CHAPTER IV
DISCUSSION

4.1 The new deproteinization process

The aim of this research is to produce a new grade of solid rubber: deproteinized solid rubber, which is free from protein allergens. The work has been successful by the coupling action of microwave energy and papain treatment.

For the optimization of this new process, both papain and Alcalase were studied. The result from microwave and papain treatment was better than microwave and Alcalase treatment, because papain is a neutral protease (pH 7.6). Thus papain is active in fresh field latex, which has pH 7-7.5, by using very small amount of chemicals to adjust the optimal pH. The deproteinized solid rubber obtained from microwave and papain treatment looks like STR5L for its light color, but low nitrogen content ($\approx$ 0.15 g% nitrogen content), good elasticity and good odor. The DPNR from microwave and Alcalase treatment was not satisfied because Alcalase is an alkali protease (pH 9.6). To adjust the fresh field latex from pH 7-7.5, to the optimal pH of Alcalase requires addition of ammonia that leads to the non-benefit chemical using and unsatisfied product. The DPNR from Alcalase treatment was too hard and the orange-red color was unsatisfied. Alcalase also caused partial coagulation resulting a lot of small popcorn particles dispersed in latex before steam coagulation. When the per cent nitrogen content was analyzed, the values fluctuated widely because in the small pieces of popcorn area, proteins were trapped and the high level of nitrogen remained as a result of heterogeneous reaction. At the 5-liter pilot scale, Alcalase was therefore not used in the process.

Deproteinization by papain is not a new method, but there was no information about the best conditions, the remaining proteins and their allergenicity.

The best conditions for field latex deproteinization by papain and microwave energy reported in this research are summarized as follows:

3 x 5 Liter fresh field latex

(≈ 30% DRC in 0.2% Ammonia)

↓

To field latex, add 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 20% latex by microwave until latex temperature is 50°C

(heating time 13 min in Sanyo, EMO-900T)

↓

Add 5% stock papain solution to the final concentration of 0.3 phr

↓ 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, 4 hrs

↓

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

* Operation time: 8-10 hrs, 76.67% Yield

Table 4.1 The cost of deproteinized natural rubber

Items	Unit cost (Baht/kg)	Consumption (kg)	Total cost (Baht)
Latex 20% DRC (15 L x 5 days = 75 liter)	17.50	15	262.50
papain	638.75	0.015	9.58
Hydroxylamine.HCl	82	0.0225	1.84
Sodium metabisulfite	25	0.0075	0.19
Wing-stay L	45	0.0075	0.34
Water for latex dilution	0.28	75	21.00
Electricity and labor expense	2.50	15	37.50
Total cost			332.95

Percentage of yield: 76.67% was calculated from the 15 liter of 20% DRC latex or 3 kg rubber

Yield of DPNR = 2.3 kg

Cost = 28.95 Baht / kg

The yield of DPNR was 76.67% from this procedure. The reduction of nitrogen content was maximum at 79-81%, which is lower than 82-90% reported by Koosakul 1994, but the enzyme treatment time was reduced from 55 minutes to 18 minutes (microwave and papain). In factory it could be applied as a continuous process, which should be more practical. When a larger batch of latex, 15 liters was used, the percentage of total nitrogen in DPNR was 0.18 g%. Although the percentage of total nitrogen was not reduced as much as in the 100 ml laboratory scale (0.15 g%), and per cent yield was only 76.67%, comparing with 90-97 per cent yield at the 100 ml laboratory scale. The yield of pilot scale DPNR was lower than the laboratory scale study, because the coagulum was shredded using the factory production line, and the rubbers were lost with in the process. The cost of deproteinized natural rubber production is another important factor for manufacturer. Table 4.1 summarizes the cost of deproteinized natural rubber production per one kilogram of solid rubber, which has been estimated from latex raw material, chemicals, water, electricity and labor. The cost of deproteinized natural rubber was 28.95 Baht per one kilogram, while the cost of STR5L was 22 Baht per one kilogram. Though the cost of deproteinized natural rubber was higher than that of STR5L, about 7 Baht per one kilogram, but the deproteinized natural rubber had higher quality than STR5L: namely lower protein content and lower allergenicity. It is expected that DPNR price should be about 10 Baht higher than STR5L from this estimation. When compare with deproteinization by saponification (Boonsook, 1999), the cost of SAP-AL was 30.04 Baht/kg excluding cost of water, electricity and labor, this new process is less expensive as the production cost per kg including water, electricity and labor is only 28.95 Baht/kg (Table 4.1). The cost of DPNR production may be reduced if fresh papain at higher specific activity can be obtained locally at lower cost than the imported papain.

4.2 Raw rubber properties of the new grade DPNR

The raw rubber properties obtained from deproteinized natural rubber show variable properties of raw rubber obtained, mostly in the acceptable range of STR5L specification, except the lower in Initial Plasticity (Po) and Plasticity Retention Index (PRI).

Initial plasticity (Po) and plasticity retention index (PRI) confer to the resistance of raw rubber to oxidative degradation before and after aging. Plasticity retention index (PRI) is expressed as a percentage of aged to initial plasticity. The removal of latex protein by enzyme treatment contributes to the drop in plasticity retention index (PRI) that means deproteinized natural rubber (DPNR) will be easily oxidized. Besides removal of protein can effect PRI. Silvabalasundevam and Nadarajah (1965) have reported anti-oxidant can improve PRI. Boonsook, 1999 reported that removal of protein by saponification of both ammoniated crumb rubber and skim crumb rubber resulted in lower PRI comparing to control non-saponified rubber, and suggested that natural anti-oxidants were washed away from the crumb rubber. In order to increase Po and PRI to the acceptable limit of STR5L specification, the researcher suggested that more anti-oxidant should be added or anti-oxidant should be changed for the future production of DPNR.

4.3 Effect of microwave and steam on papain and latex protein

The use of microwave energy in latex deproteinization is to excite the polar molecules by two mechanisms: dipole rotation and ionic conduction. Since papain and latex proteins are polar molecules, they must be affected by electromagnetic field in the same way as water. Experiment with papain only (Figure 3.10, lane 2 and 4) showed that the enzyme activity of papain as well as the native polypeptide bands at 20-28 kD were destroyed under microwave energy into smeared bands of <30 kD and created 3 new bands at 30,35 and 67 kD. However when the total papain concentration was assayed by Lowry's method, the total protein remained the same as in the initial papain solution (Table 3.2). Comparing with active papain under laboratory condition (because

only water was preheated by microwave), self-deproteinization occurred resulted in many smaller peptides visible by smear bands under 30 kD (Figure 3.10 lane 3) and increasing protein concentration about 2 fold by Lowry's method (Table 3.2).

The initial protein patterns of concentrated latex 60% (Figure 3.10 lane 5) compared with latex protein that pass through laboratory conditions (+microwave, -papain, +steam) shown in Figure 3.11 lane 2 provided evidence for the structural change and decreased protein bands of latex protein as affected by microwave and steam. These results supported the finding in Table 3.3 that the WEP from CDPNR was lower than those of concentrated latex 60% and STR5L.

The combined effect of microwave, papain and steam in the deproteinization of the latex proteins (Figure 3.11 lane 4-8) showed that papain destroyed completely all the WEP especially the major allergens (14-30 kD). However these small fragments of WEP from DPNR were still be measured by Lowry's method about one-third of CDPNR (Table 3.3). It is concluded that positive Lowry assay may not correspond with allergenicity.

In the process of DPNR production, after steam coagulation, the coagulum rubber was washed with water 3-4 times before shredding and dried at 60-120^{o}C for 4 hours. All the small fragments resulted from papain hydrolysis can dissolve in water very well, thus most of the WEP were washed away.

In the glove manufacturing process, after gel-formation and vulcanization at 120-150^{o}C, gloves were leached several minutes and therefore only a few latex protein remained associated with the glove sample as shown in Figure 3.10 lane 6,7,8. However without any protease treatment these protein bands are intact and remained active protein allergens.

4.4 Latex Allergens and prevalence of latex allergy

In Finland, U.S.A and other countries, more than 15 natural rubber protein allergens were reported from various rubber protein preparations. Those of molecular weight 10, 14, 20, and 30 kD referred to as major allergens have been demonstrated by more than one group (Turjanmaa, 1987, Turjanmaa, 1994, Turjanmaa et al., 1997, Slater et al., 1994, Morales et al., 1989, Alenius, 1991, Chamberon et al., 1992, Jaeger et al., 1992 and Alenius et al., 1994). Using glove extract, the first major latex allergen was identified by Czuppon et al., 1993, as rubber elongation factor (REF); a 14.6 kD latex protein tightly bound to rubber particles. Recently, Alenius et al.,1995 purified and identified three natural rubber latex proteins by microsequencing, namely prohevein (20 kD), hevamine (30 kD), and a 36 kD protein exhibiting high homology to several plant endo 1,3-β- glucosidases. They demonstrated that prohevein is another major latex allergen. Beezhold et al., 1994 focused their interest on a 46 kD allergen in natural latex and found a sequence homology to patatin, a major storage protein of the Solanaceae. The 27 and 14.6 kD proteins had been purified and confirmed to be the major allergens in spina bifida children (Alenius et al., 1995).

In Figure 3.10 and 3.11, concentrated latex 60% produced in Thailand shows more than 8 latex protein bands at 14.4-18, 26-30, 33-40 and 65 kD which covered the major latex allergens summarized in Table 4.2. Glove samples, CDPNR and STR5L showed protein bands in the same range as concentrated latex 60%; but smaller in number of protein bands. In contrast DPNR has no distinguished protein bands in the range of 14-30 kD.

This result is also confirmed by negative EAST with WEP from DPNR in 41 human serum samples which gave positive EAST test to standard latex allergens prepared from concentrated latex 60%, and CDPNR.

All these results indicated that the new latex deproteinization process by papain and microwave energy is successful in the production of allergen-free solid rubber. The weak point of this new process is the low Po and PRI of the raw rubber comparing to STR5L which resulting in low oxidation aging and shelf life of rubber

products. The WEP from DPNR when compare with the WEP from the gloves may be have smaller amount of proteins so when the SPT was done there was no wheal. The application of this new DPNR may be limited to disposable short-life medical products.

Table 4.2 Potential latex protein allergens

Sample	<5	10	15	20	25	30	35	40	45	50	>50
Concentrated latex 60%		< 14	14 15	18	26	33	35	40			65
STR5L				20		29				50	> 67
CDPNR		< 14	14	18		28			43		
Glove No.1			14	20							67
Glove No. 2				20		30					
Glove No. 3		< 14	16								
DPNR											

The prevalence of latex sensitivity using EAST in general healthy population observed in this research is 3% which is about the same as 2.3% reported by Porri et al, 1995, using RAST, but lower than the blood donor population 4.8% using RAST and EAST reported by Ownby et al, 1994, Merrett et al, 1995, and Harnchoreon, 1996. The overall prevalence for anti-IgE antibody in general allergic patients and general allergic healthcare workers is 8% and 30% in sample size of 100 persons from 3 hospitals. Teeraratkul et al., 1997 has reported that the prevalence of latex allergy in health care workers population at Siriraj Hospital was 3.13% by SPT and 12.4% by questionnaire. Chaiear et al, 2000 has reported positive SPT of 1.7% and 1.3% in latex glove manufacturing workers and latex-tappers in Thailand, and no positive SPT in 144 college students. The observed prevalence of latex sensitivity 30% in 100 general atopic healthcare workers is the highest value comparing to other reports summarized in Table 1.7, in which the second highest 22% prevalence was reported by Dounglas et al, 1997 in 140 nurses population in Australia.

The questionnaire used in this study on prevalence of risk factor and history of Thai general atopic patients (n=100) and atopic healthcare workers (n=100) summarized in Table 3.5 indicated that about 30% have family history of allergic

diseases, more than 50% have contact with latex gloves and more than 40% have dermal allergic symptoms and respiratory allergic symptoms.

This prevalence study on latex sensitivity suggests that there should be awareness and precautions about latex protein allergy in Thailand especially for the atopic population and atopic healthcare workers. Standard test kit for SPT of latex sensitivity should be available for high-risk populations and general atopic patients who need operation.