

## CHAPTER IV DISCUSSION



### 4.1 Operation procedures for saponification

The enzymatic deproteinization process by a combination of a proteolytic enzyme and surfactants have been reported as the favorable method to produce DPNR. However, it was economically and practically difficult to make solid DPNR form latex DPNR due to the chemical and physical stabilities of DPNR latex containing surfactants. Visessanguan, 1992 has reported the problem of coagulation of fresh field latex by papain that he sometimes failed to coagulate because of the large dilution and pH can stabilize the system.

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

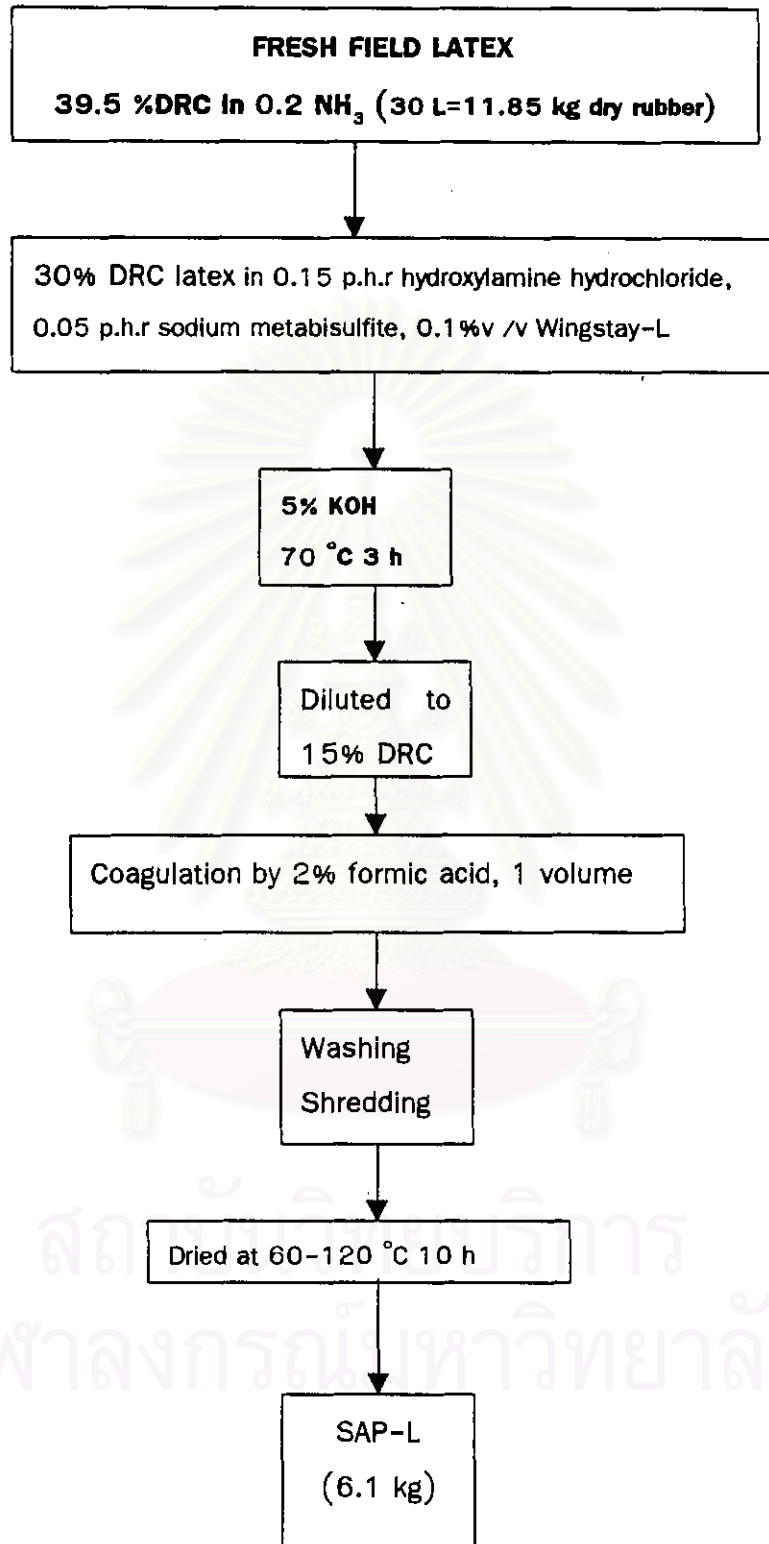
The optimal conditions for deproteinization by latex saponification of this research are as follows: 1) preparation of 30% DRC fresh latex in 0.2 %  $\text{NH}_3$ , 0.15 p.h.r hydroxylamine hydrochloride, 0.05 p.h.r sodium metabisulfite and 0.1 %v/v Wingstay-L; 2) saponification reaction in 5 % w/v KOH at 70°C, 3 hours; 3) washing of saponified latex by diluting to 15 %DRC and coagulated in equal volume of 2% formic acid, washed and passed through shredder, then dried 70°C (Figure 4.1).

These procedures are difference from latex saponification used by Tanaka, 1998 in 2 steps: 1) ten %v/v of 2-propanol was omitted in saponification reaction; and 2) saponified latex was diluted to 7.5% DRC, not 3%DRC.

Saponified rubber obtained from this procedure resulted in solid NR of 0.2 % N content (Table 3.1), which is much higher than 0.024 % obtained by Tanaka, 1998. Furthermore, when a large batch of latex, 15-liter was used, the % N not reduced.

The cost of SAP-NR production is another important factor for manufacturers. The cost of SAP-NR production per 1 kg of solid rubber has been estimated based only on the chemicals and latex consumption. The cost of SAP-L production is 54 Bath/kg, where is double to STR5L (22 Baht/kg). The high cost is partly due to very low yield (58%) when the batch size was increased form 100 ml to 15 liter. Another unsatisfied condition is the alkali solution has to be discarded as wastewater every batch of latex saponification. To minimize the loss of yield, KOH, wastewater and reduce the cost of SAP-NR, latex was coagulated and thus crumb rubber was saponified. By this way alkali solution can be reused.

Figure 4.1 The operation procedure for saponification of fresh field latex



Operation time: 30-33 h, 58% yield, cost: 54 Baht/kg.

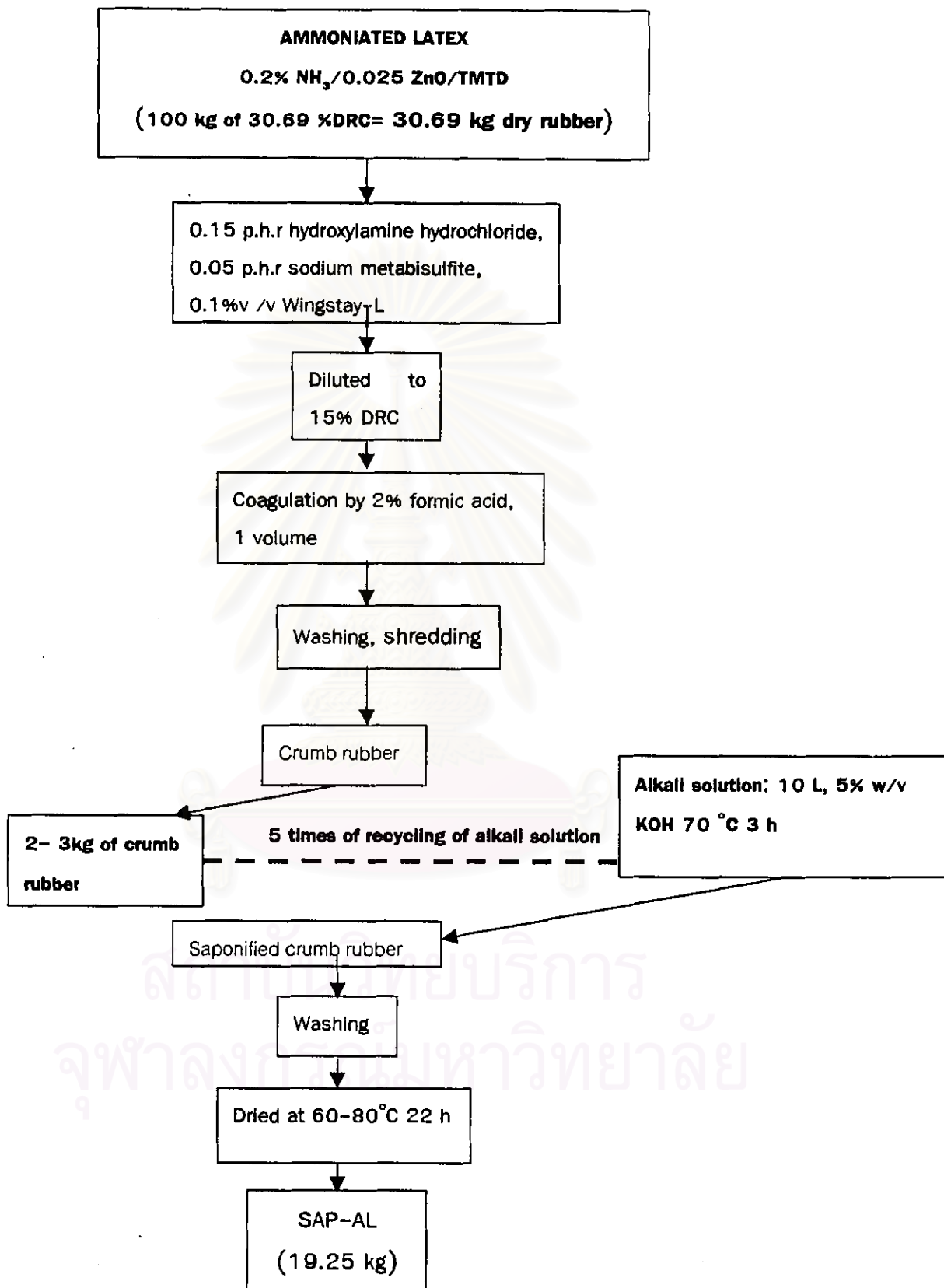
The operation procedures for ammoniated crumb saponification are summarized in Figure 4.2, the yield of solid rubber, SAP-AL is 63%, which is slightly higher than SAP-L. In contrast saponification of skim crumb as describes in Figure 4.3 resulting significant higher yield of 98%. The cost of SAP-SK production is therefore minimized to 25 Baht/kg. However it is noted that saponified rubbers are difficult to dry and more than 10 hours are required at 60-120° C.

By recycling KOH solution or alkali solution for saponification, the efficiency of alkali solution in saponification of skim crumb rubber is lower than saponification of ammoniated crumb rubber (Figure 2.1a,b). Recycling of alkali solution in the SAP-AL and SAP-SK production are 7 times and 5 times, respectively. After these limit the nitrogen content of both SAP-NR increased; these may because the alkali solution was neutralized by remaining acid in the rubber after coagulation. The lower recycling number of skim crumb saponification is caused by sulfuric acid used for skim latex coagulation has high concentration; and faster neutralized the alkali solution. While ammoniated crumb was coagulated by formic acid at milder concentration.



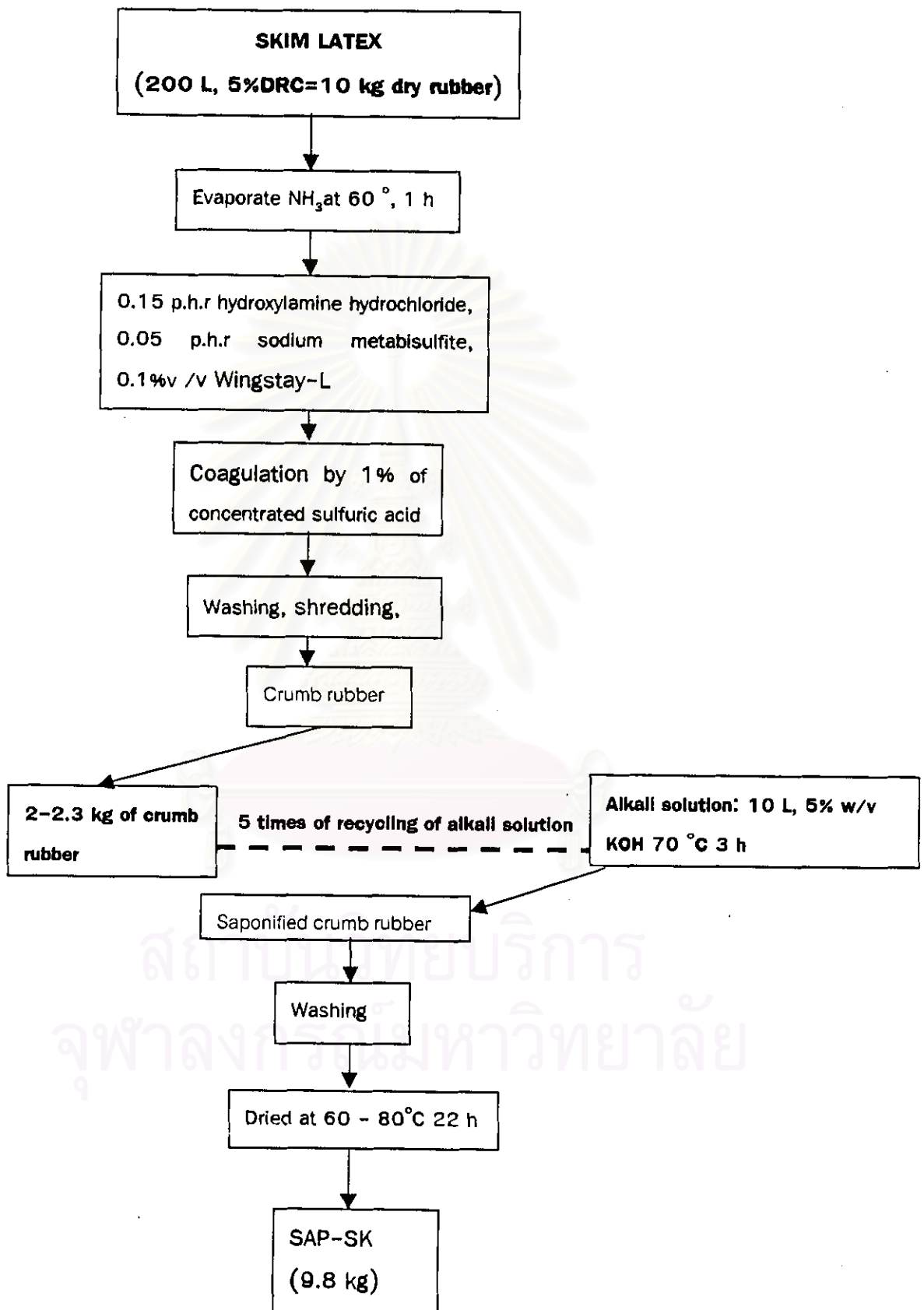
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Figure 4.2 The operation procedure for saponification of ammoniated crumb rubber



Operation time: 50-51 h, 63% yield, cost: 30 Baht/kg.

Figure 4.3 The operation procedure for saponification of skim crumb rubber



Operation time: 50-51 h, 98% yield, cost: 25 Baht/kg.

## 4.2 The properties of saponified rubber

### Raw rubber properties

Saponification of wet crumb is more effective than latex. This is supported by the reported of Rungvichaniwat et al., 1998, comparing skim latex and skim crumb treated by NaOH at various time (0-120 hours) and temperature (25-70° C). The minimized nitrogen content reported was 0.2%. In this study, the % N in SAP-SK was reduced to 0.12 % under operation procedures described in Figure 4.3.

The raw rubber properties obtained from SAP-NR of SAP-L show variable properties of raw rubber obtained, mostly in the acceptable range of STR5L specification, except the highly in ash content. Like the raw rubber properties of SAP-AL and SAP-SK that show no significantly different properties such as the dirt content, V.M, ash content. These properties are within acceptable limit of STR5L specification except ash content. The explanation of high ash content could be that the ash content in NR usually compose of  $PO_4^{3-}$ ,  $Mg^{2+}$ ,  $Ca^{2+}$  and other elements which may be retained in NR or come from the chemical in the process. For SAP-L, increase ash content may come from KOH trapped in latex before acid coagulation. Ash remaining in saponified crumb rubber is lower than latex saponification.

Initial plasticity and plasticity retention index (PRI) confer to the resistance of raw rubber to oxidative degradation before and after aging. PRI is expressed as a percentage of aged to initial plasticity. The removal of latex protein by saponification contributes to the drop in plasticity retention index (PRI) that means SAP-NR will be easily oxidized. Besides removal of protein can effect PRI. Washing crumb rubber several times can reduce antioxidants such as tocotrienols (Morimoto, 1985), phosphoaminolipids, amines and amino acids, resulting in lowered resistance of rubber to heat aging. Silvalabasundevam and Nadarajah (1965) have reported anti-oxidant can improve PRI.

Saponification of both SAP-AL and SAP-SK improve in PRI comparing to SAP-L. The results suggest that more anti-oxidant may remained in the crumb.

### Vulcanized rubber properties

In this study, the cure characteristic of SAP-AL and SAP-SK show no significant different from non-saponified rubber and STR5L from normal production used as control. Since the formulation used for curing has low NR and high accelerators, the cure rate is slightly increase for SAP-SK (Figure 3.8d). The existence of high nitrogen in NR has been believed to affect the cure behavior of rubber compound. Bloomfield (1973) has suggested that proteins can interfere with the homogenization of vulcanizing ingredients, thus reduction in cure rate of NR. Skim rubber, the by-product of latex concentrate production, containing high nitrogen content (2 g%) and high level of acetone extract show short scorch time couple with long cure time both in ACS mixing formulation (Briston, 1990) and sulphur/sulphenamide vulcanization system (Greensmith and Watson, 1969).

The presence of hydrophilic impurities such as proteins and inorganics salts can enhance the absorption of water in NR. The uptake of water by NR has effect on the processibility and physical properties of NR. The tensile strength decreased with increase in moisture content. While the heat build-up increase (Perera et al., 1985). SAP-AL and SAP-SK show no improvement in tensile strength, because the inorganic salt is still high so lower % nitrogen does not help. The results show that deproteinization by saponification has no effect on 300% modulus, tensile strength, tear strength, hardness and specific gravity except a decrease in elongation at break. The elongation at brake values reflects the cross-linking density of the rubber. The decrease in elongation value of SAP-AL and SAP-SK indicates that the cross-linking density of the SAP-AL and SAP-SK rubber decrease. This result is confirmed by decreasing in gel content of crumb rubber obtained from ammoniated and skims crumb after saponification.

#### **4.3 Molecular characteristic of rubber before and after saponification**

The molecular characteristics of saponified rubber prepared from ammoniated crumb show a slightly decrease in the  $\bar{M}_w$  and  $\bar{M}_n$  after saponification. It is clear that the  $\bar{M}_w$ ,  $\bar{M}_n$  decreased with the decreasing of the nitrogen content. This can imply that a decreasing  $\bar{M}_w$  and  $\bar{M}_n$  result from the decomposition of the branch point to form linear molecule. This result corresponding to that reported by Tanaka et al.

(1997) with after this treatment,  $\bar{M}_w$  and  $\bar{M}_n$  of saponified latex reduced to about two third of the control sample.

Table 4.1 shows the effect of saponification on SAP-SK. The molecular weight distribution of SAP-SK shows higher polydispersity, than non-saponified skim crumb rubber. The decreasing of gel content in non-saponified skim crumb rubber from 32% to 3-4% in SAP-SK support the MWD finding that the cross-link structure must be diminished together with breaking in rubber particle and protein nitrogen content. The cross-linking involved fatty acid ester also decreased as evident by the decreasing of acetone soluble content in SAP-SK. According to GPC profiles, the molecular characteristics of saponified rubber prepared from skim crumb shows a drastic decrease in the  $\bar{M}_w$  and  $\bar{M}_n$ . This less value of  $\bar{M}_w$  and  $\bar{M}_n$  suggests that SAP-SK is very small molecule. The first explanation is due the decomposition at the phospholipid branch point of the rubber to form linear molecules. The second explanation, skim is usually no antioxidant thus it prone to oxidation. The third explanation is auto-oxidation of fatty acid ester during saponification process, which lead to the degradation of rubber so the rubber was braked. The results can be supported by the reported of Keller et al., 1981 that they have examined the oxidative role of fatty acids in Grayule resin. They found that the unsaturated acids such as linoleic and linolenic acids were highly susceptible to auto-oxidation and their oxidation products (hydroperoxides) acted as initiators in polyisoprene oxidation. This result can be corresponds to the structural characteristics of SAP-SK evident by FT-IR that the band of ester diminished after saponification.

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Table 4.1 Effect of saponification on molecular characteristic of SAP-SK

Source	Effect of saponification on molecular characteristic of SAP-SK	
	Factor	Effect
Table 3.4	Nitrogen content	% N decrease from 1.85% to 0.12 %
Table 3.6	Gel content	% gel decrease from 32% to 3-4%
	Fatty acid (acetone solubility)	% acetone solubility decrease from 4.6% to 2-3%
Table 3.5	$\bar{M}_w$ , $\bar{M}_n$	$\bar{M}_w$ decrease from $3 \times 10^6$ to $0.1 \times 10^6$ , $\bar{M}_n$ decrease from $1 \times 10^6$ to $0.2 \times 10^6$ $\bar{M}_w/\bar{M}_n$ increase from 3 to 5-9

In both saponified rubber, the gel content decrease in proportion to the nitrogen content. Although the method to determine gel content was different from that reported by Tanaka et al. 1997, in the force of centrifugation of gel and filter before centrifugation that they centrifuge at 10,000 g for 30 min. In this study, gel was centrifuged at 3,500 rpm or 5,000 g for 1 hours but when comparing the force and time (g. min) there is no difference. Comparing the reported of Tanaka et al. (1997) which the gel content in the initial of FL-latex (5%) was most likely the same as of ammoniated crumb (5.7%) in this study so the method of gel determination in this research is believable. And the rubber from FL-latex was reduced from 5% to 1% by saponification. In the previous work of Tanaka et al., 1997 deproteinization by enzymatic reaction of HL-latex decreased the gel content from 42.5% to 2.2% (Eng et al., 1993).

#### 4.4 Structural characteristic of rubber before and after saponification

The presence of proteins and fatty acids can be detected by FTIR spectroscopy. It is clear that the intensity of the infrared band at  $3280 \text{ cm}^{-1}$ , which is assignable to  $\nu_{\text{N-H}}$  markedly, shifted to  $3417 \text{ cm}^{-1}$  that is terminal amino acid of oligopeptides as the nitrogen content of the SAP-SK decreased while this band remained in SAP-AL. These suggest that ammoniated saponification hydrolyze amide linkage of protein and still

consist of polypeptide because the inner N-H units of the peptide linkage is persisted.

The characteristic bands of the amide and amine bending at  $1628\text{ cm}^{-1}$  and  $1540\text{ cm}^{-1}$  were disappeared in both SAP-AL and SAP-SK. This indicates that the saponification with KOH hydrolyze amide linkage of SAP-SK better than in hydrolyzing amide linkages of SAP-AL. The structural characteristics of SAP-SK evident by FT-IR that the band of ester diminished after saponification. These can imply that skim crumb saponification hydrolyzes peptide linkage of protein that covalently linked and associated link to NR, resulting of oligopeptide. Saponification of skim crumb rubber hydrolyzes ester linkage of phospholipid that covalently-linked to NR. Saponification in ammoniated crumb rubber by these procedures, the reaction may hydrolyze only protein that associated link to NR.

#### 4.5 Total protein determination

Latex protein allergen is extractable proteins. Much of the protein in latex is not tightly bound to the rubber, and readily leaches out of the latex. The water extractable protein on NR in this research is determined by Lowry method. The amount of water extractable protein was derived from ovalbumin standard calibration graph by subtracting the  $O.D_{760}$  in the present of  $\text{CuSO}_4$  with the  $O.D_{760}$  in the absent of  $\text{CuSO}_4$ . But the  $O.D_{760}$  of SAP-AL and SAP-SK has no direct correlation with  $O.D_{760}$  in the absent of  $\text{CuSO}_4$ . These cause by the contaminants such as salt and other metal elements such as  $\text{Zn}^{2+}$  that came from rubber processing. Therefore, desalting, dialyzing and purification of protein by acid precipitation should improve protein determination by this method. However, the very low amount of water extractable protein is limitation. Yusof et al., 1992 has suggested that to concentrate the protein in the extracted glove rubber with trichloroacetic acid (TCA) and phosphotungstic acid (PTA) is a rapid and quantitative method for removing the unidentified and variable interfering substances in latex glove leaches. Based on Lowry's method in the quantity of water extractable protein, there is no significant difference water extractable protein between C-AL and SAP-AL. The results indicate that saponification of C-AL can not remove water extractable protein, while the water extractable protein of SAP-SK significantly reduced thus can imply that the water extractable protein of rubber were not removed. These can confirmed from the surface area and pore size of rubber that the surface area of SAP-SK are more smoothly than that of C-SK and the pore size of

SAP-SK are smaller than C-SK. So the higher chance of protein will be removed from the larger pore size or from C-SK.

Two of six samples of SAP-AL and three of six samples of SAP-SK show a clear major band at 45 kD (Figure 3.15a lane 4 and 6 and Figure 3.15b lane 2,3 and 5). Although each lane was loaded with 50  $\mu\text{g}$  of water extractable protein according to modified Lowry determination. There are no protein band in other SAP-AL and SAP-SK loaded lanes. This result indicates the disadvantage of modified Lowry method that;  $O.D_{750}$  may not correlate with the amount of water extractable protein.

There are several proteins band in the range of 14.2–45 kD found in non saponified rubber. This results similar to the pattern found in rubber particles isolated from fresh latex or high ammoniated latex concentrated (Hasma, 1992 and Slater et al. 1994). After crumb rubber saponification, the major protein of 45 kD persisted in SAP-AL and SAP-SK.

#### 4.6 Protein allergen

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 10,14,20,30, and 100 kD allergens have been demonstrated by more than one group (Slater et al., 1994, Morales et al., 1989, Alenius, 1991, Chamberon et al., 1992, Jaeger et al., 1992 and Alenius et al. 1994). The first major latex allergen was identified by Czuppon et al.1993, using glove extract, 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 (20kD), hevamine (30kD), and a 36 kD protein exhibiting high homology to several plant endo 1,3- $\beta$ -glucosidase. 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).

Although SAP-AL and SAP-SK contain 45 kD protein and protein lower molecular weight than 14.2 kD, allergen detection by EAST test using extractable protein of SAP-AL and SAP-SK show negative result. Whereas both non-saponified

rubber prepared from C-AL and C-SK show positive EAST. This data indicated that the 45 kD protein and protein lower than 14.2 kD observed by SDS-PAGE in both saponified rubbers may not be a significant major allergen. These result are in correspondence with the previous study by Harncharoen, 1996 that major latex allergens in Thai people are 25.5 and 30 kD protein other less frequency found in Thai people are 14,18,38, and 52 kD.

Latex allergy among healthcare workers can be considered as a serious occupation related disease. In this study, the prevalence of latex allergy in healthy blood donors, general patients and atopic health care workers was studied by using EAST test. The overall prevalence for anti-IgE antibodies in healthy blood donors is 5 %. This results is similar to the 4.5% prevalence of latex allergy in Thailand reported by Harncharoen, 1996. Teeraratkul et al.1997 has reported that the prevalence of latex allergy in population at Siriraj Hospital in health care workers was 15.2% by skin prick test. This 5-15% prevalence of latex allergy in Thai people confirmed the 5-10% prevalence of latex allergy in other countries more or less than several reports (Merrett, 1993, Turjanmaa, 1987, Ownby et al. 1994), using either SPT or latex-specific IgE antibodies. The result of this study shows the prevalence of specific IgE to latex protein antigens in 6 atopic health care workers is 66 % which is significantly higher than control healthy blood donor group (5%). The prevalence of specific IgE to latex protein in health care worker is high comparing to the report by Teeraratkul et al.1997 that the prevalence of health care workers is15.2% in 224 persons by skin prick test. The explanation are: firstly, the high prevalence may be due the small number subject tested and the difference method, secondly the health care workers reported in this study are atopic patient, therefore having more than risk factors for latex allergy; thirdly, the high prevalence of these EAST positive cases may result from cross-reactivity with some fruit allergy e.g. banana. Previous studies have shown that avocado pear, banana and chestnut can cause cross-reactivity with latex by RAST.

This prevalence of latex protein allergen suggests that there should be awareness and precaution about latex protein allergen in Thailand especially for atopic patients. If they were assigned for an operation or subject to internal rubber-based medical devices such as catheters.