CHAPTER IV

DISCUSSION

4.1 The condition of latex deproteinization by Papain and Alcalase

Latex deproteinization by enzyme has been reported as the favorable method to produce DPNR. Many kind of proteases have been subjected to latex for this purpose. But the feasibility of only two enzymes, Papain (Rubb. Res. Inst. Sri Lanka, 1986) and Alcalase (Chang et al., 1977), were reported in large scale production.

The optimized condition for deproteinization by Papain and Alcalase of this research can be specified in term of pH optimum, temperature optimum, concentration optimum and optimal reaction time. Papain, plant protease from Papaya latex, shows highest deproteinizing activity in neutral pH range, 7-8 at 50-60°C with the concentration of 0.3 p.h.r. and requires 2 h to reach the maximum nitrogen reduction. While Alcalase, microbial protease, prefers alkaline pH range 8-9 at the same optimal temperature and concentration but requires longer incubation time 8-10 h. When comparing with the common procedures for DPNR production by both enzymes in the previous works (John et al. 1977; Chang et al, 1977), the optimized conditions reported in this research require much less reaction time. Both Papain and Alcalase exhibit high potential activity to reduce proteins, the major nitrogenous contaminants in latex, markedly 70-75% of the total nitrogen reduction are the maxima in the optimal condition and processing.

4.2 Choice of starting material

The DPNR production scheme from concentrated latex 60% and fresh field latex by both enzymes are separately determined by its physical properties and composition, including the ease to process. The latex concentrate is different from the field latex in its consistency of DRC and lower non-rubber contaminants, because the C-serum fraction which is enriched with non-rubber contaminants, is partially removed by the centrifugation process and the rubber phase is preserved with high efficiency stabilizer system, ammonia/TMTD/ZnO. While fresh field latices seem to vary lot by lot in DRC and non-rubber contaminants and have the tendency in autocoagulation during transport and enzymolysis.

Deproteinization of concentrate latex is only assessed by Alcalase in 10 h by firstly evaporated and adjusted to pH optimum (8-9) accompanied with adding hydroxylamine hydrochloride to stabilize its viscosity and sodium metabisulfite to inhibit discoloration of rubber. After optimum time, the latex is then diluted 5 volume and coagulated with acid mixture. The resulted coagulum is then creeped and washed with water and finally dipped in 0.2% thiourea solution to improve its plasticity retention index (PRI) (Chang et at., 1974). In the dilution step, the large volume of water is added to wash out digested residues from the rubber and is believed to remove also the natural occurring antioxidants, like tocotrienols (Morimoto, 1985). A decrease in tocotrienols may be caused by the hydrolysis of these lipid-like antioxidants by contaminated lipase in the commercial Alcalase, resulting in lowered resistance of rubber to heat ageing (Sivabalasumderam and Nadarajah, 1965). In recent studies it has been hypothesized that this phenomenon may be related with some inorganic constituents like copper, manganese and iron (Bateman and Sekhar, 1966). Hasma and Othman (1990) proposed that these ions normally might form complex with proteins and amino acids, in latex, and in this form, it does not impart any deleterious effect on the ageing of NR. However, when the proteins or amino acids are removed by any method, releasing free ions will act as very active prooxidants and could be removed by soaking in thiourea or phosphoric acid.

In case of Papain, it is not convenient to be used with the latex concentrate due to the high ammoniation of latex and TMTD in latex stabilizer system has been reported as a strong enzyme inhibitor (John et al., 1977).

For field latex in order to remedy the autocoagulation problem, Triton X-100 is added to stabilized the latex with the optimal concentration of 0.9 p.h.r. from the rubber plantation. Triton X-100 or Nonidet P-40 is a protein solubilizing agent which has been reported by John et al. (1977) on its capabilities to further latex stabilization and enhance latex deproteinization. The optimal concentration of Triton X-100 is very critical because below this concentration, the stability of latex could not be prolonged, and at other extreme the excess amount can stabilize the latex so well that it is impossible to coagulate the latex, and steam coagulation is firstly introduced to the process because of time-saving which is an advantage over acid coagulation.

Steam coagulation of fresh field latex after enzymolysis contribute more advantage than acid coagulation in which it take only short time (3-4 min) and the resulted rubber has lower nitrogen and volatile matter values and also lighter color (RRIM Planter's Bulletin, 1987). Conversely, the acid coagulation requires at least 6 h for complete maturation of coagulum and sometimes fail to coagulate because of the large dilution of latex to remove the digested proteins. Different from fresh field latex, the coagulation of concentrated latex cannot be succeeded by steam probably because of its high dilution, and pH can stabilize the colloidal system.

4.3 Choice of enzyme

Papain seems to be more effective than Alcalase because it requires shorter time and Papain - treated rubber can be stored for upto 2 years without fungus infection under normal factory storage condition (Anandan and Loganathan, 1984).

4.4 The processibility of deproteinized rubber

The dependence of raw rubber properties on latex source and the type of enzyme treatment can be markedly observed. CV-DPNR from commercial latex concentrate reveal satisfactory properties and light color (color index <6) according to the DPNR specification of RRIM but having high viscosity (80-90 units). CV-DPNR produced from clonal field latex show variable properties of raw rubber obtained, mostly in the acceptable range, except ash content (more than 0.15 g%). The explanation could be lacking of dilution before steam coagulation. Treatment of PB 5/51 give the rubber which posses specified properties and light color although its ash content is higher than the specification. CV-DPNR produced from GT 1 perform the reliable properties but darkened in its color, especially the Alcalase-treated rubber, while RRIM 600 give the better light color of rubber but higher in total nitrogen content.

The marked difference in raw rubber properties of DPNR from Papain and Alcalase treatment are Plasticity Retention Index (PRI) inwhich Alcalase treated-rubber have lower PRI than Papain, and required thiourea treatment to boost its PRI. The color and Mooney viscosity of rubber are dependent on its clonal characteristic (Yip, 1990) CV-DPNR from each clone exhibit its specific range of Mooney viscosity which can be classified into 3 groups : soft (with a ML 1+4 of less than 50, medium (with a ML 1+4 between 50 and 75) and hard (with a ML 1+4 of higher than 75) which have been advantageous to produce the rubber in different viscosity range for different CV grade (Chin, 1969).

The removal of protein by both treatments contribute to the drop in plasticity and Mooney viscosity of bulk rubber from its initial value. These two parameters are strongly or have been correlated with each other (Yip, 1990), and in corresponding with the degree of nitrogen reduction. As previously noted by Chang et al. (1977), the maximum 15 units drop can be estimated from this investigation (Figure 3.11).

Accelerate storage hardening test (ASHT) confirms the ability of DPNR in stabilizing its viscosity versus the storage time and also indicate that soft rubber tend to be hardened easier than the hard rubber which correlate with the higher concentration of abnormal group especially aldehyde group in soft rubber. (Subramaniam, 1975)

The existence of high nitrogen in NR has been believed to affect the cure behavior of rubber compound. Skim rubber, the by-product of latex concentrate production, containing high nitrogen content (2 g%) and high level of acetone extract exhibit short scorch time couple with long cure time both in ACS 1 mixing formulation (Bristow, 1990) and sulfur/sulphenamide vulcanization system. (Greensmith and Watson, 1969). In contrast, deproteinized rubber, in this practical formulation, has not been found to be scorchy but rather increase in its cure rate which can be roughly classified into 3 groups (Figure 3.14) in corresponding to their nitrogen content; high cure rate (with % N less than 0.12 g%), moderate cure rate (with %N between 0.13 and 0.5 g%) and low cure rate (with % N of higher than 0.55 g%). These observation implies that 0.12 g%N, the maximum accepted total nitrogen value for DPNR specified by RRIM is sufficient to enhance the high cure rate characteristics of DPNR. The cure characteristic of DPNR from each clone show non-significant difference from each other. They show similar correlation between total nitrogen of the clonal rubber and their corresponding cure parameters. These may be resulted from the assumption that the removal of verifying non-rubber contaminants especially proteins in each clonal latex to the minimum and consistent amount by deproteinization process will improve the homogeneity of mixing and chemical dispersion in rubber (Bloomfield, 1973), resulting in homogeneous vulcanization. The small amount of impurities retained can not markedly influence on cure behavior and some exert no effect (Yip, 1990). Furthermore, there are compensating effects between those contaminants e.g. while ethanolamine, choline and arginine accelerate cure (Altman, 1948), other amino acids such as tyrosine, cysteine give rise to slower cure rate (Loo and Yong, 1977).

The homogeneous vulcanization of DPNR compound caused by the removal of proteins results in the improvement of stress-strain properties but lowering in hardness and 300% modulus. (Figure 3.17) These results are similar to that reported by John et al. (1977) despite DPNR was mixed in different compound formulation. However, protein has been claimed to exert an influence as reinforcing filler (Brislow, 1990) by increasing the modulus and tensile strength of skim rubber vulcanizate.

The vulcanizate of DPNR prepared from commercial concentrated latex 60% exhibit the best vulcanizate properties with the light color comparing among DPNR from field latices and two types of commercial solid rubber, crepe and TTR5L. These DPNR contain very low nitrogen content (g%) due to the removal of serum proteins during centrifugation step which has more consistency in N-content and other ingredient comparing to the field latex, and suggest for the advantage of using centrifuged latex as starting material for producing very low protein DPNR. In case of field latex, rubber clone PB 5/51 is the best among 3 clones for DPNR production, because it contains low nitrogen content and results in the low nitrogen raw rubber with light color and also end product vulcanizate of good quality. DPNR made from RRIM 600 shows light color and low Mooney viscosity (45) which is comparable to synthetic BR, therefore this clone should be good for soft rubber. DPNR made from GT 1 has intermediate Mooney viscosity (66) but provide dark raw rubber if Alcalase treated and dark vulcanizate. It is well known that the main purpose of deproteinization of NR latex is to produce a rubber having superior dynamic properties. However it has not been able to study the dynamic properties of DPNR as described, due to the non-availability of some essential instruments to do. So, the difference in rubber properties of DPNR and non-deproteinized rubber have not evidently shown.

The application of DPNR were widely reported as specialty NR for engineering purpose which require the rubber having superior quality and consistency in rubber properties (Chin et al., 1974). DPNR is also required for medical rubber, which requires higher specification in its composition (nitrogen content less than 0.05 g%) and other properties such as a well characterization of the level of local biological response following long term clinical use. (Laing, 1973).

Although the superior quality of DPNR is required to fulfil the objectives of rubber goods producer. The cost of DPNR is another important factor to determine the consumer acceptance so that the cost of DPNR production at 1 kg of rubber has been estimated as guideline at the laboratory scale, based only on the chemical consumption and with special consideration on necessary utilities, namely relative energy and water consumption (Table 4.1).

Deproteinization of concentrated latex consumes significantly more time, energy and last but not least large amount of clean water. Use of Papain is the most attractive procedure, because the process is most economic, and there are more chances to improve the technology starting from local Papain production and immobilization of the enzyme for recycling purpose. Water treatment in neutral pH range would be easier, and should be considered seriously for future industry.

Table 4.1 Process economics of DPNR

Based on 1 kg dry rubber	Concentrated latex + Alcalase	Field latex	
		+ Alcalase	+ Papain
Rubber latex	15.0	18.0	18.0
(1 kg of dry rubber)			
<u>Chemical costs</u> (Baht/kg)	6.5	7.4	28.0
<u>Relative energy consumption</u>			
Ammonia evaporation	1-2 h	O	O
Incubation (50-60^{o}C)	8-10 h	6 h	2 h
Steam coagulation	O	●	●
Drying in oven at 60^{o}C	24 h	24 h	24 h
<u>Relative water consumption</u>			
Latex dilution	2.5 l	0.7 l	0.7 l
Dilution & Coagulation	15 l	O	O
Coagulum washing	5 l	5 l	5 l

● required O not required