CHAPTER III
RESULTS

3.1. Deproteinization of Natural Rubber by Protease and Microwave Energy

3.1.1. Optimum Condition of Deproteinization

3.1.1.1 Optimum Condition for Deproteinization of Fresh Latex by Papain

The specific activity of crude papain used in this research is 0.24 CDU/mg as determined by Method 2.4.1.

Fresh field latex in this research was stabilized with 0.2% ammonia at the rubber plantation after tapping, 0.15 phr of hydroxylamine hydrocloride, 0.05 phr of sodium metabisulfite and 0.05 phr of Wingstay-L were added as viscosity stabilizer, color controller and anti-oxidant, respectively. The initial pH of latex was in the range of 8-9. By adding 2% formic acid the pH was adjusted to 7.6±0.1. The DRC of fresh latex (32-35% DRC) was adjusted to 25% by adding water. The latex was warmed up to 50°C by microwave before deproteinization by papain according to Methods 2.6.1.1. Total nitrogen content (g%) of solid rubber is used as a criterion for the efficiency of deproteinization by microwave plus various concentrations of papain. Since the initial nitrogen content of fresh latex varied from lot to lot (Appendix 2), the per cent nitrogen reduction from initial value is used to express degree of deproteinization. Figure 3.1 shows that the maximum percentage of nitrogen reduction of 74.32 ± 5.611% was obtained when 25% latex was treated by 0.3 phr of papain. Under these conditions the per cent yield of solid rubber is 90%.

The optimal time for deproteinization was therefore determined at 0.3 phr of papain. Time was varied from 0 to 60 minutes, the maximum percentage of nitrogen reduction about 77.41 ± 6.129 g% was obtained with minimum deproteinization time of 5 minutes (Figure 3.2). Under these conditions the per cent yield of solid rubber is 96%.

The optimal dilution volume was studied by fixing papain treatment at 0.3 phr for 5 minutes. By varying the dilution ratio of latex: water from 1:0.5 to 1: 3, Figure

3.3 shows that the optimum dilution is 1: 1 to obtain the maximum percentage of nitrogen reduction of 75.01 $\pm$ 5.579 % and 92% yield of solid rubber. It can be concluded that the optimum conditions for latex deproteinization are the followings: Beginning with fresh latex at 25% DRC 100 ml, pH 7.6, preheated by microwave for 5 minutes to reach 50^{o}C. The latex was deproteinized with 0.3 phr of papain at 50^{o}C for 5 minutes. To remove hydrolyzed protein, latex was diluted by equal volume of water and steam coagulated at 121^{o}C, 15 lb/in^{2}, 10 minutes. The coagulum was then milled into crepe and washed with water and dried at 60^{o}C. The yield of solid deproteinized rubber is about 90% with nitrogen content less than 0.16 g%. The solid DPNR looks like STR5L in terms of light but has better elasticity, good odor and low nitrogen content.

Figure 3.4 showed the comparison of nitrogen content (g%) among 3 lots of latex used for optimization of deproteinization by papain. Lot (A) was used for varying papain concentration, lot (B) and (C) were used for varying time and dilution fold respectively. STR5L specimens were control from production line of the factory, while CDPNR were solid rubber preheated 5 min by microwave, zero-concentration of papain (A), zero-time of papain treatment (B), and zero-dilution (C). The effect of microwave only was therefore evident by comparing %N between STR5L and CDPNR. The consistency of deproteinization by the coupling action of microwave energy and papain is obvious by comparing %N between CDPNR and DPNR histograms. These optimum conditions were used for the production in a larger scale (5-liter).

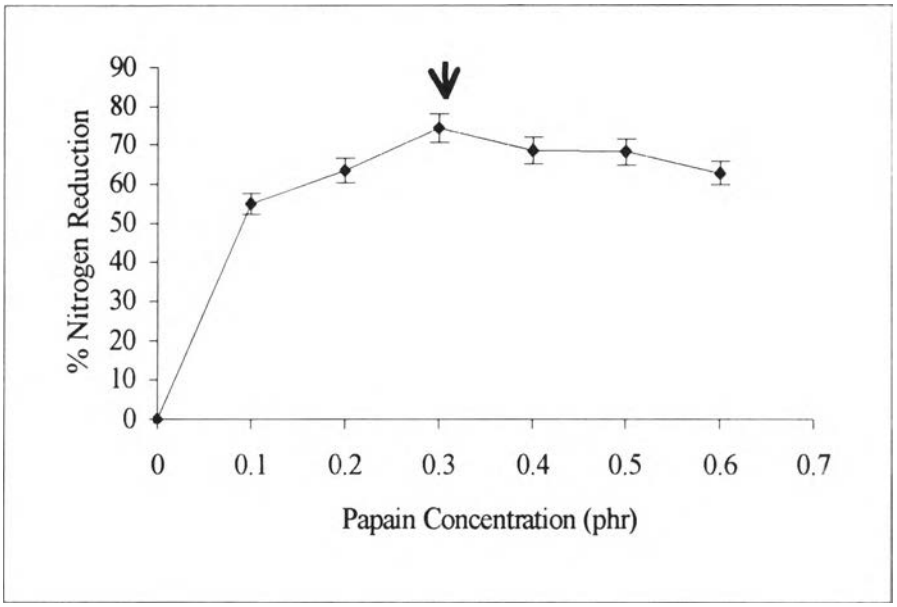

Figure 3.1 Optimum papain concentration for fresh latex deproteinization

Latex 100 ml, 25% DRC containing 0.15 phr of hydroxylamine hydrocloride, 0.05 phr of sodium metabisulfite and 0.05 phr of Wingstay-L was preheated for 5 minutes by microwave then treated with papain (0.1-0.6 phr) for 5 minutes at 50^{o}C and diluted with 100 ml water and steam coagulated. Latex, which was, treated the same way but without papain was used as control (CDPNR, 0% nitrogen reduction).

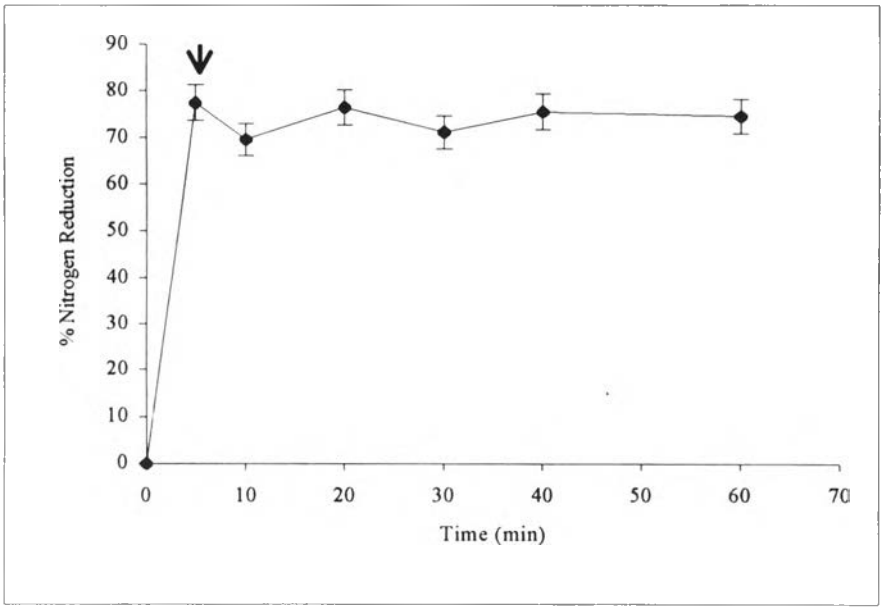

Figure 3.2 Optimum time for fresh latex deproteinization

Latex 100 ml, 25% DRC containing 0.15 phr of hydroxylamine hydrocloride, 0.05 phr of sodium metabisulfite and 0.05 phr of Wingstay-L was preheated for 5 minutes by microwave then treated with papain 0.3 phr at various time intervals (5-60 minutes), at 50°C, diluted with 100 ml water and steam coagulated. Latex, which was, treated the same way with 0.3 phr papain but zero-time of treatment was used as control (CDPNR, 0% nitrogen reduction).

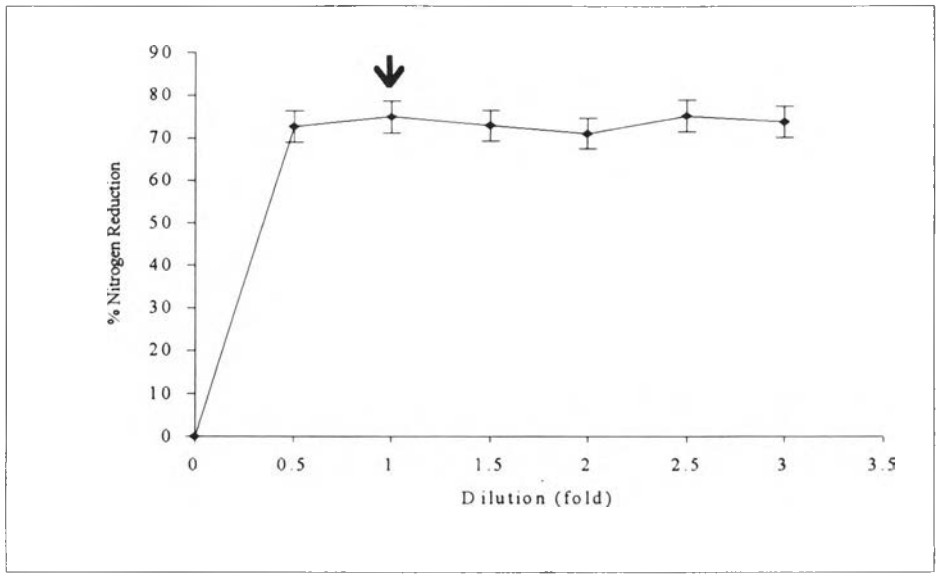

Figure 3.3 Optimum dilution for fresh latex deproteinization

Latex 100 ml, 25% DRC containing 0.15 phr of hydroxylamine hydrocloride, 0.05 phr of sodium metabisulfite and 0.05 phr of Wingstay-L was preheated for 5 minutes by microwave then treated with papain 0.3 phr at 50°C for 5 minutes, varied fold dilution with water (0.5-3 fold) and steam coagulated. Latex, which was, treated the same way with 0.3 phr papain but no dilution after 5 min treatment was used as control (CDPNR, 0% nitrogen reduction).

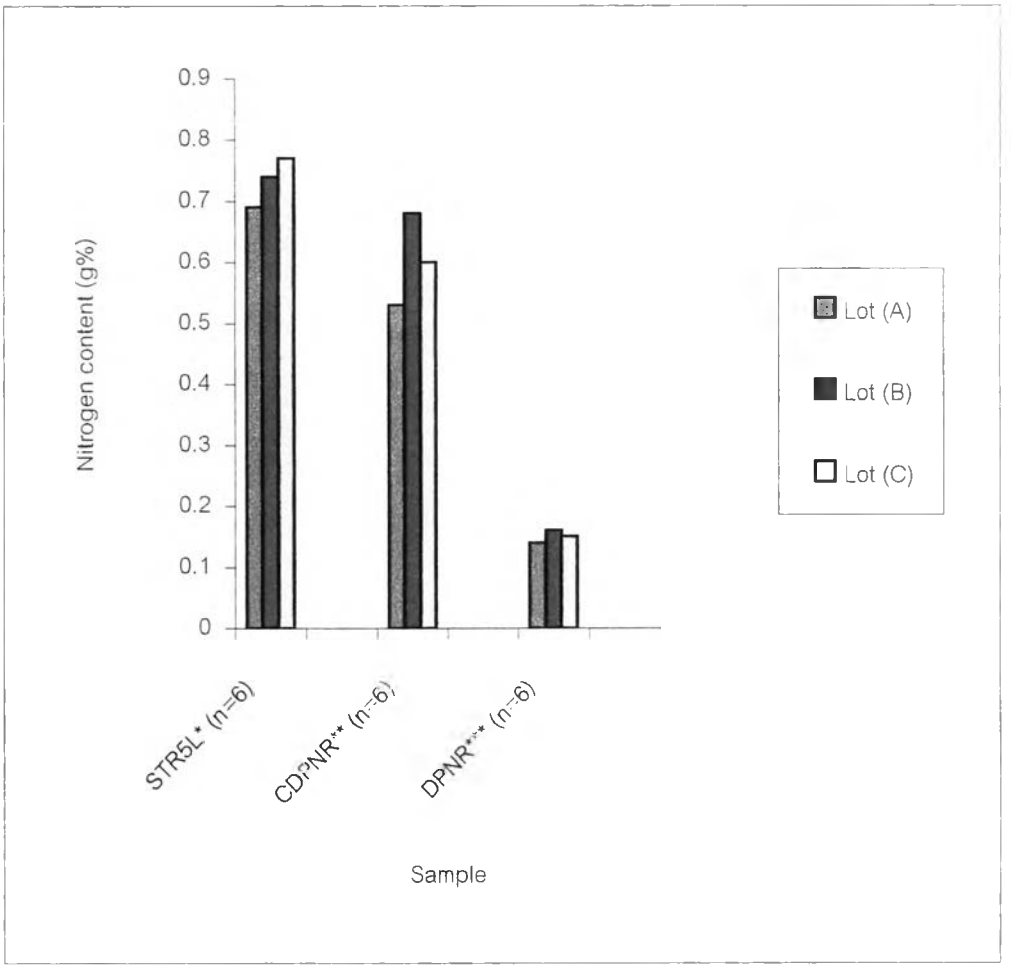

Figure 3.4 Comparison of % nitrogen content between STR5L, CDPNR and DPNR after deproteinization using optimum conditions. The enzyme used was papain.

Different lots of latex were used in the optimization:

Lot (A) : for varying papain concentration

Lot (B) : for varying time

Lot (C) : for varying dilution ratio

* : Control rubbers STR5L from production process (no treatment)

** : Control rubbers from fresh field latex, (microwave + (A) papain 0 phr; (B) papain 0.3 phr, 0 min; and (C) zero-dilution after papain treatment)

*** : Deproteinized fresh field latex (microwave + papain at optimum condition)

3.1.1.2 Optimum Condition for Deproteinization of Fresh Latex by Alcalase

The specific activity of Alcalase used in this research is 1.38 CDU/mg.

Since the optimum pH of Alcalase is 9.6, the pH of latex was therefore increased from 8-9 to 9.6 $\pm$ 0.1 by adding small amount of 20% ammonia. By varying Alcalase concentration from 0.03 to 0.30 phr (Figure 3.5), varying time from 0 to 50 minutes (Figure 3.6) and varying dilution ratio of latex: water from 1: 0.5 to 1: 2.5 (Figure 3.7). The optimum Alcalase concentration is 0.2 phr (Figure 3.5). The optimum time is 5 minutes (Figure 3.6). The optimum dilution is 1 fold (Figure 3.7).

Figure 3.8 showed the comparison of nitrogen content (g%) using 3 lots of latex fortunately with the same nitrogen content of 0.55%. Lot (a) was for varying Alcalase concentration, lot (b) and (c) for varying time and dilution respectively. The effect of microwave only was slightly observed when STR5L and CDPNR were compared at zero Alcalase (a), 0.06 phr Alcalase 0 min (b) and zero-dilution (c). The coupling action of microwave and Alcalase was observed by comparing %N between CDPNR and DPNR histograms at optimum conditions.

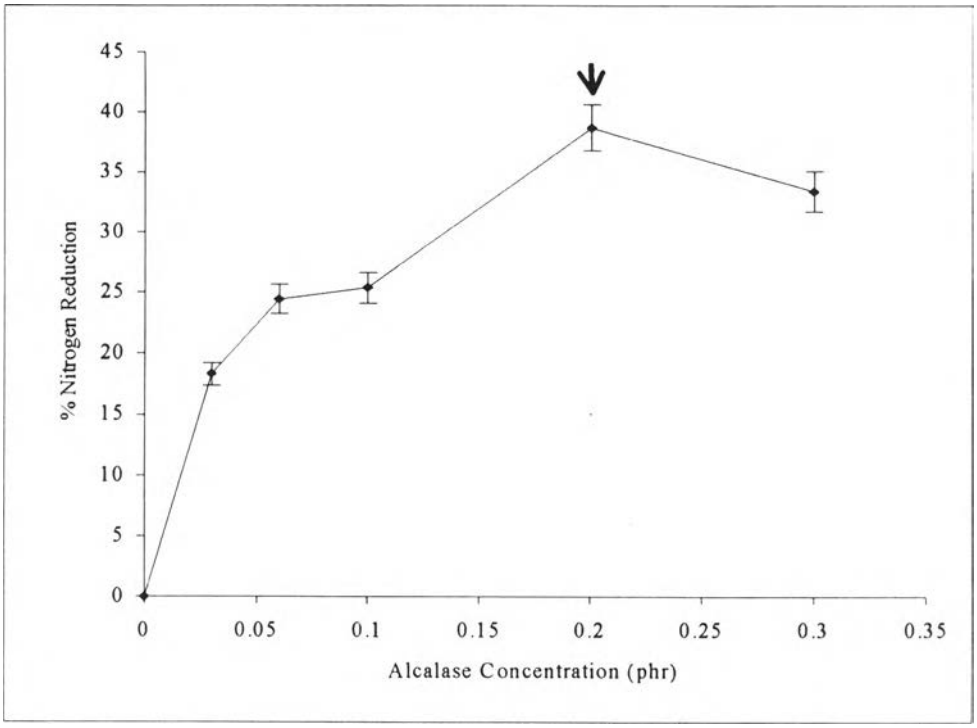

Figure 3.5 Optimum Alcalase concentration for fresh latex deproteinization

Latex 100 ml, 25% DRC containing 0.15 phr of hydroxylamine hydrocloride, 0.05 phr of sodium metabisulfite and 0.05 phr of Wingstay-L was preheated for 5 minutes by microwave then treated with Alcalase (0.03-0.3 phr) for 5 minutes at 50^{o}C and diluted with 100 ml water and steam coagulated. Latex, which was, treated the same way but without Alcalase was used as control (CDPNR, 0% nitrogen reduction).

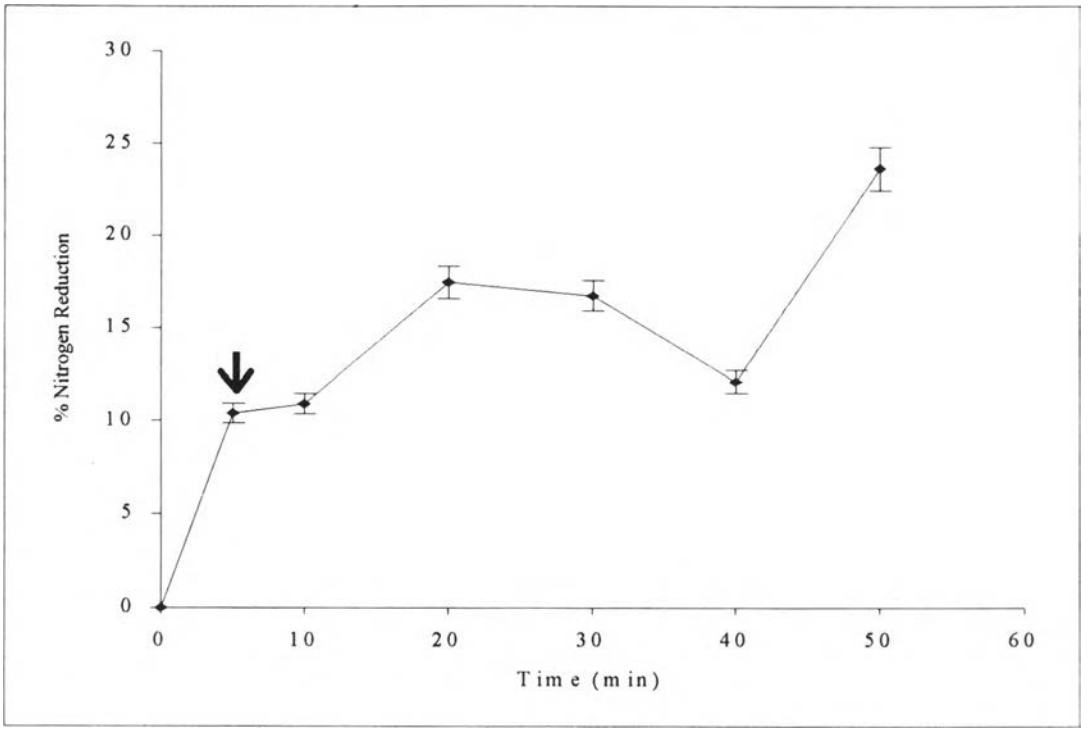

Figure 3.6 Optimum time for fresh latex deproteinization

Latex 100 ml, 25% DRC containing 0.15 phr of hydroxylamine hydrocloride, 0.05 phr of sodium metabisulfite and 0.05 phr of Wingstay-L was preheated for 5 minutes by microwave then treated with Alcalase 0.06 phr at various time intervals (5-50 minutes) at 50°C, diluted with 100 ml water and steam coagulated. Latex, which was, treated the same way with 0.06 phr Alcalase, but for 0 min was used as control (CDPNR, 0% nitrogen reduction).

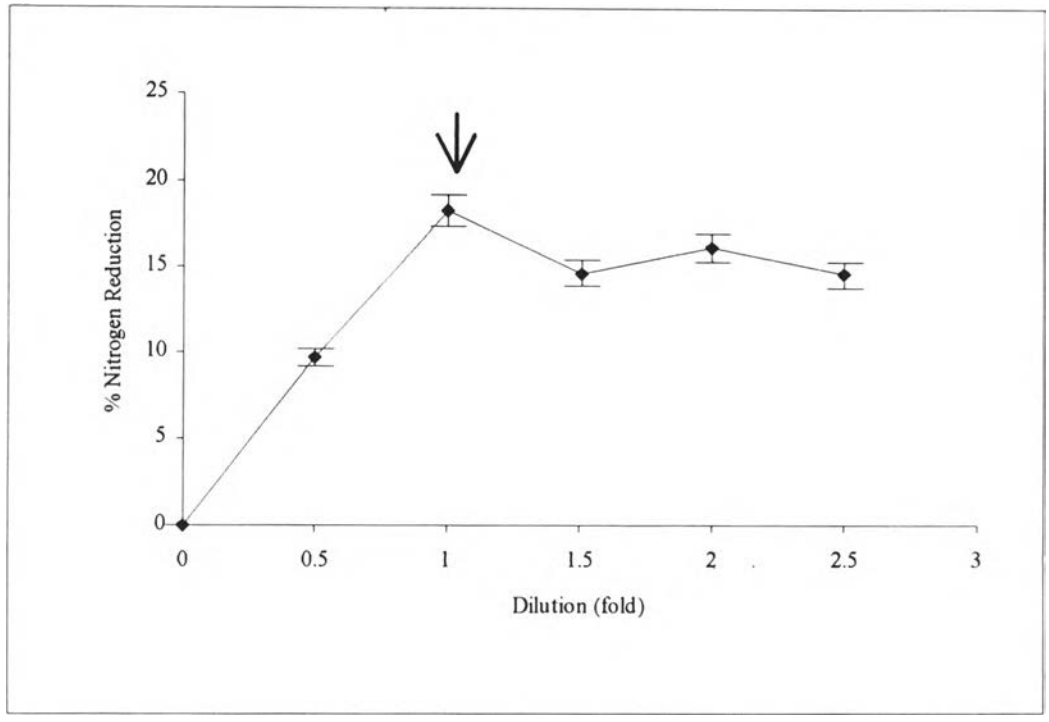

Figure 3.7 Optimum dilution for fresh latex deproteinization

Latex 100 ml, 25% DRC containing 0.15 phr of hydroxylamine hydrocloride, 0.05 phr of sodium metabisulfite and 0.05 phr of Wingstay-L was preheated for 5 minutes by microwave then treated with Alcalase 0.06 phr at 50°C for 5 minutes, varied fold dilution with water (0.5-2.5 fold) and steam coagulated. Latex, which was, treated the same way with 0.06 phr Alcalase, but without dilution was used as control (CDPNR, 0% nitrogen reduction).

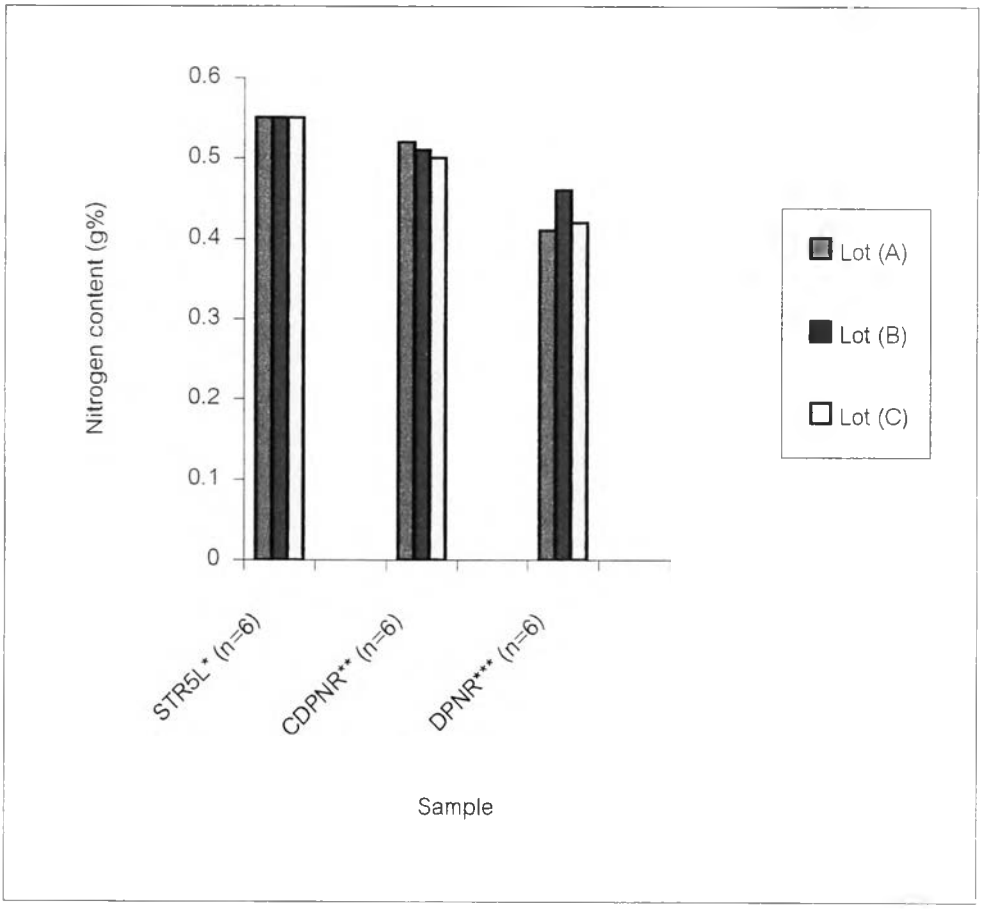

Figure 3.8 Comparison of % nitrogen content between STR5L, CDPNR and DPNR after deproteinization using optimum conditions. The enzyme used was Alcalase.

Different lots of latex were used in the optimization:

Lot (A) : for varying Alcalase concentration

Lot (B) : for varying time

Lot (C) : for varying dilution ratio

* : Control rubbers STR5L from production process (no treatment)

** : Control rubbers from fresh field latex (microwave + (A) Alcalase 0 phr; (B) Alcalase 0.06 phr 0 min; and (C) no dilution after Alcalase treatment)

*** : Deproteinized fresh field latex (microwave + Alcalase at optimum conditions)

In comparison, the result from papain treatment was better than alcalase treatment because papain is a neutral protease (pH 7.6), and its optimum pH of field latex, initial pH of latex 8-9. In contrast, the optimum pH of Alcalase is 9.6 and ammonia was required to bring fresh latex from pH 7.5 to 9.6. Alcalase-DPNR consumes more non-benefit chemical and, unsatisfied solid rubber. It also contains self-coagulated rubber and forms a lot of small popcorn-particles in the reaction mixture. After steam coagulation, the texture of rubber was too hard, and the color was orange-red. That led to the very wide variation when nitrogen content was analyzed.

Figure 3.4 and 3.8 showed that after preheating with microwave, the nitrogen content of CDPNR was lower than STR5L. This result may occur from the effect of microwave energy.

Previously, microwave was planned for reducing the time of deproteinization of natural rubber from 50 minutes (Koosakul, 1994) to 5 minutes . The other benefit of using microwave was the vibration of the rubber particles as well as the increase of latex temperature. Microwave can also breakdown the bond between protein and rubber particle and unfolds the compact structure of rubber particles. This made the proteins inside the rubber molecule better exposed to enzyme and was hydrolyzed as small peptides. The efficiency of enzyme treatment was therefore enhanced by pre-treatment with microwave.

3.1.2. Production of deproteinized rubber using papain at 5 liter scale

3.1.2.1 DPNR Production Scheme

This pilot production scale was developed using the result of optimum conditions obtained from 3.1.1.

3 x 5 Liter-Fresh Field Latex

(≈ 30% DRC in 0.2% Ammonia)

↓

Field Latex in 0.15 phr Hydroxylamine Hydrochloride, 0.05 phr Sodium Metabisulfite, 0.05 phr Wingstay-L

↓ Adjust pH to 7.6 ± 0.1 by 2% formic acid

↓ Adjust to 20% DRC by water

Preheat by microwave (Model EMO-900T, Sanyo) 100°C, 13 min until latex is 50°C

↓

0.3 phr Papain Solution

↓ Incubated at 50°C, 5 min

Dilution (latex: water = 1: 1)

↓

Steam Coagulation

(Autoclave 121°C, 15 lb/in^2, 10 min)

↓

Washing and shredding

↓

Dried at 60-120°C, 10 hrs

↓

DPNR (≈ 2.3 kg / 15 liter of 20% DRC latex)

* Operation time: 8-10 hrs, 76.67% Yield, Cost: 28.95 Baht/kg

Specific activity of crude papain used in this research was 0.24 CDU/mg as determined by Method 2.4.1. Fresh field latex was stabilized with 0.2% ammonia at the rubber plantation after tapping, 0.15 phr of hydroxylamine hydrocloride, 0.05 phr of sodium metabisulfite and 0.05 phr of Wingstay-L were added. Adjusted pH of latex to 7.6±0.1 by adding 2% formic acid and adjusted to 25% DRC by adding water. Latex was preheated with microwave (Model EMO-900T, Sanyo) at 100°C about 13 minutes until latex is 50°C. The batch size was increased from 100 ml to 15 L at 25% DRC. Latex was deproteinzed with 0.3 phr of papain solution and incubated for 5 minutes at 50°C. Dilution was done by adding water 1 fold and steam coagulation (Autoclave 121°C, 15 lb/in^2, 10 minutes). The coagulum was milled into crepe and washed with water and dried. The yield of DPNR was 76.67%. The DPNR prepared was used for further studies.

3.2. Testing of the properties of DPNR

3.2.1. Raw rubber testing (RRIM 1970)

The raw rubber properties indicate that the quality of solid rubber in term of percent non-rubber impurities (total nitrogen content, dirt, ash, volatile matter and color index) and the processibility of rubber (Po, PRI and viscosity).

Table 3.1 shows that when the batch size was increased from 100 ml to 15 L, the nitrogen content of DPNR sample was slightly higher than 100 ml. Based on STR5L specifications, nitrogen content batch size (0.18 ± 0.05 g%). Other properties were within maximum limit namely; ash: 0.31 ± 0.04%, dirt: 0.03 ± 0.06%, volatile matter: 0.18 ± 0.04%, color index: 3.6 ± 0.39 and mooney viscosity: 57.86 ± 2.94. The value of initial plasticity (Po) and plasticity retention index (PRI) were exceptionally lower than STR5L and CDPNR: initial plasticity (Po): 26.88 ± 1.88% and plasticity retention index (PRI): 63.12 ± 9.70%. The low Po and PRI indicated that DPNR has lower resistance to aging. To improve these properties, antioxidant should be added.

CDPNR have lower nitrogen content than STR5L. The percentage of nitrogen content of CDPNR was 0.39 ± 0.03 g% while the percentage of STR5L was

0.44 ± 0.01 g%. This result showed that microwave treatment could reduce the nitrogen content although without enzyme treatment. The effect of steam coagulation may reduce some protein content. The other properties of CDPNR such as ash, dirt, volatile matter, initial plasticity (Po), plasticity retention index (PRI) color index and mooney viscosity were not significant different from STR5L. CDPNR properties; ash: 0.22 ± 0.02 %, dirt: 0.02 ± 0.04 %, volatile matter: 0.34 ± 0.06 %, initial plasticity (Po): 38.53 ± 0.64%, plasticity retention index (PRI): 95.42 ± 4.49%, color index: 2.8 ± 0.49, mooney viscosity: 58.71 ± 1.81 were more or less similar to STR5L.

Significant differences in nitrogen content, volatile matter, Po and PRI suggest that beside removal of proteins, lipids and natural antioxidants were also decreased, and resulting in lower oxidation aging of DPNR. Ash and dirt were significantly higher in DPNR than STR5L and CDPNR, which may come from crude papain.

Table 3.1 Raw rubber properties of STR5L, CDPNR and DPNR *

Specification	STR5L (n=15)	CDPNR (n=15)	DPNR (n=15)
Nitrogen Content (0.60 g% max)	0.44 ± 0.01^{a}	0.39 ± 0.03^{b}	0.18 ± 0.05^{c}
Ash (0.40% max)	0.23 ± 0.02^{a}	0.22 ± 0.02^{a}	0.31 ± 0.04^{b}
Dirt (0.04% max)	0.02 ± 0.01^{a}	0.02 ± 0.04^{a}	0.03 ± 0.06^{b}
Volatile Matter (0.80% max)	0.33 ± 0.07^{a}	0.34 ± 0.06^{a}	0.18 ± 0.04^{b}
Initial Plasticity (Po, 35 min)	38.66 ± 0.61^{a}	38.53 ± 0.64^{a}	26.88 ± 1.88^{b}
Plasticity Retention Index (PRI, 60 min)	95.60 ± 4.62^{a}	95.42 ± 4.49^{a}	63.12 ± 9.70^{b}
Color Index (6 max)	2.8 ± 0.49^{a}	2.8 ± 0.49^{a}	3.6 ± 0.39^{b}
Mooney viscosity	59.10 ± 0.84^{a}	58.71 ± 1.81^{a}	57.86 ± 2.94^{a}

* Carried out at the batch size of 15 L.

CDPNR: Control of deproteinized natural rubber

DPNR: Deproteinized natural rubber

Significant difference of physical properties analyzed by F-test and Tukey's test at 95% confidence interval among STR5L, CDPNR and DPNR are marked by different alphabets (a, b, c).

3.2.2. Water extractable proteins in papain, field latex and dried solid rubber

3.2.2.1 Quantitative analysis of water extractable proteins by Lowry's Method

Since solid rubber is usually compounded and molded into various shapes of rubber product and there is no standard method for extraction and determination of water extractable proteins (WEP) as in gloves and other dipping products. In this research WEP was extracted from raw rubber specimens by cutting rubber into small pieces about 5 mm, and about 10 g specimen was extracted with 10 volume of water at 37 ^{o}C for 2 hours (2.12.1). The water extractable protein can be determined by modified Lowry method (2.12.3). This method involved determination of proteins in the presence and in the absence of $CuSO_4$. Figure 3.9 shows standard calibration graph of ovalbumin determined. The results indicated that the higher value was due to the presence of $CuSO_4$ so the optical density at 750 nm was high. Thus WEP was evaluated from standard ovalbumin after subtracting the effect from $CuSO_4$. These suggested that water extractable protein, which was determined by Lowry method, might be interfered by divalent cation contaminants, resulting in high absorbance without protein per se. Therefore to obtain the correct result, cation should be removed by dialyzing the sample or precipitation of protein before determination by Lowry's method.

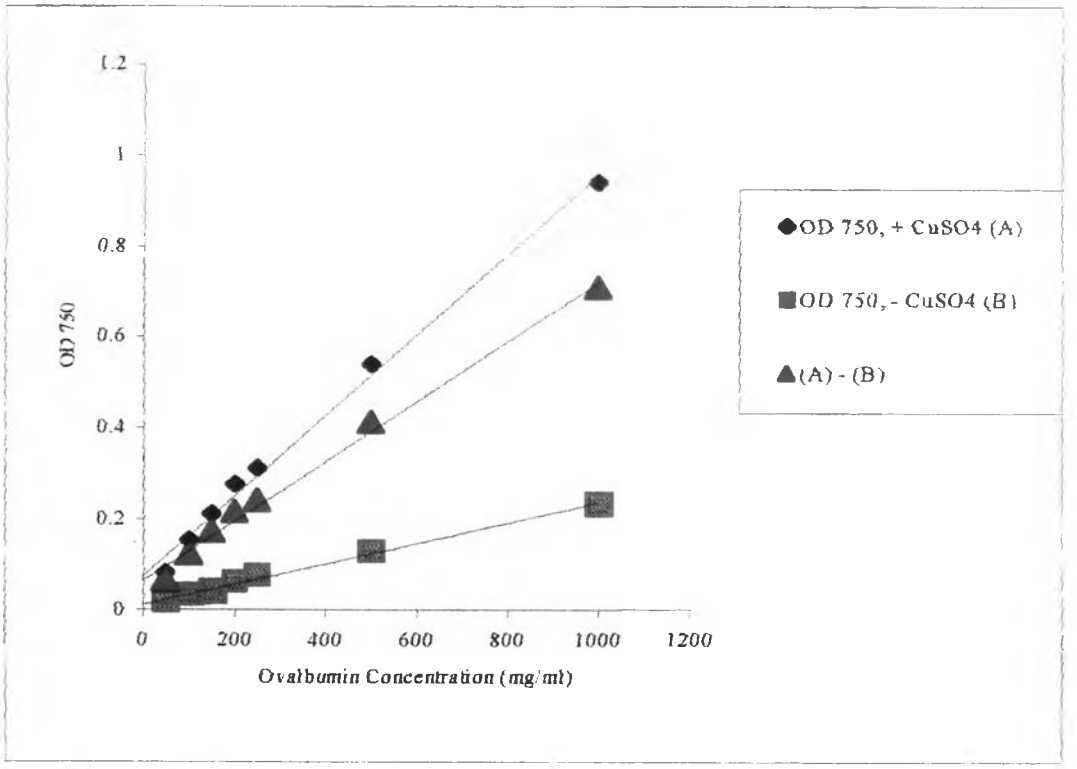

Figure 3.9 Effect of $CuSO_4$ on standard ovalbumin protein determination by Lowry Method

As papain itself is a kind of protein, at the amount of 0.3 phr (750 μg crude papain/ml, specific activity = 0.24 CDU/mg) used, the protein, may remain in the reaction mixture and in solid rubber. This experiment was performed to check the fate of papain under experimental conditions used for latex deproteinization.

1) Origin: Initial crude papain solution (0.3 phr) was prepared by adding 1.5 ml of stock 5% crude papain in water containing 3 ml 30% TCA and made up final volume to 100 ml.

2) Microwave treatment: Add 1.5 ml of 5% stock solution of papain (0.3 p.h.r: 750 μg crude papain/ml) in 98.5 ml of water, then microwave 100^{o}C for 1 minute.

3) Laboratory condition: Microwave 98.5 ml water for 5 minutes until temperature reached 50^{o}C, then add 1.5 ml of 5% stock solution of papain (0.3 p.h.r: 750 μg crude papain/ml) and incubate 5 minutes, the reaction was stopped by autoclave at 121^{o}C, 15 lb/in^{2} for 10 minutes.

Equal amount of papain (750 μg crude papain/ml) was used in all 3 conditions, the WEP was measured by modified Lowry method (2.12.3). Table 3.2 shows that the quantities of papain under the three conditions changed drastically after laboratory conditions. Papain had no activity in (1) because it was denatured and precipitated immediately by TCA whereas in (2), papain activity was destroyed completely after 1-minute treatment with microwave and the WEP concentration remained more or less similar to (1). In Laboratory condition (3) papain, having no latex protein may digest itself to smaller peptides, which were more sensitive to Lowry's reagent, resulting in higher protein concentration of 200 μg/ml. Since papain seemed to increase under laboratory condition (3), the molecular weight or form of proteins were further studied by SDS-PAGE.

Table 3.2 Protein concentration of WEP of different papain preparation

Sample (n=3)	Protein concentration (μg/ml, n=9)
Origin	110 ± 0.013
Microwave treatment	100 ± 0.021
Laboratory condition	200 ± 0.006

Origin: Initial crude papain solution (0.3 phr) was prepared by adding 1.5 ml of stock 5% crude papain in water containing 3 ml 30% TCA and made up final volume to 100 ml.

Microwave treatment: Add papain 5% stock solution 1.5 ml (0.3 p.h.r: 750 μg crude papain/ml) in the 98.5 ml water, then microwave 100°C for 1 minute.

Laboratory condition: Microwave 98.5 ml water 5 minutes until temperature reached 50° C, then add papain 5% stock solution 1.5 ml (0.3 p.h.r: 750 μg crude papain/ml) and incubate 5 minutes, the reaction was stopped by autoclave at 121°C, 15 lb/in^2 for 10 minutes.

Table 3.3 The water extractable protein prepared from STR5L, concentrated latex 60%, CDPNR, DPNR and glove samples

Sample (n=3)	The water extractable protein (μg/g, n=9)
STR5L	640 ± 0.005
Concentrated latex 60%	1120 ± 0.001
CDPNR	414 ± 0.012
DPNR	143 ± 0.028
Glove No.1	ND
Glove No.2	1150 ± 0.046
Glove No.3	7500 ± 0.041

CDPNR: Control rubber from fresh field latex

DPNR: Deproteinized fresh field latex

ND: Not determined

The next step is to identify the pattern of water extractable proteins by their molecular weight distribution.

3.2.2.2 Identification of water extractable proteins by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Figure 3.10 shows separation of water extractable proteins by SDS gel electrophoresis of papain original solution (Origin), microwave treatment (Microwave) and papain under similar laboratory condition (Lab). The original crude papain (lane 2) shows the area with dense bands between 20-28 kD. After microwave heating (lane 4), the major papain bands were destroyed into very small smear bands. The other 3 bands at 30, 35 and 67 kD may result from the larger contaminated protein bands shown in lane 2, or microwave treated papain aggregated and formed new protein bands at 30,

35 and 67 kD. Papain undergoes degradation to only one major protein band at 30 kD and many small bands in the range of 20-24 kD after steam treatment. Disappearance of papain bands (20-24 kD) corresponded with the loss of papain activity. Concentrated latex 60% (lane 5) displayed several thick bands ranging from molecular weight smaller than14.4, 14.4,15,18, 26, 33, 35, 40 and 65 kD that cover the major protein allergens; 14-30 kD. The WEP from three brands of gloves (lane 6, 7, 8) show different pattern of protein bands but, these bands are in the range of 14-30 kD, which correlated with previously reported major protein allergens. These results show evidence that different brands of gloves in the market produced from different process would result in different kinds and different protein content. The results also indicated the reason why some people, who were allergic to one brand of gloves may not be sensitive to other brands of gloves. The protein band at 67 kD found in microwave papain (lane 4) and Glove No.1 (lane 6) may occur from the heating process since this band does not exist in original papain solution and concentrated latex 60%.

Figure 3.11 shows separation of WEP by SDS gel electrophoresis from STR5L, CDPNR and DPNR in comparison with standard molecular weight markers (lane 1, 9 and Appendix 5). STR5L (lane 3) shows four major bands at molecular weight 20.1,29,50,67 kD and extended smeared band above 67 kD. CDPNR (lane 2) displays three clear bands at 14,18 and 28 kD which corresponded with major latex protein allergen. WEP from CDPNR differ from that of STR5L in the density of protein bands at 43, 30, 28, 20.1,18, 14.4 kD and smaller. Different process of STR5L and CDPNR preparation resulted in different protein pattern. After papain deproteinization, all the WEP from DPNR preparations (lane 4-8) obviously show no major latex protein allergen.

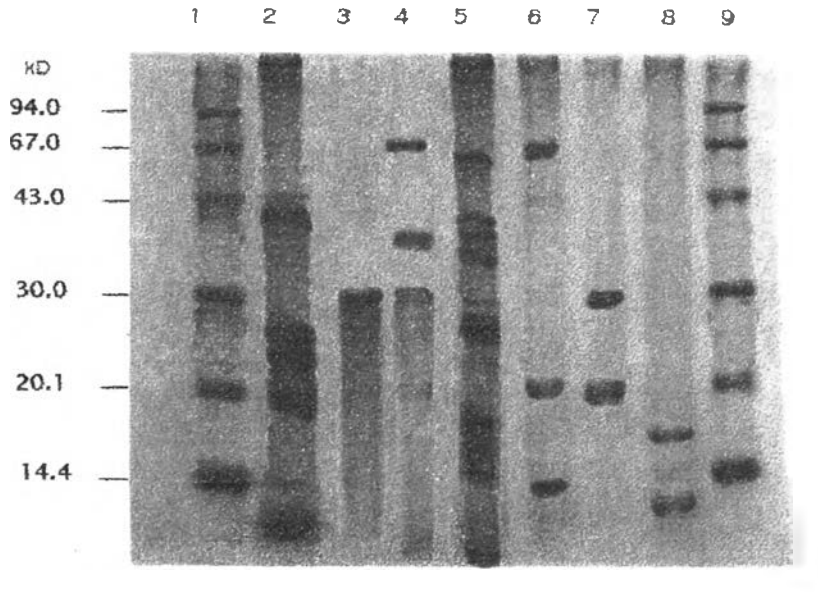

Figure 3.10 WEP pattern of papain (origin, microwave treatment, and laboratory condition), concentrated latex 60% and 3 brands of commercial gloves.

Lane 1 and 9 = Standard MW marker (14.4-94 kD)

Lane 2 = Papain (origin)

Lane 3 = Papain (lab)

Lane 4 = Papain (microwave)

Lane 5 = Concentrated latex 60 %

Lane 6 = Glove brand No.1

Lane 7 = Glove brand No.2

Lane 8 = Glove brand No.3

Each lane was loaded with protein 50 μg.

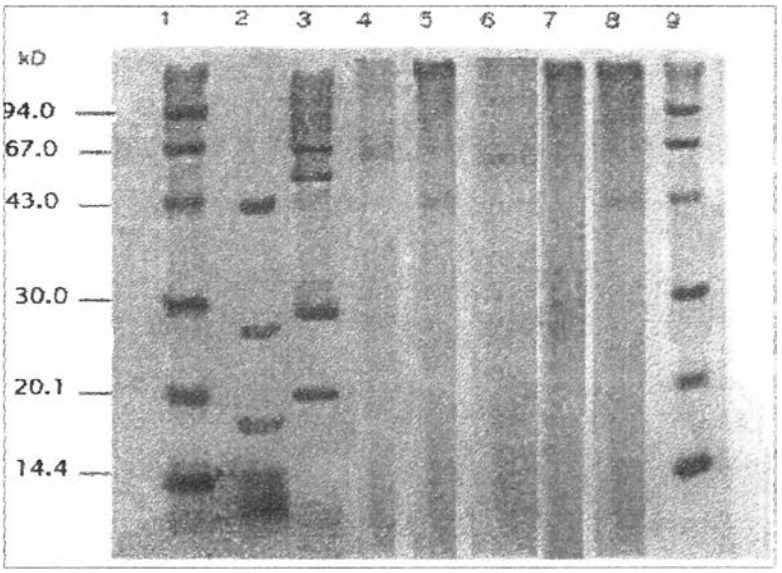

Figure 3.11 WEP pattern of STR5L, CDPNR and DPNR

Lane 1 and 9 = Standard MW marker (14.4-94 kD)

Lane 2 = Control of Deproteinized natural rubber, CDPNR

Lane 3 = STR5L

Lane 4-8 = DPNR

Each lane was loaded with protein 50 μg.

3.2.3. Prevalence of latex-specific IgE antibodies

To confirm the presence of latex protein allergens in solid rubber, and to screen for people with specific IgE for any of these latex allergens, the Enzyme Allergosorbent Test (EAST) was conducted in 300 sera, using latex protein allergens from concentrated latex 60% as standard. The serum samples, which contain latex-specific IgE, were detected according to Methods 2.8.5.1.

Positive results of EAST are evidence by the yellow color wells after incubation.

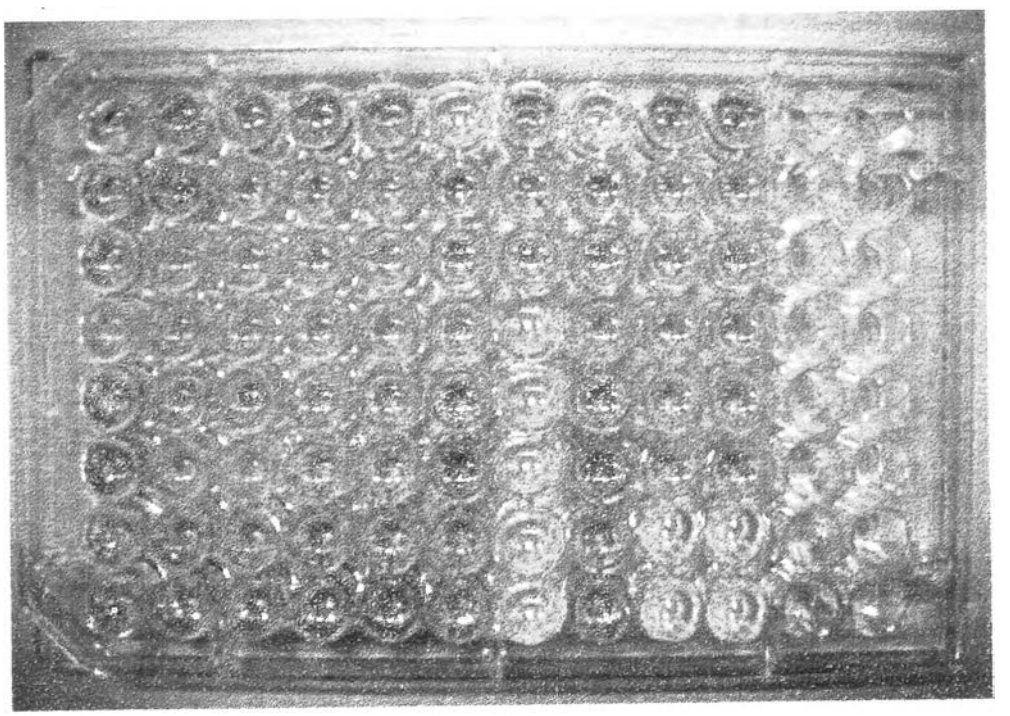

Figure 3.12 Positive EAST of the test serum: the serum that gave positive results was seen as yellow color wells.

The 300 human serum sample can be divided into 3 groups:

1. Control serums (100 samples) were collected from the volunteer healthy persons visiting the Ramathibodi Hospital and Veterans Hospital for general check-up (2.8.4.1).

2. Serum from general atopic patients (100 samples) (2.8.4.2).

3. Serum from general atopic healthcare workers (100 samples) (2.8.4.3).

Table 3.4 EAST positive with standard latex allergen from 3 groups of blood donors

Populations	Sample size	No. EAST positive (%)
1. Control	100	3 (3%)
2. Atopic patients	100	8 (8%)
3. Atopic healthcare workers	100	30 (30%)
Total	300	41 (13.7%)[a]

Table 3.4 shows 3% EAST positive in control healthy population, 8% in general atopic patients and 30% in atopic healthcare workers according to Newman_Keuls T-Test at 95% confidence (Appendix 7). The results of positive EAST evidenced for higher prevalence of latex-specific IgE antibodies in atopic group comparing to healthy people. The percentage of positive EAST, 30% in atopic healthcare workers from 3 hospitals (Ramathibodi Hospital, Veterans Hospital and School of Dentistry Mahidol University) was quite alarming and indicated for very high potential of latex allergy in this population.

All of the atopic patients and atopic healthcare workers were two hundred people. They were interviewed to study risk factors and history of atopic symptoms, which are summarized in Table 3.5.

Table3.5 Prevalence of risk factors and history of atopic patients and atopic healthcare workers with positive and negative latex-specific IgE

Characteristics	Atopic patient (n=100)				Atopic healthcare worker (n=100)			
	Positive (n=8)		Negative (n=92)		Positive (n=30)		Negative (n=70)	
	n		n		n		n	
Sex - Male	2	5.9%	32	94.1%	3	30%	7	70%
- Female	6	9.1%	60	90.9%	27	30%	63	70%
Age (Years)	8	29.88 ± 3.87	92	31.26 ± 6.36	30	32.90 ± 5.16	70	29.91 ± 4.72
		25-36		22-50		24-44		21-45
Pnor allergic diseases								
- Yes	5	7.2%	64	92.8%	24	33.8%	47	66.2%
- No	3	9.7%	28	90.3%	6	20.7%	23	79.3%
Family history of atopic								
- Present	6	8.7%	63	91.3%	15	30.6%	34	69.4%
- Absent	0	0%	7	7.61%	6	33.3%	12	66.7%
- Unknown	2	8.3%	22	91.7%	9	27.3%	24	72.7%
Working duration	8	92.75 ± 48.34	92	106.57 ± 81.15	30	127.73 ± 57.92	70	82.14 ± 53.11
(month)		38-184		7-336		25-258		8-250
Contact with latex products								
< 25 hr/wk	3	11.5%	23	88.5%	17	47.2%	19	52.8%
≥ 25 hr/wk	2	5.4%	35	94.6%	7	17.1%	34	82.9%
Unknown	3	8.1%	34	91.9%	6	26.1%	17	73.9%
No. gloves/day								
< 10 pairs	0	0%	13	100%	15	57.7%	11	42.3%
≥ 10 pairs	6	9.1%	60	90.9%	7	14.6%	41	85.4%
Unknown	2	9.5%	19	90.5%	8	30.8%	18	69.2%
Allergic symptoms								
- Skin reaction	5	13.2%	33	86.8%	21	42%	29	58%
- Urticaria/Angioedema	4	13.8%	25	86.2%	22	42.3%	30	57.7%
- Asthma	1	53%	18	94.7%	11	42.3%	15	57.7%
- Conjunctivitis	1	6.7%	14	93.3%	3	25%	9	75%
- Allergic rhinitis	4	11.1%	32	88.9%	18	36%	32	64%
- Anaphylacxis	0	0%	0	0%	0	0%	0	0%
- None	3	9.7%	28	90.3%	4	14.8%	23	85.2%

Table 3.5 shows risk factors: prior allergic diseases, family history of atopic, contact with latex product especially gloves, and allergic symptoms.

3.2.4 Allergen detection by EAST test

Positive EAST serum (n=41) from control healthy population, atopic patients and atopic healthcare workers were used for detection of latex protein allergens in the WEP from CDPNR and DPNR.

Table 3.6 Allergic response by EAST

Source of latex antigen	OD 405 (mean ±SD)		No. of Positive EAST / total No. of patient
	Negative EAST serum (n=259)	Positive EAST serum (n=41)	
Concentrated latex 60%	0.041 ± 0.021^{a}	0.207 ± 0.060^{b}	41 / 300
CDPNR	-	0.170 ± 0.044^{c}	41 / 300
DPNR	-	0.032 ± 0.018^{d}	0 / 300

CDPNR: Control of DPNR

DPNR: Deproteinized natural rubber

Table 3.6 shows significant difference of allergic response by EAST test between control and DPNR are marked by different letter (a,b,c,d) analyzed by Tukey's test and Newman-Keuls test at 95% confidence (Appendix 8). The results clearly show that WEP from DPNR contained no allergens and should be safe for all 41 persons that have latex-specific IgE.

3.2.5 Allergen detection by Skin Prick Test (SPT)

The Human Rights and Ethics Committee of the Division of Dermatology, Department of Medicine, Faculty of Medicine Chulalongkorn Hospital approved this study. Only one latex allergic patient has volunteered for SPT, kindly conducted by

Dr.Porntip Huiprasert. Table 3.7 shows the list of test solutions, total protein concentration and test results. The volunteer gave positive SPT results with latex proteins prepared from standard latex protein allergens, CDPNR and 3 brands of glove. Negative SPT results were observed with latex proteins prepared from DPNR. These results indicated that not all the latex protein bands made visible by SDS-PAGE or quantitated by modified Lowry method are allergens, therefore WEP from DPNR could not sensitize the volunteer. The WEP from the 3 different brands of gloves can tricker sensitize hypersensitivity in the volunteer although the protein patterns are different. Until now, the results cannot pointout which kind of specific protein that cause the latex hypersensitivity, but proteins that showed molecular weight distribution on SDS-PAGE in the range of 14-30 kD can be assumed to be latex allergens.

Table 3.7 Allergen detection by Skin Prick Test

Test solution	Concentration (μg/ml)	Skin prick testing (Wheal size, mm x mm)*
1. Histamine phosphate (Positive control)	10^3	++++ (12 x 15)
2. Normal saline (Negative control)	-	- (0 x 0)
3. Standard latex protein allergen	112	++++ (9 x 12)
4. CDPNR	32	+++ (6 x 6)
5. DPNR	< 1	- (0 x0)
6. Glove No.1	ND	+ (2 x 2)
7. Glove No.2	11.5	+++ (4 x 5)
8. Glove No.3	7.5	++ (3 x 3)
9. Glove No.4	ND	- (0 x 0)

* : After SPT 15 minutes

CDPNR: Control of deprotenized natural rubber

DPNR : Deprotenized natural rubber

ND : Not determined

Scoring relative to histamine wheal

++++ Strong positive, wheal size larger than 9x9 mm

+++ Clear positive, wheal size larger than 5x5 mm

++ Weak positive, wheal size larger than 3x3 mm

+, - Negative, wheal size $\leq$ 2x2 mm

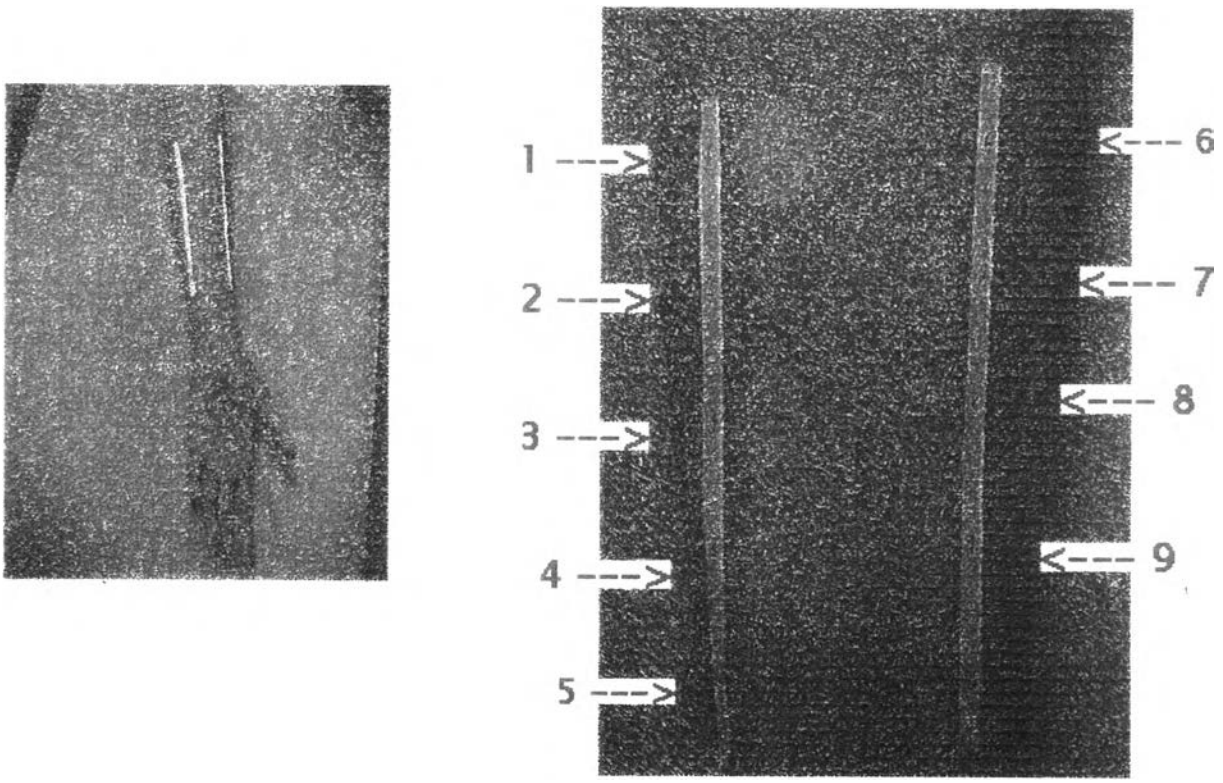

Figure 3.13 Positive SPT of protein allergen.

The samples that gave positive results show a wheal larger than 3x3 mm as similar to that of a positive control

1: Positive control (++++)

2: Negative control (-)

3: Standard latex protein allergen (++++)

4: CDPNR (+++)

5: DPNR (-)

6: Glove No.1 (+,-)

7: Glove No.2 (+++)

8: Glove No.3 (++)

9: Glove No.4 (-)

The volunteer subject has shown latex specific IgE with WEP from concentrated latex 60%, CDPNR, Glove No.2 and Glove No.3. Since the protein concentration in Glove No.2 (11.5 μg/ml) is higher than Glove No.3 (7.5 μg/ml) and protein band 30 kD were observed that should correspond to different sizes of wheal. It is indicated by the SPT that this volunteer has the IgE, which reacted with latex proteins of various molecular weights, most probably 14, 18 and 30 kD.