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Study on Protein Profiles in Commercial Examination Glove Production

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Extractable protein (EP), antigenic protein (AP) and allergenic protein (AgP) contents of examination gloves sampled at different stages of commercial examination glove production over a 3 months production period were determined. The EP, AP and AgP contents of gloves at any stage of production generally increased with increasing production time. Unexpectedly gloves collected immediately after the pre-leaching process showed higher EP contents compared to unleached gloves. Gloves taken after post-curing at 140ºC for about 30 minutes, gave the highest EP, AP and AgP contents. The EP, AP and AgP contents of gloves taken after the post-leaching and the subsequent stages were considerably lower compared to gloves taken before the post-leaching stage. There is a tendency for the AgP content of the final products to be higher than those samples taken after the post-leaching and slurry-dip stages. It is hoped that this study on protein profile of a commercial glove dipping line could provide a guideline on appropriate conditions that needs to be applied at various stages of the glove production process.

Keywords: extractable proteins; antigenic proteins; allergenic proteins; examination gloves; pre-leaching; post-leaching; protein profile; glove line

Proteins are naturally present in natural rubber latex (NRL). Some of the latex proteins are water-soluble, while others are associated with organelles such as rubber particles[1,2]. Some of the water-soluble proteins that are present in latex products such as gloves were found to cause Type 1 allergic reaction[3]. In order to deal with this problem, much effort has been put to reduce the EP content of gloves during product manufacturing, particularly during the leaching process[4,5]. In glove production, wet-gel leaching and post-cure leaching are the essential, effective and well-known methods for reduction of extractable protein content[6–8]. It has also been found that slurry-dip after drying or post-curing could initially reduce the EP content of gloves. However, the protein content of gloves was found to increase with production time in the slurry tank[8]. Recent work revealed that cornstarch slurry powder has high bound affinity towards latex proteins[9]. There are also some other factors that could contribute to the increase of EP content of gloves. One of the factors is that certain compounding materials added into the latex may raise the EP level[10].

Previous studies[7,8] on EP content reduction were carried out only at selected sections of the latex dipping line for the purpose of evaluating

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the effectiveness of certain manufacturing processes where AP and AgP contents were usually omitted. Close monitoring of protein content of gloves taken at each section of the glove dipping line has yet to be studied. Hence, this paper describes EP, AP and AgP content profiles of gloves determined at every section of glove production at a commercial glove dipping line. Knowing the stages of the production that produced gloves with high EP, AP and AgP contents, manufacturers could give more attention to these stages by taking appropriate actions in order to further reduce the protein content of gloves. Even if the protein content at several stages of the production may be high, only one or two of the stages may need to be critically looked into in order to set a cost-effective process for production of gloves with very low levels of EP, AP and AgP contents. This paper also compares the EP, AP and AgP content profiles of different batches of gloves prepared at different periods of production. It is hoped that knowledge generated in this study could help glove manufacturers to effectively reduce the EP, AP and AgP contents of their gloves without having to carry out major modification of their dipping lines and in the process help to lessen the allergy issues of natural rubber latex gloves.

EXPERIMENTAL

Preparation of Gloves

Samples of compounded latex, coagulant (L1), pre-leaching water (L2), post-leaching water (L3) and slurry (L4) were taken from a commercial glove production. These liquid

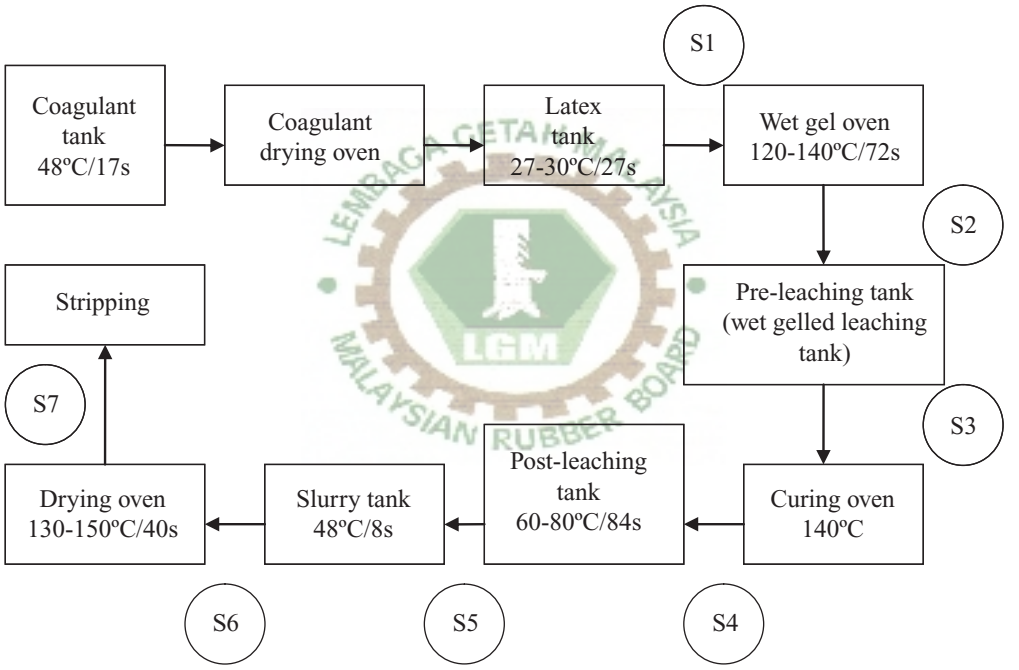

Figure 1. Flowchart of dipping process and process parameters, location of gloves produced and samples labelling at different stages.

samples were collected at day 0 (Batch 1), day 13 (Batch 2) and month 3 (Batch 3) of the production. Dipping process was carried out in the laboratory according to the similar conditions as that of the actual glove line. Gloves were produced and taken out from each stage of production where wet glove samples were taken and left to dry at RT before carrying out the protein determinations. The glove samples were labelled as S1-S7. The flowchart of dipping process and process parameters are shown in *Figure 1*.

Determination of EP, AP and AgP of Gloves

Extraction procedures. Cut pieces of gloves (palm area) of 8 cm × 8 cm were extracted in 25 mM PBS buffer (pH 7.4) at room temperature for 2 hours. Amount of buffer used was 5 mL per gram of sample. The extracts were then clarified by centrifugation at 3000x g for 15 min to remove powder or any particulate matters. Extractable protein, antigenic protein and allergenic protein contents were determined in duplicates[11–13].

Extractable protein (EP) determination. Extractable protein content was determined by Lowry method using the *ASTM D5712-99*[11]. The sample extracts were precipitated using deoxycholic acid, phosphotungstic acid and trichloroacetic acid. Protein pellets were dissolved with sodium hydroxide and treated with copper reagent to form protein-Cu^{2+} complex, which reduced Folin-Ciocalteu's phenol reagent, resulting in the development of blue colour. For the correction method, the same procedure was repeated but with the copper reagent replaced by water in alkaline tartrate solution. The colour was measured at 750 nm using micro plate reader[11].

Antigenic protein (AP) determination. For determination of the antigenic protein content, the extracts were assayed using the *ASTM D6499-00* method[12]. The procedure involves incubation of IgG antibody (raised in rabbit) and sample extracts (containing latex proteins) in dilution plate to form antibody-antigen complex. The mixtures were transferred to an assay plate. Excess IgG antibodies, which were not bound with NRL protein, will bind to the immobilised standard antigen in the assay plate. Horseradish peroxidase conjugated anti-IgG (HRP), an enzyme-substrate was added and the reaction of enzyme on the substrate resulted in a colour change. The colour was measured at 490 nm using a micro plate reader.

Allergenic protein (AP) determination. Allergenic protein content was determined using the IgE-ELISA Inhibition Method[13]. The sample extracts were incubated with IgE antibodies. IgE antibodies were recovered from a human serum pool containing specific antibodies to latex proteins. After a short incubation, the mixtures were transferred into an assay plate. The plate was washed three times. Biotinylated goat anti-human IgE and streptavidin-conjugated alkaline phosphate were used to detect immobilised antigen-bound specific antibody complexes. P-nitrophenylphosphate in carbonate buffer containing $MgCl_2$ was added and a yellow colour developed. The colour was measured at 405 nm using a micro plate reader.

Effect of Pre-leaching Time on EP Content

The wet gelled gloves were prepared by immersing formers in the compounded latex taken from the factory. According to the conditions applied in the glove factories, the wet gelled gloves were then dried in the wet gel oven at temperatures ranging between 120ºC–140ºC for 72 seconds and subsequently leached in distilled water heated at 70ºC for 5, 30, 60, 90 and 150 seconds respectively. EP content was measured for those samples.

RESULTS AND DISCUSSION

EP, AP and AgP Contents of Gloves Taken at Different Stages of Glove Production

The values of EP, AP and AgP contents for Batch 1, Batch 2 and Batch 3 gloves are shown in *Figure 2*, *Figure 3* and *Figure 4* respectively. The pattern or trend line of EP, AP and AgP content profiles over the whole processing stage was quite similar for the three batches of gloves. However, the exact EP, AP and AgP values, especially for Batch 3 gloves were noticeably different compared to those shown by Batch 1 and Batch 2 gloves.

For all batches, generally, the trend of EP, AP and AgP content of S1 glove, samples taken after the latex tank and S2 samples taken after the wet gel oven were quite similar. These samples were found to contain relatively high EP, AP, and AgP content, as these samples did not undergo the leaching process. However, for Batch 1 and Batch 2 gloves, the EP content of S3 samples taken after the pre-leaching tank was about 500 $\mu g/dm^2$ higher than that of S2 samples. As for Batch 3 gloves, the EP content of S3 samples was about 600 $\mu g/dm^2$ higher than that of S2 samples. The AP and AgP contents of S3 samples also appeared to be rather similar as those S2 samples for all batches. Furthermore, it is noteworthy that the AgP content showed high values from the beginning until S4 samples for all the three batches of gloves. The comparatively high EP content at the S3 stage could in part be due to the effect of evaporation after the pre-leaching stage. This could be explained by work done previously where after the pre-leaching stage (wet-gelled leaching stage), evaporation process of water from gloves occurred[14]. It is possible that the evaporation process mitigated the migration of soluble proteins to the glove surface and Ca^{2+} ions that remain in the glove film might have trapped the protein within the film matrix. Other contributing factors include rate of water overflow and agitation, which have a bearing on the concentration of proteins in the leaching tank.

Further increase of EP, AP and constantly high AgP content was observed for S4 samples, taken after the curing oven. It showed the highest protein content among the glove samples. This is probably due to the effect of heating over a long period of curing. Proteins may possibly degrade causing breakdown of protein chains, making them more soluble during heating where the rate of degradation which may be relatively high at the initial stage of heating as the samples contain substantial amounts of water. In addition, the heating process would promote more proteins to migrate towards the surface of glove film and be made readily extractable[14]. However, S5 samples which were post-leached samples, showed remarkably low EP, AP and AgP contents. At this stage, it is probable that most of the soluble proteins, which accumulated on the glove surface, were extracted into the post-leaching tank and hence the EP, AP and AgP contents dropped substantially.

The EP, AP and AgP contents of S5, S6 and S7 glove samples taken after the post-leaching and the subsequent stages were considerably lower compared to gloves taken before the post-leaching stage, S1-S4, for all batches of gloves.

Hence, from the pattern shown by the graphs, the processing stages from latex dipping to oven drying/curing stages are important stages as the gloves produced at these stages showed high levels of EP, AP and AgP contents. It is at these stages where process design that could enhance protein removal or suppress protein denaturation be used to produce low protein gloves.

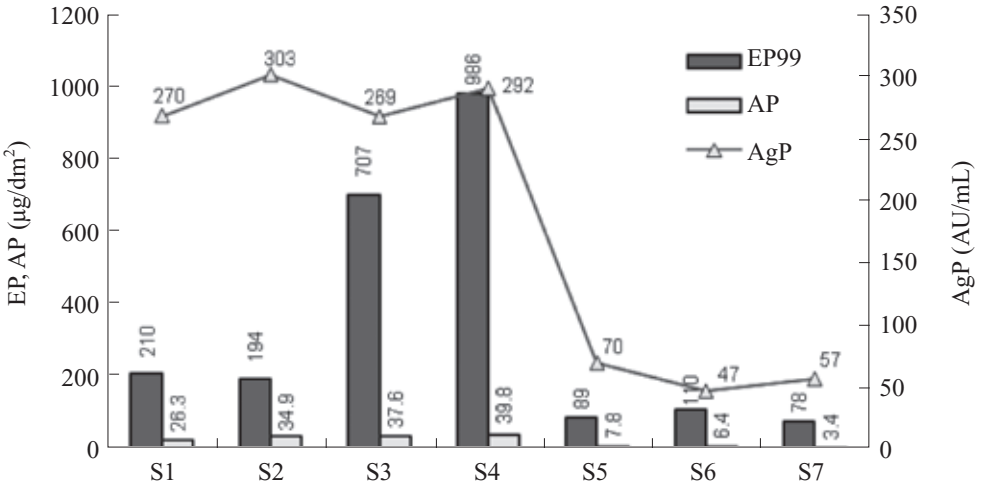

Figure 2. Extractable protein (EP), antigenic protein (AP), and allergenic protein (AgP) content of Batch 1 (0 day production)

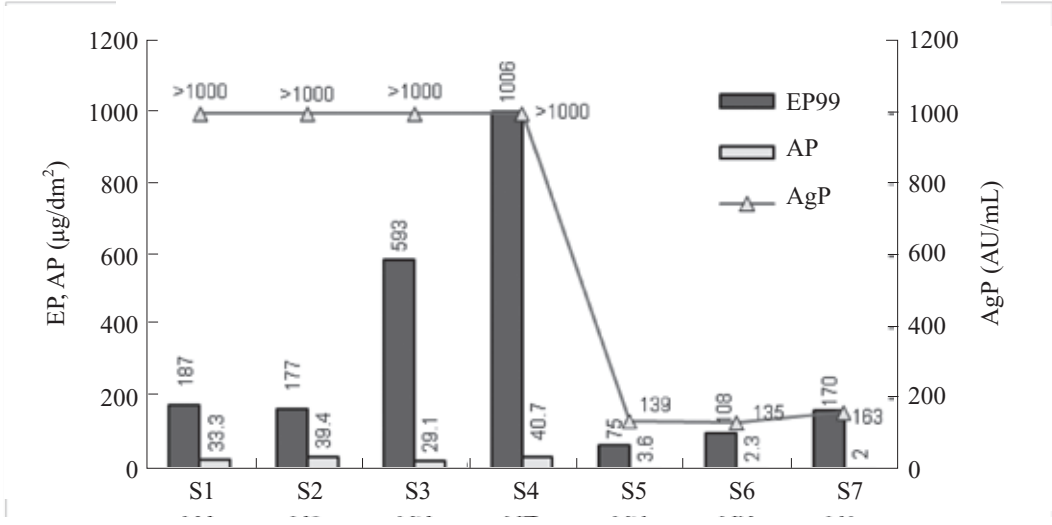

Figure 3. Extractable protein (EP), antigenic protein (AP) and allergenic protein (AgP) content of Batch 2 (13 days production)

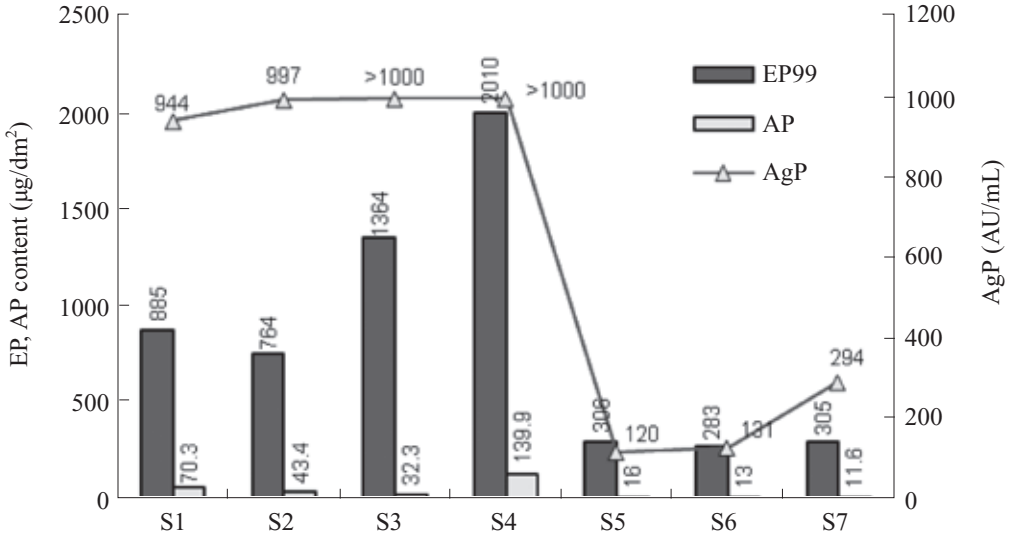

Figure 4. Extractable protein (EP), antigenic protein (AP) and allergenic protein (AgP) content of Batch 3 (3 months production).

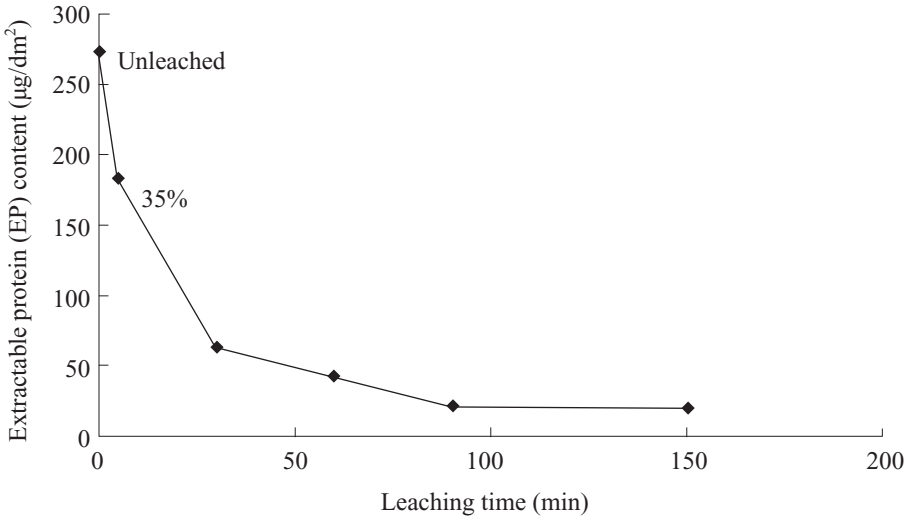

Figure 5. EP content of pre-leached film versus leaching time

Pre-leaching Time *versus* EP Content

Since the above section stated that pre-leached gloves, S3 showed high EP content compared to S2 samples taken after the wet gel oven, a study on the effect of leaching time upon EP content reduction was conducted in the laboratory with the same conditions as the glove factory. Longer leaching time extracted more proteins from the pre-leached gloves as shown in *Figure 5*. At 5 minutes of leaching time, about 35% of EP content was reduced when compared to the control (unleached) gloves. Hence, it can be said that leaching time of <50 s which is practiced by most glove factories is insufficient for proteins to be removed efficiently from the gloves. Prolonged pre-leaching time is recommended in order to reduce further EP content in the final product of gloves.

EP, AP and AgP Contents of Gloves Sampled at Different Periods (Batch) of Production

The EP and AP contents for Batch 1 and Batch 2 gloves as shown in *Figure 6* and *Figure 7* respectively, were found to be rather similar. However, the EP and AP contents of Batch 3 gloves were found to be substantially higher that those of Batch 1 and Batch 2 gloves. The differences in results between Batch 1 and 2 to Batch 3 might be due to several factors such as;

- the accumulative effect of inefficient former cleaning and build up of proteins in leaching water with time
- water in the pre-leaching and post-leaching tanks were not changed regularly but merely replenished
- the accumulation of proteins in the coagulant
- the accumulation of proteins in the latex tank associated with the old latex compound
- sedimentation of latex compound
- hydrolysis of proteins in the latex with production time.

The results for the AgP content however were quite in contrast to those of the EP and AP results as shown in *Figure 8*. Batch 1 gloves showed the lowest AgP content compared to Batch 2 and Batch 3 gloves at all stages of production. The AgP content of Batch 2 and Batch 3 gloves for all the production stages were consistently higher than Batch 1 by about 50% and 20%–30% respectively. An interesting observation is that the AgP content of S1, S2, S3 and S4 gloves for the production periods of 2 weeks (Batch 2) and 3 months (Batch 3) were similar, which was on the high side of 1000 AU/mL. Comparing results in *Figure 6* and *Figure 8* for Batch 2 gloves, it appeared that there were situations where gloves with a relatively low level of EP content could still show a significantly high AgP content. The allergenic proteins appeared to exist in the latex compound and its concentration increased rapidly to a maximum value within a relatively short production period. The localised heating of latex compound by the hot glove formers and the prevailing pH conditions perhaps promote denaturation of proteins with prolonged production time.

Protein Concentration of Coagulant, Pre-leaching water, Post-leaching water and Slurry

The coagulant (L1), pre-leaching water (L2), post-leaching water (L3), and slurry (L4) were found to show erratic values likely because several adjustments had been made to these samples. One of the adjustments made was changing or topping up of old solutions with fresh solutions.

Table 1 shows the EP, AP and AgP contents of L1, L2, L3 and L4 for all batches. Protein

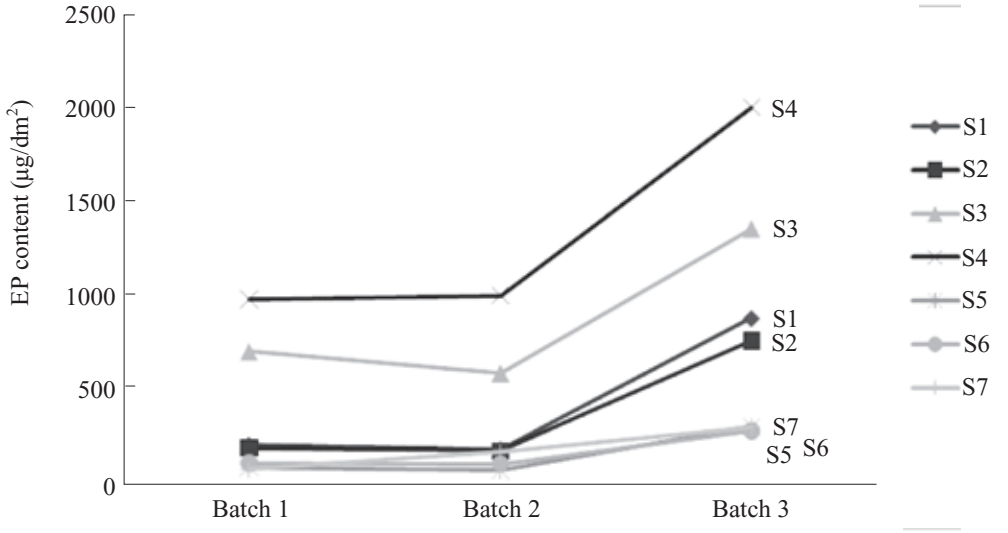

Figure 6. Extractable protein (EP) content for Batch 1, Batch 2 and Batch 3.

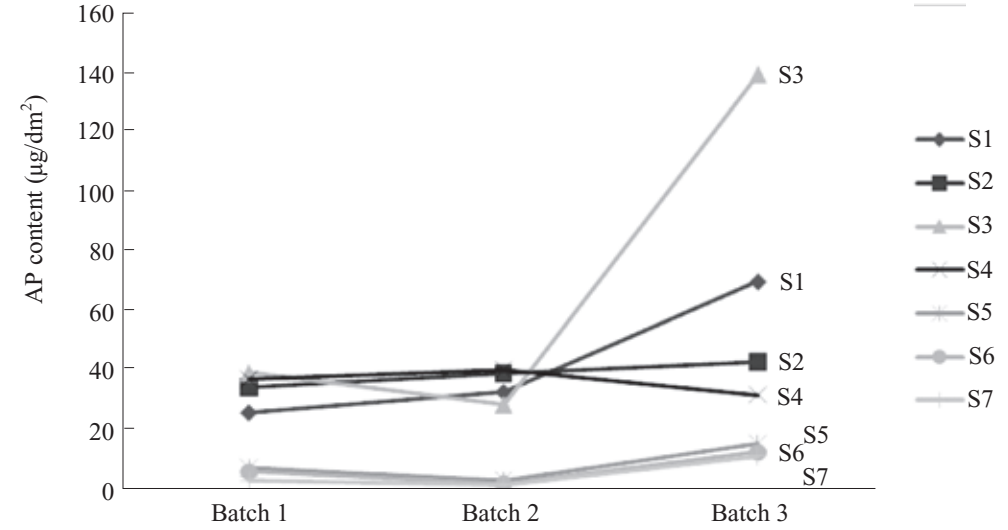

Figure 7. Antigenic protein (AP) content for Batch 1, Batch 2 and Batch 3

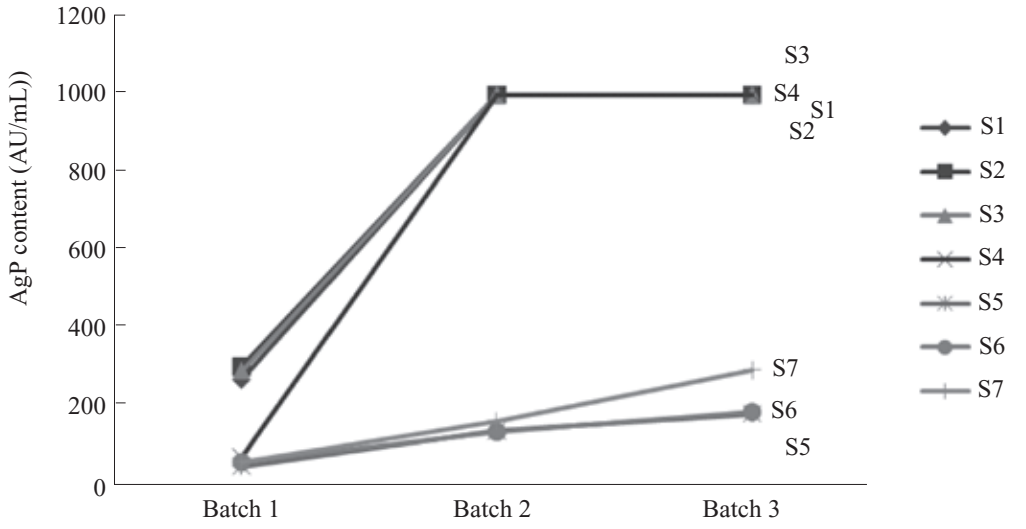

Figure 8. Allergenic protein (AgP) content for Batch 1, Batch 2 and Batch 3.

concentration of coagulant (L1) was found to be low. This could be due to the effect of calcium precipitating the proteins, and that calcium has been shown to interfere with ELISA assays[15,16].

Correction of EP determination for coagulant was carried out in this study. The corrected values showed consistently lower EP values compared to the uncorrected values. The percentage of EP reduction ranges from 90%–100%. This correction method showed that calcium nitrate was not only interfering with the ELISA assays but also with the Lowry assays[15–17].

Pre-leaching water (L2) was found to contain similar protein contents compared to post-leaching water (L3) except for Batch 3. Considering Batch 2 and Batch 3 production data, the EP, AP and AgP contents in the slurry (L4) were much lower compared to those in the post-leaching water. Despite the differences, the S5 and S6 glove samples showed comparatively similar levels of EP, AP and AgP contents. It is possible that in the samples taken after slurry tank stage, the extractable proteins in the gloves were already at relatively stable levels and it was not extracted much by the slurry.

CONCLUSIONS

A profile of EP, AP and AgP protein contents of gloves at various stages in the glove production line was obtained in this study. The results showed that distinct increase in protein contents was found on gloves collected immediately after the pre-leaching process compared with the unleached gloves. The gloves collected immediately after drying and curing stage gave the highest protein contents whilst the glove samples collected immediately after the post-leaching were at minimum levels of EP, AP and AgP contents. Moreover, the gloves taken before the post-leaching stage gave considerably higher values of EP, AP and AgP contents compared to gloves taken after post-leaching stage for all batches of production.

TABLE 1. EP-CORRECTION, AP AND AgP CONTENT OF COAGULANT (L1), PRE-LEACHING WATER (L2), POST-LEACHING WATER (L3) AND SLURRY (L4) FOR BATCH 1, BATCH 2 AND BATCH 3

Samples	Batch 1			Batch 2			Batch 3		
	EP (µg/mL)	AP (µ/mL)	AgP (AU/mL)	EP (µg/mL)	AP (µg/mL)	AgP (AU/mL)	EP (µg/mL)	AP (µg/mL)	AgP (AU/mL)
L1	12	>2	58	27	>2	178	14	>2	107
L2	5	<0.0312	6	48	0.08	208	24	0.04	24
L3	<4.7	<0.0312	5	52	0.15	516	45	0.3	271
L4	<4.7	0.04	0.4	15	0.09	248	10	0.3	94

*>2 µg/g is max conc. of std. antigen
*<0.0312 µg/g is min conc. of std. antigen
*4.7µg/mL is LOD for *ASTM D5712-99*

Through the knowledge generated from this work, it is hoped that more attention could be paid and appropriate actions on certain critical stages in the production dipping line could be considered by manufacturers in order to enhance protein removal and lessen protein allergies amongst users. Among positive measures that can be considered are prolonging the pre-leaching process, high temperature of leaching water, provide two or three short leaching tanks instead of one long tank, constant agitation in tanks, usage of clean and running water, frequent change of water and *etc*.

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