CHAPTER IV

RESULTS AND DISCUSSION

4.1 Effect of incorporation of whey protein isolate (WPI) on properties of konjac glucomannan (KGM) based films

4.1.1 Film preparation and formation

KGM solutions were partially opaque and very high in viscosity, which in turn limited the maximum concentration of KGM in casting solution to 1 g/100 g solution. KGM hydrates rapidly by absorbing up to 200 times their weight in water to form viscous, pseudoplastic dispersions (Nishinari, Kim, & Kohyama, 1987) which lend itself to be commonly used as thickening agent, stabilizer, or gelling agent (Zhang, Xie, & Gan, 2005). On the other hand, WPI quickly dissolved to form thin, transparent, slightly yellowish solution. The maximum WPI concentration was limited to 12 g/100 g solution, due to thermal gelation of WPI by heat treatment in the process of preparing film, resulting in strong elastic protein gel beyond this critical concentration.

To investigate the effect of WPI on properties of KGM film, WPI were incorporated into the casting solution. It was found that the maximum concentration of WPI in the casting solution of the blend film was lower than WPI concentration in the WPI film. The maximum total amount of biopolymer in film forming solution of the blend film was 4.2 g/100 g solution, mainly limited by strong viscosity of the blended casting solution.

The viscous KGM solution had the ability to form translucent and matt film with high-strength and flexibility, even at such low concentration (1 g/100 g solution) (Figure 1a). On the other hand, WPI films were transparent, slightly yellow-tinted and

glossy (Figure 1b). Depending on the ratio of KGM and WPI, the resulting blend film varied in visual characteristics from more translucent and matt with higher KGM, and vice versa. For example, KGM-WPI blend film prepared from 0.4 g KGM, 3.8 g WPI and 1.5 g glycerol in 100 g casting solution are shown in Figure 4.1c.

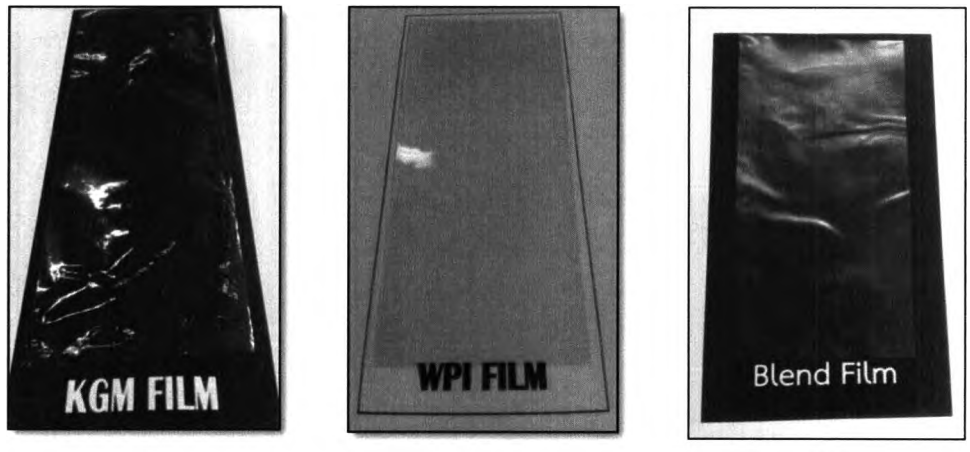


Figure 4.1 Appearance of the films (a) KGM film, (b) WPI film and (c) KGM-WPI blend film prepared from 0.4 g KGM, 3.8 g WPI and 1.5 g glycerol in 100 g casting solution

4.1.2 Film properties measurements

Thickness

The thickness of KGM, WPI and blend films are shown in Table 4.1. The thickness of the films varied between 0.112 mm to 0.128 mm. The thickness of WPI film was slightly lower than KGM film. For the blended film, KGM:WPI ratio and glycerol content did not significantly affect the film thickness ($p>0.05$). Thus, regardless of biopolymer type and glycerol content, controlling the total solids of casting solution per casting plate could effectively produce cast films with consistent thickness, which in turn kept the error in further property tests minimized.

Table 4.1 Thickness of konjac glucomannan (KGM), whey protein isolate (WPI) and blend films

(g KGM :g WPI)/ 100 g solution	Glycerol (g Gly/ 100 g solution)	Thickness (mm)
1:0	1.5	$0.128^{a} \pm 0.003$
	1.8	$0.123^{abc} \pm 0.008$
0.8:3.4	1.5	$0.128^{a} \pm 0.003$
	1.8	$0.121^{abc} \pm 0.009$
0.6:3.6	1.5	$0.121^{abc} \pm 0.012$
	1.8	$0.124^{ab} \pm 0.003$
0.4:3.8	1.5	$0.126^{ab} \pm 0.002$
	1.8	$0.124^{ab} \pm 0.006$
0:4.2	1.5	$0.115^{bc} \pm 0.006$
	1.8	$0.112^{c} \pm 0.004$

* Values are the averages ± standard deviations.

** Different superscripts (a–c) indicate significant differences between all samples ($p \leq 0.05$).

Color

L, a, b values of KGM, WPI and blend films are shown in Table 4.2. The three coordinates of hunter color space represent the lightness of the color (L= 0 yields black and L = 100 indicates diffuse white; specular white may be higher), its position between red/magenta and green (*a*, negative values indicate green while positive values indicate magenta) and its position between yellow and blue (*b*, negative values indicate blue and positive values indicate yellow). Comparing to KGM film, WPI film had significantly higher L value and lower a and b values ($p \leq 0.05$). In

another word, KGM film is darker, less green and yellower than WPI film. Incorporating WPI into KGM film, significantly increased L and decreased a and b values ($p \leq 0.05$). Range of glycerol used in this research did not significantly affect color of KGM, WPI and KGM-WPI films ($p > 0.05$).

The total color difference between KGM, WPI and KGM-WPI films and commercially available polypropylene films were calculated (Table 4.2). ΔE values of WPI film and blend film were lower than detectable threshold (above 3) by human eye (Francis, 1983; Vichi, Ferrari, & Davidson, 2004) indicating that these films were not visibly difference compared to commercial polypropylene film. On the other hand, KGM film was visually different from polypropylene ($\Delta E > 3$).

Table 4.2 Color of konjac glucomannan (KGM), whey protein isolate (WPI) and blend films, and total color difference (ΔE) between biopolymer-based samples and commercial polypropylene film

(g KGM :g WPI)/ 100 g solution	Glycerol (g Gly/ 100 g solution)	L	a	b	ΔE
1:0	1.5	$77.66^{c} \pm 0.40$	$-1.30^{a} \pm 0.03$	$4.02^{a} \pm 0.20$	$6.28^{a} \pm 0.42$
	1.8	$78.26^{c} \pm 0.35$	$-1.45^{b} \pm 0.02$	$3.70^{a} \pm 0.25$	$5.63^{a} \pm 0.38$
0.8:3.4	1.5	$81.15^{ab} \pm 0.69$	$-1.80^{cd} \pm 0.14$	$1.26^{bc} \pm 0.71$	$1.98^{bc} \pm 0.92$
	1.8	$80.84^{b} \pm 0.44$	$-1.84^{d} \pm 0.04$	$1.63^{b} \pm 0.35$	$2.44^{b} \pm 0.53$
0.6:3.6	1.5	$81.11^{ab} \pm 0.20$	$-1.76^{cd} \pm 0.02$	$1.34^{bc} \pm 0.13$	$2.03^{bc} \pm 0.21$
	1.8	$80.93^{b} \pm 0.40$	$-1.74^{cd} \pm 0.04$	$1.67^{b} \pm 0.30$	$2.41^{b} \pm 0.45$
0.4:3.8	1.5	$81.96^{a} \pm 0.85$	$-1.71^{c} \pm 0.07$	$0.93^{bc} \pm 0.67$	$1.57^{bc} \pm 0.54$
	1.8	$81.28^{ab} \pm 0.67$	$-1.73^{cd} \pm 0.05$	$1.02^{bc} \pm 0.26$	$1.74^{bc} \pm 0.49$
0:4.2	1.5	$81.81^{ab} \pm 0.55$	$-1.69^{c} \pm 0.06$	$0.65^{c} \pm 0.34$	$1.20^{c} \pm 0.40$
	1.8	$82.06^{a} \pm 0.40$	$-1.73^{c} \pm 0.06$	$0.61^{c} \pm 0.25$	$1.11^{c} \pm 0.22$

* Values are the averages ± standard deviations.

** Different superscripts (a–c) indicate significant differences within the same column ($p \leq 0.05$).

Transparency

Table 4.3 shows the transparency of the films. KGM films had the lowest transparency, compared to WPI films and blend films. The transparency value of blend films was higher than those of KGM films due to the effect of incorporation of WPI component. Moreover, this increased transparency may indicate some degree of incompatibility between KGM and WPI, resulting in heterogeneous film structures. This finding is consistent with previous research reported that WPI films had greater transparency than polysaccharides (methylcellulose, hydroxypropylmethylcellulose, sodium alginate) or polysaccharides-WPI blend films (Yoo & Krochta, 2011).

The effect of glycerol content on transparency of pure and blend films were also investigated (Table 4.3). Effect of incorporated glycerol on films' transparency was found significant ($p \leq 0.05$), except the blend film with 0.6 g KGM and 3.6 g WPI per 100 g casting solution. Overall results showed that increased glycerol concentration increased the transparency of the films.

Table 4.3 Transparency of konjac glucomannan (KGM), whey protein isolate (WPI) and blend films

(g KGM :g WPI)/ 100 g solution	Glycerol (g Gly/ 100 g solution)	Transparency (mm^{-1})
1:0	1.5	$14.724^{ef} \pm 0.031$
	1.8	$15.345^{c} \pm 0.077$
0.8:3.4	1.5	$14.419^{f} \pm 0.495$
	1.8	$15.265^{cd} \pm 0.097$
0.6:3.6	1.5	$15.578^{c} \pm 0.05$
	1.8	$15.396^{c} \pm 0.154$
0.4:3.8	1.5	$14.991^{de} \pm 0.036$
	1.8	$15.394^{c} \pm 0.204$
0:4.2	1.5	$16.743^{b} \pm 0.041$
	1.8	$17.247^{a} \pm 0.017$

* Values are the averages ± standard deviations.

** Different superscripts (a–f) indicate significant differences between all samples ($p \leq 0.05$).

Mechanical properties

The effects of incorporation of different concentrations of WPI as well as different amounts of glycerol as a plasticizer on mechanical properties were determined (Figure 4.2). KGM films exhibited significantly higher TS, EM and %E than WPI films indicating that KGM films were stronger and more ductile materials ($p \leq 0.05$).

Incorporating WPI into matrix of KGM films resulted in significantly decrease in TS and EM and significantly increase in %E, comparing to the pure KGM

film ($p \leq 0.05$) as shown in Figure 4.2 a-c. However, there was no significant difference in TS and EM between the blend films and WPI film ($p > 0.05$). It was hypothesized to be attributed to the partial incompatibility between two biopolymers. Occurrence of the denatured whey protein aggregates might result in the discontinuity of the KGM network, thereby reducing film brittleness and improving flexibility. This finding is consistent with previous work by Yoo and Krochta (2011) which reported the decrease in TS of HPMC:WPI or MC:WPI blend films with decreased concentration of polysaccharides in the blend.

Significantly increased %E (Figure 4.2c) values were also observed as WPI concentration in the blend films decreased to 3.4 g/ 100 g solution, indicating the improved stretchability compared to the pure WPI film ($p \leq 0.05$). It was hypothesized to be due to the effect of WPI content. Generally, WPI film was more brittle than KGM film; therefore, the film with lower WPI concentration tended to have higher %E.

There was a significant effect of glycerol content on %E of the blend films ($p \leq 0.05$). The films with higher glycerol concentration (at 1.8 g glycerol/ 100 g solution) tended to have higher %E due to plasticizing effect. Plasticizers can reduce internal hydrogen bonding and increase the intermolecular spacing. This finding is consistent with previous work by (McHugh & Krochta, 1994) which found that the %E of the film increased with an increase of plasticizer concentration while tensile strength and elastic modulus decreased.

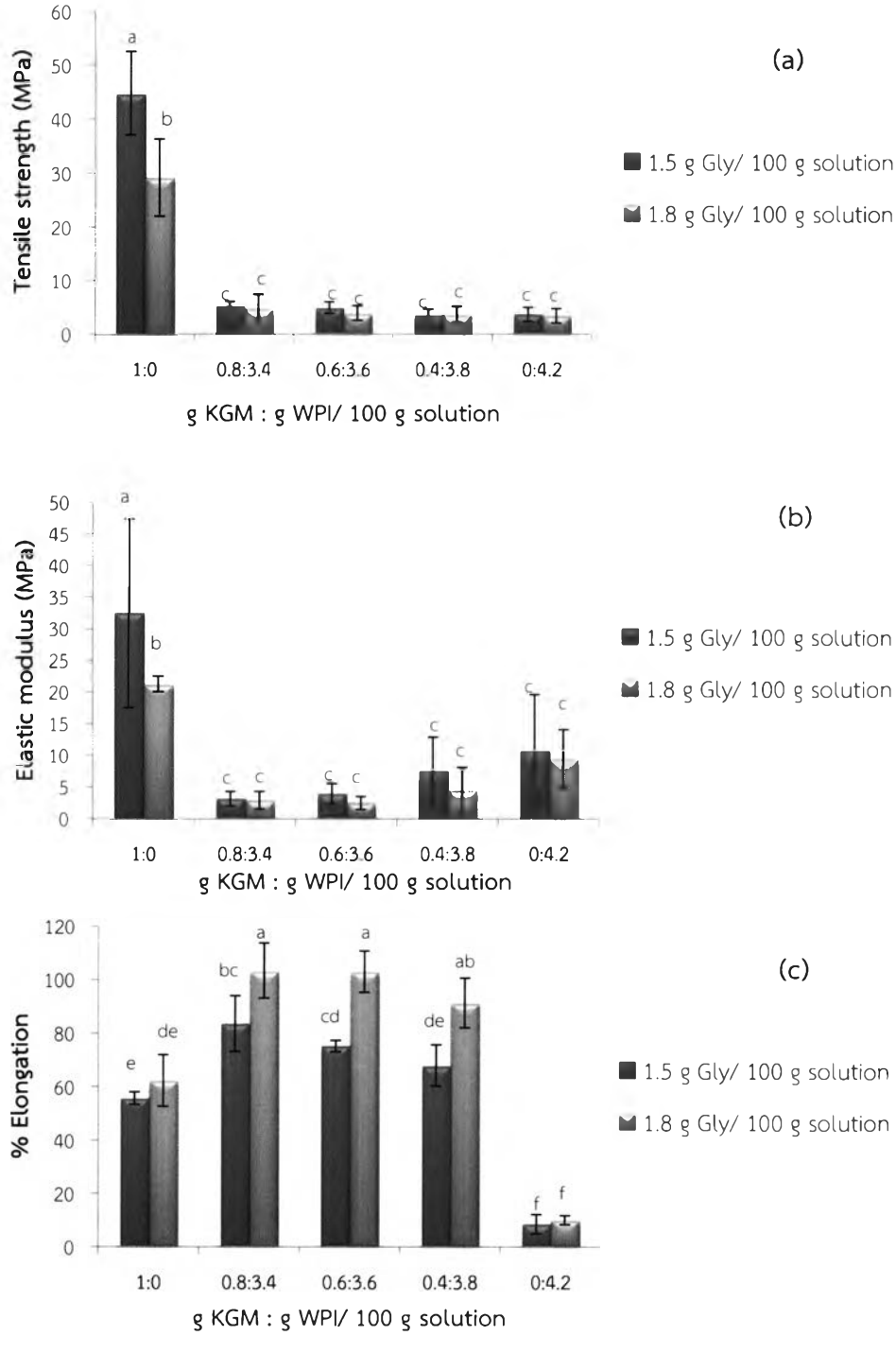


Figure 4.2 Mechanical properties; tensile strength (a), elastic modulus (b) and elongation (c), of konjac glucomannan (KGM), whey protein isolate (WPI) and blend films. Error bar shows standard deviation. Bars with different letters are significantly different at $p \leq 0.05$.

Solubility

Solubility is an important property of edible films. Typically, low water solubility property is essential in enhancing film overall integrity and water resistance. Although, certain applications require high solubility of the film in water such as dissolvable pouches for a pre-weighted single size serving instant powder.

Solubility of film is shown in Figure 4.3. The KGM film had the highest solubility value because chemical structure of KGM consists of numerous hydrophilic hydroxyl groups (Li et al., 2006). The % solubility of the WPI film was found to be the lowest indicating the strong structural cohesion of partially heat-denatured WPI matrix (Perez-Gago et al., 1999). The improved water resistance of the blend films was hypothesized to be due to the formation of covalent intermolecular disulfide bonds of whey proteins in the blend films. During heat denaturation, whey protein molecules unfold and expose free sulfhydryl groups which promote intermolecular disulfide bond formation, thus allowing formation of insoluble films (Floris et al., 2010; Janjarasskul, Tananuwong, & Krochta, 2011; Perez-Gago et al., 1999). Solubility of WPI films could be manipulated by varying the availability of disulfide bonds by changing the WPI-aggregate preparation conditions; e.g. ranging from 100% solubility of native WPI to approximately 70%, 50%, 20% solubility of WPI aggregates pretreated at 68.5 °C for 2 h, 90 °C for 30 min and 90 °C for 7 min, consecutively (Floris et al., 2010). The differences in film solubility were due to the fact that WPI was a mixture of proteins with different denaturation kinetics (Floris et al., 2010). Therefore, the incorporation of WPI decreased water solubility of KGM film, as shown by decreasing the solubility value.

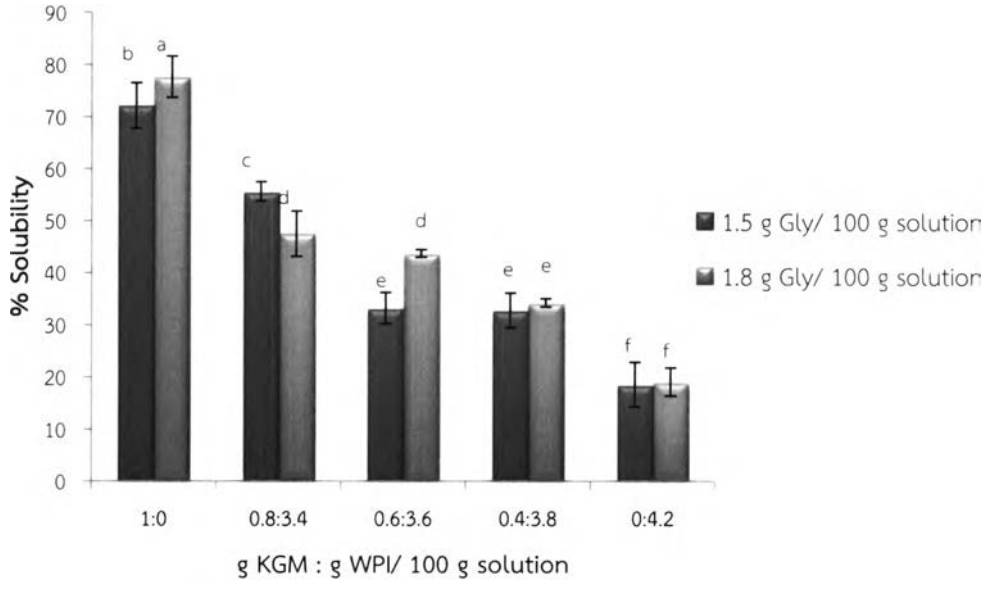


Figure 4.3 Solubility of konjac glucomannan (KGM), whey protein isolate (WPI) and blend films. Error bar shows standard deviation. Bars with different letters are significantly different at $p \leq 0.05$.

The solubility of WPI films (20%) is consistent with previous works (Brindle & Krochta, 2008; Perez-Gago et al., 1999; Sothornvit & Krochta, 2000). Glycerol content at 1.5 and 1.8 g/ 100 g solution did not apparently affect solubility of the films. Because glycerol is very hydrophilic and typically readily soluble in water, increasing glycerol in cast film is assumed to increase solubility of the films. Laohakunjit and Noomhorm (2004) reported that solubility of glycerol plasticized rice starch film was dependent on incorporated glycerol concentration and the value of the plasticized film was significantly higher than that of non-plasticized films ($p \leq 0.05$). Although the KGM and/or WPI films with higher glycerol concentration tended to have higher solubility value, this trend was not clearly shown for the blended films in this current study.

Water vapor permeability (WVP)

Water vapor permeability of KGM, WPI and blend films are shown in Figure 4.4. There was no significant effect of biopolymer composition on WVP of the films ($p>0.05$). This is due to the fact that permeability coefficient (P) is a product of the diffusion coefficient (D) and solubility coefficient (S). Although incorporating WPI improved water insolubility (decreasing solubility coefficient) by providing the intermolecular disulfide bond formation, such effect was counter-balanced with plasticizing effect of WPI molecules in KGM matrix (increasing diffusion coefficient). Thus, the overall WVP was not significantly affected by incorporation of WPI ($p>0.05$).

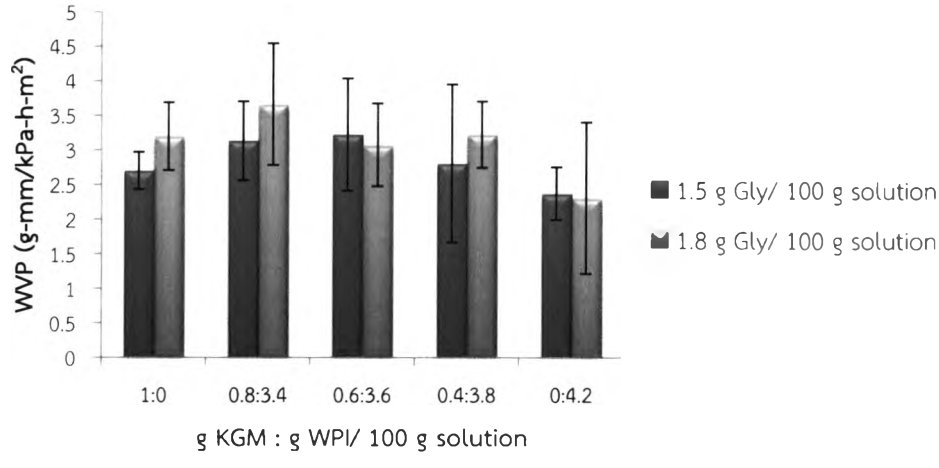


Figure 4.4 Water vapor permeability of konjac glucomannan (KGM), whey protein isolate (WPI) and blend films. Error bar shows standard deviation. Bars with different letters are significantly different at $p\leq0.05$.

Glycerol content also did not have a significant effect on WVP of the films ($p>0.05$), although the films with higher glycerol concentration tended to have higher WVP. Many research studies (Banker, 1996; Cuq et al., 1997; Janjarasskul et al., 2011; Koelsch, 1994; Sothornvit & Krochta, 2000) reported the plasticizing effect of glycerol on impairing WVP of biopolymer films by reducing intermolecular forces along polymer chains and increased the polymer free volume for water molecules to

migrate (Sothornvit & Krochta, 2000). However, the narrow glycerol contents investigated in this study did not significantly affect WVP of the films (p>0.05).

Thermal transitions

Thermal transitions of KGM, WPI and blend films were investigated (Table 4.4 and Figure 4.5). For the WPI and the blend films, the endotherm with T_o around 157-160 °C, T_p around 173-181 °C and ΔH ranged from 5.6 J/g to 19.0 J/g was detected.

Hernandez and Krochta (2008) also reported the existence of endothermic peaks with an onset temperature at the similar range (156.3 ± 1.4 °C) for both solution-cast and extruded WPI films plasticized with glycerol. They hypothesized that this endotherm represented the transformation of the film matrix to a thermoplastic-extrudable melt. In case of KGM films, there was no endotherm in this temperature range. Degradation of WPI films was reported to occur at more than 200 °C (Janjarasskul et al., 2011) while KGM film was likely to be degraded at around 326 °C (Xu et al., 2007).

Among the blend films, variation in the biopolymer concentration had no significant effect on T_o, T_p and ΔH of the films (p>0.05.). However, T_p and ΔH of WPI films were obviously higher than those of the blend films. WPI film had the highest WPI concentration and its matrix was considerably homogenous. These could enhance the interactions between whey protein molecules. Therefore, higher thermal energy was required to melt the WPI film matrix comparing to the blend films.

Table 4.4 Parameters from the endothermic transitions of konjac glucomannan (KGM), whey protein isolate (WPI) and blend films determined by differential scanning calorimetry

(g KGM :g WPI)/ 100 g solution	Endothermic transitions		
	T_o^{ns} (°C)	T_p (°C)	ΔH (J/g)
1:0	-	-	-
0.8:3.4	160.35 ± 3.14	176.84^{ab} ± 0.93	6.70^{b} ± 1.34
0.6:3.6	158.40 ± 1.26	173.62^{b} ± 3.92	7.08^{b} ± 0.76
0.4:3.8	160.30 ± 0.71	177.24^{ab} ± 2.14	5.62^{b} ± 0.85
0:4.2	157.67 ± 1.81	181.39^{a} ± 1.59	19.01^{a} ± 1.88

* T_o and ΔH represent onset temperature and enthalpy of endothermic transitions, respectively.

** Values are the averages ± standard deviations.

*** Different superscripts (a–b) indicate significant differences within the same column.

**** ns indicates no significant differences within the same column (p>0.05).

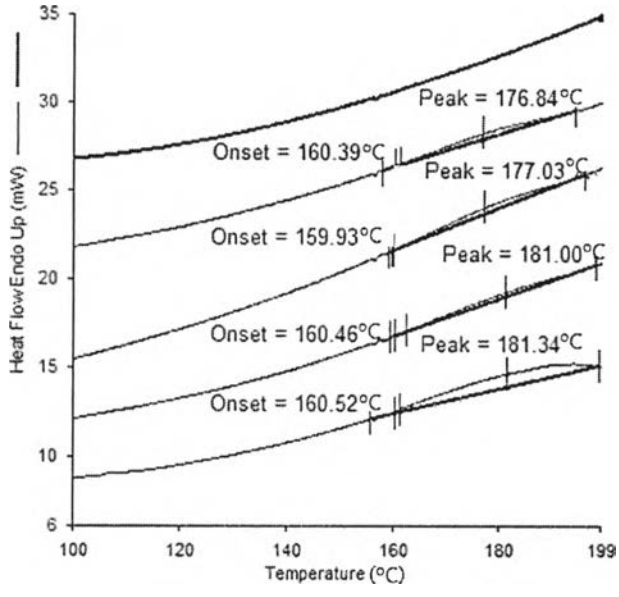


Figure 4.5 Differential scanning calorimetry thermograms of konjac glucomannan (KGM), whey protein isolate (WPI) and blend films at (a) 1.0:0 g KGM: g WPI/ 100 g solution, (b) 0.8:3.4g KGM :g WPI/ 100 g solution, (c) 0.6:3.6g KGM :g WPI/ 100 g solution, (d) 0.4:3.8g KGM :g WPI/ 100 g solution and (e) 0:4.2 g KGM or WPI/ 100 g solution.

Seal strength

Heat sealing of the films was determined near the peak temperature of the endotherm determined by DSC. Sealing temperature is considered as the most important process variable which affects seal strength. In this study, the other two heat sealing variables; jaw pressure and cooling time, were kept constant. The result showed that WPI and KGM-WPI blend film with highest WPI concentration (0.4:3.8 g KGM or WPI/ 100 g solution) could be optimally heat sealed at 175 °C. Below this optimal temperature, the seals delaminated. Distorted seals together with film discoloration and off-odor obtained at higher temperatures could hypothesize to be attributed to degradation of whey proteins matrix corresponding to thermal transitions.

During the heat sealing process, the heat melts crystalline polymer on the surfaces of two films pressed together between heated plates. The application of pressure causes the interfacial interactions to form across the joint surface. Upon cooling, a heat-sealed joint, depending on the surface chemistry of the sealing material(s), is produced due to re-crystallization of the polymer. Temperature, pressure, and dwell time are considered important process variables which affect seal strength (Mueller et al., 1998). The main forces responsible for the sealed joint formation upon heat sealing of WPI films reported to be hydrogen and covalent bonds; involving C-O-H and N-C. Polar polymers such as proteins associate by high degree of hydrogen bonding. Hydrogen bonding can occur between protein and plasticizer such as COOH side groups of amino acid. Hydrogen bonding among proteins can also occur. Covalent bond formation due to heat sealing can occur between $-NH_2$ group of lysine and carboxyl side group of asparagine (Kim & Ustunol, 2001). On the other hand, KGM films and other blend films with lower concentration of WPI were not heat-sealable. As seen from DSC thermogram (Figure 4.5), those melting endotherms were mainly be contributed to the WPI portion. Therefore, less proportion of WPI might not be sufficient to create the heat-sealable matrix. Li et al. (2006) also found that pure KGM film could not be heat sealed at 140 °C.

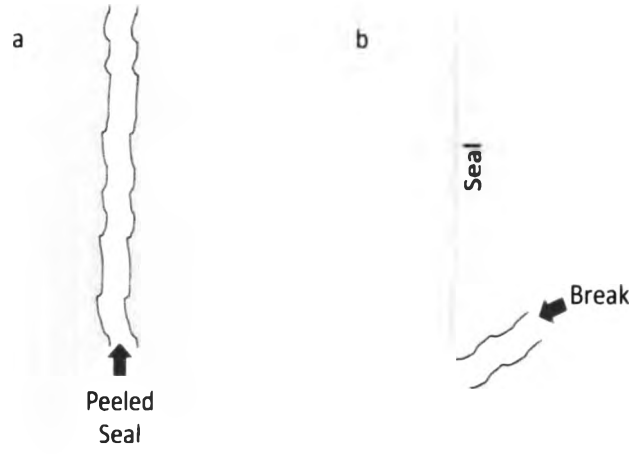


Figure 4.6 Mode of failure of heat seal during seal strength test (a) peeled seal and (b) material break

Measurement of seal strength is used as an indicator of seal quality. Seal strength is not only relevant to opening force and package integrity, but to measuring the packaging processes ability to produce consistent seals. Seal strength at some minimum level is a necessary package requirement, and at times it is desirable to limit the strength of the seal to facilitate opening. The maximum seal force is important information, but for some applications, average force to open the seal may be useful, and in those cases also should be reported. The test may be conducted on seals between flexible materials. This test method measures the force required to separate a test strip of material containing the seal.

Although WPI films and blend film with highest WPI concentration (3.8 g WPI/100 g solution) were capable to be heat sealed at 175 °C, the seal strength of such seals cannot be determined. This is due to the failure mode of the seal. To obtain correct seal strength, the seal must be peeled cohesively at sealing surface (shown in Figure 4.6a). However, in this study the sealed test strips broke at sealing edge before the sealed area could be separated from each other by the load as shown in Figure 4.6b. This means that the heat-sealed area was stronger than the film sample. Thus, the heat seal strength determination could not be achieved in this study.

Trinocular phase contrast microscope

Microstructure of blend films obtained from the trinocular phase contrast microscope is shown in Figure 4.7. Coomassie Brilliant Blue was used as dye solution to stain protein samples. The dye molecules bind to proteins to form dye-protein complex and commonly produce blue color. However, the dye-protein complex was shown in the shade of blue-green and gray color from the trinocular phase contrast microscope; therefore, darker zone implied that the area contained higher density of WPI molecules.

Overall results showed that the blend films were heterogeneous, resulting from the incompatibility between KGM and WPI. These results supported the hypothesis described in the previous sections regarding transparency and mechanical properties. Microstructure of the blend films at 0.8:3.4g KGM :g WPI/ 100 g solution and 0.6:3.6g KGM :g WPI/ 100 g solution (Figure 4.7a and b) showed the large cluster of WPI molecules while smaller WPI aggregates existed and dispersed throughout the film matrix of 0.4:3.8g KGM :g WPI/ 100 g solution (Figure 4.7c). In other words, WPI concentration greatly affected the microstructure of the blend films because WPI was the major component in the matrix due to the higher concentration comparing to KGM. Higher WPI concentration induced the formation of smaller clusters of whey protein molecules to reduce the volume fraction in the film matrix. Greater extent of self-association of biopolymers, resulting in decreasing excluded volume, could help minimize the effect of perturbation from incompatible biopolymer molecules (Tolstoguzov, 2006). Therefore, WPI might aggregate to form the small cluster in order to reduce the perturbation from KGM molecules.

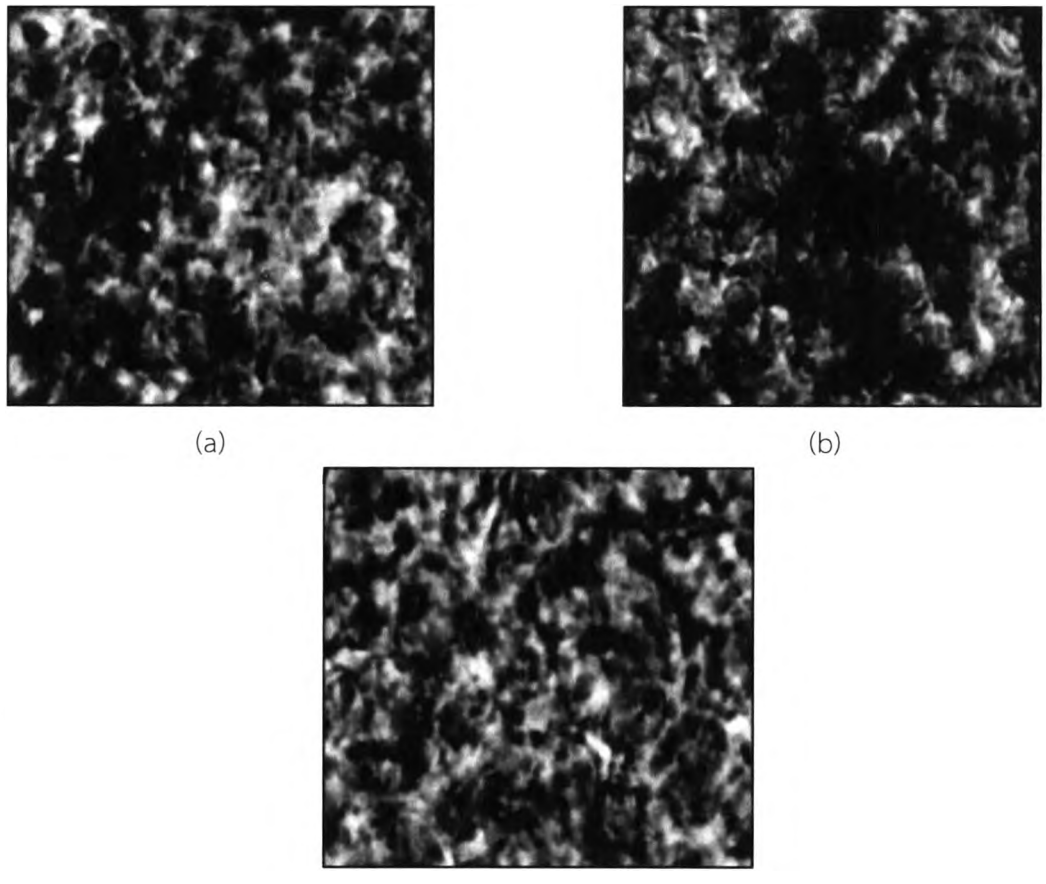


Figure 4.7 Microstructure of konjac glucomannan (KGM), whey protein isolate (WPI) and blend films at (a) 0.8:3.4g KGM :g WPI/ 100 g solution, (b) 0.6:3.6g KGM :g WPI/ 100 g solution and (c) 0.4:3.8g KGM :g WPI/ 100 g solution, obtained from trinocular phase contrast microscope using 1000X magnification. Blue green and gray area indicated the presence of WPI clusters.

4.2 Effect of drying rate on mechanical and physical properties of konjac glucomannan-whey protein isolate blend films

4.2.1 Film preparation and formation

A blend formula was selected for further investigation on effect of drying rate on properties of KGM-WPI film. KGM-WPI films with 0.4 g KGM, 3.8 g WPI and 1.5 g Gly in 100 g casting solution was selected on the basis of its superior heat sealability and water solubility, compared to other blend films, while maintaining other necessary properties; color, transparency, mechanical strength and water vapor permeability. Heat sealability of this blend film lends itself to a wider commercial feasibility in primary packaging for food and related products. Examples are preformed edible films for fabrication of a pouch or sachet used to deliver premeasured quantity, i.e. a single dose, of dry food ingredients or drugs. While providing primary protection to the premeasured portion, these edible pouches provide consumer convenience for instant foods, minimize preparation/clean up process and reduce packaging waste (Janjarasskul & Krochta, 2010). Lowest quantity of glycerol content was chosen for further research in order to minimize the utilization of plasticizer without compromising essential properties of the edible film.

Casting solution of selected blending formula was allowed to completely dry utilizing tray dryers by either fast or slow drying rates. The drying process stopped when the film sample reach constant weight. In the case of fast drying rate, inlet air temperature 50 $^{\circ}$C, velocity 3.04 ± 0.67 m/s and 19% RH, films were completely dried and able to be peeled intact off acrylic plates within 3 h while slow drying rate, inlet air temperature 50 $^{\circ}$C, velocity 0.92 ± 0.15 m/s and 18% RH, took 15 h. The appearances of films produced from fast drying rate were visually smoother and clearer when compared to films dried at slower rate.

4.2.2 Film properties measurements

Thickness

The thickness of KGM-WPI film produced from slow and fast drying rates are 0.126 ± 0.002 and 0.106 ± 0.003 mm, respectively. The difference was mostly likely caused by the velocity of the drying air. Although thickness of the films produced from two drying rates were significantly different ($p \leq 0.05$), such small difference did not affect the determination of other studies of films' properties.

Color

The color values of blend films dried by different conditions are listed in Table 4.5. There was no significant effect of drying conditions on lightness and yellowness ($p > 0.05$). However, increasing drying rate resulted in significantly increased greenness ($p \leq 0.05$). For comprehensive analysis, total color difference between film samples from two drying conditions was calculated. ΔE value of 1.16 ± 0.17 (below human eye's detectable threshold of > 3) indicated that there was no effect of drying rate on films' overall color. The total color difference between KGM-WPI films produced from slow and fast drying conditions and commercially available polypropylene films were also calculated, 1.57 ± 0.54 and 2.38 ± 0.25, respectively. ΔE values of KGM-WPI samples and commercial plastic film were lower than detectable threshold as described previously. Thus, these biopolymer-based films were not visibly difference to polypropylene sample, despite the different drying rates.

Transparency

The results showed that there was a significant effect of drying conditions on transparency value of the films ($p \leq 0.05$). Fast dried film had significantly higher transparency (17.991± 0.104) compared to the other (14.991 ± 0.036). It was hypothesized that drying rate affected the rearrangement of biopolymers in the film matrix and influenced the appearance of the film.

Table 4.5 Color and transparency of konjac glucomannan (KGM) and whey protein isolate (WPI) blend film with 0.4 g KGM, 3.8 g WPI and 1.5 g glycerol in 100 g casting solution dried by different drying rate.

Drying conditions	Film properties			
	L^{ns}	a	b^{ns}	Transparency (mm^{-1})
Slow drying	81.96 ± 0.85	-1.71^{a} ± 0.07	0.93 ± 0.67	14.991^{b} ± 0.036
Fast drying	81.51 ± 0.16	-1.85^{b} ± 0.03	1.82 ± 0.25	17.991^{a} ± 0.104

* Slow drying rate (velocity 0.92 ± 0.15 m/s and 18% RH, 15 h drying time) and fast drying rate (velocity 3.04 ± 0.67 m/s and 19% RH, 3 h drying time)

** Values are the averages ± standard deviations.

*** Different superscripts (a–b) indicate significant differences within the same column ($p \leq 0.05$).

**** ns indicates no significant differences within the same column ($p > 0.05$).

Mechanical properties

Tensile strength, % elongation and elastic modulus of KGM-WPI blend film casted with fast and slow drying methods are shown in Table 4.6. There was a significant effect of drying rate on mechanical properties of the films ($p \leq 0.05$). Fast dried film had higher TS and EM values, and lower %E than those of slow dried films. This finding is consistent with previous work which reported the increase of TS of

chitosan films as a result of reduced drying time by switching from hot air drying to vacuum drying (Mayachiew & Devahastin, 2008).

Table 4.6 Mechanical properties of konjac glucomannan (KGM) and whey protein isolate (WPI) blend film with 0.4 g KGM, 3.8 g WPI and 1.5 g glycerol in 100 g casting solution dried by different drying rate.

Drying conditions	Film properties		
	Tensile strength (MPa)	Elongation (%)	Elastic modulus (MPa)
Slow drying	$3.49^{b} \pm 1.15$	$67.97^{a} \pm 7.73$	$7.59^{b} \pm 5.26$
Fast drying	$9.74^{a} \pm 0.38$	$31.55^{b} \pm 13.12$	$100.19^{a} \pm 66.14$

* Slow drying rate (velocity 0.92 ± 0.15 m/s and 18% RH, 15 h drying time) and fast drying rate (velocity 3.04 ± 0.67 m/s and 19% RH, 3 h drying time)

** Values are the averages ± standard deviations.

*** Different superscripts (a–b) indicate significant differences within the same column ($p \leq 0.05$).

Solubility

Effect of drying rate on solubility of KGM-WPI blend film is shown in Table 4.7. It was found that drying rate did not have a significant effect on solubility of the film ($p>0.05$). This is due to the fact that the drying temperatures of both fast and slow drying methods was controlled at 50 $^{\circ}$C. Thus the drying methods did not affect the extension of protein denaturation which could in turn affect WPI solubility (Perez-Gago & Krochta, 2001; Perez-Gago et al., 1999).

Water vapor permeability (WVP)

Table 4.7 shows water vapor permeabilities of blend films. WVP of blend film dried by slow drying rate was slightly higher than the WVP of blend film dried at fast drying rate. However, there was no significant effect of drying rate on WVP of the films ($p>0.05$). Mayachiew and Devahastin (2008) and Srinivasa et al. (2004) reported that WVP of edible chitosan films were not affected by various drying technologies and conditions. On the other hands, Alcantara et al. (1998) found that shorten drying rate by elevated temperature (95 °C) improved WVP due to further denaturation of whey proteins. Therefore, this finding indicating that it is possible to dehydrate blend films with fast drying rate in order to increase production speed of wet casting for commercial scale up without impairing water vapor barrier property.

Table 4.7 Solubility and water vapor permeability of konjac glucomannan (KGM) and whey protein isolate (WPI) blend film with 0.4 g KGM, 3.8 g WPI and 1.5 g glycerol in 100 g casting solution dried by different drying rate.

Drying conditions	Film properties	
	Solubility (%)^{ns}	WVP (g-mm/kPa-h-m^2)^{ns}
Slow drying	32.66 ± 3.32	2.81 ± 1.14
Fast drying rate	37.84 ± 3.30	1.86 ± 0.38

* Slow drying rate (velocity 0.92 ± 0.15 m/s and 18% RH, 15 h drying time) and fast drying rate (velocity 3.04 ± 0.67 m/s and 19% RH, 3 h drying time)

** Values are the averages ± standard deviations.

*** ns indicates no significant differences within the same column ($p>0.05$).

Thermal transitions

Effect of drying rate on thermal transitions of the blend films is shown in Table 4.8. Slow drying rate resulted in the film with significantly higher T_o but

significantly lower ΔH ($p \leq 0.05$). Nevertheless, there was no significant difference in T_p of the blend films ($p > 0.05$). It was hypothesized that drying rate affected the rearrangement of biopolymers in the film matrix, resulting in the different melting behaviors.

Table 4.8 Endothermic transitions of konjac glucomannan (KGM) and whey protein isolate (WPI) blend film with 0.4 g KGM, 3.8 g WPI and 1.5 g glycerol in 100 g casting solution dried by different drying rate.

Drying conditions	Endothermic transitions		
	T_o (°C)	T_p^{ns} (°C)	ΔH (J/g)
Slow drying	$160.30^{a} \pm 0.71$	181.39 ± 1.59	$5.62^{b} \pm 0.85$
Fast drying	$157.00^{b} \pm 0.21$	178.06 ± 2.13	$9.02^{a} \pm 0.62$

* Slow drying rate (velocity 0.92 ± 0.15 m/s and 18% RH, 15 h drying time) and fast drying rate (velocity 3.04 ± 0.67 m/s and 19% RH, 3 h drying time)

** T_o and ΔH represent onset temperature and enthalpy of endothermic transitions, respectively.

*** Values are the averages ± standard deviations.

**** Different superscripts (a–b) indicate significant differences within the same column ($p \leq 0.05$).

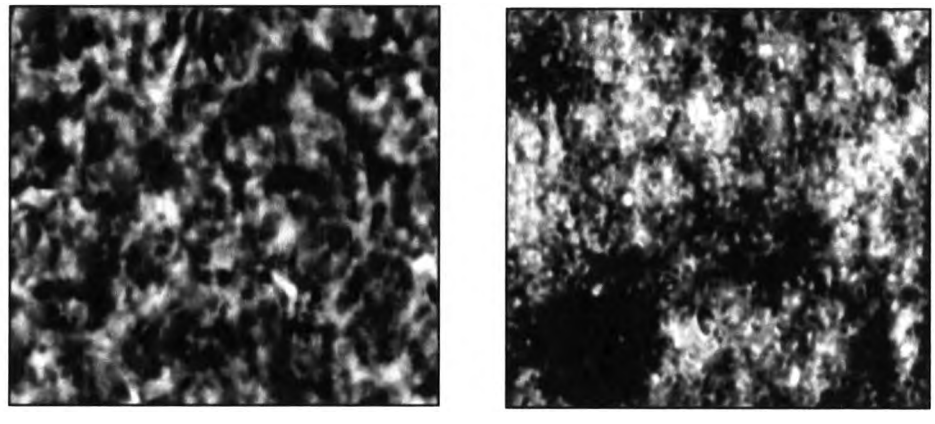
***** ns indicates no significant differences within the same column ($p > 0.05$).

Trinocular phase contrast microscope

Microstructure of blend films dried at slow and fast drying rate is shown in Figure 4.8. Fast drying rate resulted in larger WPI clusters in the film matrix while the small clusters of WPI dispersed throughout the matrix of films dried at slow drying rate. This might be due to different drying duration used. Rearrangement of biopolymer molecules could slowly occur during drying. Greater extent of the molecular rearrangement could exist in the film applied to longer drying time. Therefore, prolonged drying time could induce self-aggregation of whey protein

molecules to reduce the perturbation from incompatible KGM molecules in the slowly dried film.

These results could be used to explain the effects of drying rate on some film properties. In the matrix of the films dried at fast drying rate, there were larger clusters of whey protein molecules. This could enhance the light transmission and transparency of the quickly dried film. Moreover, higher thermal energy could be required to disrupt the large whey protein matrix in the quickly dried film, resulting in greater ΔH.



(a) (b)

Figure 4.8 Microstructure of konjac glucomannan (KGM) and whey protein isolate (WPI) blend film with 0.4 g KGM, 3.8 g WPI and 1.5 g glycerol in 100 g casting solution, dried with (a) slow drying rate (velocity 0.92 ± 0.15 m/s and 18% RH, 15 h drying time) and (b) fast drying rate (velocity 3.04 ± 0.67 m/s and 19% RH, 3 h drying time) obtained from trinocular phase contrast microscope using 1000X magnification. Blue green and gray area indicated the presence of WPI clusters.

4.3 Effect of storage temperatures on properties of konjac glucomannan-whey protein isolate blend films

4.3.1 Film properties measurements

Color

Changes of total color difference of KGM-WPI film during storage at three simulated temperatures are shown in Table 4.9. Over time, there was no significant increases in ΔE of blend film at all controlled storage temperatures (p>0.05). Although, ΔE of film sample stored at 35 °C tended to increase at a higher rate than those stored at 4 and 25 °C.

Table 4.9 Effect of storage temperatures on total color difference of konjac glucomannan (KGM) and whey protein isolate (WPI) blend film with 0.4 g KGM, 3.8 g WPI and 1.5 g glycerol in 100 g casting solution.

Storage Time (Days)	Total color difference at different Storage Temperature		
	4 °C[ns]	25 °C[ns]	35 °C[ns]
3	-	-	-
10	0.777 ± 0.15	0.720 ± 0.22	1.073 ± 0.36
17	0.787 ± 0.09	0.920 ± 0.45	1.560 ± 0.49
24	0.933 ± 0.25	1.167 ± 1.13	1.703 ± 0.33

* Values are the averages ± standard deviations.

** ns indicates no significant differences within the same column (p>0.05).

The blend film became darker over time and/or accelerated with elevated temperature. The discoloration of films has been associated with the

Maillard reactions (Kim et al., 2002b; Miller, Chiang, & Krochta, 1997; Trezza & Krochta, 2000). This finding is also consistent with previous research reported that ΔE of heat cured soy protein isolate film increased corresponding to increasing temperature (Kim et al., 2002b). Thus, the result indicated that KGM-WPI film had good color storage stability at 4, 25 and 35 °C of more than 24 days.

Transparency

Effect of storage time and temperatures on transparency of KGM-WPI film is shown in Figure 4.9. The transparency of blend film stored at all commercial simulated temperatures significantly decreased over time ($p \leq 0.05$). It was hypothesized that there might be slow molecular rearrangement, mobility or interaction of the incompatible biopolymers over storage time. The decreased transparency of blend film during storage could also be attributable to the increase of end products of non-enzymatic reaction which altered the overall transparency of the films over time.

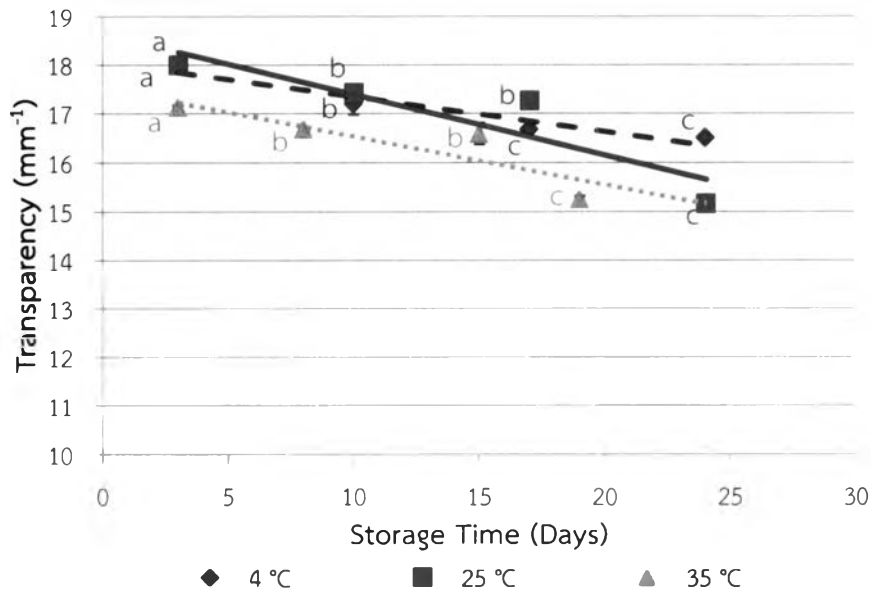


Figure 4.9 Effect of storage temperatures on transparency of konjac glucomannan (KGM) and whey protein isolate (WPI) blend film with 0.4 g KGM, 3.8 g WPI and 1.5 g glycerol in 100 g casting solution. Error bar shows standard deviation. Bars with different letters are significantly different at $p \leq 0.05$.

Mechanical properties

The effect of storage temperatures on mechanical properties of KGM-WPI film are shown in Figure 4.10. TS of blend film did not significantly change over storage time at all stored temperatures ($p>0.05$). Elongation of KGM-WPI film significantly decreased with increasing storage time at all controlled temperatures ($p \leq 0.05$). Storage at higher temperatures, 25 and 35 °C, tended to result in lower %E compared to those at 4 °C, at the same storage time. Elastic modulus of the blend film was found significantly increased with either increasing time or temperature ($p \leq 0.05$). Storage at higher temperature, 25 and 35 °C, tended to result in higher EM compared to those at 4 °C, at the same storage time. Although the changes in TS as a result of storage time and temperature were less pronounced than the %E and EM, improved mechanical properties were expected gradually over storage, especially at higher storage temperatures.

Miller et al. (1997) reported that heat curing may elicit additional cross-linking of protein, yielding increased TS and decreased %E. They also reported that increased cure temperature and reduce %RH accelerated the progress. Similar findings were also reported by Amin and Ustunol (2007); Simelane and Ustunol (2005). Improved mechanical strength was hypothesized to be due to the formation of covalent bonds between protein chains at elevated temperatures (Amin & Ustunol, 2007; Miller et al., 1997). Water evaporation during heat curing at lower %RH was also hypothesized to enhance interactions between polymer chains. Furthermore, dehydration of the film increased TS and EM while decreased %E because water, which is known to have a plasticizing effect on protein films, is reduced. The effect of heat curing on mechanical properties of soy protein isolate films was also reported (Gennadios et al., 1996). In this experiment, the mechanical changes followed the similar trends but to the lesser extent. This is due to the fact that commercial simulated temperatures used in current study are lower than heat curing temperatures (80-95 °C). Furthermore, the %RH in this study was controlled at 50 % to minimize moisture absorption or desorption during storage.

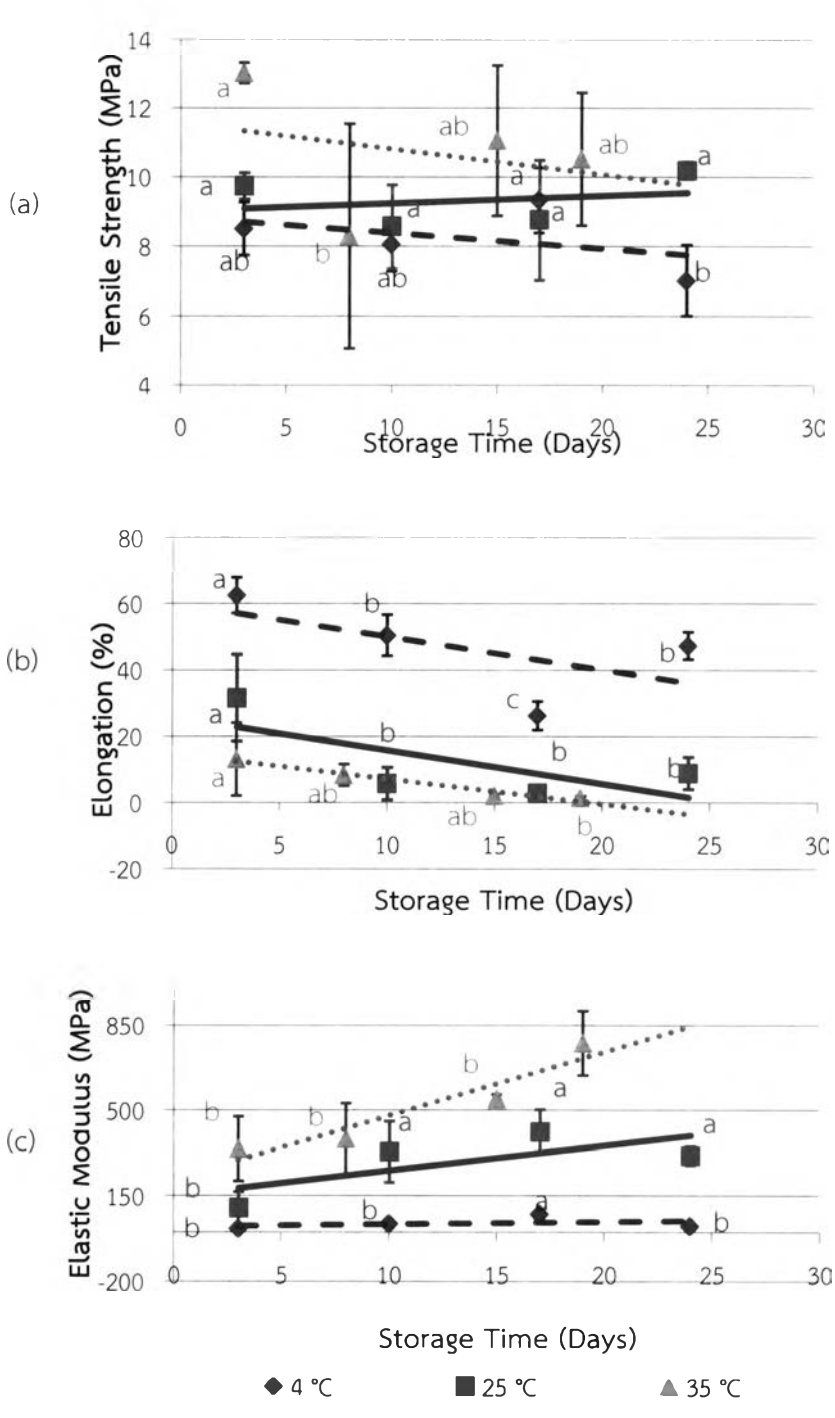


Figure 4.10 Effect of storage temperatures on mechanical properties; (a) tensile strength, (b) %elongation and (c) elastic modulus of konjac glucomannan (KGM) and whey protein isolate (WPI) blend film with 0.4 g KGM, 3.8 g WPI and 1.5 g glycerol in 100 g casting solution. Error bar shows standard deviation. Bars with different letters are significantly different at $p \leq 0.05$.

Solubility

Changes of solubility of film as a result of storage temperatures and time are shown in Table 4.10. The result of current storage stability test showed that film solubility did not significantly change over time ($p>0.05$). However, the values tended to decrease over time at higher temperatures. The decreased solubility of protein films as a result of formation of disulfide and hydrogen bonds during the heat curing process was reported (Amin & Ustunol, 2007; Gennadios et al., 1996). As previously mentioned, such molecular interactions happened to the lesser extent in this study due to lower temperature ranges and controlled %RH.

Table 4.10 Effect of storage temperatures on solubility of konjac glucomannan (KGM) and whey protein isolate (WPI) blend film with 0.4 g KGM, 3.8 g WPI and 1.5 g glycerol in 100 g casting solution.

Storage Time (Days)	Solubility (%) at different Storage Temperature		
	4 °C	25 °C^{ns}	35 °C^{ns}
3	38.67^{ab} ± 1.74	37.84 ± 3.30	34.90 ± 6.05
10	40.60^{a} ± 4.03	44.60 ± 5.06	23.91 ± 0.93
17	40.85^{ab} ± 2.79	36.02 ± 2.61	30.31 ± 15.73
24	41.63^{a} ± 4.95	30.40 ± 8.01	21.80 ± 4.07

* Values are the averages ± standard deviations.

** Different superscripts (a–b) indicate significant differences within the same column ($p\leq0.05$).

*** ns indicates no significant differences within the same column ($p>0.05$).

Water vapor permeability (WVP)

Table 4.11 shows the effect of storage temperature on WVP of KGM-WPI film. Although WVP seemed to decrease during storage, especially WVP of film stored at 35 °C, there was no significant effect of storage time on WVP ($p>0.05$).

Improved water vapor barrier of protein films as a result of heat-induced intra and intermolecular crosslinks in protein film matrix over storing at elevated temperatures were reported (Gennadios et al., 1996; Kim et al., 2002b; Miller et al., 1997). However, the decrease in WVP as a result of storage temperature was less pronounced in this current study, consistent with the result of other properties.

From the storage stability study, KGM-WPI blend film was stable under simulated commercial temperatures of 4, 25 and 35 °C for 24 days indicating potential use for further applications

Table 4.11 Effect of storage temperatures on water vapor permeabilities of konjac glucomannan (KGM) and whey protein isolate (WPI) blend film with 0.4 g KGM, 3.8 g WPI and 1.5 g glycerol in 100 g casting solution.

Storage Time (Days)	WVP (g-mm/kPa-h-m^2) at different Storage Temperature		
	4 °C ^{ns}	25 °C ^{ns}	35 °C ^{ns}
3	1.86 ± 0.30	1.89 ± 0.54	2.79 ± 0.98
10	1.76 ± 0.28	1.91 ± 0.48	2.16 ± 0.37
17	1.59 ± 0.21	1.86 ± 0.36	1.92 ± 0.35
24	2.00 ± 0.17	1.55 ± 0.21	1.81 ± 0.07

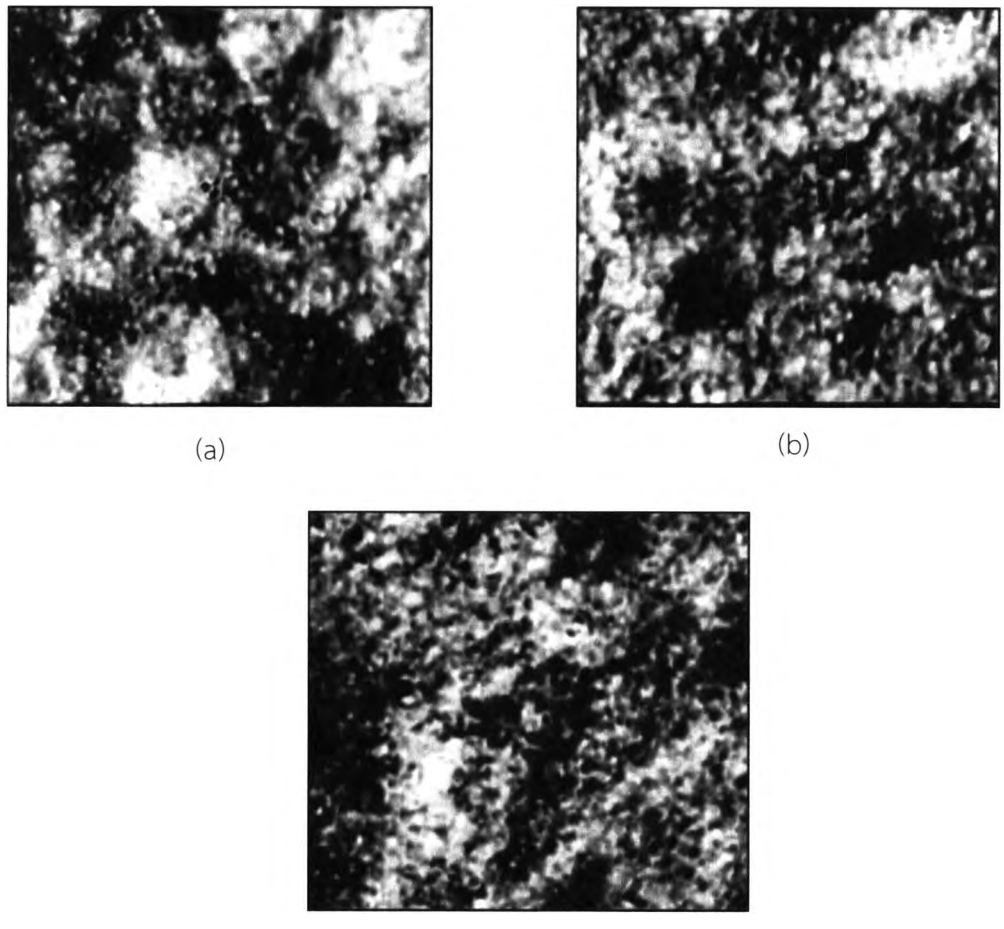
* Values are the averages ± standard deviations.

** ns indicates no significant differences within the same column ($p>0.05$).

Trinocular phase contrast microscope

The microstructure of blend films stored at three different temperatures for 24 days is shown in Figure 4.11. The results showed that the microstructure of all aged films were relatively similar to that of the freshly prepared blend films. The result supported the hypothesis described in the previous parts.

Commonly, heat curing could enhance the interactions between polymer chains; however, the simulated temperatures used in this study are lower than heat curing temperatures (80-95 °C) (Gennadios et al., 1996). Therefore, the microstructure of the films did not apparently change during storage.



(a) (b)

(c)

Figure 4.11 Microstructure of konjac glucomannan (KGM) and whey protein isolate (WPI) blend film with 0.4 g KGM, 3.8 g WPI and 1.5 g glycerol in 100 g casting solution, stored for 24 days at (a) 4 °C, (b) 25 °C and (c) 35 °C obtained from trinocular phase contrast microscope using 1000X magnification. Blue green and gray area indicated the presence of WPI clusters.