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APPENDIX

APPENDIX 1

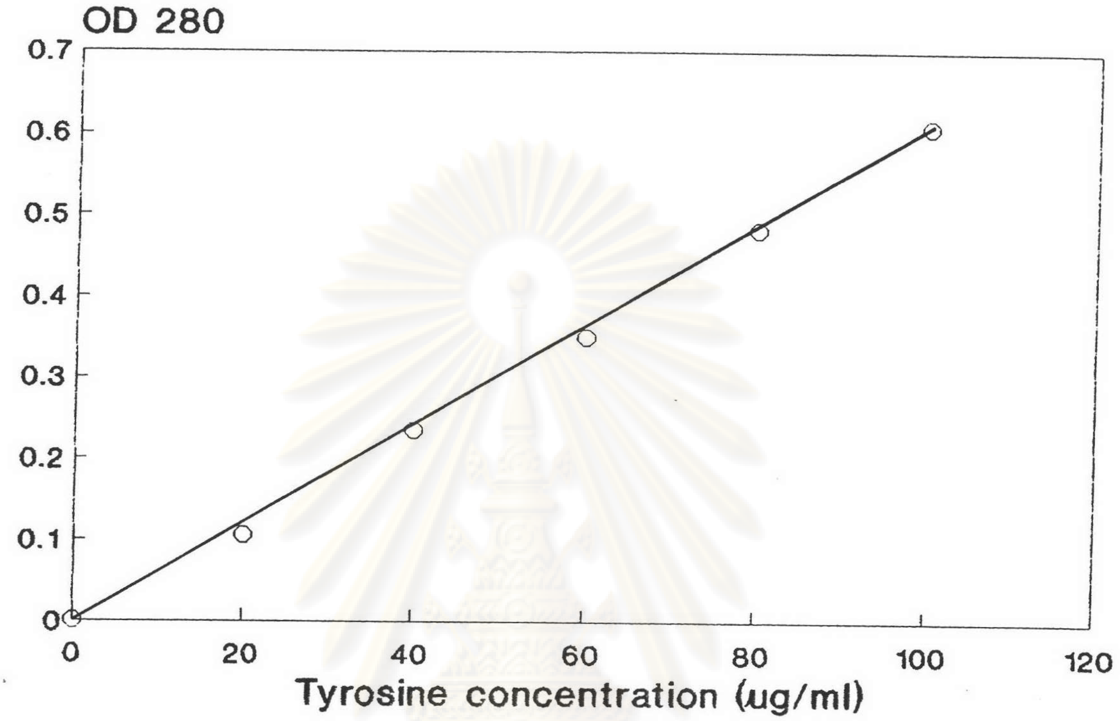


Figure 35 Tyrosine Standard curve

Slope of tyrosine standard curve = 5.833×10^{-3}

Tyrosine concentration = $OD_{280} \times 1$ / slope

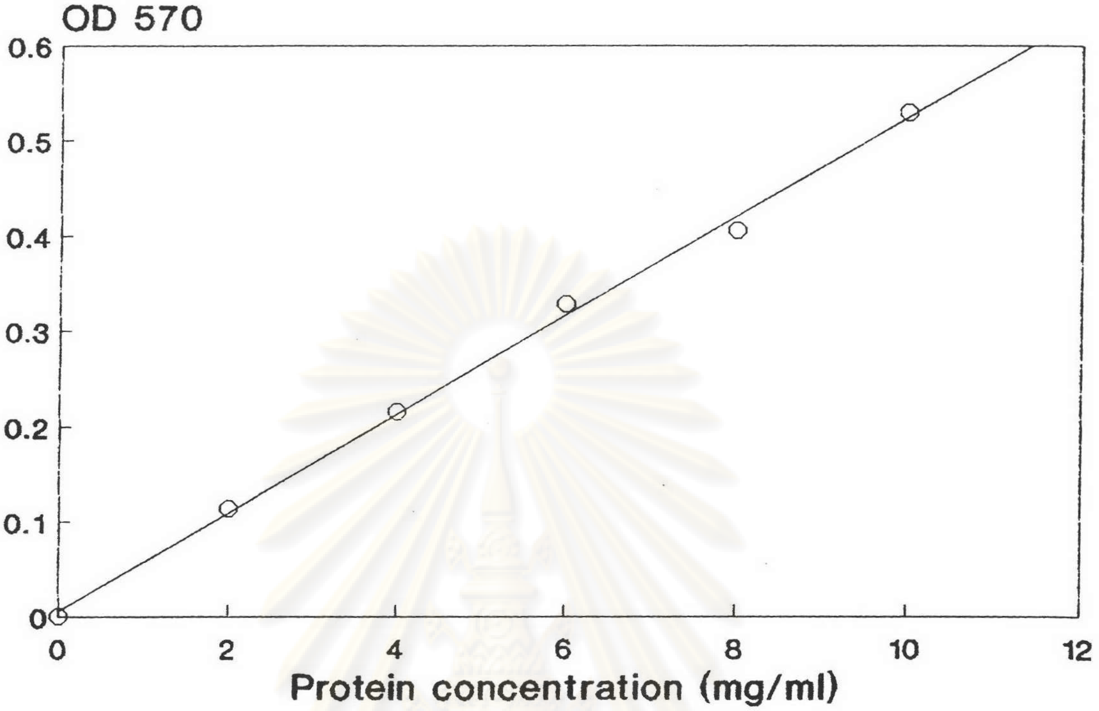


Figure 36 Biuret Standard curve

Slope of Biuret standard curve = 5.26×10^{-2}

Protein concentration = $OD_{570} \times 1 / \text{slope}$

APPENDIX 2

THE CALCULATION AND RAW DATA

1. Calculation of specific activity of immobilized papain by physical adsorption and covalent-binding

1.1 Immobilized papain by physical adsorption

1) The result as shown in Table 3.1 are that :

the activity of immobilized papain = 65-70 CDU/g wet chitin

protein bound on chitin = 0.2 mg protein/g wet chitin

the specific activity = 70 / 0.2

= 340 CDU/mg protein

2) Total activity of free papain added is about 65,000 unit per one gram of dry chitin that is equal to 12,300 unit per gram of wet chitin.

3) The specific activity of free papain (the concentration used is 7 mg/ml) is 2,100 unit/mg protein.

4) Total activity of unbound papain is about 8,100 unit

5) Calculation of % yield follows the method of Kusano (1989) :

$$\%\ \text{yield} = \frac{\text{Activity of immobilized papain} \times 100}{(\text{activity of added papain} - \text{activity of unbound papain})}$$

$$= 70 \times 100 / (12{,}300 - 8{,}100) = 1.67\ \%$$

$$\%\ \text{Bound} = \frac{\text{Specific activity of immobilized papain} \times 100}{\text{Specific activity of free papain}}$$

$$= 340 \times 100 / 2100 = 16.19\ \%$$

1.2 Immobilized papain by covalent-binding method

1) The result as shown in Table 3.1 are that :

the activity of immobilized papain = 450-470 CDU/g wet chitin
protein bound on chitin = 0.39 mg protein/g wet chitin
the specific activity = 470 / 0.39
= 1200 CDU/mg protein

2) Total activity of free papain added is about 62,000 unit per one gram of dry chitin that is equal to 12,800 unit per gram of wet chitin.

3) The specific activity of free papain (the concentration uaed is 7 mg/ml) is 2,000 unit/mg protein.

4) Total activity of unbound papain is about 10,700 unit

5) Calculation of % yield follows the method of Kusano (1989) :

$$\%\ \text{yield} = \frac{\text{Activity of immobilized papain} \times 100}{(\text{activity of added papain} - \text{activity of unbound papain})}$$

$$= 470 \times 100 / (12{,}800 - 10{,}800) = 23.50\ \%$$

$$\%\ \text{Bound} = \frac{\text{Specific activity of immobilized papain} \times 100}{\text{Specific activity of free papain}}$$

$$= 1200 \times 100 / 2000$$

$$= 60\ \%$$

2. Calculation of K_m and V_{max} of immobilized papain on chitin and free papain.

Regression equations were calculated from Lineweaver-Burk plots as shown in Figure 3.7 when casein, ovalbumin and rubber latex were used as substrate.

2.1 Physically-adsorbed papain on chitin(PIP) and free papain(FP)

1) When casein was used as substrate of PIP, K_m and V_{max} were obtained from the regression equation below :

Regression equation $Y = a + bX$

$$Y = (9.53 \times 10^{-4}) + (8.88 \times 10^{-4})\,X$$

Y-intercept = a = 9.53×10^{-4} = $1/V_{max}$

X-intercept = -a/b = -1.073 = $-1/K_m$

K_m = 0.932 g/100 ml and V_{max} = 1,049.32 CDU/mg protein

When casein was used as substrate of FP, K_m and V_{max} were obtained from the regression equation below :

$$Y = (9.47 \times 10^{-4}) + (7.24 \times 10^{-4})\,X$$

K_m = 0.764 g/100 ml and V_{max} = 1,055.97 CDU/mg protein

2) When ovalbumin was used as substrate of PIP, K_m and V_{max} were obtained from the regression equation below :

$$Y = (1.88 \times 10^{-3}) + (3.12 \times 10^{-3})\,X$$

K_m = 1.656 g/100 ml and V_{max} = 530.78 CDU/mg protein

When ovalbumin was used as substrate of FP, K_m and V_{max} were obtained from the regression equation below :

$$Y = (1.74 \times 10^{-3}) + (1.80 \times 10^{-3})\,X$$

K_m = 1.035 g/100 ml and V_{max} = 576.04 CDU/mg protein

2.2 Covalently immobilized papain on chitin (CIP) and free papain (FP)

1) When casein was used as substrate of CIP, K_m and V_{max} were obtained from the regression equation below :

$$Y = (4.62 \times 10^{-4}) + (3.35 \times 10^{-4})\,X$$

K_m = 0.725 g/100 ml and V_{max} = 2,164.50 CDU/mg protein

When casein was used as substrate of FP, K_m and V_{max} were obtained from the regression equation below :

$$Y = (4.93 \times 10^{-4}) + (3.48 \times 10^{-4})\,X$$

K_m = 0.704 g/100 ml and V_{max} = 2,026.75 CDU/mg protein

2) When ovalbumin was used as substrate of CIP, K_m and V_{max} were obtained from the regression equation below :

$$Y = (1.16 \times 10^{-3}) + (1.22 \times 10^{-3})\,X$$

K_m = 1.051 g/100 ml and V_{max} = 864.30 CDU/mg protein

When ovalbumin was used as substrate of FP, K_m and V_{max} were obtained from the regression equation below :

$$Y = (9.96 \times 10^{-4}) + (9.12 \times 10^{-4})\,X$$

K_m = 0.912 g/100 ml and V_{max} = 1,000.40 CDU/mg protein

3) When protein in rubber latex was used as substrate of CIP, K_m and V_{max} were obtained from the regression equation below :

$$Y = (1.62 \times 10^{-3}) + (0.0451) X$$

K_m = 27.85 % protein and V_{max} = 616.90 %retention protein

When protein in rubber latex was used as substrate of FP, K_m and V_{max} were obtained from the regression equation below :

$$Y = (4.23 \times 10^{-3}) + (0.0449) X$$

K_m = 10.62 % protein and V_{max} = 236.19 %retention protein

3. Optimal conditions for fresh latex deproteinization by immoblized papain on chitin

2.1 Effect of pH on latex deproteinization

Time (hr)	pH 6-7		pH 7-8		pH 8-9	
	%N	%N reduction	%N	%N reduction	%N	%N reduction
0	0.588	0	0.588	0	0.588	0
2	0.321	45.31	0.328	44.22	0.376	36.01
4	0.263	55.22	0.241	58.99	0.301	48.76
6	0.236	59.92	0.228	61.21	0.259	55.92

2.2 Effect of temperature on latex deproteinization

Time (hr)	30 C		40 C		50C	
	%N	%N reduction	%N	%N reduction	%N	%N reduction
0	0.581	0	0.581	0	0.581	0
2	0.343	40.86	0.321	44.65	0.282	52.39
4	0.313	46.12	0.266	54.11	0.253	56.34
6	0.296	48.95	0.241	58.41	0.226	59.45

2.3 Effect of immobilized papain concentration on latex deproteinization

Time (hr)	10 p.h.r.		20 p.h.r.		30 p.h.r.		40 p.h.r.	
	%N	%N reduction	%N	%N reduction	%N	%N reduction	%N	%N reduction
0	0.563	0	0.563	0	0.563	0	0.563	0
2	0.415	26.33	0.226	59.90	0.242	57.98		
4	0.311	44.77	0.22	60.89				
6	0.303	46.19	0.217	61.45				

Note : -,%N was not determined because the latex coagulated.

2.4 Effect of speed of shaking on latex deproteinization

Time (hr)	100 rpm		120 rpm		150 rpm		200 rpm	
	%N	%N reduction	%N	%N reduction	%N	%N reduction	%N	%N reduction
0	0.594	0	0.594	0	0.594	0	0.594	0
2	0.345	42.01	0.316	46.89	0.316	46.82	0.300	49.44
4	0.308	48.14	0.279	53.09	0.258	56.63	0.275	56.76
6	0.276	53.49	0.246	58.58	0.245	58.67	0.234	60.56

3. Effect of viscosity-stability, reducing agent and nonionic detergent on latex deproteinization

3.1 Effect of Hydroxylamine hydrochloride (viscosity-stabilizer)

Time (hr)	0.10 p.h.r.		0.15 p.h.r.		control	
	%N	%N reduction	%N	%N reduction	%N	%N reduction
0	0.477	0	0.520	0	0.530	0
2	0.218	54.24	0.231	55.71	0.226	57.26
4	0.207	56.59	0.212	59.49	0.221	58.19
6	0.198	58.59	0.191	63.25	0.215	59.45

3.2 Effect of sodium metabisulfite (reducing agent)

Time (hr)	0.05 p.h.r.		control	
	%N	%N reduction	%N	%N reduction
0	0.543	0	0.579	0
2	0.225	58.59	0.241	58.47
4	0.210	61.68	0.238	58.92
6	0.201	63.07	0.234	59.60

3.3 Effect of Triton X-100 (nonionic detergent)

Time (hr)	1.0 p.h.r.		1.1 p.h.r.		1.2 p.h.r.		control	
	%N	%N reduction	%N	%N reduction	%N	%N reduction	%N	%N reduction
0	0.429	0	0.429	0	0.429	0	0.579	0
2	0.208	51.38	0.211	50.87	0.220	48.65	0.241	58.47
4	0.209	51.3	0.194	54.71	0.213	50.29	0.238	58.92
6	0.205	52.17	0.205	52.30	0.209	51.27	0.234	59.60

Note : when 1.3 p.h.r. of Triton X-100 was used, the latex was not coagulated.

4. Effect of activators, metal-chelating agent of papain

4.1 Effect of thiourea (activator) on latex deproteinization

Time (hr)	0.0020 p.h.r.		0.0023 p.h.r.		control	
	%N	%N reduction	%N	%N reduction	%N	%N reduction
0	0.550	0	0.502	0	0.524	0
2	0.186	66.23	0.131	73.88	0.233	55.63
4	0.165	70.61	0.131	73.73	0.211	59.54
6	0.146	73.85	0.123	75.54	0.211	59.58

4.2 Effect of cysteine (activator) on latex deproteinization

Time (hr)	0.001 p.h.r.		control	
	%N	%N reduction	%N	%N reduction
0	0.521	0	0.524	0
2	0.217	58.74	0.233	55.63
4	0.214	59.35	0.211	59.54
6	0.207	60.49	0.211	59.58

4.3 Effect of metal-chelating (EDTA) on latex deproteinization

Time (hr)	0.015 p.h.r.		control	
	%N	%N reduction	%N	%N reduction
0	0.508	0	0.524	0
2	0.235	53.99	0.233	55.63
4	0.220	57.04	0.211	59.54
6	0.210	58.75	0.211	59.58

5. Effect of latex dilution on latex deproteinization

5.1 Effect of latex dilution before enzyme treatment

Time (hr)	10 %drc		20 %drc		control	
	%N	%N reduction	%N	%N reduction	%N	%N reduction
0			0.469	0	0.511	0
2			0.129	72.56	0.24	53.12
4			0.106	77.33	0.232	54.52
6			0.107	76.92	0.232	54.55

Note : -, %N was not determined because the latex was not coagulated.

5.2 Effect of latex dilution after enzyme treatment

Time (hr)	Dilution 1:0.5		Dilution 1:1		control	
	%N	%N reduction	%N	%N reduction	%N	%N reduction
0	0.631	0			0.685	0
2	0.174	72.45			0.321	53.21
4	0.132	79.05			0.312	54.52
6	0.141	77.71			0.291	57.53

Note : -, %N was not determined because the latex was not coagulated.

6. Overall optimal conditions for latex deproteinization

Time	Conditions for rubber latex deproteinization					
	Latex diluted to 20% DRC before CIP treatment		Latex diluted with ratio 1:0.5 after CIP treatment		Control (25 % drc)	
	%Nitrogen	% N reduction	% Nitrogen	%N reduction	% Nitrogen	%N reduction
0	0.389 ± 0.022	0	0.358 ± 0.056	0	0.406 ± 0.036	0
1	0.178 ± 0.006	56.63 ± 1.55	0.144 ± 0.019	65.08 ± 4.62	0.185 ± 0.055	58.08 ± 12.51
2	0.106 ± 0.021	74.13 ± 5.13	0.148 ± 0.039	67.69 ± 4.81	0.153 ± 0.013	65.48 ± 2.97
3	0.093 ± 0.012	77.35 ± 2.92	0.099 ± 0.045	71.71 ± 2.12	0.132 ± 0.021	70.23 ± 4.72

Note : n=6

7. Batch reusability of immobilized papain on chitin for latex deproteinization

Used time	%g Nitrogen	% N reduction	wt. of dry rubber (g)	% drc lost
1	0.165 ± 0.007	78.85 ± 1.02	4.014 ± 0.319	13.34
2	0.217 ± 0.013	68.38 ± 1.98	3.078 ± 0.257	33.30
3	0.55 ± 0.058	19.66 ± 8.51	2.304 ± 0.161	50.70

Note : n=6

8. Raw rubber properties

8.1 <u>Raw rubber properties of DPNR produced from covalently-immobilized papain (CIP) and free papain (FP) and the control rubber.</u>

Raw rubber properties	DPNR CIP	DPNR FP	Control	DPNR of RRIM Specification	Proposed specification
Nitrogen content (g%), (n=4)	0.077 ± 0.003	0.110 ± 0.006	0.498 ± 0.006	0.12	no sample > 0.12
Dirt content (g%),(n=3)	0.017 ± 0.001	0.018 ± 0.001	0.025 ± 0.004	0.005	no test value > 0.015
Ash content (g%),(n=3)	0.183 ± 0.003	0.205 ± 0.012	0.210 ± 0.012	0.13	
Volatile matter (g%),(n=3)	0.156 ± 0.005	0.190 ± 0.005	0.283 ± 0.005	0.25	no value > 0.5
Initial Plasticity (Po),(n=3)	44.00 ± 1.73	38.33 ± 1.53	39.67 ± 0.58	32	
Plasticity Retention Index (PRI),(n=3)	79.45 ± 2.11	80.01 ± 2.68	84.02 ± 2.11	85	60 (minimum)
P (PH-Po),(n=3)	2.33 ± 0.95	5.00 ± 1.65	13.33 ± 1.05	7	no value > 9
Mooney viscosity (ML1+4),100C ,(n=3)	69.37 ± 1.00	66.37 ± 0.97	75.23 ± 1.84	51	45 - 55 55 - 65
Color Index, (n=3)	2.5	3.0	6.0		

8.2 Mooney viscosity before and after storage

Time (month)	DPNR CIP	DPNR FP	Control
0	70.7	62.3	77.2
1			
2	71.0	65.2	75.2
3	70.2	65.2	75.0
4			
5	70.0	65.3	75.2

9. Cure characteristics

Cure Characteristics	Vulcanized rubber (n=3)		
	DPNR CIP	DPNR FP	Control
Scorch time (ts) (sec)	68 ± 2	83 ± 1	104 ± 1
Cure time (t90) (sec)	111 ± 3	140 ± 2	157 ± 5
Cure rate (t90-ts) (sec)	41 ± 4	57 ± 1	54 ± 4
Torque rise (MH-ML),(kg-cm)	21.58 ± 3.02	23.27 ± 2.10	18.78 ± 1.33

10. The physical properties of unaged and aged vulcanized rubber

Physical properties of vulcanizates	DPNR CIP		DPNR FP		Control	
	Unaged rubber	Aged rubber	Unaged rubber	Aged rubber	Unaged rubber	Aged rubber
Specific gravity ,(n=3)	1.12 ± 0.01	1.13 ± 0.00	1.13 ± 0.00	1.13 ± 0.00	1.13 ± 0.00	1.14 ± 0.00
Hardness (shore A) , (n=3)	52.6 ± 0.3	56.9 ± 3.2	53.2 ± 1.1	61.0 ± 2.8	59.5 ± 0.8	63.4 ± 0.3
Tensile strength (MPa) , (n=6)	27.03 ± 1.27	28.52 ± 0.67	24.86 ± 1.10	26.87 ± 0.63	21.64± 0.67	26.31 ± 0.42
%Elongation at break , (n=6)	708.6 ± 17.3	659.4 ± 7.5	731.5 ± 38.8	665.7 ± 5.9	684.7 ± 41.9	624.9 ± 10.2
300% modulus (MPa) , (n=6)	2.452 ± 0.064	5.653± 0.220	2.434 ± 0.184	6.032 ± 0.331	2.449 ± 0.259	8.403 ± 0.294

APPENDIX 3

Testing of the properties of DPNR

1 Raw rubber testing (RRIM,1970)

1.1 Determination of dirt content

About 30 g of homogenized rubber was passed twice through a cold mill with 0.330 mm nip setting. A test portion of approximately 10 g was accurately weighted, cut into small strips and placed in a 500 ml conical flask containing 250 ml of mineral turpentine and 1 ml of peptizing agent RPA No.3. (xylyl mercaptan (36%)). The flask with its contents was heated at 140 °C by infrared lamps with occasional agitation until dissolution was complete (3 hr). The hot rubber solution was filtered through a previously weighed, cleaned and dried sieve. The flask was washed twice with 30 - 50 ml of hot mineral turpentine each time and filtered through the sieve. The dirt on the sieve was, then, washed again until free of rubber solution by hot mineral turpentine, dried in an oven at 90-100°C for one hour, cooled in a desiccator and weighed to the nearest 0.1 mg. The percentage of dirt content was expressed as follows :

$$\text{Percentage of dirt (w/w)} = \frac{\text{weight of dirt (g)} \times 100}{\text{weight of rubber specimen (g)}}$$

1.2 Determination of ash content

The ash from natural rubber contains oxides in varying proportions, carbonates and phosphates of potassium,magnesium,calcium ,sodium and other trace elements. Additionally it can contain silica and silicates arising either from the rubber or from extraneous foreign matter to an extent depending on the history of the material. The ash represents as minimal figure for the amount of mineral matter present in the rubber. Weigh accurately 5-10 g test portion of the homoginized rubber. Wrap in ashless filter paper and place in a crucible which was previously ignited and weighed. Introduce the crucible into muffle furnace controlled at a temperature of $550 \pm 20\,^{\circ}\mathrm{C}$ until free from carbon (3 hr). When ashing was complete, allow the crucible to cool in a desiccator and then weigh it to the nearest 0.1 mg. The ash content was calculated as follows :

$$\text{Ash content (\% wt)} = \frac{\text{weight of ash (g)} \times 100}{\text{weight of test portion (g)}}$$

1.3 Determination of volatile matter

The volatile matter is primirily intended for the determination of moisture and the amount of any other material which is volatile at $100\,^{\circ}\mathrm{C}$ in raw rubber. The homogenized rubber was weighed approximately 10 g (to the nearest 0.1 mg) and then passed through the cold mill rolls, with nip setting at 0.5 mm. The test portion was placed on aluminum tray and heated in an oven at $100 \pm 3\,^{\circ}\mathrm{C}$ for 4 hr. After heating, each test portion was kept in a polythene bag and hung on the rack to cool down for half an hour in an air conditioned room, then weighed to the nearest 0.1 mg. The volatile matter was calculated as follows :

$$\text{Volatile matter (\%)} = \frac{(A-B) \times 100}{A}$$

where A : weight of test portion before drying (g)

B : weight of test portion after drying (g)

1.4 Determination of nitrogen content

Nitrogen occured in raw rubber chiefly as protein and it can be used to provide an indication of the protein content, by protein = 6.25 x % nitrogen content.

Rubber specimen was weighed accurately about 0.2 - 0.3 g into a semi micro - Kjeldahl tube and 0.65 g of catalyst mixture (K_2SO_4: $CuSO_4.5H_2O$: SeO = 30:4:1) and 7.5 ml of concentrated sulfuric acid were added. The mixture was boiled gently in the digestion unit until the solution becomes clear green or colorless with no yellow tint and cooled. The cool digestion was transfered to the distillation flask followed by three time washing with distilled water. Add 10 ml of boric acid solution (20 g/l) to the receiving flask and 2-3 drops of indicator solution, which is the mixture of methyl red and bromcresol green. Place the receiver and dip the end of the condenser below the surface of the H_3BO_3 solution. Add 40 % NaOH solution to the distillation flask and pass steam through the distillation apparatus until the volume of distillate in the receiving flask reaches 150 ml. Lower the receiver and wash the end of the condenser with water. Immediately titrate the distillate with standardized 0.01 M H_2SO_4. Blank can be prepared by adding all reagents but omitting the sample. Total nitrogen was calculated as follows :

$$\% \text{ Total nitrogen} = \frac{2.8 \times (V_s - V_b) \times M}{W}$$

where V_s = ml of H_2SO_4 required for titration of the content of receiving flask

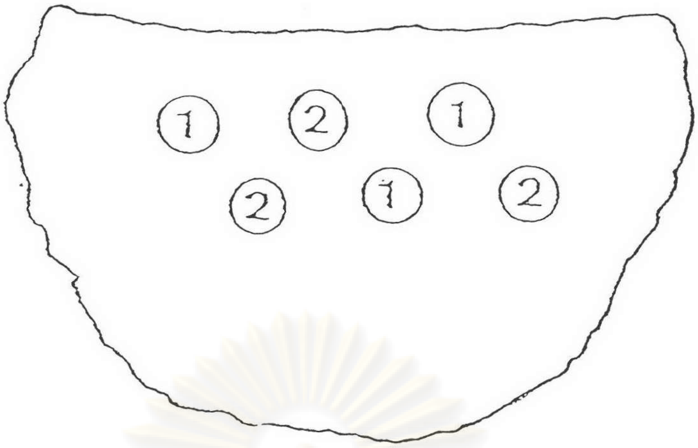
V_b = ml of H_2SO_4 required for titration of the blank

M = Molarity of H_2SO_4

W = The weight of sample used (g)

1.5 Determination of initial plasticity and plasticity retention index (PRI)

The plasticity retention index (PRI) test has been developed as a simple and rapid method for measuring the resistance of raw rubber to oxidative breakdown on heating. The test involved measurement of the rapid plasticity of the rubber test pieces before and after a short period of heating in an air oven. The rapid plasticity is measured with Wallace rapid plastimeter. A high value of PRI denotes high resistance to oxidative breakdown. A test portion of 20 ± 5 g of homogenized piece was passed twice through a two-roll mill (doubling the sheet between passes) with nip setting adjusted such that the final sheet thickness was 1.6-1.8 mm, then immediately doubled and pressed tightly together by hand. Six test pellets were cut from the doubled sheet with the Wallace punch as illustrated below.



Punched pellets for 1. initial plasticity determination
2. aged plasticity determination

Figure 37 Illustration of testpiece punching from rubber sheet

The test piece were divided into two sets each for plasticity determination before and after oven ageing. For ageing, the testpieces were heated in oven for 30 min at 140 °C. Then they were removed and allowed to cool to room temperature. The pellet piece was sandwiched between 2 pieces of cigarette paper and pressed between the two parallel plates to a fixed thickness of 1 mm with compressive force of $10 \pm 0.1\ \mathrm{kg_f}$ for 15 seconds of Wallace rapid plastimeter. The median of the three unaged and three aged test pieces were used to calculate the PRI as follow :

$$PRI = \frac{\text{Aged median plasticity value} \times 100}{\text{Unaged median plasticity value}}$$

1.6 Characterization of Viscosity-stabilized rubber

The accelerated - storage hardening test (ASHT) provides an indication of the propensity of solid natural rubber to increase its viscosity during storage as a result of the formation of cross-links between the rubber molecules. These cross-link formations are contributed largely from some condensation reactions involving aldehyde groups naturally present in the rubber molecules. ASHT involved the measurement of the Wallace rapid plasticity of test pieces before and after a short period of storage under condition which accelerated the storage-hardening reaction. The test piece was prepared by the same method as for PRI test and devided into two sets. One set of 3 pellets was placed on the platform in a weighing bottle containing 6-8 g of P_2O_5 and storage-hardened in an oven at $60 \pm 1^{o}C$ for 24 hr while another set was in normal atmosphere at room temperature. After 24 hr the plasticity of all testpieces were determined by Wallace plastimeter as well as PRI test. The median value of each set was reported and the accelerated storage-hardening (Δ P) should be expressed as

$$\Delta P = P_H - P_O$$

where P = difference in plasticity units between P_H and P_O

P_H = median plasticity value of 3 storage-hardened testpieces

P_O = median plasticity value of 3 non-storage hardened testpieces.

1.7 Determination of color index

The color of the raw rubber was compared and matched as closely as possible with that of standard colored glasses. The numerically higher index values have deeper color. The testpiece of homogenized rubber was prepared by the same procedure as PRI test. Two disc-shaped pellets were put together and pressed in the mold between two sheets of polymer or cellulose film using mold covers at not less than 3.5 MN/m^2 pressure on the cavity areas of the mold for 5 min at 150 $\pm$ 3^{o}C. The color of testpiece was determined as Lovibond index by matching as closely as possible to the approximate color standard lighting box. The result was shown as index number of color glass.

1.8 Determination of the Mooney viscosity (ASTM D1646, 1988)

The viscosity of raw rubber was determined in a Mooney viscometer SMV 201. Before loading the rubber, die cavity and roter should be heated up to the test temperature,100^{o}C. About 25 g of the homogenized rubber was cut into 2 portions. One portion was placed in the lower die cavity and the roter was placed followed by another portion placed on top of the roter and the die was closed immediately. Preheat the specimen for 1 min before starting the motor and set the running time with the motor on for 4 minutes. The viscosity was reported as Mooney unit, ML (1+4) at 100 ^{o}C (the rotor size, L for large,the number of minutes for warming up in the machine (1 min), the number of minutes of actual test (4 min) and temperature (100^{o}C).

1.9 Molecular weight averages and molecular weight distribution by gel permeation chromatography (GPC)

(ASTM D3536,1980)

Weigh 0.01 g of rubber sample into a 20 ml vial with screw cap containing 10 ml of THF. The sample was dissolved at room temperature and aided by stirring until the dissolution was complete. The solution was filtered through a membrane filter with pore size 5 µm primarily to remove lint and other materials likely to obstruct the GPC column. About 100 µl of filtrate was filled in a syringe, expeled air from the syringe and injected into the sample injection position. The solution was introduced into series of four columns packed with styragel and having nominal exclusion limits of 10^4 ^{o}A, 10^5 ^{o}A , 10^6 ^{o}A and linear (Mw range 2,000 to $>10^7$) by THF solvent at the flow rate 1 ml / min at 35 ^{o}C. The Mw and MWD of the sample was determined by using polystyrene standard calibration graph (Mw standard range $8.5 \times 10^3 - 1.06 \times 10^7$).

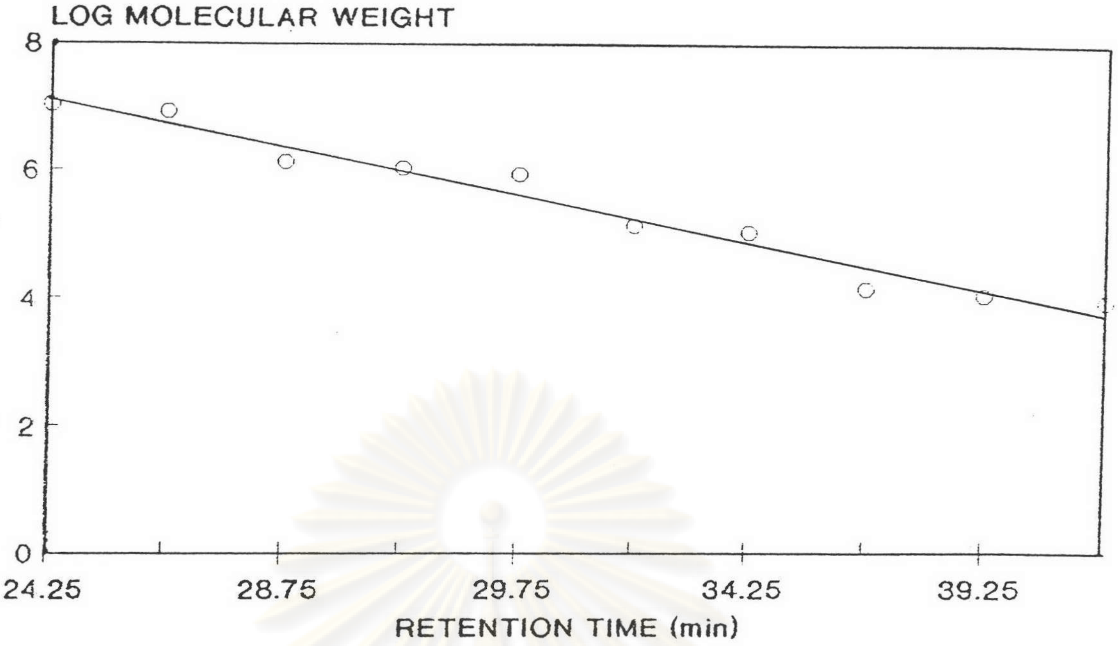


Figure 38 The molecular weight calibration curve of polystyrene standard.

2 Test of the rubber valcanization (ASTM D412 ,1989)

2.1 Preparation of rubber compounding

Preparation of rubber compound using sulfur as a vulcanizing agent is presently the most popular system. The compounding formulation in Table 2.1 to prepare compounds for comparison of the cure characteristics and physical properties of DPNR from immobilized papain and DPNR from free papain and control rubber.

Table 10 The compounding formulation chosen for assessing the cure behavior of natural rubber

Ingredient	Gram
Natural rubber	100.0
Hisil 233 S	45.0
Zinc oxide active	3.0
22 cp 46	0.3
Wax	1.0
Stearic acid	1.2
DEG	3.5
Shellflex	1.5
Sulfur	2.0
MBTS	0.78
MBT	0.2
TMTM	0.12

where

- Hisil 233S (precipitated silica) is reinforcing filler.
- ZnO active and stearic acid are the activators of vulcanization.
- MBTS(2-2 di-benzothiazyl disulfide),MBT(2-mercaptobenzothiazole) and TMTM (tetramethyl thiuram monosulfide) are accelerators of vulcanization.
- 22 CP 46 (2,2 methylene bis-4-methyl-6-p-butyl phenol) and wax are antioxidants.
- DEG (diethylene glycol) is a depressor of surface active absorption.
- Shellflex is processing oil.
- Sulfur is the crosslink agent.

compounding was carried out on a smooth two-roll mill at room temperature by adding the chemicals as follows :

1. Homogenized rubber for 3 minutes
2. Add Hisil 233S +DEG + Shellflex +Stearic acid and mix for 7 min.
3. Add wax +22 CP 46 +ZnO active and mix for 3 min and then leave until the compound cool down to room temperature.
4. Add MBTS + MBT + TMTM and mix for 3 min.
5. Finally add sulfur and mix for 2 min.

The compound was kept at room temperature for 1 day before determination of its cure characteristics.

2.2 Cure characteristics

The cure characteristics of the compound rubber was run on a

Rheometer model EK-100H (EEKORNER ,Taiwan) for 7 min at 155 ^{o}C. From the cure curve recorded (Figure A 3.3), all the necessary reading was determined and reported.

The important cure parameters were scorch time, cure rate, time to optimum cure, and maximum modulus. Scorch time was the minimum safe time for processing the compounded rubber before the rubber converted from a plastic to vulcanized state where further processing is immpossible. Cure rate determined the time to cure a given product to its optimum state.

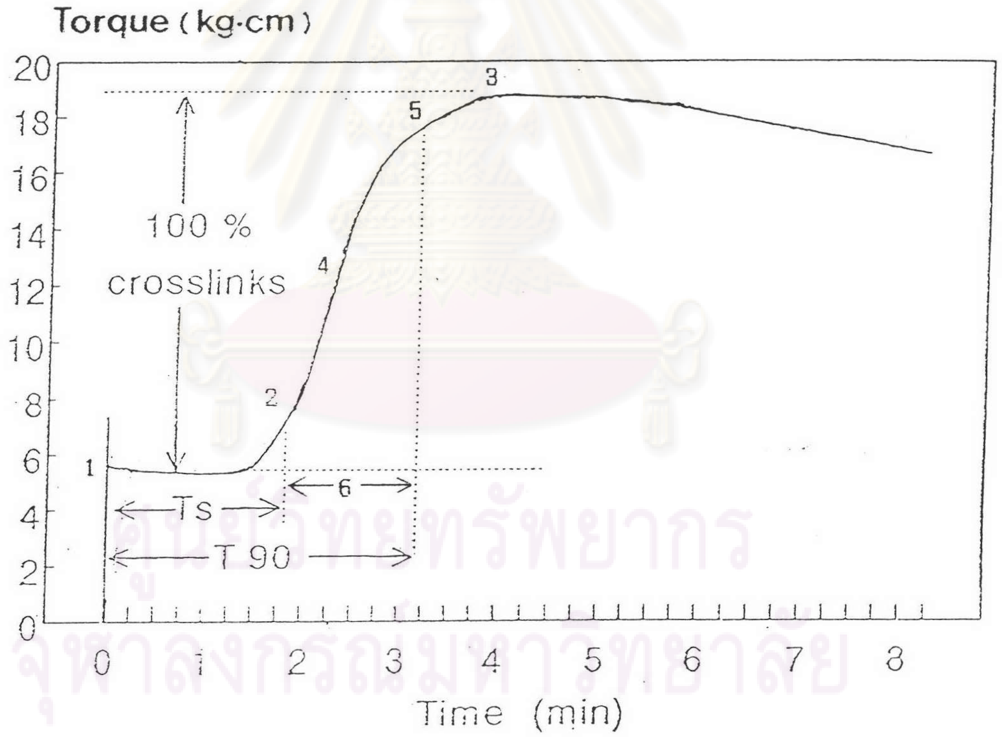


Figure 39 Rheometer curing curve

where

1 = minimum viscosity or minimum modulus, M_L

2 = scorch point (1 torque unit rise above minimum viscosity), T_s

3 = maximum modulus, M_H

4 = 50 % crosslinks, T_{50}-time to 50 % crosslink

5 = optimum cure (90% crosslinks), T_{90}-time to optimum cure

6 = cure rate, T_{90}-T_s

2.3 Testpiece preparation for measuring physical properties

The compound samples were vulcanized in a compression mold at 155 ^{o}C for a period of time to reach optimum cure indicated by rheometer graph and left for 24 hr before cutting into testpiece.

The physical properties usually measured are :Hardness (Shore A type), Specific gravity, Tensile strength, % Elongation at break and 300 % Modulus.

2.4 Test for physical properties of vulcanizates

2.4.1 Hardness (Shore A) (ASTM D1415, 1988)

The international hardness test is based on measurement of the penetration of a rigid ball into the rubber specimen under specified condition. The vulcanized rubber was prepared as a flat and smooth sheet having thickness suffucient to fit the gap of type A durometer. The plunger of durometer was pressed with the minor force on to the specimen, the scale was pointed and read as the hardness in International Rubber Hardness Degrees (IRHD) at room temperature. The median value of 5 different points distributed over the specimen was recorded.

2.4.2 Specific gravity (ASTM D3184, 1989)

This method was based on water replacement concept. The weight of rubber vulcanized testpiece was weighed in the air and recorded as W_a, and then weighed again in the water recorded as Ww. The specifoc gravity of rubber vulcanized was determineed by equation as below :

$$\text{Specific gravity} = \frac{W_a}{W_w}$$

2.4.3 Tensile strength , 300% modulus and Elongation at break (ASTM D412 ,1987)

The 6 dumb-bell testpieces (Figure A 3.4) were cut out from the vulcanized rubber by punching with die using a single stroke of a press. A reference of length 2.0 cm was marked and then thickness of the testpieces measured along the reference length by a micrometer dial guage.

The two ends of the testpiece were clamped into the two grips of the testing machine (LLOYD Instrument model LR5K). The test piece was stretched at a constant rate of moving grip of 500 ± 50 mm. The force per cross-section area of testpiece required to stretch the sample to 300 % reference mark length and to breakage were automatically recorded as 300 % Modulus and tensile strength (stress at break) respectively. The maximum stretching, 300 % modulus and % elongation at break were recorded by a graph recorder.

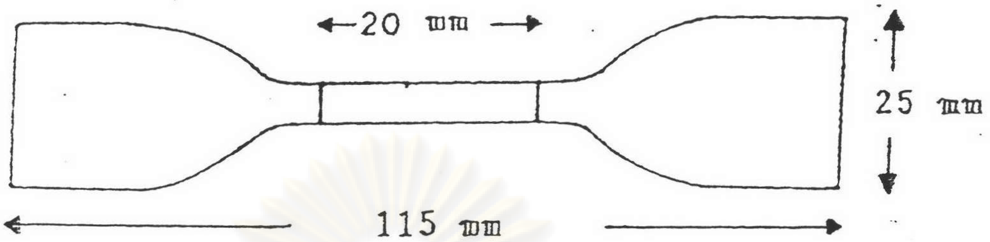


Figure 40 Shape of dumb-bell test piece

BIOGRAPHY

Alisa Vangnai was born on December 31, 1970. She graduated with A Bachelor degree in the Department of Food Technology, majoring in Food and Biotechnology from the Faculty of Science, Chulalongkorn University in 1991 and continued her study in the Master Degree in the Program of Biotechnology at the same University with the scholarship awarded by the University Development Committee (UDC) in 1991-1993.