

Use of Epoxidized Natural Rubber (ENR) for Property Improvement of Gelatin Film

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Abstract

Epoxidized Natural Rubber (ENR) was incorporated into bovine gelatin film for enhancing flexibility, water-vapor barrier and water resistance of gelatin film. The impact of gelatin/ENR (G/ENR) ratios (10/0, 8/2, 6/4, 5/5 and 0/10) and ENR types (i.e. ENR-10, ENR-25 and ENR-50 containing epoxy content of 12, 28 and 57 %mol, respectively) on properties of films from bovine gelatin was investigated. As epoxy content and level of ENR increased, the films had decreased tensile strength (TS) and transparency but increased Elongation At Break (EAB) and yellowness (b^* -value) ($p < 0.05$). Water-Vapor Permeability (WVP) of gelatin-based films decreased with ENR addition ($p < 0.05$). Incorporation of ENR-25 at the G/ENR ratio of 6/4 rendered the blend film with the increases in EAB (or flexibility) and water-vapor barrier property by approximately 1.8 and 1.3 times, respectively, compared to those of the gelatin film. The improved properties of G/ENR blend films were most likely due to the compatibility between gelatin and ENR associated with their chemical interactions, as evidenced by SEM and FTIR results.

Keywords: Biodegradable Blend Film, Epoxidized Natural Rubber (ENR), Gelatin

1. Introduction

In response to increasing concerns on environmental problem/pollution caused by non-biodegradable synthetic polymers, development of materials derived from biological and renewable resources have been received attention. For example, biodegradable films and coatings have been manufactured various bio-based materials such as polysaccharides, lipids and proteins^{1,2}. Among biopolymers, proteins from different sources have been utilized for packaging materials owing to their abundance, biodegradability and nutritive value. Moreover, agro-based materials and packaging from proteins generally have good functional properties due to the heterogeneous structure of proteins as compared to others biopolymers^{3,4}.

Gelatin is animal protein derived from hydrolyzed collagen. Bovine and porcine Skin and bone are the

major sources for extraction of gelatin⁵. Gelatin has been employed worldwide with a wide range of industrial applications. Gelatin has been proven to be a potential source for biodegradable film due to its abundant raw material, low cost and excellent film-forming ability. Film from gelatin is transparent and excellent in gas (O_2 and CO_2) barrier property⁶. However, it possesses some inferior properties such as lower strength and elasticity or flexibility as well as higher water-vapor transmission rate, as compared to synthetic films. Incorporation of typical plasticizer such as glycerol can improve flexibility of gelatin films but decrease their water-vapor barrier and water resistance^{3,4,6}. As a result, intensive studies have been focused on improving the aforementioned properties of the gelatin film by employing various strategies including chemical treatment⁷, enzymatic treatment⁸, thermal treatment⁹ and ultraviolet and gamma irradiation¹⁰. Among various approaches, polymer blend technique has been

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known as effective and simple method to enhance the properties of biopolymer-based films including protein film¹¹. Blending of polymers has been often used to obtain materials with desirable physical properties, process and cost¹². In recent years, properties of gelatin film have been improved by blending with different bio-based polymers such as polysaccharide, chitosan, soy protein and polyvinyl alcohol^{13–16}. Blending gelatin with other polymers that have high strength, elasticity and water resistance would improve the properties of the gelatin films.

Natural Rubber (NR) is a naturally derived polymer which possesses the aforementioned properties such as hydrophobic, highly elastic and water-vapor resistant. However, a polar natural rubber such as epoxidized Natural Rubber (ENR), a chemically modified natural rubber, is more promising for blending with gelatin in order to be compatible with gelatin molecules. ENR can be prepared by reacting natural rubber with organic peracid such as performic acid in either solution or latex system¹⁷. In this process, backbone double bonds of NR are converted into epoxides (oxiranes)¹⁸. Apart from highly elastic nature of rubber, ENR possesses good oil resistance due to the polarity of the epoxy group in the rubber chain^{17,18}. The polarity and properties of ENR are dependent on the epoxy content or degree of epoxidation^{17,19}. Based on the polar nature of epoxide rings, ENR of varying epoxy content have been used as reactive component to blend with other polar materials such as Poly Vinyl Chloride (PVC), chloro sulphonated polyethylene and starch, to improve their elastic property and impact resistance^{20–23}.

However, use of ENR to modify the physico-chemical properties of gelatin film has not been reported. Incorporation of rubber into gelatin film is expected to improve not only the flexibility or elasticity but also the water-vapor barrier characteristic of the gelatin film. Therefore, the present study was undertaken to investigate the impact of Epoxidized Natural Rubber (ENR) on the physico-chemical properties of glycerol-plasticized gelatin film as influenced by type of ENR (i.e. epoxy content) and gelatin/ENR blend composition.

2. Materials and Methods

2.1 Raw Materials and Chemicals

Gelatin (Type B) from bovine hide with bloom strength of approximately 250 was obtained from Halamix International Co., Ltd. (Bangkok, Thailand).

High-ammonia concentrated (~60% DRC) natural rubber (HA-NR) latex was purchased from Chalong Latex Industry Co., Ltd. (Songkhla, Thailand). Glycerol used as plasticizer was procured from Wako Pure Chemical Industry, Ltd. (Tokyo, Japan). Sodium hydroxide, potassium hydroxide, hydrogen peroxide, hydrochloric acid and formic acid were purchased from Merk (Darmstadt, Germany). Teric-N30 was procured from Orica Australia Pty Ltd. (Melbourne, Australia).

2.2 Preparation of ENR

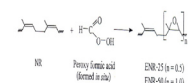
ENR latexes containing varying epoxy contents (ENR-10, ENR-25 and ENR-50) were prepared by using modified method of Jirupan²⁴. The formulation recipe used for preparation of the different ENR is shown in Table 1. HA-NR latex of 20% Dry Rubber Content (DRC) and Teric-N30, a non-ionic surfactant, were charged in a 3-neck reactor and stirred for 24 h at room temperature and after that the temperature was raised to 60°C. Formic acid was then introduced to the reaction mixture and stirred for 10 min at 60°C under nitrogen gas. Then, hydrogen peroxide was added to the reactor and stirred for 24 h. Finally, the reaction was stopped by adjusting the pH of the mixture to 6.5 – 7.0 with 10% (w/v) potassium hydroxide. The obtained ENR were subjected to structure characterization and epoxy content determination by using ¹H-NMR spectroscopic analysis as described by Burfield et al²⁵. The NMR

Table 1. Formulations used to prepare the ENR latexes containing different epoxy contents

Formulations	ENR-10	ENR-25	ENR-50*
Natural rubber latex (20% DRC), ml	1000	1000	1000
Formic acid, ml	12.34	21.60	37.00
Hydrogen peroxide, ml	100.28	175.50	300.84
Teric-N30 (non-ionic surfactant), g	6.00	6.00	6.00
Measured epoxy content, % mol **	12.28 ± 0.02	28.06 ± 0.06	57.08 ± 0.10

*Chemical structure of ENR:

**Epoxy content of ENR was determined via ¹H-NMR spectroscopic analysis.



spectra indicated the presence of characteristic signals of epoxy group which appeared at 1.29 and 2.70 ppm, assigned to methyl and methane proton of epoxide²⁶; this reconfirmed the ENR structure of ENR obtained in this study (data not shown). The epoxy contents of different ENR obtained, as measured by ¹H-NMR spectroscopy, were 12.28, 28.06 and 57.08%mol (Table 1), which referred to ENR-10, ENR-25 and ENR-50, respectively.

2.3 Preparation of Gelatin/ENR Blend Film

Gelatin was dissolved in de-ionized water to obtain the solution containing final protein concentration of 2% (w/v), followed by incubation at 60°C for 30 min in a water bath. The glycerol was then added to gelatin solution at 25% (w/w of protein). To prepare film-forming mixture containing ENR, the ENR (20% DRC) latex (including ENR-10, ENR-25 and ENR-50) was added to the gelatin solution at designated amounts to obtain the varying gelatin/ENR ratios of the blends (10/0, 8/2, 6/4, 5/5 and 0/10 (w/w of dry polymer)). All prepared film-forming solutions and mixtures contained the final polymer of 2% (w/v). The film-forming solution/mixture was further stirred gently for 30 min, followed by homogenizing at 13,000 rpm for 2 min. The film samples were manufactured by casting the film-forming solution/mixture (4 g) onto a silicone mold (5x5 cm²), followed by an air blowing for 12 h at room temperature and further drying for 24 h at 25°C and 50% Relative Humidity (RH) in a ventilated oven (WTB Binder, Tuttlingen, Germany). The obtained dried films were peeled off from the mold and subjected to analysis.

2.4 Property Determination and Characterization of Film

Physical and physico-chemical properties of obtained film samples were carried out. Prior to measurement of physical and mechanical properties, the films were kept for 48 h in a ventilated oven at 25°C and 50 ± 5% RH. The thickness, mechanical properties (including elastic modulus (E), Tensile Strength (TS) and Elongation At Break (EAB)) as well as Water Vapor Permeability (WVP) at 30°C were measured according to the methods described by Hoque et al⁹. Optical characteristics including color, light transmittance and transparency value of films were ascertained following the methods of Shiku et al²⁷. Water solubility of films was also determined according to the procedure of Gennadios et al²⁸.

Structure and molecular interaction as well as surface and cross-sectional morphologies of films were characterized by means of ATR Fourier-Transform Infrared (ATR-FTIR) spectroscopic and scanning electron microscopic techniques, respectively, in accordance with the methods described by Hoque et al⁹. Prior to characterization, films were conditioned in a desiccators containing P₂O₅ at room temperature for two weeks to obtain the most dehydrated films and to minimize the plasticizing effect due to absorbed water.

2.5 Statistical Analysis

Experiments were carried out in triplicate. Analysis of Variance (ANOVA) of the obtained data were performed and the differences between means were evaluated by using Duncan's Multiple Range Test. The SPSS statistic program (SPSS Inc., Chicago, IL, USA) was employed for data analysis.

3. Results and Discussion

3.1 Visualized Appearance of Films

Photograph of the selected film samples (gelatin, gelatin/ENR-25 (6/4) and ENR films) is shown in Figure 1. In general, the obtained gelatin film was clearer and more transparent than ENR and gelatin/ENR blend films. The gelatin/ENR blend films were homogeneous without visualized phase separation, rather transparent and flexible. Their surfaces were smooth without visualized pores and crack.

3.2 Effect of Epoxy Content of ENR and Gelatin/ENR Ratio on Properties of Gelatin-based Film

3.2.1 Thickness and Mechanical Properties

The obtained films from gelatin, gelatin/ENR (G/ENR) blend and ENR had varying thickness. In general, all ENR



Figure 1. Photographs of selected films: gelatin film, gelatin/ENR-25 (6/4) blend film and ENR-25 film.

films had lower thickness (18.04 – 18.88 μm) than did the gelatin film (26.16 μm). This was plausibly due to the difference in their molecular structures, which resulted in different molecular alignment in matrix of film²⁹. The thickness of G/ENR films tended to decrease with increasing level of ENR incorporated (data not shown).

Tensile stress-strain behavior of selected film samples was illustrated in Figure 2. In general, gelatin film was stiffer and more resistant to tensile deformation while ENR films showed more ductile behavior. As a consequence, tensile deformation of the G/ENR blend films exhibited increasing ductile behavior as indicated by large plastic deformation (Figure 2 (curves B, C and D)), as the level of ENR added increased.

Figure 3 shows film's mechanical characteristics including TS, E and EAB of gelatin-based films added with ENR having different epoxy contents (ENR-10, ENR-25 and ENR-50 containing epoxy content of 12.28, 28.06 and 57.08%mol, respectively) at various gelatin/ENR ratios (10/0, 8/2, 6/4, 5/5 and 0/10), in comparison to the control gelatin and ENR films. TS and E values of control gelatin film were greater, but EAB was lower than those of ENR films, regardless of ENR types ($p < 0.05$). This might be contributable from that gelatin molecules underwent stronger inter-molecular interaction. The result was in accordance with that of Cruz et al.³⁰, reported that the collagen film had higher TS than did the Natural Rubber (NR) film. For ENR films of all types, their TS and E decreased but EAB increased with an increase in epoxy content of ENR ($p < 0.05$).

Among G/ENR blend films, when the level of ENR incorporated increased, their TS and E decreased while

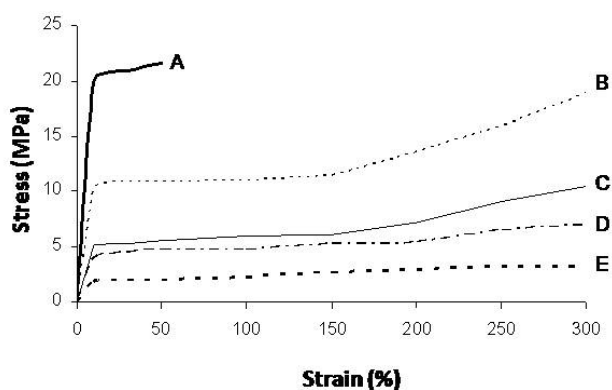


Figure 2. Representative stress-strain diagrams of selected film samples: gelatin film (A), gelatin/ENR-25 blend films at different G/ENR ratios of 8/2 (B), 6/4 (C) and 5/5 (D) and ENR-25 film (E).

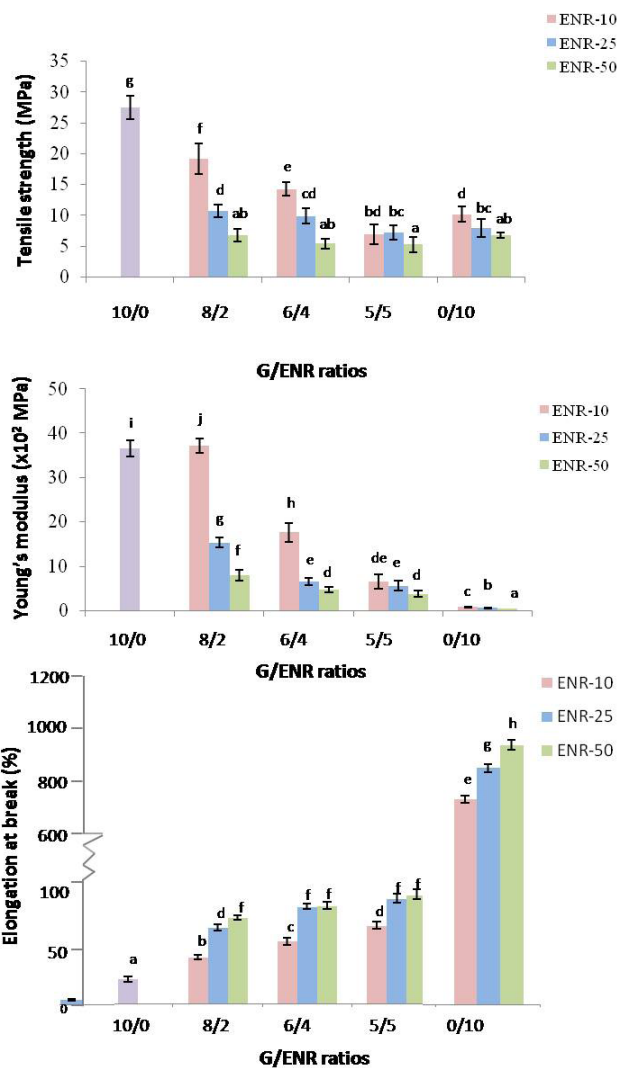


Figure 3. Mechanical properties of films fabricated from gelatin (G), different ENR and gelatin/ENR blends at various G/ENR ratios. Bars illustrate the standard deviation ($n=3$). Different lower case letters on the bars indicate the significant differences ($p < 0.05$).

EAB increased, irrespective of epoxy content of ENR. This was simply due to the additive effect caused by ENR incorporated which possesses highly elastic characteristic³¹. ENR molecules more likely inserted between and interacted with gelatin molecules. The epoxy group of ENR might interact inter-molecularly with $-\text{NH}_2$, $-\text{OH}$ or $-\text{COOH}$ groups of gelatin³⁰. Thus, inter- and intra-molecular attractive forces between gelatin molecules were decreased and thus intermolecular spacing more likely increased, due to the inserted ENR along with the glycerol added as plasticizer. As a result, chain mobility was increased. This was evidenced by the increase in EAB

and the decrease in TS and stiffness (i.e. E) of the G/ENR blend films, compared to the gelatin film. At the same level of ENR used, G/ENR-25 and G/ENR-50 blend films exhibited lower TS and E but greater EAB than did the film of G/ENR-10 blend ($p < 0.05$). No difference in EAB value of the blend films incorporated with ENR-25 and ENR-50 was observed ($p > 0.05$), except those with G/ENR ratio of 8/2. Therefore, the level of ENR incorporated as well as the epoxy content of ENR played a significant role on mechanical properties of gelatin-based films.

3.2.2 Water Vapor Permeability (WVP)

WVP value of films from bovine gelatin incorporated without and with ENR of different types (ENR-10, ENR-25 and ENR-50) at various G/ENR blend ratios is presented in Table 2. The control gelatin film exhibited higher WVP than did the ENR films ($p < 0.05$), due mainly to the greater hydrophilic nature of the gelatin molecules which contain high amount of hydrophilic groups including amino, hydroxyl and carboxyl groups³². Generally, films from proteins possess excellent barrier against oxygen and aroma permeation^{33,34}. Nevertheless, protein films are prone to absorb water especially at high Relative

Humidity (RH), owing to the hydrophilic character of proteins³⁵. Among ENR tested, ENR-10 and ENR-25 films showed similar WVP value ($p > 0.05$). However, films of ENR-50 which contains higher epoxy group had higher WVP than did those of ENR-10 and ENR-25 ($p < 0.05$), plausibly caused by higher polarity of ENR-50 as contributed from the higher amount of polar epoxy groups.

The results showed that ENR blending could decrease WVP value of gelatin-based film ($p < 0.05$). The epoxy group of ENR might interact inter-molecularly with amino, hydroxyl and carboxylic groups of gelatin, resulting in decreased reactive sites available to interact with water³⁶. From the result, for the same ENR type used, WVP of blend films seemed to increase when the level of ENR incorporated increased, especially at G/ENR ratio of 5/5. This was more likely attributable to the presence of higher degree of phase separation in the blend film. When ENR was added at the same level, the blend films with ENR-10 and ENR-25 addition showed similar WVP ($p > 0.05$). However, the G/ENR-50 blend film exhibited higher WVP than blend films of G/ENR-10 and G/ENR-25 ($p < 0.05$). An excessive amount of epoxy groups in ENR-50 might result in the increased inter-molecular

Table 2. Permeability of water vapor (WVP), color and water solubility of films from gelatin, gelatin/ENR (G/ENR) blend and ENR

Films	WVP ($\times 10^{-11}$ g.m/ m ² .s.Pa)	Color parameters			Film solubility (%)
		L*	a*	b*	
Gelatin+25% Gly	8.86 ± 0.34^b	90.21 ± 0.37^a	-1.19 ± 0.01^a	$1.35 \pm 0.03^{a,\#}$	100 ± 0.00^a
G/ENR-10 (8/2)	6.55 ± 0.19^{cde}	88.42 ± 0.93^a	-1.20 ± 0.02^a	1.34 ± 0.17^a	79.07 ± 1.06^b
G/ENR-10 (6/4)	6.12 ± 0.20^c	84.12 ± 0.67^b	-1.28 ± 0.01^b	1.45 ± 0.03^b	58.05 ± 0.83^d
G/ENR-10 (5/5)	6.25 ± 0.18^{cf}	83.66 ± 0.80^b	-1.30 ± 0.02^b	1.57 ± 0.05^c	53.12 ± 1.73^e
ENR-10	4.79 ± 0.22^a	80.17 ± 0.09^c	-1.35 ± 0.03^{cd}	1.90 ± 0.03^f	3.38 ± 0.16^f
G/ENR-25 (8/2)	6.16 ± 0.19^{cde}	81.66 ± 1.44^c	-1.28 ± 0.02^b	1.58 ± 0.05^c	73.22 ± 1.20^c
G/ENR-25 (6/4)	6.76 ± 0.58^{cd}	83.46 ± 1.03^b	-1.31 ± 0.02^c	1.57 ± 0.03^c	56.69 ± 0.89^d
G/ENR-25 (5/5)	6.82 ± 0.23^{cde}	82.63 ± 1.58^{bc}	-1.32 ± 0.02^c	1.63 ± 0.03^{cd}	50.63 ± 0.42^e
ENR-25	4.82 ± 0.18^a	81.00 ± 1.22^c	-1.40 ± 0.2^e	1.75 ± 0.04^d	3.05 ± 0.09^g
G/ENR-50 (8/2)	6.32 ± 0.19^{cdf}	82.19 ± 0.78^{bc}	-1.32 ± 0.02^c	1.81 ± 0.02^e	73.12 ± 1.70^c
G/ENR-50 (6/4)	7.17 ± 0.31^{fg}	82.39 ± 0.73^{bc}	-1.37 ± 0.03^d	1.82 ± 0.02^e	55.65 ± 1.27^d
G/ENR-50 (5/5)	7.32 ± 0.07^g	80.43 ± 0.45^c	-1.42 ± 0.04^e	1.97 ± 0.04^f	50.15 ± 0.92^e
ENR-50	5.03 ± 0.23^b	80.41 ± 0.64^c	-1.48 ± 0.03^f	2.08 ± 0.08^g	3.02 ± 0.90^g

The different superscripts within the same column represent the significant differences at $p < 0.05$.

interaction between ENR molecules with concomitantly decreased gelatin-ENR interaction. This in turn caused an increase in phase separation between gelatin and ENR in the blend, leading to increased free volume in the matrix of blend film. The presence of free volume in polymer system generally accelerates the diffusion of small molecules through the polymer matrix. This could provide the increase in WVP as a function of free-volume holes size³⁷.

When comparing WVP of the G/ENR blend films to other synthetic and protein-based films, WVP of synthetic films such as high-density polyethylene (HDPE) (1.77×10^{-14} g.m/m².s.Pa at 32°C, 90% RH) and low-density polyethylene (LDPE) (3.27×10^{-14} g.m/m².s.Pa at 32°C, 90% RH)³⁸ was much lower than the WVP of the G/ENR film in this study. However, G/ENR blend films in this study exhibited lower WVP than glutenin-rich films (7.00×10^{-11} g.m/m².s.Pa at 23°C, 50% RH)³⁹, whey protein isolate/pul-lulan blend films (10.50×10^{-11} g.m/m².s.Pa at 30°C, 80% RH)⁴⁰ and surimi films (11.25×10^{-11} g.m/m².s.Pa at 30°C, 90% RH)⁴¹.

3.2.3 Color of Films

Table 2 shows the values of L^* , a^* and b^* of films from ENR, control gelatin and gelatin incorporated with ENR of varying types at various G/ENR ratios. The gelatin film possessed lighter color than did the ENR films. As compared to the gelatin film, G/ENR blend films had increased greenness ($-a^*$) and yellowness ($+b^*$) but decreased lightness (L^*)

when the level of ENR incorporated increased ($p < 0.05$), irrespective of epoxy content of ENR. At the same level of ENR added, ENR containing higher epoxy content rendered the G/ENR blend films with increased yellowness (b^* -value) ($p < 0.05$). This might be resulted from pigments naturally present in NR raw material and also from the reaction dealing with formic acid and H_2O_2 which were added to the latex in preparing ENR. The result was in accordance with that of Ismail and Poh⁴², reported on color of PVC as influenced by ENR-25 and ENR-50 addition.

3.2.4 Light Transmission and Film Transparency

The transmission of light (%T) in the range of ultraviolet and visible light as well as the transparency value of ENR films and gelatin-based films added without and with the different ENR types (ENR-10, ENR-25 and ENR-50) at various G/ENR ratios are shown in Table 3. ENR films showed the decreased %T at 200 nm with an increase in the epoxy level of ENR used. In visible range (350 - 800 nm), ENR-10 films had higher %T than did ENR-25 and ENR-50 films while ENR-25 and ENR-50 films had similar %T. The control gelatin film possessed significantly lower UV barrier properties than did the ENR films. Addition of ENR could decrease the light transmission of bovine gelatin-based film. In general, the light transmittance of the G/ENR blend films decreased with increased ENR level and epoxy content of ENR added. For visible light in the wavelength 600 - 800 nm, the %T value of G/ENR blend films was ranged from 84% - 98%, suggested that the obtained films were consid-

Table 3. Transmission of light (%T) and transparency value of films from gelatin, ENR and G/ENR blends at various ratios

Film types	Transmittance (%T) at selected wave length (nm)								Transparency value
	200	280	350	400	500	600	700	800	
Gelatin	18.80	80.00	92.96	98.55	99.14	99.15	99.36	99.52	2.24 ± 0.08^a
G/ENR-10=8/2	16.83	51.15	87.10	85.93	96.98	96.98	97.26	98.27	3.37 ± 0.05^{bc}
G/ENR-10=6/4	15.74	53.27	87.31	86.01	92.79	92.79	93.72	94.68	3.43 ± 0.09^{cd}
G/ENR-10=5/5	14.77	43.94	86.16	82.60	89.34	89.35	89.91	90.17	3.51 ± 0.09^e
ENR-10	13.13	41.86	76.56	74.97	89.40	89.41	92.84	94.77	4.52 ± 0.01^f
G/ENR-25=8/2	14.13	42.49	77.56	75.98	90.01	90.08	93.12	95.44	3.34 ± 0.01^b
G/ENR-25=6/4	10.72	41.80	58.52	61.39	89.49	89.50	91.91	94.04	3.39 ± 0.02^{cd}
G/ENR-25=5/5	9.64	41.81	50.11	59.55	84.33	84.33	90.02	93.02	3.42 ± 0.02^d
ENR-25	10.12	41.70	55.31	61.20	82.89	82.89	90.78	93.89	4.60 ± 0.02^g
G/ENR-50=8/2	12.07	35.21	78.75	78.40	89.86	89.86	95.15	97.11	3.34 ± 0.02^b
G/ENR-50=6/4	11.04	34.91	72.39	77.96	89.04	89.04	93.16	95.77	3.36 ± 0.02^{bc}
G/ENR-50=5/5	9.60	33.99	57.48	65.40	84.60	84.59	85.92	87.43	3.38 ± 0.02^c
ENR-50	9.46	42.04	53.31	61.21	81.56	81.60	87.64	92.89	4.68 ± 0.03^h

The different superscripts within the same column represent the significant differences at $p < 0.05$.

erably clear. The addition of ENR could therefore improve barrier against UV light of the bovine gelatin-based film. This was in consistent with other studies carried out on starch/SBR blend films²⁶, graft-copolymerized starch/NR blend films⁴³ and thermoplastic starch/NR blend films⁴⁴.

The transparency value of tested films is shown in Table 3. It is noted here that the lower transparency value indicates the more transparent of the film. From the results, gelatin film was more transparent than did the ENR films and G/ENR blend films. The transparency of gelatin film obviously decreased (i.e. transparency value increased) when ENR was incorporated into the film ($p < 0.05$), irrespective of ENR type and level used. This was simply due to the more opaque nature of rubber added and also the presence of phase separation between gelatin and ENR domains. At the same level of ENR used, similar transparency value was noticeable among the blend films incorporated with ENR containing different epoxy contents ($p > 0.05$), except for that at G/ENR ratio of 5/5.

3.2.5 Water Solubility of Films

Water solubility of films from ENR and gelatin without and with the incorporation of varying types of ENR at different G/ENR ratios is shown in Table 2. Gelatin film was completely soluble in water, due to the highly hydrophilic nature of gelatin^{13,15}. In contrast, ENR films had very low water solubility (3.02- 3.38 %). Addition of ENR could significantly decrease solubility of gelatin films ($p < 0.05$). G/ENR blend films exhibited the lower solubility than control gelatin film ($p < 0.05$), regardless of ENR types. When ENR having the same epoxy content was used, solubility of G/ENR blend films declined with increasing ENR level ($p < 0.05$). The similar observation was reported in starch/NR blend film⁴³. The decrease in solubility of G/ENR blend films might be because long chain ENR molecule could form closely intermolecular interaction with gelatin molecules. As compared with other films, the films from G/ENR blend in this study had lower water solubility than did those prepared from pigskin gelatin/PVA blend¹⁵. Low solubility in water of film is important for protecting the products from water during application.

3.3 Characteristics of Selected Gelatin-based Films Incorporated with ENR

3.3.1 FTIR Spectroscopy

Figure 4 shows FTIR spectra of selected film samples (gelatin, ENR-25 and G/ENR-25 (6/4) films). From the

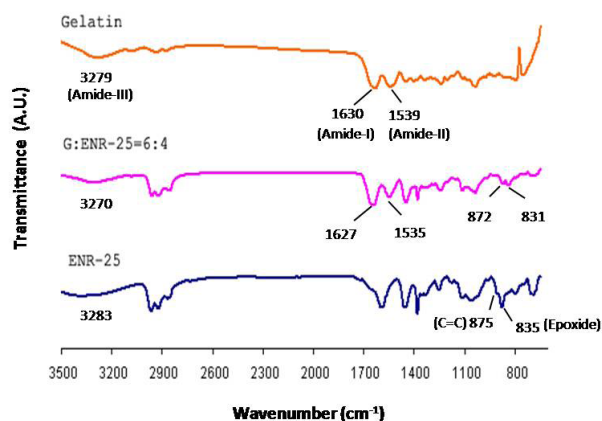


Figure 4. FTIR spectra of selected films from bovine gelatin, ENR-25 and gelatin/ENR (6/4) blend.

spectrum of ENR film, it showed absorption peaks at 835 cm^{-1} and 875 cm^{-1} , representing C=C of cis-1,4 polyisoprene and epoxy group, respectively, which are characteristic for ENR^{19,30}. The peak centered at around 3283 cm^{-1} in ENR spectra plausibly resulted from absorbed water. Gelatin-based films spectra showed strong absorption bands situated at around 3279 cm^{-1} (amide A), 1630 cm^{-1} (amide-I) and 1539 cm^{-1} (amide-II), which are typically contributed from stretching of N-H groups, C=O stretching of amide bond and N-H bending vibration of amide in protein molecules, respectively^{45,46}. For G/ENR blend film, besides amide-III, amide-I and amide-II peaks, there existed additional peaks approximately at 872, 831 cm^{-1} which represented the characteristic of the incorporated ENR on its spectra. From the result, addition of ENR to gelatin caused some shifts of peaks of amide-I, amide-II and amide-III and also the broader amide-III peak as noticed from the spectra. In addition, the intensity of the amide-II peak of gelatin and the peak at $\approx 872 \text{ cm}^{-1}$ related to epoxy group of ENR decreased with the addition of ENR. The results most likely indicated the presence of chemical interactions between gelatin and ENR molecules in the film matrix. Moreover, the shift of amide-III, amide-II and amide-I to lower wave number with broadening of the peaks (especially around 3270 cm^{-1}) observed in the spectra of G/ENR films could indicate the presence of protein-protein and protein-ENR interactions mostly due to hydrogen bonding interaction. The epoxy group of ENR might interact inter-molecularly with various moieties ($-\text{NH}_2$, $-\text{OH}$ or $-\text{COOH}$) of gelatin^{20,30}. However, no additional new absorption peak was observed in the IR spectra of G/ENR film. This suggested that no

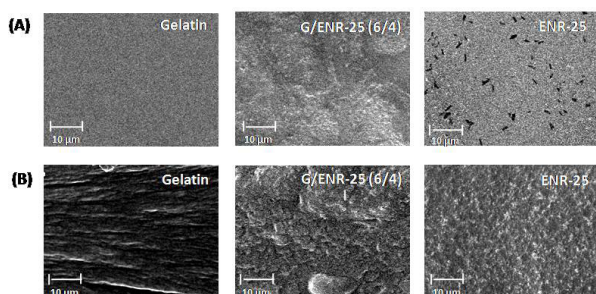


Figure 5. Micrographs from SEM analysis of films from bovine gelatin, gelatin/ENR-25 (6/4) blend and ENR-25. (A) film surface and (B) film cross-section.

inter-molecular covalent bond between gelatin and ENR was formed in the network of film from G/ENR blend. The presence of gelatin-ENR interactions most likely contributed to the improved mechanical performance as well as water vapor barrier and water resistance of the G/ENR blend films as described previously.

3.3.2 Microstructure of Films

The microstructures (surface and freeze-fractured cross-section) as observed from the SEM images of gelatin, ENR-25 and G/ENR-25 (6/4) blend films are illustrated in Figure 5. Gelatin film had smoother surface and cross-section. In contrast, ENR-25 and G/ENR-25 blend films exhibited rougher surface and cross-section, presumably resulted from differences in arrangement of gelatin and ENR molecules during film formation. This rougher fractured surface of ENR-25 film was more likely resulted from the presence of shear yielding deformation before fracture, which is a characteristic of ductile material. However, from the image of cross-section of the G/ENR-25 blend film, it suggested that gelatin and ENR-25 were still compatible since the adhesion between phases seemed to be good with no distinct separation at the interface and no visible voids of removed rubber. The compatibility of the gelatin and ENR was most likely due to interactions between those molecules as evidenced from FTIR result. The compatibility of gelatin and ENR was most likely responsible for the improved mechanical property (film flexibility) and barrier against water vapor transmission of the G/ENR blend film.

4. Conclusions

Epoxidized Natural Rubber (ENR) could be used to improve some physico-chemical properties of gelatin film.

The addition of ENR with appropriate type (i.e. epoxy content) and level could enhance the flexibility (acting like a plasticizer) and water vapor barrier of the gelatin film, mainly due to the intrinsic properties of ENR added and also to the compatibility of gelatin and ENR which resulted from the chemical interactions. The incorporation of ENR-25 at G/ENR ratio of 6/4 rendered the film with the most improved EAB or flexibility as compared to the control gelatin film. Moreover, incorporation of ENR also significantly decreased film solubility of gelatin-based film. Therefore, ENR incorporated could behave not only as an alternative plasticizer but also as a toughness modifier and a water-vapor barrier promoter for gelatin film.

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