การพัฒนาฟิล์มพอลิแล็กติกแอซิดและเวย์โปรตีนไอโซเลตเพื่อเป็นฟิล์มบรรจุภัณฑ์ชนิดใหม่ที่มีสมบัติขวางกั้นออกซิเจน

นางสาวธัญลักษณ์ ภู่พกสกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเทคโนโลยีทางอาหาร ภาควิชาเทคโนโลยีทางอาหาร
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2557

DEVELOPMENT OF POLYLACTIC ACID–WHEY PROTEIN ISOLATE FILM AS NOVEL OXYGEN BARRIER PACKAGING FILM

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Food Technology
Department of Food Technology
Faculty of Science
Chulalongkorn University
Academic Year 2014

Thesis Title	DEVELOPMENT OF POLYLACTIC ACID–WHEY PROTEIN ISOLATE FILM AS NOVEL OXYGEN BARRIER PACKAGING FILM
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ธัญลักษณ์ ภู่พกสกุล : การพัฒนาฟิล์มพอลิแล็กติกแอซิดและเวย์โปรตีนไอโซเลตเพื่อเป็นฟิล์มบรรจุภัณฑ์ชนิดใหม่ที่มีสมบัติขวางกั้นออกซิเจน (DEVELOPMENT OF POLYLACTIC ACID–WHEY PROTEIN ISOLATE FILM AS NOVEL OXYGEN BARRIER PACKAGING FILM) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: อ. ดร.ธีรนันท์ เจนจรัสสกุล, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. ดร.อนงค์นาฏ สมหวังธนโรจน์, 126 หน้า.

งานวิจัยนี้มีวัตถุประสงค์เพื่อพัฒนาฟิล์มหลายชั้นที่เป็นมิตรต่อสิ่งแวดล้อมจากพอลิเมอร์ชีวภาพโดยอาศัยความแข็งแรงเชิงกลและความสามารถในการป้องกันการซึมผ่านของความชื้นจากฟิล์มพอลิแล็กติกแอซิด (PLA) ร่วมกับความสามารถในการป้องกันการซึมผ่านของออกซิเจนจากเวย์โปรตีนไอโซเลท (WPI) ที่ใช้กลีเซอรอล (GLY) เป็นพลาสติไซเซอร์ พร้อมทั้งศึกษาผลของอัตราส่วน WPI:GLY (1:0.25, 1:0.4 และ 1:0.67) ต่อลักษณะทางกายภาพและความสามารถในการป้องกันการซึมผ่านของออกซิเจนและไอน้ำของฟิล์มสามชั้น PLA/WPI/PLA เทียบกับฟิล์มสามชั้นที่ใช้ WPI ร่วมกับฟิล์มโพลิเอทิลีนชนิดความหนาแน่นต่ำเชิงเส้นตรง (LLDPE); LLDPE/WPI/LLDPE จากการศึกษาพบว่าสามารถขึ้นรูปฟิล์มสามชั้น PLA/WPI/PLA และ LLDPE/WPI/LLDPE จากสารละลาย WPI ได้ ฟิล์มที่พัฒนาขึ้นมีลักษณะทางกายภาพที่ดี พลาสติกทั้งสองชนิดผ่านการปรับสภาพผิวด้วยการใช้ความต่างศักย์สูง (Corona discharge-treatment) ก่อนนำมาขึ้นรูปเป็นฟิล์มสามชั้นเพื่อให้มีพลังงานที่พื้นผิวเหมาะสมทำให้สามารถยึดจับกับ WPI ได้ดี ปริมาณ GLY ที่ใช้มีความเหมาะสมช่วยให้ฟิล์ม WPI ชั้นในไม่เปราะแตก ผลจากการเปรียบเทียบคุณสมบัติของฟิล์มสามชั้นกับฟิล์ม PLA หรือ LLDPE ที่เป็นโครงสร้างหลักพบว่าค่าความโปร่งใสที่ความยาวคลื่น 600 นาโนเมตร (T_{600}) ของฟิล์ม PLA/WPI/PLA และ LLDPE/WPI/LLDPE มีค่าลดลงอย่างมีนัยสำคัญ ($p \le 0.05$) เนื่องจากความไม่ต่อเนื่องกันของชั้นฟิล์ม ค่าการเปลี่ยนแปลงของสีโดยรวม (Total color difference, ΔE_{ab}) ของฟิล์มสามชั้นมีค่าต่ำกว่าระดับที่สามารถสังเกตเห็นความแตกต่างได้ด้วยตาเปล่า PLA ช่วยเพิ่มความแข็งแรงเชิงกลให้กับฟิล์มสามชั้นทำให้ฟิล์ม PLA/WPI/PLA มีความสามารถในการทนแรงดึง (Tensile strength) สูงกว่าแต่มีความสามารถในการยืดตัว (Elongation) ต่ำกว่าเมื่อเทียบกับฟิล์ม LLDPE/WPI/LLDPE ที่มีความสามารถในการยืดตัวสูงมาก ค่าการซึมผ่านของออกซิเจน (Oxygen permeability) ของฟิล์มสามชั้นลดลงอย่างมีนัยสำคัญ ($p \le 0.05$) ส่วนค่าการซึมผ่านของไอน้ำ (Water vapor permeability) นั้นมีค่าขึ้นกับชนิดของพลาสติกที่เป็นโครงสร้างหลัก จากการทดลองพบว่าสมบัติของฟิล์มสามชั้นขึ้นกับสมบัติเฉพาะตัวของพลาสติกโครงสร้างหลักมากกว่าปริมาณพลาสติไซเซอร์ที่ใช้ นอกจากนี้ยังศึกษาความเสถียรของฟิล์ม PLA/WPI/PLA ที่ใช้ GLY เป็นพลาสติไซเซอร์ที่อัตราส่วน WPI:GLY เป็น 1:0.4 เก็บรักษาที่อุณหภูมิการเก็บที่นิยมใช้ทางการค้า; 4, 25 และ 35 องศาเซลเซียส (°C) ความชื้นสัมพัทธ์ 50% เป็นระยะเวลา 21 วันเทียบกับฟิล์ม WPI และ PLA พบว่าฟิล์มหลายชั้น PLA/WPI/PLA มีลักษณะทางกายภาพและและความสามารถในการยึดเกาะกันระหว่างชั้นที่ดี ไม่สามารถสังเกตเห็นการเปลี่ยนแปลงลักษณะทางการมองเห็นของฟิล์มได้ตลอดการเก็บรักษา ค่า T_{600} ของฟิล์ม PLA/WPI/PLA มีแนวโน้มลดลงอย่างช้าๆและ ΔE_{ab} มีแนวโน้มเพิ่มขึ้นในระหว่างการเก็บ ซึ่งสมมติฐานว่ามีสาเหตุมาจากปฏิกิริยาการเกิดสีน้ำตาลแบบเมลลาร์ด (Maillard browning reaction) ของเวย์โปรตีนและน้ำตาลรีดิวซ์ที่เหลืออยู่ในชั้น WPI และการจัดเรียงตัวใหม่ของโครงสร้างโมเลกุล ส่งผลให้ความโปร่งใสและสีของฟิล์มเปลี่ยนแปลงอย่างช้าๆ การเก็บรักษาฟิล์มที่ 35 °C ทำให้ฟิล์มPLA/WPI/PLA สามารถทนต่อแรงดึงได้มากขึ้นแต่ความสามารถในการยืดตัวลดลง ซึ่งให้ผลตรงกันข้ามกับฟิล์มที่เก็บที่ 4 และ 25 °C ที่มีความแข็งแรงลดลงแต่สามารถยืดตัวได้มากขึ้น ในด้านความสามารถในการป้องกันการซึมผ่านของออกซิเจนและไอน้ำของฟิล์ม PLA/WPI/PLA นั้นมีความสามารถในการป้องกันที่ดีขึ้นอย่างช้าๆตลอดการเก็บ สังเกตจากค่าการซึมผ่านที่ลดลงโดยเฉพาะอย่างยิ่งฟิล์มที่เก็บรักษาที่ 35 °C โดยรวมแล้วสมบัติของฟิล์ม PLA/WPI/PLA มีความสอดคล้องกับฟิล์มที่เป็นโครงสร้างหลัก จากการทดลองพบว่าการเปลี่ยนแปลงของสมบัติที่เกิดขึ้นของฟิล์มสามชั้นเป็นไปตามอัตราการเกิดปฏิกิริยาอันดับศูนย์และอันดับหนึ่ง ($R^2 \ge 0.85$) ถึงแม้ว่าการเปลี่ยนแปลงที่เกิดขึ้นจะขึ้นกับอุณหภูมิแต่ก็ไม่เป็นไปตามสมการของอาร์รีเนียส อีกทั้งมีการทดสอบการยืดอายุของตัวอย่างนมผงสำหรับเด็กที่มีความไวต่อการเกิดปฏิกิริยากับออกซิเจนเก็บที่ 4, 25 และ 35 °C ระดับความชื้นสัมพัทธ์ 50% เป็นระยะเวลา 19 วัน โดยใช้ถุง PLA/WPI/PLA เทียบกับถุง PLA พบว่าถุง PLA/WPI/PLA มีความสามารถในการรักษาสี, ปริมาณน้ำอิสระ (Water activity) และปริมาณความชื้น (Moisture content) ของนมผงเทียบเท่ากับกับถุง PLA จากผลการทดสอบปริมาณคอนจูเกตไดอีน (Conjugate diene) และสารประกอบคาร์บอนิล (Carbonyl compounds) ที่เกิดขึ้นแสดงให้เห็นว่าโครงสร้างฟิล์มที่อาศัยสมบัติของ WPI นั้นช่วยชะลอการเกิดปฏิกิริยาออโตออกซิเดชัน (Autoxidation) ของไขมันในนมผงตัวอย่างได้อย่างมีนัยสำคัญ ($p \le 0.05$) จึงสามารถสรุปได้ว่าฟิล์มหลายชั้นที่ผลิตจากพอลิเมอร์ชีวภาพ; PLA/WPI/PLA สามารถใช้เป็นวัสดุบรรจุภัณฑ์ที่มีสมบัติขวางกั้นออกซิเจนซึ่งมีความเป็นไปได้ในการนำมาใช้ยืดอายุการเก็บผลิตภัณฑ์อาหารแห้งได้ที่ 4 - 35 °C ระดับความชื้นสัมพัทธ์ 50%

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5471990723 : MAJOR FOOD TECHNOLOGY
KEYWORDS: BIOPOLYMER / OXYGEN BARRIER / POLYLACTIC ACID / WHEY PROTEIN ISOLATE

THUNYALUCK PHUPOKSAKUL: DEVELOPMENT OF POLYLACTIC ACID–WHEY PROTEIN ISOLATE FILM AS NOVEL OXYGEN BARRIER PACKAGING FILM. ADVISOR: THEERANUN JANJARASSKUL, Ph.D., CO-ADVISOR: ASSOC. PROF. ANONGNAT SOMWANGTHANAROJ, Ph.D., 126 pp.

This research aims to develop a novel eco-friendly multi-layer barrier film fabricated entirely from biopolymeric materials, by employing mechanical strength and moisture barrier of poly(lactic acid) (PLA) films and oxygen barrier enhancement of glycerol (GLY) plasticized whey protein isolate (WPI). The effect of glycerol content (WPI:GLY 1:0.25, 1:0.4 and 1:0.67) on physical and barrier properties of PLA/WPI/PLA films were evaluated and compared with those of three-layer structures made of linear low-density polyethylene (LLDPE/WPI/LLDPE). The results showed that composite structures of PLA/WPI/PLA and LLDPE/WPI/LLDPE can be obtained by simple solution-casting process. The transparent WPI layer could successfully be formed thinly between two base layers of corona discharge-treated PLA or LLDPE films. The resulting multi-layer films, both PLA/WPI/PLA and LLDPE/WPI/LLDPE showed no visible optical change and possessed good flexibility and layer adhesion. The range of GLY contents used in this study was sufficient to prevent the films from cracking and curling. Transparency at 600 nm (T_{600}) of multi-layer films were significantly reduced ($p \leq 0.05$) comparing to their parental substrates as a result of discontinuous layers. The resulting total color difference (ΔE_{ab}) was lower than the detectable threshold by the human eye. This indicated that the composite structures were not visibly different from their single substrates. PLA enhanced tensile strength of the composite structures. PLA/WPI/PLA films exhibited a higher tensile strength (TS) but lower percentage of elongation (%E) compared to highly-extensible LLDPE/WPI/LLDPE films. The oxygen permeability of the three-layer structures were significantly reduced ($p \leq 0.05$). However, the water vapor permeability of the structure relied mainly on the base films. Overall properties of the three-layer films tended to depend on the intrinsic trait of the substrate films more than the GLY content. To examine shelf stability of PLA/WPI/PLA structure, the multi-layer structure with WPI:GLY = 1:0.4 was stored in the simulated commercial storage temperature; 4, 25 and 35°C, at 50% relative humidity (%RH), for 21 days, along with single-layer WPI and PLA substrates. The multi-layer film showed no visible optical change and maintained good handling ability and layer adhesion throughout the storage test. It was found that transparency of PLA/WPI/PLA film gradually decreased over time, corresponding to the increasing trends of ΔE_{ab}. Non-enzymatic Maillard browning of whey proteins and trace reducing sugar in the middle-layer and molecular re-arrangement were hypothesized to slowly cause changes in transparency and color of the film. Storage at 35 °C caused PLA/WPI/PLA film to become stronger and less extendible. On the other hand, the multi-layer structure showed lower TS and higher %E over storage at 4 and 25 °C. Oxygen and water vapor barrier abilities of PLA/WPI/PLA gradually improved over time as evident by the decreased permeabilities, especially at 35 °C. Overall, the PLA/WPI/PLA structure generally had properties in between its parental substrates. The changes in properties of laminate structure can be empirically fitted with either zero or first order reaction kinetics, with overall $R^2 \geq 0.85$. Although such changes were temperature dependent, they did not follow Arrhenius behavior. The shelf life extension of oxygen-sensitive baby formula by using PLA/WPI/PLA pouch was also assessed at 4, 25 and 35 °C, 50% RH for 19 days, in comparison with PLA pouch. The result showed that PLA/WPI/PLA pouch did not improve color stability of packaged baby formula. The a_w and moisture content of packaged baby formula indicated that PLA/WPI/PLA pouch provided equal protection against ingress water vapor to PLA packaging. The result of conjugate diene and carbonyl compound of baby formula concluded that WPI-enhanced composite structure could effectively retard autoxidation of lipid in dry food. In conclusion, the results suggested a novel multi-layer barrier film made entirely from biodegradable materials; PLA/WPI/PLA, could work successfully as a “green” oxygen barrier alternative. The results also indicated that PLA/WPI/PLA film could be stored and used to package dry food properly at 4-35 °C, 50% RH, for extended period of inventory or storage time.

Department:	Food Technology	Student's Signature	
Field of Study:	Food Technology	Advisor's Signature	
Academic Year:	2014	Co-Advisor's Signature	

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my adviser, Dr. Theeranun Janjarasskul, and my co-adviser, Assoc. Prof. Anongnat Somwangthanaroj, for their critical suggestion, valuable assistance and all the encouragement to carry out my research.

I am grateful to Asst. Prof. Pasawadee Pradipasena, Dr. Thanachan Mahawanich and Asst. Prof. Lerpong Jarupan for constructive comments as thesis committees.

I wish to acknowledge the financial support for this work by the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund). I am also grateful for assistance of Polymer Laboratory, Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University for facilitating fabrication of polylactic acid film as well as permeability measurements. The assistance of Prepack Thailand Co., Ltd. for corona surface treatment and advice is also greatly appreciated. I am also very thankful to staff of Food Technology Department, Faculty of Science, Chulalongkorn University, who assisted me with various laboratory equipments, administrative and documentary problems.

My heartfelt thanks also go to my friend, Manusawee Leuangsukrerk, for her advice on research techniques and unconditional support and affection. Moreover, I am also thankful to my friends here at the Food Technology Department for their friendliness and encouragement. Lastly, I would like to express a deep sense of gratitude to my parents and family, who have always been standing by me in times of need and gave me all the supports and blessings.

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CHAPTER I
INTRODUCTION

The main functions of food packaging are containment, communication, convenience and protection (Robertson, 2012a). These days, particular focus has been placed upon preservation of food quality due to consumer trends for high quality products with extended shelf-life. Food products contain several complex compositions which may suffer from biological, chemical and physical deteriorations during distribution and storage. Water vapor and oxygen protections are important prerequisite packaging properties for such complex biological systems like food products. Moisture exchange between surrounding atmosphere and food product not only results in mechanical damage and loss of textural properties of food but also water activity (a_w) change which leads to microbial growth and/or chemical reactions with or without enzyme catalysis. Oxygen is one of the most critical compounds that can strongly react with food components. The oxidation reaction may cause undesirable permanent changes such as rancidity, off-color, off-odor and nutrition loss in food product. By using a proper packaging material, these two critical environmental factors can be blocked or minimized.

The conventional barrier packaging is usually composed of petroleum-based polymer which forms a multi-layer film structure. This packaging often uses expensive polyvinylidene chloride (PVDC) or water sensitive ethylene vinyl alcohol (EVOH) as an oxygen barrier layer laminated with other polymers to provide other desirable functions such as structural integrity, moisture barrier, heat sealability and printability (Hong and Krochta, 2004). Although, most of plastics are recyclable they often lack high purity. The complicated layer identification and separation of these multi-layer structures can be difficult and limit their reusability and recyclability (Marsh and Bugusu, 2007).

Advanced research studies have been reporting the use of biopolymer for oxygen barrier function, especially the potential uses of whey protein (Stuchell and Krochta, 1995; Maté, Frankel, and Krochta, 1996; Janjarasskul and Krochta, 2010a; Janjarasskul, Tananuwong, and Krochta, 2011). Whey protein isolate (WPI) is a by-product of the cheese industry and has a protein purity of more than 90% on a dry basis. WPI films and coatings are reported to be glossy and transparent with excellent barrier properties to oxygen, aroma and oil (Sothornvit and Krochta, 2000). The oxygen permeability (OP) of properly plasticized heat-denatured WPI films is reported to be lower than that of synthetic polyethylene (PE) films and to be comparable to that of PVDC or EVOH at low relative humidity conditions (McHugh and Krochta, 1994a; Miller and Krochta, 1997). Exploration of the use of WPI coatings, which possess excellent oxygen-barrier properties, in combination with common synthetic moisture barrier polymers, e.g. polypropylene (PP), low density polyethylene (LDPE), and polyvinyl chloride (PVC), to produce bi-layer structures have been reported (Hong and Krochta, 2003; Hong, Han, and Krochta, 2004; Hong and Krochta, 2004, 2006; Lee, Son, and Hong, 2008; Schmid *et al.*, 2012). The results showed that smooth and transparent WPI coating can be formed on a plastic surface. WPI coated films give effective oxygen barrier properties as well as visual and mechanical properties.

Although, the WPI coatings could easily be separated chemically or enzymatically from synthetic substrate layer in preparation for recycle or reutilization of the plastic layer, it would be a novel idea to develop oxygen barrier films completely fabricated from sustainable and bio-degradable materials to reduce the use of synthetic materials and to simplify the disposal process. Furthermore, the oxygen barrier of WPI of these reported bi-layer structures need moisture protection from the environment to maintain low OP.

Polylactic acid (PLA) is a biodegradable thermoplastic, which has recently become commercially available, produced from renewable bio-based materials, i.e. from corn milling or sugar and starch processing. PLA can be formed into films or moulded containers (Zhang and Sun, 2005). It is expected to be a replacement material for conventional plastics such as PE, polyethylene terephthalate (PET) and PVC (Komatsuka, Kusakabe, and Nagai, 2008). However, PLA is reported to show lower moisture and oxygen barrier properties than PET (Auras *et al.*, 2003; Drieskens *et al.*, 2009).

In addition, properties of the packages are one of the main factors which controlling the food product shelf life, beside product characteristics and distribution environment. The difference chemical composition and structure of polymer influence the different properties of various polymeric materials. The plasticizer incorporation was generally reported as necessary to overcome the intrinsic brittleness of WPI by enhancing the mobility of polymer chains through a decrease in intermolecular force of polymer. However, use of plasticizer pose an effect on lowering barrier properties (Khwaldia *et al.*, 2004; Lee *et al.*, 2008). Furthermore, the packaging functionality can perform variously in different surrounding environment. Ambient environment may affect the stability of the package properties during the storage. For this reason, the stability of the multi-layer structure barrier film during storage needs to be identifying to achieve the limitation of realistic usage.

It was hypothesized that a novel three-layer barrier structure PLA/WPI/PLA made entirely from environmentally friendly biopolymers can be developed by employing the mechanical strength and moisture barrier of PLA films and the oxygen barrier enhancement of WPI films. The developed multi-layer PLA/WPI/PLA film was hypothesized to have reasonable storage stability in simulated commercial storage temperatures.

The objectives of this study were to explore a feasibility to develop a novel multi-layer barrier film structure constituted entirely from biopolymeric materials; PLA and WPI compared to the combination of linear low-density polyethylene (LLDPE) and WPI structure, as well as to examine effect of storage temperature on the storage stability of PLA/WPI/PLA film. The shelf life extension of baby formula as oxygen-sensitive food model by using PLA/WPI/PLA pouch was also assessed.

CHAPTER II

LITERATURE REVIEWS

2.1 Edible films and coatings

2.1.1 Definition

Edible films and coatings can be defined as any types of edible material used for enrobing various food to extend shelf life of the product, or for other purposes, that may be eaten together with food with or without further removal (Pavlath and Orts, 2009).

2.1.2 Films preparation and application

Edible films and coatings can be obtained in many ways. The first method was to deposit the film directly on food surface by dipping the food into, or by brushing or spraying with coating solutions (Gontard and Guilbert, 1994). The food will adsorb a certain amount of coating solution to form the protective layer on its surface when the coating material dried. The second method was by creating standalone film for covering food surface through evaporation of film solution on a casting surface or through thermo-formation. The films and coatings were applied mainly to provide the ability to safely maintain various gas or liquid transmission rates, to reduce the environmental impact or to carry any desirable ingredients such as antioxidant, antimicrobial agent, flavor, fragrance or nutrient in order to prevent the packaged food from the premature deterioration as well as to fulfill consumer demands.

2.1.3 Films forming materials

Generally, edible films and coatings compose of food components. For example, polysaccharides and proteins can be applied to provide gas barriers or to provide mechanical stability in film applications. On the other hand, edible fats can be used as water transmission reducing agents.

I) Protein-based films and coatings

Protein-based films and coatings can be composed from either plant-proteins (e.g., corn zein, soy protein, wheat gluten) or animal-proteins (e.g. collagen, milk casein, whey protein). Protein-based films not only have good mechanical and optical properties, but also have good barrier ability against oxygen, carbon dioxide, aroma and lipid. The main disadvantage of protein-based films is their poor water vapor barrier ability because of their hydrophilic nature (Janjarasskul and Krochta, 2010a).

II) Polysaccharide-based films and coatings

Various types of polysaccharide, such as alginate, carrageenan, cellulose, dextrin, pectin and starch, have been used to produce polysaccharide-based films and coatings. The major advantage of polysaccharide-based films and coatings are their structural stability. The polysaccharide-based films possess good mechanical and gas barrier properties (Baldwin, Nisperos-Carriedo, and Baker, 1995). They also provide an efficient barrier against oils and lipids but provide poor barrier against water vapor as a result of their intrinsic hydrophilic functional structure (Janjarasskul and Krochta, 2010a).

III) Lipid-based films and coatings

Natural originate waxes and synthetic acetylated fat, monoglycerides are the oldest known edible films. Lipid-based films and coatings have been legally applied to meat, fish and poultry, under FDA approval, to provide an excellent water vapor barrier ability and gas sacrificing ability (Pavlath and Orts, 2009) due to their low polarity structure (Greener and Fennema, 1989). However, the critical disadvantage of lipid-based films is their waxy taste and texture, greasy surface and potential rancidity.

2.2 Biodegradable films and coatings

2.2.1 Definition

Films or coatings that can be completely degraded by microorganism in a composting process ultimately to carbon dioxide, water, methane and some biomass residue are defined as biodegradable films and coatings (Steuteville, 1993). Edible films and coatings can also be classified as biodegradable material. However, the eatable ability of the films or coatings are lost if they reacted or mixed with non-edible components in the process of fabrications (Krochta, 2002).

2.2.2 Biodegradable films and coatings application

Biodegradable films and coatings have provided many useful functions for food applications, such as barrier ability and protective ability to preserve the food product. There are potential uses of biodegradable films and coatings to partially or totally replace the conventional synthetic packaging. Biodegradable materials are essential complementary approaches to the success of reducing the use of non-renewable resources and solving the disposal of packaging wastes and environmental problems.

2.2.3 Classification of biodegradable polymer

The biodegradable polymers can be classified according to their chemical composition, synthetic method, processing method, economic importance, application, etc. One of the simplest ways to classify biodegradable polymers is by their origins; natural and synthetic polymers (Zhang and Sun, 2005).

I) Natural biopolymer

Natural biopolymers are derived from natural resources. They can be sub-divided in accordance with chemical composition into

- Polysaccharides polymers (e.g. starch, cellulose, lignin, chitin)

- Proteins polymers (e.g. gelatin, casein, wheat gluten, silk and wool)
- Micro-organism and plants-produced polyesters (e.g. polyhydroxy-alcanoates, poloy-3-hydroxybutyrate)
- Bio-derived monomer-synthesized polyesters (e.g. polylactic acid)
- Miscellaneous polymers (e.g. natural rubbers, composites)

II) Synthetic biopolymer

Synthetic biopolymers are the polymers that synthesized from mineral and crude oil. These polymers can be divided into four sub-groups

- Aliphatic polymers (e.g. polyglycolic acid, polybutylene succinate, polycaprolactone)
- Aromatic or two-typed blended polyester (e.g. polybutylene succinate terephthalate)
- Polyvinyl alcohols
- Modified polyolefins (e.g. polyethylene (PE) or polypropylene (PP) with specific agents sensitive to temperature or light)

2.3 Whey protein-based films

2.3.1 Chemical structure

Whey protein is a remaining protein in milk serum after the coagulation of casein in cheese or casein industry. Whey protein is a mixture of five main proteins including of α-lactalbumins, β-lactoglobulins, bovine serum albumin, immunoglobulins and proteose-peptones. The approximate composition in whey protein, molecular weight component, number of disulfide bond (S-S) and sulfhydryl group (SH) per molecule are shown in *Table 2.1*. Depending on protein purity, there are two commercial types of whey protein; whey protein concentrate (WPC) and whey protein isolate (WPI) which contains protein purity of 25 - 80% and more than 90% of dry matters, respectively (Perez-Gago and Krochta, 2002).

Table 2.1: Protein components of whey protein

Protein Type	Fraction (%)	Molecular Weight (Da)	Disulfide Bonds	Sulfhydryl Groups
β-lactoglobulins	57	18,300	2	1
α-lactalbumins	19	14,200	4	0
Immunoglobulins	13	150,000-1,000,000	nv	nv
Bovine serum albumin	7	66,300	17	1
Proteose-peptones	4	4,100-22,000	0	0

* nv = numerous variable.

Source : Perez-Gago and Krochta (2002).

2.3.2 Whey protein films formation and properties

2.3.2.1 Whey protein films forming

Native globular whey proteins contain most of the hydrophobic and SH groups in the interior of the molecule. Heat denaturation (*Figure 2.1*) is essential step in whey protein film formation. Heating process helps exposing those internal hydrophobic and SH groups (Shimada and Cheftel, 1989) to form the S-S intermolecular bonding and hydrophobic interaction when dried (McHugh and Krochta, 1994b).

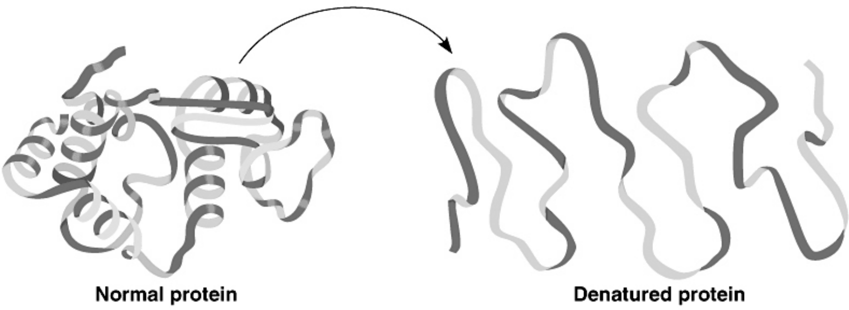

Figure 2.1: Heat-denature protein

Source : Priest (2010)

There was a reported that WPI solution concentration down to 5% (w/w) shown to form good WPI films (Perez-Gago, Nadaud, and Krochta, 1999) while the concentration more than 12% (w/w) gelled upon heating. McHugh, Aujard, and Krochta (1994) reported the minimum heat treatment required for formation of intact whey protein films to be 75 °C for 30 min. This heating temperature is close to the denaturation temperature (78 °C) of β-lactoglobulin, the major component of whey protein. McHugh *et al.* (1994) also reported that heat treatment at 90 °C for 30 min was an optimum condition for 10% (w/w) WPI solution at neutral pH to form a film with consistent structure.

Native whey protein which has not undergone heat treatment was reported to also have good film-forming ability (Perez-Gago *et al.*, 1999). Cohesion of native whey protein films rely on intermolecular hydrogen bonding created upon film coacervation while hydrophobic and SH groups still bury in the interior of the molecule. Both native and heat-denature whey protein films reported to be similar transparent and water vapor permeability (WVP), but they possessed different solubility and mechanical properties. The covalent S-S bonding between unfolded structure of heat denatured whey protein produces the water-insoluble and stronger films, while lower energy bonding between globular structure of native whey protein produces the completely water soluble and poorer mechanical films (Perez-Gago *et al.*, 1999).

Hernández-Izquierdo and Krochta (2008) reported that WPI displays thermoplastic behavior at low moisture level. WPI was successfully formed into films and sheets using thermoplastic processes such as compression molding and extrusion (Sothornvit *et al.*, 2007).

2.3.2.2 Plasticizers

Plasticizers are extensively used in plastic industry to improve processability, flexibility and ductility of glassy polymer (Sears and Darby, 1982). They are normally hygroscopic and attract water molecules. Many kinds of plasticizer are available such as water, glycerol (GLY), sorbitol, sucrose, urea, propylene glycol (PG), polyethylene glycol (PEG), phospholipids, etc.

Protein-based films and coatings are usually stiff and brittle due to the extensive interaction between protein chains through hydrogen bonding, electrostatic forces, hydrophobic bonding and/or disulfide cross-linking, so the small amount of hydrophilic plasticizer is added. Plasticizers interact with polymer chain by competing with the neighboring protein chain for hydrogen bonding and electrostatic interactions. Therefore, the protein chain-to-chain intermolecular forces are reduced, thus increasing chain mobility free volume and leading to the lowering glass transition temperature (T_g) and improving film flexibility. Plasticized films typically have higher gas permeabilities (Krochta, 2002; Perez-Gago and Krochta, 2002).

Sucrose ($C_{12}H_{22}O_{11}$) and sorbitol ($C_6H_{14}O_6$) have also been studied for their plasticizing effects, including plasticizing fish myofibrillar proteins to produce biopackaging materials by thermal compression-molding (Cuq, Gontard, and Guilbert, 1997).

Water can also be used as an effective plasticizer in biopolymer materials but it is easily lost due to dehydration. Water enables biopolymers to undergo glass transition, facilitates deformation, and processability of the biopolymer matrix. Without water addition, the temperature region of thermal degradation would be easily reached before films could be formed. However, an excessive amount of water during protein extrusion would decrease melt viscosity, resulting in low product temperature that could reduce the degree of protein transformation and interactions (Hernández-Izquierdo and Krochta, 2008).

GLY ($C_3H_8O_3$) is a low molecular weight, hydrophilic plasticizer that has been widely used in the thermoplastic processing of proteins (Redl *et al.*, 1999; Cunningham *et al.*, 2000; Pommet *et al.*, 2003; Sothornvit *et al.*, 2003, 2007; Hernández-Izquierdo and Krochta, 2008). Its high plasticizing effect has been attributed to the ease of GLY can insert and position itself within the three-dimensional biopolymer network (Di Gioia and Guilbert, 1999). GLY is much less volatile than water. However, the presence of GLY attracts additional moisture and often causes additional plasticizing effect on film properties (Krochta, 2002).

Pommet *et al.* (2005), stated that critical factors for a good plasticizer are low melting point, low volatility, and protein compatibility. In addition to these characteristics, permanence in the film and amount of plasticizer needed should be taken into account when choosing a good plasticizer (Di Gioia and Guilbert, 1999; Sothornvit and Krochta, 2001).

Plasticizer composition, size, shape, and ability to attract water have been shown to affect solution-cast whey protein film barrier properties. Maté and Krochta (1996) studied the effect of GLY amount on WVP and oxygen permeability (OP) for WPI and β-lactoglobulins films, and reported that increase in GLY levels led to a higher permeability values with no significant difference between type of films.

2.3.2.3 Whey protein films properties

Plasticized whey protein films are glossy, transparent, bland, and flexible. Also, they possess excellent barrier to oxygen, aroma and oil, but poor barrier to moisture because of their hydrophilic character.

The OP of properly plasticized heat denatured WPI films reported to be lower than synthetic PE films and be comparable to polyvinylidene chloride (PVDC) or ethylene vinyl alcohol (EVOH) polymer at low relative humidity conditions (McHugh and Krochta, 1994a; Miller and Krochta, 1997).

2.3.2.4 Storage stability of whey protein films and coatings

Physical condition and climatic condition during storage may influence the properties of bio-based films which originate from food elements. The polymer properties may change due to the effect of temperature, % relative humidity (%RH) over time.

The color stability of edible coatings during prolonged storage was studied by Trezza and Krochta (2000). The yellowing rates of edible coatings were determined at 23, 40, and 55 °C at 75% RH. It was reported that WPI coatings had lower yellowing rates than WPC. Activation energy and Q_{10} values for the yellowing of whey protein coatings were similar to those previously reported for the browning of whey powder (LaBuza and Saltmarch, 1982). The milk fat content of WPC also contributes to its yellow color.

The effect of temperature (15, 23, 30, and 37 °C) on the OP of WPI and β-lactoglobulins edible films at three different levels of GLY content were studied by Maté and Krochta (1996). It can be concluded that β-lactoglobulins and the WPI fractions likely contributed equally to film barrier properties. Increased amounts of GLY in the film formulation resulted in exponential increases of OP. The temperature had an exponential effect on the OP of WPI and β-lactoglobulins films. The OP also increased as the determinating temperature increased. Results fitted the Arrhenius model with activation energy in the 10.5 - 13.5 kcal/mol.

Amin and Ustunol (2007) investigated the solubility and mechanical properties of heat-cured WPI films (at 80, 90 or 100 °C for 12, 24, 48 and 72 h). It was reported that increase in heat-curing temperature and time decreased water solubility, but increased tensile strength and wet strength of the films due to the additional covalent bonds formed during the heat-curing process.

Simelane and Ustunol (2005) also investigated the mechanical properties of heat-cured WPI films (at 90 °C for 12 h and at 80 °C for 24 h) under 3 different sausage manufacturing conditions (stage 1; 57 °C/ 60 min/ 36% RH, stage 2; 65 °C/ 90 min/ 60% RH, and stage 3; 77 °C/ 30 min/ 80% RH). It was found that there were no significant differences between the WPI-based films heat-cured at 80 °C for 24 h and those heat-cured at 90 °C for 12 h during the multi-stage cooking process. Although, tensile strength and apparent modulus of WPI films decreased with the increased temperature, time, and %RH, while percentage of elongation remained the same.

Miller, Chiang, and Krochta (1997) reported that heat curing time linearly affected tensile stress and Young's Modulus, while influencing elongation at break and WVP exponentially. Increased curing temperature and reduced %RH accelerated the increase of tensile stress, decrease of elongation at break and Young's Modulus and improvement in water vapor barrier properties. These data supported the hypothesis that heat curing may elicit additional crosslinking of protein, yielding increased tensile stress, decreased elongation and decreased WVP.

Moreover, the effects of environmental relative humidity and plasticizers on the WVP and OP of WPI-based films were examined by McHugh *et al.* (1994) and McHugh and Krochta (1994a). Due to their hydrophilic nature, these films exhibited higher WVP and OP with increased %RH.

2.4 Polylactic acid-based films

2.4.1 Chemical structure and preparation

Polylactic acid or PLA (*Figure 2.2*) is a biocompatible and biodegradable polyester. It is a linear high molecular weight polyester based on lactic acid which consists of both hydroxyl and carboxyl groups.

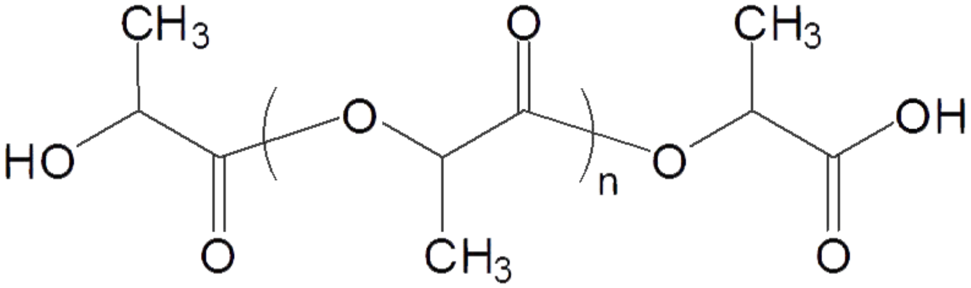

Figure 2.2: Polylactic acid $(C_3H_4O_2)_n$ chemical structure

Source : Eustathios *et al.* (2013)

Lactic acid can be produced via a biological starch fermentation or chemical method (*Figure 2.3*). PLA is generally prepared by a ring-opening polymerization of lactide, which is obtained by the microbial fermentation of a renewable sugar-based materials, i.e. from corn milling or sugar and starch processing (Kricheldorf, Berl, and Scharnagl, 1988; Nijenhuis, Grijpma, and Pennings, 1992; Zhang and Sun, 2005). Starch is converted into dextrose sugar, and then passed through the fermentation process to form lactic acid which can be found in two enantiomers: L-lactic acid and D-lactic acid, or their mixtures, before took part in the ring formation, to form lactide monomer (*Figure 2.4*). There are three stereoisomers of lactide: L-, D- and meso-lactide. The lactides are then purified and polymerized into the forms of poly(L-lactic acid); L-PLA and/or poly(D,L-lactic acid); D,L-PLA and processed into pallets ready to be fabricated for end-use applications (Lehermeier, Dorgan, and Way, 2001; Vink *et al.*, 2003; Bhattacharya *et al.*, 2005; Nobuyuki *et al.*, 2005; Drieskens *et al.*, 2009).

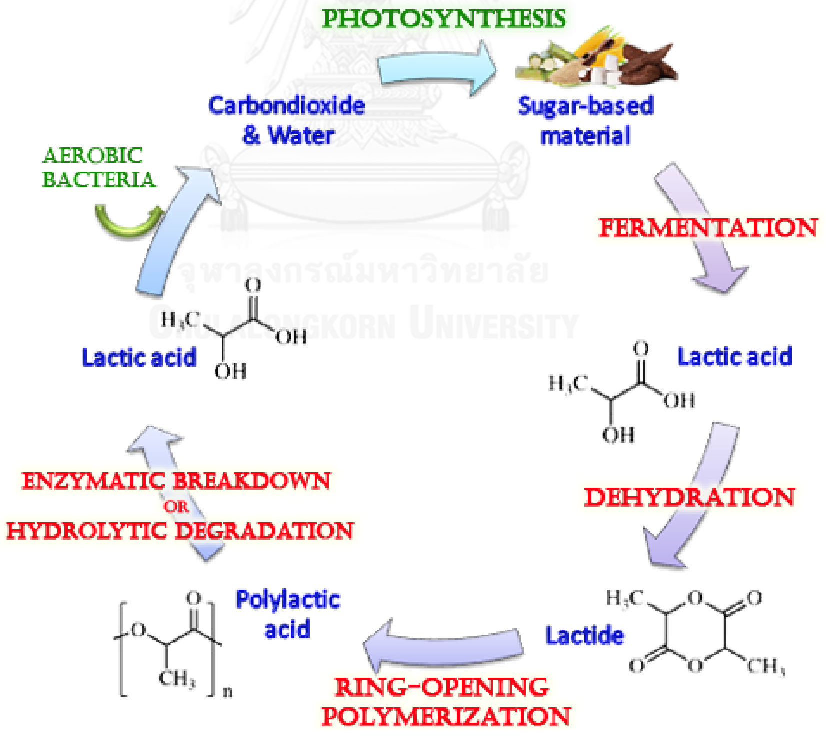

Figure 2.3: PLA cycle

Source : Richard *et al.* (2012)

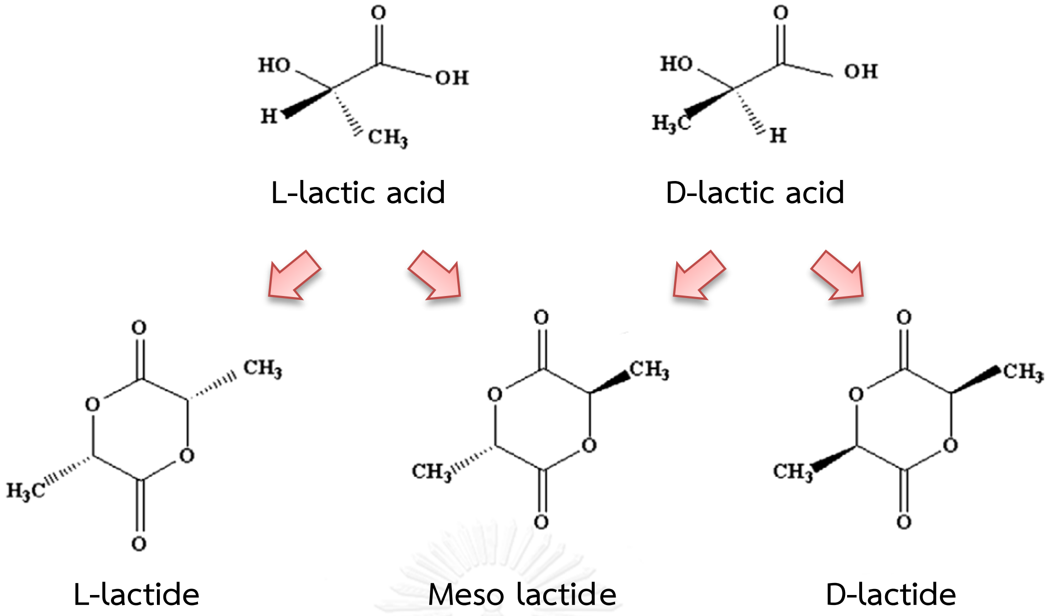

Figure 2.4: Stereoforms of lactides

Source : Madhavan, Nair, and John (2010)

2.4.2 Polylactic acid-based biopolymer properties

2.4.2.1 Thermal characteristic

The physical properties of PLA are affected by thermic condition. Heat can induce the change in crystalline/amorphous ratio and glassy amorphous phase in PLA due to its intrinsic characteristic as a semi-crystalline polymer (Celli and Scandola, 1992). L-PLA is a crystalline polymer while D,L-PLA is an amorphous polymer (Bhattacharya *et al.*, 2005). The equilibrium melting point ant the glass transition temperature are about 215 °C and 55 °C, respectively (Kalb and Pennings, 1980).

2.4.2.2 Mechanical properties

PLA is rigid, brittle and likely to deform at the excessive temperatures over its glass transition temperature. Physical and mechanical properties of PLA largely depend on the L/D ratios, molecular weight, crystallinity, orientation and preparation method. The typical tensile strength of PLA film were approximately about 28 - 60

MPa and its tensile modulus were in range of 1200 - 3000 MPa, while the elongation at break were about 2.0 - 6.0% (Lu and Mikos, 1999; Bhattacharya *et al.*, 2005).

2.4.2.3 Barrier properties

This biodegradable PLA reported to show moderate moisture barrier behavior comparable to synthetic polyethylene terephthalate (PET) (Auras *et al.*, 2003; Drieskens *et al.*, 2009) however its OP needs further improvement (Svagan *et al.*, 2012).

2.4.2.4 Aging and biodegradation

Physical aging is a thermo-reversible process that is characteristic of the glassy state of materials. Aging normally occurs in the glassy state as an effect of room temperature storage. It develops at a faster rate as the aging temperature (T_a) approaches T_g where the molecular relaxation occurs toward equilibrium. Physical aging can dramatically influence polymer properties. For example, impact strength significantly reduces as a result of increasing relaxation time, as well as migration and loss of plasticizers (Shogren, 1992; Van Soest *et al.*, 1994). Ljungberg, Andersson, and Wesslén (2003) stated that the crytallinity of PLA increased after stored at temperature near T_g. Wang, Sun, and Seib (2003) reported that the thermal transition behavior of PLA/starch/MDI blends is more obvious with increase aging time as a result of free volume relaxation during sub-T_g aging. Mechanical properties were distorted, the tensile strength decreases from 63.6 MPa to 51.3 MPa while, the elastic modulus decrease from 1.75 GPa to 1.56 GPA after being aged at room temperature.

PLA degrades in moisture and at elevated temperatures. The degradation of PLA environmentally consists of two-step process. At first, a high-molecular weight chain is slowly hydrolyzed to lower molecular weight oligomers. The chemical structure change process is affected by temperature and moisture level and can be accelerated by acids or bases (Mason, Miles, and Sparks, 1981; Ogawa *et al.*, 1988a; Ogawa *et al.*, 1988b). Then, the microorganism will convert these oligomers to carbon dioxide and water or methane rely on the presence or the absent of oxygen, respectively (Narayan, 1993; Drumright, Gruber, and Henton, 2000).

2.4.3 Polylactic acid-based biopolymer application

PLA is a thermoplastic that is insoluble in water. The main application of the biaxial oriented PLA film is for food packaging (*Figure 2.5*) such as thermoformed container and beverage bottle due to its excellent flavor barrier ability and its heat sealability. Also, the lactide residue can be hydrolysed to lactic acid, which naturally exists in food and body and listed as an approved monomer for food contact application in EU national regulatory status (Vlieger, 2003). Therefore, it is designed for food contact and expected to be a replacement material for conventional plastics such as PE, PET and polyvinyl chloride (PVC) (Komatsuka *et al.*, 2008).

Figure 2.5: Polylactic acid (PLA) films and containers

Source : Lui (2006)

2.5 Multi-layer packaging for food application

2.5.1 Multi-layer films formation

2.5.1.1 Multi-layer films forming

Single-layer plastics are insufficient for most food application packaging, which could be resolved by coating, making laminates, and coextrusion. Coatings can be melt extrusion of a polymer on the base film or they can be made by applying solutions or dispersions of polymer to base film. The laminates generally consist of two or more layers of material bonded with an adhesive or a lower-melting plastic. Normally plastic is bonded to aluminum foil, paper or another plastic. While, coextrusion is a process whereby several layers of plastic are extruded at once. Multi-layer films are fabricated to improve the mechanical properties, heat-sealability, printability and/or barrier abilities to gases and water vapor (Jenkins and Harrington, 1991).

2.5.1.2 Films surface treatments

Many plastics require oxidation of the film surface to achieve sufficient adhesion of ink, adhesives or laminating substance. For example, polyolefin films generally have relatively low surface energy and must be treated to increase the probability of satisfactory bond to others substances. This is accomplished by electrical corona discharged-treatment, gas plasma treatment, flame treatment or etching with chemical.

Corona discharge-treatment is the process which impinges a high electrical discharge (10 - 40 kV and 1 - 40 kHz) on a surface, resulting in a change in character of the surface to be ready to adhere to other substances. Before coating or laminating operation, the film is passed between a grounded roll with a high voltage source. A corona is created which produces oxidation and formation of high polarity functional group (e.g. carbonyl) at the polymer surface (Jenkins and Harrington, 1991; Brown, 1992; Baghdachi, 1996).

2.5.2 Combination of biopolymer and petroleum-based plastic films

Exploration of the use of protein-based polymer such as WPI, soy protein isolate (SPI) and corn zein coating, which possesses excellent oxygen-barrier ability, in combination with common synthetic moisture barrier polymers, i.e., PP, PE, low density polyethylene (LDPE), PVC, to produce laminated structures have been reported by many observations.

Hong and Krochta (2003) investigated the oxygen permeation characteristics of WPI coatings on PP films to examine the feasibility of WPI coating as a novel biopolymer oxygen barrier for food packaging applications. Heat-denatured aqueous solutions of WPI with several plasticizers including GLY, sorbitol, sucrose, PG, and PEG were applied on the surfaces of PP films previously treated with corona discharge, in order to achieve well adhering WPI coatings. Overall oxygen-barrier property of the WPI-coated PP films with different plasticizers significantly decreased in the order of sucrose > sorbitol, GLY, and PG > PEG. Among plasticizers used, sucrose conferred the best oxygen barrier property to the WPI-coated films but tended to crystallize after storage for 2 month at ambient temperature and low %RH, because sucrose is solid phase at this condition.

Hong *et al.* (2004) reported that composite film structures of common plastic polymers including PP or PVC with WPI coatings may be obtained by a casting method. The nonpolar nature of PP films requires a surface treatment such as corona discharge to modify their surfaces to accomplish better adhesion between WPI coatings and the substrate. Regardless of the substrates, WPI-coated films possessed very high gloss, as well as no color, comparable to that of the synthetic polymer substrates. In addition, hydrophilic WPI coatings confer increased surface energy on the substrate films, along with increased water affinity. As a replacement for existing synthetic oxygen-barrier polymers, WPI coatings with a proper plasticizer have great potential for improving the visual characteristics of the polymeric substrate and for enhancing water wettability of the coated plastic films.

Hong and Krochta (2004) examined the feasibility of WPI coating as an alternative oxygen barrier for food packaging, heat-denatured aqueous solutions of WPI with various levels of GLY (16.7%, 25.9%, 33.3% or 41.2% (w/w) on dry basis) as a plasticizer were applied on corona discharge-treated LDPE films. The resulting WPI-coated films have excellent oxygen-barrier properties, much better than uncoated LDPE film, at low to intermediate relative humidity. The OP values of WPI-coated

LDPE films increased linearly with GLY content, but the increase was not found to be significant. Also, the OP of the coated films increased significantly with increasing %RH. The coated films also have excellent gloss, as well as low haze and color.

Hong and Krochta (2006) examined the oxygen permeation properties of whey-protein-coated plastic films to compare their oxygen-barrier performance as affected by temperature, %RH, base film (PE and PP), and protein type (WPI and WPC). The resulting whey-protein-coated films showed increase in OP as temperature increased, regardless of types of whey proteins and base films. Relative humidity level had an exponential effect on OP of the coated films, with excellent oxygen-barrier properties at low to intermediate %RH. Overall OP values of the whey-protein-coated PP were significantly lower than those of the coated PE, mainly due to the intrinsic permeation characteristics of the base films. No remarkable differences could be observed in the oxygen-barrier performance between WPI and WPC coatings.

Lee *et al.* (2008) reported that composite structure of PP film coated with SPI, WPI and corn zein, with various plasticizers (PG, GLY, PEG, sorbitol, sucrose) could be obtained by a simple casting method. Among the proteins used, corn zein appeared not to be a good coating material for forming a composite structure with PP film. However, WPI as well as SPI could be used as an excellent coating material to produce composite films when a proper plasticizer was applied. High glossy surfaces were observed on the coated films with WPI and corn zein, with the sucrose-plasticized WPI coating giving the highest gloss. Proteins as well as plasticizers exerted a noticeable effect on color of the coated films. WPI coated films also showed greater transparency and tensile strength than the other coated films. It was also suggested that WPI or SPI coatings on plastic films have great potential for acting as an efficient carrier for bioactive compounds in active food packaging systems. Nisin-incorporated WPI coatings on PP film exhibited significant bacterial growth. Results suggest that WPI coatings with a proper plasticizer possess excellent visual and mechanical characteristics and have great potential for application in active packaging systems.

Tihminlioglu, Atik, and Özen (2010) evaluated an affected by coating formulation, corn zein with different amounts (5% and 15%) was dissolved in 70% and 95% aqueous ethanol solution at 50 °C, respectively. Solutions of corn zein plasticized by PEG and GLY with various levels (20% and 50%) were applied on corona discharged- treated PP by using solvent-casting method. The significant improvements in water vapor and oxygen barrier properties of uncoated PP films were obtained with coating. WVP of the coated films decreased significantly with increasing corn zein concentration. The application of plasticized corn zein coating on PP films showed nearly more than three order of reduction in OP. The high water vapor and oxygen-barriers were obtained for films coated with higher amounts of corn zein plasticized by GLY.

Tihminlioglu, Atik, and Özen (2011) developed a novel film structure of corn zein coated (with different amounts of 5% and 15%) plasticized with PEG and GLY (with different levels of 20% and 50%) on corona discharge-treated PP films for food packaging applications. It was reported that a significant improvement in the coated film’s mechanical properties was observed compared to those of the uncoated PP. The effect of the plasticization of the coating solutions was also quite significant. The corn zein and plasticizer concentrations and plasticizer type used in the coating formulations were more effective parameters and had significant effects on the mechanical behavior of the coated PP films. The plasticization degree of the coating solutions increased considerably with elongation of the coated PP films, and the tensile strength decreased with increasing plasticizer content used.

2.6 Shelf life of foods in polymeric packaging material

2.6.1 Definition

For the majority of food products in which the quality decreases with time, it follows that there will be a finite length of time before the product becomes unacceptable. This time from production to unacceptability is referred to as shelf life (Robertson, 2012b).

2.6.2 Factors controlling shelf life

The product shelf life is typically controlled by three main factors of product intrinsic characteristic (i.e. perishability, bulk density and concentration), distribution environment (i.e. physical condition and climatic condition which respectively related to a transportation damage and mass or heat transfer, which are an important influence on rate of food deterioration, and packaging properties (i.e. water vapor transfer, gas and odor transfer and package-product interaction).

2.6.3 Permeability of polymeric packaging material

The protecting capacity of polymeric packaging mainly originated from its gas and vapor permeabilities that are deleterious to the quality of food product. The moisture gaining and losing lead to either a physical or biological defect. Also, the permeated oxygen can irreversibly causes oxidation of susceptible food components and rancidity.

2.6.3.1 Parameter affecting permeability

Jasse, Seuvre, and Mathlouthi (1994) reviewed that the permeability coefficient depends on the structure of polymer and characteristic of permeant. The polymer properties affecting permeability includes free volume, crystallinity, tacticity, cross-linking, orientation and thickness.

Apart from polymeric film structure, diffusive solubility flow is greatly affected by the size, shape and polarity of the penetrant molecules. Also, it was extent by relative humidity, temperature and pressure. The diameter or molar volume of permeant molecules mainly influences solubility coefficient (S) and diffusion coefficient (D). The increase in solubility with permeant size induces increase in S and decrease in D values. Also, the shape of permeant was found to show a linear relationship in function of molar volume (Rogers, 1985). Due to the interaction of polar groups in polymeric material with polar molecule (such as, alcohol, water, etc.), the polar group-contained polymer may be good for their non-polar gas barrier properties but poor for water vapors, which may be the influence of relative humidity. The package may absorb moisture and promotes its swelling/plasticizing, then reduces its oxygen barrier properties. When there are no polar groups in polymer, the OP is not influenced by relative humidity (Jasse *et al.,* 1994). The structural changes which occur at T_g greatly affect the gas diffusion and low molecular weight organic vapors. For gases and vapors which do not interact with polymer, the permeability coefficient is independent of the pressure of penetrant gas (Ashley, 1985). But for the case of interaction, the permeability increase with the increase in pressure due to the increasing of D values under the plasticizing effect of permeant as well as the increasing of S value (Cairns, Oswin, and Paine, 1974).

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

Whey protein isolate (WPI) (BiPRO®, 97.0% dry basis protein) provided by Davisco Food Intl. (Le Sueur, Minnesota, U.S.A.) was used to make the whey coating and film-forming solution, plasticized with glycerol (GLY) (QReC™, 99.5% purity) obtained from QReC chemical Co., Ltd. (Chonburi, Thailand). The Ingeo™ Biopolymer 2003D polylactic acid (PLA) used in this study was supplied by Natureworks LLC. (Minnetonka, Minnesota, U.S.A.). PLA resins were dried at 80 °C for 4 h prior to extrusion. Dried PLA resins were extruded through single-screw extruder which was attached to blown-film die (Blow film line 180/400E, Dr. Collin GmbH, Ebersberg, Hesse, Germany) at the facilities of Chemical Engineering Department, Faculty of Engineering, Chulalongkorn University (Bangkok, Thailand), with a temperature profile of 220 - 223 °C and a screw speed of 70 rpm to fabricate PLA film samples of 35 - 45 µm thickness. Surface modification of PLA film was done to achieve a surface energy of > 40 mN/m. Corona discharge-treatment (20 kV, 20 kHz) was performed on film surface using corona treater (IN TEN Co., Ltd., Taipei, Taiwan) equipped with 4 rolls of 45 cm x 0.2 cm electrodes at a speed of 5 m/min. Commercial surface treated linear low-density polyethylene (LLDPE) films of 65 - 75 µm thickness supplied by Prepack Thailand Co., Ltd. (Bangkok, Thailand) were also used as a substrate for WPI coating.

3.2 Methods

3.2.1 Characterization of multi-layer barrier film structures constituted from biopolymeric materials; polylactic acid (PLA) and whey protein isolate (WPI)

3.2.1.1 Film and coating formation

The WPI solution of 100 g/kg were prepared by dissolving WPI powder in distilled water and heated at 90 °C for 30 min in a water bath (NESLAB™ EX-10, Themo Fisher Scientific Inc., Newington, New Hampshire, U.S.A.) to partially denature proteins. The WPI solutions were cooled to room temperature and GLY was added as a plasticizer. Three ratios of WPI:GLY; 1:0.25, 1:0.40 and 1:0.67 (GLY 20%, 29% and 40% dry basis, respectively) were explored.

To cast single-layer WPI film, film-forming solutions containing 5 g of total solids (in order to maintain film thickness of approximately 80 - 100 μm) were poured onto acrylic plates with dimensions of 15 cm x 30 cm. After the cast films were allowed to dry in a tray dryer (Contherm Thermotec 2000 Oven, Contherm Scientific Ltd., Lower Hutt, New Zealand) at 50 °C for 15 h, the films were peeled off intact from the surface of the plates.

To compose three-layer films, PLA/WPI/PLA and LLDPE/WPI/LLDPE, a 1.5 ml amount of coating solution was applied and spread evenly on both surface-treated PLA or LLDPE- based film (dimension of 7.5 cm x 15 cm) before being covered by the top layer. The films were then dried (3 days for PLA-based films and 7 days for LLDPE-based films) at ambient temperature; 23 - 25 °C and 35 - 45% relative humidity (%RH). A total of three replications of film preparation were used to determine each property.

The film samples were conditioned at 25 °C, 50% RH in a desiccator with saturated salt solution of magnesium nitrate hexahydrate (QReC chemical Co., Ltd., Chonburi, Thailand) at least 48 h before measurements.

3.2.1.2 Film properties measurements

I) Thickness

The thickness of the films was measured with a digital micrometer (Model ID-C112, Mitutoyo Manufacturing Co., Ltd., Kanagawa, Japan) at five random positions before testing properties.

II) Transparency

Film transparency was determined according to standard method ASTM D1746-97 (AOAC, 1995). The films were cut into rectangular shapes (1 cm x 4 cm) then placed on the internal side of a cuvette cell. An empty cuvette was used as a reference. The percentage of light transmission (%T) through the films was measured by using a UV-visible spectrophotometer (Model Genesys 10, Thermo Fisher Scientific Inc., Rochester, New York, U.S.A.) at 600 nm. Transparency at 600 nm (T_{600}) was calculated from equation *(Eq. 3.1)* (Han and Floros, 1997) where *b* is the film thickness (mm). Five measurements were done for each film replication. The analysis was replicated three times.

$$T_{600} = (\log \%T)\ /b \qquad (Eq.\ 3.1)$$

III) Color

The color of the composite film was evaluated using a Chroma meter (Model CR-400, Minolta Co., Ltd., Tokyo, Japan) with pulsed xenon lamp D_{65} as a light source and 2 degree closely matches CIE 1931 standard ($\bar{x}2\lambda$, $\bar{y}\lambda$ and $\bar{z}\lambda$) as an observer. A white standard color plate (L^* = 97.29, a^* = -1.28, b^* = 1.26) was used for the instrument calibration and a white color plate (L^*= 92.96, a^* = -2.21, b^* = 1.97) was used as a background for color measurements of the films. The L^* (Lightness, ranging from 0 = black to 100 = white), a^* (ranging from $-a^*$ = greenness to $+a^*$ = redness) and b^* (ranging from $-b^*$ = blueness to $+b^*$ = yellowness) values were averaged from five random readings, for each film sample. The total color difference (ΔE_{ab}) was calculated using the equation (*Eq. 3.2)* (Lee *et al.*, 2008). The results were expressed as ΔE_{ab} values that used the substrate PLA or LLDPE as a reference. Ten measurements were done for each film replication. The analysis was repeated three times.

$$\Delta E_{ab} = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \qquad (Eq.\ 3.2)$$

IV) Mechanical properties

An Instron universal testing machine (Model 5565, Instron Engineering Corp., Canton, Massachusetts , U.S.A.) equipped with a 5 kg load cell was used to measure mechanical properties of the films, according to standard method ASTM D882-90 (ASTM, 1997). Test films were cut into rectangular strips (1 cm x 12 cm). Initial grip separation and cross-head speed were set at 70 mm and 50 mm/min, respectively. Tensile strength (TS) is the largest stress (force/area) in which a film is able to sustain before breakage. Percentage of elongation (%E) is the maximum change in length in which a film is able to sustain before breakage. Elastic modulus (EM) is the stress/strain ratio in elastic region, indicating resistance to elastic deformation. Ten measurements were done for each film replication. The analysis was replicated three times.

V) Oxygen permeability

An Ox-Tran 2/21 ST modular system (MOCON Inc., Minneapolis, Minnesota, U.S.A.) was used to measure oxygen transmission rate (OTR) according to standard method ASTM D3985-05 (ASTM, 2001). The OTR test range of ST modular system is 775 to 155,000 cc/m^2/day. OTRs were determined at 23 °C and 0% RH. Film samples were covered with an aluminum foil mask with an open testing area of 5 cm^2. Oxygen permeability (OP) was calculated by dividing the OTR by oxygen partial pressure and multiplying by the film thickness. Two measurements were done for each film replication. The analysis was replicated three times.

VI) Water vapor permeability

The water vapor transmission rate (WVTR) was determined according to ASTM E398-03 (ASTM, 2003) using a Permatran-W model 398 (MOCON Inc., Minneapolis, Minnesota, U.S.A.). The film sample was masked with an aluminum foil with an open testing area of 5 cm^2. Testing was performed at constant temperature, 23 °C and at a 90 - 10% RH gradient. Water vapor permeability (WVP) was evaluated the same way as the OP by replacing oxygen partial pressure value with water vapor pressure. Two measurements were done for each film replication. The analysis was replicated three times.

VII) Statistical analysis

A completely randomized experimental design was used in this experiment. All tests were replicated three times. Analysis of variance (ANOVA) and Duncan's new multiple comparison range test were utilized at a confidence level of 95%.

3.2.2 Effect of storage temperature on stability of PLA/WPI/PLA film

3.2.2.1 Film and coating formation

The single-layer WPI and three-layer PLA/WPI/PLA films were prepared by the same method as listed in 3.2.1.1. Only one optimized WPI:GLY ratio (selected from section 3.2.1) of 1:0.40 (GLY 29% dry basis) was examined. A total of three replications of film preparation were used to determine each property. Also, single-layer PLA with thickness of 70 - 80 μm (which obtained from the clinging of the original PLA film with 35 - 45 μm thickness) was used for comparison.

3.2.2.2 Film conditioning and storage

To study the effect of storage temperature on stability of PLA/WPI/PLA film, testing films were stored in the dark at three controlled temperatures, simulating common commercial storage conditions; 4, 25 and 35 °C, at 50% RH for 21 days. The constant %RH was maintained by saturated magnesium nitrate (QReC chemical Co., Ltd., Chonburi, Thailand) in the bottom of desiccators. %RH and temperature were monitored by a hygrometer (Thermo-Hygro, Fisher Scientific Inc., Fair Lawn, New Jersey, U.S.A.).

3.2.2.3 Film properties measurements

Incubated samples were evaluated on day 1, 7, 15 and 21 for thickness, transparency, color, mechanical properties, OP and WVP as done in section 3.2.1.2. ΔE_{ab} values were calculated using the data of corresponding one-day-old sample as reference.

Ox-Tran 2/21 MD modular system (MOCON Inc., Minneapolis, Minnesota, U.S.A.) with OTR test range 0.5 to 2,000 cc/m^2/day was used to determine OP in this section.

All the tests were replicated three times except OP test which was done for duplicated. Analysis of variance (ANOVA) and Duncan's new multiple comparison range test were utilized at a confidence level of 95%.

3.2.3 Storage stability of baby formula packaged in PLA/WPI/PLA pouch

3.2.3.1 Baby formula packing

The same three-layer PLA/WPI/PLA film as used in section 3.2.2 was prepared. After conditioned, the film was fabricated into pouch with dimension of 6.5 cm x 12 cm. Baby formula (Dumex Dugro$^®$ 1 Plus Super mix: Regular flavor, Dumex Co., Ltd., Samutprakarn, Thailand; *Appendix A.1*) of 15 g was packaged in four-side-seal pouch of PLA/WPI/PLA by using an impulse heat sealer with heating temperature at 50 ± 2.3 °C with heating time and dwelling time of 3 sec. Head space was minimized before finishing the top seal.

Baby formula packaged in four-side-seal pouch made of single-substrate PLA was prepared for comparison.

A total of 3 replications of PLA/WPI/PLA- and PLA-packaged baby formula were used to determine each property.

3.2.3.2 Controlled storage

The packaged baby formula samples were stored in a dark controlled %RH condition of 50% and controlled temperatures at 4, 25 or 35 °C for 19 days. The constant %RH was maintained by saturated magnesium nitrate (QReC chemical Co., Ltd., Chonburi, Thailand) in the bottom of desiccators. %RH and temperature were monitored by a hygrometer (Thermo-Hygro, Fisher Scientific Inc., Fair Lawn, New Jersey, U.S.A.)

3.2.3.3 Properties measurements

After storage for 0, 5, 12, and 19 days, the packaged baby formula samples were taken out from incubation. The dry baby formula samples were separated from their packaging.

The pouch were dust off all the remaining baby formula for measuring the thickness, transparency, color as mentioned in section 3.2.1.2. ΔE_{ab} values were calculated using the data of corresponding fresh film sample as reference.

The powdered baby formula samples were determined for water activity (a_w) by using a a_w meter, AquaLab Series 3 TE (Decagon Devices Inc., Pullman, Washington, U.S.A.) while, moisture content (%MC) were determined by using a HB43-S Halogen Moisture Analyze (Mettler-Toledo (Schweiz) GmbH, Greifensee, Switzerland). Moreover, the baby formula samples were also determined for oxidation stability over time. The extraction of lipid from baby formula was done by using the modified method from AOAC 905.02 Rose-Gottlieb (*Appendix A.2*). The extraction was measured for the conjugated diene hydro peroxide (***Appendix A.3***) and total carbonyl compound content (*Appendix A.4*) by using spectrophotometric method.

All the tests were replicated three times. Analysis of variance (ANOVA) and Duncan's new multiple comparison range test were utilized at a confidence level of 95%.

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Characterization of multi-layer barrier film structures constituted from biopolymeric materials; polylactic acid (PLA) and whey protein isolate (WPI)

4.1.1 Film formation

The transparent WPI layer could successfully be formed thinly (9.89 ± 2.73 μm) between PLA outer layers, as well as between linear low-density polyethylene (LLDPE) layers, as shown in *Figure 4.1*. and *Table 4.1*. The resulting multi-layer films, both PLA/WPI/PLA and LLDPE/WPI/LLDPE, showed no visible optical change and possessed good flexibility and layer-adhesion. The range of glycerol (GLY) contents used in this study gave sufficient plasticizing effect to the WPI film within the sandwiched structures preventing the films from cracking or curling. A good adhesion of multi-layer films was achieved via surface treatment of the base substrates; PLA or LLDPE. By passing the polymer substrate through the electromagnetic fields in corona-treatment, the air between the two surfaces ionizes and excited species are introduced onto the surface of polymer film. The attachment of polar functionalities on polymer surfaces via corona-treatment promotes adhesion and wettability (Rangavajhyala, Ghorpade, and Hanna, 1997; Ozdemir, Yurteri, and Sadikoglu, 1999).

(*a*) (*b*)

Figure 4.1: Multi-layer structures made of glycerol-plasticized whey protein isolate (WPI) sandwiched between two substrates, polylactic acid (PLA) and linear low-density polyethylene (LLDPE). PLA/WPI/PLA and LLDPE/WPI/LLDPE film samples were displayed on a dark **(*a*)** and a white **(*b*)** background.

4.1.2 Film properties measurements

I) Transparency

Percentage of light transmission (%T) and transparency at 600 nm (T_{600}) of single-layer films; WPI, PLA, LLDPE and multi-layer structures; PLA/WPI/PLA and LLDPE/WPI/LLDPE are shown in *Table 4.1*. The result shows that there was no significant effect of the GLY content ($p > 0.05$), used in this study, on transparency of WPI films. Transparencies of single substrate films were significantly reduced ($p \leq 0.05$) when formed into laminate structures. The reduction trend on transparency was also observed in the previous study (Lee *et al.*, 2008) which reported that the transparency of polypropylene (PP) film (38.2 ± 0.3 1/mm) was reduced when coated with plasticized WPI (17.8 to 28.6 1/mm). This is probably because of non-homogenous multi-layer structure is formed. The discontinuous layer of the films could affect the light transmission behavior and leads to the reduction of transparency value. However, all the %T that were obtained from all PLA composite structures were comparable to a transparent polyethylene terephthalate (PET) plastic (> 90%) (Schmid *et al.*, 2012).

II) Color

The color coordinates of the single and multi-layer films are shown in *Table 4.2.* Small values of *–a** and *+b** color dimensions are relatively very small compared to the full scale of CIE*Lab* color space, indicating that the films tend to show light neutral color. A summation of the differences in hue, lightness, and chroma between composite films and their parental substrate films were evaluated as total color difference (ΔE_{ab}) value. ΔE_{ab} values of PLA/WPI/PLA and LLDPE/WPI/LLDPE films were lower than the detectable threshold (above 3) of the human eye (Francis, 1983; Vichi, Ferrari, and Davidson, 2004) indicating that composite structures were not visibly different from PLA or LLDPE substrate films. Increasing GLY content did not result in ΔE_{ab} values greater than 3, thus there was no significant effect of GLY content on total color of composite structures ($p > 0.05$).

Table 4.1: Thickness, % light transmission (%T) and transparency at 600 nm (T_{600}) of single-layer films; linear low-density polyethylene (LLDPE), polylactic acid (PLA), glycerol (GLY)-plasticized whey protein isolate (WPI), and WPI-enhanced composite structures; PLA/WPI/PLA and LLDPE/WPI/LLDPE.

Film	WPI:GLY ratio	Thickness (mm)	%T (%)	T_{600} (1/mm)
LLDPE	-	0.066 ± 0.001^{Ba}	80.57 ± 0.14^{Bb}	29.07 ± 0.49^{Db}
LLDPE/WPI/LLDPE	**1:0.25**	0.138 ± 0.001^{Eb}	73.37 ± 0.50^{Aa}	13.51 ± 0.08^{Aa}
	1:0.40	0.142 ± 0.002^{Ec}	73.87 ± 0.08^{Aa}	13.12 ± 0.16^{Aa}
	1:0.67	0.143 ± 0.001^{Ec}	73.82 ± 0.44^{Aa}	13.09 ± 0.07^{Aa}
PLA	-	0.040 ± 0.001^{Aa}	91.35 ± 0.31^{Eb}	48.78 ± 0.92^{Ec}
PLA/WPI/PLA	**1:0.25**	0.086 ± 0.001^{Cb}	90.25 ± 0.46^{Da}	22.80 ± 0.33^{Cb}
	1:0.40	0.089 ± 0.002^{Cc}	90.08 ± 0.43^{Da}	22.09 ± 0.57^{BCab}
	1:0.67	0.091 ± 0.000^{Cc}	90.81 ± 0.79^{DEab}	21.43 ± 0.13^{BCa}
WPI	**1:0.25**	0.088 ± 0.009^{Ca}	87.44 ± 0.14^{Ca}	22.70 ± 2.42^{Ca}
	1:0.40	0.092 ± 0.009^{Ca}	87.93 ± 0.74^{Ca}	21.64 ± 2.04^{BCa}
	1:0.67	0.097 ± 0.008^{Da}	87.07 ± 0.79^{Ca}	20.26 ± 1.79^{Ba}

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–c) indicate significant differences between GLY content used among the same plastic substrate ($p \leq 0.05$).

***Different superscript upper-case letters (A-E) indicate significant differences between all samples ($p \leq 0.05$).

Table 4.2: *CIELab* color space and total color difference (ΔE_{ab}) of single-layer films; linear low-density polyethylene (LLDPE), polylactic acid (PLA), glycerol (GLY)-plasticized whey protein isolate (WPI) and WPI-enhanced composite structures; PLA/WPI/PLA and LLDPE/WPI/LLDPE.

Film	WPI:GLY ratio	*L**	*a**	*b**	ΔE_{ab}
LLDPE	-	$92.44^{Eb} \pm 0.04$	$-1.22^{Aa} \pm 0.02$	$2.58^{Aa} \pm 0.04$	-
LLDPE/ WPI/LLDPE	**1:0.25**	$91.54^{ABa} \pm 0.02$	$-1.23^{Aa} \pm 0.01$	$2.79^{BCb} \pm 0.03$	$0.90^{Ca} \pm 0.05$
	1:0.40	$91.44^{ABa} \pm 0.19$	$-1.24^{Aa} \pm 0.01$	$2.83^{BCDb} \pm 0.04$	$1.03^{Ca} \pm 0.09$
	1:0.67	$91.38^{Aa} \pm 0.32$	$-1.23^{Aa} \pm 0.02$	$2.86^{BCDb} \pm 0.15$	$1.07^{DCa} \pm 0.21$
PLA	-	$92.27^{DEc} \pm 0.09$	$-0.69^{Ca} \pm 0.00$	$2.67^{ABa} \pm 0.03$	-
PLA/WPI/ PLA	**1:0.25**	$91.94^{CDb} \pm 0.09$	$-0.69^{Ca} \pm 0.00$	$2.84^{BCDb} \pm 0.02$	$0.38^{Aa} \pm 0.09$
	1:0.40	$91.96^{CDb} \pm 0.06$	$-0.69^{Ca} \pm 0.00$	$2.90^{CDb} \pm 0.06$	$0.41^{Aa} \pm 0.09$
	1:0.67	$91.75^{BCa} \pm 0.05$	$-0.68^{Ca} \pm 0.00$	$2.99^{Dc} \pm 0.03$	$0.62^{Bb} \pm 0.05$
WPI	**1:0.25**	$92.19^{DEa} \pm 0.29$	$-1.05^{Bb} \pm 0.06$	$3.35^{Ea} \pm 0.15$	-
	1:0.40	$92.22^{DEa} \pm 0.29$	$-1.05^{Bb} \pm 0.06$	$3.35^{Ea} \pm 0.16$	-
	1:0.67	$91.98^{CDa} \pm 0.24$	$-1.25^{Aa} \pm 0.04$	$3.71^{Fb} \pm 0.19$	-

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–c) indicate significant differences between GLY content used among the same plastic substrate ($p \leq 0.05$).

***Different superscript upper-case letters (A-F) indicate significant differences between all samples ($p \leq 0.05$).

III) Mechanical properties

The tensile properties of single substrates (PLA or LLDPE), and composite films are shown in *Figure 4.2a*. The result showed that tensile strength (TS) of PLA and PLA/WPI/PLA were significantly higher than those of single LLDPE and LLDPE/WPI/LLDPE composites ($p \leq 0.05$). It is due to the fact that PLA films have the highest strength among testing films.

The single WPI film was insufficiently plasticized by WPI:GLY ratio of 1:0.25. The resulting single film was too brittle to measure its tensile properties. In contrast, the same GLY amount was sufficient to increase flexibility of WPI in middle-layer of composite structures. Entrapped water molecules in WPI layer between PLA or LLDPE were assumed to facilitate/enhance plasticization of GLY in composite structures. GLY bonds with water molecules, thereby further decreasing intermolecular interactions and increasing intermolecular distances between WPI molecules (Shaw *et al.*, 2002).

Increasing the GLY content in WPI film significantly resulted in reduction of TS of PLA/WPI/PLA structures ($p \leq 0.05$). Nevertheless, such a plasticizing effect was not significant in LLDPE/WPI/LLDPE samples ($p > 0.05$). It is probably because of the plasticization effect of the entrapped water (which was originally used as a solvent during the preparation of the WPI solution via coacervation) migrated into hydrophilic PLA structures. The remaining water, after completing the drying procedure, not only plasticized the WPI layer, but also the PLA films. A similar result was reported in the case of soy protein isolate (SPI) films coated with PLA, both water in the SPI film layer and solvent in the PLA coating layer seemed to work as plasticizers to result in increased film extensibility (Rhim, Lee, and Ng, 2007). Plasticizing effect of increasing GLY and/or relative humidity were reported to decrease TS of protein-based films (Gennadios, Park, and Weller, 1993; Lim, Mine, and Tung, 1999; Janjarasskul and Krochta, 2010b). Therefore, there were synergistic plasticizing effects of increasing GLY and associated water molecules on both WPI and PLA layers which leads to the significant reduction of tensile strength. On the other hand, GLY and the entrapped water molecule hardly migrate into hydrophobic LLDPE as compared to films containing PLA. The solubility coefficient of water molecules in LLDPE is much lower than that of PLA. The activation energy of water vapor permeation in low density polyethylene (LDPE) films was also reported to be much higher than that of PLA films (Auras, 2007). Hence the plasticization of either GLY or associated water molecules to LLDPE base films was limited compared to PLA.

Figure 4.2b shows elastic modulus (EM) of the films. The resulting modulus values, indicating film stiffness, conformed to associated TS values. EM of PLA-based films; single PLA and PLA/WPI/PLA, were significantly higher than those of LLDPE-based films; single LLDPE and LLDPE/WPI/LLDPE ($p \leq 0.05$).

The percentages of film elongation (%E) which expressed a film's extensibility are shown in *Figure 4.2c*. The value of %E of single LLDPE film and LLDPE/WPI/LLDPE structures could not be obtained because the film specimens did not break in the testing range. However, the maximum %E values, at the end of measurement, of LLDPE-based films were 330.15 ± 0.48% to 330.95 ± 0.26%. These values are significantly higher than those of single WPI film, single PLA substrate and PLA/WPI/PLA structure ($p \leq 0.05$).

As can be seen from the results, mechanical properties of the developed films mostly rely on intrinsic characteristic of the base films. PLA was reported to have higher TS and %E (Martin and Avérous, 2001; Auras *et al.*, 2003) than WPI films (Janjarasskul and Krochta, 2010a). Therefore, PLA could be used to improve TS of the composite structure. On the contrary, LLDPE is well known as flexible packaging material. The inherent high extensibility of LLDPE film contributed to the increased %E of the composite structure.

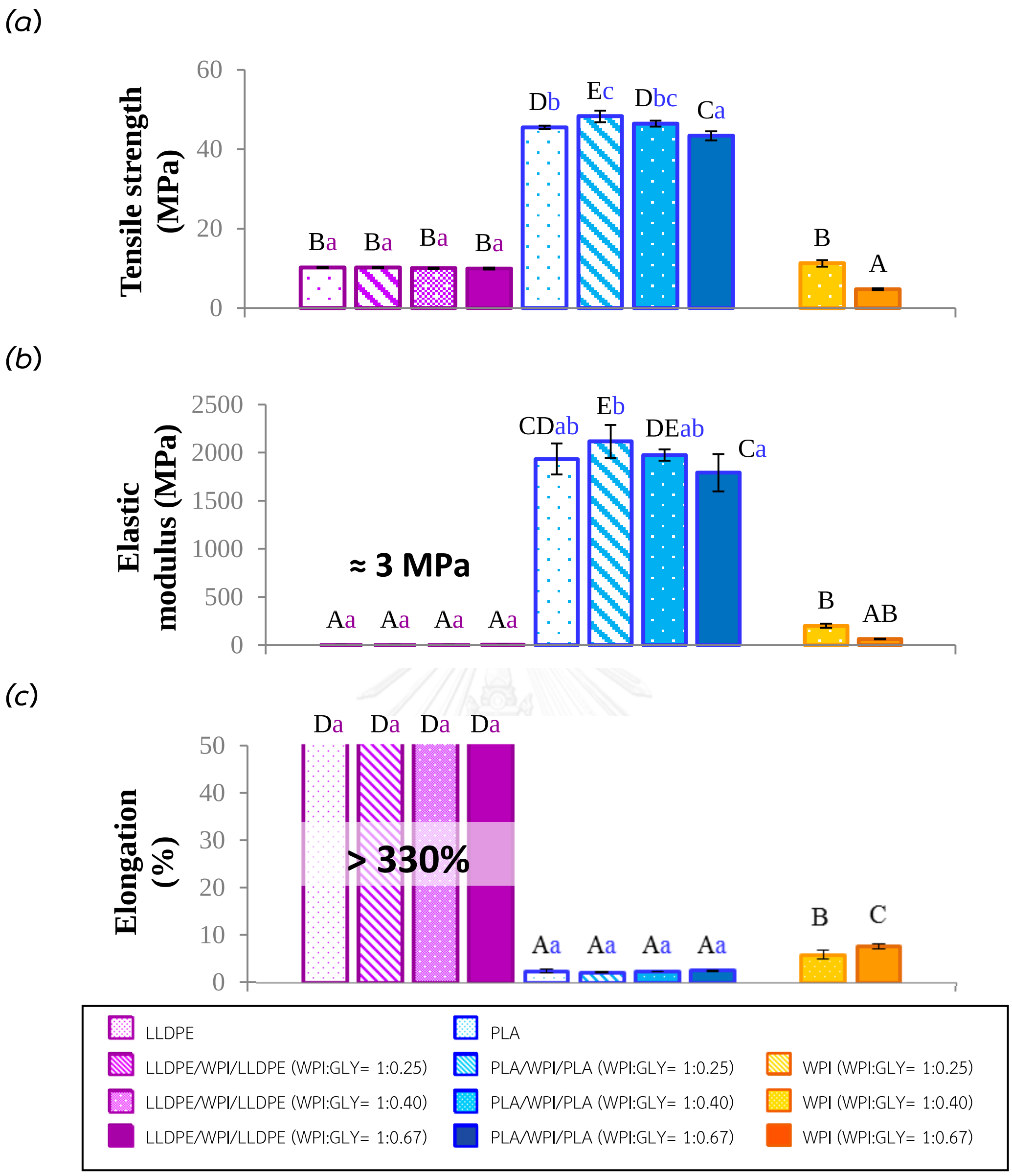

Figure 4.2: Mechanical properties: tensile strength ***(a)***, elastic modulus ***(b)*** and % elongation ***(c)***, of single-layer films; linear low-density polyethylene (LLDPE), polylactic acid (PLA), glycerol (GLY)-plasticized whey protein isolate (WPI), and WPI-enhanced composite structures; PLA/WPI/PLA and LLDPE/WPI/LLDPE.

* Each bar represents the average value. Error bars show standard deviations.

** Different lower-case letters (a–c) indicate significant differences between GLY content used among the same plastic substrate ($p \leq 0.05$).

*** Different upper-case letters (A-E) indicate significant differences between all samples ($p \leq 0.05$).

****The percentage of elongation data from LLDPE and LLDPE/WPI/LLDPE films could not be obtained because the specimens did not break in the testing range.

IV) Oxygen permeability (OP)

OPs of single substrate plastics and multi-layer structures are shown in *Figure 4.3*. The result showed that there was a trend of the slightly increasing OP with the increasing plasticizer content in the WPI layer between either PLA or LLDPE layers. However, there was no significant effect of GLY concentration in this study (WPI:GLY = 1:0.25, 1:0.40 and 1:0.67) on OPs of either PLA/WPI/PLA or LLDPE/WPI/LLDPE at 95% confidence level.

Nonetheless, proper range of incorporated plasticizer must be defined. GLY is a commonly used plasticizer incorporated into casting solution to increase flexibility of the film by reducing intermolecular hydrogen bonding between polymer chains. Researchers often reported that the increase molecular mobility resulted from GLY plasticizing effects increases the diffusivity coefficient and thus the permeability coefficient of oxygen (McHugh and Krochta, 1994a; Coupland *et al.*, 2000; Sothornvit and Krochta, 2000; Shaw *et al.*, 2002).

As shown in *Figure 4.3*, the OP of PLA single-layer was significantly reduced ($p \leq 0.05$) when laminated by thin WPI middle-layer into PLA/WPI/PLA. A much greater reduction trend could also be obtained from OP of stand-alone LLDPE film in which it was significantly reduced ($p \leq 0.05$) when formed into laminate LLDPE/WPI/LLDPE structures at 95% confidence level.

To compare the OP of the WPI between two lamination systems, the following equations for the three-layer model, were used.

$$l = l_1 + l_2 + l_3 \qquad (Eq.\ 4.1)$$

$$l/P = (l_1/P_1) + (l_2/P_2) + (l_3/P_3) \qquad (Eq.\ 4.2)$$

Where P represents the OP (cc·μm/m^2·day·kPa) and l represents the thickness (μm) of the layers, while the subscripts 1 and 3 represent the outer layers and 2 represents the WPI coating layer. By substituting values of l, l_1, l_2, l_3, P, P_1 and P_3, the OP of WPI coatings (P_2) can be calculated from equation *(Eq. 4.2).*

The calculated OPs of WPI layer sandwiched between PLA films are 77.23, 96.24 and 144.87 cc·μm/m^2·day·kPa at WPI:GLY of 1:0.25, 1:0.40 and 1:0.67, respectively. These calculated values are in the range of reported (Sothornvit and Krochta, 2000) OP of single cast WPI film of approximately 40.92 to 133.73 cc·μm/m^2·day·kPa at WPI:GLY of 1:0.25, 1:0.40 and 1:0.67. On the other hand, the calculated OPs of inner WPI layer between LLDPE substrates are 303.59, 326.65 and 363.93 cc·μm/m^2·day·kPa at WPI:GLY of 1:0.25, 1:0.40 and 1:0.67, respectively. The higher OPs of WPI in LLDPE/WPI/LLDPE, compared to those of WPI in PLA/WPI/PLA, were probably due to plasticizing effect of the entrapped water molecules remaining from casting solution in the lamination process. This is due to the fact that the solubility coefficient of water molecules in LLDPE is much lower than that of PLA. Although most of the water in casting solution could be removed in the drying process following the film forming procedures, the moisture transferring across LLDPE decreased as drying progressed and as the concentration gradient lessened.

V) Water vapor permeability (WVP)

Figure 4.4 shows WVPs of stand-alone substrate films and multi-layer structures measured at 23 °C, 90 to 10% RH. WVPs of PLA/WPI/PLA films were not significantly different from that of single PLA layer ($p > 0.05$). On the other hand, WVPs of LLDPE/WPI/LLPDE films were significantly higher than that of LLDPE single-layer film ($p \leq 0.05$). Similar to OP, there was no significant effect of the plasticizer content on WVPs of both laminated structures, at 95% confidence level. In 1994, McHugh, Aujard and Krochta reported much greater WVPs values of WPI films (119.8 - 154.6 g·mm/m^2·day·kPa for WPI:GLY ratios of 1:0.63 - 1:1 used at 25 °C, 0 - 100% RH and 70.0 g·mm/m^2·day·kPa for WPI:GLY ratio of 1:0.25 used at 25 °C, 0 - 77% RH) than those of PLA, PLA/WPI/PLA, LLDPE and LLDPE/WPI/LLDPE films. Thus, hydrophilic WPI layer did not contribute to moisture barrier ability of the multi-layer systems.

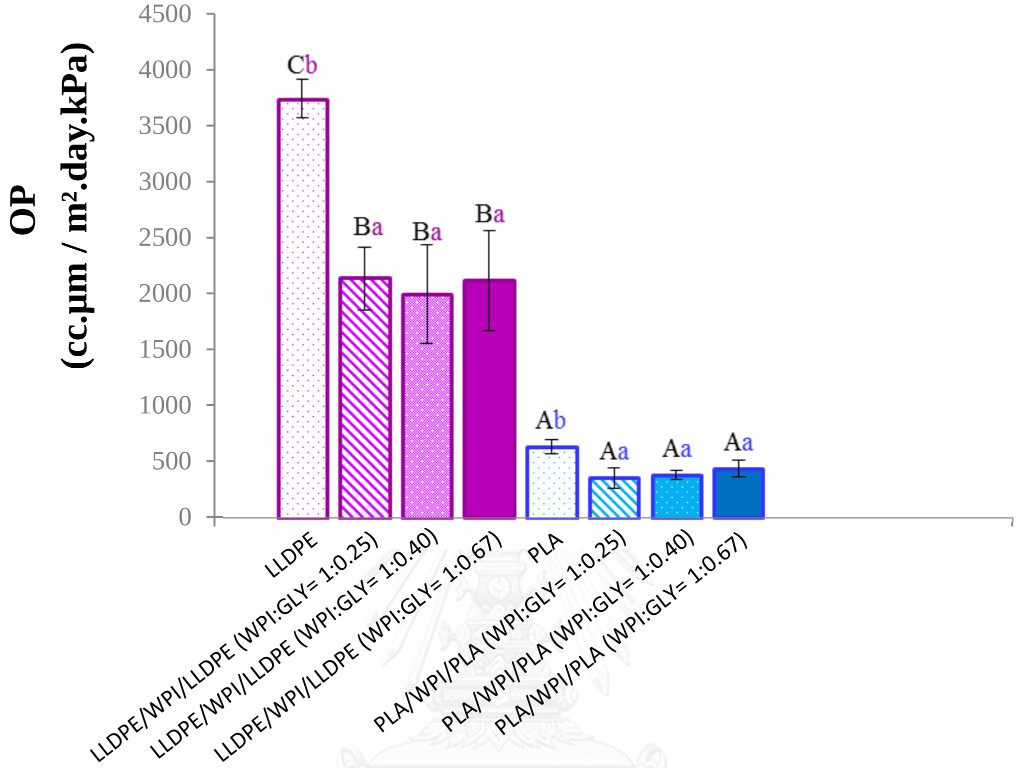

Figure 4.3: Oxygen permeability (OP) of single-layer films; linear low-density polyethylene (LLDPE), polylactic acid (PLA), glycerol (GLY)-plasticized whey protein isolate (WPI), and WPI-enhanced composite structures; PLA/WPI/PLA and LLDPE/WPI/LLDE, determined at 23 °C, 0% relative humidity.

* Each bar represents the average value. Error bars show standard deviations.

** Different lower-case letters (a-b) indicate significant differences between GLY content used among the same plastic substrate ($p \leq 0.05$).

***Different upper-case letters (A-C) indicate significant differences between all samples ($p \leq 0.05$)

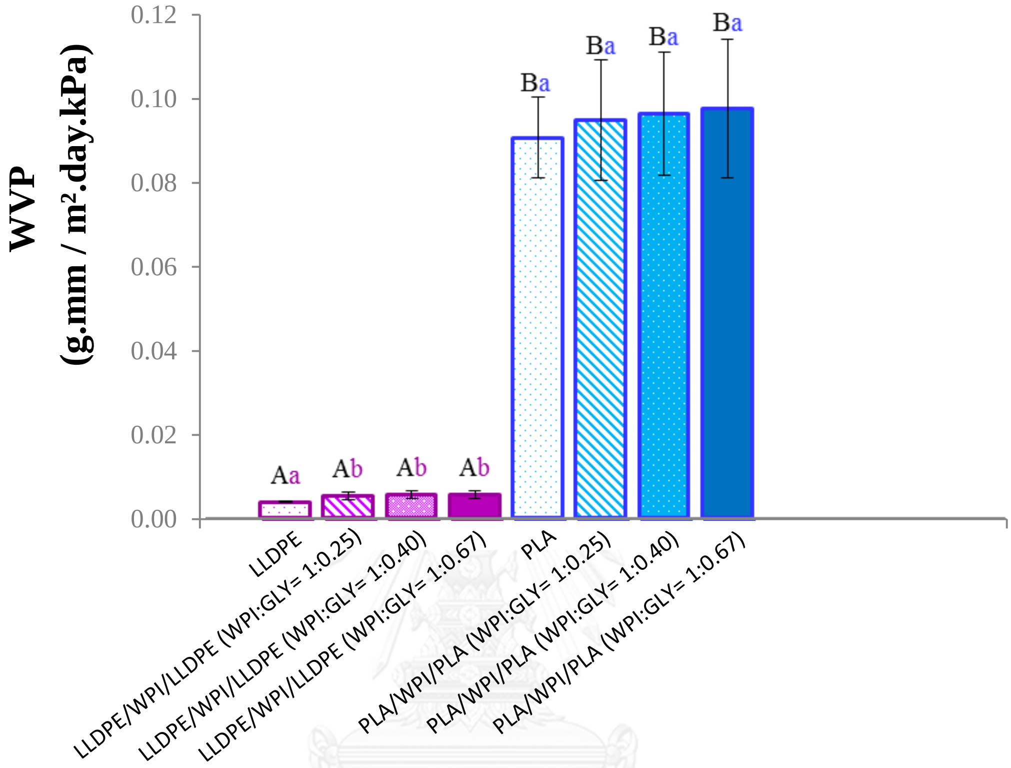

Figure 4.4: Water vapor permeability (WVP) of single-layer films; linear low-density polyethylene (LLDPE), polylactic acid (PLA), glycerol (GLY)-plasticized whey protein isolate (WPI), and WPI-enhanced composite structures; PLA/WPI/PLA and LLDPE/WPI/LLDPE, determined at 23 °C, 90 - 10% relative humidity.

* Each bar represents the average value. Error bars show standard deviations.

** Different lower-case letters (a-b) indicate significant differences between GLY content used among the same plastic substrate ($p \leq 0.05$).

***Different upper-case letters (A-B) indicate significant differences between all samples ($p \leq 0.05$)

4.2 Effect of storage temperature on stability of PLA/WPI/PLA film

According to the results in section 4.1, increases in GLY level led to the higher permeability values. There was no significant effect of GLY level used on the overall properties of WPI and PLA/WPI/PLA films accept mechanical properties. Thus, among the three ratios used, WPI:GLY ratio of 1:0.40 were selected to use in the study of film stability. Because this ratio was the minimum amount of GLY to produce the film that possessed proper plasticizing effect for both films.

4.2.1 Film formation

Figure 4.5 displays single-layer substrates; PLA and WPI, and their composite structure PLA/WPI/PLA (thickness of 0.074 ± 0.001 to 0.078 ± 0.001 mm, 0.091 ± 0.001 to 0.093 ± 0.001 mm and 0.086 ± 0.002 to 0.089 ± 0.001 mm, respectively) on a white background. Overall, the laminate structure has visible properties between its parental substrates. The multi-layer film showed no visible optical change and maintained good handling ability and layer adhesion throughout the storage test.

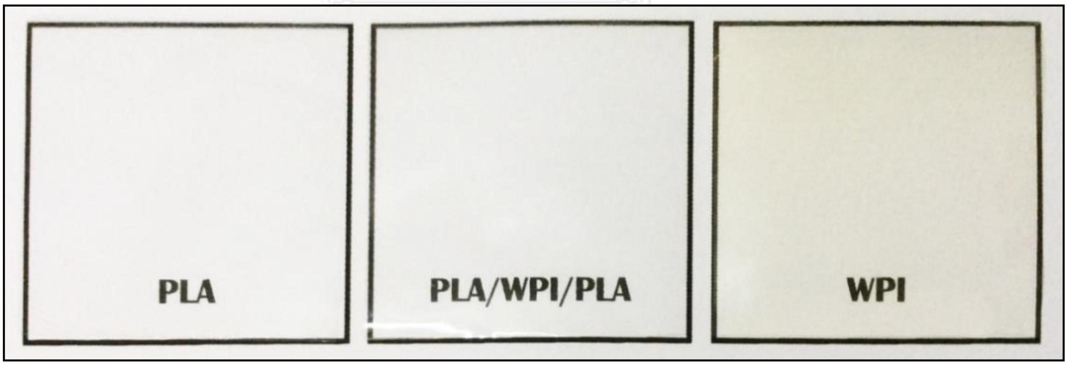

Figure 4.5: Single-layer polylactic acid (PLA) film, whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) and glycerol-plasticized WPI single cast film.

4.2.2 Film properties measurements

I) 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 4.6*. The result showed that T_{600} of PLA was stable over 21 days at all storage temperatures. Although, PLA biopolymer decomposes via hydrolytic degradation into smaller polymer molecules/fragments, and finally lactic acid, which can later be consumed by microorganisms as nutrients into carbon dioxide, water and biomass, the degradation process is temperature and humidity dependent (Piemonte, Sabatini, and Gironi, 2013). High molecular weight PLA plastic films were reported to have good storability at 28, 40 and 55 °C at 50% RH (Ho *et al.*, 1999).

On the other hand, T_{600} of WPI film slowly decreased at 25 and 35 °C over 21 days of storage. This is probably due to 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, 40 and 55 °C at 75% RH were reported to cause by Maillard reaction (Trezza and Krochta, 2000). Slow molecular rearrangement over storage time is hypothesized to also happen over time. GLY, used to plasticize WPI film, could migrate from the bulk of the film matrix to the surface due to weak interaction between protein molecules and GLY. Park *et al.* (1994) reported the visible change of cast zein film from initially transparent to appear greasy and cloudy because of glycerol sweating out.

T_{600} of composite PLA/WPI/PLA gradually decreased at 35 °C over storage time. As expected, composite structure has transparency stability between its parental materials.

These decreasing trends in T_{600} of all films can be fitted by either zero-order or first-order reaction models (with $R^2 \geq 0.90$) as shown in *Table 4.3*. The rate constants were temperature dependent. However, changes of film transparency did not follow Arrhenius behavior.

Overall, all of the films have rather small rates of change in transparency at these testing conditions suggesting that these biopolymer films have a good visual stability during storage prior to being used as food packaging.

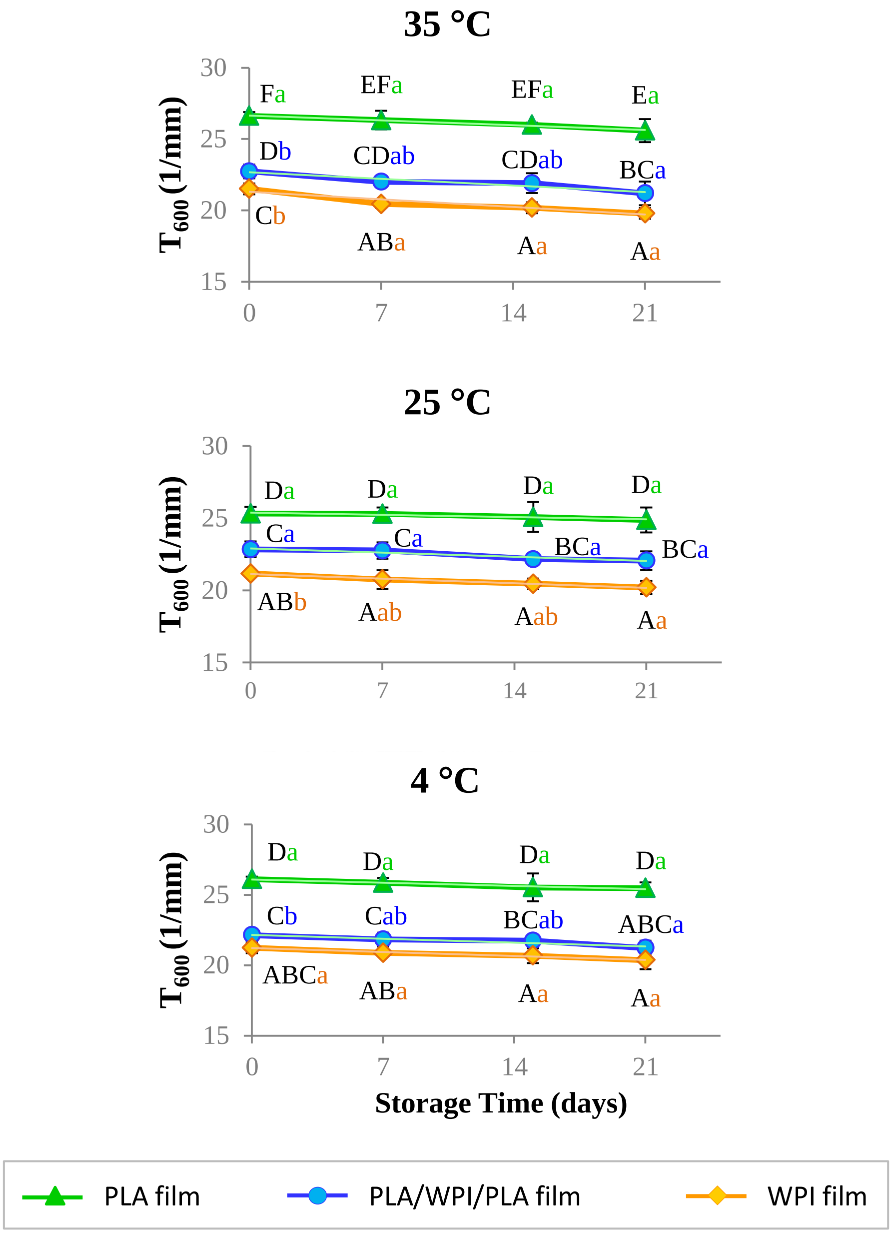

Figure 4.6: Effect of time and temperature on transparency at 600 nm (T_{600}) of single-layer polylactic acid (PLA) film, whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) and glycerol-plasticized WPI single cast film during controlled storage 50% relative humidity at 4, 25 and 35 °C for 21 days.

* Each data point represents average value. Error bars show standard deviations.

** Different lower-case letters (a-b) indicate significant differences in each film samples during storage ($p \leq 0.05$).

*** Different upper-case letters (A-F) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Table 4.3: Linear-model kinetics parameters for T_{600} : transparency at 600 nm (1/mm) 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 50% relative humidity at 4, 25 and 35 °C for 21 days.

Storage Tempe-rature (°C)	Film	Zero-order kinetics model	R^2	First-order kinetics model	R^2
35	PLA	$T_{600} = -0.0481t + 26.658$	0.99	$\ln T_{600} = -0.0019t + 3.2822$	0.98
	PLA/WPI/PLA	$T_{600} = -0.0656t + 22.667$	0.91	$\ln T_{600} = -0.0030t + 3.1194$	0.93
	WPI	$T_{600} = -0.0762t + 21.302$	0.90	$\ln T_{600} = -0.0037t + 3.0552$	0.91
25	PLA	$T_{600} = -0.0220t + 25.381$	0.92	$\ln T_{600} = -0.0009t + 3.2328$	0.94
	PLA/WPI/PLA	$T_{600} = -0.0418t + 22.908$	0.92	$\ln T_{600} = -0.0020t + 3.1305$	0.95
	WPI	$T_{600} = -0.0449t + 21.126$	0.99	$\ln T_{600} = -0.0024t + 3.0439$	1.00
4	PLA	$T_{600} = -0.0321t + 26.085$	0.96	$\ln T_{600} = -0.0013t + 3.2610$	0.97
	PLA/WPI/PLA	$T_{600} = -0.0389t + 22.153$	0.90	$\ln T_{600} = -0.0018t + 3.0975$	0.90
	WPI	$T_{600} = -0.0395t + 21.213$	0.98	$\ln T_{600} = -0.0018t + 3.0477$	0.97

II) Color

Figure 4.7 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 ($p \le 0.05$). 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 increases of a^* (negative values indicate green while positive values indicate magenta) and b^* (negative values indicate blue and positive values indicate yellow) values (*Appendix B.9 - Appendix B.11*) The color change of WPI film (water activity; a_w = 0.593 ± 0.003 to 0.647 ±

0.002) was hypothesized to be mainly by non-enzymatic Maillard browning of whey proteins and trace reducing sugar which can slowly occur during storage even at room temperature (McHugh and Krochta, 1994b; Miller, Upadhyaya, and Krochta, 1998). Trezza and Krochta (2000) 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). Lin and Krochta (2003) 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 ± 5% RH ($p \leq 0.05$).

These increasing trends of ΔE_{ab} of all films can be fitted by either zero-order (with $R^2 \geq 0.95$) or first-order reaction models (with $R^2 \geq 0.90$), as shown in *Table 4.4*. 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 (Trezza and Krochta, 2000). However, the increasing ΔE_{ab} of WPI film did not follow Arrhenius equation. This is probably due to 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 (Trezza and Krochta, 2000).

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 ($\Delta E_{ab} > 3$) (Francis, 1983; Vichi *et al.*, 2004) 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), are 84, 237 and 209 days, respectively.

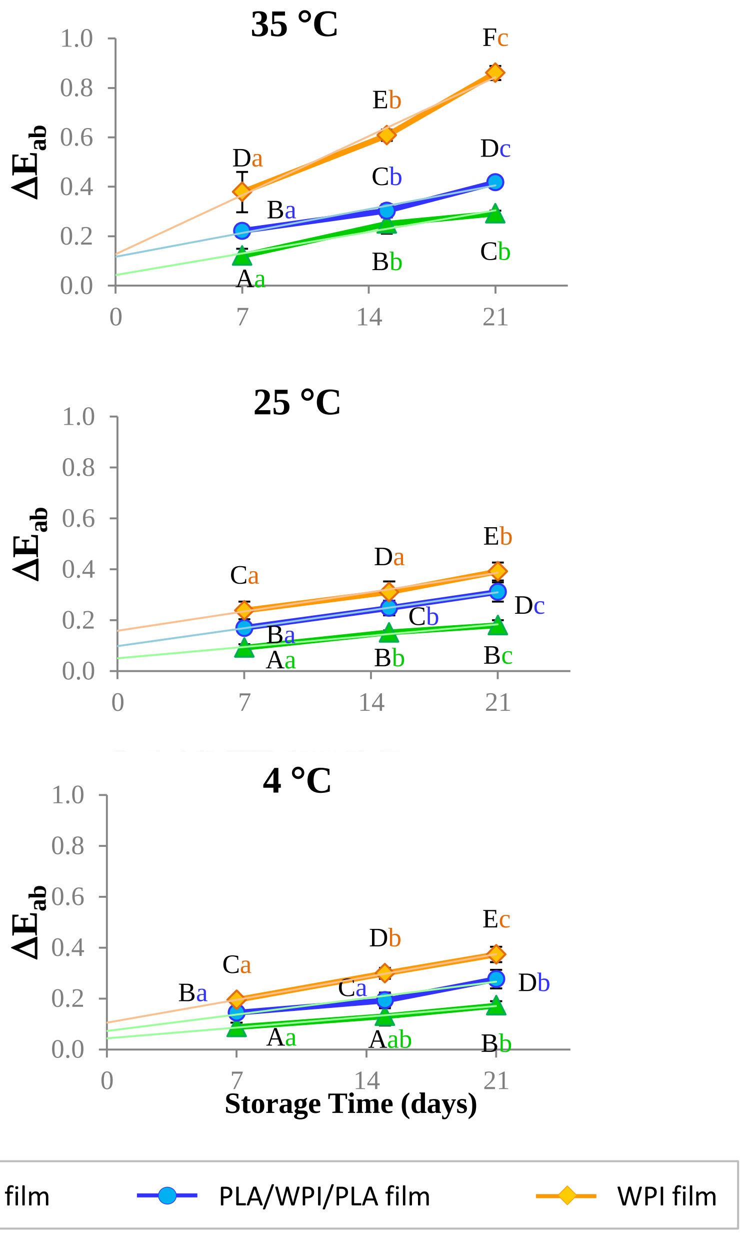

Figure 4.7: Effect of time and temperature on total color difference (ΔE_{ab}) of single-layer polylactic acid (PLA) film, whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) and glycerol-plasticized WPI single cast film during controlled storage 50% relative humidity at 4, 25 and 35 °C for 21 days.

* Each data point represents average value. Error bars show standard deviations.

** Different lower-case letters (a-c) indicate significant differences in each film samples during storage ($p \leq 0.05$).

*** Different upper-case letters (A-F) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Table 4.4: Linear-model kinetics parameters for 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 50% relative humidity at 4, 25 and 35 °C for 21 days.

Storage Tempe-rature (°C)	Film	Zero-order kinetics model	R^2	First-order kinetics model	R^2
35	PLA	$\Delta E_{ab} = 0.0125t + 0.0429$	0.96	$\ln \Delta E_{ab} = 0.0667t - 2.6380$	0.90
	PLA/WPI/PLA	$\Delta E_{ab} = 0.0138t + 0.1166$	0.97	$\ln \Delta E_{ab} = 0.0471t - 1.9661$	0.97
	WPI	$\Delta E_{ab} = 0.0341t + 0.1272$	0.99	$\ln \Delta E_{ab} = 0.0616t - 1.4826$	1.00
25	PLA	$\Delta E_{ab} = 0.0064t + 0.0497$	0.99	$\ln \Delta E_{ab} = 0.0575t - 2.9203$	0.94
	PLA/WPI/PLA	$\Delta E_{ab} = 0.0101t + 0.0977$	1.00	$\ln \Delta E_{ab} = 0.0364t - 2.0765$	0.97
	WPI	$\Delta E_{ab} = 0.0109t + 0.1578$	0.99	$\ln \Delta E_{ab} = 0.0412t - 1.8704$	1.00
4	PLA	$\Delta E_{ab} = 0.0060t + 0.0439$	0.99	$\ln \Delta E_{ab} = 0.0537t - 2.9164$	1.00
	PLA/WPI/PLA	$\Delta E_{ab} = 0.0092t + 0.0722$	0.95	$\ln \Delta E_{ab} = 0.0429t - 2.2836$	0.99
	WPI	$\Delta E_{ab} = 0.0128t + 0.1053$	1.00	$\ln \Delta E_{ab} = 0.0484t - 2.0306$	0.99

III) 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. The results are shown in *Figure 4.8, Figure 4.9 and Figure 4.10,* respectively. The results show 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 4.8 - Figure 4.10* showed that there were decreases in TS and EM and increase in %E of all testing films during storage at 4 and 25 °C, 50% RH. Although such changes were statistically significant ($p \leq 0.05$), the rates of changes are minute *(Table 4.5 - Table 4.7)*. Osés *et al.* (2009) 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. Gennadios *et al.* (1993) reported that effect of temperature on storage (23 - 75% RH, 5 – 45 °C) corn 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. At constant %RH storage, the amount of water absorbed by food materials typically decreases with an increases in temperature (LaBuza, 1968, 1984). Thus, more water was bound at lower temperature. The increasing bound water in protein-based film matrix could plasticize and cause weakening of protein-network. On the contrary, Anker, Stading, and Hermansson (2001) 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. 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 seem 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 is reported to increase the formation of covalent bonds between protein chains and as water is evaporated, closer interaction occurs between the protein chains, resulting in increased TS (Miller *et al.*, 1997). In addition, the similar mechanical properties improvement of protein-based films as effect of heat-curing was reported by many researchers (Gennadios *et al.*, 1996; Miller *et al.*, 1997; Kim *et al.*, 2002; Amin and Ustunol, 2007).

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 $R^2 \geq 0.85$) as shown in *Table 4.5, Table 4.6* and *Table 4.7.* 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, 25 °C, 50% RH.

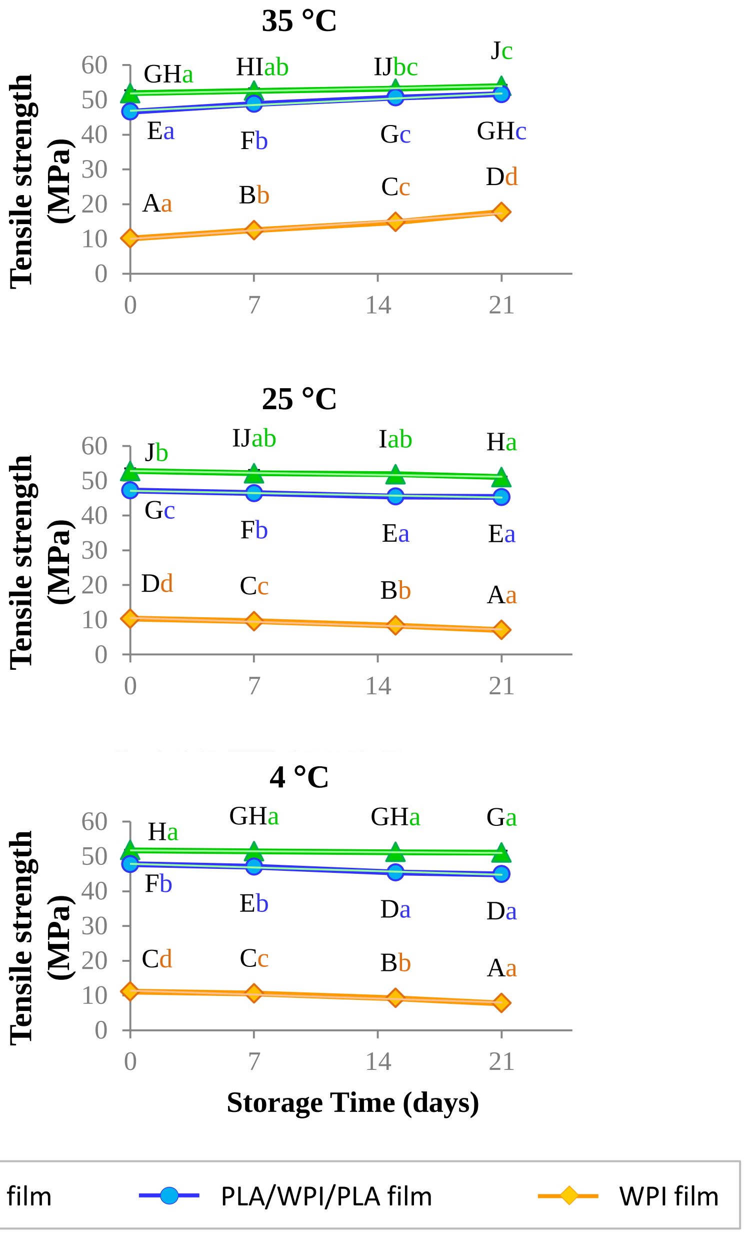

Figure 4.8: Effect of time and temperature on tensile strength of single-layer polylactic acid (PLA) film, whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) and glycerol-plasticized WPI single cast film during storage for 21 days.

* Each data point represents average value. Error bars show standard deviations.

** Different lower-case letters (a-d) indicate significant differences in each film samples during storage ($p \leq 0.05$).

*** Different upper-case letters (A-J) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

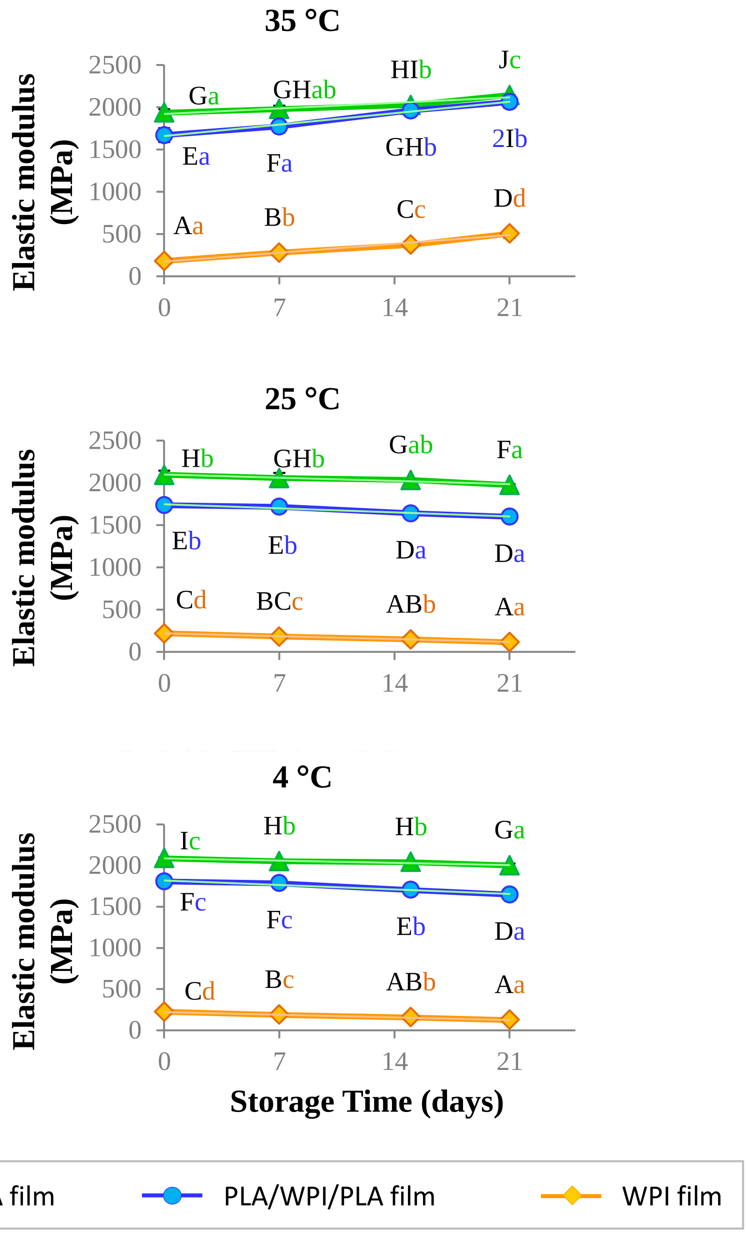

Figure 4.9: Effect of time and temperature on elastic modulus of single-layer polylactic acid (PLA) film, whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) and glycerol-plasticized WPI single cast film during controlled storage 50% relative humidity at 4, 25 and 35 °C for 21 days.

* Each data point represents average value. Error bars show standard deviations.

** Different lower-case letters (a-d) indicate significant differences in each film samples during storage (*p* ≤ 0.05).

*** Different upper-case letters (A-J) indicate significant differences between all samples among the same storage temperature used (*p* ≤ 0.05).

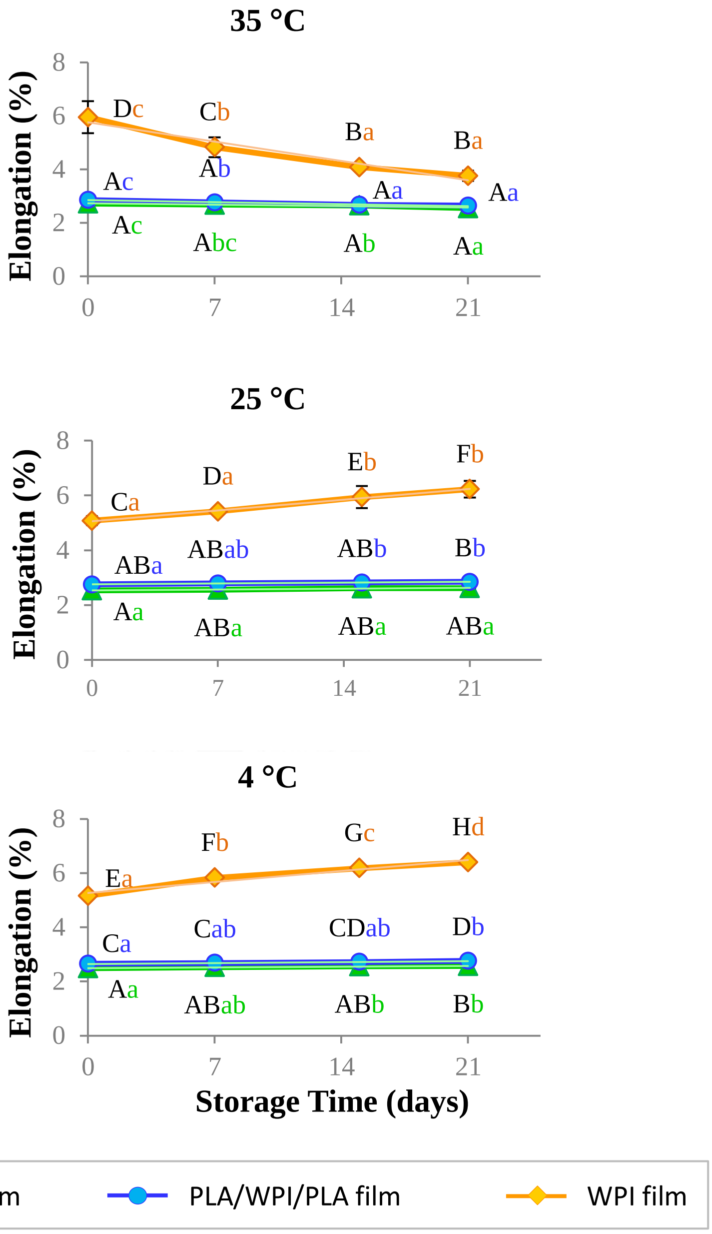

Figure 4.10: Effect of time and temperature on percentage of elongation of single-layer polylactic acid (PLA) film, whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) and glycerol-plasticized WPI single cast film during controlled storage 50% relative humidity at 4, 25 and 35 °C for 21 days.

* Each data point represents average value. Error bars show standard deviations.

** Different lower-case letters (a-d) indicate significant differences in each film samples during storage ($p \leq 0.05$).

*** Different upper-case letters (A-H) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Table 4.5: Linear-model kinetics parameters for TS : tensile strength (MPa) 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 50% relative humidity at 4, 25 and 35 °C for 21 days.

Storage Tempe-ratur (°C)	Film	Zero-order kinetics model	R^2	First-order kinetics model	R^2
35	PLA	TS = 0.0968t + 51.886	0.99	ln TS = 0.0015t + 3.9493	0.99
	PLA/WPI/PLA	TS = 0.2395t + 46.857	0.98	ln TS = 0.0052t + 3.8364	0.99
	WPI	TS = 0.3525t + 10.007	0.99	ln TS = 0.0269t + 2.3022	0.99
25	PLA	TS = -0.0767t + 52.758	0.95	ln TS = -0.0014t + 3.9638	0.94
	PLA/WPI/PLA	TS = -0.0938t + 47.107	0.96	ln TS = -0.0022t + 3.8508	0.92
	WPI	TS = -0.1560t + 10.509	0.98	ln TS = -0.0182t + 2.3511	0.97
4	PLA	TS = -0.0327t + 51.745	0.97	ln TS = -0.0007t + 3.9457	0.90
	PLA/WPI/PLA	TS = -0.1476t + 47.859	0.98	ln TS = -0.0034t + 3.8659	0.95
	WPI	TS = -0.1596t + 11.389	0.96	ln TS = -0.0169t + 2.4366	0.94

Table 4.6: Linear-model kinetics parameters for EM : elastic modulus (MPa) 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 50% relative humidity at 4, 25 and 35 °C for 21 days.

Storage Tempe-ratur (°C)	Film	Zero-order kinetics model	R^2	First-order kinetics model	R^2
35	PLA	EM = 9.2772t + 1922.5	0.90	ln EM = 0.0043t + 7.5550	0.96
	PLA/WPI/PLA	EM = 19.334t + 1659.8	0.99	ln EM = 0.0096t + 7.4051	0.99
	WPI	EM = 14.897t + 175.29	0.98	ln EM = 0.0483t + 5.1826	0.99
25	PLA	EM = -5.3568t + 2097.2	0.95	ln EM = -0.0029t + 7.6431	0.98
	PLA/WPI/PLA	EM = -6.9009t + 1747.3	0.96	ln EM = -0.0039t + 7.4530	0.99
	WPI	EM = -4.8782t + 217.90	1.00	ln EM = -0.0287t + 5.3636	0.99
4	PLA	EM = -3.7522t + 2085.8	0.94	ln EM = -0.0022t + 7.6410	0.96
	PLA/WPI/PLA	EM = -7.8739t + 1819.6	0.96	ln EM = -0.0049t + 7.5010	0.98
	WPI	EM = -4.6139t + 222.90	1.00	ln EM = -0.0270t + 5.3996	0.99

Table 4.7: Linear-model kinetics parameters for %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 50% relative humidity at 4, 25 and 35 °C for 21 days.

Storage Temperature (°C)	Film	Zero-order kinetics model	R^2	First-order kinetics model	R^2
35	PLA	%E = -0.0078t + 2.7375	0.90	ln %E = -0.0028t + 0.9995	0.88
	PLA/WPI/PLA	%E = -0.0104t + 2.8477	0.98	ln %E = -0.0044t + 1.0365	0.98
	WPI	%E = -0.1035t + 5.7683	0.96	ln %E = -0.0215t + 1.7247	0.99
25	PLA	%E = 0.0044t + 2.5355	0.96	ln %E = 0.0015t + 0.9258	1.00
	PLA/WPI/PLA	%E = 0.0045t + 2.7550	1.00	ln %E = 0.0017t + 1.0029	0.91
	WPI	%E = 0.0560t + 5.0629	1.00	ln %E = 0.0104t + 1.5926	1.00
4	PLA	%E = 0.0039t + 2.4963	0.95	ln %E = 0.0015t + 0.9098	0.96
	PLA/WPI/PLA	%E = 0.0050t + 2.6498	0.98	ln %E = 0.0015t + 0.9701	0.85
	WPI	%E = 0.0576t + 5.2737	0.95	ln %E = 0.0101t + 1.6422	0.93

IV) Oxygen permeability

Values of OP of the PLA, WPI and PLA/WPI/PLA films as a function of storage time and temperature are shown in *Figure 4.11*. The result showed that all of the testing films were able to maintain their oxygen barrier properties at 4 and 25 °C, 50% RH, throughout the storage time.

At 35 °C, PLA film did not significantly changed 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 $R^2 \geq 0.94$) as shown in *Table 4.8*.

Although, OP of PLA film did not significantly changed over time ($p > 0.05$), but its decreasing trend could also be fitted by either zero-order or first-order reaction models with lower R^2 (0.89 and 0.88, respectively), (*Table 4.8*).

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-liking and polymerization of proteins (Miller *et al.*, 1997).

Although the films were stored at controlled 50% RH in order to minimize moisture absorption and desorption, the moisture loss occurred as biopolymer molecules rearranged themselves to their equilibrium over time or due to intrinsic instability of their raw material (Wittaya, 2012). Moisture loss of protein-based films during storage was reported even in controlled humidity environments (Anker *et al.*, 2001; Hernández-Muñoz *et al.*, 2004). Water decreases glass transition temperature of protein-based films induced by a plasticization phenomena (Wittaya, 2012). 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 (Sothornvit and Krochta, 2005).

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.

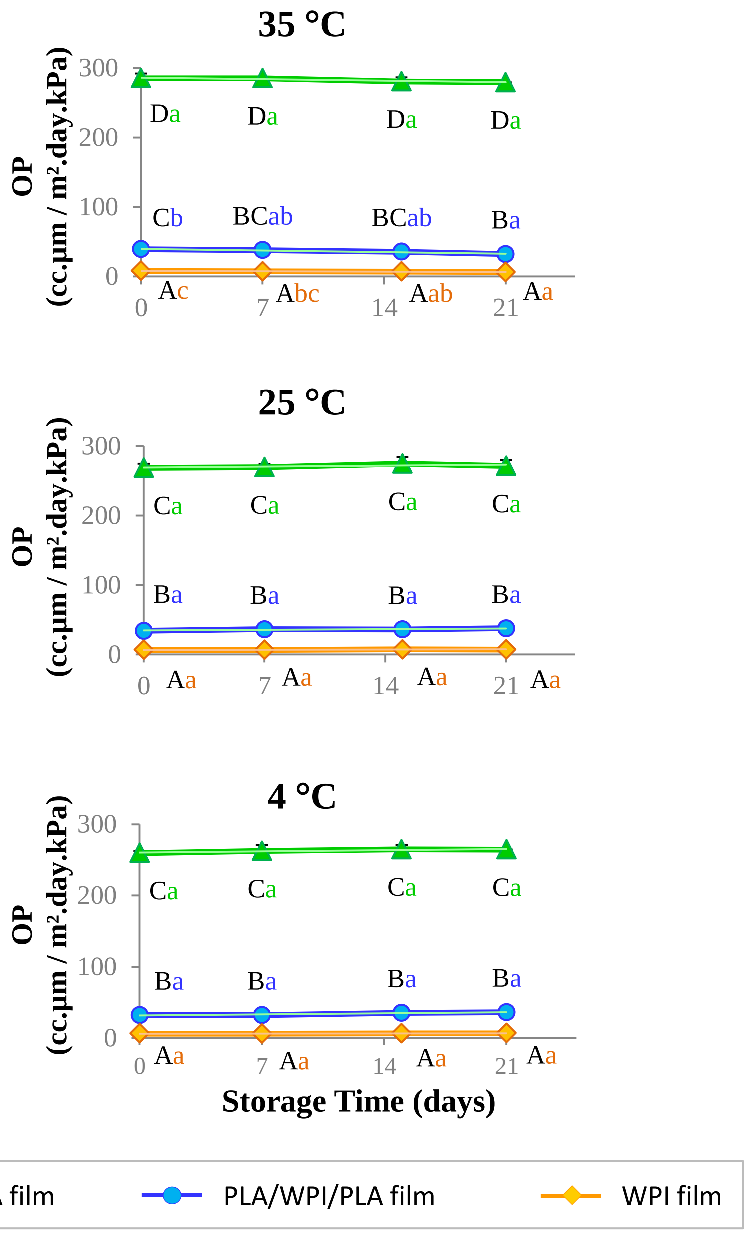

Figure 4.11: Effect of time and temperature on oxygen permeability (OP) of single-layer polylactic acid (PLA) film, whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) and glycerol-plasticized WPI single cast film during controlled storage 50% relative humidity at 4, 25 and 35 °C for 21 days.

* Each data point represents average value. Error bars show standard deviations.

** Different lower-case letters (a-c) indicate significant differences in each film samples during storage ($p \le 0.05$).

***Different upper-case letters (A-D) indicate significant differences between all samples among the same storage temperature used ($p \le 0.05$).

Table 4.8: Linear-model kinetics parameters for OP : oxygen permeability (cc.µm/m².day.kPa) 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 50% relative humidity at 35 °C for 21 days.

Storage Tempe-rature (°C)	Film	Zero-order kinetics model	R^2	First-order kinetics model	R^2
35	PLA	OP = -0.3151t + 286.0925	0.89	ln OP = -0.0011t + 5.6562	0.88
	PLA/WPI/PLA	OP = -0.3321t + 39.8423	0.95	ln OP = -0.0093t + 3.6874	0.94
	WPI	OP = -0.0681t + 7.9449	0.99	ln OP = -0.0095t + 2.0745	0.99

V) Water vapor permeability

The effect of storage time and temperature on WVP of the films are illustrated in *Figure 4.12*. 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, as shown in *Table 4.9*. 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 4.10*, are useful prediction of film WVP stability 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 crosslinks formation of amino acid residues, resulting in the increasing of protein hydrophobicity (Cheftel, Cuq, and Lorient, 1985; Gennadios *et al.*, 1996; Miller *et al.*, 1997; Kim *et al.*, 2002). 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.* (2001) 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. 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 to -45 °C. Similar finding in other protein-based film plasticized with GLY have also been reported (Hernández-Muñoz *et al.*, 2004).

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 (Ljungberg *et al.*, 2003). Hassouna *et al.* (2012) investigated the aging of PLA/ATBC systems at 20 °C (almost at T_g) for 6 months. 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. 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 (Dobircau *et al.*, 2014).

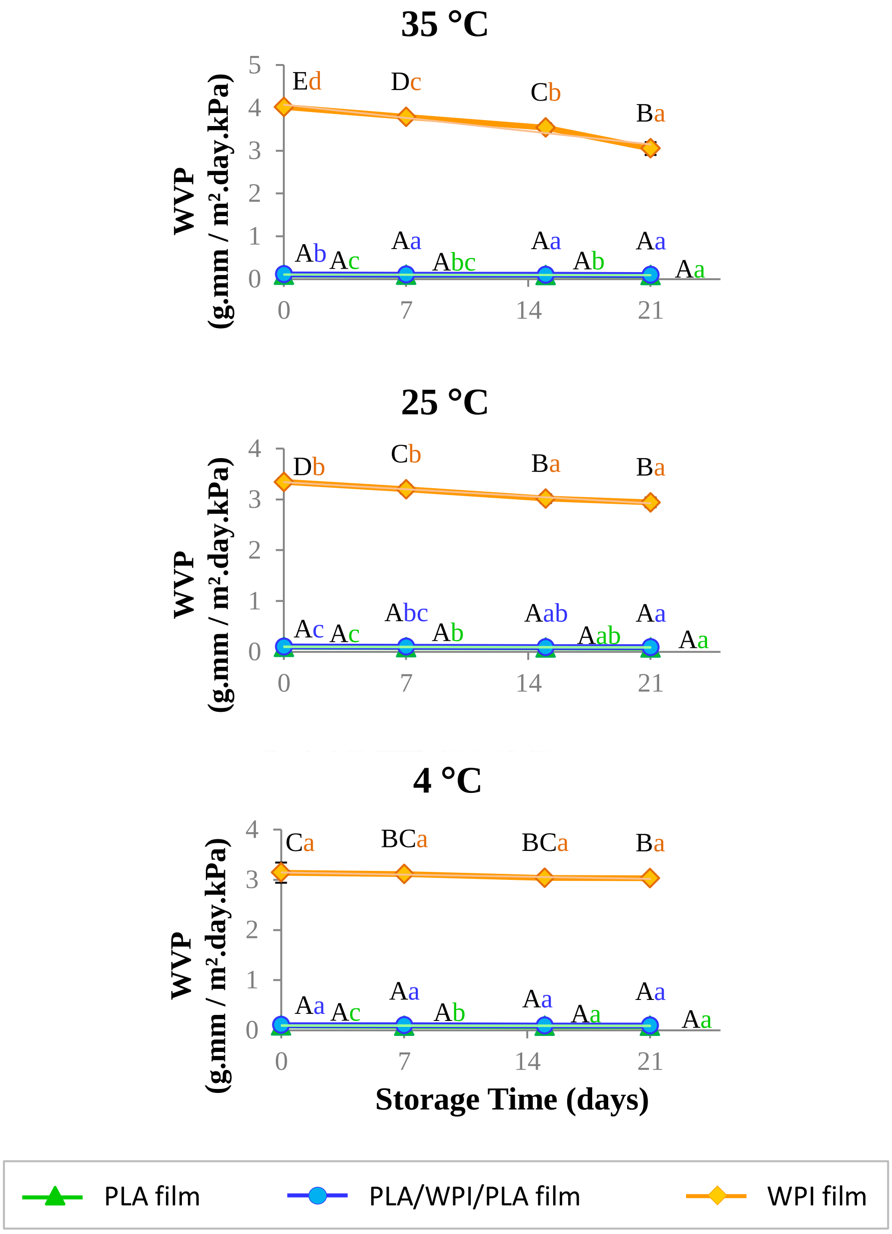

Figure 4.12: Effect of time and temperature on water vapor permeability (WVP) of single-layer polylactic acid (PLA) film, whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) and glycerol-plasticized WPI single cast film during controlled storage 50% relative humidity at 4, 25 and 35 °C for 21 days.

* Each data point represents average value. Error bars show standard deviations.

** Different lower-case letters (a-d) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different upper-case letters (A-E) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Table 4.9: Linear-model kinetics parameters for WVP : water vapor permeability (g.mm/m².day.kPa) 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 50% relative humidity at 4, 25 and 35 °C for 21 days.

Storage Temperature (°C)	Film	Zero-order kinetics model	R^2	First-order kinetics model	R^2
35	PLA	WVP = -0.0005t + 0.1006	0.97	ln WVP = -0.0056t - 2.2963	0.96
	PLA/WPI/PLA	WVP = -0.0007t + 0.1109	0.94	ln WVP = -0.0072t - 2.1993	0.94
	WPI	WVP = -0.0440t + 4.0726	0.94	ln WVP = -0.0125t + 1.4084	0.93
25	PLA	WVP = -0.0006t + 0.0958	0.99	ln WVP = -0.0070t - 2.3462	0.99
	PLA/WPI/PLA	WVP = -0.0007t + 0.1047	0.96	ln WVP = -0.0071t - 2.2568	0.96
	WPI	WVP = -0.0198t + 3.3374	0.99	ln WVP = -0.0065t + 1.2020	0.99
4	PLA	WVP = -0.0004t + 0.0904	0.93	ln WVP = -0.0049t - 2.4047	0.93
	PLA/WPI/PLA	WVP = -0.0005t + 0.1028	0.98	ln WVP = -0.0056t - 2.2780	0.99
	WPI	WVP = -0.0060t + 3.1449	0.95	ln WVP = -0.0018t + 1.1386	0.82

Table 4.10: 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 50% relative humidity at 4, 25 and 35 °C for 21 days.

Kinetic model	Film	Water vapor permeability			
		$\log k = -(E/2.3RT) + \log k_0$ $[k = k_0 e^{-E/RT}]$	Arrhenius constant (k_0)	Ea (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

4.3 Storage stability of baby formula packaged in PLA/WPI/PLA pouch

Figure 4.13 displays fortified instant formula packaged in four-side-seal pouch made from composite PLA/WPI/PLA films on a white background. Baby formula packaged in the four-side-seal pouch made of PLA with the same dimension was prepared for comparison. The thickness of PLA/WPI/PLA and PLA films were 0.088 ± 0.004 to 0.089 ± 0.001 mm and 0.072 ± 0.001 to 0.079 ± 0.002 mm, respectively. The OTR of PLA/WPI/PLA and PLA films were 35.53 ± 2.80 to 44.38 ± 2.19 cc/m^2.day and 349.72 ± 6.39 to 374.23 ± 1.22 cc/m^2.day, respectively. The packaged formula samples were stored in a dark environmental chamber with controlled 50% RH, at 4, 25 or 35 °C for 19 days. Overall, the packaged dry baby formula packaged in multi-layer pouch showed no visible optical change. The layer adhesions were in good condition and the heat seal had good integrity throughout the storage test.

Figure 4.13: Baby formula packed in polylactic acid (PLA) and whey protein isolated (WPI)-enhanced composite structures (PLA/WPI/PLA) four-side-seal pouches.

l) Transparency of packaging film

The effect of time and temperature on transparency value at 600 nm (T_{600}) of PLA and PLA/WPI/PLA pouches used to package baby formula, stored in a dark controlled 50% RH at 4, 25 or 35 °C are shown in *Figure 4.14*. The result showed that T_{600} of the sachets were insignificantly changed over storage time ($p > 0.05$), except the decreasing trend of PLA/WPI/PLA sachet stored at 35 °C. These decreasing trends in T_{600} of all sachets can be fitted by either zero-order (with $R^2 \geq 0.89$) or first-order reaction models (with $R^2 \geq 0.90$) as shown in *Table 4.11.*

The stability of sachet transparency were in accordance to the result from previous films storage experiment shown in *Table 4.3*. Overall, transparency of PLA pouch remained higher than those of PLA/WPI/PLA pouch at all storage conditions. The rates of transparency change of PLA pouch were found to be slower than those of PLA/WPI/PLA sample at all storage conditions, as indicated by smaller slopes (*Table 4.11).*

Packing the baby formula in the sachet did not accelerate the change in film transparency. Overall, the sachets seemed to have a good stability of transparency during the storage.

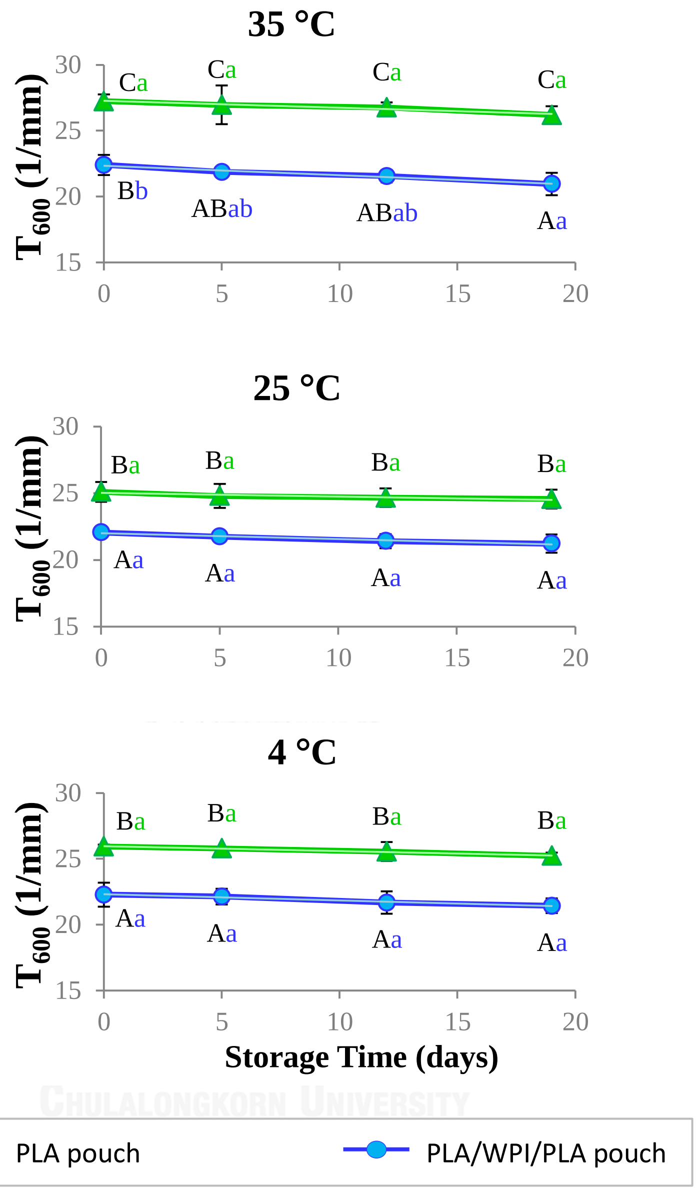

Figure 4.14: Effect of time and temperature on transparency at 600 nm (T_{600}) of polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch used to package baby formula. The packaged baby formula samples were stored in a dark environmental chamber with controlled 50% relative humidity, at 4, 25 or 35 °C for 19 days.

* Each data point represents average value. Error bars show standard deviations.

** Different lower-case letters (a-b) indicate significant differences in each film samples during storage ($p \leq 0.05$).

*** Different upper-case letters (A-C) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Table 4.11: Linear-model kinetics parameters for T_{600} : transparency at 600 nm (1/mm) changes of polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch used to package baby formula over time (t) during controlled storage 50% relative humidity at 4, 25 and 35 °C in a dark environmental chamber for 19 days.

Storage Tempe-rature (°C)	Pouch	Zero-order kinetics model	R^2	First-order kinetics model	R^2
35	PLA	$T_{600} = -0.0522t + 27.262$	0.96	$\ln T_{600} = -0.0019t + 3.3028$	0.90
	PLA/WPI/PLA	$T_{600} = -0.0721t + 22.342$	0.98	$\ln T_{600} = -0.0033t + 3.1043$	0.99
25	PLA	$T_{600} = -0.0271t + 25.024$	0.89	$\ln T_{600} = -0.0012t + 3.2191$	0.95
	PLA/WPI/PLA	$T_{600} = -0.0443t + 22.004$	0.97	$\ln T_{600} = -0.0021t + 3.0903$	0.98
4	PLA	$T_{600} = -0.0367t + 25.959$	0.99	$\ln T_{600} = -0.0015t + 3.2563$	0.98
	PLA/WPI/PLA	$T_{600} = -0.0470t + 22.302$	0.98	$\ln T_{600} = -0.0021t + 3.1023$	0.97

II) Color of packaging films and packaged baby formula

The effects of time and temperature on ΔE_{ab} of PLA and PLA/WPI/PLA sachet and of correspondant packaged baby formulas, stored in a dark controlled 50% RH at 4, 25 or 35 °C, are shown in *Figure 4.15* and *Figure 4.16,* respectively. The result showed that ΔE_{ab} of all sachet types and those of corresponding baby formula packed in each type of sachet were significantly changed over storage time ($p \leq 0.05$). The ΔE_{ab} values significantly increased at all commercial simulated temperatures used during storage evaluation ($p \leq 0.05$). These increasing trends in ΔE_{ab} of all sachets can be fitted by either zero-order (with $R^2 \geq 0.98$) or first-order reaction models (with $R^2 \geq 0.94$) as shown in *Table 4.12.* The ΔE_{ab} of baby formula packed in different kind of sachet can also be fitted by either zero-order (with $R^2 \geq 0.92$) or first-order ($R^2 \geq 0.97$) as shown in *Table 4.13*.

The sachet color changes were in accordance to the result from previous films storage test in *Table 4.4*. The ΔE_{ab} of PLA/WPI/PLA samples remained higher than PLA throughout the stability evaluation. The smaller slopes of the rates of ΔE_{ab} change of PLA sample than those of PLA/WPI/PLA sample, at all storage temperatures, indicated that the PLA sample had higher color stability than PLA/WPI/PLA. By comparing the rates of ΔE_{ab} change of the films (*Table 4.4*) and those of the film from baby formula sachet, packaging baby formula did not significantly accelerate the rates of color change of PLA and PLA/WPI/PLA sachets.

The total color change of packaged baby formula in biopolymer sachets significantly increased over storage time and temperatures ($p \leq 0.05$). However, the type of packaging films did not give significant effect on the color change of baby formula ($p > 0.05$). The baby formula tended to be darker and browner as indicating by decreasing L^* and increasing a^* and b^* value (*Appendix B.24 - Appendix B.26*). It was hypothesized that the underlying cause of color change of baby formula was Maillard reaction. Infant formulas typically have a high level of lysine which can react with reducing sugars causing undesirable browning during storage (Guerra-Hernández *et al.*, 2002). Although the browning rate is rather slow at this low range of baby formula's initial water activity (0.245 ± 0.002 to 0.249 ± 0.002) and relatively low temperature of the current experiment (4 - 35 °C), the reactive carbonyl group of sugar could gradually react with the nucleophilic amino group of the amino acids to form a complex mixture of molecules responsible for browning color (LaBuza, 1980). Other possible causes of color change of baby formula are degradation of nutrients fortified in the formula (*Appendix A.1*).

Although, PLA/WPI/PLA sachet did not improve color stability of packaged baby formula, comparing to those packaged in PLA sachet. The color changes of all packaged baby formula in this stability test were relatively low as demonstrated by much lower ΔE_{ab} than threshold detectable by human eye ($\Delta E_{ab} > 3$). The estimated detectable color change, by human eye, of infant formula packed in PLA and PLA/WPI/PLA sachets stored at 35 °C are 74 and 75 days, respectively.

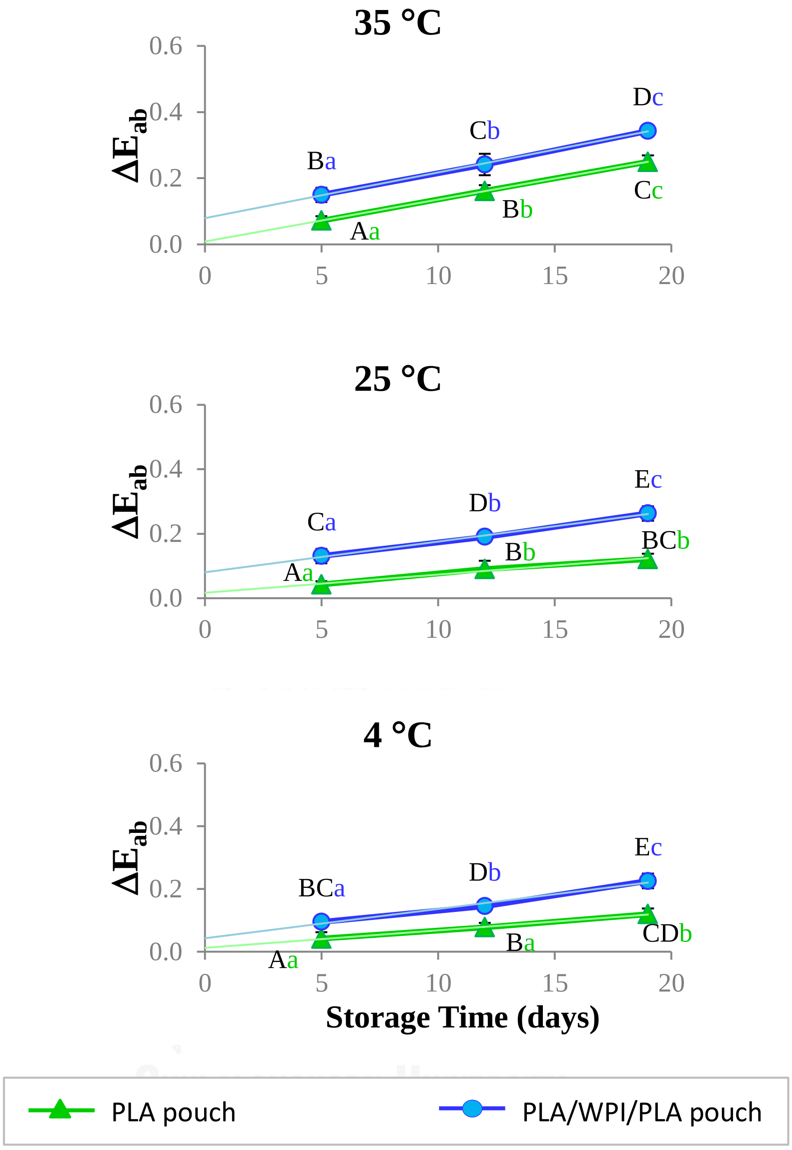

Figure 4.15: Effect of time and temperature on total color differrence (ΔE_{ab}) of polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch used to package baby formula. The packaged baby formula samples were stored in a dark environmental chamber with controlled 50% relative humidity, at 4, 25 or 35 °C for 19 days.

* Each data point represents average value. Error bars show standard deviations.

** Different lower-case letters (a-c) indicate significant differences in each film samples during storage (*p* $\le$ 0.05).

*** Different upper-case letters (A-E) indicate significant differences between all samples among the same storage temperature used (*p* $\le$ 0.05).

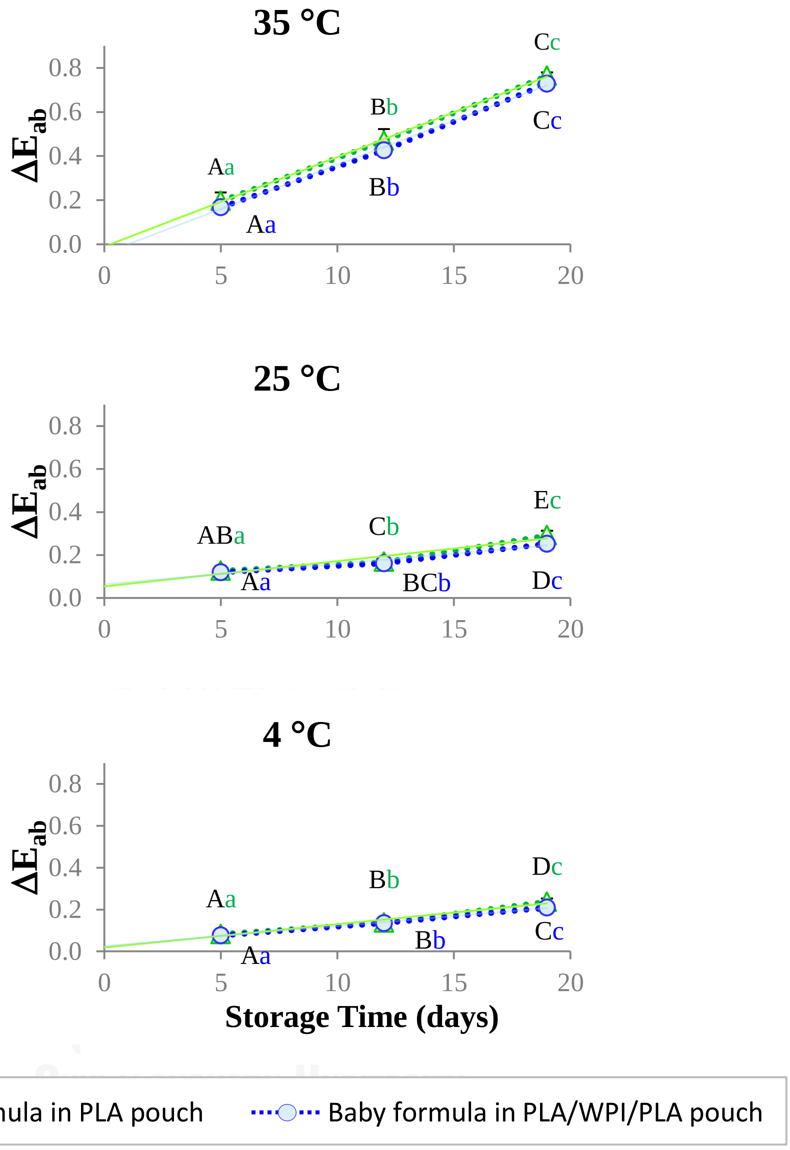

Figure 4.16: Effect of time and temperature on total color differrence (ΔE_{ab}) of baby formula packaged in polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch. The packaged baby formula samples were stored in a dark environmental chamber with controlled 50% relative humidity, at 4, 25 or 35 °C for 19 days.

* Each data point represents average value. Error bars show standard deviations.

** Different lower-case letters (a-c) indicate significant differences in each baby formula samples among the same pouch type used ($p \leq 0.05$).

***Different upper-case letters (A-E) indicate significant differences between all baby formula samples among the same storage temperature used ($p \leq 0.05$).

Table 4.12: Linear-model kinetics parameters for total color difference (ΔE_{ab}) changes of polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch used to package baby formula over time (t) during controlled storage 50% relative humidity at 4, 25 and 35 °C in a dark environmental chamber for 19 days.

Storage Tempe-rature (°C)	Pouch	Zero-order kinetics model	R^2	First-order kinetics model	R^2
35	PLA	$\Delta E_{ab} = 0.0127t + 0.0089$	1.00	$\ln \Delta E_{ab} = 0.0859t - 3.0191$	0.96
	PLA/WPI/PLA	$\Delta E_{ab} = 0.0138t + 0.0790$	1.00	$\ln \Delta E_{ab} = 0.0669t - 2.3636$	1.00
25	PLA	$\Delta E_{ab} = 0.0056t + 0.0175$	0.98	$\ln \Delta E_{ab} = 0.0742t - 3.4778$	0.94
	PLA/WPI/PLA	$\Delta E_{ab} = 0.0095t + 0.0811$	1.00	$\ln \Delta E_{ab} = 0.0530t - 2.3480$	1.00
4	PLA	$\Delta E_{ab} = 0.0056t + 0.0131$	1.00	$\ln \Delta E_{ab} = 0.0742t - 3.6041$	0.94
	PLA/WPI/PLA	$\Delta E_{ab} = 0.0093t + 0.0437$	0.98	$\ln \Delta E_{ab} = 0.0607t - 2.7151$	1.00

Table 4.13: Linear-model kinetics parameters for total color difference (ΔE_{ab}) changes of baby formula packaged in polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch used to package baby formula over time (t) during controlled storage 50% relative humidity at 4, 25 and 35 °C in a dark environmental chamber for 19 days.

Storage Tempe-rature (°C)	Pouch	Zero-order kinetics model	R^2	First-order kinetics model	R^2
35	PLA	$\Delta E_{ab} = 0.0406t - 0.0104$	1.00	$\ln \Delta E_{ab} = 0.0980t - 2.0675$	0.97
	PLA/WPI/PLA	$\Delta E_{ab} = 0.0401t - 0.0403$	1.00	$\ln \Delta E_{ab} = 0.0980t - 2.0675$	0.97
25	PLA	$\Delta E_{ab} = 0.0117t + 0.0542$	0.92	$\ln \Delta E_{ab} = 0.0621t - 2.4584$	0.98
	PLA/WPI/PLA	$\Delta E_{ab} = 0.0095t + 0.0637$	0.96	$\ln \Delta E_{ab} = 0.0609t - 2.5577$	1.00
4	PLA	$\Delta E_{ab} = 0.0111t + 0.0197$	0.97	$\ln \Delta E_{ab} = 0.0855t - 3.0578$	1.00
	PLA/WPI/PLA	$\Delta E_{ab} = 0.0095t + 0.0256$	1.00	$\ln \Delta E_{ab} = 0.0776t - 3.0397$	1.00

III) Water activity and Moisture content of packaged baby formula

Figure 4.17 shows the effect of time and temperature on water activity (a_w) and moisture content (%MC) of baby formula packed inside polylactic acid (PLA) pouches, and PLA/WPI/PLA pouches. Initial a_w of packaged baby formula ranged from 0.245 ± 0.002 to 0.249 ± 0.002, corresponding with 4.37 ± 0.09 to 4.54 ± 0.08 %MC, which are typical for commercial baby formula. Commonly, the a_w of whole milk powder varies from 0.25 to 0.35 (Baechler *et al.*, 2005) and for skimmed milk powder from 0.32 to 0.43 (Shrestha *et al.*, 2008).

The result showed that a_w and %MC of baby formula in both types of sachet significantly increased over storage time at all testing temperatures ($p \leq 0.05$). There seemed to be a good correlation between the increasing a_w and %MC. However, the result suggested that the storage temperature and packaging materials did not significantly affect a_w and %MC of the packaged baby formula ($p > 0.05$).

The rise in experimental a_w data can be fitted by either zero-order (with $R^2 \geq 0.93$) or first-order reaction models (with $R^2 \geq 0.91$) as shown in *Table 4.14,* as well as those of %MC (with $R^2 \geq 0.92$) as shown in *Table 4.15*.

The resulting moisture sorption happened over time due to the moisture ingress through both PLA and PLA/WPI/PLA films, consistent with previous reports with other polymeric packaging (Kumar and Mishra, 2004; Koç *et al.*, 2010; Jena and Das, 2012). Although the previous experiment showed that WVPs of either PLA or PLA/WPI/PLA films decreased over time, at corresponding controlled storage conditions, the improved water vapor barrier ability of the films could not compensate with the permeating water vapor. Nevertheless, it would take 197 and 185 days for the permeating water vapor through PLA and PLA/WPI/PLA, respectively, to increase a_w of packaged baby formula to 0.6, a critical value to minimize microbial growth and activities, at 25 °C, 50% RH.

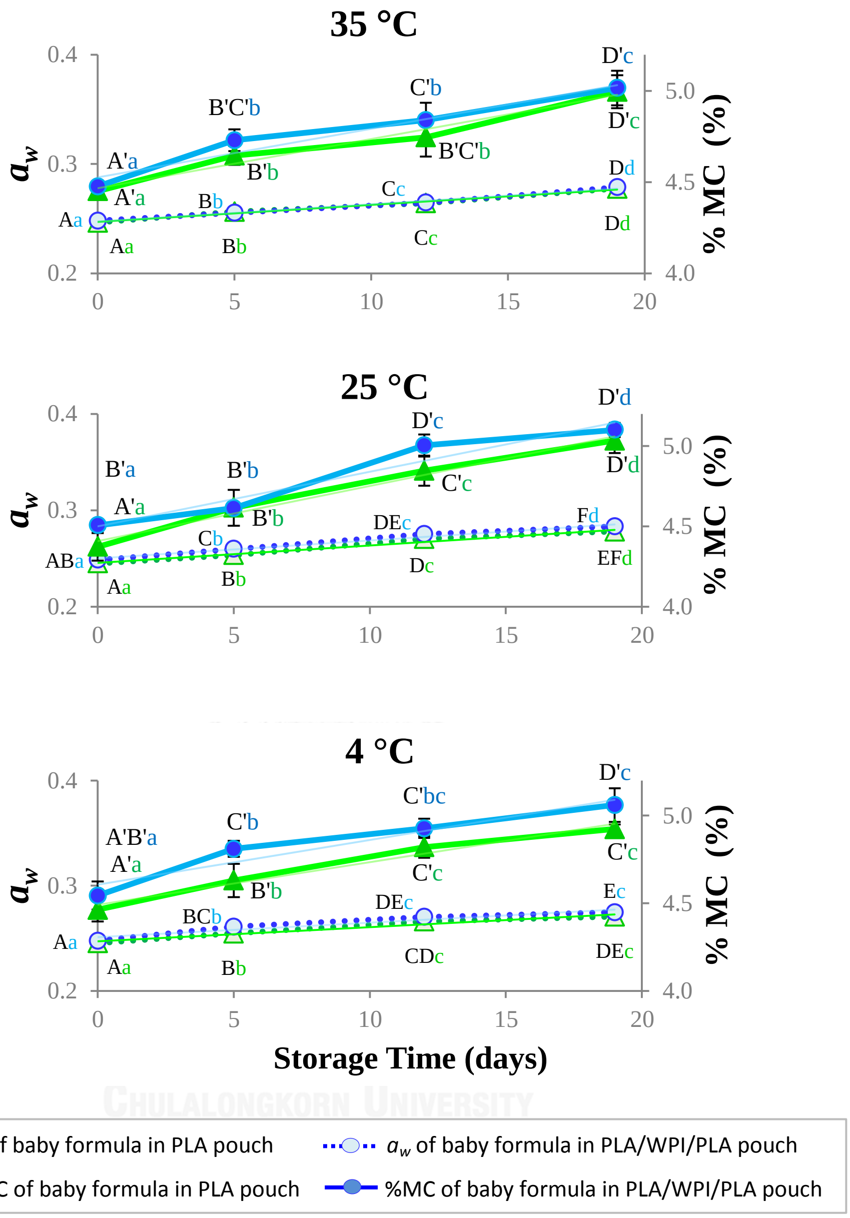

Figure 4.17: Effect of time and temperature on water activity (a_w) and moisture content (%MC) of baby formula packaged in polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch. The packaged baby formula samples were stored in a dark environmental chamber with controlled 50% relative humidity, at 4, 25 or 35 °C for 19 days.

* Each data point represents average value. Error bars show standard deviations.

** Different lower-case letters (a-d) indicate significant differences in each baby formula samples among the same pouch type used ($p \leq 0.05$).

*** Different upper-case letters (A-F) and (A'-D') respectively indicate significant differences of a_w and %MC between all baby formula samples among the same storage temperature used ($p \leq 0.05$).

Table 4.14: Linear-model kinetics parameters for water activity (a_w) changes of baby formula packaged in polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch used to package baby formula over time (t) during controlled storage 50% relative humidity at 4, 25 and 35 °C in a dark environmental chamber for 19 days.

Storage Tempe-rature (°C)	Pouch	Zero-order kinetics model	R^2	First-order kinetics model	R^2
35	PLA	a_w = 0.0016t + 0.2469	0.99	ln a_w = 0.0060t - 1.3981	0.99
	PLA/WPI/PLA	a_w = 0.0016t + 0.2474	0.99	ln a_w = 0.0062t - 1.3978	0.99
25	PLA	a_w = 0.0018t + 0.2455	0.98	ln a_w = 0.0069t - 1.4037	0.98
	PLA/WPI/PLA	a_w = 0.0019t + 0.2502	0.98	ln a_w = 0.0070t - 1.3850	0.98
4	PLA	a_w = 0.0013t + 0.2470	0.93	ln a_w = 0.0052t - 1.3981	0.96
	PLA/WPI/PLA	a_w = 0.0014t + 0.2510	0.95	ln a_w = 0.0053t - 1.3824	0.91

Table 4.15: Linear-model kinetics parameters for %MC : moisture content (%) changes of baby formula packaged in polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch used to package baby formula over time (t) during controlled storage 50% relative humidity at 4, 25 and 35 °C in a dark environmental chamber for 19 days.

Storage Tempe-rature (°C)	Pouch	Zero-order kinetics model	R^2	First-order kinetics model	R^2
35	PLA	%MC = 0.0270t + 4.4674	0.97	ln %MC = 0.0057t + 1.4973	0.97
	PLA/WPI/PLA	%MC = 0.0266t + 4.5266	0.95	ln %MC = 0.0056t + 1.5102	0.94
25	PLA	%MC = 0.0342t + 4.4096	0.98	ln %MC = 0.0073t + 1.4843	0.98
	PLA/WPI/PLA	%MC = 0.0340t + 4.5002	0.94	ln %MC = 0.0071t + 1.5048	0.94
4	PLA	%MC = 0.0243t + 4.4906	0.97	ln %MC = 0.0052t + 1.5022	0.97
	PLA/WPI/PLA	%MC = 0.0255t + 4.6056	0.92	ln %MC = 0.0053t + 1.5273	0.92

IV) Lipid oxidation of packaged baby formula

Baby formula was selected as food model for investigation of efficiency of PLA and PLA/WPI/PLA films as oxygen barrier over common storage temperatures because it is highly susceptible to lipid oxidation. Baby formula is rich in linoleic acid (LA, C 18:2, n-6), as the majority polyunsaturated fatty acids (PUFAs), and supplemented with arachidonic acid (ARA, C 20:4, n-6) and docosahexaenoic acid (DHA, C 22:6, n-3). These PUFAs are sensitive to oxidative reactions. Hence, infant formula supplemented with PUFA is highly sensitive to oxidation, despite the low fatty acid content. The effect of storage time and temperature on conjugate diene (CD) content and total carbonyl compound (C_T) of baby formula packed inside PLA sachets, and PLA/WPI/PLA are shown in *Figure 4.18* and *Figure 4.19,* respectively. The result showed that both CD and C_T of baby formula in all sachets significantly increased over storage time at all simulated temperatures ($p \leq 0.05$). These raising trends of CD can be fitted by either zero-order or first-order reaction models (with $R^2 \geq 0.92$) as shown in *Table 4.16,* as well as those of C_T contents (with $R^2 \geq 0.91$ and $R^2 \geq 0.89$, respectively) as shown in *Table 4.17*.

The rate constants of lipid oxidation of packaged baby formula increased as the storage temperature increased. The result was consistent with the previous research reported that the oxidation in milk product could be negatively influenced by the high storage temperature (Cluskey *et al.*, 1997; Thomsen *et al.*, 2005). Furthermore, these augmentations obeyed the Arrhenius relationship (with $R^2 \geq 0.93$) as shown in *Table 4.18* and *Table 4.19.*

The result also indicated that the type of sachet had a significant effect on the lipid oxidation of packaged baby formula ($p \leq 0.05$). The PLA/WPI/PLA packaging can effectively retard degradation of lipids due to oxidation comparing to single-layer PLA sachet at all storage conditions. The efficiency of PLA/WPI/PLA to reduce autoxidation was hypothesized to be the result of the improved ability of the PLA/WPI/PLA to reduce permeating atmospheric oxygen into packaging headspace. This result is in agreement with the findings from previous experiments (*Figure 4.3*, *Figure 4.11* and *Table 4.8*) denoted that laminated WPI layer significantly enhanced oxygen barrier ability of PLA film ($p \leq 0.05$).

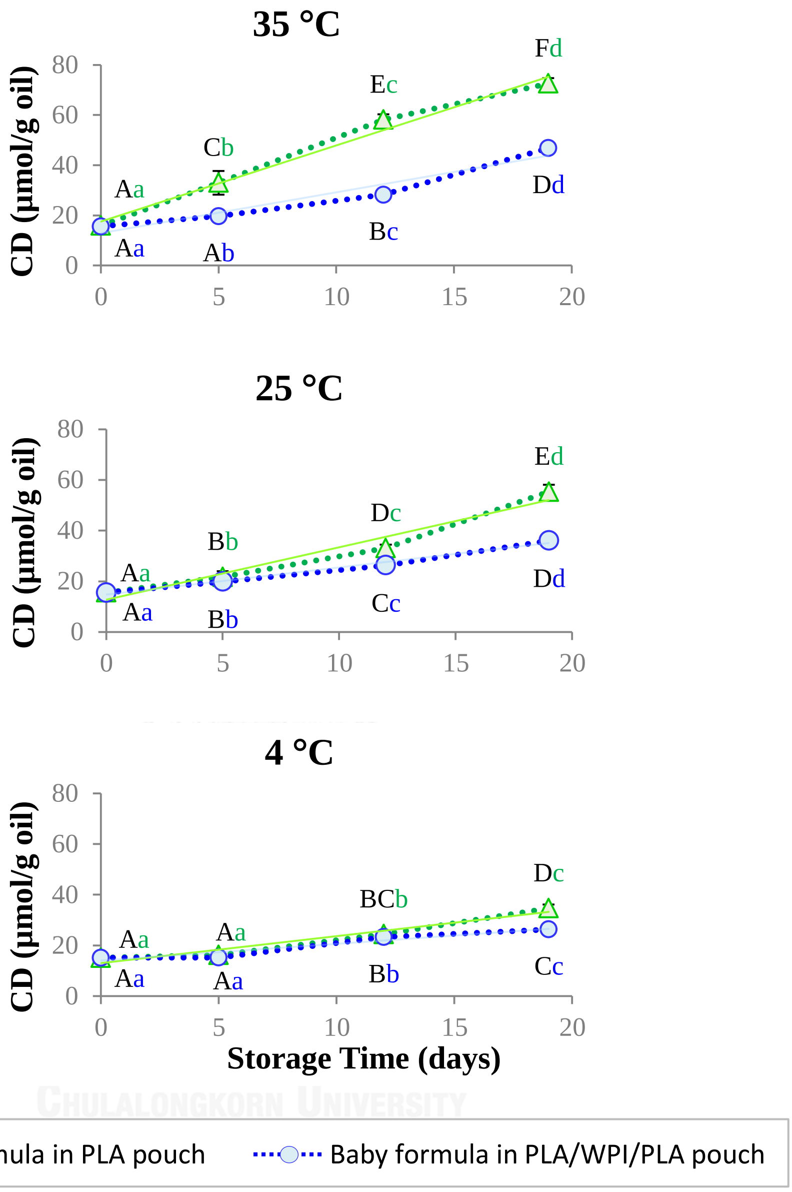

Figure 4.18: Effect of time and temperature on conjugate diene (CD) content of baby formula packaged in polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch. The packaged baby formula samples were stored in a dark environmental chamber with controlled 50% relative humidity, at 4, 25 or 35 °C for 19 days.

* Each data point represents average value. Error bars show standard deviations.

** Different lower-case letters (a-d) indicate significant differences in each baby formula samples among the same pouch type used ($p \leq 0.05$).

*** Different upper-case letters (A-F) indicate significant differences between all baby formula samples among the same storage temperature used ($p \leq 0.05$).

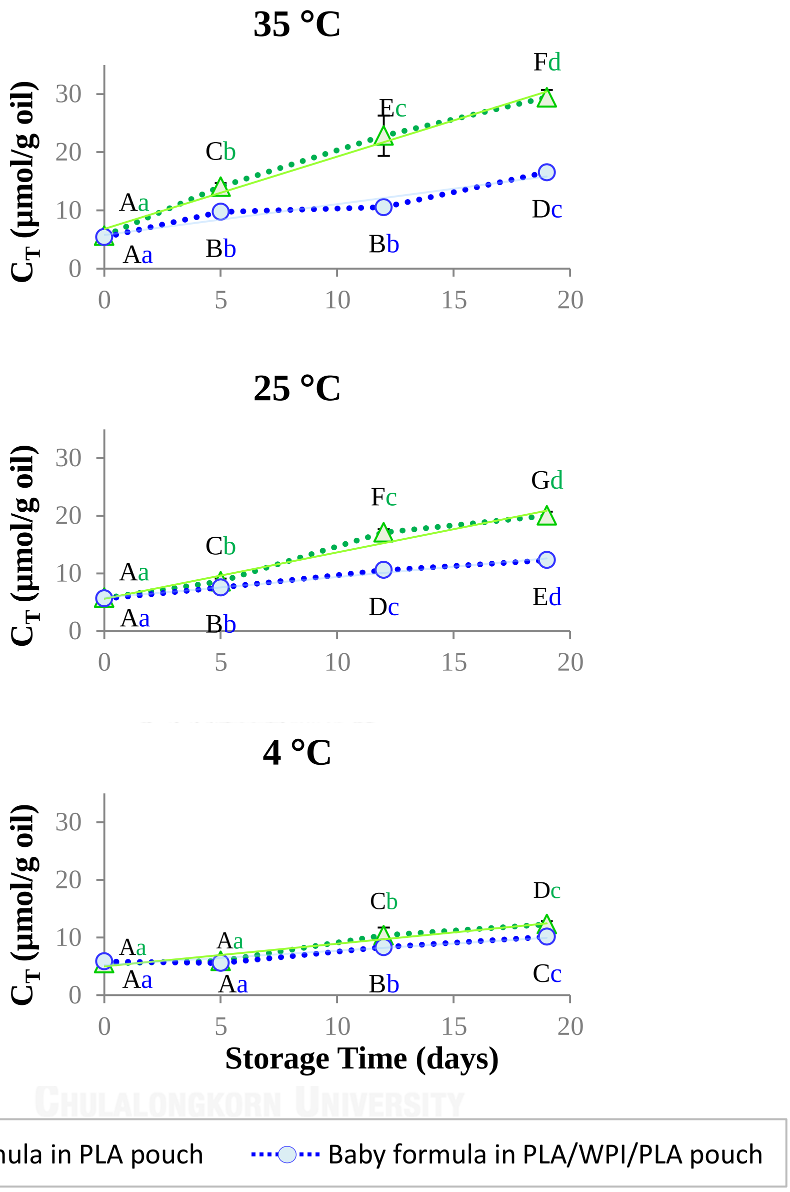

Figure 4.19: Effect of time and temperature on total carbonyl compound (C_T) content of baby formula packaged in polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch. The packaged baby formula samples were stored in a dark environmental chamber with controlled 50% relative humidity, at 4, 25 or 35 °C for 19 days.

* Each data point represents average value. Error bars show standard deviations.

** Different lower-case letters (a-d) indicate significant differences in each baby formula samples among the same pouch type used ($p \leq 0.05$).

*** Different upper-case letters (A-G) indicate significant differences between all baby formula samples among the same storage temperature used ($p \leq 0.05$).

Table 4.16: Linear-model kinetics parameters for CD : conjugate diene content (μmol/g oil) changes of baby formula packaged in polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch used to package baby formula over time (t) during controlled storage 50% relative humidity at 4, 25 and 35 °C in a dark environmental chamber for 19 days.

Storage Tempe-rature (°C)	Pouch	Zero-order kinetics model	R^2	First-order kinetics model	R^2
35	PLA	CD = 3.0343t + 17.581	0.99	ln CD = 0.0790t + 2.9332	0.92
	PLA/WPI/PLA	CD = 1.6217t + 12.984	0.94	ln CD = 0.0571t + 2.7042	0.98
25	PLA	CD = 2.0659t + 12.807	0.96	ln CD = 0.0663t + 2.7331	1.00
	PLA/WPI/PLA	CD = 1.0691t + 14.799	0.99	ln CD = 0.0468t + 2.7000	1.00
4	PLA	CD = 1.0634t + 13.004	0.95	ln CD = 0.0459t + 2.6441	0.97
	PLA/WPI/PLA	CD = 0.6599t + 14.004	0.92	ln CD = 0.0336t + 2.6496	0.92

Table 4.17: Linear-model kinetics parameters for C_T : total carbonyl compound content (μmol/g oil) changes of baby formula packaged in polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch used to package baby formula over time (t) during controlled storage 50% relative humidity at 4, 25 and 35 °C in a dark environmental chamber for 19 days.

Storage Tempe-rature (°C)	Pouch	Zero-order kinetics model	R^2	First-order kinetics model	R^2
35	PLA	C_T = 1.2416t + 6.8174	0.99	ln C_T = 0.0826t + 1.9701	0.89
	PLA/WPI/PLA	C_T = 0.5306t + 5.7863	0.92	ln C_T = 0.0526t + 1.8049	0.90
25	PLA	C_T = 0.8026t + 5.6307	0.96	ln C_T = 0.0687t + 1.8128	0.94
	PLA/WPI/PLA	C_T = 0.3576t + 5.7825	0.99	ln C_T = 0.0415t + 1.7768	0.96
4	PLA	C_T = 0.3920t + 4.9976	0.95	ln C_T = 0.0467t + 1.6586	0.93
	PLA/WPI/PLA	C_T = 0.2494t + 5.2101	0.91	ln C_T = 0.0339t + 1.6620	0.91

Table 4.18: Arrhenius parameters for conjugate diene content changes of baby formula packaged in polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch during controlled storage 50% relative humidity at 4, 25 and 35 °C in a dark environmental chamber for 19 days.

Kinetic model	Pouch	Conjugate diene content			
		log k = -(E/2.3RT) + log k_0 [$k = k_0 e^{-E/RT}$]	Arrhenius constant (k_0)	Ea (kJ/mol)	R^2
Zero-order	PLA	y = -1232.0x + 4.4686	87.23	23558.55	0.99
	PLA/WPI/PLA	y = -1030.2x + 3.5263	34.00	19699.69	0.97
First-order	PLA	y = -645.22x + 0.9901	2.69	12338.03	0.99
	PLA/WPI/PLA	y = -621.72x + 0.7675	2.15	11888.65	0.99

Table 4.19: Arrhenius parameters for total carbonyl compound content changes of baby formula packaged in polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch during controlled storage 50% relative humidity at 4, 25 and 35 °C in a dark environmental chamber for 19 days.

Kinetic model	Pouch	Total carbonyl compound content			
		log k = -(E/2.3RT) + log k_0 [$k = k_0 e^{-E/RT}$]	Arrhenius constant (k_0)	Ea (kJ/mol)	R^2
Zero-order	PLA	y = -1350.50x + 4.4614	86.61	25824.53	0.99
	PLA/WPI/PLA	y = -851.41x + 2.4567	11.62	16280.83	0.94
First-order	PLA	y = -677.58x + 1.1144	3.05	12956.82	1.00
	PLA/WPI/PLA	y = -493.18x + 0.3020	1.35	9430.69	0.93

CHAPTER V

CONCLUSIONS AND SUGGESTIONS

5.1 CONCLUSIONS

It was possible to form biodegradable multi-layer barrier film made of whey protein isolate (WPI)-enhanced polylactic acid (PLA) composite structure; PLA/WPI/PLA via simple casting process after treatment with corona discharge to modify PLA film surface in order to achieve well-adhering multi-layer structures. The developed films also showed a good appearance with no noticeably visible change. Mechanical properties of the composite structures mostly rely on substrate films. The resulting multi-layer structures, with proper plasticizers, have excellent oxygen barrier properties at dry condition. The water vapor barrier of the multi-layer films was not significantly reduced by hydrophilic WPI inner layer ($p > 0.05$).

Also, the composite PLA/WPI/PLA films have reasonable storage stability in simulated commercial storage temperatures. The multi-layer film maintained good handling ability and layer adhesion more than 21 days with the small rates of decreasing in transparency and increasing total color difference (ΔE_{ab}) occurred, suggesting that these films have a good visual and transparency stability during storage. The mechanical properties gradually differently changed as a function of storage time and conditions depending on the storage temperature. PLA/WPI/PLA films tend to be more extended during storage at 4 and 25 °C, 50% RH, while be more strength and loss their extensibility during storage at 35 °C, 50% RH. For their barrier abilities, PLA/WPI/PLA films were able to maintain their oxygen and water vapor barrier properties throughout the storage time. The oxygen barrier ability of biopolymer films improved as a function of time at 35 °C, 50% RH, while the improvements of water vapor barrier were found to be a function of increasing storage time and temperature Arrhenius relationship.

Moreover, PLA/WPI/PLA can be 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 and 25 °C, 50% RH.

5.2 SUGGESTIONS

The main goal of this research was to develop multi-layer structure barrier films made entirely from sustainable PLA and WPI have potential as a ‘green’ alternative to existing synthetic oxygen-barrier polymers. Although, new knowledge has been developed and many hypotheses were confirmed, many other hypotheses and new ideas arose along the course of the research. Below are possible research suggestions that could help 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 as biodegradable oxygen-barrier films.

- Further research must be done to address the feasibility of scaling up the production for commercial applications. WPI has been shown to exhibit thermoplastic behavior lending itself to extrusion lamination or preformed film formation which can later be adhesively laminated into multi-layer structures.
- PLA/WPI/PLA is a potential good carrier for active ingredients. Investigation of incorporation of antioxidants, antimicrobial agents, nutraceuticals or combinations of functional compounds into the structure of the film and the ability to control-release such beneficial compounds is appropriated.
- Other aspects of utilizing PLA/WPI/PLA, such as consumer perception, biodegradability or laws and regulations should also be explored.

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APPENDIX

APPENDIX A
PRODUCT SPECIFICATION AND ASSAY TECHNIQUES

Appendix A.1: Baby formula supplementary information

Nutritional information

- Serving size : 44 g in 225 ml of water
- Total energy per serving : 190 kcal (Calories from Fat 60 kcal)

Amount per serving	% Daily value
Total Fat 6 g	9%
Saturated Fat 3 g	15%
Cholesterol 10 mg	3%
Protein 6 g	
Total Carbohydrates 19 g	10%
Dietary Fiber lower than 1 g	2%
Sugar 23 g	
Sodium 70 mg	3%
Potassium 290 mg	8%

Vitamin A	20%	Vitamin B1	20%	Vitamin B2	20%
Calcium	30%	Iron	15%	Vitamin D	35%
Vitamin C	30%	Vitamin B12	30%	Folic acid	25%
Phosphorus	20%	Vitamin E	20%	Vitamin K	20%
Iodine	20%	Pantothenic acid	15%	Niacin	10%
Zinc	10%	Vitamin B6	10%	Biotin	10%
Magnesium	6%	Copper	6%	Chloride	4%

Amount per serving			
Oleic acid (Omega-9)	1960 mg	*lc*FOS	600 mg
Linoleic acid (Omega-6)	990 mg	β-carotene	70 mg
Linolenic acid (Omega-3)	115 mg	Choline	50 mg
DHA	25 mg	Inositol	4 mg
Taurine	20 mg		

* Percent daily values are based on 2,000 calories diet

Ingredients

• Baby formula (Skimmed Milk) 32.4% • Lactose 29.9% • Molto dextrin 20.1% • Baby formula (Whole Milk) 4.8% • Vegetable Oil Blend 10.22% (Palm Olein 2.58%, Coconut Oil 2.99%, Canola Oil 2.07%, Corn Oil 2.58%) • Long Chain Fructo-Oligosaccharides (*lc*FOS)/Polyfructose (Plant) 1.4% • Docosahexaenoic Acid (DHA) from Fish Oil 0.5%, • Dietary Mineral 0.48% • Multi-Vitamin 0.28% • Mixed Vegetable Fruit Powder 0.2% • Soya Lecithin 0.1% • Carrot Extract 0.1% • Taurine 0.05% • β-carotene 0.01%

Preparation information

Add 6 level scoops (44g) of baby formula to 225 ml of boiled lukewarm (approx. 40°C) water, and then stir well before drinking.

Appendix A.2: Milk fat extraction

AOAC 905.02-Rose-Gottlieb modified method (AOAC, 1995)

Reagents

- Ammonia 25% (v/v)
- Ethyl alcohol 95% (v/v)
- Diethyl ether
- Petroleum ether (Boiling range 40 - 60 °C)
- Phenolphthalein indicator 0.5% (w/v) in alcohol

All the reagents were analytical reagent grade, obtained from QReC chemical Co., Ltd. (Chonburi, Thailand).

Procedure

Ten grams of baby formula were dissolved in distilled water to make 20% w/v liquid milk as stated in the label of baby formula.

To dissolve and precipitate the milk protein before extracting the milk fat, the liquid milk samples were treated with ammonia and alcohol by weighing 30 g of liquid milk into a glass stoppered flask. Then added 3.75 ml of ammonia and mixed thoroughly, also added 3 drops of phenolphthalein indicator to help sharpen visual appearance of interface between ether and aqueous layers during extraction. After that, 30 ml of ethyl alcohol were added and mixed together.

For first extraction, 75 ml of diethyl ether were added and shaken vigorously for 2 min. Then added 73 ml of petroleum ether and shaken again vigorously for 1 min. the mixed solution were transferred to separating funnel, let it stand until the ethereal layer were clear and completely separated from the aqueous (bright pink) phase, afterward decanted off the ethereal phase into an evaporating flask. The remaining solutions were repeatedly twice extracted using 45 ml of each solvent every time. All the ethereal extracts were evaporated off by using a rotary evaporator (Rotavapor® R-114, BÜCHI, Flawil, Switzerland) under vacuum at 50 °C for 1 h.

Appendix A.3: Determination of conjugated diene hydro peroxides

(Wrolstad *et al.*, 2005a)

Reagents

- 2,2,4-Trimethylpentane (Grade AR, Fisher Scientific Inc., Loughborough, UK)

Procedure

Ten to thirty milligrams of milk lipid extract sample were accurately weighed into 25 ml volumetric flask. Then added 2,2,4-Trimethylpentane (isooctane) and brought to volume and mixed thoroughly. The absorbance of the dissolved lipid samples were measured via a quartz cuvette by using the spectrometer (Lambda 25 UV/VIS spectrometer, PerkinElmer Inc., San Jose, California, U.S.A.) at 233 nm. Isooctane was used as solvent blank.

Conjugated diene (CD) content as μmol/g oil was calculated from the following equation (*Eq. A.1* and *Eq. A.2*)

$$C_{CD} = A_{233}/(\varepsilon \times l) \qquad (Eq.\ A.1)$$

$$CD\ content = \frac{[C_{CD} \times (2.5 \times 10^4)]}{W} \qquad (Eq.\ A.2)$$

where C_{cd} is the CD concentration (mmol/ml), A_{233} is the absorbance of sample solution at 233 nm, the molar extinction coefficients (ε) is the molar absorptivity of linoleic hydro peroxide (2.525×10^4 $M^{-1}.cm^{-1}$), l is the cuvette path length (1 cm), while 2.5×10^4 is a factor that include a volume of isooctane (25 ml) used to dissolved the lipid sample as well as the unit conversion (1000 μmol/mmol) so that the CD content can be expressed in μmol, and *W* is the weight of lipid extract sample (g).

Appendix A.4: Determination of carbonyl compounds

(Wrolstad *et al.*, 2005b)

Reagents

- Benzene
- Ethyl alcohol
- Trichloroacetic acid (TCA) 4.3% (w/v) in benzene
- 2,4-Dinitrophenyl hydrazine (DNPH) 0.05% (w/v) in benzene
- KOH solution

All the reagents were analytical reagent grade. Benzene and ethyl alcohol were obtained from QReC chemical Co., Ltd. (Chonburi, Thailand), TCA was obtained from Fisher Scientific Inc. (Loughborough, UK), DNPH was obtained from LOBA Chemie Pvt. Ltd., (Mumbai, India) and KOH was obtained from Ajex Finechem Pty. Ltd., New South Wales, Australia).

KOH solution preparation

Four grams of KOH were dissolved in 100 ml ethanol and stirred to get the KOH into solution. The solution was filtered through a fine glass wool with suction. The KOH solution was freshly prepared before used.

Procedure

Fifty to eighty milligrams of milk lipid extract sample were accurately weighed into 50 ml volumetric flask, then dissolved with 5 ml benzene and mixed thoroughly. After showed that pipetted 3.0 ml of 4.3% TCA and 0.05% DNPH at the same time, mix thoroughly and heated in 60 °C water bath (NESLAB™ EX-10, Themo Fisher Scientific, Newington, New Hampshire, U.S.A.) for 30 min before cooling to room temperature. A reagent blank was prepared at the same time by using 5.0 ml each of benzene 4.3% TCA and 0.05% DNPH. The KOH solution 10 ml was added and absolute ethanol was used to dilute to volume then mixed thoroughly and incubated for 10 min, aiming to develop the color.

The absorbance of the mixture solutions were exactly measured after 10 min via a glass cuvette by using the spectrometer (Lambda 25 UV/VIS spectrometer, PerkinElmer Inc., San Jose, California, U.S.A.) at 430 and 460 nm.

The total carbonyl compounds (C_T) content as μmol/ g oil was calculated from the following equation (*Eq. A.3*, *Eq. A.4* and *Eq. A.5*)

$$C_U = \frac{3.861 \times A_{460} - 3.012 \times A_{430}}{0.854} \qquad (Eq.\ A.3)$$

$$C_S = 3.861 \times A_{460} - 2.170 \times C_U \qquad (Eq.\ A.4)$$

$$C_T = C_U + C_S \qquad (Eq.\ A.5)$$

where A_{460} and A_{430} are the absorbance of sample solution at 460 and 430 nm respectively, while C_U, C_S and C_T are the unsaturated, saturated and total carbonyl contents respectively. The absorbance of DNPH derivatives of several purified carbonyl compound were measured to express the moles of carbonyl obtained from this method. The molar extinction coefficients of 16,000, 21,350, 12,450 and 28,100 were determined for the saturated and unsaturated carbonyl compound at 430 nm and 460 nm, respectively.

APPENDIX B
ADDITIONAL DATA

Appendix B.1: Mechanical properties: tensile strength (TS), elastic modulus (EM) and percentage of elongation (%E) of single-layer films; linear low-density polyethylene (LLDPE), polylactic acid (PLA), glycerol (GLY)-plasticized whey protein isolate (WPI), and WPI-enhanced composite structures; PLA/WPI/PLA and LLDPE/WPI/LLDPE.

Film	WPI:GLY ratio	TS (MPa)	EM (MPa)	%E (%)
LLDPE	-	10.24 ± 0.14^{Ba}	3.10 ± 0.04^{Aa}	330.85 ± 0.28^{Da}
LLDPE/WPI/LLDPE	1:0.25	10.18 ± 0.15^{Ba}	3.08 ± 0.04^{Aa}	330.95 ± 0.26^{Da}
	1:0.40	10.02 ± 0.15^{Ba}	3.03 ± 0.05^{Aa}	330.51 ± 0.48^{Da}
	1:0.67	9.97 ± 0.24^{Ba}	3.02 ± 0.07^{Aa}	330.54 ± 0.21^{Da}
PLA	-	45.49 ± 0.42^{Db}	$1932.98 \pm 161.65^{CDab}$	2.47 ± 0.30^{Aa}
PLA/WPI/PLA	1:0.25	48.28 ± 1.46^{Ec}	2117.76 ± 171.31^{Eb}	2.29 ± 0.14^{Aa}
	1:0.40	46.44 ± 0.76^{Dbc}	1975.17 ± 58.98^{DEab}	2.38 ± 0.07^{Aa}
	1:0.67	43.39 ± 1.12^{Ca}	1790.87 ± 194.52^{Ca}	2.50 ± 0.23^{Aa}
WPI	1:0.25	-	-	-
	1:0.40	11.29 ± 0.82^{Bb}	201.34 ± 20.68^{Bb}	5.94 ± 0.96^{Ba}
	1:0.67	4.78 ± 0.23^{Aa}	63.39 ± 2.58^{ABa}	7.72 ± 0.53^{Cb}

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–c) indicate significant differences between GLY content used among the same plastic substrate ($p \leq 0.05$).

***Different superscript upper-case letters (A-E) indicate significant differences between all samples ($p \leq 0.05$).

Appendix B.2: Oxygen transmission rate (OTR), oxygen permeability (OP), water vapor transmission rate (WVTR) and water vapor permeability (WVP) of single-layer films; linear low-density polyethylene (LLDPE), polylactic acid (PLA), glycerol (GLY)-plasticized whey protein isolate (WPI), and WPI-enhanced composite structures; PLA/WPI/PLA and LLDPE/WPI/LLDPE.

Film	WPI:GLY ratio	OTR cc/[m^2.day]	OP cc.µm / [m^2.day].kPa	WVTR g/[m^2.day]	WVP g.mm / [m^2.day].kPa
LLDPE	-	^{Cb} 5578.52 ± 412.87	^{Cb} 3742.85 ± 171.74	^{Ab} 5.42 ± 0.31	^{Aa} 0.004 ± 0.000
LLDPE-/WPI/LLDPE	**1:0.25**	^{Ba} 1488.89 ± 226.38	^{Ba} 2141.94 ± 280.86	^{Aa} 3.59 ± 0.59	^{Ab} 0.005 ± 0.001
	1:0.40	^{Ba} 1369.38 ± 337.80	^{Ba} 2000.19 ± 445.74	^{Aa} 3.77 ± 0.57	^{Ab} 0.006 ± 0.001
	1:0.67	^{Ba} 1463.31 ± 335.65	^{Ba} 2125.86 ± 446.18	^{Aa} 3.75 ± 0.55	^{Ab} 0.005 ± 0.001
PLA	-	^{Bb} 1503.81 ± 56.60	^{Ab} 637.10 ± 59.29	^{Cb} 208.69 ± 22.19	^{Ba} 0.090 ± 0.010
PLA/WPI/PLA	**1:0.25**	^{Aa} 381.35 ± 90.22	^{Aa} 357.67 ± 88.54	^{Ba} 102.56 ± 15.73	^{Ba} 0.095 ± 0.014
	1:0.40	^{Aa} 405.51 ± 44.66	^{Aa} 386.45 ± 43.53	^{Ba} 103.36 ± 15.67	^{Ba} 0.096 ± 0.015
	1:0.67	^{Aa} 460.42 ± 85.80	^{Aa} 441.59 ± 79.13	^{Ba} 105.09 ± 17.96	^{Ba} 0.097 ± 0.017

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–b) indicate significant differences between GLY content used among the same plastic substrate ($p \leq 0.05$).

***Different superscript upper-case letters (A-D) indicate significant differences between all samples ($p \leq 0.05$).

Appendix B.3: Weight change, water activity (a_w) and moisture content (%MC) 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 50% relative humidity at 35 °C for 21 days.

Film	Storage time (days)	Weight change (g)	a_w	%MC (%)
PLA	0	-	0.542 ± 0.002^{Ac}	1.44 ± 0.06^{Aa}
	7	-0.0001 ± 0.0002^{Aa}	0.539 ± 0.001^{Aa}	1.41 ± 0.08^{Aa}
	15	-0.0001 ± 0.0002^{Aa}	0.539 ± 0.002^{Aab}	1.44 ± 0.06^{Aa}
	21	-0.0001 ± 0.0002^{Aa}	0.542 ± 0.001^{Abc}	1.38 ± 0.04^{Aa}
PLA/WPI/PLA	0	-	0.541 ± 0.001^{Aa}	1.75 ± 0.11^{Aa}
	7	-0.0077 ± 0.0015^{Ca}	0.541 ± 0.004^{Aa}	1.84 ± 0.16^{Aa}
	15	-0.0086 ± 0.0068^{Ca}	0.546 ± 0.005^{Aa}	1.79 ± 0.69^{Aa}
	21	-0.0080 ± 0.0054^{Ca}	0.540 ± 0.007^{Aa}	1.75 ± 0.65^{Aa}
WPI	0	-	0.593 ± 0.003^{Ba}	11.25 ± 0.48^{Ba}
	7	-0.0108 ± 0.0040^{Ba}	0.610 ± 0.009^{Cb}	11.52 ± 0.50^{Ba}
	15	-0.0115 ± 0.0041^{Ba}	0.599 ± 0.005^{Ba}	11.33 ± 0.68^{Ba}
	21	-0.0122 ± 0.0043^{Ba}	0.600 ± 0.001^{Bab}	11.22 ± 0.49^{Ba}

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–c) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-C) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.4: Weight change, water activity (a_w) and moisture content (%MC) 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 50% relative humidity at 25 °C for 21 days.

Film	Storage time (days)	Weight change (g)	a_w	%MC (%)
PLA	0	-	0.551 ± 0.002^{Aa}	1.41 ± 0.09^{Aa}
	7	0.0000 ± 0.0001^{Aa}	0.552 ± 0.003^{Aa}	1.33 ± 0.08^{Aa}
	15	- 0.0001 ± 0.0001^{Aa}	0.553 ± 0.004^{Aa}	1.42 ± 0.09^{Aa}
	21	0.0000 ± 0.0002^{Aa}	0.551 ± 0.004^{Aa}	1.42 ± 0.04^{Aa}
PLA/WPI/PLA	0	-	0.552 ± 0.001^{Aa}	1.59 ± 0.30^{Aa}
	7	- 0.0008 ± 0.0002^{Ba}	0.555 ± 0.006^{Aa}	1.52 ± 0.37^{Aa}
	15	- 0.0017 ± 0.0002^{Bc}	0.555 ± 0.008^{Aa}	1.53 ± 0.19^{Aa}
	21	- 0.0012 ± 0.0002^{Bb}	0.554 ± 0.004^{Aa}	1.58 ± 0.15^{Aa}
WPI	0	-	0.625 ± 0.007^{Ba}	11.43 ± 0.18^{BCa}
	7	- 0.0084 ± 0.0020^{Ca}	0.639 ± 0.007^{Cb}	11.54 ± 0.50^{Ba}
	15	- 0.0097 ± 0.0036^{Ca}	0.637 ± 0.005^{Aab}	11.05 ± 0.14^{BCa}
	21	- 0.0097 ± 0.0025^{Ca}	0.643 ± 0.008^{Cb}	11.79 ± 0.44^{Ca}

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–c) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-C) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.5: Weight change, water activity (a_w) and moisture content (%MC) 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 50% relative humidity at 4 °C for 21 days.

Film	Storage time (days)	Weight change (g)	a_w	%MC (%)
PLA	0	-	0.561^{ABa} ± 0.003	1.43^{Aa} ± 0.11
	7	- 0.0000^{Ca} ± 0.0001	0.559^{ABa} ± 0.003	1.41^{Aa} ± 0.08
	15	- 0.0001^{Ca} ± 0.0002	0.560^{ABa} ± 0.004	1.42^{Aa} ± 0.06
	21	- 0.0001^{Ca} ± 0.0001	0.560^{ABa} ± 0.007	1.39^{Aa} ± 0.05
PLA/WPI/PLA	0	-	0.559^{Aa} ± 0.003	3.34^{Cc} ± 0.26
	7	- 0.0001^{Cc} ± 0.0002	0.563^{ABab} ± 0.008	2.45^{Bb} ± 0.04
	15	- 0.0013^{Aa} ± 0.0001	0.567^{BCab} ± 0.003	1.79^{Aa} ± 0.26
	21	- 0.0009^{Bb} ± 0.0002	0.572^{Cb} ± 0.006	2.00^{ABa} ± 0.22
WPI	0	-	0.647^{Da} ± 0.002	11.24^{Da} ± 0.18
	7	0.0000^{Ca} ± 0.0002	0.660^{Eb} ± 0.001	12.77^{Eb} ± 1.09
	15	0.0011^{Db} ± 0.0003	0.668^{Fc} ± 0.004	14.37^{Fc} ± 0.12
	21	0.0009^{Db} ± 0.0002	0.689^{Gd} d± 0.002	16.23^{Gd} ± 0.17

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–d) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-G) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.6: Thickness, % light transmission (%T) and transparency at 600 nm (T_{600}) 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 50% relative humidity at 35 °C for 21 days.

Film	Storage time (days)	Thickness (mm)	%T (%)	T_{600} (1/mm)
PLA	0	$0.074^{Aa} \pm 0.001$	$92.63^{Ea} \pm 0.23$	$26.64^{Fa} \pm 0.27$
	7	$0.075^{Aa} \pm 0.002$	$92.62^{Ea} \pm 0.40$	$26.33^{EFa} \pm 0.65$
	15	$0.076^{Aa} \pm 0.001$	$92.41^{Ea} \pm 0.38$	$26.00^{EFa} \pm 0.16$
	21	$0.077^{Aa} \pm 0.003$	$92.31^{Ea} \pm 0.18$	$25.60^{Ea} \pm 0.80$
PLA/WPI/PLA	0	$0.086^{Ba} \pm 0.002$	$89.74^{Da} \pm 0.61$	$22.73^{Db} \pm 0.48$
	7	$0.089^{BCab} \pm 0.001$	$89.60^{Da} \pm 0.19$	$22.01^{CDab} \pm 0.20$
	15	$0.089^{BCab} \pm 0.002$	$89.19^{Da} \pm 0.08$	$21.91^{CDab} \pm 0.69$
	21	$0.092^{CDc} \pm 0.004$	$88.95^{Da} \pm 0.61$	$21.19^{BCa} \pm 0.82$
WPI	0	$0.091^{Ca} \pm 0.001$	$87.75^{Cb} \pm 0.54$	$21.51^{Cb} \pm 0.39$
	7	$0.095^{DEb} \pm 0.001$	$86.11^{Ba} \pm 0.96$	$20.45^{ABa} \pm 0.29$
	15	$0.096^{Ebc} \pm 0.001$	$85.83^{ABa} \pm 0.51$	$20.19^{Aa} \pm 0.38$
	21	$0.098^{Ec} \pm 0.001$	$85.05^{Aa} \pm 0.86$	$19.79^{Aa} \pm 0.38$

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–c) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-F) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.7: Thickness, % light transmission (%T) and transparency at 600 nm (T_{600}) 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 50% relative humidity at 25 °C for 21 days.

Film	Storage time (days)	Thickness (mm)	%T (%)	T_{600} (1/mm)
PLA	0	0.078 ± 0.001^{Aa}	91.98 ± 0.25^{Da}	25.33 ± 0.45^{Da}
	7	0.078 ± 0.002^{Aa}	91.94 ± 0.59^{Da}	25.30 ± 0.45^{Da}
	15	0.078 ± 0.003^{Aa}	91.78 ± 0.53^{Da}	25.08 ± 1.03^{Da}
	21	0.079 ± 0.003^{Aa}	91.63 ± 0.62^{Da}	24.88 ± 0.86^{Da}
PLA/WPI/PLA	0	0.086 ± 0.002^{Ba}	90.64 ± 0.69^{CDa}	22.84 ± 0.54^{Ca}
	7	0.086 ± 0.003^{Ba}	90.33 ± 0.10^{Ca}	22.76 ± 0.57^{Ca}
	15	0.088 ± 0.002^{Ba}	90.23 ± 1.16^{Ca}	22.17 ± 0.32^{BCa}
	21	0.089 ± 0.003^{BCa}	89.97 ± 0.63^{Ca}	22.06 ± 0.65^{BCa}
WPI	0	0.093 ± 0.001^{CDa}	86.60 ± 0.28^{Ba}	21.17 ± 0.12^{ABb}
	7	0.094 ± 0.003^{Da}	86.45 ± 1.02^{Ba}	20.75 ± 0.64^{Aab}
	15	0.096 ± 0.003^{Da}	85.22 ± 0.39^{ABa}	20.46 ± 0.37^{Aab}
	21	0.098 ± 0.004^{Da}	85.11 ± 0.62^{Aa}	20.20 ± 0.46^{Aa}

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–b) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-D) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.8: Thickness, % light transmission (%T) and transparency at 600 nm (T_{600}) 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 50% relative humidity at 4 °C for 21 days.

Film	Storage time (days)	Thickness (mm)	%T (%)	T_{600} (1/mm)
PLA	0	0.075 ± 0.001^{Aa}	92.35 ± 0.33^{Fa}	26.12 ± 0.17^{Da}
	7	0.076 ± 0.001^{Aa}	92.18 ± 0.19^{Fa}	25.85 ± 0.36^{Da}
	15	0.077 ± 0.003^{Aa}	92.18 ± 0.16^{Fa}	25.52 ± 0.99^{Da}
	21	0.077 ± 0.001^{Aa}	92.02 ± 0.34^{Fa}	25.47 ± 0.41^{Da}
PLA/WPI/PLA	0	0.089 ± 0.001^{BCa}	91.46 ± 0.16^{Eb}	22.14 ± 0.31^{Cb}
	7	0.090 ± 0.002^{BCab}	91.31 ± 0.27^{DEab}	21.83 ± 0.49^{Cab}
	15	0.090 ± 0.001^{BCab}	91.25 ± 0.13^{DEab}	21.74 ± 0.14^{BCab}
	21	0.092 ± 0.002^{CDb}	90.92 ± 0.23^{Da}	21.23 ± 0.51^{ABCa}
WPI	0	0.093 ± 0.001^{CDa}	87.13 ± 0.02^{Cc}	21.24 ± 0.39^{ABCa}
	7	0.094 ± 0.001^{DEa}	86.82 ± 0.64^{CBc}	20.87 ± 0.24^{ABa}
	15	0.095 ± 0.002^{DEa}	86.29 ± 0.22^{Bb}	20.68 ± 0.53^{Aa}
	21	0.096 ± 0.003^{Ea}	85.44 ± 0.02^{Aa}	20.36 ± 0.64^{Aa}

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–c) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-F) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.9: CIELab color space and 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 controlled storage 50% relative humidity at 35 °C for 21 days.

Film	Storage time (days)	*L**	*a**	*b**	ΔE_{ab}
PLA	0	92.48^{Gc} ± 0.04	-0.77^{BCa} ± 0.02	2.69^{Ab} ± 0.02	-
	7	92.43^{Gb} ± 0.01	-0.75^{BCa} ± 0.01	2.67^{Aab} ± 0.01	0.12^{Aa} ± 0.03
	15	92.28^{Fa} ± 0.03	-0.67^{DEb} ± 0.00	2.70^{Ab} ± 0.00	0.25^{Bb} ± 0.04
	21	92.25^{Fa} ± 0.03	-0.68^{DEb} ± 0.00	2.66^{Aa} ± 0.02	0.29^{Cb} ± 0.01
PLA/WPI/PLA	0	91.90^{DEb} ± 0.24	-0.71^{CDEb} ± 0.01	2.84^{Ba} ± 0.02	-
	7	91.88^{CDb} ± 0.04	-0.72^{CDa} ± 0.01	2.97^{Cb} ± 0.01	0.22^{Ba} ± 0.02
	15	91.78^{CDab} ± 0.05	-0.67^{DEc} ± 0.01	3.04^{Dbc} ± 0.07	0.30^{Cb} ± 0.03
	21	91.62^{Ba} ± 0.03	-0.67^{DEc} ± 0.00	3.12^{Ec} ± 0.05	0.42^{Dc} ± 0.02
WPI	0	92.04^{Ed} ± 0.01	-0.91^{Aa} ± 0.06	3.54^{Fa} ± 0.02	-
	7	91.88^{Dc} ± 0.07	-0.80^{Bab} ± 0.06	3.74^{Gb} ± 0.01	0.38^{Da} ± 0.08
	15	91.74^{Cb} ± 0.07	-0.72^{CDb} ±c 0.04	3.84^{Hc} ± 0.02	0.61^{Eb} ± 0.02
	21	91.48^{Aa} ± 0.07	-0.65^{Ec} ± 0.04	4.02^{Id} ± 0.02	0.86^{Fc} ± 0.03

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–d) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-I) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.10: CIELab color space and 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 controlled storage 50% relative humidity at 25 °C for 21 days.

Film	Storage time (days)	*L**	*a**	*b**	ΔE_{ab}
PLA	0	$92.33^{Ga} \pm 0.08$	$-0.77^{CDa} \pm 0.02$	$2.66^{Aab} \pm 0.01$	-
	7	$92.32^{FGa} \pm 0.01$	$-0.77^{CDa} \pm 0.01$	$2.65^{Aba} \pm 0.01$	$0.09^{Aa} \pm 0.01$
	15	$92.33^{Ga} \pm 0.04$	$-0.68^{Fb} \pm 0.00$	$2.66^{Ab} \pm 0.01$	$0.15^{Bb} \pm 0.01$
	21	$92.29^{FGa} \pm 0.04$	$-0.66^{Fc} \pm 0.02$	$2.62^{Aa} \pm 0.02$	$0.18^{Bc} \pm 0.02$
PLA/WPI/PLA	0	$92.24^{EFb} \pm 0.05$	$-0.79^{Ca} \pm 0.02$	$2.85^{Ba} \pm 0.03$	-
	7	$92.20^{Eb} \pm 0.03$	$-0.77^{CDa} \pm 0.01$	$2.84^{Ba} \pm 0.00$	$0.17^{Ba} \pm 0.01$
	15	$92.09^{Da} \pm 0.04$	$-0.72^{Ec} \pm 0.01$	$2.87^{Ba} \pm 0.04$	$0.25^{Cb} \pm 0.03$
	21	$92.05^{CDa} \pm 0.09$	$-0.75^{DEb} \pm 0.00$	$2.93^{Bb} \pm 0.03$	$0.31^{Dc} \pm 0.04$
WPI	0	$92.08^{Dc} \pm 0.02$	$-1.01^{Aa} \pm 0.01$	$3.56^{CDab} \pm 0.06$	-
	7	$91.98^{BCb} \pm 0.02$	$-0.99^{Aa} \pm 0.06$	$3.62^{Dab} \pm 0.05$	$0.24^{Ca} \pm 0.03$
	15	$91.95^{Bb} \pm 0.02$	$-0.98^{ABa} \pm 0.02$	$3.49^{Ca} \pm 0.15$	$0.31^{Da} \pm 0.04$
	21	$91.86^{Aa} \pm 0.03$	$-0.96^{Ba} \pm 0.01$	$3.74^{Eb} \pm 0.15$	$0.39^{Eb} \pm 0.03$

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–c) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-G) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.11: CIELab color space and 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 controlled storage 50% relative humidity at 4 °C for 21 days.

Film	Storage time (days)	*L**	*a**	*b**	ΔE_{ab}
PLA	0	$92.49^{Ea} \pm 0.06$	$-0.75^{BCa} \pm 0.04$	$2.68^{Ab} \pm 0.03$	-
	7	$92.46^{Ea} \pm 0.01$	$-0.72^{BCDab} \pm 0.01$	$2.67^{Aab} \pm 0.02$	$0.09^{Aa} \pm 0.02$
	15	$92.45^{Ea} \pm 0.04$	$-0.69^{CDbc} \pm 0.01$	$2.64^{Aab} \pm 0.03$	$0.13^{Aab} \pm 0.04$
	21	$92.46^{Ea} \pm 0.02$	$-0.65^{Dc} \pm 0.01$	$2.63^{Aa} \pm 0.00$	$0.17^{Bb} \pm 0.02$
PLA/WPI/PLA	0	$92.09^{Dc} \pm 0.08$	$-0.78^{Ba} \pm 0.04$	$2.87^{Ba} \pm 0.06$	-
	7	$92.03^{BCDbc} \pm 0.02$	$-0.75^{BCa} \pm 0.01$	$2.87^{Ba} \pm 0.01$	$0.14^{Ba} \pm 0.13$
	15	$91.95^{BCab} \pm 0.01$	$-0.71^{BCDb} \pm 0.01$	$2.89^{Ba} \pm 0.03$	$0.19^{Ca} \pm 0.15$
	21	$91.92^{ABCa} \pm 0.01$	$-0.72^{BCDb} \pm 0.01$	$2.91^{Ba} \pm 0.01$	$0.28^{Db} \pm 0.15$
WPI	0	$92.05^{CDb} \pm 0.20$	$-0.96^{Aa} \pm 0.15$	$3.50^{Ca} \pm 0.05$	-
	7	$91.97^{BCDab} \pm 0.03$	$-0.92^{Aa} \pm 0.04$	$3.53^{Ca} \pm 0.05$	$0.19^{Ca} \pm 0.01$
	15	$91.90^{ABab} \pm 0.07$	$-0.89^{Aa} \pm 0.02$	$3.63^{Db} \pm 0.02$	$0.30^{Db} \pm 0.02$
	21	$91.80^{Aa} \pm 0.05$	$-0.90^{Aa} \pm 0.02$	$3.70^{Db} \pm 0.06$	$0.37^{Ec} \pm 0.03$

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–c) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-E) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.12: Mechanical properties: tensile strength (TS), elastic modulus (EM) and percentage of elongation (%E) 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 50% relative humidity at 35 °C for 21 days.

Film	Storage time (days)	TS (MPa)	EM (MPa)	%E (%)
PLA	0	$51.90^{GHa} \pm 0.85$	$1937.58^{Ga} \pm 41.56$	$2.73^{Ac} \pm 0.01$
	7	$52.61^{HIab} \pm 0.69$	$1979.89^{GHab} \pm 39.00$	$2.69^{Abc} \pm 0.02$
	15	$53.19^{IJbc} \pm 0.59$	$2026.42^{HIb} \pm 27.77$	$2.65^{Ab} \pm 0.01$
	21	$54.01^{Jc} \pm 0.30$	$2144.98^{Jc} \pm 23.32$	$2.55^{Aa} \pm 0.03$
PLA/WPI/PLA	0	$46.60^{Ea} \pm 0.82$	$1679.89^{Ea} \pm 82.63$	$2.86^{Ac} \pm 0.04$
	7	$48.82^{Fb} \pm 0.92$	$1772.97^{Fa} \pm 68.80$	$2.76^{Ab} \pm 0.04$
	15	$50.68^{Gc} \pm 0.19$	$1963.03^{GHb} \pm 33.73$	$2.68^{Aa} \pm 0.01$
	21	$51.63^{GHc} \pm 0.86$	$2063.85^{Ib} \pm 71.24$	$2.64^{Aa} \pm 0.05$
WPI	0	$10.13^{Aa} \pm 0.58$	$182.06^{Aa} \pm 33.44$	$5.95^{Dc} \pm 0.60$
	7	$12.48^{Bb} \pm 0.23$	$279.91^{Bb} \pm 20.86$	$4.83^{Cb} \pm 0.37$
	15	$14.83^{Cc} \pm 0.77$	$374.26^{Cc} \pm 20.63$	$4.09^{Ba} \pm 0.04$
	21	$17.74^{Dd} \pm 0.98$	$505.51^{Dd} \pm 48.16$	$3.76^{Ba} \pm 0.19$

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–d) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-J) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.13: Mechanical properties: tensile strength (TS), elastic modulus (EM) and percentage of elongation (%E) 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 50% relative humidity at 25 °C for 21 days.

Film	Storage time (days)	TS (MPa)	EM (MPa)	%E (%)
PLA	0	52.76 ± 0.74^{Jb}	2094.43 ± 50.66^{Hb}	2.54 ± 0.03^{Aa}
	7	52.12 ± 0.96^{IJab}	2056.90 ± 59.96^{GHb}	2.56 ± 0.03^{ABa}
	15	51.85 ± 0.33^{Iab}	2033.37 ± 12.65^{Gab}	2.61 ± 0.07^{ABa}
	21	51.01 ± 0.11^{Ha}	1973.93 ± 11.21^{Fa}	2.62 ± 0.01^{ABa}
PLA/WPI/PLA	0	47.20 ± 0.19^{Gc}	1736.14 ± 20.06^{Eb}	2.75 ± 0.04^{ABa}
	7	46.41 ± 0.54^{Fb}	1717.31 ± 31.43^{Eb}	2.79 ± 0.01^{ABab}
	15	45.46 ± 0.40^{Ea}	1640.17 ± 31.09^{Da}	2.82 ± 0.01^{ABb}
	21	45.32 ± 0.31^{Ea}	1598.88 ± 16.05^{Da}	2.85 ± 0.05^{Bb}
WPI	0	10.34 ± 0.20^{Dd}	219.08 ± 19.97^{Cd}	5.08 ± 0.16^{Ca}
	7	9.59 ± 0.19^{Cc}	181.21 ± 8.52^{BCc}	5.42 ± 0.14^{Da}
	15	8.35 ± 0.17^{Bb}	146.55 ± 6.79^{ABb}	5.94 ± 0.40^{Eb}
	21	7.05 ± 0.15^{Aa}	115.01 ± 5.24^{Aa}	6.23 ± 0.31^{Fb}

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–d) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-J) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.14: Mechanical properties: tensile strength (TS), elastic modulus (EM) and percentage of elongation (%E) 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 50% relative humidity at 4 °C for 21 days.

Film	Storage time (days)	TS (MPa)	EM (MPa)	%E (%)
PLA	0	51.80 ± 0.15^{Ha}	2088.06 ± 11.97^{Ic}	2.49 ± 0.01^{Aa}
	7	51.45 ± 0.24^{GHa}	2051.17 ± 24.09^{Hb}	2.53 ± 0.04^{ABab}
	15	51.22 ± 0.53^{GHa}	2040.97 ± 8.80^{Hb}	2.56 ± 0.03^{ABb}
	21	51.10 ± 0.55^{Ga}	2001.56 ± 23.28^{Ga}	2.57 ± 0.02^{Bb}
PLA/WPI/PLA	0	47.78 ± 0.39^{Fb}	1805.41 ± 6.73^{Fc}	2.65 ± 0.02^{Ca}
	7	47.06 ± 0.25^{Eb}	1784.85 ± 43.21^{Fc}	2.68 ± 0.05^{Cab}
	15	45.39 ± 0.43^{Da}	1703.38 ± 11.94^{Eb}	2.72 ± 0.07^{CDab}
	21	44.86 ± 0.47^{Da}	1646.04 ± 37.42^{Da}	2.76 ± 0.04^{Db}
WPI	0	11.13 ± 0.03^{Cd}	224.27 ± 1.61^{Cd}	5.16 ± 0.04^{Ea}
	7	10.55 ± 0.49^{Cc}	187.25 ± 6.21^{Bc}	5.83 ± 0.05^{Fb}
	15	9.25 ± 0.15^{Bb}	156.73 ± 4.84^{ABb}	6.18 ± 0.09^{Gc}
	21	7.76 ± 0.09^{Aa}	124.96 ± 2.82^{Aa}	6.40 ± 0.01^{Hd}

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–d) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-I) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.15: Oxygen transmission rate (OTR), oxygen permeability (OP), water vapor transmission rate (WVTR) and water vapor permeability (WVP) 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 50% relative humidity at 35 °C for 21 days.

Film	Storage time (days)	OTR cc/[m².day]	OP cc.μm / [m².day].kPa	WVTR g/[m².day]	WVP g.mm / [m².day].kPa
PLA	0	$374.23^{DEa} \pm 1.22$	$285.30^{Da} \pm 6.98$	$132.33^{Ab} \pm 4.43$	$0.100^{Ac} \pm 0.000$
	7	$381.86^{Ea} \pm 9.20$	$285.30^{Da} \pm 1.49$	$131.93^{Ab} \pm 6.35$	$0.\ 097^{Abc} \pm 0.003$
	15	$365.81^{Da} \pm 0.81$	$280.72^{Da} \pm 5.77$	$122.44^{Aa} \pm 4.02$	$0.094^{Ab} \pm 0.002$
	21	$373.94^{DEa} \pm 7.45$	$279.45^{Da} \pm 0.30$	$117.85^{Aa} \pm 4.23$	$0.089^{Aa} \pm 0.001$
PLA/WPI/PLA	0	$44.38^{BCab} \pm 2.19$	$39.37^{Cb} \pm 1.94$	$133.47^{Ab} \pm 4.77$	$0.112^{Ab} \pm 0.003$
	7	$46.12^{Cb} \pm 2.30$	$37.93^{BCab} \pm 2.22$	$132.62^{Ab} \pm 8.36$	$0.103^{Aa} \pm 0.006$
	15	$42.29^{BCab} \pm 3.97$	$35.57^{BCab} \pm 2.45$	$125.69^{Ab} \pm 3.96$	$0.101^{Aa} \pm 0.002$
	21	$36.74^{Ba} \pm 3.35$	$32.22^{Ba} \pm 2.94$	$108.88^{Aa} \pm 5.11$	$0.095^{Aa} \pm 0.005$
WPI	0	$8.27^{Ab} \pm 0.08$	$7.95^{Ac} \pm 0.10$	$3834.70^{Ca} \pm 753.39$	$4.021^{Ed} \pm 0.071$
	7	$8.23^{Ab} \pm 0.36$	$7.43^{Abc} \pm 0.38$	$4030.71^{Ca} \pm 679.86$	$3.788^{Dc} \pm 0.089$
	15	$7.57^{Aab} \pm 0.34$	$7.01^{Aab} \pm 0.10$	$3542.99^{Ba} \pm 353.15$	$3.539^{Cb} \pm 0.104$
	21	$7.13^{Aa} \pm 0.13$	$6.47^{Aa} \pm 0.08$	$3145.23^{Ba} \pm 121.20$	$3.051^{Ba} \pm 0.148$

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–c) indicate significant differences in each film samples during storage ($p \le 0.05$).

***Different superscript upper-case letters (A-E) indicate significant differences between all samples among the same storage temperature used ($p \le 0.05$).

Appendix B.16: Oxygen transmission rate (OTR), oxygen permeability (OP), water vapor transmission rate (WVTR) and water vapor permeability (WVP) 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 50% relative humidity at 25 °C for 21 days.

Film	Storage time (days)	OTR cc/[m².day]	OP cc.µm / [m².day].kPa	WVTR g/[m².day]	WVP g.mm / [m².day].kPa
PLA	0	^{Ca} 354.62 ± 7.94	^{Ca} 268.60 ± 6.01	^{Ab} 123.57 ± 0.76	^{Ac} 0.096 ± 0.002
	7	^{DEa} 365.61 ± 6.38	^{Ca} 269.63 ± 4.71	^{Aab} 116.47 ± 7.28	^{Ab} 0.091 ± 0.004
	15	^{CDa} 360.31 ± 2.24	^{Ca} 274.72 ± 9.33	^{Aa} 113.72 ± 3.92	^{Aab} 0.086 ± 0.001
	21	^{Ea} 370.66 ± 8.02	^{Ca} 271.53 ± 8.48	^{Aa} 109.81 ± 4.96	^{Aa} 0.083 ± 0.002
PLA/WPI/PLA	0	^{Ba} 43.30 ± 0.09	^{Ba} 34.07 ± 1.87	^{Aa} 115.89 ± 7.69	^{Ac} 0.104 ± 0.005
	7	^{Ba} 46.42 ± 5.50	^{Ba} 36.34 ± 6.75	^{Aa} 113.16 ± 3.82	^{Abc} 0.101 ± 0.005
	15	^{Ba} 45.76 ± 5.59	^{Ba} 36.11 ± 5.05	^{Aa} 106.91 ± 6.24	^{Aab} 0.093 ± 0.005
	21	^{Ba} 47.57 ± 1.83	^{Ba} 37.69 ± 1.46	^{Aa} 117.54 ± 6.31	^{Aa} 0.091 ± 0.006
WPI	0	^{Ab} 8.07 ± 0.23	^{Aa} 6.77 ± 0.31	^{Ba} 3354.25 ± 849.90	^{Db} 3.345 ± 0.075
	7	^{Aab} 7.86 ± 0.24	^{Aa} 6.75 ± 0.13	^{Ba} 3371.34 ± 767.01	^{Cb} 3.199 ± 0.082
	15	^{Aa} 7.35 ± 0.06	^{Aa} 7.40 ± 0.44	^{Ba} 3258.04 ± 429.94	^{Ba} 3.014 ± 0.083
	21	^{Aa} 7.41 ± 0.26	^{Aa} 7.13 ± 0.01	^{Ba} 3328.42 ± 430.17	^{Ba} 2.941 ± 0.095

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–c) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-E) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.17: Oxygen transmission rate (OTR), oxygen permeability (OP), water vapor transmission rate (WVTR) and water vapor permeability (WVP) 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 50% relative humidity at 4 °C for 21 days.

Film	Storage time (days)	OTR cc/[m².day]	OP cc.µm / [m².day].kPa	WVTR g/[m².day]	WVP g.mm / [m².day].kPa
PLA	0	349.72 ± 6.39^{Ea}	259.59 ± 2.65^{Ca}	120.36 ± 2.94^{Ab}	0.091 ± 0.001^{Ac}
	7	346.55 ± 1.97^{Ea}	262.45 ± 8.27^{Ca}	112.47 ± 1.08^{Aab}	0.086 ± 0.002^{Ab}
	15	349.67 ± 1.82^{Ea}	264.86 ± 6.31^{Ca}	110.33 ± 3.86^{Aa}	0.084 ± 0.001^{Aa}
	21	340.24 ± 0.30^{Da}	264.48 ± 0.24^{Ca}	111.48 ± 2.04^{Aa}	0.082 ± 0.001^{Aa}
PLA/WPI/PLA	0	35.53 ± 2.80^{Ba}	32.22 ± 2.54^{Ba}	117.51 ± 5.71^{Aa}	0.103 ± 0.008^{Aa}
	7	35.60 ± 2.44^{Ba}	32.10 ± 1.95^{Ba}	115.77 ± 1.45^{Aa}	0.098 ± 0.002^{Aa}
	15	38.85 ± 1.39^{BCa}	35.42 ± 1.00^{Ba}	116.73 ± 12.63^{Aa}	0.094 ± 0.010^{Aa}
	21	41.46 ± 3.53^{Ca}	36.29 ± 1.34^{Ba}	108.30 ± 4.90^{Aa}	0.092 ± 0.003^{Aa}
WPI	0	7.41 ± 0.06^{Ab}	6.29 ± 0.06^{Aa}	3409.36 ± 619.14^{Ba}	3.144 ± 0.199^{Ca}
	7	7.11 ± 0.48^{Aab}	6.13 ± 0.30^{Aa}	3432.80 ± 316.35^{Ba}	3.112 ± 0.058^{BCa}
	15	6.91 ± 0.31^{Aab}	6.55 ± 0.39^{Aa}	3249.30 ± 289.80^{Ba}	3.038 ± 0.039^{BCa}
	21	6.37 ± 0.08^{Aa}	6.79 ± 0.15^{Aa}	3323.90 ± 160.59^{Ba}	3.029 ± 0.017^{Ba}

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–c) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-E) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.18: **Thickness, % light transmission (%T) and transparency at 600 nm (T_{600}) of** polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch used to package baby formula. The packaged baby formula samples were stored in a dark environmental chamber with controlled 50% relative humidity, at 35 °C for 19 days.

Pouch	Storage time (days)	Thickness (mm)	%T (%)	T_{600} (1/mm)
PLA	0	$0.072^{Aa} \pm 0.001$	$92.27^{Ba} \pm 0.73$	$27.24^{Ca} \pm 0.52$
	5	$0.074^{Aa} \pm 0.003$	$92.25^{Ba} \pm 0.23$	$26.96^{Ca} \pm 1.47$
	12	$0.073^{Aa} \pm 0.001$	$92.16^{Ba} \pm 0.06$	$26.77^{Ca} \pm 0.38$
	19	$0.075^{Aa} \pm 0.002$	$92.06^{Ba} \pm 0.35$	$26.20^{Ca} \pm 0.66$
PLA/WPI/PLA	0	$0.088^{Ba} \pm 0.003$	$90.85^{Aa} \pm 0.72$	$22.40^{Bb} \pm 0.76$
	5	$0.090^{BCa} \pm 0.000$	$90.74^{Aa} \pm 0.43$	$21.87^{ABab} \pm 0.60$
	12	$0.090^{BCa} \pm 0.003$	$90.58^{Aa} \pm 0.18$	$21.55^{ABab} \pm 0.48$
	19	$0.094^{Ca} \pm 0.004$	$90.13^{Aa} \pm 0.23$	$20.96^{Aa} \pm 0.85$

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–b) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-C) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.19: **Thickness, % light transmission (%T) and transparency at 600 nm (T_{600}) of** polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch used to package baby formula. The packaged baby formula samples were stored in a dark environmental chamber with controlled 50% relative humidity, at 25 °C for 19 days.

Pouch	Storage time (days)	Thickness (mm)	%T (%)	T_{600} (1/mm)
PLA	0	0.079^{Aa} ± 0.002	92.38^{Ba} ± 0.57	25.11^{Ba} ± 0.74
	5	0.079^{Aa} ± 0.003	92.27^{Ba} ± 0.49	24.97^{Ba} ± 0.91
	12	0.080^{Aa} ± 0.002	91.99^{Ba} ± 0.05	24.66^{Ba} ± 0.70
	19	0.081^{Aa} ± 0.002	92.08^{Ba} ± 0.28	24.56^{Ba} ± 0.70
PLA/WPI/PLA	0	0.089^{Ba} ± 0.001	90.78^{Ab} ± 0.25	22.05^{Aa} ± 0.30
	5	0.090^{Ba} ± 0.001	90.48^{Aab} ± 0.45	21.75^{Aa} ± 0.14
	12	0.091^{Ba} ± 0.002	90.41^{Aab} ± 0.12	21.41^{Aa} ± 0.52
	19	0.092^{Ba} ± 0.003	90.16^{Aa} ± 0.25	21.21^{Aa} ± 0.68

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–b) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-B) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.20: **Thickness, % light transmission (%T) and transparency at 600 nm (T_{600}) of** polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch used to package baby formula. The packaged baby formula samples were stored in a dark environmental chamber with controlled 50% relative humidity, at 4 °C for 19 days.

Pouch	Storage time (days)	Thickness (mm)	%T (%)	T_{600} (1/mm)
PLA	0	$0.075^{Aa} \pm 0.001$	$92.56^{Ba} \pm 0.24$	$25.93^{Ba} \pm 0.15$
	5	$0.076^{Aab} \pm 0.000$	$92.48^{Ba} \pm 0.34$	$25.79^{Ba} \pm 0.05$
	12	$0.077^{Aab} \pm 0.002$	$92.27^{Ba} \pm 0.28$	$25.55^{Ba} \pm 0.71$
	19	$0.078^{Ab} \pm 0.000$	$92.29^{Ba} \pm 0.08$	$25.24^{Ba} \pm 0.23$
PLA/WPI/PLA	0	$0.088^{Ba} \pm 0.004$	$90.53^{Aa} \pm 0.59$	$22.228^{Aa} \pm 0.92$
	5	$0.089^{Ba} \pm 0.002$	$90.42^{Aa} \pm 0.37$	$22.13^{Aa} \pm 0.60$
	12	$0.090^{Ba} \pm 0.003$	$90.20^{Aa} \pm 0.39$	$21.68^{Aa} \pm 0.84$
	19	$0.091^{Ba} \pm 0.002$	$90.03^{Aa} \pm 0.63$	$21.43^{Aa} \pm 0.56$

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–b) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-B) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.21: CIELab **color space and total color difference (ΔE_{ab}) of** polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch used to package baby formula. The packaged baby formula samples were stored in a dark environmental chamber with controlled 50% relative humidity, at 35 °C for 19 days.

Pouch	Storage time (days)	*L**	*a**	*b**	ΔE_{ab}
PLA	0	92.39^{Dc} ± 0.07	-0.78^{Aa} ± 0.00	2.72^{Aa} ± 0.03	-
	5	92.34^{CDbc} ± 0.02	-0.74^{Bb} ± 0.01	2.70^{Aa} ± 0.01	0.07^{Aa} ± 0.01
	12	92.28^{BCab} ± 0.02	-0.68^{Cc} ± 0.01	2.70^{Aa} ± 0.02	0.16^{Bb} ± 0.02
	19	92.21^{Ba} ± 0.07	-0.68^{Cc} ± 0.02	2.68^{Aa} ± 0.01	0.25^{Cc} ± 0.02
PLA/WPI/PLA	0	92.25^{Bc} ± 0.03	-0.75^{Ba} ± 0.01	2.71^{Aa} ± 0.03	-
	5	92.23^{Bc} ± 0.02	-0.73^{Ba} ± 0.02	2.81^{Bb} ± 0.04	0.15^{Ba} ± 0.02
	12	92.13^{Ab} ± 0.03	-0.69^{Cb} ± 0.00	2.87^{Cb} ± 0.04	0.24^{Cb} ± 0.03
	19	92.08^{Aa} ± 0.03	-0.69^{Cb} ± 0.02	2.93^{Dc} ± 0.03	0.34^{Dc} ± 0.01

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–c) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-D) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.22: CIELab **color space and total color difference (ΔE_{ab})** of polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch used to package baby formula. The packaged baby formula samples were stored in a dark environmental chamber with controlled 50% relative humidity, at 25 °C for 19 days.

Pouch	Storage time (days)	*L**	*a**	*b**	ΔE_{ab}
PLA	0	$92.41^{DEa} \pm 0.04$	$-0.77^{Aa} \pm 0.00$	$2.68^{Bc} \pm 0.01$	-
	5	$92.42^{Ea} \pm 0.02$	$-0.77^{Aa} \pm 0.01$	$2.65^{ABb} \pm 0.01$	$0.04^{Aa} \pm 0.01$
	12	$92.40^{DEa} \pm 0.02$	$-0.70^{Bb} \pm 0.01$	$2.65^{ABb} \pm 0.01$	$0.09^{Bb} \pm 0.03$
	19	$92.37^{Da} \pm 0.01$	$-0.69^{Bb} \pm 0.02$	$2.62^{Aa} \pm 0.02$	$0.12^{BCb} \pm 0.02$
PLA/WPI/PLA	0	$92.26^{Cc} \pm 0.03$	$-0.77^{Aa} \pm 0.01$	$2.84^{Cab} \pm 0.03$	-
	5	$92.24^{Cc} \pm 0.03$	$-0.76^{Aa} \pm 0.01$	$2.84^{Ca} \pm 0.02$	$0.13^{Ca} \pm 0.02$
	12	$92.12^{Bb} \pm 0.02$	$-0.73^{Bab} \pm 0.02$	$2.84^{Cab} \pm 0.05$	$0.19^{Db} \pm 0.02$
	19	$92.02^{Aa} \pm 0.02$	$-0.71^{Bb} \pm 0.03$	$2.90^{Db} \pm 0.02$	$0.26^{Ec} \pm 0.02$

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–c) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-E) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

***Appendix B.23: CIELab* color space and total color difference (ΔE_{ab}) of** polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch used to package baby formula. The packaged baby formula samples were stored in a dark environmental chamber with controlled 50% relative humidity, at 4 °C for 19 days.

Pouch	Storage time (days)	*L**	*a**	*b**	ΔE_{ab}
PLA	0	$92.42^{Cb} \pm 0.02$	$-0.76^{Aa} \pm 0.01$	$2.68^{Aa} \pm 0.02$	-
	5	$92.41^{Bb} \pm 0.01$	$-0.76^{Aa} \pm 0.01$	$2.66^{Aa} \pm 0.02$	$0.04^{Aa} \pm 0.02$
	12	$92.41^{Ab} \pm 0.02$	$-0.71^{CDb} \pm 0.01$	$2.66^{Aa} \pm 0.02$	$0.08^{Ba} \pm 0.01$
	19	$92.37^{Aa} \pm 0.02$	$-0.74^{ABa} \pm 0.02$	$2.68^{Aa} \pm 0.01$	$0.12^{CDb} \pm 0.02$
PLA/WPI/PLA	0	$92.27^{Dc} \pm 0.01$	$-0.75^{ABa} \pm 0.01$	$2.84^{Ba} \pm 0.02$	-
	5	$92.27^{Dbc} \pm 0.04$	$-0.73^{BCab} \pm 0.02$	$2.84^{Ba} \pm 0.01$	$0.10^{BCa} \pm 0.01$
	12	$92.17^{Dab} \pm 0.05$	$-0.71^{CDbc} \pm 0.02$	$2.86^{BCab} \pm 0.01$	$0.15^{Db} \pm 0.01$
	19	$92.13^{Da} \pm 0.02$	$-0.69^{Dc} \pm 0.01$	$2.88^{Cb} \pm 0.01$	$0.23^{Ec} \pm 0.02$

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–c) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-D) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.24: CIELab **color space and total color difference (ΔE_{ab}) of** baby formula packaged in polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch used to package baby formula. The packaged baby formula samples were stored in a dark environmental chamber with controlled 50% relative humidity, at 35 °C for 19 days.

Pouch	Storage time (days)	*L**	*a**	*b**	ΔE_{ab}
PLA	0	$96.64^{CDc} \pm 0.12$	$-2.48^{ABa} \pm 0.03$	$12.68^{Ba} \pm 0.03$	-
	5	$96.54^{Cc} \pm 0.04$	$-2.36^{Cb} \pm 0.04$	$12.79^{Cb} \pm 0.04$	$0.20^{Aa} \pm 0.04$
	12	$96.34^{Bb} \pm 0.05$	$-2.21^{Dc} \pm 0.02$	$12.92^{Dc} \pm 0.05$	$0.47^{Bb} \pm 0.05$
	19	$96.14^{Aa} \pm 0.02$	$-2.12^{Ed} \pm 0.02$	$13.13^{Fd} \pm 0.03$	$0.76^{Cc} \pm 0.02$
PLA/WPI/PLA	0	$96.68^{Dc} \pm 0.11$	$-2.51^{Aa} \pm 0.05$	$12.61^{Aa} \pm 0.06$	-
	5	$96.58^{CDc} \pm 0.06$	$-2.42^{Bb} \pm 0.04$	$12.73^{Bb} \pm 0.03$	$0.17^{Aa} \pm 0.02$
	12	$96.35^{Bb} \pm 0.03$	$-2.26^{Dc} \pm 0.01$	$12.87^{Dc} \pm 0.01$	$0.43^{Bb} \pm 0.03$
	19	$96.14^{Aa} \pm 0.03$	$-2.10^{Ed} \pm 0.04$	$13.04^{Ed} \pm 0.02$	$0.73^{Cc} \pm 0.01$

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–d) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-F) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.25: CIELab **color space and total color difference (ΔE_{ab}) of** baby formula packaged in polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch used to package baby formula. The packaged baby formula samples were stored in a dark environmental chamber with controlled 50% relative humidity, at 25 °C for 19 days.

Pouch	Storage time (days)	*L**	*a**	*b**	ΔE_{ab}
PLA	0	96.67 ± 0.03^{Ec}	-2.52 ± 0.02^{Aa}	12.67 ± 0.05^{ABa}	-
	5	96.62 ± 0.07^{DEbc}	-2.48 ± 0.03^{ABCab}	12.66 ± 0.05^{ABa}	0.13 ± 0.01^{ABa}
	12	96.54 ± 0.05^{BCab}	-2.45 ± 0.03^{BCb}	12.72 ± 0.03^{BCa}	0.17 ± 0.02^{Cb}
	19	96.46 ± 0.02^{Aa}	-2.39 ± 0.02^{Dc}	12.82 ± 0.00^{Db}	0.29 ± 0.02^{Ec}
PLA/WPI/PLA	0	96.69 ± 0.01^{Ec}	-2.49 ± 0.04^{ABa}	12.65 ± 0.04^{Aa}	-
	5	96.66 ± 0.01^{DEc}	-2.47 ± 0.02^{BCa}	12.66 ± 0.01^{ABa}	0.12 ± 0.03^{Aa}
	12	96.60 ± 0.01^{CDb}	-2.44 ± 0.03^{CDab}	12.72 ± 0.02^{Cb}	0.16 ± 0.01^{BCb}
	19	96.52 ± 0.01^{ABa}	-2.39 ± 0.02^{Db}	12.81 ± 0.02^{Dc}	0.25 ± 0.00^{Dc}

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–c) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-E) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.26: CIELab **color space and total color difference (ΔE_{ab}) of** baby formula packaged in polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch used to package baby formula. The packaged baby formula samples were stored in a dark environmental chamber with controlled 50% relative humidity, at 4 °C for 19 days.

Pouch	Storage time (days)	*L**	*a**	*b**	ΔE_{ab}
PLA	0	96.66^{DEc} ± 0.05	-2.51^{Aa} ± 0.05	12.68^{Aa} ± 0.03	-
	5	96.61^{BCbc} ± 0.01	-2.50^{Aab} ± 0.01	12.68^{Aab} ± 0.00	0.08^{Aa} ± 0.02
	12	96.58^{Bb} ± 0.03	-2.48^{ABCab} ± 0.02	12.73^{ABb} ± 0.03	0.14^{Bb} ± 0.01
	19	96.50^{Aa} ± 0.01	-2.45^{BCDb} ± 0.01	12.80^{Cc} ± 0.03	0.24^{Dc} ± 0.02
PLA/WPI/PLA	0	96.69^{Ed} ± 0.03	-2.49^{ABa} ± 0.02	12.68^{Aa} ± 0.08	-
	5	96.63^{CDc} ± 0.01	-2.47^{ABCa} ± 0.01	12.69^{Aab} ± 0.02	0.08^{Aa} ± 0.00
	12	96.58^{Bb} ± 0.01	-2.44^{CDb} ± 0.01	12.72^{ABab} ± 0.01	0.13^{Bb} ± 0.01
	19	96.52^{Aa} ± 0.01	-2.42^{Db} ± 0.01	12.77^{BCb} ± 0.01	0.21^{Cc} ± 0.01

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–c) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-E) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.27: **Water activity (a_w), moisture content (%MC), conjugate diene (CD) content and total carbonyl compound (C_T) of** baby formula packaged in polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch used to package baby formula. The packaged baby formula samples were stored in a dark environmental chamber with controlled 50% relative humidity, at 35 °C for 19 days.

Pouch	Storage time (days)	a_w	%MC (%)	CD (μmol/g oil)	C_T (μmol/g oil)
PLA	0	0.246 ± 0.001^{Aa}	4.45 ± 0.04^{Aa}	15.88 ± 1.45^{Aa}	5.63 ± 0.13^{Aa}
	5	0.256 ± 0.004^{Bb}	4.65 ± 0.05^{Bb}	32.99 ± 4.73^{Cb}	14.07 ± 0.62^{Cb}
	12	0.264 ± 0.001^{Cc}	4.75 ± 0.11^{BCb}	58.13 ± 2.22^{Ec}	22.84 ± 3.46^{Ec}
	19	0.277 ± 0.001^{Dd}	5.00 ± 0.09^{Dc}	72.56 ± 2.16^{Fd}	29.42 ± 1.25^{Fd}
PLA/WPI/PLA	0	0.248 ± 0.001^{Aa}	4.48 ± 0.03^{Aa}	15.59 ± 0.86^{Aa}	5.40 ± 0.03^{Aa}
	5	0.255 ± 0.002^{Bb}	4.73 ± 0.06^{BCb}	19.66 ± 0.99^{Ab}	9.76 ± 0.77^{Bb}
	12	0.265 ± 0.002^{Cc}	4.84 ± 0.10^{Cb}	28.25 ± 2.29^{Bc}	10.54 ± 0.73^{Bb}
	19	0.279 ± 0.001^{Dd}	5.02 ± 0.10^{Dc}	48.82 ± 0.65^{Dd}	16.54 ± 0.39^{Dc}

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–d) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-F) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.28: **Water activity (a_w), moisture content (%MC), conjugate diene (CD) content and total carbonyl compound (C_T) of** baby formula packaged in polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch used to package baby formula. The packaged baby formula samples were stored in a dark environmental chamber with controlled 50% relative humidity, at 25 °C for 19 days.

Pouch	Storage time (days)	a_w	%MC (%)	CD (μmol/g oil)	C_T (μmol/g oil)
PLA	0	0.245 ± 0.002^{Aa}	4.37 ± 0.09^{Aa}	15.54 ± 1.56^{Aa}	5.73 ± 0.26^{Aa}
	5	0.254 ± 0.006^{Bb}	4.62 ± 0.11^{Bb}	21.70 ± 2.33^{Bb}	8.60 ± 0.48^{Cb}
	12	0.270 ± 0.005^{Dc}	4.85 ± 0.09^{Cc}	33.05 ± 1.42^{Dc}	17.10 ± 0.54^{Fc}
	19	0.278 ± 0.001^{EFd}	4.03 ± 0.08^{Dd}	55.31 ± 2.76^{Ed}	20.00 ± 0.73^{Gd}
PLA/WPI/PLA	0	0.249 ± 0.002^{ABa}	4.51 ± 0.04^{Ba}	15.49 ± 1.65^{Aa}	5.63 ± 0.29^{Aa}
	5	0.260 ± 0.004^{Cb}	4.61 ± 0.03^{Bb}	19.87 ± 1.96^{Bb}	7.53 ± 0.09^{Bb}
	12	0.275 ± 0.005^{DEc}	5.00 ± 0.07^{Dc}	26.30 ± 2.54^{Cc}	10.56 ± 0.38^{Dc}
	19	0.283 ± 0.003^{Fd}	5.10 ± 0.04^{Dd}	36.02 ± 1.28^{Dd}	12.28 ± 0.73^{Ed}

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–d) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-F) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

Appendix B.29: **Water activity (a_w), moisture content (%MC), conjugate diene (CD) content and total carbonyl compound (C_T) of** baby formula packaged in polylactic acid (PLA) pouch and whey protein isolate (WPI)-enhanced composite structure (PLA/WPI/PLA) pouch used to package baby formula. The packaged baby formula samples were stored in a dark environmental chamber with controlled 50% relative humidity, at 4 °C for 19 days.

Pouch	Storage time (days)	a_w	%MC (%)	CD (μmol/g oil)	C_T (μmol/g oil)
PLA	0	$0.245^{Aa} \pm 0.002$	$4.46^{Aa} \pm 0.07$	$15.11^{Aa} \pm 0.85$	$5.51^{Aa} \pm 0.07$
	5	$0.255^{Bb} \pm 0.005$	$4.63^{Bb} \pm 0.10$	$16.16^{Aa} \pm 0.77$	$5.93^{Aa} \pm 0.66$
	12	$0.266^{CDc} \pm 0.006$	$4.82^{Cc} \pm 0.06$	$24.38^{BCb} \pm 0.42$	$10.37^{Cb} \pm 1.36$
	19	$0.271^{DEc} \pm 0.004$	$4.92^{Cc} \pm 0.03$	$34.65^{Dc} \pm 1.45$	$12.30^{Dc} \pm 0.55$
PLA/WPI/PLA	0	$0.248^{Aa} \pm 0.004$	$4.54^{ABa} \pm 0.08$	$15.10^{Aa} \pm 0.99$	$5.84^{Aa} \pm 0.55$
	5	$0.261^{BCb} \pm 0.004$	$4.81^{Cb} \pm 0.05$	$15.15^{Aa} \pm 1.18$	$5.55^{Aa} \pm 0.37$
	12	$0.270^{DEc} \pm 0.004$	$4.93^{Cbc} \pm 0.06$	$23.23^{Bb} \pm 0.66$	$8.30^{Bb} \pm 0.58$
	19	$0.275^{Ec} \pm 0.002$	$5.06^{Dc} \pm 0.10$	$26.28^{Cc} \pm 1.82$	$10.12^{Cc} \pm 0.64$

* Values are the average ± standard deviation. Means with the same superscript within the same column are not significantly different from each other ($p > 0.05$).

** Different superscript lower-case letters (a–c) indicate significant differences in each film samples during storage ($p \leq 0.05$).

***Different superscript upper-case letters (A-E) indicate significant differences between all samples among the same storage temperature used ($p \leq 0.05$).

VITA

Ms. Thunyaluck Phupoksakul was born on October 31, 1988 in Bangkok, Thailand. In 2010, she graduated Bachelor of Science from Food Science and Technology Program, Faculty of Science, King Mongkut's University of Technology, Thonburi (KMUTT). She enrolled in Master of Science in Food Technology Program, Faculty of Science, Chulalongkorn University in 2011.

PUBLICATION AND PRESENTATION

- Publication paper in Journal of the Science of Food and Agriculture. Phupoksakul T, Leuangsukrerk M, Numpiboonmarn P, Somwangthanaroj A, and Janjarasskul T. (2014). Properties of poly(lactide)–whey protein isolate laminated films. Journal of the Science of Food and Agriculture, doi: 10.1002/jsfa.6775

- Abstract accepted for poster section at the Institute of Food Technologists (IFT) Annual Meeting & Food Expo® (June 21st – 24th, the New Orleans Morial Convention Center, New Orleans, LA, U.S.A.), in the topic of "The effect of temperature on color, mechanical properties, and water vapor permeability of polylactic acid-whey protein isolate laminated films during storage".

- Oral Presentation at 5th International Conference on Fermentation Technology for Value Added Agricultural Product (August 21st – 23rd 2013, Centara Hotel & Convention Centre, Khon Kaen, Thailand), in the topic of "Barrier properties of polylactic acid-whey protein isolate laminated films".