

Effect of storage temperature and time on stability of poly(lactide)-whey protein isolate laminated films

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ABSTRACT: The stability of biopolymeric multilayer film fabricated from poly(lactide) (PLA) and glycerol-plasticized whey protein isolate (WPI), PLA/WPI/PLA, at 4 °C, 25 °C, and 35 °C were examined. The PLA/WPI/PLA film showed small rates of decreasing in transparency and increasing total color difference. Storage at 35 °C caused the film to become stronger and less extendible. The multilayer structures showed lower tensile strength and higher elongation over storage at 4 °C and 25 °C. Oxygen and water vapor barrier abilities of PLA/WPI/PLA gradually improved over time, especially at 35 °C. The changes in properties of laminate structure can be empirically fitted with either zero- or first-order reaction kinetics, with overall $R^2 \geq 0.90$. The results suggested that PLA/WPI/PLA film could be stored at 4–35 °C, 50–59% RH, for extended period of inventory time. © 2016 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* 2016, 133, 43547.

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INTRODUCTION

Food industry has been under growing pressure to feed exponentially increasing world population and challenge to meet rigorous consumer's demand for safety and sustainability, organic and wholesome product with "clean" label. The requirement of barrier abilities containing packaging material has become an attention for reducing the food deterioration causing factors. To further complicate the research and development in food packaging, ecological consciousness of limited natural resources and the environmental impact of packaging waste triggered the use of renewable bio-based packaging materials as a "green" alternative.^{1–3}

Oxygen barrier of packaging material is one of the most essential elements when it comes to extending the shelf life of food products. To minimize oxygen permeation, single-layer plastics are, for the most part, insufficient for many food applications. Multilayer plastic films/sheets are fabricated by coating, making laminates, and coextrusion to improve properties, especially barrier abilities.⁴ These conventional multilayer flexible packaging are not only made from nonrenewable petroleum-based materials but also practically not reusable or recyclable.

Although most plastic packaging materials are recyclable, the complicated layer identification and separation of multilayer structures to achieve high purity, necessary for recycling process, are not economically feasible.

A novel multilayer barrier structure made entirely from environmentally friendly biopolymers was developed by employing the mechanical strength and moisture barrier of poly(lactide) (PLA) films and the oxygen barrier enhancement of whey protein isolate (WPI) film; PLA/WPI/PLA, aiming to minimize waste management and environmental problem.⁵ WPI is derived from a by-product or waste from cheese production. By employing WPI films with inherent odorless, transparent, and glossy properties, the developed multilayer also possess these desirable properties. Edible films from whey protein were chosen owing to its excellent oxygen barrier which reported to be comparable to those of polyvinylidene chloride or ethylene vinyl alcohol at low relative humidity (RH) conditions.^{6,7} The globular whey protein unfolds during coacervation and promotes combinations of intermolecular hydrophobic interactions as well as hydrogen and disulfide bonds upon solvent evaporation. Such cohesive forces in protein matrices contribute to the oxygen barrier ability. However, WPI films are sensitive to water because of

their hydrophilic character.⁸ In order to maintain such low oxygen permeability, WPI film needs moisture protection from ambient environment by being sandwiched in between outer PLA layers. PLA is a thermoplastic produced from bio-based materials expecting to be a replacement material of polyethylene terephthalate (PET).^{9–11}

One of the most challenging obstacles restricting commercial applications of bio-based packaging materials is the limited knowledge on the storage stability. In general, biopolymers are inherently biodegradable, thus physical and climatic conditions may play important roles on the packaging properties and performance of bio-based films which originate from food elements. The critical storage temperature of the composite film was limited by the glass transition temperature of PLA. For this reason, the stability of PLA/WPI/PLA film must be studied in order to predict the changes of properties during inventory storage prior to being used to function as food packaging.

It was hypothesized that the developed multilayer PLA/WPI/PLA film has reasonable storage stability in simulated commercial storage temperatures. The objectives of this study were to examine effect of storage temperature on the storage stability of a three-layer oxygen barrier film structure constituted entirely from biopolymeric materials; PLA/WPI/PLA film.

MATERIALS AND METHODS

Materials

Whey protein coating and film-forming solutions were prepared using WPI (97.0% dry basis protein, BiPRO, Davisco Food Intl., Le Sueur, MN, USA), plasticized with 99.5% purity glycerol (GLY) (QReCTM, QReC Chemical, Chonburi, Thailand). The PLA resin (IngeoTM Biopolymer 2003D, Natureworks LLC., Minnetonka, MN, USA) was dried at 80 °C for 4 h prior to extrusion. The PLA resin has a melt flow rate at 6 g/10 min (210 °C, 2.16 kg). Dried PLA resins were extruded through a single-screw extruder which was attached to a blown-film die (Blow film line 180/400E, Dr. Collin GmbH, Ebersberg, Hesse, Germany), with a temperature profile of 220–223 °C and a screw speed of 70 rpm to fabricate PLA film samples. Corona discharge treatment (20 kV, 20 kHz) was performed on PLA film surface using corona treater (IN TEN, Taipei, Taiwan), equipped with four rolls of 45 cm × 0.2 cm electrodes at a speed of 5 m/min. The surface modification of PLA film was done to achieve a surface energy of > 40 mN/m.

Film Formation and Storage

PLA/WPI/PLA film was prepared according to the method of Phupoksakul *et al.*⁵ Briefly, single-layer WPI film was prepared from 10% (w/w) heat-denatured WPI solution containing GLY as a plasticizer to improve film flexibility at WPI:GLY ratio of 1:0.40 (29% dry basis). The film forming solution containing 5 g of total solid was poured onto 15 cm × 30 cm acrylic plates, and dried at 50 °C for 15 h in a tray dryer (Contherm Thermotec 2000 Oven, Contherm Scientific, Lower Hutt, New Zealand). To compose PLA/WPI/PLA film, 1.5 mL of WPI coating solution was applied and spread evenly on surface-treated PLA base film (7.5 cm × 15 cm) before being covered by the top PLA layer. The films were then dried at ambient tempera-

ture (23–25 °C and 35–45% RH). Single-layer PLA was used for comparison. A total of three replications of film preparation were used to determine each property.

To study the effect of storage temperature on stability of PLA/WPI/PLA film, testing films were stored in desiccators containing saturated solution of magnesium nitrate (QReC Chemical, Chonburi, Thailand). The desiccators were placed in incubators providing three different controlled temperatures simulating common commercial storage conditions; chilling at 4 °C, ambient at 25 °C and tropical ambient 35 °C for 21 days. Equilibrium relative humidity provided by the saturated solution at 4 °C, 25 °C, and 35 °C was approximately 59%, 53%, and 50%, respectively.¹²

Film Properties Measurement

Film thickness was measured by digital micrometer (Model ID-C112, Mitutoyo Manufacturing, Kanagawa, Japan). Film transparency was determined according to standard method ASTM D1746-97,¹³ using UV-visible spectrophotometer (Model Genesys 10, Thermo Fisher Scientific, Rochester, NY, USA) to measure the percentage of light transmission (%*T*) through the films at 600 nm. Transparency at 600 nm (*T*₆₀₀) was calculated from the below equation,¹⁴ where *b* is the film thickness:

$$T = (\log \%T) / b \quad (1)$$

A Chroma meter (Model CR-400, Minolta, Tokyo, Japan) was used to evaluate the color of the composite film in CIELAB system. The total color difference (ΔE_{ab}) was calculated using the following equation.¹⁵ The results were expressed as ΔE_{ab} values calculated by using the one-day-old sample as a reference:

$$\Delta E = \sqrt{\Delta L^*2 + \Delta a^*2 + \Delta b^*2} \quad (\text{Eq. 2})$$

Mechanical properties of the films, tensile strength (TS), percentage of elongation (%*E*), and elastic modulus (EM), were measured using Instron universal testing machine (Model 5565, Instron Engineering, Canton, MA, USA) equipped with a 5 kg load cell, according to standard method ASTM D882-90.¹⁶ Rectangular film strip (1 cm × 12 cm) was used for the measurement. Initial grip separation and cross-head speed were set at 70 mm and 50 mm/min, respectively.

Oxygen transmission rate (OTR) was measured according to standard method ASTM D3985-05.¹⁷ OTRs were determined using Ox-Tran 2/21 MD modular system (MOCON, Minneapolis, MN, USA) at 23 °C and 0% RH. Water vapor transmission rate (WVTR) was determined according to ASTM E398-0324¹⁸ using a PERMATRAN-W model 398 (MOCON). Testing was performed at 23 °C and at a 90% to 10% RH gradient. Oxygen permeability (OP) and water vapor permeability (WVP) were calculated by dividing the corresponding transmission rate by oxygen partial pressure and water vapor pressure, respectively, and multiplying by the film thickness.

Additional details on each property determination were listed in Phupoksakul *et al.*⁵

Statistical Analysis

Completely randomized design was used in this experiment. All tests were replicated three times, except OP test which was

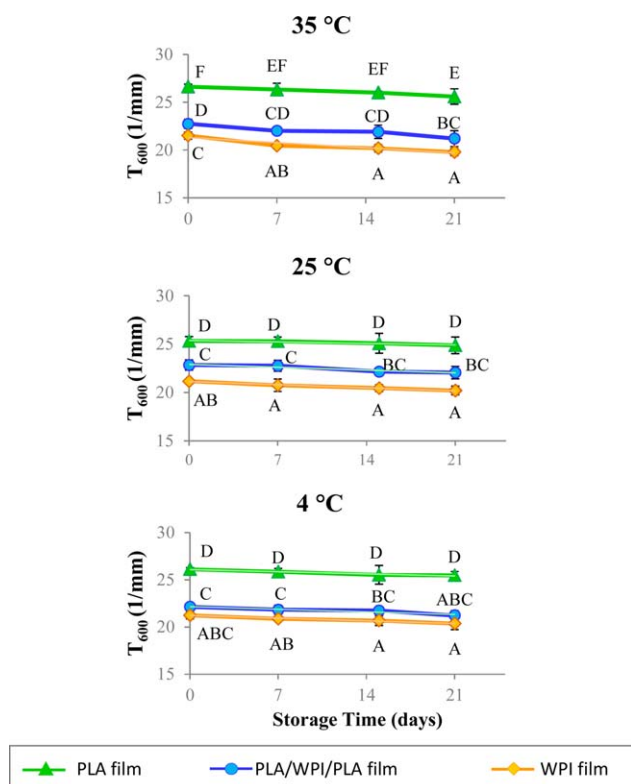


Figure 1. Effect of time and temperature on transparency at 600 nm (T_{600}) of single-layer poly(lactic acid) (PLA) film, whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA), and glycerol-plasticized WPI single-cast film during storage for 21 days. The equilibrium relative humidity at 4 °C, 25 °C, and 35 °C was approximately 59%, 53%, and 50%, respectively. *Each data point represents average value. Error bars show standard deviations. **Different upper-case letters (A–F) indicate significant differences between all samples among the same storage temperature ($P \leq 0.05$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

duplicated. Analysis of variance (ANOVA) and Duncan's new multiple comparison range test were utilized at a confidence level of 95%.

RESULTS AND DISCUSSION

Film Formation

The thicknesses of PLA, PLA/WPI/PLA, and WPI films were 0.076 ± 0.002 , 0.087 ± 0.002 , and 0.088 ± 0.001 mm, respectively. The laminate structure was visually glossy, transparent and colorless, of which the thicknesses of PLA and WPI layers are 0.036 ± 0.004 and 0.009 ± 0.004 mm, respectively.

Transparency

The effect of time and temperature on T_{600} of single substrate PLA, composite PLA/WPI/PLA and WPI films are shown in Figure 1. The result showed that T_{600} of PLA was stable over 21 days at all storage temperatures. Although, PLA biopolymer decomposed via hydrolytic degradation into smaller polymer molecules/fragments, and finally lactic acid, which could later be consumed by microorganisms as nutrients into carbon dioxide, water, and biomass, the degradation process was tempera-

ture and humidity dependent.¹⁹ High molecular weight PLA plastic films were reported to have good storability at 28 °C, 40 °C, and 55 °C at 50% RH.²⁰

On the other hand, T_{600} of WPI film slowly decreased at 25 °C and 35 °C over 21 days of storage. This was probably because of Maillard reaction of whey proteins and small amount of left over lactose in WPI films creating small molecular products that alternate T_{600} of WPI film. The yellowing of WPI films at 23 °C, 40 °C, and 55 °C at 75% RH were reported to be caused by Maillard reaction.²¹ Slow molecular rearrangement over storage time was hypothesized to also happen over time. GLY, used to plasticize WPI film, could migrate from the bulk of the film matrix to the surface because of weak interaction between protein molecules and GLY. Park *et al.* reported the visible change of cast zein film from initially transparent to appear greasy and cloudy because of glycerol sweating out.²²

T_{600} of composite PLA/WPI/PLA gradually decreased at 35 °C over storage time. As expected, composite structure had transparency stability in between its parental materials. These decreasing trends in T_{600} of all films could be fitted by either zero-order or first-order reaction models (with $R^2 \geq 0.90$). The conservative zero-order equations are as shown in Table I. The rate constants were temperature dependent. However, changes of film transparency did not follow Arrhenius behavior. Overall, all of the films had rather small rates of change in transparency at these testing conditions suggesting that these biopolymer films had a good visual stability during storage prior to being used as food packaging.

Color

Figure 2 shows the effect of storage time and temperature on ΔE_{ab} of single PLA, WPI, and their composite structure; PLA/WPI/PLA films. The result showed that ΔE_{ab} of all films significantly increased over storage time at all storage temperatures ($P \leq 0.05$). The color of WPI changed at a highest rate followed by PLA/WPI/PLA and PLA, respectively. During the storage, the discoloration of WPI film occurred. The film became darker, redder, and yellower as evidenced by the decrease of L^* value ($L^* = 0$ yield black and $L^* = 100$ indicates diffuse white) and the increases of a^* (negative values indicate green while positive values indicate magenta) and b^* (negative values indicate blue and positive values indicate yellow) value (data not shown).

The color change of WPI film (water activity; $a_w = 0.593 \pm 0.003$ to 0.647 ± 0.002) was hypothesized to be mainly by nonenzymatic Maillard browning of whey proteins and trace reducing sugar which could slowly occur during storage even at room temperature.^{23,24} Trezza and Krochta²¹ reported that activation energy for the yellowing of WPI films were similar to those previously reported for the browning of whey powder by Labuza and Saltmarch (1982).²⁵ Lin and Krochta (2003)²⁶ reported that when sucrose was used as plasticizer, the yellow index value became significantly higher than GLY-plasticized-WPI film during storage at 25 °C, 35% RH ($P \leq 0.05$).

These increasing trends of ΔE_{ab} of all films could be fitted by either zero-order (with $R^2 \geq 0.95$) or first-order reaction models

Table I. Linear-Model Kinetics Parameters for T_{600} : Transparency at 600 nm (1/mm), Total Color Difference (ΔE_{ab}) Changes of Single-Layer Polylactic Acid (PLA) Film, Whey Protein Isolate (WPI)-Enhanced Composite Structure (PLA/WPI/PLA), and Glycerol-Plasticized WPI Single Cast Film Over Time (t) During Controlled Storage 59%, 53%, and 50% Relative Humidity at 4 °C, 25 °C, and 35 °C for 21 Days, Respectively

Storage temperature (°C)	Film	Transparency	R^2	Total color difference	R^2
35	PLA	$T_{600} = -0.0481t + 26.658$	0.99	$\Delta E_{ab} = 0.0125t + 0.0429$	0.96
	PLA/WPI/PLA	$T_{600} = -0.0656t + 22.667$	0.91	$\Delta E_{ab} = 0.0138t + 0.1166$	0.97
	WPI	$T_{600} = -0.0762t + 21.302$	0.90	$\Delta E_{ab} = 0.0341t + 0.1272$	0.99
25	PLA	$T_{600} = -0.0220t + 25.381$	0.92	$\Delta E_{ab} = 0.0064t + 0.0497$	0.99
	PLA/WPI/PLA	$T_{600} = -0.0418t + 22.908$	0.92	$\Delta E_{ab} = 0.0101t + 0.0977$	1.00
	WPI	$T_{600} = -0.0449t + 21.126$	0.99	$\Delta E_{ab} = 0.0109t + 0.1578$	0.99
4	PLA	$T_{600} = -0.0321t + 26.085$	0.96	$\Delta E_{ab} = 0.0060t + 0.0439$	0.99
	PLA/WPI/PLA	$T_{600} = -0.0389t + 22.153$	0.90	$\Delta E_{ab} = 0.0092t + 0.0722$	0.95
	WPI	$T_{600} = -0.0395t + 21.213$	0.98	$\Delta E_{ab} = 0.0128t + 0.1053$	1.00

(with $R^2 \geq 0.90$). The zero-order equations are shown in Table I. The color change of WPI film was found to be temperature dependent corresponding to the previous report that browning of WPI film occurred at higher rates at elevated temperatures.²¹ However, the increasing ΔE_{ab} of WPI film did not follow Arrhenius equation. This was probably because of the other co-existing degradation reactions such as lipid oxidation of the residual milk fat (less than 5% dry basis in WPI powder) or degradation of proteins which in turn altering the total color change of WPI films at higher temperatures.²¹

Although the changes were significant ($P \leq 0.05$), ΔE_{ab} of all films were well below 1 at the end of 21 days. Such color changes at all storage temperatures, during 3 weeks of storage, were much lower than threshold detectable by human eye ($\Delta E_{ab} > 3$)^{27,28} indicating that these stored films were not visibly different compared to the freshly prepared samples. The estimated detectable color change by human eye of WPI, PLA, and PLA/WPI/PLA stored at the most influential temperature (35 °C), were 84, 237, and 209 days, respectively.

Mechanical Properties

The effect of time and temperature on mechanical properties including TS, EM, and %E of single-substrate PLA, single-cast WPI and composite PLA/WPI/PLA films are investigated (Figure 3). The results showed that mechanical properties of all biopolymeric films gradually changed as a function of storage time and temperature. Mechanical properties of WPI film were found to be the most sensitive to storage temperature followed by PLA/WPI/PLA and PLA films, respectively.

Depending on the storage temperature, biopolymer films behaved differently. Figure 3 showed that there were decreases in TS and EM and increase in %E of all testing films during storage at 4 °C and 25 °C, 59% and 53% RH, respectively. Although such changes were statistically significant ($P \leq 0.05$), the rates of changes were minute (Table II). Osés *et al.* reported that there were no significant changes in TS, EM, and %E of GLY-plasticized WPI film kept at room temperature, 50% and 75% RH as long as 180 days.²⁹ Gennadios *et al.* reported that effect of temperature on storage (23–75% RH, 5–45 °C) corn

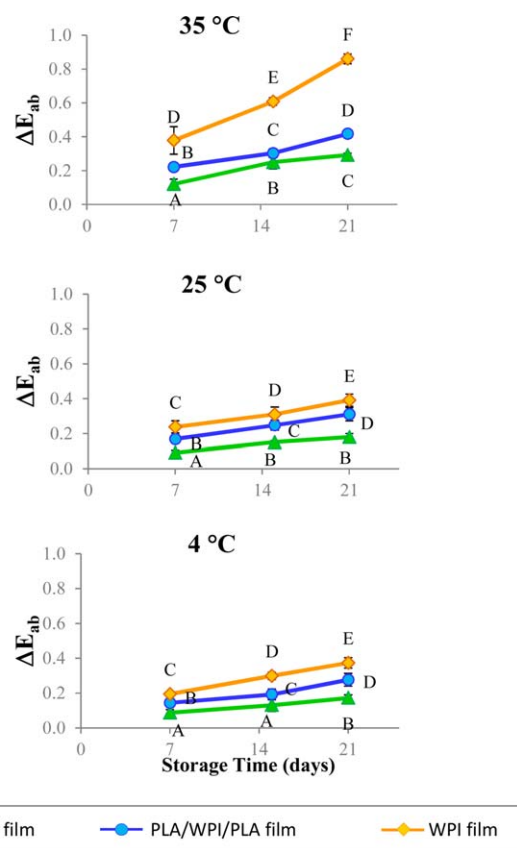


Figure 2. Effect of time and temperature on total color difference (ΔE_{ab}) of single-layer polylactic acid (PLA) film, whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA), and glycerol-plasticized WPI single cast film during storage for 21 days. The equilibrium relative humidity at 4 °C, 25 °C and 35 °C was approximately 59%, 53%, and 50%, respectively. *Each data point represents average value. Error bars show standard deviations. **Different upper-case letters (A–F) indicate significant differences between all samples among the same storage temperature ($P \leq 0.05$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

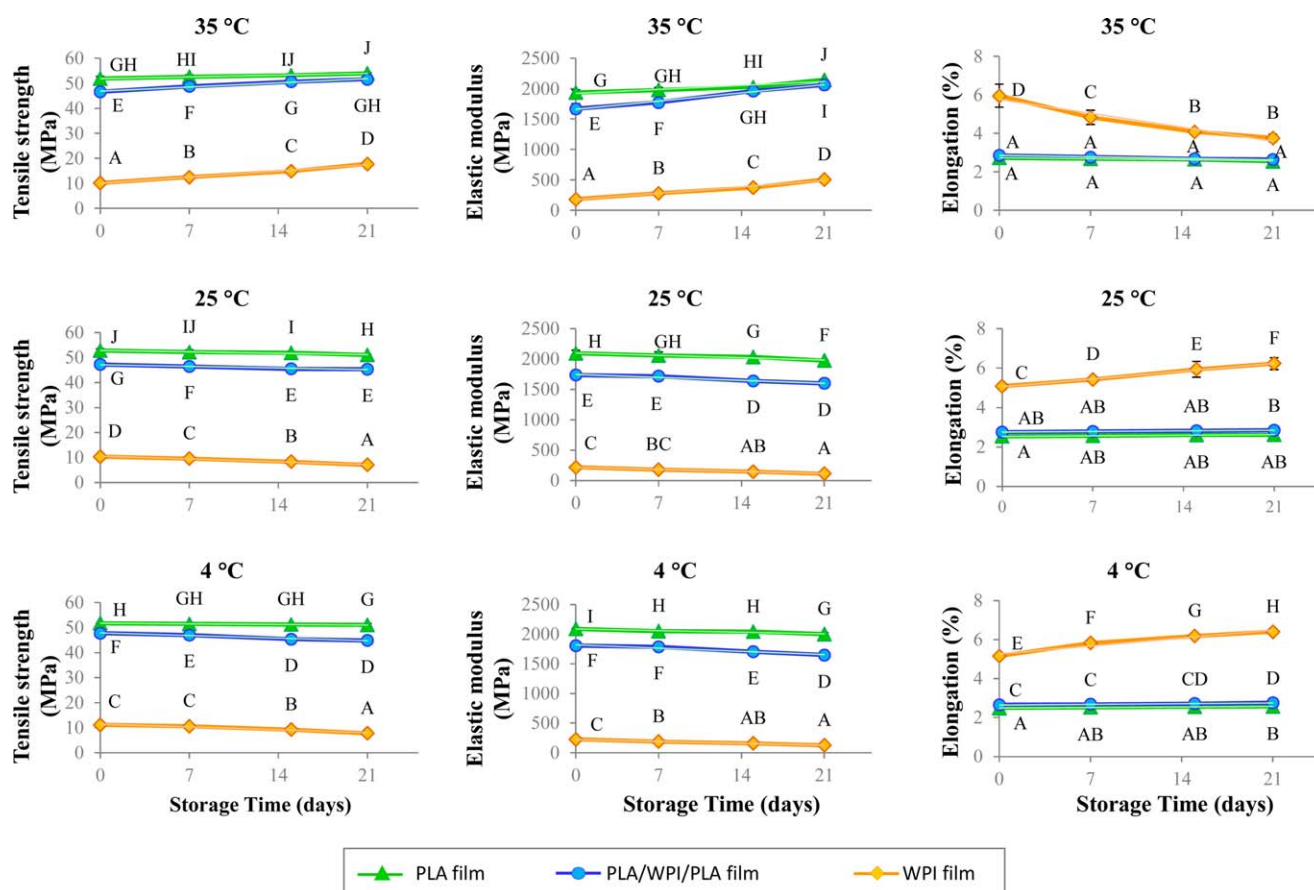


Figure 3. Effect of time and temperature on tensile strength, elastic modulus, and percentage of elongation of single-layer poly(lactic acid) (PLA) film, whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA), and glycerol-plasticized WPI single cast film during storage for 21 days. The equilibrium relative humidity at 4 °C, 25 °C, and 35 °C was approximately 59%, 53%, and 50%, respectively. *Each data point represents average value. Error bars show standard deviations. **Different upper-case letters (A–J) indicate significant differences between all samples among the same storage temperature ($P \leq 0.05$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

zein, wheat gluten, methylcellulose, and hydroxypropyl cellulose films was opposite to that of %RH. The effect of temperature on tensile properties was attributed to film moisture content.³⁰ At constant %RH storage, the amount of water absorbed by food materials typically decreases with an increase in temperature.^{31,32} Thus, more water was absorbed at lower temperature. The increasing water content in protein-based film matrix could plasticize and cause weakening of protein-network. On the contrary, Anker *et al.* reported that their GLY-plasticized WPI film became stiffer and less extendable as a result of storage in climate room at 23 °C, 50% RH for 120 days.³³ The authors explained that the changes in mechanical properties were a result of moisture loss and migration of plasticizer.

On the other hand, TS and EM of all films increased with decreased %E during storage at 35 °C, 50% RH. The changes of mechanical properties over time seemed to be related to the change in polymer structures. WPI film was reported to become stronger and less extendible as a result of heat curing which additional protein chain cross-linking occurred. The increase in heat-curing temperature was reported to increase the formation

of covalent bonds between protein chains and as water evaporated, closer interaction occurs between the protein chains, resulting in increased TS.³⁴ In addition, the similar mechanical properties improvement of protein-based films as effect of heat-curing was reported by many researchers.^{34–37}

These modifications in mechanical properties of all films, at each storage temperature, can be fitted by either zero-order (with $R^2 \geq 0.90$) or first-order reaction models (with $R^2 \geq 0.85$). The zero-order equations are shown in Table II. These temperature-dependent behaviors did not follow Arrhenius relationship.

From the result, it can be suggested that PLA, PLA/WPI/PLA, and WPI can be stored and used to package dry to intermediate moisture food properly at 35 °C, 50% RH, for extended period of inventory or storage time. However, low to ambient commercial storage temperature may induce the mechanical weakness of the films resulting in the minimized protective function of packaging. To maintain integrity of packaging fabricated from these films, shorter storage time is suggested at 4 °C and 25 °C, 59% and 53% RH, respectively.

Table II. Linear-Model Kinetics Parameters for TS: Tensile Strength (MPa), EM: Elastic Modulus (MPa) and %E: Percentage of Elongation (%) Changes of Single-Layer Polylactic Acid (PLA) Film, Whey Protein Isolate (WPI)-Enhanced Composite Structure (PLA/WPI/PLA) and Glycerol-Plasticized WPI Single Cast Film Over Time (*t*) During Controlled Storage 59%, 53%, and 50% Relative Humidity at 4 °C, 25 °C, and 35 °C for 21 Days, Respectively

Storage temperature (°C)	Film	Tensile strength	R^2	Elastic modulus	R^2	% Elongation	R^2
35	PLA	$TS = 0.0968t + 51.886$	0.99	$EM = 9.2772t + 1922.5$	0.90	$\%E = -0.0078t + 2.7375$	0.90
	PLA/WPI/PLA	$TS = 0.2395t + 46.857$	0.98	$EM = 19.334t + 1659.8$	0.99	$\%E = -0.0104t + 2.8477$	0.98
	WPI	$TS = 0.3525t + 10.007$	0.99	$EM = 14.897t + 175.29$	0.98	$\%E = -0.1035t + 5.7683$	0.96
25	PLA	$TS = -0.0767t + 52.758$	0.95	$EM = -5.3568t + 2097.2$	0.95	$\%E = 0.0044t + 2.5355$	0.96
	PLA/WPI/PLA	$TS = -0.0938t + 47.107$	0.96	$EM = -6.9009t + 1747.3$	0.96	$\%E = 0.0045t + 2.7550$	1.00
	WPI	$TS = -0.1560t + 10.509$	0.98	$EM = -4.8782t + 217.90$	1.00	$\%E = 0.0560t + 5.0629$	1.00
4	PLA	$TS = -0.0327t + 51.745$	0.97	$EM = -3.7522t + 2085.8$	0.94	$\%E = 0.0039t + 2.4963$	0.95
	PLA/WPI/PLA	$TS = -0.1476t + 47.859$	0.98	$EM = -7.8739t + 1819.6$	0.96	$\%E = 0.0050t + 2.6498$	0.98
	WPI	$TS = -0.1596t + 11.389$	0.96	$EM = -4.6139t + 222.90$	1.00	$\%E = 0.0576t + 5.2737$	0.95

Oxygen Permeability

The values of OP of the PLA, WPI, and PLA/WPI/PLA films as a function of storage time and temperature are shown in Figure 4. The result showed that all of the testing films were able to maintain their oxygen barrier properties at 4 °C and 25 °C, 59% and 53% RH, respectively, throughout the storage time. At 35 °C, OP of PLA film did not significantly change over time ($P > 0.05$). However, OPs of WPI and PLA/WPI/PLA films significantly decreased at 35 °C ($P \leq 0.05$). During the whole of storage time and temperatures, WPI film maintained the lowest OPs followed by PLA/WPI/PLA and PLA films, respectively. The improvement trend of oxygen barrier of WPI and PLA/WPI/PLA films could be fitted by either zero-order (with $R^2 \geq 0.95$) or first-order reaction models (with $R^2 \geq 0.94$). The conservative zero-order model is shown in Table III.

The oxygen barrier ability of biopolymer films improved as a function of time at 35 °C, 50% RH. It was hypothesized that storage at 35 °C, 50% RH, may elicit additional cross-linking of proteins and cause moisture loss resulting in improvement of oxygen barrier of WPI-based films. Storing films at elevated temperatures induced sulfhydryl-disulfide interchange resulting in a cross-linking and polymerization of proteins.³⁴ Although the films were stored at controlled RH (50–59%) in order to minimize moisture absorption and desorption, the moisture loss occurred as biopolymer molecules rearranged themselves to their equilibrium over time or because of intrinsic instability of their raw material.³⁸ Moisture loss of protein-based films during storage was reported even in controlled humidity environments.^{33,39} Water decreases glass transition temperature of protein-based films induced by a plasticization phenomena.³⁸ Reduction of water in film matrix decreased molecular mobility and diffusion of permeant across film matrix, thus decreased gas permeability. Extent of effect of plasticizers on barrier properties of edible films and coatings was well documented.^{40,41}

The stability of oxygen barrier ability of PLA, WPI, and PLA/WPI/PLA films suggested that these films can be used properly at commercial storage temperatures. Furthermore, the result suggested that oxygen barrier properties of WPI and PLA/WPI/PLA films can be improved further by aging at 35 °C, 50% RH.

Water Vapor Permeability

The effect of storage time and temperature on WVP of the films are illustrated in Figure 5. As expected, WPI film had significantly higher WVP during storage at all temperatures ($P \leq 0.05$). There was no significant difference between WVP of PLA and PLA/WPI/PLA films ($P > 0.05$). The result also showed that WVP of all films tended to slowly decreased over storage time at all commercial-simulated temperatures. These decreasing trends of WVP could be fitted by either zero-order (with $R^2 \geq 0.93$) or first-order reaction models (with $R^2 \geq 0.82$) at all storage temperatures. Zero-order kinetic models are shown in Table III. The improvements of water vapor barrier of WPI and PLA/WPI/PLA were found to be a function of temperature. Furthermore, these temperature-dependent WVP obeyed Arrhenius relationship. Arrhenius equations, constants, and activation energies of decreasing WVP of WPI and PLA/WPI/PLA films, shown in Table IV, are useful predictions of film WVP stability

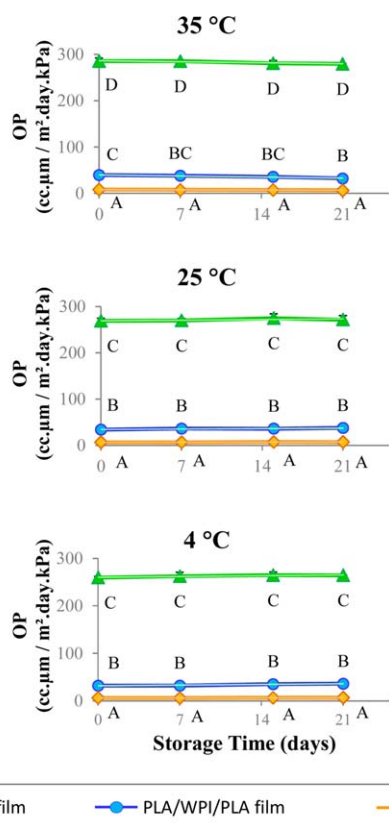


Figure 4. Effect of time and temperature on oxygen permeability (OP) of single-layer poly(lactic acid) (PLA) film, whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA), and glycerol-plasticized WPI single cast film during storage for 21 days. The equilibrium relative humidity at 4 °C, 25 °C, and 35 °C was approximately 59%, 53%, and 50%, respectively. *Each data point represents average value. Error bars show standard deviations. **Different upper-case letters (A–D) indicate significant differences between all samples among the same storage temperature ($P \leq 0.05$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

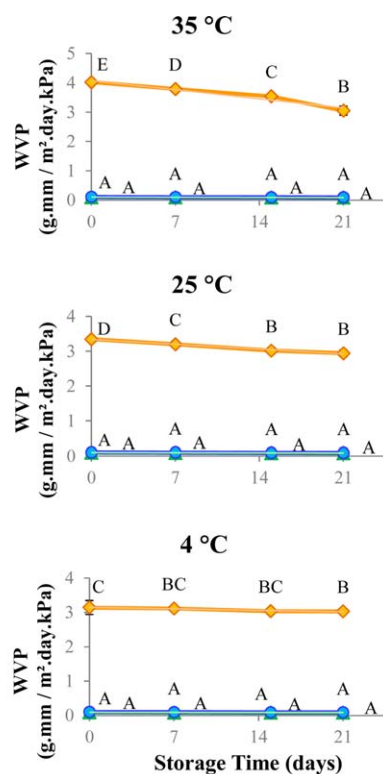


Figure 5. Effect of time and temperature on water vapor permeability (WVP) of single-layer poly(lactic acid) (PLA) film, whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA), and glycerol-plasticized WPI single cast film during storage for 21 days. The equilibrium relative humidity at 4 °C, 25 °C, and 35 °C was approximately 59%, 53%, and 50%, respectively. *Each data point represents average value. Error bars show standard deviations. **Different upper-case letters (A–E) indicate significant differences between all samples among the same storage temperature ($P \leq 0.05$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table III. Linear-Model Kinetics Parameters for OP: Oxygen Permeability ($\text{cc } \mu\text{m}^2 \text{ day kPa}$) and WVP: Water Vapor Permeability ($\text{g mm}^2 \text{ day kPa}$) Changes of Single-Layer Poly(lactic Acid) (PLA) Film, Whey Protein Isolate (WPI)-Enhanced Composite Structure (PLA/WPI/PLA), and Glycerol-Plasticized WPI Single Cast Film Over Time (t) During Controlled Storage 59%, 53%, and 50% Relative Humidity at 4 °C, 25 °C, and 35 °C for 21 days, respectively

Storage temperature (°C)	Film	Oxygen permeability	R^2	Water vapor permeability	R^2
35	PLA			$\text{WVP} = -0.0005t + 0.1006$	0.97
	PLA/WPI/PLA	$\text{OP} = -0.3321t + 39.8423$	0.95	$\text{WVP} = -0.0007t + 0.1109$	0.94
	WPI	$\text{OP} = -0.0681t + 7.9449$	0.99	$\text{WVP} = -0.0440t + 4.0726$	0.94
25	PLA			$\text{WVP} = -0.0006t + 0.0958$	0.99
	PLA/WPI/PLA			$\text{WVP} = -0.0007t + 0.1047$	0.96
	WPI			$\text{WVP} = -0.0198t + 3.3374$	0.99
4	PLA			$\text{WVP} = -0.0004t + 0.0904$	0.93
	PLA/WPI/PLA			$\text{WVP} = -0.0005t + 0.1028$	0.98
	WPI			$\text{WVP} = -0.0060t + 3.1449$	0.95

Table IV. Arrhenius Parameters for Water Vapor Permeability Changes of Single-Layer Polylactic Acid (PLA) Film, Whey Protein Isolate (WPI)-Enhanced Composite Structure (PLA/WPI/PLA), and Glycerol-Plasticized WPI Single Cast Film During Controlled Storage 59%, 53%, and 50% Relative Humidity at 4 °C, 25 °C, and 35 °C for 21 days, respectively

Kinetic model	Film	Water vapor permeability			
		$\log k = -(E/2.3RT) + \log k_0$ [$k = k_0 e^{-E/RT}$]	Arrhenius constant (k_0)	E_a (kJ/mol)	R^2
Zero-order	PLA/WPI/PLA	$y = -433.16x - 1.7298$	0.18	8282.97	0.91
	WPI	$y = -2322.9x + 6.1431$	465.49	44418.96	0.99
First-order	PLA/WPI/PLA	$y = -319.29x - 1.0947$	0.33	6105.53	0.94
	WPI	$y = -2296.6x + 5.5358$	253.61	43916.04	1.00

as a function of temperature as well as potential aging application in order to improve water vapor barrier of the films.

The improvement of vapor barrier ability of WPI as a function of increasing storage time and temperature was hypothesized to be obtained by the more stiffness of polymer chain at higher temperature. Thermal treatments could promote the intra- and intermolecular covalent cross-links formation of amino acid residues, resulting in the increasing protein hydrophobicity.^{33,35,37,42} Along with the increasing protein interactions, the decreased WVP of WPI was also hypothesized to cause by loss of water from film matrix. Anker *et al.* reported the loss of moisture content of GLY-plasticized WPI from 22% to 15% after storage in the climate room (23 °C, 50% RH) for 45 days.³³ The loss of hard-to-control water, which is known to behave as protein-based film plasticizer, increased glass transition temperature (T_g) from -56 °C to -45 °C. Similar finding in other protein-based film plasticized with GLY have also been reported.³⁹

The improvement of water vapor barrier abilities of PLA was hypothesized to cause by molecular mobility and physical aging. It has been observed that the storage of PLA at room temperature showed cold crystallization.⁴³ Hassouna *et al.* investigated the aging of PLA and Acetyl tri-*n*-butyl citrate (ATBC) systems at 20 °C (almost at T_g) for 6 months.⁴⁴ They found that T_g decreases during aging and explained that crystallization of PLA during the aging period might lead to the expulsion of the plasticizer from the crystalline lamellae and thereby to its enrichment in the amorphous phase.⁴⁴ The determination of the recovery function from the physical aging study shows the role of the plasticizer during physical aging. Even in the glassy state, the molecular mobility is accelerated by the plasticizer.⁴⁵

CONCLUSIONS

The biodegradable multilayer PLA/WPI/PLA film showed reasonable storage stability in simulated commercial storage temperatures. The composite film maintained good visual and transparency stability during storage. The mechanical properties gradually changed depending on the storage temperature. PLA/WPI/PLA films tended to increasingly gain tensile strength during storage at 35 °C, 50% RH. On the other hand, chill to ambient commercial storage temperature may induce the mechanical weakness of the films resulting in the reducing protective function of packaging. To maintain integrity of packaging fabricated

from these films, shorter storage time is suggested at 4 °C and 25 °C, 59% and 53% RH, respectively. Oxygen and water vapor barrier abilities of PLA/WPI/PLA were found to gradually improve over time, especially at 35 °C. The temperature-dependent changes in properties of laminate structure can be empirically fitted with either zero- or first-order reaction kinetics. These linear models are useful in predicting the stability and shelf life of laminating structure.

The study suggested that developed multilayer structure barrier films made entirely from sustainable PLA and WPI have potential as a “green” alternative to existing synthetic oxygen-barrier polymers to extend shelf life of oxygen-sensitive dry to intermediate moisture food properly at 4–35 °C and approximately 50–59% RH. Nevertheless, further application tests on stability and shelf life extension of oxygen-sensitive dry food must also be investigated to further develop the body of knowledge necessary in order for the food industry and the consumer to fully benefit from the application of WPI-enhanced composite structures.

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