CHAPTER IV

DISCUSSION

4.1 Seasonal variation of DRC and WEP of fresh field latex and concentrated latex

Positive relationship between DRC and WEP of FFL was observed during 3 tapping seasons in 2001 – 2003. In the beginning of tapping season which depend on rainfall period (May – October) the % DRC of FFL were about 40 % which is significantly higher than 30% DRC in the dry season and end of tapping season (November – December) when rubber trees drop their leaves. Similarly WEP of FFL varied from about 50 – 70 mg/g during the beginning of tapping season or during the rainy season and decreased to the 20 – 30 mg/g at the end of tapping season or during the dry season (Figure 3.1 – 3.2). Jacob *et al.*, 1989 and Rao *et al.*, 1998 also reported that the lowest rubber yield or % DRC coincided with a time of rain-lagging period or during the dry season, and the good yield of rubber is after a good spell of rainfall.

Seasonal variation of WEP of FFL from 20 – 73 mg/g rubber (Figure 3.2) show wider range comparing to 30 – 50 mg/g reported by Hasma, 1992. Hevea latex consists of about 35 % rubber particles and 15 % of non-rubber particle. Protein constitute approximately 1-1.5 %, of non-rubber components, which distributed in 3 major fraction; the rubber phase (27%), the B-serum or lutoid fraction (25%) and as soluble proteins in the C-serum phase (48%) (Tata, 1980).

By centrifugation of FFL to high ammoniated concentrated latex, the WEP reduces significantly to 0.7-0.5 mg/g (Figure 3.3). Hasma, 1992 reported that CL 60% contained 16 – 26 mg / g rubber which distributed in two main fractions ; the proteins associated with rubber particles and the serum phase. The major WEP of CL are 14 and 24 kDa which were reported as major allergens (Hev b1, Hev b8 and Hev b3) and rubber particle proteins (Slater and Chhabra, 1992; Alenius *et al.*, 1993; 1994).

4.2 Effect of irradiation on latex proteins and standard BSA

FFL used in this research, was preserved with only 0.3–0.4 % NH_3. Tetramethylthiuram disulfide, TMTD was not used as preservative because it was reported as a radical scavenger, and can form carcinogenic nitrosamines under some physiological conditions (Makuuchi, 2003). TMTD can also Type IV chemical allergic reaction (Bryand and Keith, 1998). Zinc oxide is a heavy metal and excess amount is considered as a water pollutant, and therefore not used in this research to keep the concept of an environmental friendly process.

When samples of FFL were irradiated with low dose (1-3 kGy) before centrifugation, WEP of irradiated rubber film increased slightly from control. In contrast at higher dose in the range of 20-120 kGy, WEP content increased to 40-90 mg/g rubber even higher than original FFL (48 mg/g rubber) (Figure 3.5). These results agree with Varghese (2000) using radiation dose of 10-50 kGy; Rogero *et al.* (2003) 10-50 kGy and Makuuchi (2003) 7.5-30 kGy that the WEP from irradiated FFL increased with increasing irradiation dose.

When standard BSA, a soluble protein MW 66 kDa was irradiated at dose 1-10 kGy, the SDS-PAGE (Figure 3.8) show protein disintegration since 1 kGy evident by smeared bands which increased with the increasing irradiation dose.

WEP of fresh field latex was compared with irradiated concentrated latex, Figure 3.5 that WEP of low dose irradiated latex reduced from 48 mg/g rubber to less than 3 mg/g rubber but when irradiated at high dose the WEP increase higher than WEP fresh field latex. It is possible that insoluble proteins associated with rubber particles were degraded into water soluble form (Makuuchi, 2003).

The MW distribution of these WEP analyzed by SDS PAGE (Figure 3.7) indicated that the major protein of low dose irradiated latex is 45 and 14 kDa, but increasing irradiation dose to 20-120 kGy, protein MW 45, 30 and 14 kDa are the major bands. The smear at the bottom of the gel may be due to the presence of low molecular weight protein caused by the disintegration of higher one (Varghese *et al.*, 2000). Up to 20 kGy irradated, the protein bands are almost the same pattern but the smear at the bottom increased. This result indicated that the smear bands are broken fragments of high MW of proteins. The major latex allergens such as 14 and 30 kDa remained but the intensity of these bands were decreased when irradiated at dose 120 kGy. This indicated that the major allergen protein (30 and 14 kDa) was prominent in the irradiated latex (Figure 3.7). The previous study reported that the rubber associated with rubber particles are mainly the 14 kDa protein with a minor amount of 24 kDa protein. A greater proportion of these proteins are tightly bound to the rubber particles and any factor that break the bands influence migration of proteins out of the latex products (Hasma, 1992).

4.3 The effect of natural polysaccharide (NP) on irradiated natural rubber latex

It is well known that WEP can bind to cornstarch added in the process of glove production for donning assistance. Shaking of powdered gloves enhanced protein bound to starch tiny particles to became airborne and can behave as aeroallergen. Since starch powder can bind with latex proteins allergens, the use of alginate and carrageenan as latex proteins carrier was conducted to facilitate removal of WEP by leaching process.

Alginates which are produced by brown seeweeds (*Phaeophyceae* mainly *Laminaria*) are linear unbranched polymers containing β-(1-4)- linked D- mannuronic acid (M) and α-(1-4)- linked L-glucuronic acid (G) residues. Carrageenan is a collective term for polysaccharides prepared by alkaline extraction from red seaweed (*Rhodophycae*). It is polymer of α–(1-3)-β-D-galactopyranose-2-sulfate-(1-4)-α-D-galactopyranose-2, 6- disulfate-(1-3)-. It has been reported that alginate and carrageenan can be degraded due to scission of glycosidic bond by irradiation (Nagasawa, 2000). Alginate has been used as a creaming agent in the process of CL production (Makuuchi, 2003). In this research the recovery yield of rubber in alginate added FFL before centrifugation was higher than carrageenan (Table 3.1). The results suggest that carrageenan can associate with both of rubber particle and protein so they were remained in the serum phase after centrifugation.

The single addition of either alginate or carrageenan (1-2 phr) resulted in decreasing WEP, 4.38-11.25 % remained for AGCL (Table 3.2) and 3.54 % for CACL (Table 3.3) suggesting interaction of NP with WEP, resulting in enhancing the migration of proteins into serum phase.

The combined effect of irradiation, NP and centrifugation (IR-AG-CL or IR-CA-CL) shows synergistic effect on WEP reduction. One kilo Gray irradiation plus 1 or 2 phr NP and followed by centrifugation were enough for reducing WEP over 95%. It is not necessary to increase dose, because it increases cost. It is clear that the addition of NP to irradiated latex followed by centrifugation is effective to reduce WEP. This is probably due to the interaction of WEP and NP. The NP and WEP have polar sites along their chains, may form protein-NP complexes. It is postulated that protein adsorbed on the surface of rubber particles are taken away by NP into serum phase. Therefore, the NP-complexes were discharged together with water by centrifugation. Moreover WEP remained in the rubber film can be removed to the non-detectable level by leaching for 10 min in distilled at water 70°C (Table 3.4), and confirmed by SDS PAGE profiles that show no band of protein (Figure 3.10 and 3.11).

4.4 Effect of irradiation on WEP of alginate added FFL.

From this research we know that radiation and alginate addition cooperated to reduce WEP and the previous studies reported that alginate can be degraded by radiation (Nagesawa, 2000). So the alginate was added to FFL before irradiation and centrifuged to improve efficiency for protein reduction.

The recovery yield of NP-IR-CL latex is better than IR-NP-CL latex. Because alginate acts as creaming agent to concentrate latex before irradiation and centrifugation. Addition of alginate 1 phr followed by irradiation 1 kGy and centrifugation was enough to reduce WEP to 0.2-0.4 mg/g rubber (Table 3.6). The results suggest that both alginate and rubber proteins were disintegrated by irradiation to smaller molecules, which affected better interaction between alginate and proteins, because size of alginate were smaller and can interact with small fragments of protein. Centrifugation can discharge both degraded rubber proteins, and alginate-protein complex together into serum phase.

4.5 Effect of irradiation after centrifugation

The effect of irradiation after centrifugation in this research was consisted of 3 types of concentrated latex, DPCL, AGCL, and control CL. Production of DPCL was similar to Ngamlert (2002) except that FFL used in this research was preserved with only NH_3 no TMTD and ZnO. The DPCL produced can not meet specifications of 60% DRC because of the minimum volume of FFL used for centrifugation in the factory scale (200 L). The magnitude of the VFA number, which is a good index of the preservation quality of the latex concentrate increased significantly in DPCL comparing to CCL before irradiation and during maturation, however the values were in acceptable range (<0.05).

The MST of DP was lower than 300 sec and it was not increased after irradiation and during incubation (Table 3.8, 3.9 and Figure 3.17). Irradiation of both CCL and DPCL on day 3 after centrifugation at MST about 200 sec did not result in increasing the MST to > 2000 sec as reported by Lukhovitskii (1983) and Makuuchi (2003), where irradiation was performed at MST 800, and minimum maturing period of 6 weeks. Since too high MST (>2000 sec) causes uneven film in dipping products, it could be avoided by early irradiation 3 days after centrifugation. The nitrogen content of DPCL (0.09 ± 0.01 %) was significantly lower than 0.16 ± 0.005% of CCL (Table 3.9) indicating that enzyme deproteinization disintegrate both WEP and insoluble proteins which bound to rubber particles and stabilize them in suspension, thereby reducing the MST of DPCL. Removal of WEP by adding alginate followed by centrifugation and radiation did not affect both nitrogen content, and MST, hence this procedure has higher advantage for processability.

From the determination of WEP by modified lowry method. It was shown in Table 3.8 that WEP in control CL were varied in the wide range (2411 ± 998 μg/g rubber). The deproteinized process can reduce WEP to 82 ± 28 μg/g rubber, which is about 97% reduction. After irradiation, WEP of all control CL and DPCL were increased to 2655 ± 998 and 373 ± 72 μg/g rubber respectively. This results agrees with a previous study that the irradiation of concentrated latex increased the WEP content because it enhances the dissolution of insoluble proteins previously bound to rubber particles by breaking the polypeptide bonds and turn them to soluble proteins (Makuuchi, 2003; Sebastian, 1999).

4.6 Effect of irradiation and NP addition on latex concentrates

From 2 lots of AGCL production in this research the recovery yield of rubber in AGCL (69.5%) (Table 3.10) was higher than control CL (58.5%) because of the creaming effect of alginate added before centrifugation. The % DRC of AGCL was also higher than control CL. The VFA of control and AGCL latex were lower than 0.03 %, but after irradiation, VFA increased to 0.06-0.08% (Table 3.12). It was reported that VFA are formed by the decomposition of carbohydrates in the NRL, caused by contamination of acid producing bacteria. The major form of volatile acids are acetic acid and formic acid. Since these acids are strong coagulants of the NR latex, microflocculation tends to occur. NRL with a VFA number of less than 0.02 is recommended for RVNRL (Makuuchi, 2003). However irradiation has been used for elimination of microbial contamination in the range of 1-6 kGy, therefore the increasing VFA may not be due to microbial contamination, but may be resulted from decomposition of some organic fatty acids.

Mg content of AGCL was higher than CCL indicating that alginate may not be effective in the reduction of Mg by centrifugation.

The nitrogen content of CCL and AGCL before and after irradiation were more or less similar although a small decrease of 0.01% was observed with AGCL (Table 3.12-3.13). The WEP of AGCL was obviously lower than control in both lots. The results suggest that alginate addition follow by irradiation and centrifugation can reduce only WEP but not insoluble proteins. Since irradiation can also degrade alginate and enhance dissolution, there should be more carrier molecules to interact with degraded proteins especially proteins that have polar amino acid residues in the peptide chain and form WEP alginate complexes. These complexes can be removed by centrifugation. These results have the same trend as of Ratnayake (2001) that added water soluble polymers such as polyvinyl alcohol and polyvinyl pyrolidone into RVNRL, followed by centrifugation resulting in effective reduction of the WEP. Moreover chemically-modified cellulose such as hydroxy ethyl cellulose (HEC) and hydroxy propyl cellulose (HPC) addition before irradiation resulted in degradation of polymer to small fragments suitable for use as protein scavengers in the RVNRL (Makuuchi, 2003).

4.7 The effect of irradiation on MST of control, DPCL and AGCL

The control and DPCL of lot No. 11/7/03 and 21/7/03 were irradiated on days 3 after centrifugation. Figure 3.17 shows that irradiation had no effect on MST. This result is the same with Control and AGCL lot no 21/9/03 was irradiated on 17 (Figure 3.18). CCL and AGCL lot no 21/9/03 was irradiated on days 143 at MST 894 and 630 for CCL and AGCL and 150 days after centrifugation at initial MST 823 and 769 for control and AgCL respectively. Irradiation at high MST and long maturation time resulting in transient increase of MST (Figure 3.17). Similar profiles were observed with AGCL lot No. 4/11/03, the increasing MST after irradiation may result from the increasing WEP after irradiation at 10 kGy (Table 3.12). It should be noted that the four lots of CCL were irradiated at various MST values ranging from 200-800 sec, none of them show increasing MST of RVNRL upto 2000-3000 sec reported by Makuuchi (2003).

4.8 The effect of leaching on WEP of rubber films

Table 3.13 shows that leaching film with 70°C water can reduce WEP of CCL film to 125-279 μg/g rubber which were in the range of non-powdered gloves, and DPCL film to < 50 μg/g rubber as reported by Ngamlert ,2002; Ghazaly, 1994. Leaching of cured rubber CCL-IRR, DPCL-IRR and AGCL-IRR or RVNRL film can remove the WEP lower than leaching of non-irradiated rubber film (CCL, DPCL and AGCL). This result shows that degraded WEP are easy to be washed out with increasing leaching time.

4.9 Effect of centrifugation, deprotenization and irradiation on molecular weight distribution of WEP

From SDS-PAGE analysis of CCL of all lot shows several protein bands in the range 45-14.4 kDa and lower MW than the 14.4 kDa (Figure 3.19-3.22). The major WEP band is about 14 kDa. This result agrees with Alenius *et al.*, 1992; Slater and Trybul, 1994; Jaeger *et al.*, 1992.; Ngamlert ,2002) that also found protein bands in the range 45-14.4 kDa. DPCL shows the remaining of protein band at 28.5 kDa. After irradiation control CL still display the major latex allergens previously bound to the rubber particle, which are 30, 35 and 45 kDa proteins, and many smeared bands of randomly degraded proteins. These results indicated that the over-detected value of WEP from irradiated latex by modified Lowry method may come from small peptides produced by irradiation (Ngamlert, 2002; Varghese *et al.*, 2000; Sebastian, 1999; Ratnayake *et al.*, 2001; Rogero *et al.*, 2003). AGCL and control show the same pattern of protein bands, indicating that alginate dose not disintegrate WEP(Figure 3.21-3.22). Leaching can remove all of the WEP confirm by no protein band.

4.10 Latex protein allergen detection by skin prick test (SPT)

The skin prick test (SPT) is known to be a rapid test of high sensitivity for Ig-E mediated or Type I allergy (Turjanmaa *et al.*, 1996; Hadjiliadis *et al.*, 1995). Beside being used to identify sensitized patients, it can also be used to detect the presence of protein allergens in latex products (Turjanmaa *et. al.*,1988). In this research there were 2 volunteered persons for SPT who are sensitized with latex gloves and show positive wheal when challenged by WEP parpared from commercial latex concentrated. The ability to produce a positive wheal response in the two volunteers was shown by the positive histamine wheals. The SPT results show that the volunteers positive with commercial concentrated latex, show negative SPT with control CL-IR leaching and AGCL-IR leaching. From previous report, the 2 volunteers were allergic to proteins of MW 17, 30 and 33 kDa, but the control CL- IR and AGCL-IR do not have these 3 protein allergen, so these 2 volunteers showed negative SPT. Due to ethical problem and limitation of volunteered persons allergic to latex allergens, SPT was performed in only two cases.

4.11 The effect of irradiation on physical properties of rubber films

The tensile strength of all RVNRL (CL-IRR, DPNR-IRR and AGCL-IRR) were 7.16 – 8.04 Mpa (Table 3.16) that meets the requirement for NR latex for radiation vulcanization (7 MPa) (Makuuchi, 2003). The 300% modulus and tear strength of RVNRL were slightly higher than dental dam. It is indicates that rubber dam or SVNRL has higher resistance to tear forces than RVNRL's because of the crosslinking of rubber molecule by radiation vulcaniation is carbon to carbon linkage but the crosslinking of the conventional vulcanization system is achieved through sulfur linkages (Jacob and

Vijayakumar, 1997) that is the sulfur-sulfur linkage is stronger than carbon-carbon linkage Therefore, the strength of SVNRL is higher than RVNRL's.