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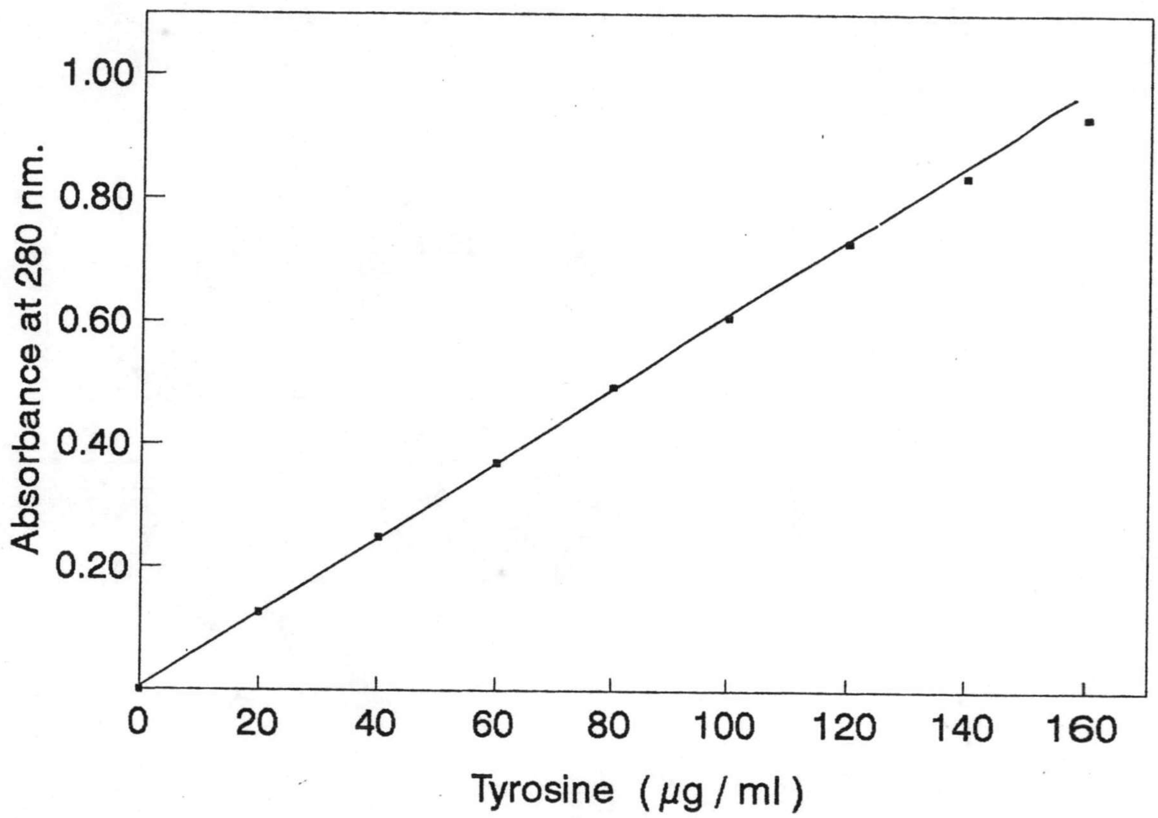
APPENDIX

APPENDIX 1

Tyrosine standard curve



APPENDIX 2

Optimal condition for Alcalase activity ,using casein as substrate

1. Effect of pH on Alcalase activity

pH	Alcalase activity (CDU /mg)	% Activity
7.2	184.8 ± 6.8	25.6
7.6	353.1 ± 19.2	48.9
8.0	488.7 ± 21.9	67.5
8.4	553.0 ± 16.4	76.5
8.8	611.9 ± 5.5	84.7
9.4	707.7 ± 13.7	97.9
9.8	722.8 ± 8.2	100.0
10.0	710.4 ± 13.7	98.3
10.6	532.5 ± 7.4	73.7

2. Effect of temperature on Alcalase activity

Temperature °C	Alcalase activity (CDU /mg)	% Activity
25	25.7 ± 4.8	5.9
30	84.3 ± 12.7	19.3
35	138.1 ± 12.8	31.6
40	168.6 ± 1.6	38.5
45	282.1 ± 22.7	64.4
50	376.2 ± 9.7	85.9
55	437.8 ± 27.6	100.0
60	405.3 ± 50.3	92.6
65	49.6 ± 6.6	11.3
70	0	0

APPENDIX 3

Optimal condition for Papain activity ,using casein as substrate

1. Effect of pH on Papain activity

pH	Papain activity (CDU /mg)	% Activity
5.7	456.0 ± 22.5	74.0
6.0	552.4 ± 22.5	89.6
6.5	600.6 ± 9.6	97.4
7.0	616.6 ± 3.2	100.0
7.5	497.8 ± 6.4	80.7
8.0	449.6 ± 22.5	72.9
8.6	427.1 ± 25.7	69.5
9.0	391.8 ± 19.3	63.5

2. Effect of temperature on Papain activity

Temperature ^{o}C	Papain activity (CDU /mg)	% Activity
30	114.6 ± 7.1	54.2
35	115.3 ± 9.9	54.5
40	147.9 ± 8.5	69.9
45	157.8 ± 0.7	74.6
50	191.1 ± 4.2	90.3
55	186.8 ± 17.0	88.3
60	211.6 ± 7.1	100.0
65	148.6 ± 5.0	70.2
70	101.2 ± 9.0	47.8

APPENDIX 4

Optimal condition for concentrated latex deproteinization by Alcalase

1) Effect of pH on latex deproteinization

Time (h)	At pH 8-9		At pH 9-10		At pH 10-11	
	g%N	% Reduction	g%N	% Reduction	g%N	% Reduction
0	0.148	18.2	0.150	17.1	0.187	0
2	0.081	55.2	0.094	48.1	0.128	29.3
4	0.065	64.1	0.089	50.8	0.121	33.1
6	0.058	68.0	0.093	48.6	0.120	33.7
9	0.051	71.8	0.077	56.9	0.122	32.6
12	0.058	68.0	0.072	60.2	0.117	35.4
16	0.051	71.8	0.077	57.4	0.120	33.7
20	0.060	66.9	0.067	63.0	0.119	34.2
24	0.058	68.0	0.065	64.1	0.126	30.4

2) Effect of temperature on latex deproteinization

Time (h)	At 40 ^oC		At 50 ^oC		At 60 ^oC	
	g%N	% Reduction	g%N	% Reduction	g%N	% Reduction
0	0.129	28.7	0.148	18.2	0.119	34.2
2	0.083	54.1	0.081	55.2	0.062	65.7
4	0.073	59.7	0.065	64.1	0.068	62.4
6	0.058	68.0	0.058	68.0	0.063	65.2
9	0.053	70.7	0.051	71.8	0.066	63.5
20	0.034	81.2	0.058	68.0	0.060	66.8

3) Effect of Alcalase on latex deproteinization

Time (h)	Alcalase 0.1 p.h.r.		Alcalase 0.2 p.h.r.		Alcalase 0.3 p.h.r.	
	g%N	% Reduction	g%N	% Reduction	g%N	% Reduction
0	0.165	8.8	0.156	13.8	0.148	18.2
2	0.102	43.6	0.094	48.1	0.081	55.2
4	0.091	49.7	0.078	56.9	0.065	64.1
6	0.087	51.9	0.075	58.6	0.058	68.0
9	0.079	56.4	0.066	63.5	0.051	71.8
12	0.072	60.2	0.070	61.3	0.058	68.0
16	0.072	60.2	0.066	63.5	0.051	71.8
20	0.075	58.6	0.062	65.7	0.060	66.9
24	0.076	58.0	0.064	64.6	0.058	68.0

APPENDIX 5

Concentrated latex deproteinization by Papain

Time (h)	At pH 8-9		At pH 9-10		At pH 10-11	
	g%N	% Reduction	g%N	% Reduction	g%N	% Reduction
2	0.153	24.2	0.178	11.9	0.197	2.5
4	0.159	21.3	0.179	11.4	0.199	1.5
6	0.161	20.3	0.180	10.9	0.208	0
8	0.167	17.3	0.179	11.4	0.214	0
10	0.165	18.3	0.183	9.4	0.209	0
24	0.161	20.3	0.191	5.4	0.204	0

APPENDIX 6

Optimization of field latex deproteinization by Alcalase

Time (h)	% Nitrogen reduction								
	pH			Temperature (^{o}C)			Alcalase (p.h.r.)		
	8-9	9-10	10-11	40 ^{o}C	50 ^{o}C	60 ^{o}C	0.2	0.3	0.4
2	31.4	23.7	13.1	25.2	31.4	59.2	30.4	31.4	43.8
4	38.3	41.6	51.8	26.9	38.3	60.9	40.0	38.3	60.2
6	55.8	53.7	55.0	25.4	55.8	58.6	65.6	55.8	68.3
8	62.4	58.8	54.0	44.2	62.4	64.5	56.7	62.4	63.0
10	59.8	58.1	58.8	-	-	-	-	-	-

APPENDIX 7

Optimization of field latex deproteinization by Papain

Time (h)	% Nitrogen reduction									
	pH			Temperature (^{o}C)			Alcalase (p.h.r.)			
	7-8	8-9	9-10	40 ^{o}C	50 ^{o}C	60 ^{o}C	0.1	0.2	0.3	0.4
2	67.4	55.3	29.7	65.6	67.4	65.4	26.6	47.8	67.4	64.4
4	66.8	52.6	28.2	63.7	66.8	62.2	27.9	50.8	66.8	65.6
6	67.6	59.7	24.4	65.8	67.6	62.8	22.0	50.6	67.6	67.1
8	61.8	58.6	31.2	-	-	-	27.9	44.9	61.8	65.0

APPENDIX 8

Optimal time for latex deproteinization by enzyme of both concentrated latex 60 % and field latex

Time (h)	Conc.latex 60%		Field latex			
	Alcalase treatment		Papain treatment		Alcalase treatment	
	g%N	%Reduction	g%N	%Reduction	g%N	%Reduction
2	0.120	31.0	0.110	79.6	0.162	69.9
4	0.105	37.4	0.172	68.1	0.120	77.7
6	0.090	48.3	0.146	72.9	0.121	77.7
8	0.075	56.1	0.169	68.6	0.120	77.7
10	0.065	62.6	0.160	70.3	0.111	79.4
12	-	-	0.169	68.6	0.105	80.5
15	-	-	-	-	0.106	80.3
24	0.060	65.5	-	-	-	-

APPENDIX 9

Accerelated storage hardening test of deproteinized rubber and its control

Sample		P_O	P_H	$\Delta P(P_H-P_O)$	Sample		P_O	P_H	$\Delta P(P_H-P_O)$
	PCT1	61.5	88.5	27.0		GCT1	48.9	85.9	37.0
	PAT1	55.0	59.5	4.5		GAT1	40.3	45.5	5.2
	PPT1	56.0	57.0	1.0		GPT1	43.9	45.0	1.1
	PCT2	60.0	89.0	29.0		GCT2	51.5	89.5	38.0
PB 5/51	PAT2	54.5	57.5	3.0	GT 1	GAT2	38.5	41.5	3.0
	PPT2	56.5	59.0	2.5		GPT2	41.5	44.5	3.0
	PCT3	57.7	81.4	23.7		GCT3	51.3	84.9	33.6
	PAT3	58.6	64.8	6.2		GAT3	41.3	48.0	6.7
	PPT3	60.3	62.2	1.9		GPT3	40.3	45.7	3.4
	RCT1	30.4	73.5	43.1		C1	66.0	82.0	16.0
	RAT1	21.1	24.6	3.5		C2	63.0	80.0	17.0
	RPT1	23.1	28.2	5.1	Latex 60%	D1	43.0	51.0	8.0
	RCT2	21.0	77.0	56.0		D2	52.5	55.0	2.5
RRIM600	RAT2	22.0	24.5	2.5		D3	58.0	60.0	2.0
	RPT2	24.5	30.5	6.0					
	RCT3	42.5	67.1	24.6					
	RAT3	31.3	35.8	4.5					
	RPT3	31.5	36.6	5.1					

P_O : Initial plasticity ; P_H : Aged plasticity

-CT- : Untreated rubber as a control of that experiment

-AT- : Alcalase-treated rubber ; -PT- : Papain-treated rubber

C- : Control rubber of concentrated latex 60 %

D- : Enzyme-treated rubber

APPENDIX 10

Summary of cure parameters of compound rubber from various sources in outsole mixing formulation

Sample	Scorch time (min)	Cure time (min)	Torque rise (in.lb.)	Cure rate (in.lb./min)
PB 5/51				
Control	1.52 ± 0.10	2.82 ± 0.21	32.05 ± 0.96	0.91 ± 0.13
+ Alcalase	1.78 ± 0.19	3.22 ± 0.41	31.98 ± 2.36	1.03 ± 0.24
+ Papain	1.78 ± 0.24	3.20 ± 0.42	33.55 ± 3.01	1.04 ± 0.21
GT 1				
Control	1.30 ± 0.43	3.30 ± 0.35	28.95 ± 1.90	0.49 ± 0.17
+ Alcalase	1.60 ± 0.16	2.78 ± 0.57	28.70 ± 3.12	0.90 ± 0.13
+ Papain	1.65 ± 0.17	3.02 ± 0.34	30.80 ± 2.42	0.89 ± 0.07
RRIM 600				
Control	1.67 ± 0.06	3.60 ± 0.10	30.60 ± 3.80	0.69 ± 0.11
+ Alcalase	1.88 ± 0.43	3.62 ± 0.46	29.20 ± 1.32	0.66 ± 0.10
+ Papain	1.52 ± 0.08	3.00 ± 0.20	29.00 ± 1.80	0.92 ± 0.10
Latex 60 %				
Control	1.60 ± 0.10	2.70 ± 0.40	34.60 ± 1.30	1.12 ± 0.28
+ Alcalase	1.53 ± 0.06	2.50 ± 0.25	35.80 ± 1.00	1.29 ± 0.06
Crepe rubber	1.53 ± 0.12	3.00 ± 0.62	34.80 ± 2.67	1.06 ± 0.28
TTR5L	1.50 ± 0.05	2.60 ± 0.10	34.85 ± 2.05	0.98 ± 0.04

APPENDIX 11

Physical properties of vulcanizate rubber from various sources

1) From concentrated latex 60 % and commercially used rubber, TTR 5L and crepe rubber

Physical test	Concentrated latex 60%		CREPE	TTR5L
	Control	DPNR		
Hardness(Type A)	52 ± 1	52 ± 1	56 ± 1	55 ± 1
300 % Modulus (kg/cm^2)	18.0 ± 2.1	17.0 ±0.2	18.4 ±1.7	19.9 ±0.5
Elongation at break (%)	827.0±23.9	839.3±17.8	798.3±21.1	761.0±1.7
Tensile strength (kg/cm^2)	201.7±20.9	211.8±12.7	176.1±35.5	165.8±1.6
Specific gravity	1.11	1.11	1.11	1.11

2) From clonal field latex

Physical test	PB 5/51			GT 1			RRIM 600		
	Control	+Alcalase	+Papain	Control	+Alcalase	+Papain	Control	+Alcalase	+Papain
Hardness(Type A)	58 ± 1	53 ± 2	52 ± 2	56 ± 1	52 ± 1	49 ± 2	51 ± 2	48 ± 2	50 ± 2
300 % Modulus (kg/cm^2)	18.0±1.3	16.6±1.9	15.0±2.0	16.9±1.6	15.4±1.5	14.4±0.8	17.9 ±1.2	13.7 ±1.4	14.2 ±1.4
Elongation at break (%)	730.8±29.0	784.8±19.9	820.0±31.2	713.4±49.4	755.9±55.8	741.8±58.9	747.3±5.5	833.8±20.8	839.3±22.5
Tensile strength (kg/cm^2)	127.5±17.2	130.8±15.8	135.7±23.1	105.7±9.9	129.8±21.7	128.3±21.0	125.2±24.5	142.4±25.3	155.8±20.5
Specific gravity	1.12	1.12	1.12	1.12	1.12	1.11	1.11	1.11	1.10

BIOGRAPHY

Wonnop Visessanguan was born on November 18, 1968. He conferred his Bachelor degree of Science with second class honours in Biochemistry from Chulalongkorn University in 1989 and continued his study in Master program at the same department with the fellowship awarded by the Science and Technology Development Board (STDB) in 1989-1991. He received the award from the Tab Nilanidhi foundation as the first rank in Biochemistry program of Chulalongkorn University in 1990 and was one of the representative youths from Thailand to participate in Ship for South East Asian Youth Program 1991.