ORIGINAL PAPER

Determination and detection of water-extractable protein from latex gloves

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Abstract. Natural rubber latex (NRL) from the rubber tree (*Hevea brasiliensis*) is used in various products. However, the NRL protein is generally of concern to medical personnel and regular users. In this study, a phosphate buffer saline (PBS) extraction was introduced to facilitate the evaluation of water-extractable proteins (WEPs). The six WEPs, five different gloves, and NRL samples were determined and compared using Bradford and modified Lowry methods, respectively. An appropriate incubation time for protein extraction was at least 60 min. The standard curve of protein determination showed a good linear correlation (R^2 = 0.99). Samples had higher WEP contents when determined by the modified Lowry method. In addition, WEP patterns were detected by polyacrylamide gel electrophoresis with sodium dodecyl sulphate (SDS-PAGE). The SDS-PAGE showed 7–9 bands with molecular weights ranging from 9.0 to 66.6 kDa. This indicates that PBS extraction can be efficiently used to evaluate allergies.

Keywords: Bradford method, Latex glove, Modified Lowry method, Natural rubber latex (NRL), Water-extractable protein (WEP).

1. Introduction

Natural rubber latex (NRL) is a mixture of several substances, including 30%–40% of rubber particles, 1%–2% of protein and 55%–65% water (Sussman et al., 2002; Perera and Perera, 2017). Latex proteins are a group of water-soluble, thermostable or glycoproteins with molecular weights between 5 and 200 kDa (Fisher et al., 1993). Notably, NRL contained 17 allergens and isoforms with molecular weights ranging from 4.7 to 60 kDa [IUIS-International Union of Immunological Societies]. Latex-induced protein allergy is a concern for healthcare workers and anyone who comes into frequent contact with rubber products (Ranta and Ownby, 2004). Skin contact or inhalation of latex particles can cause latex allergy. Moreover, latex allergy is an IgE-mediated hypersensitivity to NRL that causes various clinical symptoms including angioedema, swelling, cough, asthma and anaphylactic reactions (Deval et al., 2008). Numerous products made of NRL are the result of allergies in medical and regular users. Thus, reducing total protein in NRL products to reduce allergenic potential requires methods to accurately estimate protein levels (Lucas and Tomazic, 2000). One strategy to reduce additional sensitizations and reactions to these allergens is to remove or reduce water-soluble proteins from the final NRL products (Tomazic and Lucas, 2002). Deproteinised natural rubber latex was protein-free which formed a nice elastic film and had poor skin adherence by showing no significant response in a rabbit skin irritation test (Pichayakorn et al., 2015). However, the water-extractable protein (WEP) content in NRL products varies widely depending on

the manufacturer and the type of rubber products. These problems need to be addressed, thus a new and reliable process to reduce residual WEP needs to be developed to improve the quality of finished rubber products.

An accurate protein measurement method is required to evaluate the safety of the allergenic rubber latex protein. However, several published and widely used methods for protein measurement in finished NRL products have significant inconsistencies in quantifying extractable NRL proteins as compared with the Kjeldahl nitrogen assay and the gravimetrically determined reference (Tomazic et al., 1999). Therefore, researchers examined several protein assays and the standard proteins to determine the most appropriate approach to quantify soluble glove latex proteins. Of the two protein assays studied (Bradford and Lowry), the modified Lowry assay had better consistency than the Bradford assay and was highly consistent with the Kjeldahl nitrogen assay (Lucas and Tomazic, 2000). This study aimed to investigate the determination of total protein content and examine the pattern of protein distribution in rubber glove samples using a suitable, reliable, and non-complex method.

2. Materials and Methods

2.1. Materials

Five different latex glove samples (A–E) and natural rubber latex (NRL) were obtained from Rayong Bangkok Rubber Co., Ltd.

2.2. Extraction procedure

Glove and NRL samples were cut into small pieces of 1 cm^2 in size. They were extracted in 0.1 M phosphate-buffered saline, PBS, pH 7.4 (1:5 w/v). The mixtures were shaken at 150 rpm at room temperature for 2 h and mixed in a peak mixer for an additional 30 min. Then, the large latex pieces were removed, and the extract solution was centrifuged at 1,000*x*g for 20 min (Baur et al., 1997).

2.3. Precipitation procedure

In a polypropylene test tube, 0.1 mL of 0.15% sodium deoxycholate (DOC) was mixed with 1.0 mL of standard bovine serum albumin (BSA), and the solution was extracted. After shaking and incubating the test tube for 10 min, 0.1 mL of 72% trichloroacetic acid (TCA) and 0.1 mL of 72% phosphotungstic acid (PTA) were added. Following vigorous shaking, the test tubes were allowed to stand at room temperature for 30 min. Proteins were then precipitated by centrifugation at 6,000*x*g for 30 min (Lucas and Tomazic, 2000). Finally, the protein pellet can be used for protein determination, followed by decantation in 0.1 N sodium hydroxide (NaOH).

2.4. Protein determination

The protein content of the five glove samples and NRL was determined using Bradford microplate protein assay and modified Lowry methods. The absorbance of the Bradford and modified Lowry assays was measured at wavelengths of 595 and 750 nm, respectively (Yusof and Yeang, 1992; Tomazic et al., 1999).

2.5. SDS-PAGE electrophoresis and gel analysis

SDS-PAGE was performed in 5% acrylamide/bis-acrylamide stacking gel and 10% acrylamide/bis-acrylamide resolving gel (3.3% C). Stacking and resolving gels were run at 15 mA/gel for 15 min and 20 mA/gel for 50 min, respectively. The latex

protein samples were loaded with 10 µg of WEP. Standard proteins (Bio-Rad 161-0304) were loaded into a well, including phosphorylase b (97.4 kDa), serum albumin (66.2 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (31.0 kDa), soybean trypsin inhibitor (21.5 kDa) and lysozyme (14.4 kDa). The molecular weights of protein bands found on the SDS-PAGE gels were assigned using the standard lane. Then, gels were stained with Coomassie R-250 and double-stained with silver. Subsequently, gels were scanned using an Epson Expression 1680 scanner. Image analysis software 'CLIQS' (http://totallab.com/cliqs/) was used to analyze gels from SDS-PAGE to automatically detect protein bands (Srisomboon et al., 2021).

2.6 Statistical analysis

All experiments were performed in triplicate. Data were analyzed using analysis of variance (ANOVA) with the significant difference due to treatment; Tukey's multiple range test at $p \leq 0.5$. SPSS (version 18.0) was used for all statistical analyses.

3. Results

Five latex gloves and NRL extracts were prepared using PBS at a concentration of 0.137 M NaCl (Tomazic et al., 1999). All extract proteins were stable after 60 min. For gloves A, C and NRL, the protein content was highest after 30 min and remained constant from 60 to 120 min. Meanwhile, for gloves B, D and E, the highest protein content was observed at 60 min (Figure 1). This result suggests a sufficient incubation period of at least 60 min for protein extraction. Most proteins were rapidly extracted at the beginning of the extraction period, which was consistent with that of Koch (1997) and Yagami et al. (1993). In addition, using PBS for protein extraction has been recommended since it is acceptable for a wide range of soluble proteins and is not specific to each protein concentration (Koch, 1997 and Yagami et al., 1993). Even though the determination and detection process using PBS for protein extraction was supposed to be completed in 24 h (Perera and Perera, 2017), it did not obstruct this experiment which found the protein contents remained greatest at 60 min.

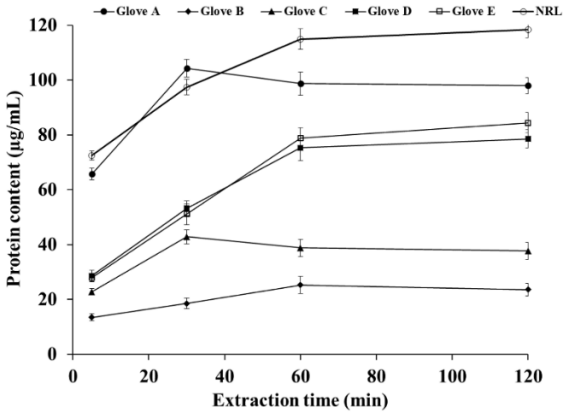

Figure 1. Extraction time of water-extractable protein from latex glove samples (mean ± SD).

TCA, DOC and PTA were used to precipitate the WEP. The precipitated standard bovine serum albumin (BSA) was resuspended in 0.1 N NaOH and compared with the non-precipitated to evaluate protein recovery after precipitation (Figure 2). Upon protein recovery, the precipitated protein was between 92%–96% when 5–80 µg/ml of BSA was used. The standard protein BSA has been widely used to determine protein in natural rubber latex. This experiment found that the BSA precipitated with TCA, DOC and PTA could be completely recovered which is consistent with the report of Tomazic et al. (1999).

The Bradford and Lowry methods are generally recommended for high-sensitivity assays (Lucas and Tomazic, 2000). Extracts from five gloves and NRL samples (Figure 1) were prepared using the standard method

(Tomazic et al., 1999). The WEP of latex glove samples was determined using a calibration curve of the absorbance of BSA. The absorbance of the standard protein BSA was higher when determined by the Bradford method (Figure 3).

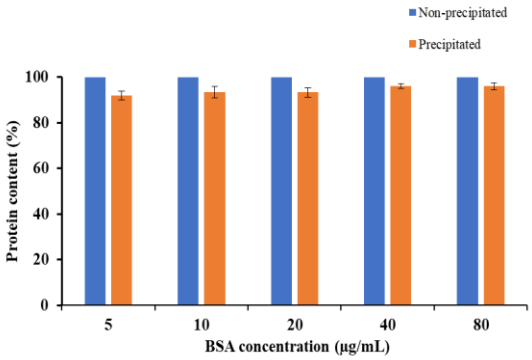

Figure 2. The protein content of non-precipitated and precipitated proteins. BSA was precipitated with TCA, DOC and PTA (mean ± SD).

This result was in contradiction with the concentration of WEP from the latex gloves and NRL. T-test analysis showed that the average protein content was significantly higher when determined by the modified Lowry method than by the Bradford method (Table 1). This is related to Tomazic et al. (1999), who reported that the Bradford method determined a lower protein content in NRL than in BSA when the same amount of protein was used. Kalapat et al. (2009) suggested that the nitrogen content of the NRL is lower than that of the BSA based on the analysis of CHN. This may be due to NRL containing both protein and non-protein components. In addition, BSA and NRL contain different types of amino acids. BSA comprises a high content of lysine and arginine amino acids with $–NH_3^+$ groups in their side chains. Some non-proteins that absorb UV light have the potential to interfere with the protein. This caused a lower protein content in NRL when compared to BSA.

In this research, the protein content determined by the modified Lowry method was significantly higher when compared to the Bradford method (Table 1). Because of the reliance of the Coomassie stain on specific amino acids, the Bradford approach revealed higher variability in protein detection (Tomazic et al., 1999). In accordance with the modified Lawry method (Tomazic et al., 1999), the protocol used in this research was accurate and appeared to have higher sensitivity. In addition, the differences between these two assays lead to different values. Bradford method, the Coomassie G-250 dye reacts with the ionizable groups of the protein, thereby breaking the tertiary structure and exposing the hydrophobic pockets. Then, the dye binds to the hydrophobic amino acids to form stable complexes detectable at 595 nm. The modified Lowry method combines the biuret method, in which copper ions react with peptide bonds in the protein, with a reaction involving the Folin-Ciocalteu reagent and the aromatic amino acid ring structure. The overall reaction produces a stable dark blue complex at 650–750 nm (Mæhre et al., 2018).

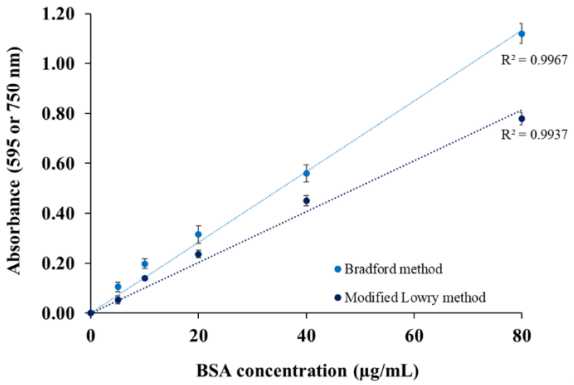

Figure 3. Standard curve of protein determination for Bradford and modified Lowry methods (mean ± SD).

An investigation of WEP levels in various products is shown in Table 1. All latex glove samples examined in this study were made from 100% NRL. The protein

content of the latex glove samples ranged from 62.31–298.64 µg/g and 117.13–485.60 µg/g based on Bradford and modified Lowry methods, respectively. In addition, NRLs contained protein levels of 364.54 µg/g and 592.74 µg/g based on Bradford and modified Lowry methods, respectively. Of the five glove samples, glove A had the highest protein content of 485.60 µg/g and glove B had the lowest protein content of 117.13 µg/g when determined by the modified Lowry method (Table 1). Rubber products currently on the market contain allergic proteins. Docena et al. (2000) indicated higher protein levels in gloves and condoms of up to 740 g/g and 200 g/g, respectively, while Yip and Cacioli (2002) found 674 g/g and showed the presence of various allergenic proteins. US Food and Drug Administration (FDA) allows products with a protein content of 50 µg protein/g glove. Furthermore, latex gloves should have a protein content of less than 30 µg protein/g glove based on the German technical standards for hazardous substances (TRGS406 and 540, 2000). These results indicate that all five glove samples have a high content of latex proteins, which could cause allergies in consumers.

Table 1. Protein determination of latex glove samples using Bradford and modified Lowry methods.

Samples	Protein concentration (µg protein/g sample)	
	Bradford method	**Modified Lowry method**
Glove A	298.64 ± 6.82^{b}	485.60 ± 16.48^{b}
Glove B	62.31 ± 5.81^{e}	117.13 ± 13.50^{e}
Glove C	108.91 ± 8.40^{d}	188.75 ± 20.14^{d}
Glove D	228.38 ± 6.97^{c}	395.80 ± 15.57^{c}
Glove E	260.28 ± 8.90^{bc}	423.22 ± 20.27^{c}
NRL	364.54 ± 7.04^{a}	592.74 ± 16.98^{a}

The value represents means ± SD followed by different letters in each column that are significantly different from one another ($P \leq 0.05$) based on Tukey's test at a 5% probability level.

The protein pattern was analyzed using the SDS-PAGE. An automatic detection method (the software 'CLIQS') was used to ensure that all bands were identified on all gels under the same conditions and to avoid bias caused by manual processing (Srisomboon et al., 2021). Figure 4 shows an image analysis of the SDS-PAGE profile. Total protein bands of 10 bands were detected with molecular weights ranging from 9.0 to 66.6 kDa. Seven bands were found in all samples: Bands # 9, 8, 7, 5, 4, 2 and 1 at 10.3, 14.5, 17.4, 37.9, 52.0, 62.3 and 66.6 kDa, respectively. The protein profiles showed only minor differences. The protein patterns of NRL allergic subjects were analyzed at SDS-PAGE. For example, two significant surface-bound proteins, 14 and 24 kDa were found in the rubber particle phase after SDS-PAGE analysis of NRL proteins (Hasma et al., 1997). The soluble proteins in sera C and B ranged from less than 7 to about 133 kDa and less than 14 to about 45 kDa, respectively (Yip and Cacioli, 2002). The 66 kDa NRL protein band was found in products such as gloves and condoms (Makinen-Kiljunen et al. 1992). Three protein bands with molecular weights of 66, 78 and 116 kDa were found in skin gum (Kalapat et al. 2009). In addition, Srisomboon et al. (2021)

identified 13 protein bands in air-dried minibranes (ADS) with molecular weights ranging from 14.4 to 98.9 kDa at the SDS-PAGE. Thus, NRL comprises 17 allergens and isoforms that range in molecular weight from 4.7–60 kDa (according to IUIS-International Union of Immunological Societies). Interestingly, a protein band with a molecular weight of 69 kDa in NRL products may be a novel allergen (Docena et al. 2000). In the present result, the molecular weight of the protein ranged from 9.0 to 66.6 kDa, which could be indicative of an allergen. Table 2 summarizes the major bands identified in five latex gloves and NRL extracts based on their molecular weight distribution. These latex extracts contained 7–9 protein bands with a similar distribution.

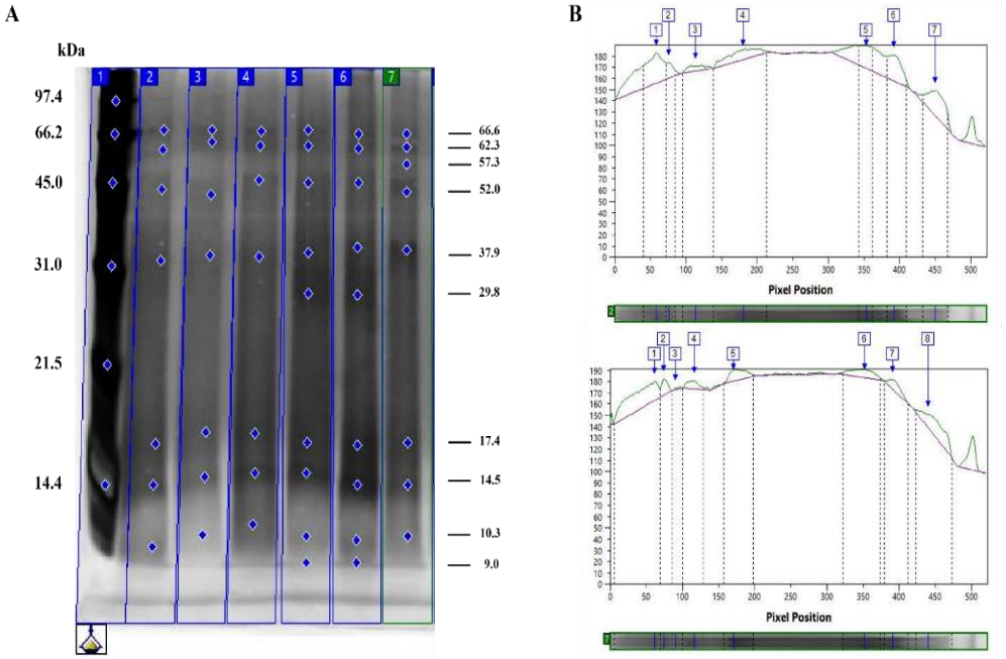

Figure 4. SDS-PAGE of the WEP pattern from latex gloves and NRL samples: lane 1; standard proteins, lane 2; glove A, lane 3; glove B, lane 4; glove C, lane 5; glove D, lane 6; glove E and lane 7; NRL (A). Then, the gel was stained with Coomassie R-250 and double-stained with a silver stain. Image of WEP from the latex glove A (top) and NRL (bottom) was analyzed using the software 'CLIQS' (B).

Table 2. Distribution of protein molecules in different latex extracts observed at SDS-PAGE.

Samples	Protein bands	Molecular weight range (kDa)									
		9.0	10.3	14.5	17.4	29.8	37.9	52.0	57.3	62.3	66.6
Glove A	7		+	+	+		+	+		+	+
Glove B	7		+	+	+		+	+		+	+
Glove C	7		+	+	+		+	+		+	+
Glove D	9	+	+	+	+	+	+	+		+	+
Glove E	9	+	+	+	+	+	+	+		+	+
NRL	8		+	+	+		+	+	+	+	+

+ Protein band was detected by using the software 'CLIQS'.

4. Conclusion

Natural rubber latex allergy is a serious health problem affecting people who use rubber products worldwide. The phosphate buffer was suitable for the WEP extraction with an incubation time was at least 60 min. The modified Lowry method was suggested for protein analysis in natural rubber latex since it yields more protein than the Bradford method. Furthermore, SDS-PAGE showed 7–9 protein bands in all samples with molecular weight distributions ranging from 9.0 to 66.6 kDa. The WEP concentration in all glove samples tested was significantly higher than the FDA and TRGS allowed value.

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