REFERENCES

Aamir, R., Safadi, G.S., Melton, A.L., Wagner, W.O., Pien, L.C., Cornish, K., et al. 1996. Shared IgE-binding sites among separated components of natural rubber latex. Int Arch Allergy Immunol. 111:48-54.

Akasawa, A., Hsieh, L., Lin, Y. 1995. Serum reactivities to latex proteins (*Hevea brasiliensis*). J.Allergy Clin.Immunol. 95:1196-1205.

Akasawa, A., Matsumoto, K., Saito, H. 1993. Incidence of latex allergy in atopic children and hospital workers in Japan. Int.Arch.Allergy Immnol. 101:177-181.

Alenius, H., Kalkkinen, N., Lukka, M., Reunala, T., Turjanmaa, K., and Makinen-Kiljunen, S. 1995. Prohevein from the rubber tree (*Hevea brasiliensis*) is a major latex allergen. Clin Exp. Allergy. 25:65-665.

Alenius, H., Kalkkinen, N., Lukka, M., Reunala, T., Turjanmaa, K., Reunala, T., and Makinen-Kiljunen, S. 1995. Purification and partial amino acid sequencing of a 27 kD natural rubber allergen recognized by latex allegic children with spina bifida. Int Arch Allergy Immunol. 106:258-262.

Alenius, H., Kalkkinen, N., Yip, E., Hamin, H., Turjanmaa, K., Makinen-Kiljunen, S., and et al. 1995. Significance of rubber elongation factor as a latex allergen. Int Arch Allergy Immunol. 109:362-368.

Alenius, H., Reunala, T., Turjanmaa, K., and Palossuo, T. 1992. Detection of IgG4 and IgE antibodies to rubber proteins by immunoblotting in latex allergy. Allergy Proc. 13:75-77.

Alenius, H., Palossuo, T., Kelly, K., Kurup, V., Reunala, T., Makinen-Kiljunen. S., et al. 1993. IgE reactivity to 14 kD and 27 kd natural rubber proteins in latex-allergic children with spina bifida and other congenital anomalies. Int.Arch Allergy Immunol. 102:61-66.

Alenius, H., Turjanmaa, K., Makinen-Kiljunen, S., Reunala, T., 1994. IgE immune response to rubber proteins in adult patients with latex allergy. J.Allergy Clin.Immunol. 93:859-863.

Alenius, H., Turjanmaa, K., Palossuo, T., Makinen-Kiljunen, S., Reunala, T., 1991. Surgical latex glove allergy. Characterization of rubber protein allergens by immunoblotting. Int.Arch.Allergy Appl.Immunol. 96:376-380.

Altman, R.F.A. 1948. Natural vulcanization accelerators in Hevea latex. Ind. Eng.

Chem. 40:24.

American Society for testing and Materials –D624. 1986. Standard test method for rubber property-tear resistance. Annual book of ASTM 09.01:113-116.

American Society for testing and Materials-1D412.1987.Stand test method for rubber property in tension. Annual book of ASTM 09.01:39-50.

American Society for testing and Materials-D415. 1988. Stands test method for rubber property-International hardness. Annual book of ASTM 09.01:223-226.

American Society for testing and Materials- D415. 1646. Standard test method for rubber viscosity and vulcanization characteristic (Mooney viscometer). Annual book of ASTM 09.01:317-324.

Archer, B.L. 1976. Hevamine: a crystalline basic protein form *Hevea brasiliensiss* latex. Phytochemistry. 15:297-300.

Allanyake, D.P. Kekwick, R.G., and Franklin, F.C. 1991.Molecular cloning and nucleotide sequencing of the rubber elongation factor gene from *Hevea brasiliensis*. Plant. Mol. Biol. 16:1079-1081.

Axelsson, IGK., Eriksson, M., and Wrangsjo, K. 1987., IgE-mediated anaphylactoid reactions to rubber. Allergy. 42:46-50.

Bloomfield. G.F. 1973. Super rubbers for engineers. Rubber Developments. 26:74-77.

Bristow, G.M. 1990. Composition and cure behavior of skim block natrual rubber. J.nat.Rubb.Res. 5:114-134.

Chambeyron, C., Dry. J., Leynadier, F., Pecquet, C., and Thao,T.X. 1992. Allergy. 47:92-97.

Chang, W.P., Lau, C.M., and Nambiar, J. 1977. Deproteinised natural rubber from field latex. Proceddings of RRIM Planter's Conference. :295-306.

Chen, Z., Czuppon, A.B., and Baur, X. 1995. Analysis of continue allergenic B-cell epitopes on latex allergen Hev B1 Elisa using biotinylated peptides (abstract). J. Allergy Clin Immunol. 95:154.

Chiu, A.M., Murali, P.S., Fink, J.N., Kelly, K.J., and Kurup, V.P. 1997. Vrliulst immune response to purified latex proteins (abstract). J. Allergy Clin. Immunol. 99(Suppl):S343.

Czuppon, A.B, Chen, Z., Rennert, S., Engelke, T., Meyer, H.E., Heber, M., and Baur, X. 1993. The elongation factor of rubber trees (*Hevea brasiliensis*) is the

major allergen in latex. J.Allergy Clin. Immunol. 92:690-697.

Dennis, M., Henzel, W., Bell, J., Kohr, WI, and Light, D.R. 1989. Amino acid sequence of rubber elongation factor protein associated rubber particles in *Hevea* latex. J. Biol Chem. 264:18618-18726.

Downing, J.G. 1993. Dermatitis from rubber gloves. J. Med. 208:196.

Eng, A.H., and Tanaka, Y. 1992. Short communication FTIR studied on amino groups in purified *Hevea* rubber. J.nat.Rubb.Res. 7(2):152-155.

Eng, A.H., and Tanaka, Y. 1993. Properties of deproteinsed natural rubber latex. Proc.Int.Rubb.Tech.Conf. 101-110.

Firestone Tyre Rubber Company. 1955. Improvements in/or relating to high grade rubber and method of making the same. Br.Pat.No.739:750.

Gonzales, E. 1992. Latex hypersensitivity: a new and unexpected problem. Hospital Practices. 27:137-140.

Greensmith, H.W., and Watson, A.A. 1969. Studies on the curing characterics of natural rubber. J.nat.Rubb.Res.Inst.Malaya. 22:120-130.

Harncharoen, K. 1996. Development of immunoassay for the detection of protein allergens in natural rubber products. Doctor's thesis, Philosophy in Biological Sciences, Graduate School, Chulalongkorn University.

Hasma, H. 1992. Proteins of natural rubber latex concentrate. J.nat.Rubb. 7 (2): 102-112.

Heese, A., Hintzenstern, J.V., Peters, K.P, et al. 1995. Allergic and irritant reactions to rubber gloves in medical health services. J.Ann.Acad.Dermatal. 101:597.

Jacob, J.L, d'Auzac J., and Prevot J.C. 1993. The composition of latex from *Hevea brasiliensiss*. Cli.Rev.Al. 11:327-337.

Jaeger, D., Kleinhans, D., Czuppon, A.B., and Baur, X. 1992. Latex-specific proteins causing immediate-type cutaneous, nasal, bronchial, and systemic reactions. J.Allergy Clin.Immunol. 89(3):759-768.

John, C.K. 1971. Coagulation of Hevea latex with surfactant an salt I. Development of the process and its effect on raw rubber properties. J. Rubb. Inst. Malaya. 23(2): 147.

John, C.K., Nadarajah, M., and Chan, B.L. 1977. Enzyme treatment of Hevea latex to obtain superior quality rubber. J. Rubb. Inst. Sri Lanka. 54: 610-629.

Kekwick, R.G.O. 1993. Origin and source of latex protein allergy. Latex protein allergy: the present position. UK: Rubber Consultants. pp. 21-24.

Loo, C.T., and Yong. W.M. 1977. Influence of non-rubber separated from *Hevea* latex on heat build up and related dynamic properties. Rubb.Res.Inst. Malaysia.

Merrett, T.G., Merrett, J. ,Bhambri, S., and Kekwick, R. 1993. The prevalence of anti-IgE antibodies in the UK (abstract). J. Allergy Clin.Immunol. 92:668-677.

Moir, G.F.J. 1959. Ultracentrifugation and staining of *Hevea* latex. J.nat.Rubb.Res. 9:127-130.

Moneret-Vautrin D., Beaudoin E., Widmer S., outon, C., Kanny G., Prestat, F., et al. 1993.. Prospectives study of risk factors in natural rubber latex hypersensitivity. J. Allergy Clin.Immunol. 92:668-677.

Morales, C., Bassomba, A., Carrieira, J., Sastre, A. 1989. Anaphylaxis produced by rubber glove contact. Case reports and immunological identification of antigens involve. Cli.Exp.Allergy. 19:425.430.

Nakade, S., Kuga, A., Hayashi, M., and Tanaka, Y. 1997. Highly purified natural rubber IV. Preparation and characteristics of gloves and condoms. J.nat.Rubb. 12(1):33-28.

Nutter, A.F. 1979. Contact urticaria to rubber. Br.J.Dermatology. 101:597.598.

Ownby DR, Ownby, H.E., McCullough, B.A., and Shafer, A.W. 1994. The prevalence of anti-IgE antibodies in 100 volunteer blood donors (abstract). J. Allergy Clin.Immunol. 93:282.

Porri, F., Lemiere, C., Birnbaum, J., Guilloux, L., Didelot, R., Vervloet, D., et al. 1995. Prevalence of anti-IgE antibodies in atopic and non-atopic subjects from the general population. J. Allergy Clin.Immunol. 95:154.

Raulf-Heimsoth, M., Chen.Z., Zliebers, V., Allmers, H., and Baur, X. 1996. Lymphocyte proliferation response to extracts from different latex materials and to the purified latex allergen Hev b 1 (rubber elongation factor). Int Arch Allergy Immunol. 106:258-262. 98:640-651.

Rubber Research Institute department of Agriculture. 1999. Thailand Rubber Statistic. 28(1-2): 1-29.

Rungvichaniwat, D., Nithi-Uthai, B., Nithi-Uthai, P., 1998. The color and nitrogen content of skim rubber. The 7th international seminar on elastomer.1-8.

Sivabalsunderam, J., and Nadarajat, M. 1965. Fator affecting the plasticity retention index. Proc. Int. Rubb. Conf. Sri Lanka. 13-28.

Slater, J.E. 1989. Rubber anaphylaxis. N.Engl.J.Med. 320:1126-1131.

Slater, J.E., and Chhabra, S. 1989. Latex antigens. J. Allergy Clin. Immunol. 89:673-678.

Slater, J.E., Makinen-Kiljunen, S., Suvilehto, K.,Juntunen-Backman, K., and Haahtela, T. 1994. Affinity purification of latex antigens. J.Allergy Clin. Immunol. 93:644-649.

Slater, J.E., Mostello, L.A., Dhaer, C., and Honsinger, R.W. 1990. Type I hypersensitivity to rubber. Ann. of allergy. 65:411-414.

Smith, J.F. 1974. Compounding NR for improved performance. Rubber in Engineering Conf. (Kaula Lumpur) (Preprint)

Subramaniam, A. 1975. Molecular and other properties of natural rubber: A study of clonal variation. Proc.Int.Rubb.Conf. 4:110.

Subramaniam, A. 1980. RRIM Technol.Bull. Molecular weigh and molecular weight distribution of natural rubber. Rubber Research Institute of Malaysia.

Tanaka, Y. 1989. Structure and biosynthesis mechanism of natural polyisoprene. Prog. Polym. Sci. 14:339-371.

Tanaka, Y. 1998. Preparation and application of DPNR, recovery of small rubbre particles from skim and deproteinization of latex by saponification. pp. 1-5. (Unpublished manuscript).

Tanaka, Y., Eng, A.H., and Ichikawa, N. 1992. Proc.6th Elastomer symposium. Japan. 63.

Tanaka, Y., Kawahara, S., and Tangpakdee, J. 1997. Structural characterization of natural rubber. KGK Kautschuk Gummi Kunststoffe 50.Jahragang. 6-10.

Tangpakdee, J., and Tanaka, Y., 1997. Purification of natural rubber. J.nat.Rubb.Res. 12(2): 112-119.

Teeraratkul, A., Dangsuwan, T., Wittisuwannakul, R., Kerdsomnuk, S., et al. 1997. Epidemiology of latex allergy among healthcare personnel at Siriraj Hospital. Siriraj Hosp Gaz. 49:832-845.

Tomazic, V.J., Withrow, T.J., Fisher, B.R., and Dillard, S.F. 1992. Latex associated allergies and anphylactic reactions. Clin.Immunol.Immunpathol. 64:89-97.

Turjanmaa, K. 1987. Incidence of immediate allergy in hospital personnel. Contact Dermatitis. 17:270-275.

Turjanmaa, K. 1994. Allergy to natural rubber latex problem. Ann Med. 26:297-300.

Turjanmaa, K., Palosur, T., Alenius, H., et al. 1997. Latex allergy diagnosis :in vivo and in vitro standardization of a natural rubber latex extract. Allergy. 52:41-45.

Visessanguan, W. 1992. Optimization of natural rubber latex deproteinization by enzymes. Master' s thesis. Chulalongkorn University.

Wilkinson, S.M, and Beck, M.H. 1996. Allergic contact dermatitis from latex rubber. Br. J. Dermatology. 134:910-914.

Wilson, H.T. 1960. Rubber glove dermatitis. Br.Med.J. 20:5191.

Yagami, T., Sato, M., Nakamura, A., and Shono, M. 1995. One of the rubber latex allergens is a lysozyme. J.Allergy Clin.Immunol. 96:677-686.

Yapa, P.A.J. 1975. The preparation and properties of low nitrogen constant viscosity rubber. J.Rubb.Inst. Sri. Lanka. 52:1-9.

Yapa, P.A.J. 1977. Enzyme deproteinization of Hevea latex. I. Preparation and properties of DPNR and viscosity stabilized DPNR. J.Rubb.Inst. Sri. Lanka. 54:509-519.

Yapa, P.A.J., and Balasingham, C.G. 1974. Proteolytic action of papain on Hevea latex. J.Rubb.Inst. Sri. Lanka. 51:1.

Yapa, P.A.J., Nadarajah, M., and Lioel, W.A. 1978. High quality low protein rubber from skim latex. IRRDB Symposium Kuala Lumpur (preprint).

Yapa, P.A.J., and Yapa, S. 1984. Recent development in the manufacture of deproteinized natural rubber. Proc. Int. Rubb. Conf. 2:145-306.

Yeang, H.Y., Cheong, K.F., Sunderasan, E., Hamid, S., and Cardosa, M.J. 1995. The 14.6 kd rubber elongation factor (Hev b1) and 24 kd (Hev b3) rubber particle proteins are recognized by IgE patients with spina bifida and latex allergy. J.All.Clin. Immunol. 98(3):628-639.

APPENDICES

APPENDIX 1

Determination of % N content by Kjeldahl method

1. Effect of KOH concentration on % N

KOH (%w/v)	% Total nitrogen	% Nitrogen reduction
0	0.48±0.02	0
1	0.29±0.06	40
3	0.12±0.01	77
5	0.09±0.01	81
7	0.10±0.02	79
10	0.10±0.02	81

2. Effect of Isopropanol concentration on %N

Isopropanol (%v/v)	% Total nitrogen	% Nitrogen reduction
0	0.09±0.01	81
1	0.11±0.02	81
2	0.11±0.02	81
3	0.13±0.03	75
4	0.11±0.01	77
5	0.11±0.01	77

3. Effect of Temperature on %N

Temperature (°C)	% Total nitrogen	% Nitrogen reduction
40	0.51±0.03	30
50	0.20±0.01	60
60	0.15±0.02	71
70	0.11±0.01	80
80	0.11±0.01	80

4. Effect of time on %N

Time (hour)	% Total nitrogen	% Nitrogen reduction
0	0.27±0.02	51
1	0.18±0.01	67
2	0.13±0.02	76
3	0.11±0.01	80
4	0.12±0.03	77
5	0.11±0.01	80

APPENDIX 2

Efficiency of alkali solution for saponification of solid crumb rubber

1. Efficiency of alkali solution for saponification of ammoniated crumb rubber

Number of recycling alkali solution (time)	% Total nitrogen (n=4)
0	0.54±0.04
2	0.25±0.01
3	0.25±0.02
3	0.24±0.02
4	0.27±0.07
5	0.23±0.01
6	0.24±0.03
7	0.23±0.02
8	0.37±0.03
9	0.52±0.01
10	0.55±0.01

2. Efficiency of alkali solution for saponification of skim crumb rubber

Number of recycling alkali solution (time)	% Total nitrogen (n=4)
0	2.58±0.03
2	0.22±0.02
3	0.21±0.01
3	0.21±0.02
4	0.23±0.04
5	0.24±0.02
6	0.36±0.02
7	1.25±0.12
8	1.60±0.06
9	2.42±0.04
10	2.51±0.01

APPENDIX 3

Summary of cure parameters of compound rubber from SAP-NR

Sample	Scorch time (min)	Cure time (min)	Torque rise (Kg-cm)	Cure rate (Kg-cm/min)
C-P	3.19	4.13	15.44	15.56
C-AL	3.23	4.19	15.83	14.77
SAP-AL1	3.53	4.12	15.99	13.97
SAP-AL2	3.20	3.56	16.16	13.18
SAP-AL3	3.48	4.32	16.19	13.80
SAP-AL4	3.23	4.18	16.19	13.27
SAP-AL5	3.29	3.92	16.00	14.60
C-SK	3.40	3.53	15.81	13.50
SAP-SK1	3.20	4.40	16.37	16.95
SAP-SK2	3.32	4.31	15.68	15.75
SAP-SK3	3.22	3.97	16.59	16.69
SAP-SK4	3.19	4.14	16.66	15.81
SAP-SK5	3.21	4.11	16.14	14.60

APPENDIX 4

Physical properties of valcanizate rubber from SAP-NR

1. From ammoniated crumb rubber and commercially used, TTR 5L

Physical test	C-P (STR 5L)	C-AL	SAP-AL1	SAP-AL2	SAP-AL3	SAP-AL4	SAP-AL5
Hardness (Type A)	65	64	64	65	65	63	66
300% Modulus $(kg/cm)^2$	45		41	50	56	49	47
Elongation at break (%)	663	750	656	678	654	698	666
Tensile strength (Kg/cm^2)	134	130	117	128	116	133	121
Tear strength (kg/cm)	69	65	66	68	59	62	62
Specific gravity	1.11	1.14	1.13	1.13	1.12	1.14	1.13

2. From ammoniated crumb rubber and commercially used, TTR 5L

Physical test	C-P (STR 5L)	C-AL	SAP-SK1	SAP-sk2	SAP-SK3	SAP-SK4	SAP-SK5
Hardness (Type A)	65	65	64	63	63	64	62
300% Modulus $(kg/cm)^2$	45	43	46	44	49	44	48
Elongation at break (%)	663	688	667	623	654	683	626
Tensile strength (Kg/cm^2)	134	121	104	118	119	113	125
Tear strength (Kg/cm)	69	66	62	56	58	63	54
Specific gravity	1.11	1.14	1.14	1.14	1.12	1.11	1.12

APPENDIX 5

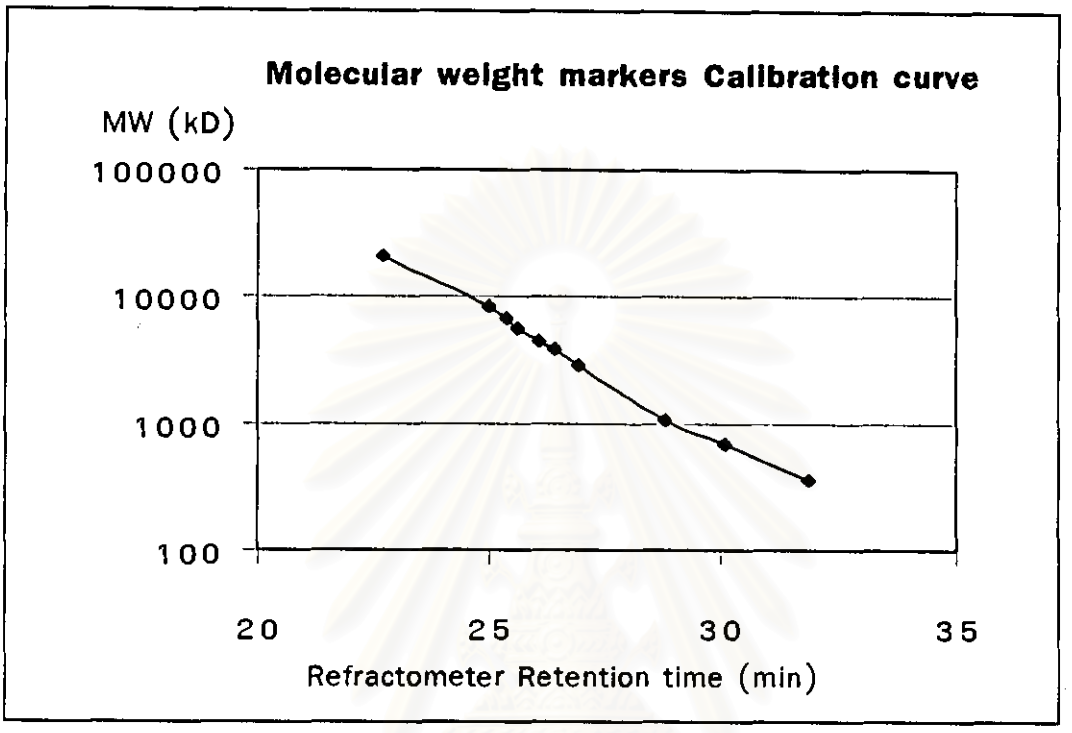

Figure A1: Molecular weight markers calibration curve of GPC

APPENDIX 6

Protein determination-Modified Lowery ' s method

Solution for Modified Lowery method

Solution A : Alkali copper sulfate (10 parts of C: 0.2 part of D)
Solution B : Dilute Folin Reagent
Solution C : 6 % w/v of sodium carbonate
Solution D : 1.5% w/v of copper sulfate in 3%w/v of sodium citrate
Solution DA : Alkali copper sulfate (10 parts of DC: 0.2 part ofDD)
Solution DC : 6 % w/v of sodium carbonate
Solution DD : 3%w/v of sodium citrate

Measurement water extractable protein with $CuSO_4$ by modified Lowry method

Protein samples were assayed by the micro -plate modified Lowry method as followed: solution A 200 µl was added to 50 µl of sample solution in each wells of micro titer plate, mixed and allowed for 15 min at room temperature. Then 50 µl of solution B was added and measured for absorbance at 750 nm after 15-20 min. The $O.D_{750}$ was subtracting from the blank, which was water. Protein contents were evaluated from standard ovalbumin. The standard protein was done like this method except 50 µl of 0-50 µg ovalbumin was used instead of sample solution.

Measurement water extractable protein without $CuSO_4$ by modified Lowry method

Protein samples were assayed by the micro -plate modified Lowry method as followed: solution DA 200 µl was added to 50 µl of sample solution in each wells of micro titer plate, mixed and allowed for 15 min at room temperature. Then 50 µl of solution DB was added and measured for absorbance at 750 nm after 15-20 min. The $O.D_{750}$ was subtracting from the blank, which was water. Protein contents were evaluated from standard ovalbumin. The standard protein was done like this method except 50 µl of 0-50 µg ovalbumin was used instead of sample solution.

The absorbance $O.D_{750}$ of protein sample

Sample	$O.D_{750}$	$O.D_{750}$, $CuSO_4$	$O.D_{750}$, no $CuSO_4$	Protein, µg	Protein, µg/ml
C-AL	0.406	0.343	0.063	2.6	3.25
SAP-AL1	0.116	0.070	0.046	2.4	1.53
SAP-AL2	0.502	0.267	0.235	12	9.00
SAP-AL3	0.491	0.271	0.220	11	4.40
SAP-AL4	0.240	0.126	0.114	5.5	1.60
SAP-AL5	0.469	0.242	0.227	11.3	7.06
C-SK	0.740	0.525	0.215	10.5	13.12
SAP-SK1	0.189	0.177	0.012	1	0.07
SAP-SK2	0.249	0.172	0.077	3.5	0.245
SAP-SK3	0.293	0.221	0.072	3	1.00
SAP-SK4	0.290	0.230	0.060	2.5	0.412
SAP-SK5	0.299	0.200	0.099	4.7	0.783

Water extractable proteins of non-saponified and saponified rubber

Sample	Weight, g	Extracted volume, ml	Redissovled volume, µl	Protein, µg/g
C-AL	0.80	8	500	32.5
SAP-AL1	4.70	47	1,500	15.3
SAP-AL2	4.00	40	1,500	90
SAP-AL3	3.00	30	600	44
SAP-AL4	5.50	55	800	16
SAP-AL5	4.80	48	1,500	70
C-SK	0.80	8	500	131.2
SAP-SK1	6.0	60	210	7.0
SAP-SK2	6.0	60	210	2.5
SAP-SK3	6.0	60	1,000	10
SAP-SK4	6.0	60	500	4.1
SAP-SK5	6.0	60	500	7.8

Calculation

C-AL: $O.D_{750}$ = 0.063, Protein evaluated from standard protein ovalbumin = 2.6 μg
Extraction: 0.8 g of C-AL / 8 ml of water then the solution was lyophilized and re-dissolved of 500 μl water

There fore water extractable protein = 2.6 x 500 / 50 = 26 μg

Total water extractable protein = 26 μg

= 26 / 8 = 3.25 μg/ml

= 32.5 μg/g

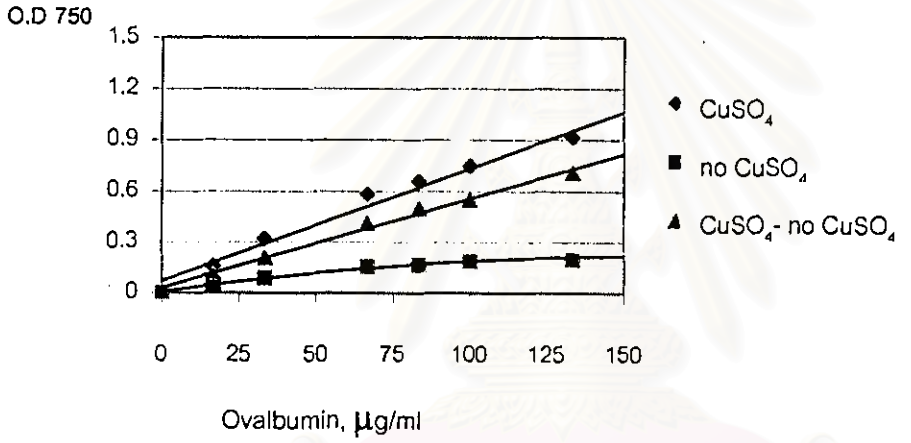

Figure A5: Standard curve of Ovalbumin measured by modified Lowry

APPENDIX 7

Solution for SDS-PAGE

1. Tris glycine eletrode buffer

   (25 mM Tris, 192 mM glycine)

Tris	3.0	g
Glycine	14.4	g
SDS	5.0	g
H_2O	1 l	

2. Tris-HCl stock solution pH 8.8

   (2 M Tris)

Tris	24.2	g
H_2O	100 ml	

   (adjust pH to 8.8 with $HCl_{conc.}$ Or 0.1 M NaOH)

3. Tris-HCl stock solution pH 6.8

   (1 M Tris)

Tris	12.2	g
H_2O	100 ml	

   (adjust pH to 8.8 with $HCl_{conc.}$ Or 0.1 M NaOH)

4. Sample buffer

Tris-HCl stock solution pH 6.8	0.6	ml
10 % SDS	2	ml
2-mercaptoethanol	0.5	ml
1% bromophenol blue	1	ml
H_2O	0.9	ml

5. Acrylamide stock (30%)

Acrylamide	30	g
Bis	0.8	g
H_2O	100	ml

6. Ammonium persulfate 0.1 g/ml

7. 15% Separating gel

Stock gel (30%)	5	ml

Stock buffer pH8.8	2.5	ml
H_2O	2.5	ml
Ammonium persulfate	50	µl
TEMED	5	µl

8. Stacking gel

Stock gel (30%)	2.3	ml
Stock buffer pH 6.8	1.0	ml
H_2O	2.3	ml
Ammonium persulfate	30	µl
TEMED	5	µl

9. Staining solution

Commassie Blue R-250	1.0	g
Methanol	450	ml
Glacial acetic acid	100	ml
H_2O	450	ml

10. Destain solution

Glacial acetic acid	100	ml
Methanol	100	ml
H_2O	800	ml

APPENDIX 8

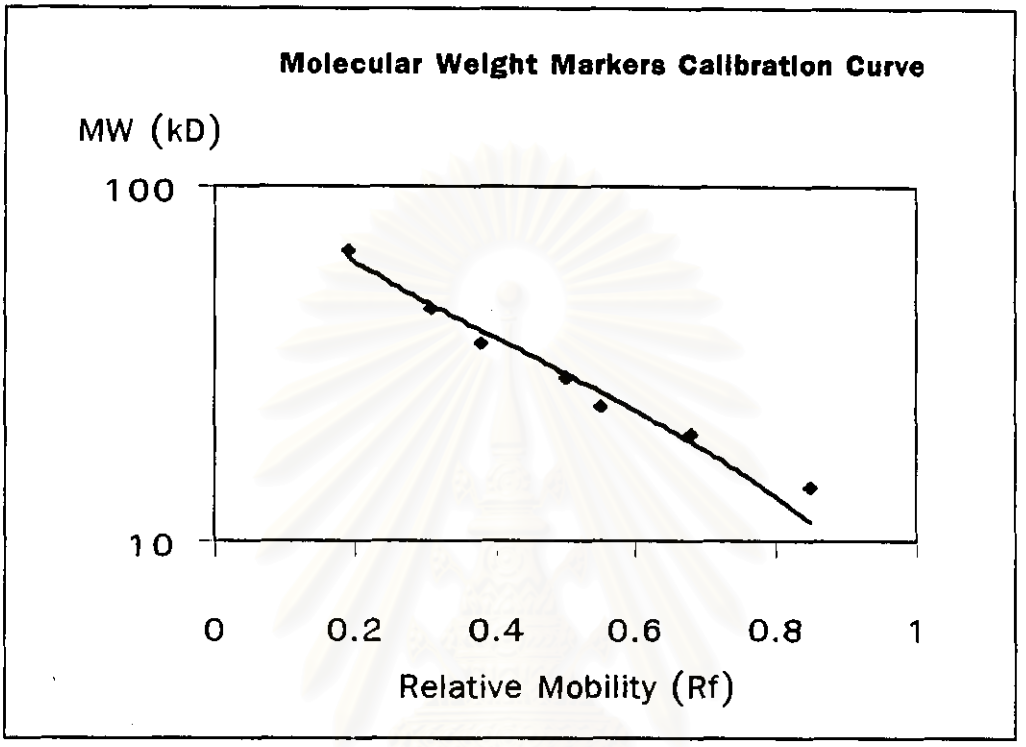

Figure: A3 Molecular weight markers calibration curve of SDS-PAGE

APPENDIX 9

Statistical calculation:

Statistical analysis of variance of nitrogen of ammoniated crumb

Sample	DF	SS_1	SS_2	F	$\bar{X}$	S.D	t
C	3	$2x10^{-4}$	-	0.016	0.49	9.5710^{-3}	6.05
S	9	-	0.373		0.29	0.048	

$F_{0.95}$-value from table at 3,9 df. Is 8.81

$t_{0.01}$ – value from table at df.12 is 3.055

Reject $H_0: \mu_1 = \mu_2$

Statistical analysis of variance of nitrogen of skim crumb

Sample	DF	SS_1	SS_2	F	$\bar{X}$	S.D	t
C	3	$6x10^{-4}$	-	0.011	1.85	0.13	71.9
S	9	-	0.0193		0.12	0.04	

$F_{0.95}$-value from table at 3,9 df. Is 8.81

$t_{0.01}$ – value from table at df.12 is 3.055

Reject $H_0: \mu_1 = \mu_2$

Statistical analysis of variance of ash from ammoniated crumb

Sample	DF	SS_1	SS_2	F	$\bar{X}$	S.D	t
C	3	$6x10^{-4}$	-	0.011	0.14	0.012	12
S	9	-	0.116		0.85	0.11	

$F_{0.95}$-value from table at 3,9 df. Is 8.81

$t_{0.01}$ – value from table at df.12 is 3.055

Reject $H_0: \mu_1 = \mu_2$

Statistical analysis of variance of ash from skim crumb

Sample	DF	SS_1	SS_2	F	$\bar{X}$	S.D	t
C	3	$5x10^{-4}$	-	0.012	0.29	0.012	3.07
S	9	-	0.1172		0.65	0.11	

$F_{0.95}$-value from table at 3,9 df. Is 8.81

$t_{0.01}$ – value from table at df.12 is 3.055

Accept $H_0: \mu_1 = \mu_2$

Statistical analysis of variance of dirt from ammoniated crumb

Sample	DF	SS_1	SS_2	F	$\bar{X}$	S.D	t
C	3	$2x10^{-4}$	-	0.057	0.09	0.001	1.13
S	9	-	$1.04x10^{-4}$		0.011	0.011	

$F_{0.95}$-value from table at 3,9 df. Is 8.81

$t_{0.01}$ – value from table at df.12 is 3.055

Accept $H_0: \mu_1 = \mu_2$

Statistical analysis of variance of dirt from skim crumb

Sample	DF	SS_1	SS_2	F	$\bar{X}$	S.D	t
C	3	$2x10^{-6}$	-	0.002	0.014	0.001	0.45
S	9	-	$2.68x10^{-3}$		0.018	0.017	

$F_{0.95}$-value from table at 3,9 df. Is 8.81

$t_{0.01}$ – value from table at df.12 is 3.055

Accept $H_0: \mu_1 = \mu_2$

Statistical analysis of variance of volatile matter from ammoniated crumb

Sample	DF	SS_1	SS_2	F	$\bar{X}$	S.D	t
C	3	$8x10^{-4}$	-	$4.85x10^{-4}$	0.76	0.011	1.45
S	9	-	4.97		1.33	0.74	

$F_{0.95}$-value from table at 3,9 df. Is 8.81

$t_{0.01}$ – value from table at df.12 is 3.055

Accept $H_0: \mu_1 = \mu_2$

Statistical analysis of variance of volatile matter from skim crumb

Sample	DF	SS_1	SS_2	F	$\bar{X}$	S.D	t
C	3	$5x10^{-4}$	-	$5.21x10^{-4}$	0.63	0.013	1.14
S	9	-	2.91		0.96	0.57	

$F_{0.95}$-value from table at 3,9 df. Is 8.81

$t_{0.01}$ – value from table at df.12 is 3.055

Accept $H_0: \mu_1 = \mu_2$

Statistical analysis of variance of color from ammoniated crumb

Sample	DF	SS_1	SS_2	F	$\bar{X}$	S.D	t
C	3	2	-	0.56	5	0.58	0.06
S	9	-	10.5		5	1.05	

$F_{0.95}$-value from table at 3,9 df. Is 8.81

$t_{0.01}$ – value from table at df.12 is 3.055

Accept $H_0: \mu_1 = \mu_2$

Statistical analysis of variance of color from skim crumb

Sample	DF	SS_1	SS_2	F	$\bar{X}$	S.D	T
C	3	1	-	0	5	0.5	17.9
S	9	-	0		5	0	

$F_{0.95}$-value from table at 3,9 df. Is 8.81

$t_{0.01}$ – value from table at df.12 is 3.055

Reject $H_0: \mu_1 = \mu_2$

Statistical analysis of variance of P_0 from ammoniated crumb

Sample	DF	SS_1	SS_2	F	$\bar{X}$	S.D	t
C	3	8	-	1.04	20	1.63	8.41
S	9	-	23		28	1.71	

$F_{0.95}$-value from table at 3,9 df. Is 8.81

$t_{0.01}$ – value from table at df.12 is 3.055

Accept $H_0: \mu_1 = \mu_2$

Statistical analysis of variance of P_0 from skim crumb

Sample	DF	SS_1	SS_2	F	$\bar{X}$	S.D	t
C	3	20	-	0.063	80	1.91	0.75
S	9	-	946		76	20.6	

$F_{0.95}$-value from table at 3,9 df. Is 8.81

$t_{0.01}$ – value from table at df.12 is 3.055

Accept $H_0: \mu_1 = \mu_2$

Statistical analysis of variance of Mooney from ammoniated crumb

Sample	DF	SS_1	SS_2	F	$\bar{X}$	S.D	t
C	3	8	-	0.10	40	1.63	0.06
S	9	-	232		55	4.93	

$F_{0.95}$-value from table at 3,9 df. Is 8.81

$t_{0.01}$ – value from table at df.12 is 3.055

Accept $H_0: \mu_1 = \mu_2$

Statistical analysis of variance of Mooney from skim crumb

Sample	DF	SS_1	SS_2	F	$\bar{X}$	S.D	t
C	3	47	-	0.05	33	3.40	2.46
S	9	-	317.34		55.7	17.8	

$F_{0.95}$-value from table at 3,9 df. Is 8.81

$t_{0.01}$ – value from table at df.12 is 3.055

Accept $H_0: \mu_1 = \mu_2$

Statistical analysis of variance of PRI from ammoniated crumb

Sample	DF	SS_1	SS_2	F	$\bar{X}$	S.D	t
C	3	2	-	0.001	99	0.58	3.28
S	9	-	3879		64	4.93	

$F_{0.95}$-value from table at 3,9 df. Is 8.81

$t_{0.01}$ – value from table at df.12 is 3.055

Reject $H_0: \mu_1 = \mu_2$

Statistical analysis of variance of PRI from skim crumb

Sample	DF	SS_1	SS_2	F	$\bar{X}$	S.D	t
C	3	20	-	0.063	80	1.91	0.75
S	9	-	946		76	20.6	

$F_{0.95}$-value from table at 3,9 df. Is 8.81

$t_{0.01}$ – value from table at df.12 is 3.055

Accept $H_0: \mu_1 = \mu_2$

Statistical analysis allergenic response: Significant difference allergenic response by EAST test between control and saponified crumb rubber analyzed by Wilcoxon Signed-Rank Test at 99 % confidence.

Statistical analysis of variance of latex allergen from non-saponfied ammoniated crumb

T	σ	μ	Z
60	7.5	95	4.6

$t_{0.01}$ – value from table is 18

Reject $H_0: \mu_1 = \mu_2$

Statistical analysis of variance of latex allergen from non-saponified skim crumb

T	σ	μ	Z
60	7.5	95	4.6

$t_{0.01}$ – value from table is 18

Reject $H_0: \mu_1 = \mu_2$

Statistical analysis of variance of latex allergen from saponified ammoniated crumb

T	σ	μ	Z
60	7.5	95	0

$t_{0.01}$ – value from table is 18

Reject $H_0: \mu_1 = \mu_2$

Statistical analysis of variance of latex allergen from non-saponified ammoniated crumb

T	σ	μ	Z
60	7.5	95	0

$t_{0.01}$ – value from table is 18

Reject $H_0: \mu_1 = \mu_2$

APPENDIX 10

The cost of saponified latex (SAP-L)

Items	Price/unit (Baht)	Quatity used	Total cost (Baht)
Starting material 39.5 % DRC latex, rubber content = 11.85 kg	16.5/kg	30 kg	195.52
Chemicals			
Formic acid	29/kg	0.48 kg	13.92
KOH	65/kg	1.5 kg	97.5
Sodium metabisulfite	25/kg	5.92 g	0.15
Hydroxylamine hydrochloride	82/kg	17.86 g	1.45
Wing-Stay L	45/kg	30 g	1.35
Water for latex dilution	0.28/L	60 L	16.80

Total cost = 326.68 Baht

Yield :6.1 kg of SAP-L, **Cost 54 Baht/ kg**

The cost of saponified crumb rubber

Items	Price/unit (Baht)	Quatity used	Total cost (Baht)
Starting material 30.69 % DRC ammoniated latex, rubber content = 30.69 kg	16.5	100 kg	506
Chemicals			
Formic acid	29/kg	0.19 kg	5.51
KOH	65/kg	1 kg	65
Sodium metabisulfite	25/kg	3 g	0.08
Hydroxylamine hydrochloride	82/kg	9 g	0.74
Wing-Stay L	45/kg	20 g	0.90
Total 578.23 Baht/19.25 kg			
Yield: 19.25 kg SAP-AL	**Cost =30.04 Baht/ kg**		

Items	Price/unit Baht	Quality used	Total cost (Baht)
Starting material 5% DRC skim latex,rubber content = 10 kg Rubber	17.50	200 kg	175
Chemicals			
Sulphuric acid	3.5/kg	2.3 kg	8.05
KOH	65/kg	1 kg	65
Sodium metabisulfite	25/kg	3 g	0.08
Hydroxylamine hydrochloride	82/kg	9 g	0.74
Wing-Stay L	45/kg	20 g	0.9
Total cost 249.77 Baht			
Yield: 9.8 kg SAP-SK	**Cost = 25.49 Baht / kg**		

SAP-AL: saponified ammoniated crumb rubber, SAP-SK: saponfied skim crumb rubber

BIOGRAPHY

Siriwan Boonsook was born on April 16, 1971. She conferred her Bachelor degree of Science in Biochemistry and Biochemical Technology from Chiang Mai University in 1993. In 1994, she had worked in the Immunology Department, Chulalongkorn Hospital, Faculty of Medicine, Chulalongkorn University in the position of a Medical Scientist. In 1995-1997, she had worked in a position of a Scientist in the Industrial Chemistry Department of King Mongkut' s Institute of Technology North Bangkok. In 1997, she continued her study in the Master Program of Biochemistry at the Biochemistry Department Chulalongkorn University.