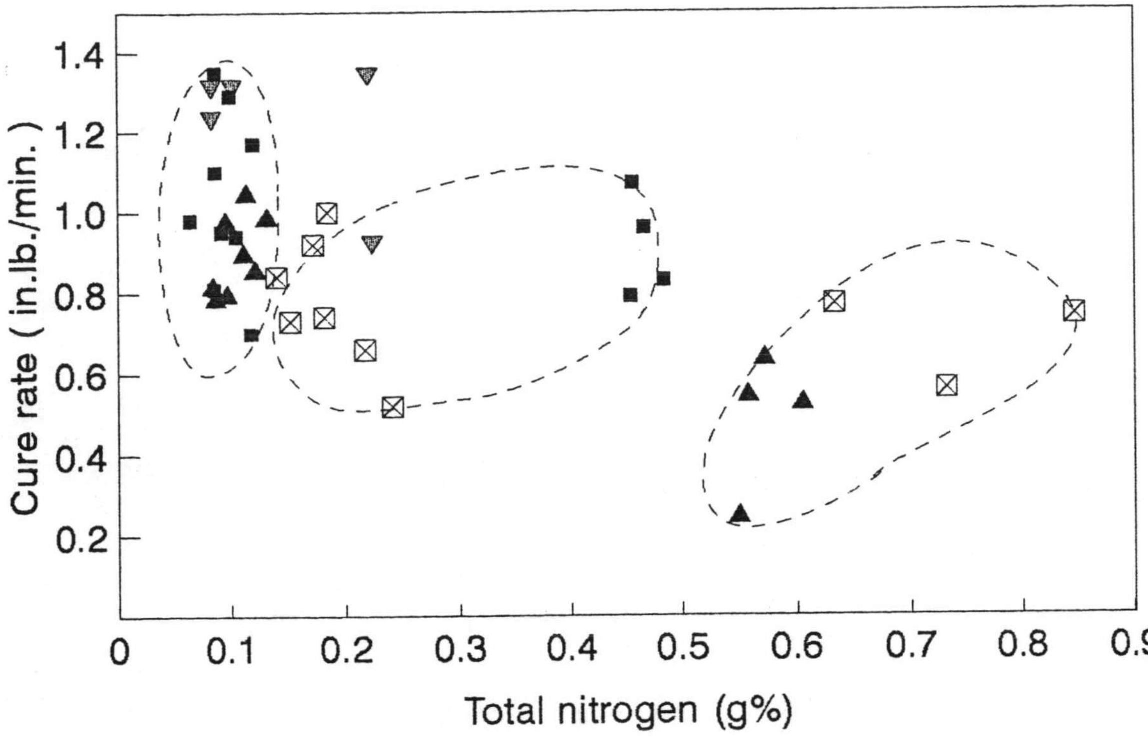


Figure 3.14 Scattered plot between total nitrogen and its cure rate

PB 5/51 (■), GT 1 (▲)

RRIM 600(⊠), Concentrated latex 60 % (▼)

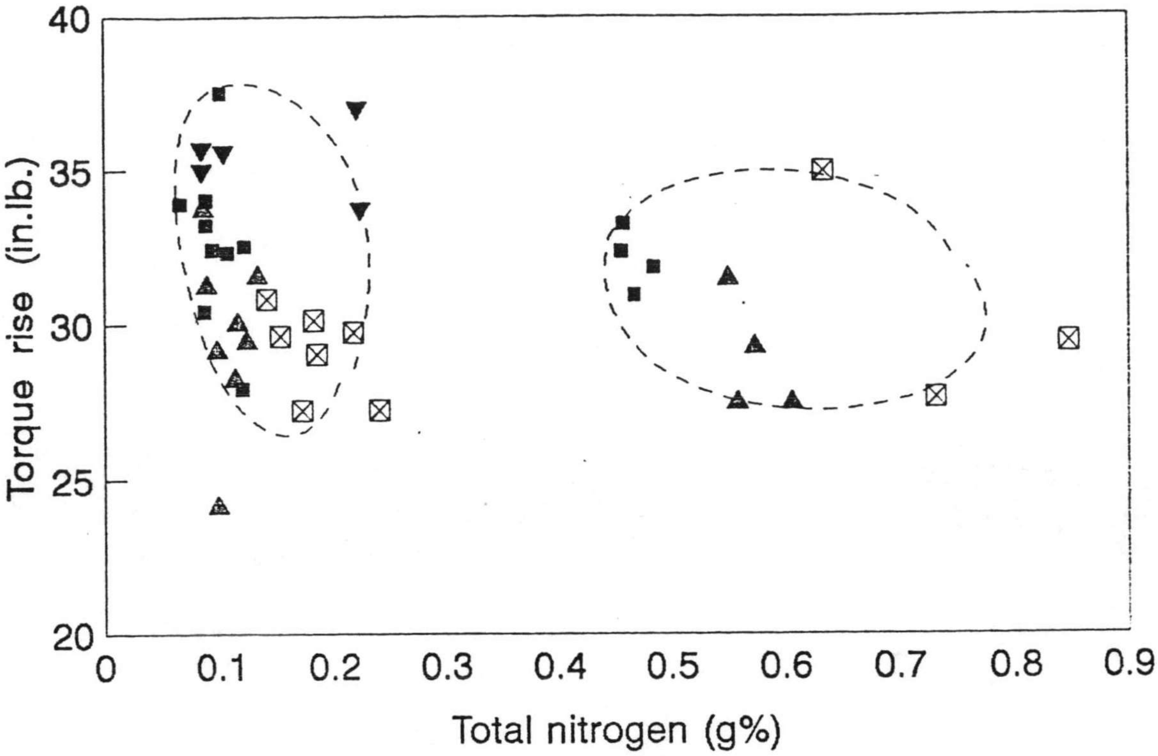


Figure 3.15 Scattered plot between total nitrogen and torque rise

PB 5/51 (■), GT 1 (▲)

RRIM 600(⊠), Concentrated latex 60 % (▼)

When the color of compound rubber of deproteinized rubber were compared with its control, the color of compound rubber depend partially on the color of the original raw rubber clone and partially on the interaction with the chemical ingredients added in compound formulation. Figure 3.16 a-d show the color of compound rubber from DPNR. It is noted that the color of compound DPNR produced from different clone or different enzyme treatment are slightly lighter than the control except GT 1,the Alcalase-treated compound rubber show the distinct dark color more than its original control and Papain-treated rubber.

Technological properties of deproteinized NR vulcanizates have been compared with its control and commercial crepe rubber and TTR 5L as shown in Figure 3.17, Hardness and 300% modulus of vulcanized DPNR were decreased while other stress-strain properties, tensile strength and ultimate elongation show increasing trend when compared with the control rubber of the experiment. But the physical properties of vulcanized DPNR of concentrated latex 60% were not evidently changed after Alcalase treatment.

Source and type of rubber latex, including rubber processing, also affected to the physical properties of vulcanized rubber. DPNR produced from concentrated latex 60% showed higher stress-strain properties than DPNR from each clone of field latex and also higher than the commercially used rubber, TTR 5L and crepe.

The color of vulcanized DPNR from different clones of rubber are shown in Figure 3.18 to be dependent on both rubber clone and process. DPNR vulcanized from RRIM 600 and PB 5/51 exhibit lighter color than GT 1. It is noted that the color of low protein rubbers are lighter than non-

Figure 3.16 Color of rubber compound

DPNR from each clone of rubber were mixed with chemicals in the same formulation

a) From concentrated latex 60%

1. Non-treated rubber
2. Alcalase- treated rubber
3. CREPE
4. TTR 5L

b) From fresh latex clone PB 5/51

1. Non-treated rubber
2. Alcalase-treated rubber
3. Papain-treated rubber

c) From fresh latex clone GT 1

1. Non-treated rubber
2. Alcalase-treated rubber
3. Papain-treated rubber

d) From fresh latex clone RRIM 600

1. Non-treated rubber
2. Alcalase-treated rubber
3. Papain-treated rubber

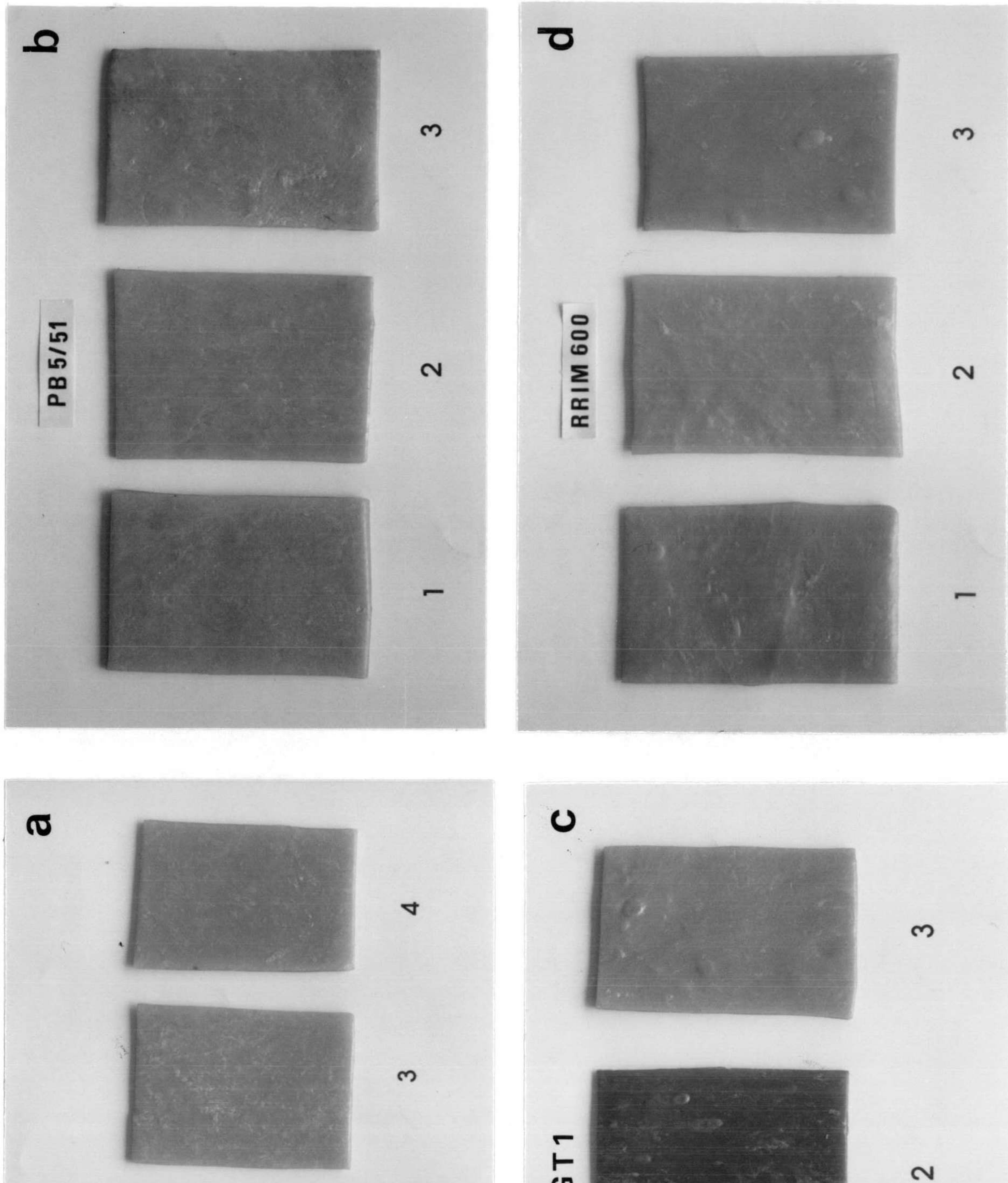

a
1
2
3
4
b
PB 5/51
1
2
3
c
GT1
1
2
3
d
RRIM 600
1
2
3

Figure 3.17 Vulcanizate properties of DPNR produced from various sources

Control untreated vulcanized rubber ☐ , Papain-treated rubber ▥ ,and Alcalase-treated rubber ▩ from field latex /concentrated latex 60% were compared for

a) Hardness (shore A) b) 300% Modulus

c) Ultimate elongation d) Tensile strength

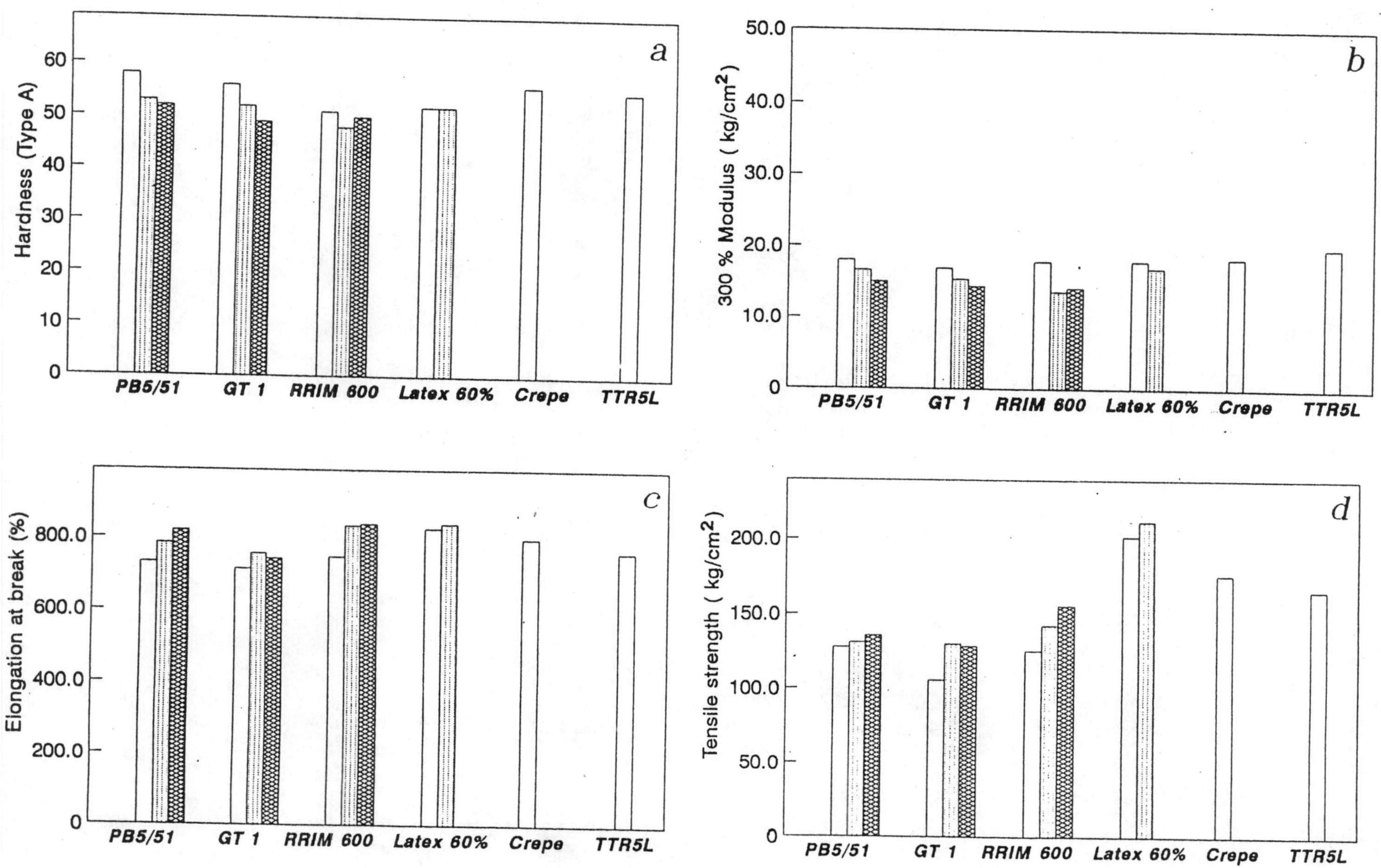

a
Hardness (Type A)
0
10
20
30
40
50
60
PB5/51
GT 1
RRIM 600
Latex 60%
Crepe
TTR5L
b
300 % Modulus (kg/cm^2)
0
10.0
20.0
30.0
40.0
50.0
PB5/51
GT 1
RRIM 600
Latex 60%
Crepe
TTR5L
c
Elongation at break (%)
0
200.0
400.0
600.0
800.0
PB5/51
GT 1
RRIM 600
Latex 60%
Crepe
TTR5L
d
Tensile strength (kg/cm^2)
0
50.0
100.0
150.0
200.0
PB5/51
GT 1
RRIM 600
Latex 60%
Crepe
TTR5L

Figure 3.18 Color of rubber vulcanizate

Compound rubber from each clone either deproteinized or control, were cured in similar compression molds at 155°C for optimal cure time and compared for color of the testpieces.

a) From concentrated latex 60%

1. Non-treated rubber
2. Alcalase- treated rubber
3. CREPT
4. TTR 5L

b) From fresh latex clone PB 5/51

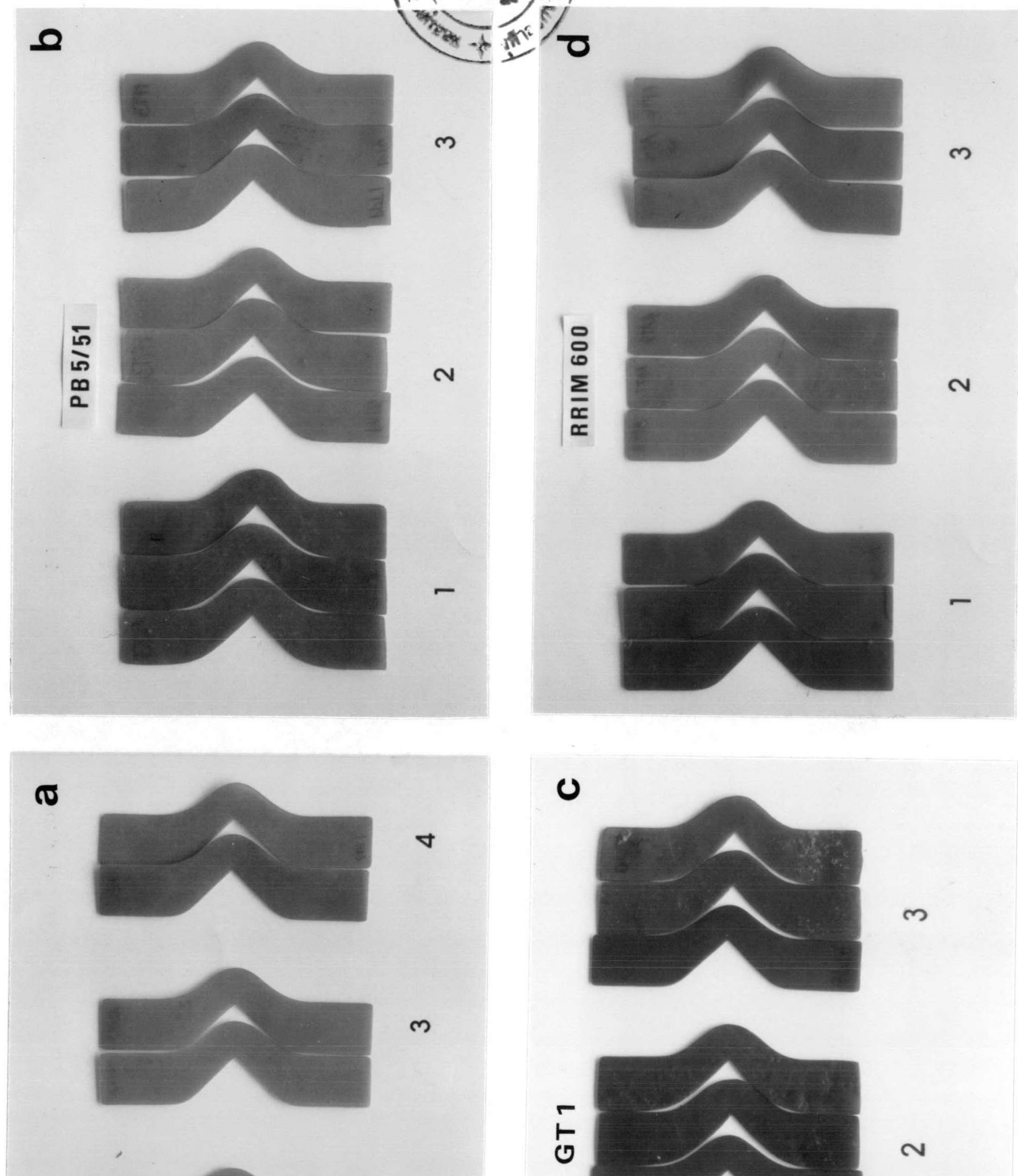
1. Non-treated rubber
2. Alcalase-treated rubber
3. Papain-treated rubber

c) From fresh latex clone GT 1

1. Non-treated rubber
2. Alcalase-treated rubber
3. Papain-treated rubber

d) From fresh latex clone RRIM 600

1. Non-treated rubber
2. Alcalase-treated rubber
3. Papain-treated rubber


a
1
2
3
4
b
PB 5/51
1
2
3
c
GT1
1
2
3
d
RRIM 600
1
2
3

enzyme-treated rubber, except Alcalase-treated rubbers produced from GT 1 fresh latex. Vulcanized rubber prepared from concentrated latex 60%, both of enzyme treated and non-treated rubber show light color as well as the commercially used TTR 5L and crepe rubber.