Effect of Epoxidized Natural Rubber (ENR) on Properties of Bovine Gelatin Film

Pornsawan Chamnanvatchakit

A Thesis Submitted in Partial Fulfillment of the Requirementsfor the Degree of
Master of Science in Packaging Technology
Prince of Songkla University
2010

Thesis Tiltle	Effect of Epoxidized Natural Rubber (ENR) on Properties of Bovine Gelatin Film
Author	Miss Pornsawan Chamnanvatchakit
Major Program	Packaging Technology

Major Advisor	**Examining Committee:**
……………………..…………….	….…………..………..……Chairperson
(Asst. Prof. Dr. Thummanoon Prodpran)	(Dr. Supachai pisatpen)
	…………………...…………………
Co-advisor	(Asst. Prof. Dr. Thummanoon Prodpran)
……………………..…………….	……………………..…………….
(Prof. Dr. Soottawat Benjakul)	(Prof. Dr. Soottawat Benjakul)
	……………………..………………
	(Asst. Prof. Dr. Sureurg Khongtong)

The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for the Master of Science Degree in Packaging Technology.

..……………………………………..……

(Assoc. Prof. Dr. Krerkchai Thongnoo)

Dean of Graduate School

ชื่อวิทยานิพนธ์ ผลของความเข้มข้นกลีเซอรอลและการเติมยางธรรมชาติอิพอกไซด์ (ENR) และยางธรรมชาติ (NR) ต่อสมบัติของฟิล์มเจลาตินจากวัว
ผู้เขียน นางสาว พรสวรรค์ ชำนาญเวชกิจ
สาขาวิชา เทคโนโลยีบรรจุภัณฑ์
ปีการศึกษา 2553

บทคัดย่อ

จากการศึกษาผลของความเข้มข้นกลีเซอรอลที่ระดับต่างๆ (0,15, 20, 25 และ 30 เปอร์เซ็นต์ โดยน้ำหนักของโปรตีน) ต่อสมบัติของฟิล์มเจลาตินจากวัว พบว่า ฟิล์มเจลาตินมีความแข็งแรงและความแข็งตึงลดลงเมื่อปริมาณกลีเซอรอลที่ใช้เพิ่มขึ้น ซึ่งบ่งบอกจากค่าความต้านทานแรงดึง (TS) และค่ายังส์มอดุลัส (E) ที่เพิ่มขึ้น ($p<0.05$) ค่าการยืดตัวเมื่อขาด (EAB) ที่แสดงถึงความสามารถในการยืดตัวหรือความอ่อนตัวของฟิล์ม มีค่าเพิ่มขึ้นตามปริมาณกลีเซอรอลที่เพิ่มขึ้น ($p<0.05$) รวมทั้งปริมาณความชื้นและค่าการซึมผ่านไอน้ำ (WVP) ของฟิล์มเจลาตินมีค่าสูงขึ้นเมื่อปริมาณกลีเซอรอลเพิ่มขึ้น ($p<0.05$) อันเป็นผลมาจากปริมาณหมู่ที่ชอบน้ำที่เพิ่มมากขึ้นในฟิล์มเจลาติน นอกจากนี้ พบว่า การเติมกลีเซอรอลมีผลให้การส่องผ่านแสงในช่วงยูวี (350 – 800 nm) ของฟิล์มเจลาตินเพิ่มขึ้นเล็กน้อย แต่ไม่มีผลต่อสีของฟิล์มเจลาตินที่ได้ ดังนั้นการเติมกลีเซอรอลในปริมาณที่เหมาะสมสามารถปรับปรุงความยืดหยุ่นหรือความอ่อนตัวของของฟิล์มเจลาตินได้ แต่ฟิล์มที่ได้มีสมบัติการป้องกันการซึมผ่านไอน้ำลดลง ดังนั้นการเติมกลีเซอรอลทำให้เกิดสภาพพลาสติกในฟิล์มเจลาติน อันเป็นผลเนื่องมาจากการลดลงของแรงดึงดูดภายในโมเลกุลและระหว่างโมเลกุลของโปรตีน รวมทั้งการเพิ่มขึ้นของระยะห่างระหว่างโมเลกุลของโปรตีน ทำให้ความสามารถในการเคลื่อนไหวตัวของสายโซ่โมเลกุลเพิ่มขึ้น

จากการศึกษาผลของอัตราส่วนระหว่างเจลาตินต่อยาง ENR (G/ENR = 10/0, 8/2, 6/4, 5/5 และ 0/10) และชนิดของ ENR (ENR-10, ENR-25 และ ENR-50 ที่มีปริมาณหมู่อิพ็อกซีประมาณ 10, 25 และ 50 เปอร์เซ็นต์โมล ตามลำดับ) ต่อสมบัติของฟิล์มผสมที่ได้ พบว่า เมื่อระดับของหมู่อิพ็อกซีและปริมาณการเติม ENR ที่สูงขึ้น ฟิล์มที่ได้มีค่า TS และความโปร่งใสลดลง แต่มีค่า EAB และความเหลือง (ค่า b^*) สูงขึ้น ($p<0.05$) และพบว่า ฟิล์มเจลาตินที่เติมยาง ENR มีค่า WVP ลดลง ($p<0.05$) การเติม ENR-25 ที่อัตราส่วน G/ENR = 6/4 ให้ฟิล์มผสมที่มีสมบัติที่ดีขึ้นมากที่สุด โดยฟิล์มผสมดังกล่าวมีค่า EAB (หรือความอ่อนตัวของฟิล์ม) และการป้องกันการซึมผ่านไอน้ำเพิ่มขึ้นประมาณ 1.8 และ 1.3 เท่า ตามลำดับ เมื่อเปรียบเทียบกับฟิล์มเจลาตินที่ไม่เติม ENR จากผลการวิเคราะห์ด้วยเทคนิค SEM และ FTIR แสดงให้เห็นว่า สมบัติที่ดีขึ้นของฟิล์มผสมระหว่าง

G/ENR ดังกล่าว อาจเกิดจากความเข้ากันได้ดีระหว่างเจลาตินและ ENR อันเป็นผลมาจากการเกิดอันตรกิริยาทางเคมีระหว่างโมเลกุลของเจลาตินและ ENR

จากการศึกษาผลของการใช้ยาง ENR-25 ร่วมกับการลดปริมาณกลีเซอรอลที่เติมต่อสมบัติของฟิล์มเจลาตินจากวัว พบว่า ฟิล์มผสมที่เตรียมจากเจลาตินและ ENR-25 ที่อัตราส่วน G/ENR = 6/4 มีค่า TS และค่า E เพิ่มขึ้น แต่มีค่า EAB และค่า WVP ลดลง เมื่อปริมาณกลีเซอรอลที่ใช้ลดลง ($p<0.05$) ฟิล์มผสมที่เตรียมจาก G/ENR-25 = 6/4 และไม่เติมกลีเซอรอลมีสมบัติการป้องกันการซึมผ่านไอน้ำดีขึ้นมากที่สุด ซึ่งมากกว่าฟิล์มผสมที่เตรียมจาก G/ENR (6/4) ที่เติมกลีเซอรอล 25 เปอร์เซ็นต์ ประมาณ 1.4 เท่า และมากกว่าฟิล์มเจลาตินที่เติมกลีเซอรอล 25 เปอร์เซ็นต์ ประมาณ 2 เท่า อย่างไรก็ตามฟิล์มผสมดังกล่าวมีความอ่อนตัว (ค่า EAB) ลดลงมาก เมื่อเทียบกับฟิล์มผสมที่มีการเติมกลีเซอรอล 25 เปอร์เซ็นต์

เพื่อปรับปรุงความอ่อนตัวและสมบัติการป้องกันการซึมผ่านไอน้ำของฟิล์มผสมระหว่าง G/ENR (6/4) ที่ไม่เติมกลีเซอรอลดังกล่าวให้ดีขึ้น จึงได้ทำการศึกษาการใช้ยางธรรมชาติ (NR) เพื่อทดแทนปริมาณ ENR บางส่วนในฟิล์มผสมดังกล่าว พบว่า เมื่อศึกษาสมบัติของฟิล์มผสม (G/ENR) ที่ไม่เติมกลีเซอรอล การเติมยาง NR ทำให้ได้ฟิล์มผสม (G/ENR/NR) ที่มีค่า EAB สูงขึ้น ($p<0.05$) แต่มีค่า TS และ E ที่ไม่แตกต่างกัน ($p>0.05$) ฟิล์มผสมที่เตรียมจาก G/ENR/NR ในอัตราส่วน 6/2/2 และไม่เติมกลีเซอรอล มีค่า EAB สูงกว่า ฟิล์มผสม G/ENR/NR ที่อัตราส่วน 6/3/1 ฟิล์มผสม G/ENR อัตราส่วน 6/4 ที่ไม่เติมกลีเซอรอล และฟิล์มเจลาตินที่เติมกลีเซอรอล 25 เปอร์เซ็นต์ ($p<0.05$) นอกจากนี้การเติม NR ทำให้ฟิล์มผสมที่ได้มีค่า WVP ที่ลดลง ($p<0.05$) โดยฟิล์มผสมจาก G/ENR/NR ที่อัตราส่วน 6/2/2 และไม่เติมกลีเซอรอล มีค่า WVP ต่ำที่สุด ฟิล์มผสมจาก G/ENR/NR ที่อัตราส่วน 6/2/2 และไม่เติมกลีเซอรอลมีสมบัติที่ดีขึ้นมากที่สุด โดยมีค่า TS ค่า EAB และการป้องกันการซึมผ่านไอน้ำ สูงขึ้นประมาณ 2 , 5 และ 4 เท่า ตามลำดับ เมื่อเปรียบเทียบกับฟิล์มควบคุม (ฟิล์มเจลาตินที่เติมกลีเซอรอล 25 เปอร์เซ็นต์) นอกจากนี้ยังพบว่า ฟิล์มผสมดังกล่าวมีความต้านทานน้ำและความคงตัวทางความร้อน (อุณหภูมิการสลายตัวสูงขึ้น) เพิ่มสูงขึ้น โดยสมบัติด้านต่างๆที่ดีขึ้นของฟิล์มผสมดังกล่าว น่าจะเป็นผลมาจากสมบัติเฉพาะตัวของยาง NR ที่เติม รวมทั้งการกระจายตัวอย่างสม่ำเสมอของ NR ในเมทริกซ์ของฟิล์ม ที่เกิดจากยาง ENR ที่เติมซึ่งทำหน้าที่ช่วยเพิ่มสภาพความเข้ากันได้ระหว่างยางกับเจลาติน อย่างไรก็ตามฟิล์มเจลาตินที่เติมยาง NR และ ENR มีความโปร่งใสลดลงและมีสีเหลืองเพิ่มขึ้นเล็กน้อย

ดังนั้น การใช้ยาง ENR และ NR โดยไม่เติมกลีเซอรอล สามารถปรับปรุงสมบัติของฟิล์มเจลาตินจากวัวได้อย่างมีประสิทธิภาพ

Thesis Title Properties of bovine gelatin-based films as influenced by glycerol concentration and the incorporation of epoxydized natural rubber (ENR) and natural rubber (NR)

Author Miss Pornsawan Chamnanvatckatit

Major program Packaging Technology

Academic Year 2010

ABSTRACT

Effect of glycerol, a protein miscible plasticizer, at different concentrations (0, 15, 20, 25 and 30% (w/w) of protein) on properties of bovine gelatin-based film was studied. With increasing glycerol content, the strength and stiffness of the films decreased as evidenced by the decreased tensile strength (TS) and Young's modulus (E), respectively ($p<0.05$). Elongation at break (EAB), representing the film stretchability or flexibility, of the films increased with increasing glycerol content ($p<0.05$). Moisture content and water vapor permeability (WVP) of gelatin films increased as glycerol content increased ($p<0.05$), mainly due to the increase in hydrophilic groups in gelatin film. Addition of glycerol slightly increased transmission of light in the visible range (350-800 nm) of the gelatin films. However, glycerol added showed no impact on color of the resulting films. Therefore, glycerol at an appropriate amount could improve flexibility but decreased water-vapor barrier property, of bovine gelatin films. This plasticizing effect most likely resulted from the decrease in inter- and intra molecular attractive forces, and the increase in intermolecular spacing, thereby increasing chain mobility.

The impact of gelatin/ENR (G/ENR) ratios (10/0, 8/2, 6/4, 5/5 and 0/10) and ENR types (ENR-10, ENR-25 and ENR-50 containing epoxy content ≈ 10, 25 and 50 %mol, respectively) on properties of bovine gelatin-based films was investigated. With increasing epoxy content and level of ENR used, TS and transparency of the films decreased but EAB and yellowness (b*-value) of the films increased ($p<0.05$). WVP of the gelatin-based films decreased with ENR incorporation ($p<0.05$). Incorporation of ENR-25 at the G/ENR ratio of 6/4 rendered the blend film with the increases in EAB (or flexibility) and water-vapor barrier by approximately 1.8 and 1.3

times, respectively, compared to those of the gelatin film. The improved properties of G/ENR blend films were most likely due to the compatibility between gelatin and ENR associated with their chemical interactions, as evidenced by SEM and FTIR results.

Effect of ENR addition along with reduced glycerol content on properties of bovine gelatin-based film was investigated. Blend films incorporated with ENR-25 at G/ENR ratio of 6/4 showed the increased TS and E but decreased EAB and WVP when glycerol content decreased ($p<0.05$). Films from G/ENR-25 (6/4) blend without glycerol added had the increase in water-vapor barrier property by 1.4 times, compared with the same blend film with 25% glycerol. It also had the increase in water-vapor barrier property by 2 times, compared with the gelatin film. However, this blend film had much decreased flexibility (i.e., EAB) as compared to that added with 25% glycerol.

To further improve the flexibility as well as the water-vapor barrier property of the G/ENR-25 (6/4) blend film without glycerol, the incorporation of natural rubber (NR) as partial ENR substitute was also studied. Among blend films without glycerol added, those incorporated with NR had the higher EAB ($p<0.05$) but similar TS and E ($p>0.05$). Film prepared from the blend of G/ENR/NR at 6/2/2 without glycerol exhibited the higher EAB than did that at 6/3/1, G/ENR (6/4) blend film without glycerol and gelatin film with 25% glycerol ($p<0.05$). Incorporation of NR resulted in a marked decrease in WVP of the blend films obtained ($p<0.05$). The blend film of G/ENR/NR at 6/2/2 without glycerol had the lowest WVP. The blend film of G/ENR/NR at 6/2/2 without glycerol exhibited the greatest improvement in TS, EAB and water-vapor barrier by approximately 2, 5 and 4.3 times, respectively, compared to the gelatin film added with 25% glycerol. Moreover, this blend film had increased water resistance and thermal stability as evidenced by the increased degradation temperature. The great property improvement of this blend film was due to intrinsic characteristics of the NR and also presumably to a uniform dispersion of NR in the film matrix by the aid of added ENR, acting as a compatibilizer. However, films incorporated with NR and ENR had the decreased transparency and slightly increased yellowness.

Therefore, the properties of bovine gelatin-based film could be improved by the incorporation of ENR and NR without glycerol addition.

CONTENTS

Page

Chapter

CONTENTS (Continued)

Chapter **Page**

CONTENTS (Continued)

Chapter **Page**

CONTENTS (Continued)

Chapter **Page**

CONTENTS (Continued)

Chapter **Page**

LIST OF TABLES

Table **Page**

LIST OF TABLES

LIST OF FIGURES

Figure **Page**

LIST OF FIGURES (Continued)

Figure **Page**

LIST OF FIGURES (Continued)

CHAPTER 1

INTRODUCTION

In recent years, there is an increasing interest in the development of materials from renewable resources, which are environmental friendly. Various types of biodegradable films and coatings have been prepared from different renewable materials including polysaccharides, lipids and proteins (Irissin-Mangata *et al.*, 2001; Baudoin *et al.*, 2001). Gelatin is one type of protein found mainly in animal skin and bone. Bovine and porcine wastes are the most frequent sources to obtain gelatin of good quality. Other sources of gelatin are becoming increasingly relevant, such as fish bones and skins. Gelatin is a protein with a wide range of industrial applications employed worldwide. It enhances the functional properties of food products by improving their elasticity, consistency and stability, and it may also be used as an outer film to protect food against drying, light and oxygen, especially in those cases where oxidative and microbiological deterioration occurs (Arvanitoyannis, 2002). Gelatin is one of the first materials employed in formation of biomaterials (Gennadios *et al.*, 1994), and has been subjected in many patents (Torres, 1994). Gelatin continues to be used in studies on biodegradable/edible film because it is an abundant raw material, produced in the whole world at low cost and has excellent film-forming properties.

The making of gelatin films generally needs the incorporation of the minimal content of plasticizer to reduce its brittleness. As a result, the film properties would depend on type and amount of plasticizer used. The most common plasticizers used are polyols and mono-, di- and oligosaccharides (Sobral *et al.*, 2001). Plasticizer selection is normally based on the compatibility between plasticizer and protein, performance in the film and amount necessary to plasticization (Sothornvit and Krochta, 2001). Despite different possibilities of additive that can be used as plasticizer in protein-based film, glycerol is more used as plasticizers in gelatin-based film. Glycerol, as a plasticizer, has been incorporated into most hydrocolloid films. It is a high boiling point plasticizer, water soluble, polar, nonvolatile, protein miscible and has a low molecular weight and one hydroxyl group on each carbon (Sobral *et al.*, 2001). These properties make glycerol a suitable plasticizer for use with a compatible water-soluble polymer, especially protein.

Gelatin films are very transparent and excellent in gas (O_2 and CO_2) barrier property. However, they have lower strength and elasticity or flexibility as well as poorer water-vapor barrier property, compared to synthetic films (Bogdanovic *et al.*, 2008). Blending gelatin with other polymers possessing better strength, elasticity and water resistance would improve the properties of the gelatin films. Natural rubber may be a polymer of choice which possesses the aforementioned properties. However, to be compatible with gelatin molecules, a polar natural rubber such as epoxidized natural rubber (ENR) is more promising to be used.

ENR is a chemically modified natural rubber which can be prepared in solution or latex stage by reacting natural rubber with performic acid (Tangpakdee *et al.,* 1998). Apart from the highly elastic nature of rubber, ENR possesses good oil resistance due to the polarity of the epoxy group in the rubber chain (Mishra *et al.,* 2006). ENR has recently been used for reactive blending with starch and other polar polymers to improve their elastic property and impact resistance (Nakason *et al.,* 2001; Rouilly *et al.,* 2004; Qi *et al.,* 2006).

Incorporation of rubber into gelatin film is expected to improve not only the mechanical properties but also the water-vapor barrier property of the gelatin film. However, use of ENR to modify the properties of gelatin film has not been reported. Therefore, this work was aimed at the study of effect of ENR incorporation on properties of bovine gelatin-based film.

Review of Literatures

1. Collagen and gelatin

1.1 Collagen

Collagen is the most abundant protein of animal origin and comprises approximately 30% of total animal protein (Muyonga *et al*., 2004). Collagen is a main part in skin, bone, tendon, the vascular system of animals and the connective tissue sheath surrounding muscle. About 10% of mammalian muscle protein is collagen (Bailey and Light, 1989). Some of the collagen is soluble in neutral salt solution; some is soluble in acid and some is insoluble (Foegding and Lanier, 1996).

The collagen monomer is a long cylindrical protein about 2,800 – 3,000 Å long and 14-15 Å in diameter. It consists of three polypeptide chains wound around each other in a superhelical fashion (Foegding and Lanier, 1996; Belitz and Grosch, 1999), called tropocollagen as shown in Figure 1 (Griffiths *et al.*, 2000). The α-chains form a left-handed helix due to the presence of Pro and Hyp, which give the chain links and turns, enhancing the stabilization of secondary structure of single helix.

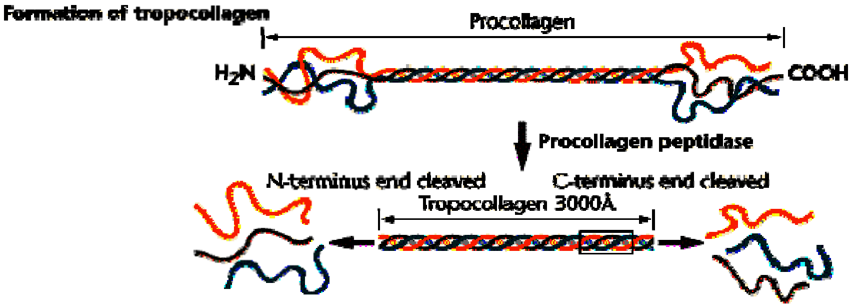


Figure 1 Tropocollagen conformation.
Source: Griffiths et al. (2000)

Tropocollagen, a basic structural unit, arranges in a specific way to form collagen fibers, as presented schematically in Figure 2 (Wong, 1989). The association of adjacent rows is displaced by about one-fourth of tropocollagen length. During maturation or aging, collagen fibers strengthened and stabilized primarily by covalent cross-linkages mainly in the terminal (non-helical regions). Cross-link formation involves an oxidation of lysine and hydroxylysine residue (Belitz and Grosch, 1999).

Figure 2 Arrangement of tropocollagen.

Source: Wong (1989)

The general amino acid sequence in the α-chains is Gly-X-Y, where X is often proline and Y is normally hydroxyproline (Haug *et al*., 2004). The amino acid composition of collagen is unique in that it is exceptionally high glycine (33%), proline (12%) and alanine (11%). Two amino acids that are not commonly present in many other proteins include hydroxyproline (12%) and hydroxylysine (1%) (Wong, 1989). Glycine represents nearly one-third of the total residues, and it is distributed uniformly at every third position throughout most of the first collagen molecule. The repetitive occurrence of glycine is absent in the first 14 amino acid residues from N-terminus and the 10 amino acid residues from C-terminus. These end portions are termed "telopeptides" (Hultin, 1976; Foegeding and Lanier, 1996).

There are at least 19 variants of collagen, named type I to type XIX (Bailey *et al*., 1998). Types I, II, III and V are the fibrous collagen (Muyonga *et al*., 2004). The most common collagen type is type I collagen is found in all connective tissue, including bones and skins. It is a heteropolymer of two α_1-chains and one α_2-chain. Each chain has a molecular mass about 100,000 Da, yielding a total molecular mass approximately 300,000 Da for collagen. Three chains are hold together by hydrogen bonding. Since it contains no tryptophan or cysteine and is very low in tyrosine and histidine (Muyounga *et al*., 2004), its nutritive value is low (Sikorsiki, 1990; Foegding and Lanier, 1996).

1.2 Gelatin

Gelatin is a denatured protein derived from collagen by thermo-hydrolysis and has a rheological property of thermo-reversible transformation between sol and gel (Cho *et al.,* 2005). There are two main types of gelatin. Type A, with isoelectric points at pH 6-9, is derived from collagen with exclusively acid pretreatment of pig skins. Type B, with isoelectric points at pH 5, is the result of an alkaline pretreatment of collagen from hides and bones of cattle (Stansby, 1987). Conversion of collagen to gelatin involves the shrinkage at some particular temperature (T_s), which veries with species (Belitz and Grosch, 1999). The shrinkage includes a disassembly of fibers and a collapse of the triple-helical arrangement of polypeptide subunits in the collagen molecule at a critical temperature. The midpoint of the collagen-to-gelatin transition is defined as the melting temperature (Bremner, 1992). Generally, at heating temperature more than T_s, the triple-stranded helix of collagen is also destroyed to a great extent and exists as the random coils (Figure 3). Heating energy breaks many non-covalent bonds with some covalent inter-and intra-molecular bonds (Schiff base and also condensation bonds) and few peptide bonds. This results in the conversion of the helical collagen structure to a more amorphous form, known as "gelatin" (Foegding and Lanier, 1996) (Figure 3). For fish collagen, T_s is 45°C, while collagen from mammals has T_s of 60-65°C (Belitz and Grosch, 1999).

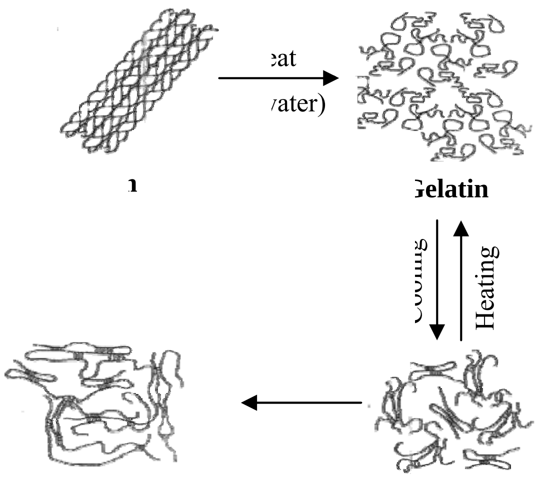


Figure 3 Collagen conversion into gelatin.
Source: Griffiths (2000)

Gelatin contains approximately 1,050 amino acids per α-chain with left-handed protein helix conformation. The protein is made up of peptide triplets Gly-X-Y, where X and Y can be any one of the amino acids but proline has a preference for the X position and hydroxyproline for the Y position (Figure 4) (Bailey and Ligth, 1989). For the N-terminal amino acid composition, it depends more on the pretreatment received by the collagen than its source. Serine, threonine, aspartic acid and glutamic acid predominate in alkaline-processed gelatins and alanine is dominant in acid processed ones (Eastoe and Laech, 1977). The carboxyl-terminal amino acids of gelatin are mainly composed of glycine (0.5 mole/10^5 g protein), alanine (0.8mole/10^5 g protein), valine (0.2 mole/10^5 g protein) and leucine/isoleucine (1.9 mole/10^5 g protein) (Eastoe and Laech, 1977). Table 1 presents the amino acid composition of the gelatins from different sources. Both the bovine-hide and the tuna-skin gelatins exhibited typical type I collagen. Gly content presented approximately 1/3 of the total amino acids. As described by Asghar and Henrickson (1982), 50–60% of α-chains consist of tripeptides having the general formula Gly-X-Y. The proline plus hydroxyproline (imino acids) content was higher in the bovine-hide gelatin than in the tuna-skin one (210 vs. 185 residues/1000, respectively). Gelatins made from warm-blooded animal tissues have been reported to have a higher imino acid content, hydroxyproline in particular (Norland, 1990), and this promotes triple helix formation and stabilization of the gelatin at low temperatures (Burjandze, 1979) due to the hydrogen bonding ability of the –OH group on the hydroxyproline. Gelatins from warm-water fishes have a higher imino acid content than gelatins from cold-water fish species, closer to mammalian ones (Gilseman and Ross-Murphy, 2000; Avena-Bustillos *et al*., 2006). Cold-water fish gelatins are typically more hydrophobic.

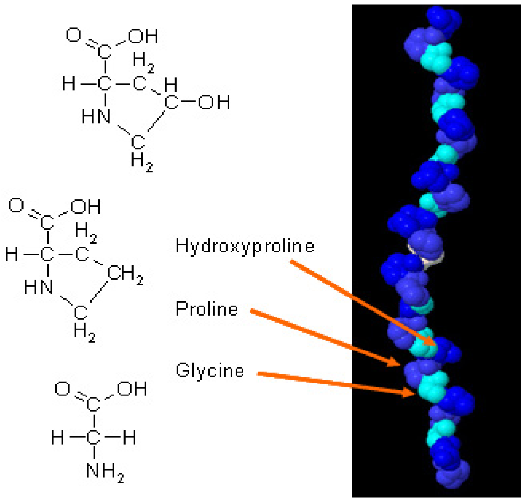


Figure 4 Gelatin chain structure.
Source: Bailey and Ligth (1989)

Processing of collagen into gelatin involves three major steps. First is the removal of noncollagenous components from stock (skin and bones), then the conversion of collagen to gelatin by heating in the presence of water, and finally recovery of gelatin in the final form (Foegeding and Lanier, 1996). For raw material constituting high content of lipid, it is more important to degrease before another pretreatment and extraction (Holzer, 1994). During the liming stage, the higher lipid content in raw material causing processing problem due to fat or calcium soaps formation and then clog the capillary spaces in the deminiralized bone (ossein) and impede the penetration of alkaline solution. Fat in gelatin liquor may become partially emulsified during extraction, resulting in the difficulty of filtration (Jones, 1987). Gelatin with a high grease or fatty acid content may show localize non-wettability, resuting in *fish eye* in photographic film or *window* in hard gelatin capsules (Jones, 1987).

Gelatin extraction normally takes place under either acid or neutral conditions at the minimum temperature needed to give a reasonable extraction rate and a high yield of gelatin (Jones, 1987). The type of acids used, ionic strength and pH that the acid produces strongly influences swelling properties and solubilization of collagen as well as the extraction of gelatin (Giménez *et al*., 2005). The type of chemical pretreatment and parameters of extraction can influence the length of polypeptide chains and the functional properties of gelatin. Increasing H^+ ions favors the access of water to the collagen fibers, and this water is held by electrostatic forces between charged polar groups and negative atoms (lyotropic hydration) (Gustavson, 1956; Giménez *et al*., 2005). According to Asghar and Henrickson (1982), the lyotropic effect of carboxylic acids on collagen seems to dominate the swelling capacity, rather than a specific ion effect. It is the non-ionized acid that acts as the swelling agent by competing with the peptide group involved in intermolecular linking of the protein chain, mainly of the hydrogen bonding power of the acid. The type of acid used influenced the gelatin viscoelastic and gelling properties. Gòmez-Guillèn *et al*. (2001) compared the pretreatment with different acids (formic, acetic, propionic, lactic, malic, tartaric and citric acid) on the properties of gelatins, and found that acetic acid- and propionic acid-pretreatment yielded the mergrim *(lepidorhombus boscii)* skin gelatin with the highest elastic modulus, viscous modulus, melting temperature and gel strength, especially when skins were previously treated with dilute NaOH. However, the propionic acid led to turbid gelatin than those obtained from citric acid. Commercially, citric acid is widely used for the manufacture of food grade gelatin from fish gelatin since it does not introduce undesirable color or order to the gelatin (Giménez *et al*., 2005).

Table 1 Amino acid composition of the bovine-hide and the tuna-skin gelatins.

Amino acid residues	Number of residues/1000	
	Bovine-hide	Tuna-skin

Hyp	83	78
Asp	46	44
Thr	83	21
Ser	39	48
Glu	74	71
Pro	127	107
Gly	342	336
Ala	113	119
Val	19	28
Met	4	16
Ile	11	7
Leu	24	21
Tyr	4	4 3
Phe	12	13
His	4	7
Lys	25	25
Arg	47	52
Hyl	5	6

Source: Gòmez-Estaca *et al*. (2009)

2. Biodegradable films

Although synthetic polymers are extensively used for multi-purpose applications because of their satisfactory mechanical and thermal properties, their lack of biodegradability has recently complicated and hindered their use (Wool, 1989; Peanasky *et al*., 1991; Albertson and Karlsson, 1994; Griffin, 1994; Wool, 1995). The developments of packaging polymers using renewable resources which are naturally biodegradable and the possibility of combining their biodegradability with cost reduction and market needs have been the object of intensive academic and industrial research. In recent years, biodegradable or edible films have attracted much attention in food and drug packaging. This is because biodegradable or edible films could partly substitute for the traditional non-biodegradable plastic films. Biodegradable or edible films could provide protection as moisture, gas and lipid barriers to enhance food quality (Kim and Ustunol, 2001). Biodegradable films could be prepared from proteins, polysaccharides, lipid or their blends such as starch (Bertuzzi *et al*., 2006), cassava starch (Famá *et al*., 2006), potato starch (Talja *et al*., 2006), hydroxypropyl methylcellulose (Villalobos *et al*., 2006), blue marlin muscle protein (Hamaguchi et al., 2007), protein from lentil (*Lens cukinaris*) (Bamdad *et al*., 2006), etc. The mixtures or blends of different polymers have been used for film preparation such as caseinate-pullulan films (Kristo *et al*., 2007), glucomannan-chitosan-nisin ternary antimicrobial blend film (Li *et al*., 2006) and chitosan-ovalbumin films (Pierro *et al*., 2006). Among them, biodegradable films of protein are supposed to provide nutritional value, also have impressive mechanical properties and gas barrier property (Ou *et al*., 2004). Among protein films, gelatin obtained by partial degradation of collagen has gained more attention as edible films for its abundance and biodegradability (Jongjareonrak *et al*., 2006). Gelatin is unique among hydrocolloids in forming thermo-reversible film with a melting point close to the body temperature, which is particularly significant in edible and pharmaceutical applications (Achet and He, 1995).

3. Protein-based films

Development of biopolymer films and coatings from protein has received increasing interest (Gennadios *et al*., 1994; Choi and Han, 2002; Bigi *et al.,* 2001; Shiku *et al*., 2004). Two major promising applications of such films are the replacement of short-lie plastic in food packing and use as excellent oxygen, lipid, and aroma barriers; however, due to their hydrophilic nature, they have poor moisture barrier properties (Chen, 1995; Chick and Ustunol, 1998; Gennadios *et al,* 1994; Krochta and DeMulder-Johnston, 1997; Miller and Krochta, 1997). This property was improved by the incorporation of hydrophobic material such as lipids (McHugh and Krochta, 1994; Perez-Gago and Krochta, 1999; Shellhammer and Krochta. 1997).

Proteins cover a broad range of polymeric compounds that provide structure or biological activity in plants or animals. Various proteins can be used as film-forming materials (Alexy et *al*., 2003) such as soy protein isolate (Hang Wan *et al*., 2005; Tang *et al*., 2003; Rhim *et al*., 1999). Whey protien isolate (Stuchell and Krochta, 1995), wheat gluten (Zhang *et al*., 2004), eggwhite (Gennadios *et al*., 1996) and fish myofibrillar protein (Cuq *et al*., 1997; Shiku *et al*., 2003; Chinnabark *et al*., 2007). Protein-based films generally have the superior mechanical and barrier properties to polysaccharide-based films. Proteins consisting of about 20 amino acids have a specific structure which confers a wider variety of functional properties, compared with polysaccharides which are mostly homopolymers. Furthermore, inter-and intra-interaction between protein molecules, such as hydrogen bonds, ionic-ionic interaction, hydrophobic interaction and covalent bonds, could be formed during drying condition (Chinnabark *et al*., 2007; Iwata *et al*., 2000). Properties of protein-based films are most likely dependent on the protein sources which are different in sequential order of the amino acids, protein structure and the degree of molecular extension (Iwata *et al*., 2000).

3.1 Approaches for protein film formation

Several approaches can be used to form protein films (Stuchell and Krochta, 1995) as follows:

3.1.1 Simple coacervation

A single hydrocolloid is driven from aqueous suspension or caused to undergo a phase change by evaporation of solvent, addition of a water-miscible nonelectrolyte in which the hydrocolloids is not soluble (e.g., alcohol), adiition of an electrolyte to cause out or crosslink, or alteration of pH.

3.1.2 Complex coacervation

Two solutions of oppositely charged hydrocolloids are combined, causing interaction and precipitation of the polymer complex.

3.1.3 Thermal gelation or precipitation

A sol-gel transformation can occur by heating of protein to cause denaturation followed by gelation (e.g., egg albumin) or precipitation, or simple cooling of a warm hydrocolloid suspension.

3.2 Protein film formation

Protein based-film can be formed in three steps (Figure 5) (Marquie and Guilbert, 2002):

3.2.1 Break intermolecular bonds (non-covalent and covalent bonds) that stabilize polymers in their native forms by using chemical or physical rupturing agents (by solubilization or thermal treatment). Polymer chains become mobile.

3.2.2 Arrange and orient mobile polymer chains in the desired shape.

3.2.3 Allow the formation of new intermolecular bonds and interactions to stabilize the three-dimensional network. The shape obtained in step 2 is maintained by eliminating agents used in step 1 (e.g., solvent removal or cooling).

Based on these three steps, solvent process is based on dispersing and solubilizing the proteins in various solvents and then casting, spaying or dipping followed by drying. This process has been extensively studied and applied to produce films from various proteins (Cuq *et al*., 1995).

Figure 5 Mechanism of film formation via solution casting process.

Source: Adapted from Marquie and Guilbert (2002)

3.3 Important properties of protein-based films

Protein films possess different properties depending upon the source of protein, protein concentration, extrinsic factors, etc.

3.3.1 Barrier properties

Barrier property refers to the ability of film to resist the prenetration of small molecules (gas, vapor or aroma) through the film. Protein films provide the advantage of being excellent oxygen and carbondioxide barriers (Gennadios *et al*., 1993), but their hydrophilic nature makes them rather ineffective moisture barrier (McHugh and Krochta, 1994; Roy *et al*., 2000). Barrier property of protein films can be varied depending on the source of protein, which can be associated with amino acid composition (Table 2) (Cuq *et al*., 1995).

Table 2 Water vapor permeability of various protein films.

Film	Water vapor permeability ($\times10^{-12}$ mol.m/m^2.s.Pa)	Temp. (°C)	RH (%) conditions	Thickness ($\times10^{-6}$m)
Sodium caseinate film	24.7	25	100 – 00	-

Soy protein film (pH 3)	23.0	25	100 – 50	83
Corn zein film	6.45	21	85 – 00	200
Wheat gluten film	5.08	30	100 – 00	50
Myofibrillar protein film	3.91	25	100 – 00	60

Source: Adapted from Cuq *et al.* (1995)

3.3.2 Mechanical properties

Mechanical properties of protein films are generally poorer than synthetic films (Cuq, 2002; Gennadios *et al*., 1994). Several factors, including surface charges, hydrophobicity, polymer chain length, etc., may significantly affect the mechanical properties of protein films (Kester and Fennema, 1986). Hydrogen bonds are considered important in contributing to the tensile strength (TS) of protein films (Meirer, 1990). Type and level of plasticizer have a dramatic effect on film properties (Shellhammer and Krochta, 1997; Cuq, 2002). Lim *et al.* (1998) reported that egg white films with higher glycerol contents had greater elongation at break (EAB) values. The distribution and concentration of inter- and intra-molecular interactions allowed by primary and spatial structures most likely affected the mechanical properties of myofibrillar protein-based films.

3.3.3 Solubility property

Film solubility is an important property that relates to intended use. Some high-molecular-weight proteins are insoluble or slightly soluble in water and thus have potential for forming water-resistant films (Cuq, 2002). Low-molecular- weight protein chain such as monomer and small peptides, formed during the film-forming solution and immobilized in the film network, could thus constitute the water-soluble proteinic component of the films (Cuq *et al*., 1995). Regardless of plasticizer type (glycerol, sorbital or sucrose), the increase in plasticizer content in the film normally increased the water-soluble dry matter content. In general, hydrophilic plasticizers enhance water solubility of the protein film (Cuq, 2002; Shiku *et al*., 2004).

3.4 Protein-based films from different sources

3.4.1 Wheat gluten films

Wheat gluten is defined as the water-soluble protein of wheat flour. Wheat gluten contains the prolamine and glutelin fractions of wheat flour protein, typically referred to as gliadin and glutenin, respectively (Krochta, 2002). Gliadin is soluble in 70% ethanol, but glutenin is not. Both gliadin and glutenin fractions of wheat gluten contain intra molecular disulfide bonds. Intermolecular disulfide bonds, which link individual glutenin protein chains, result in the larger polymers with high molecular weight. The extensive intermolecular interactions in wheat gluten result in quite brittle films with poor water-vapor barrier properties (Gennadios and Weller, 1990). Herald *et al.* (1995) reported that films prepared from spray-dried (SD) and flash-dried (FD) wheat gluten had differences in properties. Films from wheat gluten are comparable to plastic wrap for most properties except water vapor permeability. SD wheat gluten film exhibited a higher tensile strength (TS) than did the FD wheat gluten film and plastic wrap.

3.4.2 Casein films

Casein, which comprises 80% of milk protein, precipitates when skim milk is acidified to the isoelectric pH, approximately of 4.6 (McHugh and Krochta, 1994). Film formation of aqueous casein solution without heat treatment was due to their random-coil nature. Interactions in the film matrix likely include hydrophobic, ionic and hydrogen bonding (Avena-Bustillos and Krochta, 1993).

3.4.3 Whey protein films

Whey protein comprising 20% of milk protein is the protein that remains soluble after casein is precipitated at pH 4.6. Whey protein consists of several proteins, which are globular and heat labile in nature (McHung *et al*., 1994). Because of the globular nature of whey proteins, the formation of films requires heat denaturation to open the globular structure, break exisiting disulfide bonds, and form new intermolecular disulfide and hydrophobic interaction (McHung *et al,* 1994). McHung *et al*. (1994) suggested that the best film formation condition were 10% (w/w) protein solutions with neutral pH and heated for 30 min at 90°C.

3.4.4 Corn zein films

The zein, which is prolamine, is soluble in 70% ethanol. In term of the amino acid composition, zein has high content of nonpolar hydrophobic amino acids such as leucine, alanine and proline. Zein also contains a high level of glutamic acid (about 20-22%), which exists mostly as glutamine. Glutamine contributes to the insolubility of zein in water (Gennadios and Weller, 1990). Therefore, zein films are generally cast from alcohol solution (Gennadios *et al*., 1993). The interactions formed in the film matrix likely include hydrophobic interaction, hydrogen bonding and disulfide bond (Ghanbarzadeh *et al*., 2007).

3.4.5 Muscle protein film

Muscle proteins consist of sarcoplasmic protein, myofibrillar protein and stroma protein. These proteins are capable of forming a continuous films matrix (Garcia and

Sobral *et al*., 2005; Sobral *et al*., 2005). The edible films or biodegradable films based on fish myofibrillar protein have been developed by solution casting process (Sobral *et al*., 2005; Cuq *et al*., 1995; Vanin *et al*., 2004; Carvalho and Grosso, 2004). Cuq *et al*. (1995) found that the pH and protein concentration had strong interactive effects on viscosity of film-forming solution (FFS) from Atlantic sardines myofibrillar protein. During FFS storage before casing, partial degradation of high-molecular-weight protein components led to decreased viscosity allowing thin layer casting.

3.4.6 Other protein-based films

Various other proteins can be used to prepare biodegradable films or edible film such as collagen, gelatin and sarcoplasmic protein (Iwata *et al*., 2000; Tanaka *et al*., 2001). Fish sarcoplasmic protein film from marin meat had better flexibility and lower water vapor permeability compared with most of the other proteins films. Properties of protein-based films are most likely dependent on the sequential order of the amino acids and protein structure.

4. Gelatin film

Gelatin has been known to possess the film forming ability. However, gelatin film still needs the improvement to reach the maximized uses. Mechanical properties of gelatin films were improved by including segmental orientation in gelatin films though uniaxial stretching and successive drying (Bigi *et al*., 1998). Carvalho and Grosso (2004) studied the effect of the crosslinking agents (formaldehyde, glyoxal and transglutaminase) on some properties of its films plasticized with the glycerol. The film solubility in water was reduced, but the other functional properties were not necessarily improved because the amount of plasticizer necessary to maintain these films flexible was increased. Bertan *et al*. (2005) studied the incorporation of ‘Barzilian elemi’, highly hydrophobic resinous exudates of the botanical family *Burseraceae*, into gelatin films, using a blend of stearic and palmitic acid to dissolve the elemi, and subsequent emulsification of the filmoginic solution using triacetin as plasticizer. Film with the addition of acid and the elemi presented better water vapor barrier properties as compared to the gelatin/triacetin film. The mechanical resistance decreased with the addition of the lipid, while the optical and soluble matter increased. Grosso (2006) reported the addition of TGase to produce cross-linked casein, gelatin and casein-gelatin blend (100:0, 75:25, 50:50, 25:75 and 0:100) edible films. Enzymatic cross-linking also induced a substantial increase in the high molecular weight protein components in the film forming solution. The casein-gelatin film shown significant greater elongation value with or without TGase treatment, as compared to films made from gelatin or casein alone. Mixture of casein and gelatin produced a synergistic effect elongation improvement, while no improvement was detected for tensile strength and water vapor barrier properties.

5. Plasticizer used in biodegradable films

Plasticizers with characteristics such as small size, high polarity, more polar group per molecule, and greater distance between polar groups within a molecule generally impart greater plasticizing effects on a hydrophilic polymeric system (Sothornvit and Krochta, 2001). The selection of a plasticizer for a specified system is normally based on the compatibility of the plasticizer, the amount necessary for plasticization and the desired physical properties of the films (Cheng *et al*., 2006). In addition to the film-forming biopolymer, a major component of edible films is the plasticizer. The addition of a plasticizing agent to edible film is required to overcome film brittleness caused by intensive intermolecular forces. The increase in mobility of polymer chain can improve the flexibility and extensibility of the films (Gontarard *et al*., 1993). A variety of common plasticizers used in edible films include glycerol, polyethylene glycol (PEG), sorbitol, proplylene glycol (PG) and ethylene glycol (EG), monosaccharide, disaccharide or oligosaccharide, lipid and their derivatives (Yang and Paulson, 2000; Irissin-Mangata *et al*., 2001; Gontard *et al*., 1993). In general, addition of plasticizer, especially polyols, decreases the inter- and intra-molecular attractive forces (Sánchez *et al*., 1998; Irissin-Mangata *et al*., 2001) and increases the flexibility and water vapor permeability of the films (Pascholick *et al*., 2003; Irissin-Mangata *et al*., 2001). According to Jangchud and Chinnan (1999) and Vanin *et al*. (2005), glycerol is one of the best plasticizers that can be used in protein films, because it is water soluble, polar, non-volatile, protein miscible and has a low molecular weight and one hydroxyl group on each carbon. Recently, Menegalli *el al*. (1999), Sobral (1999), and Sobral *et al*. (2001) studied various properties of gelatin-based edible films plasticized by sorbitol as a function of plasticizer concentration (20, 40 and 60%). The increase of plasticizer concentration increased flexibility and decreased resistance and water vapor barrier of the film. Arvanitoyannis *et al*. (1997) studied the thermal and functional properties of edible films made from blend of gelatin and starch as a function of various plasticizers, Addition of water or polyol to the gelatin/starch blend resulted in plasticization of the polymer matrix as reflected by lower T_m and T_g. Bertan *et al*. (2005) studied the influence of the addition of laulic acid to film made from gelatin. The addition of 2, 5 and 10% laulic acid was sufficient to make a significant difference in the water-vapor permeability (WVP) at the higher level used and reduced TS but increased elongation.

6. Natural rubber (NR)

Natural rubber is cis-1,4 polyisoprene (Figure 6) and is present as latex in a large variety of plants in many regions of the world. The most important source is the tree of *Hevea brasiliensis*. Latexes from the other sources suffer from disadvantages such as low rubber content, high resin content and difficulties in extraction (Vijayakumer *et al*., 2000).

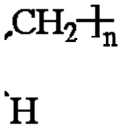


Figure 6 Structure of natural rubber (cis-1, 4 polyisoprene).
Source: Vijayakumer *et al*. (2000)

6.1 Composition of natural rubber latex

Natural rubber latex is a colloid with a specific gravity of 0.96 to 0.98 and a pH in the range of 6.5 to 7.0. The dispersed phase is mainly rubber and the dispersion medium is water. However, in addition to rubber and water, latex contains small quantities of protein, resins including fats, fatty acids, other lipid, sterol and sterol ester, carbohydrates and mineral matter. The composition of field latex (latex as obtained from the tree) is given in Table 3 (Vijayakumer *et al*., 2000).The dominant particulate constituent of fresh latex is the rubber hydrocarbon with a size ranging from 0.02 to 3.0 µm and shape mostly spherical and strongly protected in suspension by a film of adsorbed proteins and phospholipids (Archer *et al*., 1963). The other particles in latex comprise lutoids and Frey-Wyssling particles. The lutoids are subcellular membrane bound bodies ranging in size from 2 to 5 µm, containing a fluid serum known as B-serum,which is a destabiliser for rubber particles. Frey-Wyssling particles are spherical, larger in size and are yellow coloured. Quebrachitol (methyl-l-inositol), sucrose and glucose are the major carbohydrates in latex. Of the total protein content of fresh latex about 20% is adsorbed on the rubber particles, an equal quantity found in the B-serum and the remainder in the latex-serum. The adsorbed protein and the phospholipids on the rubber particles impart a net negative charge, thereby contributing to the colloids stability of latex. Lipids in fresh latex consist of fats, waxes, sterols, sterol esters and phospholipids. Lipids associated with the rubber and non-rubber particles in latex play a key role in the stability and colloidal behavior of latex. Most of the classic amino acids have been found in latex. Nucleotides contained in latex are important as cofactors and intermediates in the biosynthesis of rubber. Low molecular weight thiols such as glutathione and cysteine and ascorbic acid determine the redox potential of latex.

Table 3 The composition of field NR latex (latex as obtained from the tree)

Constituent	Percentage
Rubber	30-40
Proteins	1-1.5
Resins	1.5-3.0
Minerals	0.7-0.9
Carbohydrates	0.8-1.0
Water	55-60

Source: Vijayakumer *et al.* (2000)

6.2 Modified forms of natural rubber (NR)

Natural rubber can be modified by physical and/or chemical means (Figure 7) (Chmpbell, 1992).

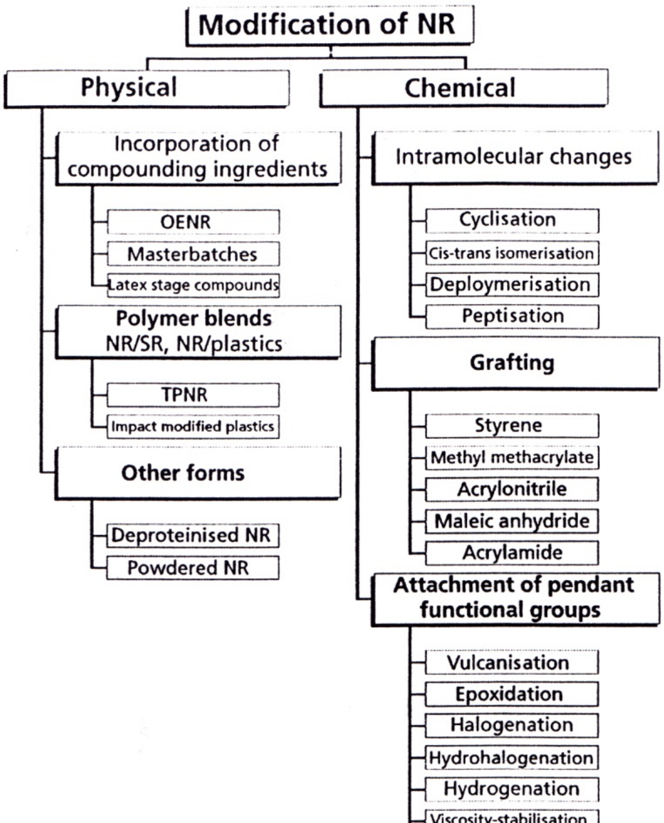


Figure 7 Modification of natural rubber.

Source: Chmpbell (1992)

6.2.1 Physically modified forms of NR

The physical methods of modification of NR involve incorporation of additives which do not chemically react with rubber. The additives include various rubber compounding ingredients and polymers such as synthetic rubbers and thermoplastic. Some of the commercially important in this group are as following:

6.2.1.1 Oil extended natural rubber (OENR)

This contains 20 to 25 phr of aromatic or naphthenic oil and is produced either in the latex stage (Thomas *et al*., 1983) or in the dry rubber stage (Baker *et al*., 1985). Extension in the latex stage is affected by adding an aqueous emulsion of the oil into latex followed by acid coagulation and processing into block rubber. In general, increasing oil content reduces tensile strength and resilience, but the vulcanisates retain good tear resistance and possess high wear resistance when blended with butadiene rubber. OENR also shows good skid resistance on wet surfaces when used in tread.

6.2.1.2 Thermoplastic natural rubber (TPNR)

Thermoplastic natural rubber blends are prepared by blending NR and polyolefins, particularly polypropylene and polyethylene in varying proportions. As the ratio varies, materials with a wide range of properties are obtained. Rubber-rich blends are thermoplastic elastomers while those with lower rubber content are impact-modified plastic. The mechanical properties of rubber-thermoplastic blend depend on the proportion of the rubber-thermoplastic components. In order to get reasonably good elastic properties, the hard phase must be continuous. The soft phase, providing elastic properties, need not be continuous, providing it is sufficiently small and adheres to the matrix under stress. The elastic properties of TPNR are considerably improved if the rubber phase is partially crosslinked during mixing. This process is called dynamic vulcanization. TPNR is more resistance to heat aging than NR vulcanisate and ozone resistance of TPNR is also very high (Elliot, 1982).

6.2.1.3 Deproteinised natural rubber (DPNR)

This is a purified form of NR with low protein and mineral content. The proteins and other hydrophilic non-rubbers can absorb moisture leading to reduction in modulus and electrical resistance and increase in stress relaxation and creep. Absorption of water by unvulcanised rubber can affect its degree of crosslinking and other cure characteristic. Deproteinisation of NR reduces moisture sensitivity thereby improving consistency and modulus. The general method of deproteinisation involves treating the latex with an enzyme preparation followed by dilution and coagulation. The enzyme hydrolyses the proteins into water soluble forms which are then washed away during subsequent processing (Chin *et al.,* 1974).

6.2.2 Chemically modified forms of NR

Being an unsaturated organic compound, natural rubber is highly reactive and several chemical reactions can be carried out in NR, resulting in materials having entirely different properties. These reactions can take place by the attachment of pendant functional groups, grafting of different polymers along the rubber molecule or through intra-molecular changes. The products of the reactions possess properties which are different from those of NR and therefore, can find use in applications where other materials are being used. Some of the important chemically modified forms of NR are as following:

6.2.2.1 Constant viscosity (CV) rubber

NR undergoes hardening during storage especially under low humidity. The increase in viscosity is caused by a crosslinking reaction involving the randomly distributed carbonyl group on the rubber molecule (Montes and White, 1982). The reaction can be prevented by the addition of small quantities of hydroxylamine salts to the latex before coagulation. Thus, the rubber treated retains its original viscosity for a long time and is marketed as CV rubber. The controlled and stable viscosity favours easy and uniform processing. Premastication can be minimized or even avoided. This form of rubber is available mostly in the Mooney viscosity range of 60-65. Stabilisation of viscosity is evaluated by an accelerated storage hardening test. The increase in initial plasticity should be less than eight units.

6.2.2.2 Graft copolymer rubbers

Graft copolymers are prepared from natural rubber by polymerizing vinyl monomers either in latex or in solution. Methyl methacrylate (MMA) and styrene are the common monomer used for grafting onto NR. Poly(methyl methacrylate) (PMMA)-graft-NR has been commercially produced since the mid-195s in Malaysia. Generally, grafting can be achieved using free radical initiation by chemical method or by irradiation.

6.2.2.3 Epoxidized natural rubber (ENR)

The mechanical properties of NR are superior to those of most synthetic rubbers. However, with regard to special properties such as oil resistance and gas permeability, NR is inferior to the special purpose synthetic rubbers. George *et al.* (1992) reported that epoxidation of NR in latex stage, under controlled condition, gave epoxidized NR with improved resistance to hydrocarbon oils, low permeability to air, increase damping and good bonding properties while retaining the high strength properties of NR. Improvement in these properties depends on the degree of epoxidation. Two grades of ENR of 25 and 50 mole% epoxidation (ENR-25 and ENR-50) have gained commercial importance.

7. Epoxidized natural rubber (ENR)

Natural rubber consists of a long chain with an unsaturated double bond at every five carbon atoms (Tanaka, 1985). The unsaturated double bond is the functional group of the rubber molecule which can be reacted to form a new copolymer or branching. There are a number of processes which have been used commercially for the production of chlorinated, hydrochlorinated, cyclised, methylmethacrylate grafted and epoxidized (ENR) natural rubbers. The commercial production of ENR was started in the late 80s by a Malaysian company called Kumpulan Guthrie Berhad. The research work had been carried out by the Malaysian

Rubber Producers' Research Association (MRPRA), Tun Abdul Razak Laboratory, U.K. with the cooperation of the Rubber Research Institute of Malaysia (RRIM) since the 1970s.The production involves chemical modification of natural rubber field latex under controlled conditions. NR is converted to an epoxidized form by the *in- situ* generation of peroxyformic acid from hydrogen peroxide and formic acid (Figure 9), prior to coagulation and drying (Roy *et al*., 1993; Gelling, 1991; Bradbury and Perera, 1985; Roy *et al*., 1990; Fong, 1985). The advantage of this process is that only hydrogen peroxide is consumed (Figure 8) (Gelling, 1991). The commercial production route of ENR and reaction of epoxy ring opening can be represented in Figure 9 and 10, respectively (Baker and Gelling, 1987).

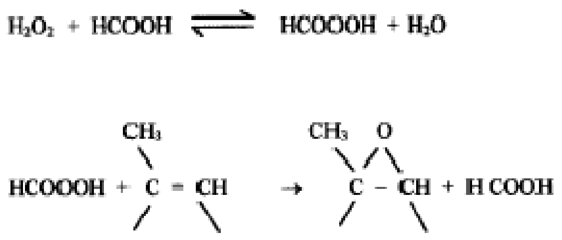


Figure 8 Schematic reaction for ENR preparation (only hydrogen peroxide process).

Source: Gelling (1991)

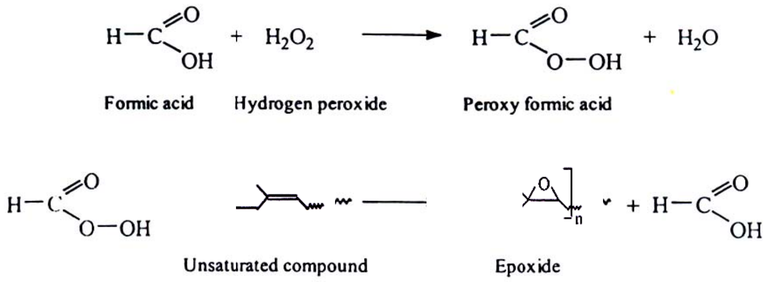


Figure 9 Schematic reaction for ENR preparation by hydrogen peroxide and formic acid process.

Source: Baker and Gelling (1987)

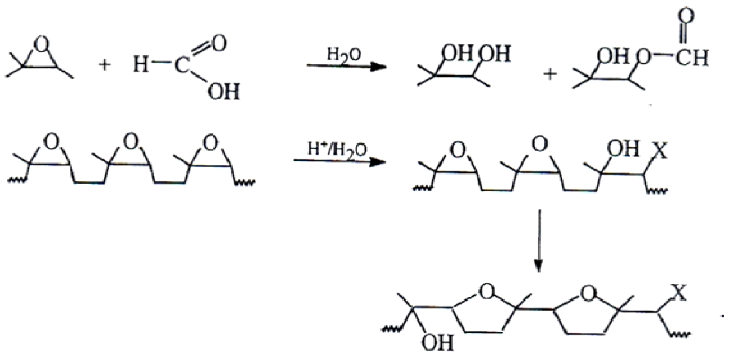


Figure 10 Schematic reaction for epoxy ring opening process.

Source: Baker and Gelling (1987)

Apart from the highly elastic nature of rubber, ENR possesses good oil resistance due to the polarity of the epoxy group in the rubber chain (Mishra *et al*., 2007). Two commercial grades of ENR are currently produced under the trade name Epoxyprene, i.e., Epoxyprene-25 and Epoxyprene-50. The numbers denote the mole percentage level of epoxidation produced. Epoxyprene-50 shows a typical Mooney viscosity (ML 1 ± 4 at 100 °C) of about 80 units, a gel content of 30-40% (Wong and Ong, 1992). The storage stability of the products has been reported to be fairly good and consistent. However, on extended aging, Epoxyprene-50 undergoes some significant changes which are reflected in an increase in the carbonyl absorption in its infrared spectra. This increase in carbonyl absorption is attributed to oxidation of the rubber through chain scission (Roy *et al*., 1993). Roy *et al.* (1993), working on laboratory-prepared ENR, concluded that during thermal ageing of ENR, carbonyl, alcohol, tetrahydrofuran and ether cross-links were formed presumably due to traces of acid present in the rubber. Addition of a phenolic antioxidant such as 2,4-dinitrophenyl hydrazine (DPH) ensures adequate storage stability of ENR. Increasing level of epoxidation changes certain physical properties of ENR. These changes are attributed to an increase in its polarity and glass transition temperature (T_g) (Gelling, 1987). Its T_g has a direct relationship with the level of epoxidation of ENR as depicted in Figure 11 (Gelling, 1987). The T_g increases by approximately 1°C for every 1 mol% epoxidation (Baker and Gelling, 1987).

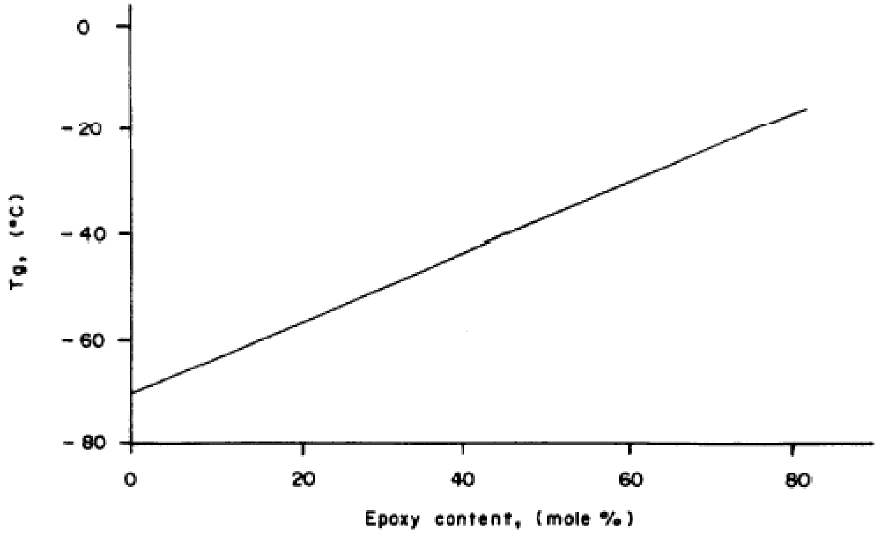


Figure 11 Effect of epoxy content on the glass transition temperature, T_g, of ENR.

Source: Gelling (1987)

7.1 Determination of epoxy content of ENR

Various methods have been used for determination of epoxy content of ENR including chemical titration and instrumental analyses (Chrimisinoff *et al.*, 1993).

7.1.1 Titration method

In chemical titration method, the epoxy group can be titrated by using bromic acid (Burfield *et al.*, 1984). The reaction between epoxy group and bromic acid is shown in Figure 12. This method is fairly accurate and has not been affected

by other chemicals, such as carboxylic acid, aldehyde, ether, ester and peroxide. However, this method is more appropriate for analysis of ENR containing the epoxy content lower than 15 %mol.

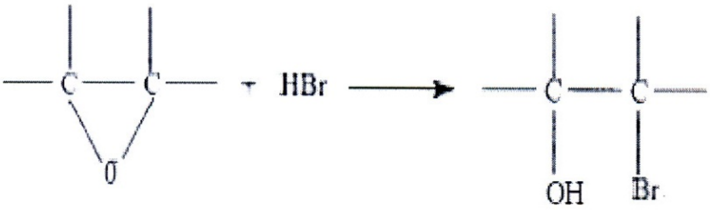


Figure 12 The reaction between epoxy group and bromic acid.

Source: Derbetaki *et al*. (1956)

7.1.2 FTIR technique

FTIR is widely used for qualitative and quantitative analysis of ENR. From FTIR spectrum, relative absorbance ratio (Ar) is calculated by the following equation (Davey and Loadman, 1984)

$$Ar = a_{870} / (a_{835}+a_{870}) \qquad \text{.... ...... (Eqn.1)}$$

Where a_{870} is absorbance at wave number of 870 cm^{-1} due to the epoxy group and a_{835} is absorbance at wave number of 835 cm^{-1} due to C=C of cis-1,4 polyisoprene.

The epoxy content of ENR is then obtained by using the calibration curve which is a plot between Ar and %mol of epoxy group (Figure 13) (Chrimisinoff *et al.*, 1993)

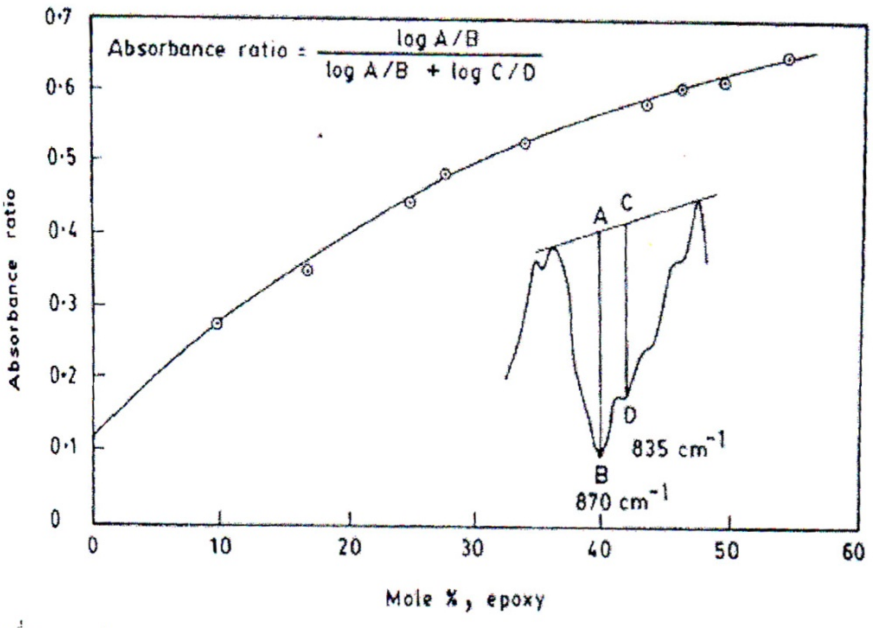


Figure 13 The calibration curve showing relationship between absorbance ratio (Ar) and % mol of epoxy group in ENR.

Source: Chrimisinoff *et al*. (1993)

7.1.3 ^{1}H–NMR technique

^{1}H–NMR technique is also used to elucidate the structure and determine the epoxy content of ENR. ENR exhibites characteristic signals at the chemical shift of 5.11 ppm due to olefin proton and the chemical shift of 2.70 ppm due to the epoxy ring proton. The epoxy content of ENR (%mol epoxy) is determined by using the following equation (Burfied *et al.*, 1984):

$$\%\text{mol epoxy} = [A_{2.70} / (A_{5.14}+A_{2.70})] \times 100 \qquad \ldots\ldots\ldots\ (\text{Eqn.}2)$$

Where $A_{2.70}$ and $A_{5.14}$ are intensity of the signals at the chemical shift of 2.70 ppm and 5.14 ppm, respectively.

8. Polymer blend containing NR or ENR

8.1 Polymer blend

Polymer blend, in general, has been prepared commercially by melt mixing, solution blending and latex blending. Polymer blending is a well-used technique when ever modification of polymer properties is required, because it had an easy and straightforward procedure and it is low cost. Polymer blends are expected to produce materials with better properties compared to similar materials made from the respective pure polymers. Polymer blend can be classified in various ways using different indicators such as miscibility (miscible and immicible blends) compatibility (compatible and incompatible blends), production method (mechanical blend and chemical blend), nature of polymer architecture (block and graft polymers), etc. The importance of the interface in multiphase polymer system has been long recognized. Physical and chemical interactions across the phase boundaries are known to control the overall performance of both immisible polymer blend and polymer composites. Interfacial adhesion is one of the factors which control the mechanical properties, rheological and processing characteristics. There exist two general rotes to improve compatibility of the blend system (Utracki, 2002):

(1) Addition of compatibilizer which is capable of producing specific interactions with the blend. Various compatibilizers can be used such as block and graft copolymers and a variety of low-molecular-weight reactive chemicals. The choice of block or graft copolymer as compatibiliser is based on the miscibility or reactivity of its segments with at least one of the blend components.

(2) Blending suitably funtionalized polymers capable of enhancing specific interaction and/or chemical reaction. The compatibilizer has segments that are chemically identical to those in the respective unreacted homopolymer and are thought to be located preferentially at the interface. Thus, they may be considered equivalent to the block or graft copolymers that are added separately.

8.2 Use of NR or ENR in polymer blend

ENR has recently been used for reactive blending with starch and other polar synthetic polymers to improve their elastic property and impact resistance. Nakason *et al.* (2001) studied properties of composites of starch and ENR. The elastic modulus of the composites was greatly decreased and stain at break greatly increased, and T_g increased from -48°C to -32°C. More recently, Carvaho *et al.* (2002) reported the properties of starch/NR blend. The dispersion of rubber in the starch matrix was homogeneous. The results revealed a reduction in modulus and tensile strength, and SEM results showed a good dispersion of the NR in the continuous phase of starch matrix. Cruz *et al.* (2002) studied the mechanical properties of collagen/NR blend films. For the blend with 25% of NR, it had the highest denaturation temperature (109°C). The presence of NR led to the decrease in the organization of the microscopic structure of the films, which resulted in the decrease in the piezoelectricity. Qi *et al.* (2006) investigated some properties of starch/ENR blend. The TS and tear resistance of the blend decreased, but EAB increased.

Objectives

1. To study the effect of glycerol content on properties of bovine gelatin film.
2. To prepare and investigate the properties of bovine gelatin film as influenced by the addition of epoxidized natural rubber (ENR).
3. To study the effect of ENR containing different epoxy contents at various gelatin/ENR ratios on properties of bovine gelatin-based film.
4. To study the influence of using ENR incombination with reduced glycerol content on properties of bovine gelatin-based film.

CHAPTER 2

RESEARCH METHODOLOGY

1. Materials

1.1 Raw materials

Bovine hide gelatin (Type B) (Figure 14) with a bloom strength of approximately 250 was obtained from Halamic company (Bangkok, Thailand). High-ammonia concentrated (~60% DRC) natural rubber (HA-NR) latex was purchased from Chalong Latex Industry (Songkhla, Thailand).

Figure 14. Photograph of Bovine gelatin.

1.2 Chemicals

Glycerol was purchased from Wako Pure Chemical Industry, Ltd. (Tokyo, Japan). Sodium hydroxide, potassium hydroxide, hydrogen peroxide and hydrochloric acid were obtained from Merk (Darmstadt, Germany). Chloroform, benzene, methanol and isopropanol were purchased from Labscan Asia Co., Ltd. (Bangkok, Thailand). Teric-N30 was purchased from Orica Australia Pty Ltd. (Melbourne, Australia).

2. Equipments

Equipments	Model	Company/Country
pH meter	pH/Ion 510	Eutech Instruments Pte Ltd., Singapore
Magnetic stirrer	Ro 15 power	IKA labortechnik, Stanfen, Germany
Vortex mixer	G-560E	Scientific Indrustries Inc., NY, USA
Shaker	Heidolth Inkubator 10000	Schwabach, Germany
Water bath	W350	Memmert, Schwabach, Germany
Hot air oven	Binder EFD115	Tuttlingen, Germamny
Microcentrifuge	MIKRO20	ZENTRIFUGEN, Hettich, Germany
Universal testing machine	LR 30 K	LLOYD Instruments Ltd., Hampshire, UK
Environmental chamber	KBF 115	WTB Binder, Tuttlingen, Germany
Double-beam spectrophotometer	UV-16001	Shimadzu, Kyoto, Japan
CIE colorimeter	Color Flex	HunterLab Reston, Virginia, USA
Fourier transform infrared spectrometer	Bruker Model Equinox 55	Bruker Co., Ettlingen, Germany
Scanning electron microscope	JSM-5800 LV	JEOL, Tokyo, Japan
Differential scanning calorimeter	DSC 7	Perkin Elmer, Norwalk, CT, USA
Texture analyzer	TA-XT2	Stable Micro System, Surrey, UK
Thermo-gravimetric analyzer	TGA 7	Perkin Elmer, Norwalk, CT, USA
Dynamic mechanical thermal analyzer	DMTA 5	Rheometric, New Jersey, USA
Nuclear magnetic resonance spectrometer	Unity Inova 500 MHz	Varian Inc., Germamny

3. Methods

3.1 Compositional and property analyses of bovine gelatin

3.1.1 Proximate composition

Protein, lipid, moisture and ash of gelatin were determined according to the method of AOAC (1999).

3.1.2 Determination of hydroxyproline content

Hydroxyproline content was analyzed according to the method of Berman and Loxley (1963) with a slight modification. The samples were hydrolyzed with 6 M HCL at 100°C for 24 h in an oil bath. The hydrolysate was clarified with activated carbon and filtered though Whatman No. 4 filter paper. The filtrate was neutralized with 10 M and 1 M NaOH to obtain the pH of 6.0-6.8. The neutralized sample (0.1 ml) was transferred into a test tube and isopropanol (0.2 ml) was added and mixed well; 0.1ml of oxidant solution (mixture of 7% (w/v) chlororamine T and acetate/citrate buffer (0.42 M sodium acetate, 0.13 M trisodium citrate, 0.03 M citric acid and 38.5% isopropanol) pH 6, at a ratio of 1:4 (v/v)) was added and mixed thoroughly; 1.3 ml of Ehrlich's reagent solution (mixture of solution A; 2 g of p-dimethylamino-benzaldehyde in 3 ml of 60% (v/v) perchloric acid (w/v)) and isopropanol at a ratio of 3:13 (v/v)) was added. The mixture was mixed and heated at 60°C for 25 min in a temperature-controlled water bath (Memmert, Schwabach, Germany) and then cooled for 2-3 min in the running water. The solution was diluted to 5 ml with isopropanol. Absorbance was measured against water at 558 nm. Hydroxyproline standard with the concentrations ranging from 10 to 60 ppm were prepared. Hydroxyproline content was calculated and expressed as mg/g sample.

3.1.3 Determination of bloom strength

Gelatin gel was prepared following the method of Fernadez-Diaz *et al.* (2001) with a slight modification. Dried gelatin (2 g) was dissolved with de-ionized water (30 ml) to obtain 6.67% protein content in 50 ml beaker. The gelatin solution was then heated at 60°C for 15 min in a temperature-controlled water bath and cooled in a refrigerator (4°C, maturation temperature) for 16-18 h.

The bloom strength was determined by the method of Fernádez - Diaz *et al.* (2001) with a slight modification. The bloom strength was determined using a texture analyzer (Stable Micro System, Surrey, UK) with a load cell of 5 kN, cross-head speed of 1 mm/s, equipped with a 1.27 diameter flat-faced cylindrical Teflon® plunger. The dimensions of the sample were 3.8 cm diameter and 2.7 cm height. The maximum force (in grams), taken when the plunger had penetrated 4 mm into the gelatin gels, was recorded. The measurement was preformed in triplicate determinations.

3.1.4 Color

The color of gelatin powder and gelatin gel (6.67% (w/v)) was measured by using colorimeter (model ColorFlex, HunterLab Reston, Virginia, USA), and reported in CIE color parameters of L^*, a^* and b^*.

3.2 Study on effect of glycerol concentration on properties of bovine gelatin film

3.2.1 Preparation of film-forming solution (FFS)

Bovine gelatin was dissolved with de-ionized water to obtain the final protein concentration of 2% (w/v). The solution was incubated at 60°C for 30 min. The glycerol was added to gelatin solution with different concentrations (0, 15, 20, 25 and 30% (w/w) of protein). The film-forming solution was stirred gently for 30 min.

3.2.2 Film casting and drying

The FFS obtained (4 g) was cast onto a rimmed silicone plate (5x5 cm^2) and air blown for 12 h at room temperature prior to further drying at 25°C and 50% relative humidity (RH) for 24 h in an environmental chamber (WTB Binder, Tuttlingen, Germany). Finally, the resulting films were manually peeled off and used for analysis.

3.2.3 Determination of film properties

3.2.3.1 Film thickness

The thickness of film was measured using a micrometer (Mitutoyo Absolute, Tokyo, Japan). Five random positions of each film of five films were used for thickness determination.

3.2.3.2 Mechanical properties

Prior to the measurement of mechanical properties, the films were conditioned for 48 h in a ventilated oven at 25°C and 50±5% RH. Elastic modulus (E), tensile strength (TS) and elongation at break (EAB) of films were determined as described by Iwata *et al.* (2000) with a slight modification using a Universal Testing Machine (Lloyd Instruments, Hampshire, UK) equipted with tensile load cell of 100 N. Ten samples (2x5 cm^2) with initial grip length of 3 cm were used for testing. The samples were clamped and deformed under tensile load with the cross-head speed of 30 mm/min until the samples were broken. The maximum load and the final extension at break were used for calculation of TS and EAB, respectively. The elastic modulus (E) was calculated as the initial slope of the linear portion of stress-strain curve.

3.2.3.3 Water vapor permeability (WVP)

WVP of films was determined using a modified ASTM D-882 method (1989) as described by Shiku *et al.* (2004). The film was sealed on an aluminum cup containing silica gel (0% RH) with silicone vacuum grease and rubber gasket. The cup was placed at 30°C in a desiccator containing the distilled water. The cup was weighed at 1 h intervals over a 10 h period. WVP of the films was calculated as follows:

$$WVP\ (\mathrm{g\ m^{-1} s^{-1}\ Pa^{-1}}) = wlA^{-1}t^{-1}(P_2\text{-}P_1)^{-1}$$

Where w is the weight gain of the cup (g); l is the film thickness (m); A is the exposed area of film (m^2); t is the time of gain (s); (P_2-P_1) is the vapor pressure difference across the film (Pa). Five films were used for WVP testing.

3.2.3.4 Color, light transmittance and transparency value

Color of films was determined using a CIE colorimeter (Hunter associates laboratory, Inc., Reston, Virginia, USA) and expressed as L*-, a*- and b*-values. The light transmittance of films was measured at the ultraviolet and visible range (200 – 800 nm) using UV-Vis spectrophotometer (Jasco V530, Tokyo, Japan) as described by Shiku *et al.* (2004). The transparency value of films was calculated by the following equation (Han and Floros, 1997):

$$\text{Transparency value} = -\log T_{600}/x$$

Where T_{600} is the fractional transmittance at 600 nm and x is the film thickness (mm).
The higher value represents the lower transparence of the film.

3.2.3.5 Film solubility

Film solubility was determined according to the method of Gennadios *et al.* (1998). A portion of the film (2x4 cm^2) was weighed and immersed in 10 mL of distilled water containing sodium azide (0.1% w/v) to prevent microbial growth. The mixture was shaken at a speed of 250 rpm using a shaker (Heidolth Inkubator 10000, Schwabach, Germany) at 30ºC for 24 h. Undissolved debris was removed by centrifugation at 3000xg for 20 min. The pellet was dried at 105ºC for 24 h using hot air oven (Binder FED115, Tuttlingen, Germany). Film solubility was calculated by subtracting the weight of unsolubilized dry matter from initial weight of dry matter and expressed as a percentage of the total weight.

The condition provided the film which had the highest mechanical properties and water barrier property was chosen for further study.

3.3 Preparation and characterization of epoxidized natural rubber

3.3.1 Preparation of epoxidized natural rubber (ENR) latex

ENR latexes with different epoxy contents of ≈ 10%, 25% and 50% mol (ENR-10, ENR-25 and ENR-50, respectively) were prepared according to the method of Jirupan (2003) with slight modification. The formulation recipe used for preparation of the different ENR$_s$ is shown in Table 4. HA-NR latex of 20% dry rubber content (DRC) and Teric-N30, a non-ionic surfactant, were charged in a 3-neck reactor and stirred at room temperature for 24 h. The temperature of the reactor was raised to 60°C. Formic acid was then introduced to the reaction mixture and stirred for 10 min at 60°C under nitrogen gas. Then, hydrogen peroxide was added to the reactor and stirred for 24 h. Finally, the reaction was stopped by adjusting the pH of the mixture to 6.5 – 7.0 with 10% (w/v) potassium hydroxide. The obtained latexes were subjected to analyses (section 3.3.2) and used for film preparation (section 3.5).

Table 4 Formulations used to prepare the ENR latexes of different epoxy contents

Ingredients	ENR-10*	ENR-25*	ENR-50*
NR latex (20% DRC), ml	1000	1000	1000
Formic acid, ml	12.34	21.60	37.00
Hydrogen peroxide, ml	100.28	175.50	300.84
Teric-N30 (non-ionic surfactant), g	6.00	6.00	6.00

*ENR-10, ENR-25 and ENR-50 refer to ENR with epoxy content approximately 10, 25 and 50 %mol, respectively.

3.3.2 Structural analysis and epoxy content determination of ENR

ENR latexes (section 3.3.1) (10 ml) were precipitated in 20 ml of methanol and then washed with distilled water. The ENR$_s$ were dried at 50^{o}C in vacuum oven for 24 h. Dried ENR samples were subjected to FTIR and ^{1}H-NMR analyses.

3.3.2.1 Analysis of ENR by FT-IR spectroscopy

Dried ENR (1 g) was dissolved with chloroform and then coated on ZnSe cell. The sample was dried at 50^{o}C to evaporate the solvent. The sample was then scanned with a Bruker Model Equinox 55 FTIR spectrometer (Bruker Co., Ettlingen, Germany). The spectra were performed in the 4000 – 650 cm^{-1} regions. To determine the epoxy content, relative absorbance ratio (Ar) was calculated using Equation 1 and compared the obtained value to the standard curve (Figure 13). (Davey and Loadman, 1984; Cheremisinoff *et al.*, 1993).

3.3.2.2 Analysis of ENR by ^{1}H-NMR spectroscopy

Dried ENR (15 mg) was dissolved with 10 ml deuterated chloroform ($CDCl_3$). The ENR solution was placed into the NMR tube and scanned with NMR spectrometer (Varian Unity Inova 500 MHz, Varian Inc., Germamny). The epoxy content of ENR was estimateted from the intensity ratio of the signals at 2.7 and 5.1 ppm, using Equation 2 (Burfied *et al.*, 1984).

3.4 Study on effect of epoxy content and concentration of incorporated ENR on properties of bovine gelatin films

Bovine gelatin powder was dissolved with de-ionized water to obtain the final protein concentration of 2% (w/v). The solution was incubated at 60°C for 30 min. The glycerol was added to gelatin solution with 25% (w/w) of protein. To prepare film-forming mixture containing ENR, the ENR (20% DRC) latex (ENR-10, ENR-25 or ENR-50) was added to the gelatin solution at varying amounts to obtain the different gelatin/ENR ratios (10:0, 8:2, 6:4, 5:5 and 0:10 (w:w of dry polymer)). All film-forming solutions/mixtures prepared had the final polymer concentration of 2% (w/v). The film-forming solution/mixture was stirred gently for 30 min and homogenized at 13,000 rpm for 2 min. The film-forming solution/mixture obtained (4 g) was cast onto a rimmed silicone plate (5x5 cm^2) and air blown for 12 h at room temperature prior to further drying at 25°C and 50% relative humidity (RH) for 24 h in an environmental chamber. The resulting films were manually peeled off and used for analysis as mentioned in section 3.2.3.

The condition provided the film which had the highest mechanical properties and water barrier property was chosen for further study.

3.5 Study on effect of ENR in combination with decreased plasticizer content on properties of bovine gelatin film

To further improve the water-vapor barrier property of the gelatin/ENR blend film, the blend films added with glycerol at reduced amounts were prepared.

First, the gelatin solutions added with different glycerol contents (25%, 6.25%, 2.5% and 0% of protein) were prepare in the same way as described in section 3.2.1. The ENR latex (containing 25%mol of epoxy content, as selected from section 3.4.2.2) was then added to the gelatin solution at designate amount to obtain the gelatin/ENR ratio of 6/4 (w/w) (selected from section 3.4.2.2). The mixture was mixed and used to prepare film in the same manner as described in section 3.4.1 The properties of resulting films were determined as mentioned in section 3.2.3.

3.6 Study on effect of incorporation of natural rubber compatibilized with ENR on properties of bovine gelatin film

To further improve the flexibility and water-vapor barrier property of the gelatin/ENR blend film, natural rubber was incorporated for partial substitution of ENR component in the blend.

The gelatin solution without glycerol was prepared as mentioned in section 3.2.1. The NR and ENR-25 latexes were added at designate amounts to the gelatin solution to obtain the different gelatin/ENR/NR ratios (6/3/1, 6/2/2 and 6/1/3). The films were prepared and subjected to analyses as described in section 3.2.3.

The film which had the highest mechanical properties and water barrier property was chosen for further analyses in comparison with the control films as following:

- Film solubility

The solubility in water of the selected film samples was determined as described in section 3.2.3.5.

- Thermal property

Samples were determined for thermal degradation using thermogravimetric analyzer (TGA7, Perkin Elmer, Norwalk, CT, USA). Films were scanned from room temperature to 600ºC at a rate of 10ºC/min. Nitrogen was used as the purge gas with a flow rate of 20 ml/min.

- Film morphology

Morphology of surface and freeze-fractured cross section of the film samples were visualized using a scanning electron microscope (SEM) (JSM-5800LV, JEOL, Tokyo, Japan) at an acceleration voltage of 10 kV. For cross section, samples were fractured under liquid nitrogen prior to morphology visualization. Then, the samples were mounted on bronze stub and sputtered with gold (Sputter coater SPI-Module, PA, USA) in order to make the sample conductive, and photographs were taken at selected magnification.

4. Statistical analysis

Experiments were run in triplicate. Data were subjected to Analysis of Variance (ANOVA) and the differences between means were evaluated by Duncan's Multiple Range Test (Steel and Torrie, 1980). SPSS statistic program (SPSS Inc., Chicago, IL, USA.) was used for data analysis.

CHAPTER 3

RESULTS AND DISCUSSION

1. Composition and some properties of bovine gelatin

1.1 Proximate composition and hydroxyproline content

The proximate composition and hydroxyproline content of commercial bovine gelatin are shown in Table 5. Protein was found as a major constituent (at 85.02%) of the gelatin sample. The composition of gelatin depended on raw material and extraction processes (Jones, 1997). Bovine gelatin sample contained hydroxyproline content of 162.50 mg/g dried sample.

Bovine gelatin was high in hydroxyproline content. Hydroxyproline is the unique imino acid in collagen and gelatin (Kittiphattanabawan *et al*., 2004). Hydroxyproline content has been reported to contribute to the bloom strength and thermal stability of gelatin (Haug *et al*., 2004).

Table 5. Proximate composition and hydroxyproline content of bovine gelatin used in this study.

Compositions	Amount (%)
Moisture	12.61±0.14*
Protein	85.02±0.82
Lipid	0.18±0.01
Ash	2.31±0.04
Hydroxyproline (mg/g dry sample)	162.50±2.74

*Mean ± SD (n=3).

1.2 Bloom strength and color

Bloom strength and color of bovine gelatin are shown in Table 6. Bloom strength of gel from gelatin was high, approximately 248. The bloom strength of gelatin depended on its composition, particularly in terms of amino acid composition, and size of protein chains (Muyonga *et al*., 2004). Hydroxyproline, a unique imino acid of gelatin, had the marked influence on gel strength due to its hydrogen bonding ability, via the hydroxyl group (Ledward, 1986). High bloom strength of gelatin correlates in part to the large size of protein molecules, which is one of the prerequisites for the formation of polymeric film with high strength.

L^*, a^* and b^*-values of powder and gel from bovine gelatin are shown in Table 6. Bovine gelatin gel showed the high L^*-value than did bovine gelatin powder ($p<0.05$). A commercial gelatin powder is usually dehydrated by progressive increases in air temperature, but a gelatin gel is formed by cooling of heated gelatin solution. The yellow color of gelatin powder and gel might be developed during thermal process. A small amount of sugar and carbohydrate added in a commercial gelatin possibly interacted with amino group of gelatin via Maillard reaction, resulted in yellowing.

Table 6. Bloom strength and color of the powder and gel from commercial bovine gelatin

Physical properties	Gelatin powder	Gelatin gel
L^*	27.36 ± 1.11^a	$34.76\pm1.76^{b\#}$
a^*	-1.18 ± 0.34^a	-0.95 ± 0.24^a
b^*	11.71 ± 2.30^a	13.51 ± 1.52^a
Bloom strength (g)		247.90 ± 1.21

$^\#$Means ± SD (n=3).

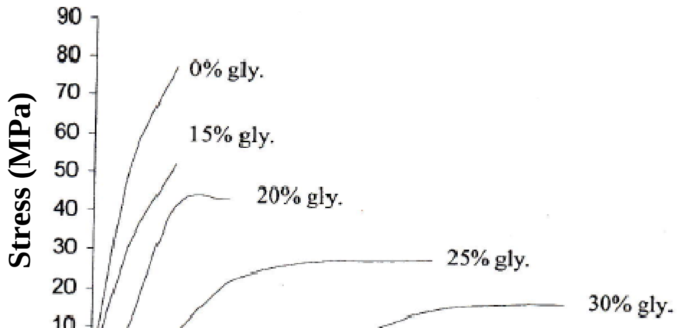
The different superscripts in the some row indicate the significant differences ($p<0.05$).

2. Effect of glycerol concentration on properties of bovine gelatin film

2.1 Thickness and mechanical properties

The average thickness of bovine gelatin film without glycerol (0% glycerol) and gelatin films added with glycerol at different concentrations (15, 20, 25 and 30% (w/w) of protein) was 0.030 mm. The thickness of the films was not influenced by glycerol concentrations ($p<0.05$).

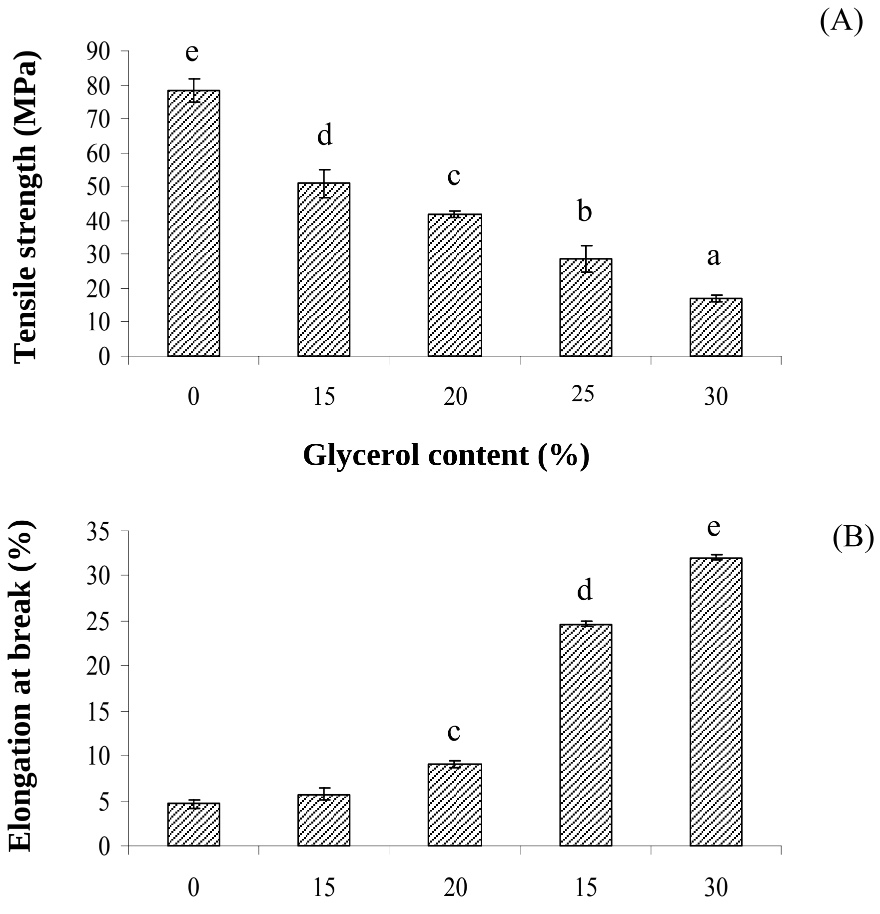
Figure 15 illustrates tensile stress-strain curves of representative film samples. Gelatin film without glycerol showed rather brittle characteristic and more resistant to tensile deformation, probably due to the higher aggregation of the protein chains (Moore *et al*., 2006). The films with glycerol addition exhibited ductile behavior in which highly plastic deformation was observed. This behavior was more pronounced when the increasing amount of glycerol was added. This was contributed from the plasticizing effect, most likely resulted from the decrease in inter- and intra molecular attractive forces (Bergo and Sobral, 2007; Rodriguéz *et al*., 2006; Sothornvit and Krochta, 2001).



Strain (%)

Figure 15. Representative tensile stress-strain diagrams of selected bovine gelatin films incorporated with different glycerol concentrations.

TS, EAB and E of the films prepared from film-forming solution (FFS) added with different glycerol concentrations are shown in Figure 16(A), 16(B) and 16(C), respectively. Gelatin film without glycerol exhibited the higher TS and E but lower EAB than did the films added with glycerol. The result was in agreement with that of Moore *et al*. (2006) who reported that TS and E of keratin films without glycerol was higher than the films added with glycerol. TS and E of the film decreased and EAB increased when glycerol concentration increased ($p<0.05$). The result agreed with other studies concerning the effect of plasticizer concentrations on protein–based films from wheat gluten and soy proteins (Gennadios *et al*., 1994), peanut proteins (Jangohod and Chinnan, 1999) and fish muscle proteins (Sobral *et al*., 2005). The results showed that glycerol could improve the flexibility and decreased the stiffness of gelatin films. This was contributed mainly from the plasticizing effect of glycerol added, which increased intermolecular spacing, and thereby increased chain mobility (Audic and Chaufer, 2005).



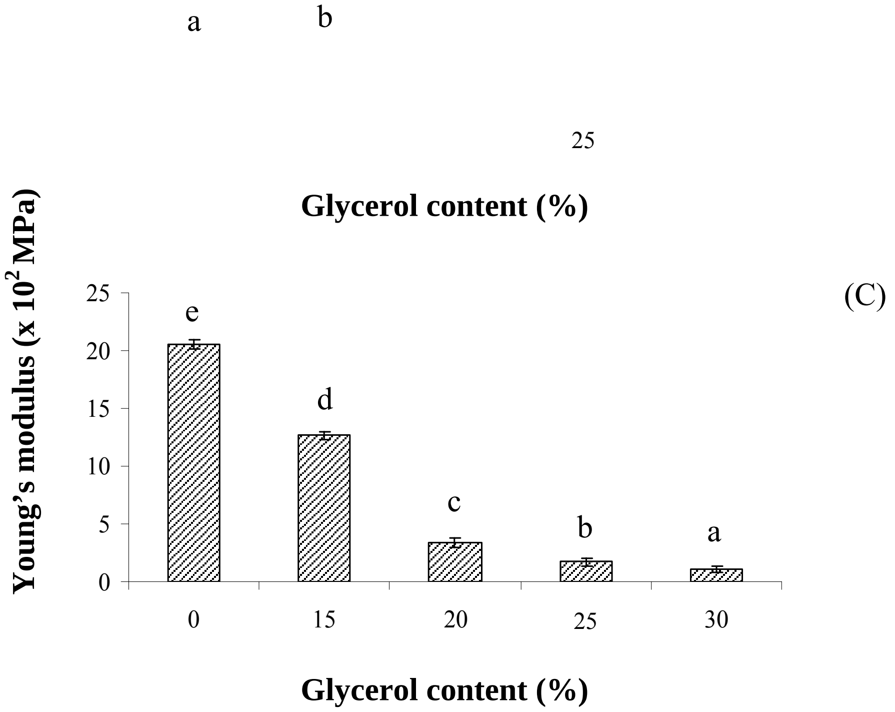
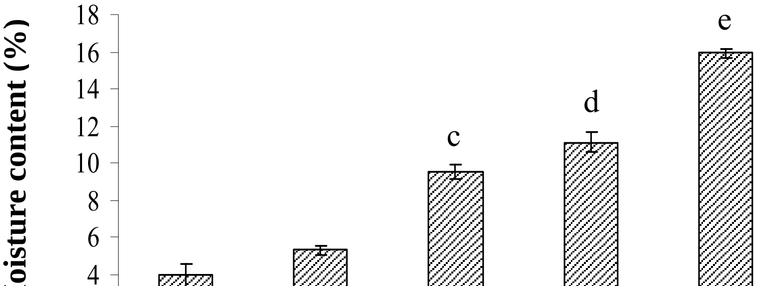


Figure 16. Mechanical properties of bovine gelatin films incorporated with different glycerol concentrations: (A) tensile strength, (B) elongation at break and (C) Young's modulus. Bars represent the standard deviation (n=3). Different letters indicate the significant differences ($p<0.05$).

2.2 Moisture content and water vapor permeability

Moisture content (MC) and water vapor permeability (WVP) of the gelatin films with different glycerol concentrations are shown in Figure 17 and 18, respectively. In general, the gelatin films without glycerol had lower MC and WVP than did those added with glycerol. The increase in glycerol concentration increased the values of MC and WVP of the resulting films ($p<0.05$). The result was in agreement with other studies (Jongjareonruk *et al.*, 2006; Sobral *et al.*, 2001; Thomazine *et al.*, 2005; Vanin *et al.*, 2005). The addition of glycerol of low molecular mass reduced protein–protein interactions, resulted in an increase in molecular mobility, and thus facilitating migration of water molecules (Oliare *et al.*, 2003; Rudriguéz *et al.*, 2006; Subral *et al.*, 2001; Thomazine *et al.*, 2005). In addition, it might provoke a reorganization of protein network which become less dense with a larger free volume (Cuq *et al.*, 1997; Claire *et al.*, 2003), enhancing the ease of water diffusion. As a consequence, greater water-vapor permeation through the film matrix was obtained.



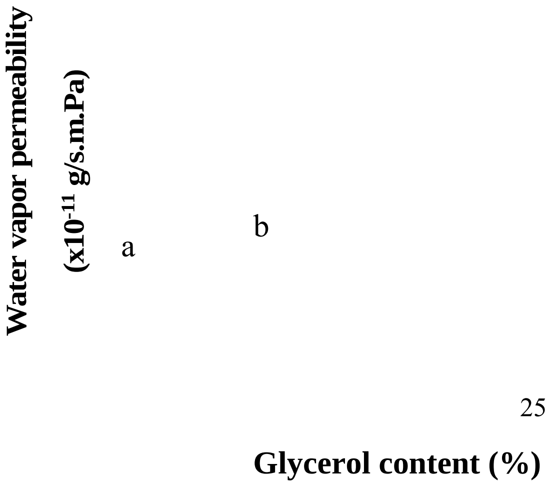


Figure17. Moisture content of bovine gelatin films incorporated with different glycerol concentrations. Bars represent the standard deviation (n=3). Different letters indicate the significant differences ($p<0.05$).

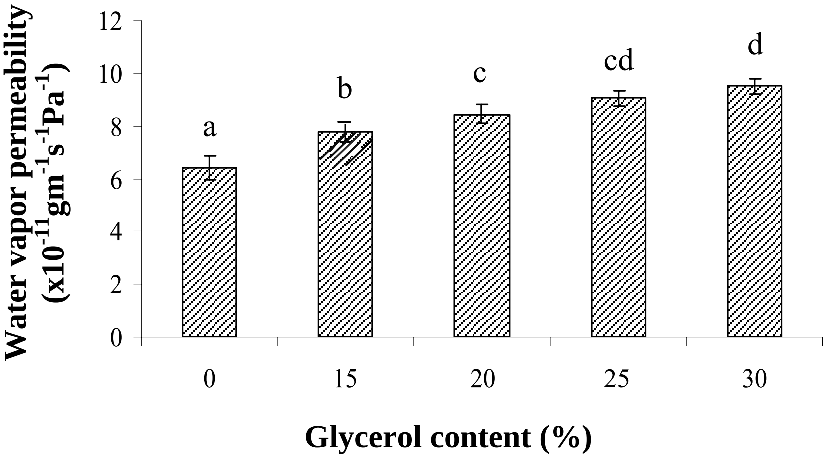


Figure 18. Water vapor permeability of bovine gelatin films incorporated with different glycerol concentrations. Bars represent the standard deviation (n=3). Different letters indicate the significant differences ($p<0.05$).

2.3 Optical properties

2.3.1 Light transmission and film transparency

Transmission of UV and visible light at selected wavelength in the range of 200 – 800 nm of films from gelatin with different glycerol concentrations is shown in Table 7. Transmission in visible length (350 – 800 nm) of the films varied from 44.05 to 77.46%.

The transmission of UV light at 280 nm was in the range of 26.25–36.61%. Very low transmission (1.18–3.48%) was found at 200 nm. Therefore, those gelatin films effectively prevented the UV light and potentially prevented the retardation of lipid oxidation induced by the UV light. This advantage of protein films has been reported for the films from fish and bovine gelatins (Intarasirisawat, 2006), surimi (Shiku *et al*., 2004), whey protein (Fang *et al*., 2002), fish myofibrillar protein (Shiku *et al*., 2006), etc. Gelatin constituents low content of tyrosine and phenylalanine (aromatic amino acids) (Jongjareonruk *et al*., 2005). In general, tyrosine and phenylalanine are well known to be sensitive chromophores, which absorb light at the wavelength below 300 nm (Li *et al*., 2004). The aromatic amino acids of protein might play an important role in the UV barrier properties of protein films. Gelatin film without glycerol had the higher barrier for light transmission in UV range than did the gelatin films added with glycerol. Light transmission of the films slightly increased with increasing glycerol content.

For transparency value, all films obtained had similar transparency value ($p>0.05$) (Table 7). Therefore, the glycerol added had no impact on transparency of bovine gelatin film. The gelatin films had comparable transparency value to some synthetic films such as polyvinylidene chloride (PVDC: 4.58) and low-density polyethylene (LDPE: 3.05) (Shiku *et al*., 2003). Therefore, the resulting gelatin film was transparent and clear enough for use as a see-through packaging.

Table 7. Light transmittance (%) and transparency value of bovine gelatin films incorporated with different glycerol concentrations.

Films	**Wave length (nm)**								**Transparency value**[¥]
	200	**280**	**350**	**400**	**500**	**600**	**700**	**800**	
Control	1.18	26.30	44.05	66.01	67.16	67.72	68.02	68.48	3.35±0.17^{a}[*]
15% Glycerol	2.15	36.61	54.39	70.78	70.78	71.88	72.95	74.14	3.38±0.09^{a}
20% Glycerol	2.56	32.98	52.95	72.56	72.56	73.21	74.15	75.93	3.41±0.06^{ab}
25% Glycerol	2.91	35.80	54.87	74.35	74.35	75.70	76.65	77.22	3.42±0.04^{ab}
30% Glycerol	3.47	34.31	55.21	73.44	73.44	75.37	76.95	77.47	3.42±0.09^{ab}

[*] Mean ± SD (n=3). The difference superscripts in the same column indicate the significant differences ($p<0.05$).

[¥] Transparency value = (-logT_{600})/ L, where T_{600} is fractional transmittance at 600 nm and L is film thickness (mm). The lower transparency value, the more transparent of the film.

2.3.2 Color of films

L^*, a^* and b^*-values of bovine gelatin films with different glycerol concentrations are shown in Table 8. The addition of glycerol at different concentrations had no impact on color of the resulting films ($p>0.05$). From the results, besides being transparent, the gelatin films were light in color. The gelatin films were lighter in color as compared to some other protein films such as myofibrillar protein films (L^* = 88.69, a^*= -1.37, b^*= 2.91) (Limpan *et al.,* 2010) and wheat gluten film (L^* = 62.37, a^*= -2.35, b^*=5.26) (Zhang *et al*., 2004).

Table 8. L^*, a^* and b^*-values of bovine gelatin films at different glycerol concentrations

Films	**L^***	**a^***	**b^***
Control	$90.59 \pm 0.14^{a¥}$	-1.16 ± 0.01^{a}	$1.46 \pm 0.01^{a\#}$
15% Glycerol	90.30 ± 0.23^{a}	-1.20 ± 0.05^{a}	1.50 ± 0.13^{a}
20% Glycerol	90.17 ± 0.20^{a}	-1.20 ± 0.07^{a}	1.44 ± 0.06^{a}
25% Glycerol	90.27 ± 0.75^{a}	-1.12 ± 0.11^{a}	1.39 ± 0.11^{a}
30% Glycerol	90.64 ± 0.56^{a}	-1.15 ± 0.06^{a}	1.47 ± 0.08^{a}

[#] Mean ± SD (n=3). The difference superscripts in the same column indicate the significant differences ($p<0.05$).

From the results, the film incorporated with 25% glycerol (of protein) had sufficient flexibility (i.e., EAB) to be handled without being easily broken. Thus, addition of glycerol at this level was chosen to prepare the films in next studies.

3. Characterization of ENR

ENR latexes containing different epoxy contents (ENR–10, ENR–25, ENR–50) were prepared and subjected to characterization via FTIR and ^1H –NMR. The results are as followed:

3.1 Characterization of ENR by FTIR

Figures 19-22 show FTIR spectra of NR and ENR with different epoxy contents. The NR exhibited FTIR spectra similar to that reported by other studies (Jirupan, 2003; Cruz *et al*., 2002; Gunasekaran *et al*., 2006). For the IR spectra of NR, it presented the absorption band at 836.43 cm^{-1} which associated with C=C bond of cis–1,4–polyisoprene. The strong asymmetrical (ν_{as} CH_2) and symmetrical (ν_s CH_2) stretching of methylene group vibrations present in NR were observed near 2926.73 and 2858.03 cm^{-1}, respectively (Arroyo *et al*., 2003; Gunasekaran *et al*., 2006). Spectra of NR which contains methyl groups also showed two bands occurring at around 2962.80 and 3033.57 cm^{-1}. The first band results from the C-H bonds of methyl group and the second band arises from =C-H bonds (Chaudhry and Billinghum, 2001).

In the case of ENR, the absorption bands at about 870 and 1250 cm^{-1} shown in the spectra (Figures 20-22) were characteristic of epoxy group in the ENR molecule (Jirupan, 2003; Cruz *et al*., 2002; Gunasekaran *et al*., 2006). The increase in intensity of the peak of epoxy group represented the increase in epoxy content, simultaneously with the decrease in C=C group. The broad absorption band at 3500 cm^{-1} was observed in the FTIR spectra of ENR. This was plausibly due to the presence of –OH group, resulting from epoxide ring opening reaction that might take place especially at high epoxy content (Takayuki *et al.*, 2006; Jirupan, 2003).

By applying the method of Davey and Loadman (1984), the epoxy content of ENR could be determined. The obtained ENR-10, ENR-25 and ENR-50 had the average epoxy content of 12.08, 30.08 and 57.41 %mol, respectively (Table 9), as measured by FTIR technique.

Table 9. Epoxy content of different ENRs prepared in this work

	Epoxy content (%mol)		
	ENR-10	ENR-25	ENR-50
FTIR	12.08 ± 0.06^{a}	30.08 ± 0.15^{a}	$57.41 \pm 0.25^{a*}$
^{1}H-NMR	12.28 ± 0.02^{a}	28.06 ± 0.06^{a}	57.08 ± 0.10^{a}

[*] Mean ± SD (n=3). The difference superscripts in the same column indicate the significant differences ($p<0.05$).

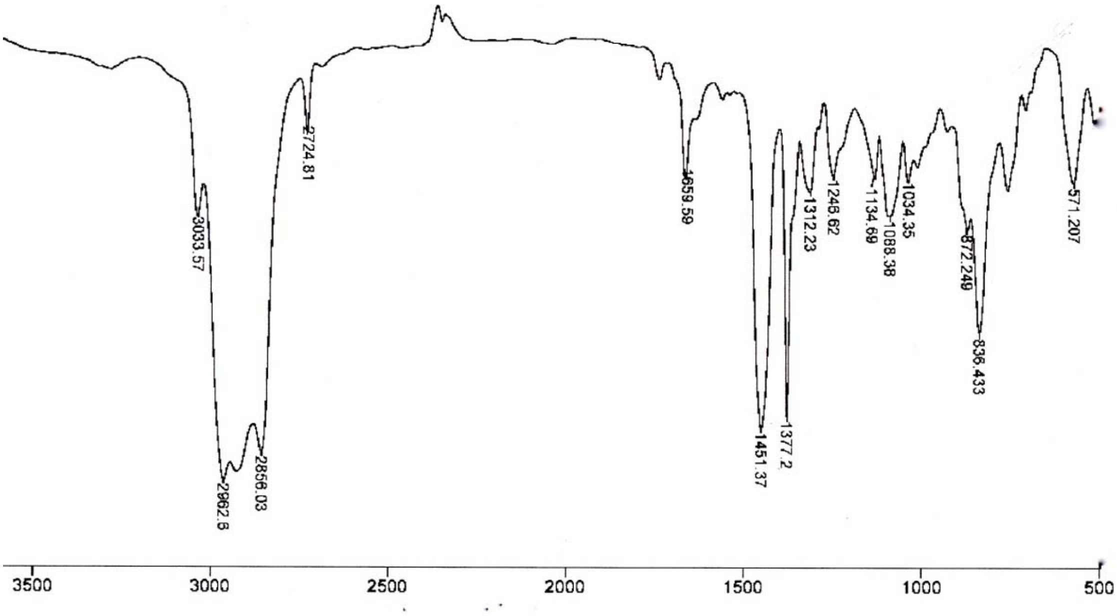


Figure 19. FTIR spectra of natural rubber (NR).

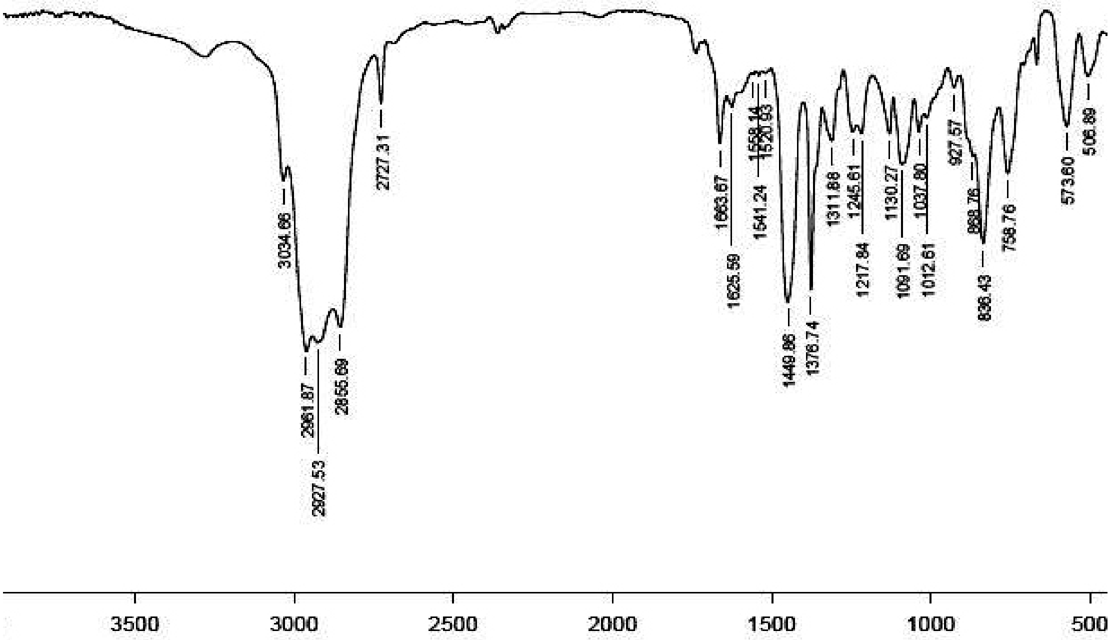


Figure 20. FTIR spectra of epoxidized natural rubber (ENR - 10).

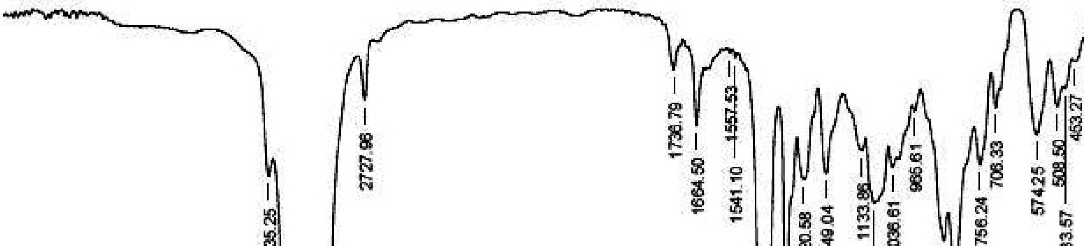


Figure 21. FTIR spectra of epoxidized natural rubber (ENR - 25).

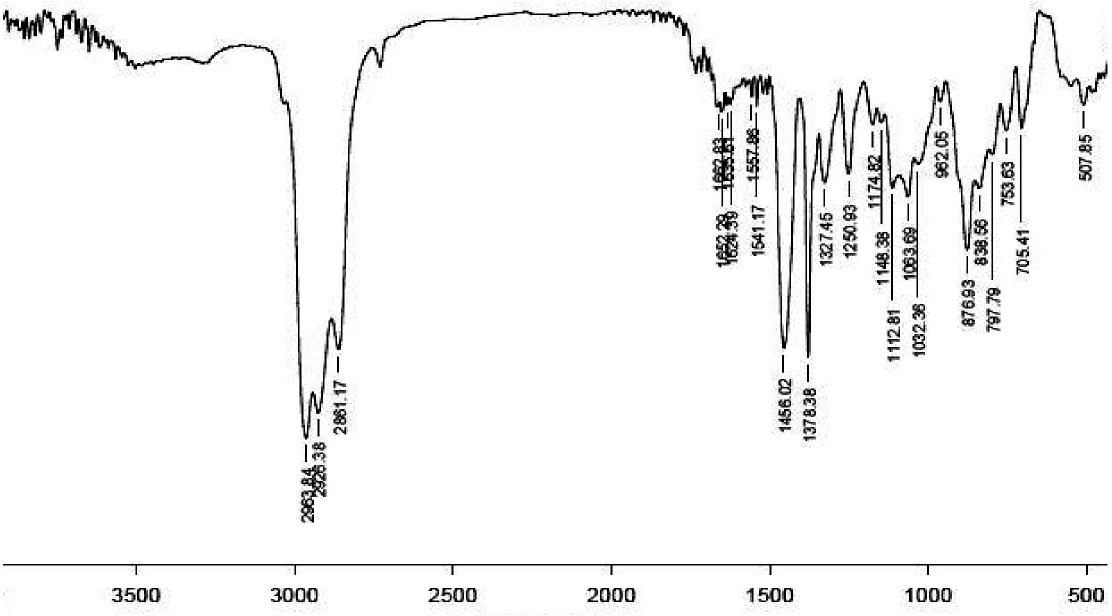


Figure 22. FTIR spectra of epoxidized natural rubber (ENR - 50).

3.2 Characterization of ENR by ^{1}H-NMR

Figures 23-26 show the typical ^{1}H–NMR spectra of NR, ENR–10, ENR–25 and ENR–50. As for NR, three signals (chemical shift: δ) characteristic of methyl, methylene and unsaturated methane proton of cis–1,4–polyisoprene unit appeared at 1.68, 2.05 and 5.1

ppm, respectively. In contrast, for ENR, two additional signals characteristic of epoxy group appeared at 1.29 and 2.70 ppm (Figures 24–26), which were assigned to methyl and methane proton of resulting epoxy group, respectively (Takayuki *et al.*, 2006; Jirupan, 2003; Gunasekaran *et al.*, 2006). It was noted that there were small signals appeared at δ = 1.55 and 2.15 ppm, which were overlapping with the signals at 1.68 and 2.05 ppm. These overlapping signals (δ = 1.55 and 2.15 ppm) typically observed in ^{1}H-NMR spectra of ENR have been reported to be due to various combinations of triad sequences of epoxidized isoprene unit (E) and unepoxidized isoprene unit (C) (Figure 27) (Bradbury and Perera, 1985).

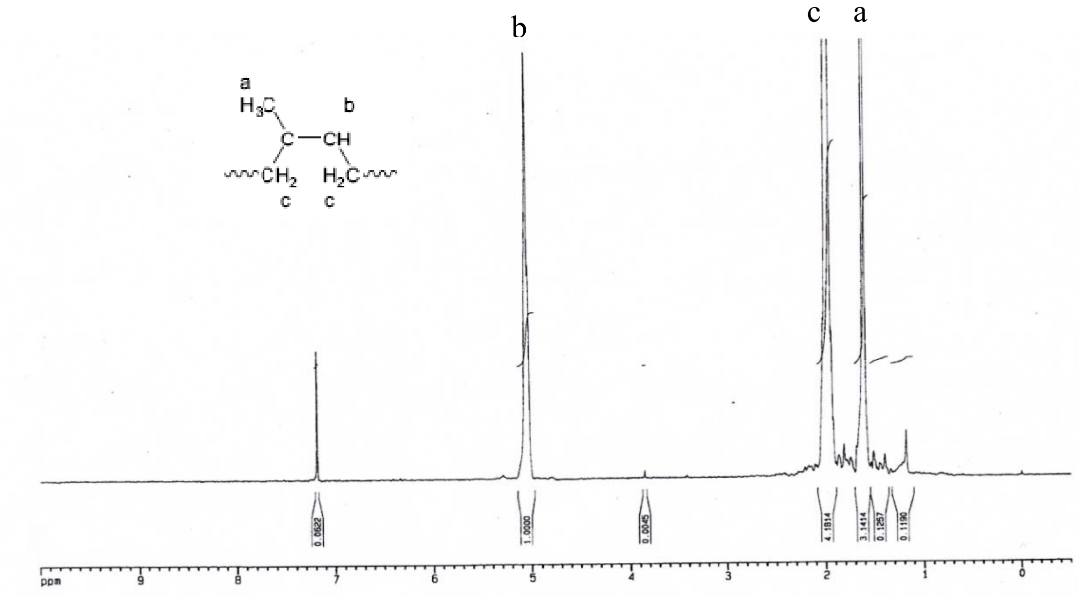
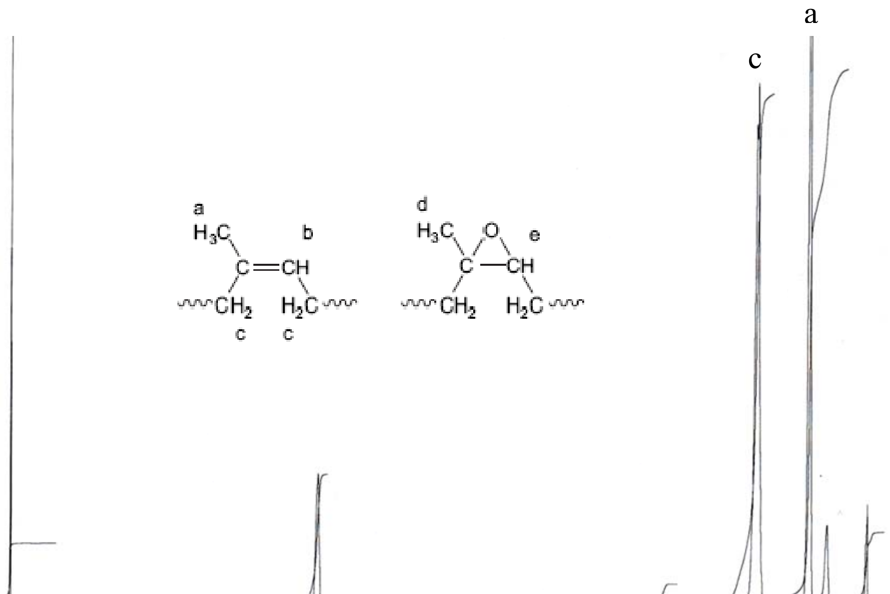


Figure 23. ^{1}H – NMR spectra of natural rubber (NR).



b

d

e

Figure 24. ¹H – NMR spectra of epoxidized natural rubber (ENR-10).

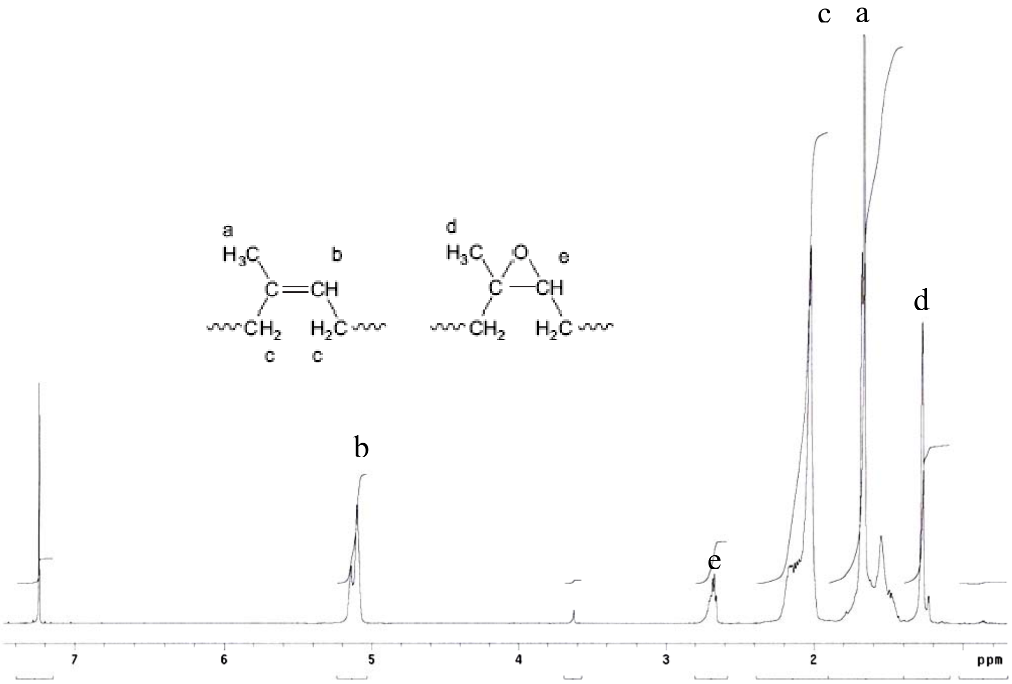
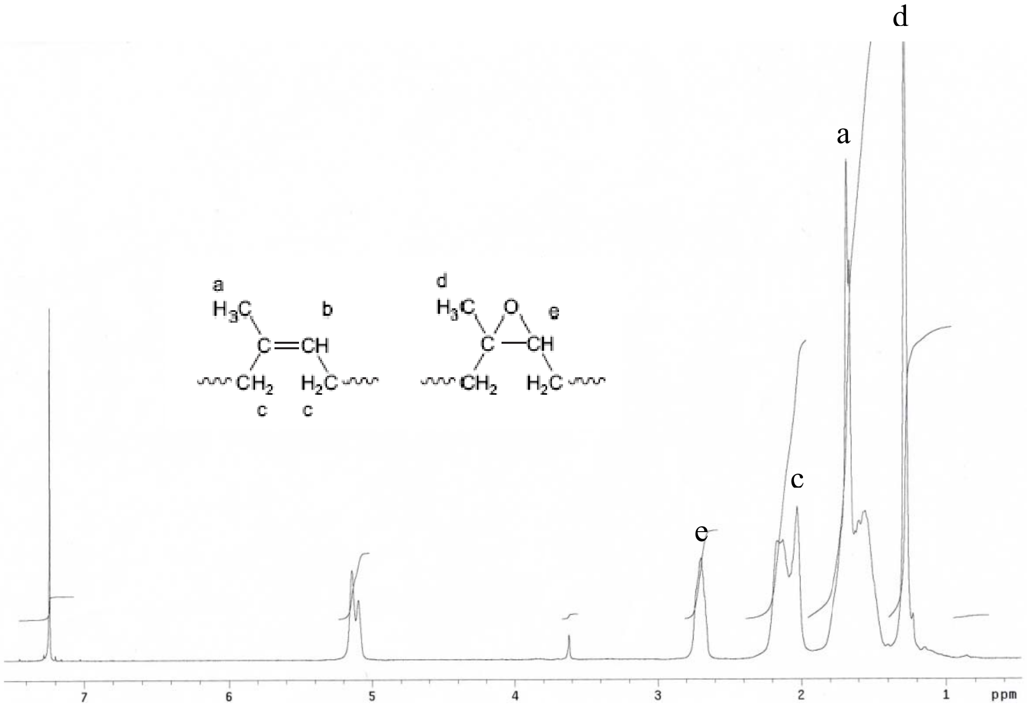


Figure 25. ¹H – NMR spectra of epoxidized natural rubber (ENR-25).



b

Figure 26. ^{1}H – NMR spectra of epoxidized natural rubber (ENR-50).

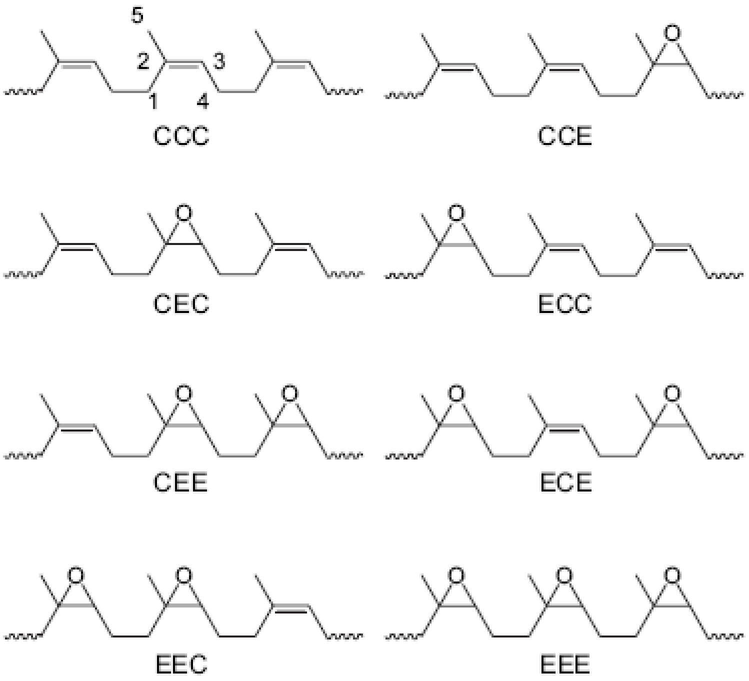


Figure 27. All triad sequences of epoxidized units (E) and of isoprene units (C) in partially epoxidized natural rubber.

Source: Bradbury and Perera (1985)

From the ^{1}H-NMR spectra, the epoxy content of the ENR was calculated by using Equation 2. By this technique, the obtained ENR-10, ENR-25 and ENR-50 had the average epoxy content of 12.28, 28.06 and 57.08 %mol, respectively (Table 9). Similar values of the epoxy content of coresponding ENR were obtained from FTIR and NMR techniques ($p>0.05$).

Therefore, ENR latexes with desinage epoxy contents were successfully prepared and were used in next studies.

4. Effect of epoxy content of ENR and gelatin/ENR ratio on properties of gelatin-based film

4.1 Visualized appearance of films

Figure 28 shows photograph of the selected film samples (gelatin,

gelatin/ENR-25 (6/4) and ENR films). All films could be easily separated from the casting plates and easy to handle. Gelatin film was clearer and more transparent than ENR and gelatin/ENR blend films. For gelatin/ENR blend films, they were homogeneous without visualized phase separation, rather transparent and flexible. Their surfaces were smooth without pores and crack.

Gelatin **Gelatin/ENR=6/4** **ENR-25**

Figure 28. Photographs of selected resulting films: gelatin film, gelatin/ENR-25 (6/4) blend film and ENR-25 film.

4.2 Thickness and mechanical properties

Thickness of gelatin films, gelatin/ENR (G/ENR) blend films and ENR films is shown in Figure 29. The thickness varied depending on film types. All ENR films had lower thickness (18.04 – 18.88 μm) than did the gelatin film (26.16 μm). This was plausibly due to the difference in their molecular structures, which resulted in different molecular alignment in the film matrix (Ibrahim and Dahlan, 1995). The thickness of the G/ENR blend films decreased with increasing amount of ENR incorporated.

Figure 30 shows tensile stress–strain curves of selected film samples. Gelatin film was stiffer and more resistant to tensile deformation while ENR films showed the more ductile behavior. As a consequence, tensile deformation of the G/ENR blend films exhibited increasing ductile behavior (more observed plastic deformation) when increased amount of ENR was added (Figure 30 (curves B, C and D)).

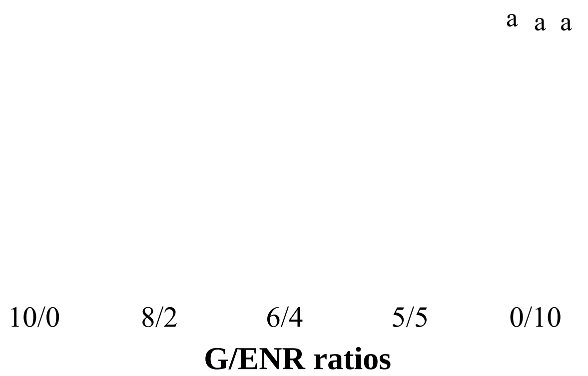


Figure 29. Thickness of gelatin films, gelatin/ENR (G/ENR) blend films and ENR films.

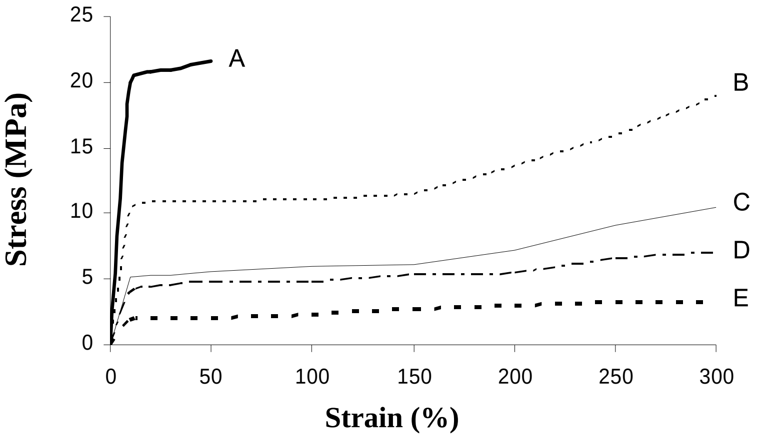


Figure 30. Representative tensile stress-strain diagrams of selected film samples: Gelatin film (A), gelatin/ENR-25 blend films at different ratios 8/2 (B), 6/4 (C) and 5/5 (D)) and ENR-25 film (E).

Figure 31 shows TS, EAB and E of bovine gelatin-based films added with glycerol at 25% (w/w of protein) and ENR of different epoxy contents (ENR-10, ENR-25 and ENR-50) at various gelatin/ENR ratios (10/0, 8/2, 6/4, 5/5 and 0/10). The gelatin film had higher TS and E but lower EAB than did the ENR films ($p<0.05$). This was more likely due to higher interaction between gelatin molecules. The result was similar to that of Cruz *et al.* (2000) who reported that the collagen film had higher TS than did the natural rubber (NR) film. Among ENR films (ENR-10, ENR-25 and ENR-50), TS and E of the films decreased but EAB increased with increasing epoxy content of ENR ($p<0.05$).

For G/ENR blend films, when the amount of incorporated ENR increased, their TS and E decreased while EAB increased, regardless of epoxy content of ENR. This additive effect observed was simply because of the highly elastic characteristic of ENR incorporated (Ibrahim and Dahlan, 1998). ENR molecules more likely inserted between and interacted with gelatin molecules. The epoxy group of ENR might interact inter-molecularly with -NH_2 group, -OH groups or -COOH group of gelatin molecules (Cruz *et al.*, 2002). Thus, inter- and intra-molecular attractive forces between gelatin molecules were decreased and thus intermolecular spacing most likely increased, due to the inserted ENR together with glycerol added. Thereby, chain mobility was increased. This was evidenced by the increase in EAB and decrease in TS and stiffness (i.e., E) of the G/ENR blend films, compared to the gelatin film. For the same level of ENR used, blend films added with ENR-10 had higher TS and E but lower EAB than did those added with ENR-25 and ENR-50. No difference in EAB of the blend films incorporated with ENR-25 and ENR-50 was observed, excepted at G/ENR ratio of 8/2.

4.3 Water vapor permeability (WVP)

WVP of bovine gelatin-based films incorporated without and with ENR of different types (ENR-10, ENR-25 and ENR-50) at various G/ENR ratios is shown in Figure 32. The gelatin film had higher WVP than did the ENR films ($p<0.05$), due mainly to the greater hydrophilic nature of the gelatin molecules which contain amino, hydroxyl and carboxyl groups (Skiest, 1990; McHugh *et al.*, 1994). In general, protein films are the excellent oxygen and aroma barriers (Bigi *et al.*, 2001; Bialopiotrowicz and Jańczuk, 2001; Yasuda *et al.*, 1994). However, due to their inherent hydrophilic nature, such films tend to absorb quantities of water at elevated relative humidity (RH) (Lim *et al.*, 1998). Among ENR films, there was no difference in WVP of those from ENR-10 and ENR-25 ($p>0.05$). However, films from ENR-50 which contained higher epoxy content had higher WVP than did those from ENE-10 and ENR-25, plausibly due to higher polarity contributed from the polar epoxy group.

Incorporation of ENR into the gelatin film resulted in the decrease in WVP of the G/ENR blend films compared to the control film ($p<0.05$). Epoxy group of ENR might interact inter-molecularly with amino group and hydroxyl group of gelatin, resulted in lowered reactive sites available to interact with water (Rouilly *et al.*, 2004). At the same level of ENR used, blend films added with ENR-10 and ENR-25 showed similar WVP ($p>0.05$). However, blend film incorporated with ENR-50 had higher WVP than did the films incorporated with ENR-10 and ENR-25 at the same level. From the result, WVP of the G/ENR blend films seemed to decrease when the amount of ENR added was increased up to 40% for ENR-10 and ENR-25 and up to 20% for ENR-50. At higher amount of ENR added, the blend films showed increased WVP. An excessive amount of epoxy group of ENR-50 might result in the presence of intermolecular interaction between ENR molecules. This plausibly caused an increase in phase separation between gelatin and ENR of the blend, which resulted in increased free volume in the film matrix. The increase in free volume of the polymer system enhances the diffusion of small molecules in the polymer matrix. This could provide the increase in WVP as a function of free-volume holes size (Wang *et al*., 2003).

When comparing WVP of the G/ENR blend films to that of other films (synthetic films and protein films). The synthetic films such as high-density polyethylene (HDPE) and low-density polyethylene (LDPE) exhibited much lower WVP (Krochta and Johnston, 1997). WVP of G/ENR blend films was lower than that of glutenin–rich films (Hernandez–Munoz *et al*., 2004), whey protein isolate and pullulan blend films (Gounga *et al.*, 2007), surimi films (Chinnabhark *et al.*, 2007) and cod gelatin and sunflower oil blend film (Perez-Mateos *et al.*, 2009).

4.4 Optical properties

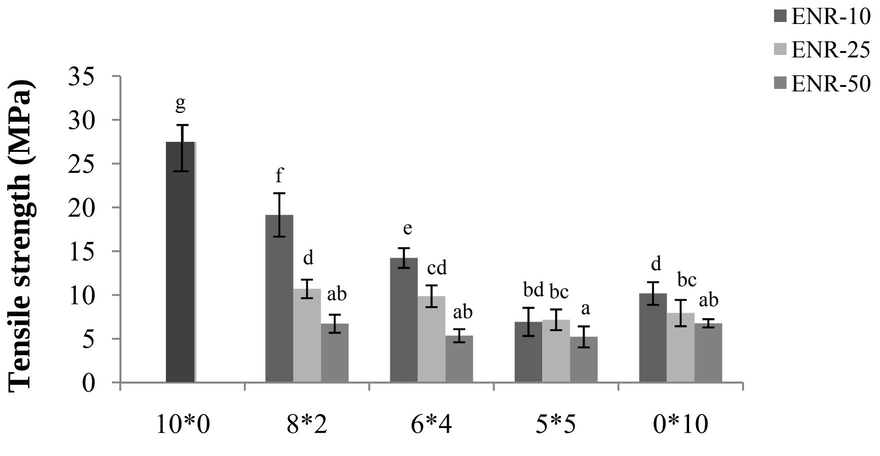
4.4.1 Light transmission and film transparency

Light transmittance (%T) in UV-Visible range and transparency value of ENR films and gelatin films without and with the different ENR types (ENR-10, ENR-25 and ENR-50) at various G/ENR ratios are shown in Table 10. ENR films showed the decreased %T at 200 nm as the epoxy content of ENR increased. In visible range (350 – 800 nm) ENR-10 films had higher %T than did ENR-25 and ENR-50 films while ENR-25 and ENR-50 films had similar %T. Gelatin film had much lower barrier properties against UV light, in comparison with ENR films. Addition of ENR decreased %T of the bovine gelatin–based film. In general, the light transmittance of the G/ENR blend films decreased with increased ENR concentration and epoxy content of ENR added. For visible light in the wavelength 600 – 800 nm, the %T value of G/ENR blend films were ranged from 84% - 98%. This result indicated that the resulting films were quite clear. Therefore, addition of ENR cloud improve barrier against UV light of the bovine gelatin-based film. This was in consistent with other studies carried out on starch/SBR blend films (Qi *et al*., 2006), graft-copolymerized starch/NR blend films (Lui *et al*., 2008) and thermoplastic starch/NR blend films (Cavalho *et al*., 2002; Röper and Koch, 1990; Shogren *et al*., 1993).

Transparency value of tested film samples is shown in Table 10. The lower transparency value indicates the more transparent of the film. From the results, gelatin film was more transparent than did the ENR films and G/ENR blend films. Addition of ENR in gelatin film resulted in the lower transparency of the G/ENR blend films as compared to the gelatin film ($p<0.05$). At the same level of ENR used, similar transparency value was noticeable among the blend films incorporated with ENR containing different epoxy contents, excepted at G/ENR ratio of 5/5.

4.4.2 Color of films

Table 11 shows L^*, a^* and b^*- values of ENR films and gelatin films without and with the incorporation of different ENR types at various G/ENR ratios. The gelatin film possessed lighter color than did the ENR films. As compared to the gelatin film, G/ENR blend films had increased greenness ($-a^*$) and yellowness ($+b^*$) but decreased lightness (L^*) when the amount of ENR added increased, regardless of ENR types. At the same level of ENR used, ENR containing higher epoxy content rendered the G/ENR blend films with increased yellowness. This plausibly resulted from pigments naturally present in NR raw material and also from the reaction dealing with formic acid and H_2O_2 which were added to the latex in preparing ENR (Okwu and Okieimen, 1998; Gelling, 1991). The result was in agreement with that of Ismail and Poh (2000) who reported that addition of ENR-25 and ENR-50 had an impact on the color of PVC.



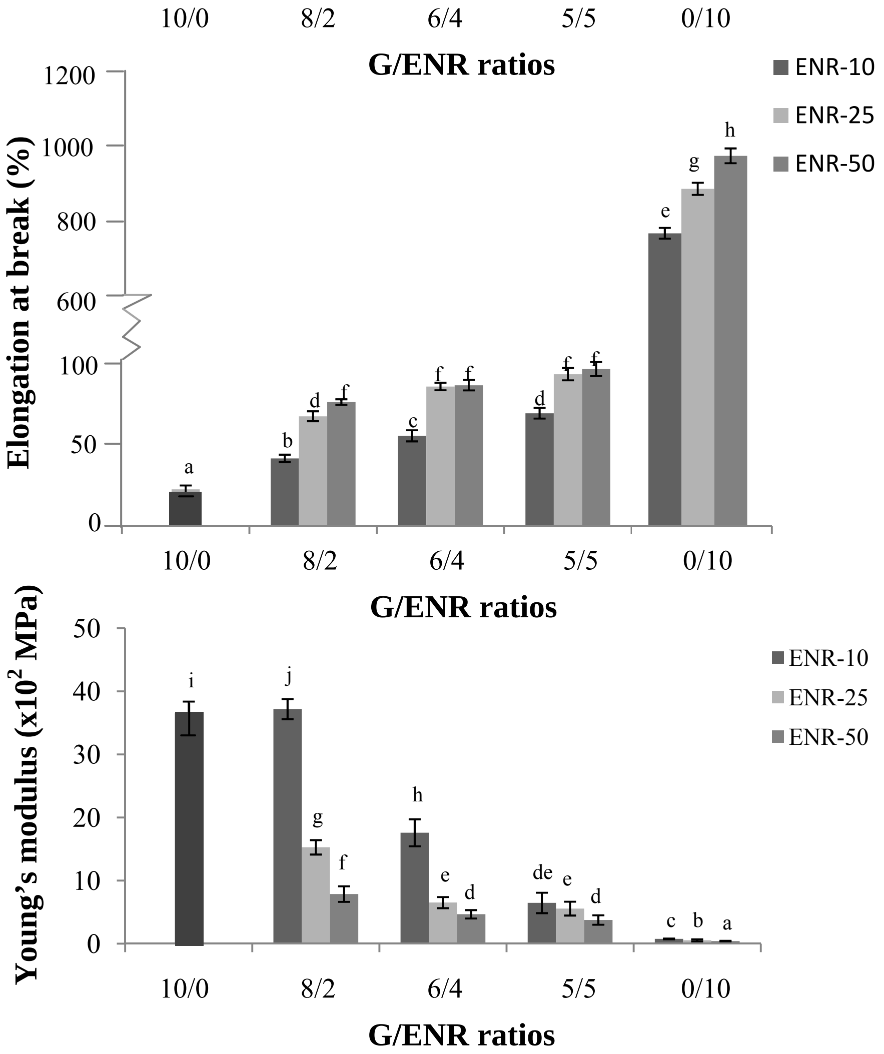
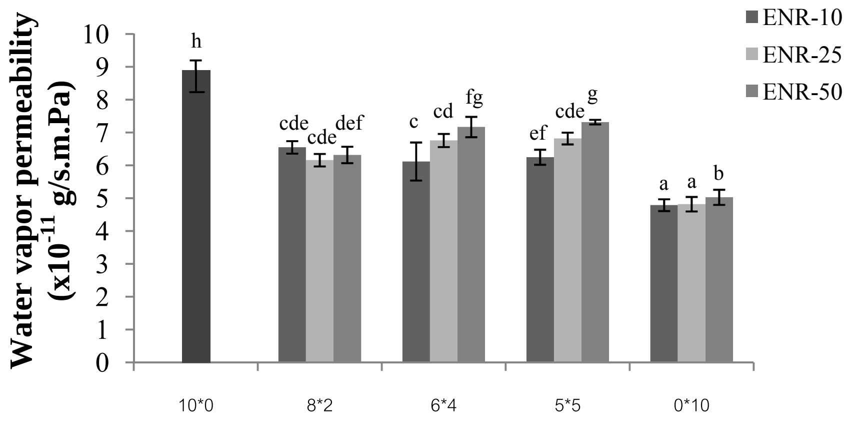


Figure 31. Mechanical properties of films from gelatin (G), different ENR and G/ENR blends at various ratios. Bars represent the standard deviation (n=3). Different letters indicate the significant differences ($p<0.05$).



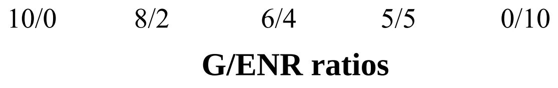


Figure 32. Water vapor permeability (WVP) of gelatin film and Gelatin/ ENR blend films. Bar represent the standard deviation from three determinations. Different letters indicate the significant differences (P<0.05)

Table 10. Light transmittance (%T) and transparency value of films from gelatin, different ENR and G/ENR blends at various ratios.

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

[*] Mean ± SD (n=3). The different superscripts in the same column indicated the significant differences (p≤0.05).

[¥] Transparency value = (-logT_{600})/ L, where T_{600} is fractional transmittance at 600 nm and L is film thickness (mm). The lower transparency value, the more transparent of the film.

Table 11. L*, a* and b*-values of films from gelatin, different ENR and G/ENR blends at various ratios.

Film types	**L***	**a***	**b***
Gelatin	90.21±0.37^{a}	-1.19±0.01^{a}	1.35±0.03$^{a,\#}$
G/ENR-10=8/2	88.42±0.93^{a}	-1.20±0.02^{a}	1.34±0.17^{a}
G/ENR-10=4/6	84.12±0.67^{b}	-1.28±0.01^{b}	1.45±0.03^{b}
G/ENR-10=5/5	83.66±0.80^{b}	-1.30±0.02^{b}	1.57±0.05^{c}
ENR-10	80.17±0.09^{c}	-1.35±0.03^{cd}	1.90±0.03^{f}
G/ENR-25=8/2	81.66±1.44^{c}	-1.28±0.02^{b}	1.58±0.05^{c}
G/ENR-25=6/4	83.46±1.03^{b}	-1.31±0.02^{c}	1.57±0.03^{c}

G/ENR-25=5/5	82.63±1.58^{bc}	-1.32±0.02^{c}	1.63±0.03^{cd}
ENR-25	81.00±1.22^{c}	-1.40±.0.2^{e}	1.75±0.04^{d}
G/ENR-50=8/2	82.19±0.78^{bc}	-1.32±0.02^{c}	1.81±0.02^{e}
G/ENR-50=6/4	82.39±0.73^{bc}	-1.37±0.03^{d}	1.82±0.02^{e}
G/ENR-50=5/5	80.43±0.45^{c}	-1.42±0.04^{e}	1.97±0.04^{f}
ENR-50	80.41±0.64^{c}	-1.48±0.03^{f}	2.08±0.08^{g}

$^{\#}$ Mean ± SD (n=3). The different superscripts in the same column indicated the significant differences ($p<0.05$).

4.5 Film solubility

Solubility in water of ENR films and gelatin films without and with incorporation of different types of ENR at various G/ENR ratios is shown in Table 12. Gelatin film was completely soluble in water, due to the highly hydrophilic nature of gelatin (Cuq *et al*., 1997; Shiku *et al*., 2003). In contrast, ENR films had very low water solubility (3.02 – 3.38%). Addition of ENR could decrease solubility of gelatin films. G/ENR blend films had the lower solubility than did gelatin film ($p<0.05$), regardless of ENR types. For the same type of ENR used, solubility of G/ENR blend films decreased with an increase in the level of ENR incorporated. Similar results were reported in starch/NR blend film (Liu *et al*., 2008). The decrease in solubility of G/ENR blend films might be because long chain ENR molecule could form the greater intermolecular interaction with gelatin molecules. As compared with other films, the solubility of G/ENR blend films was lower than that of pigskin gelatin/PVC blend films (Carvlhu *et al*., 2009). Low water solubility of the film is important to protect products from water.

Table 12. Solubility in water of films from gelatin, different ENR and G/ENR blends at various ratios.

Film types	**Film solubility** (%)
Gelatin	100 ± 0.00^{a*}
G/ENR-10=8/2	79.07 ± 1.06^{b}
G/ENR-10=6/4	58.05 ± 0.83^{d}
G/ENR-10=5/5	53.12 ± 1.73^{e}
ENR-10	3.38 ± 0.16^{f}
G/ENR-25=8/2	73.22 ± 1.20^{c}

G/ENR-25=6/4	56.69 ± 0.89^{d}
G/ENR-25=5/5	50.63 ± 0.42^{e}
ENR-25	3.05 ± 0.09^{g}
G/ENR-50=8/2	73.12 ± 1.70^{c}
G/ENR-50=6/4	55.65 ± 1.27^{d}
G/ENR-50=5/5	50.15 ± 0.92^{e}
ENR-50	3.02 ± 0.90^{g}

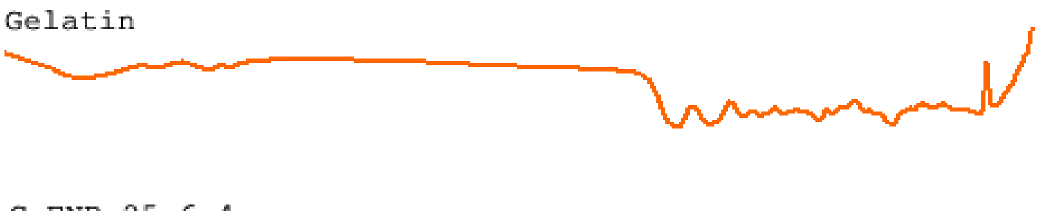
*Mean ± SD (n=3). The different superscripts in the same column indicated the significant differences ($p < 0.05$).

4.6 Fourier-transform infrared (FTIR) spectroscopy

Figure 33 shows FTIR spectra of selected film samples (gelatin, ENR-25 and G/ENR-25 (6/4) films). From the spectrum of the ENR film, it showed the absorption bands associated with C=C for cis-1,4 polyisoprene at 835 cm^{-1} and epoxy group at 875 cm^{-1}, which is characteristic for ENR (Cruz *et al.*, 2002). The peak centered at 3384 cm^{-1} in ENR spectra plausibly resulted from absorbed water in the film. In the spectra of gelatin-based films, there were strong absorption bands situated at around 3277, 1629 and 1539 cm^{-1}, corresponding to Amide-III and free water, Amide-I and Amide-II, respectively (Bergo and Sobral, 2007). The Amide-I arises from stretching of C=O of amide in protein; the amide-II arises from bending vibration of N-H group and stretching vibration of C-N group. Amide-III is related to the vibrations in plane of C-N and N-H groups of bound amide or vibrations of CH_2 group of glycine (Schmidt *et al.*, 2005). For G/ENR blend film, basides Amide-III, Amide-I and Amide-II peaks, there existed additional peaks (≈875, 835 cm^{-1}) characteristic of the incorporated ENR on its spectra. From the result, addition of ENR to gelatin caused some shifts in the Amide-I, Amide-II and Amide-III bands as well as the broader Amide-III peak of the spectra. In addition, the intensity of the Amide-II peak of gelatin and the peak at ≈870 cm^{-1} related to epoxy group of ENR decreased with the addition of ENR. This most likely indicated the presence of chemical interactions between gelatin and ENR molecules in the film matrix.

Moreover, the shift of Amide-III, Amide-II, Amide-I to lower wevenumber and broadening of the peaks (especialy around 3270 cm^{-1}) in G/ENR spectra suggested the presence of protein–protein and protein–ENR interactions possibly via hydrogen bond.

Gelatin



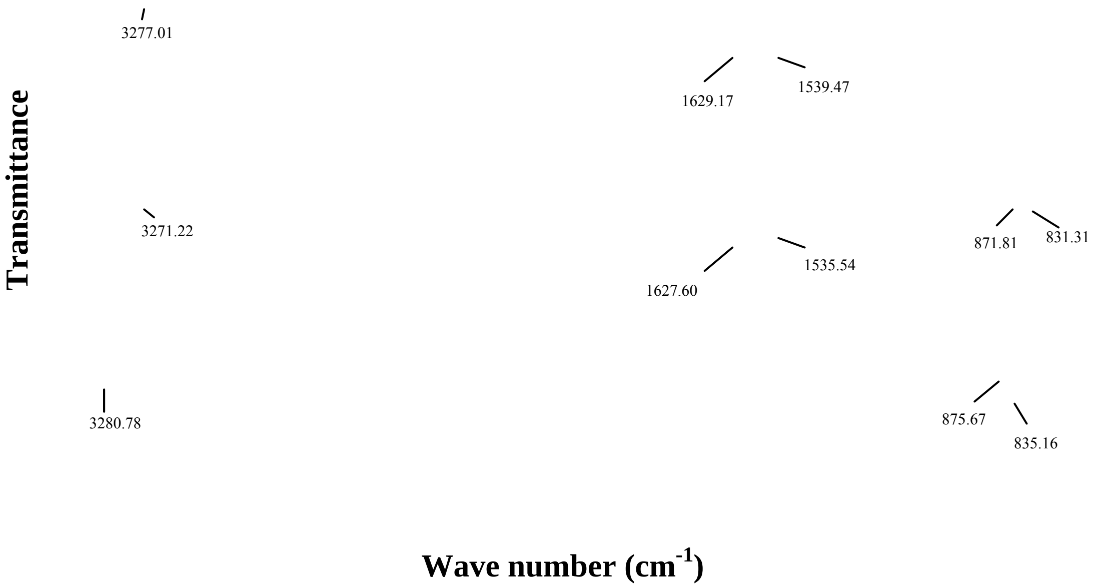


Figure 33. FTIR spectra of selected films from gelatin, ENR-25 and G/ENR (6/4) blend.

4.7 Film morphology

SEM images of surface and freeze-fractured cross-section of bovine gelatin, ENR-25 and G/ENR-25 (6/4) blend films are illustrated in Figure 34. Gelatin film had smoother surface and cross-section. In contrast, ENR-25 and G/ENR-25 blend films exhibited rougher surface and cross-section, which might result from differences in molecular alignment of gelatin and ENR molecules. The rougher fractured surface of ENR-25 film was more likely resulted from the presence of shear yielding deformation before fracture which is a characteristic of ductile material. However, from the image of cross-section of the G/ENR-25 blend film, it suggested that gelatin and ENR-25 were still compatible since the adhesion between phases seemed to be good with no distinct separation at the interface and no visible voids of removed rubber. The compatibility of the gelatin and ENR was most likely due to specific interfacial interactions between epoxy groups in ENR and hydroxyl and compatibility of gelatin and ENR was most likely responsible for the improved mechanical property (film flexibility) and water-vapor barrier property of the gelatin film.

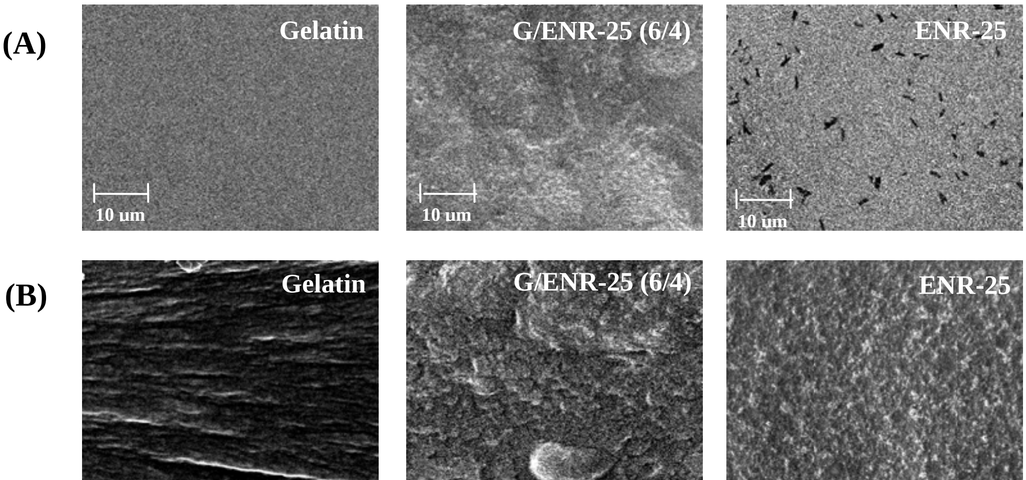


Figure 34. SEM micrographs of the surface (A) and cross-section (B) of the gelatin film, G/ ENR-25 (6/4) blend film and ENR-25 film.

From the results, incorporation of ENR-25 at 6/4 (w/w of G/ENR) rendered the film with the most improved mechanical properties (flexibility) and water-vapor barrier property. Thus, ENR-25 was chosen for further study.

5. Effect of incorporated ENR in combination with decreased glycerol content on properties of bovine gelatin film

Properties of G/ENR-25 (6/4) blend films containing different glycerol contents (25, 6.26. 2.5 and 0% of protein) were compared with those of gelatin films (without and with 25% glycerol) and ENR-25 film. The results are as following:

5.1 Thickness and mechanical properties

The thickness of gelatin-based films was in the rage of 0.029 – 0.032 mm (Table 13). All gelatin-based films had similar thickness ($p>0.05$). TS, EAB and E of the tested films are shown in Table 13. The gelatin films without glycerol exhibited the highest TS and E but the lowest EAB, compared to the other films. Without plasticizer added, gelatin film was quite brittle as evidenced by very low extensibility (EAB), which limited its use as packaging film. The result was in agreement with that of Moure *et al*. (2006) who reported that TS and E of keratin films without glycerol was higher than those of the films added with glycerol, due to highly aggregation of protein in the film matrix. As compared to gelatin film containing 25% glycerol, all G/ENR-25 (6/4) blend films had higher EAB, suggesting the greater flexibility or extensibility. In case of G/ENR-25 (6/4) blend films, the decreasing glycerol content (from 25% to 0% of protein) yielded the blend films with increased TS (from 10.38 to 54.79 MPa) and E (from 1112 to 3254 MPa) but decreased EAB (from 76.54 to 26.16%). The decrease in glycerol content might allowed gelatin molecules to undergo more interaction among themself, thereby decreased chain mobility (Audic and Chaufer, 2005). Therefore, even without glycerol addition, incorporation of ENR-25 at this level could improve the flexibility/extensibility (i.e., EAB) as well as toughness of the gelatin film.

Table 13. Thickness and mechanical properties of films from gelatin and G/ENR-25 (6/4) blends containing different glycerol contents.

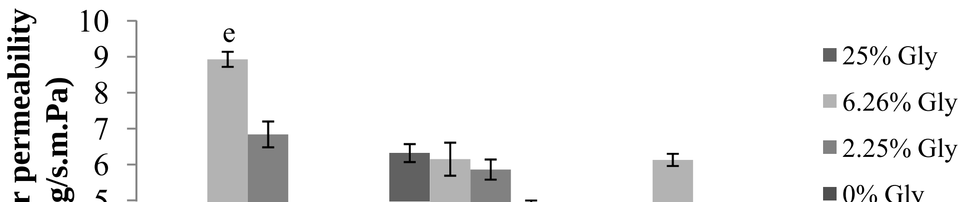
Films	Thickness (µm)	TS (MPa)	EAB (%)	E (x10^{2}MPa)
Gelatin+25% Gly$^{¥}$	30.56±0.1^{b}	26.66±1.27^{c}	17.69±1.08^{b}	42.42±1.21^{f*}
Gelatin+0% Gly	32.02±016^{b}	72.08±1.32^{h}	4.47±0.24^{a}	47.45±0.09^{g}
G/ENR+25% Gly	29.43±0.13^{b}	10.83±0.86^{b}	76.54±2.10^{f}	11.12±0.54^{b}
G/ENR+6.25% Gly	29.46±0.09^{b}	33.84±1.21^{d}	57.18±0.99^{e}	17.34±1.32^{c}
G/ENR+2.5% Gly	29.50±0.03^{b}	49.60±0.72^{e}	54.55±1.87^{d}	25.31±1.12^{d}
G/ENR+0% Gly	30.12±0.03^{b}	54.79±1.19^{f}	22.16±0.85^{c}	32.54±.32^{e}
ENR-25	18.26±0.03^{a}	7.46±0.17^{a}	730.12±3.17^{g}	1.36±0.07^{a}

*Mean ± SD (n=3).The different superscripts in the same column indicated the significant differences ($p< 0.05$).

$^{¥}$%glycerol content added based on protein.

5.2 Water vapor permeability (WVP)

WVP of the tested film samples is shown in Figure 35. Among all film tested, the gelatin film with 25% glycerol had higher WVP (8.93 x10^{-11} g/s.m.Pa) than did that without glycerol (6.84 x10^{-11} g/s.m.Pa) and G/ENR-25 (6/4) blend films (4.64-6.32x10^{-11} g/s.m.Pa). For G/ENR-25 (6/4) blend films, as expected, the WVP of the films seemed to decrease with the decrease in the amount of glycerol added, especially for G/ENR-25 blend film without glycerol which possessed the lowest WVP. The reduction of glycerol content might enhanced interactions of protein–protein and protein–rubber, resulting in decreased hydrophilic functional groups as well as molecular mobility, thus reducing absorption and diffusion of water molecules in the film matrix (Oliare *et al*., 2003; Rudriguéz *et al*., 2006; Subral *et al*., 2001; Thomazine *et al*., 2005). From the result, incorporation of ENR-25 to gelatin at G/ENR-25 ratio of 6/4 and without glycerol added could improve the water-vapor barrier property of gelatin-based films by about 2 times.



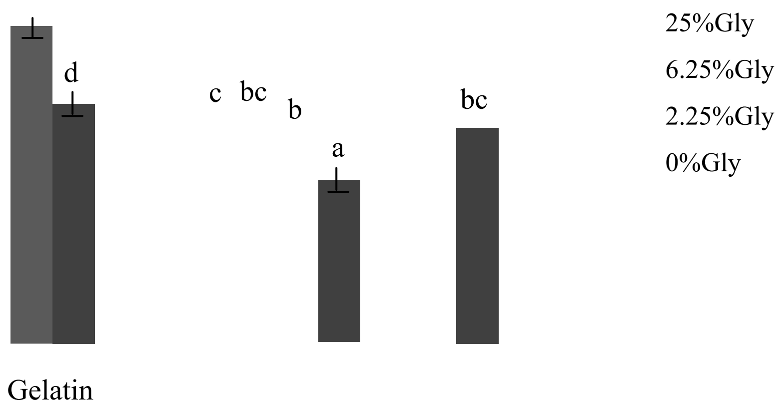


Figure 35. Water vapor permeability of films from gelatin and G/ENR-25 (6/4) blends containing different glycerol contents. Bars represent the standard deviation (n=3). Different letters indicate the significant differences ($p<0.05$)

5.3 Optical properties

5.3.1 Light transmission and film transparency

Transmission of UV and visible light at selected wavelength in the range of 200 – 800 nm of all tested films is shown in Table 14. ENR-25 film showed the lowest light transmission. For all gelatin-based films, transmission in visible length (350 – 800 nm) of films varied from 64.10 to 97.52%. The transmission of UV light at 280 nm was in the range of 52.21 – 55.72%. Very low transmission (9.53 – 10.91%) was found at 200 nm. This was in agreement with that of other protein-based films from fish and bovine gelatins (Intarasirisawat, 2006), surimi (Shiku *et al*., 2004), whey protein (Fang *et al*., 2002), fish myofibrillar protein (Shiku *et al*., 2006), etc. Gelatin film without glycerol had lower %T than that with 25% glycol added, both in UV and visible ranges. Gelatin-based films had decreased %T when ENR-25 at 40% was incorporated. For G/ENR-25 (6/4) blend films, the %T was slightly different among the blend films with different glycerol contents.

For transparency value, G/ENR-25 (6/4) blend films had higher transparency value than did gelatin films, regardless of the amount of glycerol added. The higher transparency value represented the lesser transparent film. However, G/ENR-25 blend films containing different glycerol contents showed similar transparency value ($p>0.05$). The G/ENR-25 blend films had transparency value (3.18-3.22) comparable to some synthetic films such as polyethylene (LDPE: 3.05) (Shiku *et al*., 2003).

5.3.2 Color of films

L^*, a^* and b^*-values of the film samples are shown in Table 15. Gelatin films without and with glycerol had higher L^* and a^*-values but lower b^*-value than did ENR-25 and G/ENR-25 blend films. The more color of G/ENR-25 blend films most likely resulted from the ENR-25 incorporated as previously described in section 4.1. G/ENR-25 blend films with different glycerol contents had similar L^*, a^* and b^*-values ($p>0.05$).

From the results, G/ENR-25 (6/4) blend without glycerol added rendered the film with the most improved water-vapor barrier property, compared to the gelatin film. However, this film showed much decrease in flexibility as compared to that with glycerol added. Thus, to further improve its flexibility as well as water-vapor barrier property, natural rubber (NR) was incorporated into the blend for partial substitution of ENR-25, and the properties of the resulting films were investigated in next study.

Table 14. Light transmission (%) and transparency value of films from gelatin and G/ENR-25 (6/4) blends containing different glycerol contents.

Film types	**Wave length (nm)**								**Transparency value$^{¥}$**
	200	**280**	**350**	**400**	**500**	**600**	**700**	**800**	
Gelatin+25% Gly	10.91	55.72	64.32	78.55	85.14	95.75	96.36	97.52	$2.74 \pm 0.08^{a*}$
Gelatin+0% Gly	9.53	52.21	67.35	72.31	74.64	80.65	92.42	94.51	2.91 ± 0.05^{a}
G/ENR+25% Gly	15.32	39.45	58.56	76.16	78.04	80.54	84.14	91.17	3.22 ± 0.02^{b}
G/ENR+6.25% Gly	14.43	39.99	56.45	72.40	78.98	80.59	85.57	90.56	3.20 ± 0.06^{b}
G/ENR+2.5% Gly	14.01	32.67	46.12	56.63	74.78	80.76	82.24	90.72	3.20 ± 0.05^{b}
G/ENR+0% Gly	13.32	32.56	43.78	51.60	69.49	79.60	84.91	91.64	3.18 ± 0.04^{b}
ENR-25	8.32	30.43	41.78	56.83	61.54	68.78	73.43	75.56	3.43 ± 0.06^{c}

*Mean ± SD (n=3). The difference superscripts in the same column indicate the significant differences ($p<0.05$).

$^{¥}$ The lower transparency value, the more transparent of the film.

Table 15. L^*, a_* and b^*-values of films from gelatin and G/ENR-25 (6/4) blends containing different glycerol contents.

Films	**L^***	**a^***	**b^***
Gelatin+25% Gly	90.30 ± 1.59^{c}	-1.16 ± 0.02^{c}	$1.38 \pm 0.09^{a\#}$
Gelatin+0% Gly	90.69 ± 0.38^{c}	-1.17 ± 0.02^{bc}	1.43 ± 0.06^{a}
G/ENR+25% Gly	85.42 ± 1.16^{b}	-1.29 ± 0.02^{a}	1.58 ± 0.03^{b}
G/ENR+6.25% Gly	84.64 ± 0.58^{b}	-1.29 ± 0.02^{a}	1.55 ± 0.03^{b}
G/ENR+2.5% Gly	85.18 ± 1.00^{b}	-1.29 ± 0.02^{a}	1.57 ± 0.02^{b}
G/ENR+0% Gly	85.87 ± 1.65^{b}	-1.28 ± 0.02^{a}	1.61 ± 0.03^{b}
ENR-25	81.58 ± 0.17^{a}	-1.30 ± 0.17^{a}	2.24 ± 0.17^{c}

$^{\#}$ Mean ± SD (n=3). The difference superscripts in the same column indicate the significant differences ($p<0.05$).

6. Effect of NR compatibilized with ENR on properties of bovine gelatin film

6.1 Thickness and mechanical properties

Thickness of films from gelatin, ENR-25, NR, G/ENR (6/4) blend and G/ENR/NR blends without glycerol was in the range of 0.018 – 0.032 mm (Table 16). The NR and ENR-25 films had lower thickness (0.018 mm) than did the gelatin and the blend films (0.029 – 0.032 mm). The difference was more likely due to the differences in their molecular structures and molecular alignments in the film matrix (Ibrahim and Dahlan, 1998).

TS, EAB and E of the tested films are shown in Table 16. Among them, gelatin film added with 25% glycerol had the highest E but the lowest EAB. In the case of rubber films, ENR-25 film showed higher TS and E but lower EAB than did the NR film. Among blend films without glycerol added, those incorporated with NR had higher EAB ($p<0.05$) but similar TS and E ($p>0.05$). Film prepared from the blend of G/ENR/NR at 6/2/2 exhibited higher EAB than did that at 6/3/1 ($p<0.05$). From the results, films of G/ENR/NR blend at 6/2/2 had higher EAB (or flexibility) than did the G/ENR (6/4) blend films without glycerol and the gelatin film with 25% glycerol by about 4 times and 5 times, respectively. The great increase in flexibility of gelatin film by incorporation of NR simply because of the additive effect of NR which possesses highly elasticity or flexibility in nature. This improvement would more likely due to the dispersed phases of NR which were compatibilized by ENR in the blend film matrix. Generally, gelatin and ENR-25 are both polar (however, ENR-25 has non-polar part in polymer chain too) whereas NR is non-polar. With the addition of ENR in gelatin/NR blend, partial compatibility might occur due to the interaction of matrix plausibly through hydrogen bonding which improve the interaction between protein and rubber, thus resulting in an increase in mechanical properties of the blend films (Iamail and Hairunezam, 2001; Poh *et al.*, 2002). The results were in agreement with those of Dahlan *et al.* (2002) who reported the improvement of properties of NR/LLDPE blend when LNR was added as a compatibiliser and those of Dahlan (1998) who presented the roles of LNR in the compatibilisation of NR/PE blend.

Table 16. Thickness and mechanical properties of films from gelatin, ENR-25, NR, G/ENR-25 blends and G/ENR-25/NR blends.

Films	Thickness (µm)	TS (MPa)	EAB (%)	E ($\times10^{2}$MPa)
Gelatin (G)+25% Gly	30.56±0.1	26.66 ± 1.27^{d}	17.69 ± 1.08^{a}	$42.42\pm1.21^{e*}$
G/ENR (6/4)+25% Gly	29.02±016	10.83 ± 0.86^{c}	76.54 ± 2.10^{c}	11.12 ± 0.54^{c}

G/ENR (6/4)+0 % Gly	29.32±0.13	54.79±1.19^{f}	22.16±0.85^{b}	32.54±0.^{32d}
G/ENR/NR (6:3:1)	28.93±0.13	51.90±1.71^{ef}	75.29±1.81^{c}	33.45±0.09^{de}
G/ENR/NR (6:2:2)	28.85±0.09	54.60±1.08^{f}	88.38±1.58^{d}	31.13±0.54^{d}
ENR-25	18.26±0.03	7.46±0.17^{b}	730.12±3.17^{e}	1.36±0.07^{b}
NR	18.24±0.03	4.62±0.12^{a}	967.69±3.23^{f}	1.01±1.12^{a}

*Mean ± SD (n=3). The different superscripts in the same column indicate the significant differences ($p < 0.05$).

6.2 water vapor permeability (WVP)

The WVP of tested film samples is shown in Table 17. The gelatin film had the highest WVP ($p<0.05$). Among blend films without glycerol added, incorporation of NR resulted in the decreased in WVP of the films obtained ($p<0.05$). Blend film of G/ENR/NR at 6/2/2 (without glycerol) had the lowest WVP (2.02 x10^{-11} g/s.m.Pa) which was approximately 4.3 times lower than that of the gelatin film added with 25% glycerol (control film). Addition of hydrophobic NR most likely resulted in the decrease in overall contents of hydrophilic moieties present in the blend film, resulting in the lower hydrophilic sites for water to be absorbed in the film matrix. Moreover, the added ENR which acted as a compatibilizer could enhance the interaction between protein and rubber (Abdullah and Ahmad, 1992), producing the decrease in molecular mobility, and thus reducing the diffusion of water molecules through the film matrix (Oliare *et al*., 2003; Rodriguéz *et al*., 2006; Subral *et al*., 2001; Thomazine *et al*., 2005).

Table 17. Water vapor permeability (WVP) of films from gelatin, ENR-25, NR, G/ENR-25 blends and G/ENR-25/NR blends.

Films	**WVP (x10^{-11} g/s.m.Pa)**
Gelatin (G)+25% Gly	8.93±0.21^{f*}
G/ENR (6/4)+25% Gly	6.32±0.25^{e}
G/ENR (6/4)+0 % Gly	4.64±0.36^{c}
G/ENR/NR (6:3:1) +0 % Gly	3.22±0.41^{b}
G/ENR/NR (6:2:2) +0 % Gly	2.09±0.16^{a}
ENR-25	6.13±0.17^{e}
NR	5.10±0.17^{d}

*Mean ± SD (n=3). The different superscripts in the same column indicate the significant differences ($p< 0.05$).

6.3 Optical properties

6.3.1 Light transmission and film transparency

Light transmittance (%T) in UV (200-280 nm) and visible (350-800 nm) ranges and transparency value of the tested films are shown in Table 18. Gelatin-based films showed higher %T in UV range than did ENR-25 and NR films. For blend films with and without NR, the %T in visible range (350-800 nm) of films ranged from 55.79 – 90.56%. The %T of the films slightly decreased with increasing NR added. For transparency value, gelatin film exhibited the lowest transparency value (2.74) while ENR-25 (3.57) and NR (3.59) films had higher transparency values than the other films. The higher transparency value suggested the lesser transparent of the film. For G/ENR-25 blend films (with 0% glycerol) with and without NR, the transparency value of the films increased when NR was added to the blends. However, no difference of the transparency value of the films was observed when different amounts of NR was incorporated.

6.3.2 Color of films

L^*, a^* and b^*-values of the tested film samples are shown in Table 20. Gelatin film had higher L^* and a^*-values but lower b^*-value than the other tested films. Films from rubbers (ENR-25 and NR) had higher b^*-value than the gelatin film and the blend films. Natural pigments present in NR and ENR more likely contributed to the increased yellowness (b^*) of the film observed (Okwu and Okieimen, 1998; Gelling, 1991). Among blend films incorporated with NR, the increasing NR content in the blend resulted in slightly increased b^*-value of the films, while it showed no impact on L^* and a^*-values. Therefore, incorporation of NR in combination with ENR as compatibilizer had an impact on light transmission and transparency of the gelatin-based films.

Table 18. Light transmittance (%) and transparency value of films from gelatin, ENR-25, NR, G/ENR-25 blends and G/ENR-25/NR blends.

Film types	Wave length (nm)								Transparency value
	200	280	350	400	500	600	700	800	
Gelatin +25% Gly	10.91	55.72	64.32	78.55	85.14	95.75	96.36	97.52	2.74 ± 0.08^{a*}
G/ENR+25% Gly	15.32	39.45	58.56	76.16	78.98	80.59	85.57	90.56	3.20 ± 0.06^{b}
G/ENR+0% Gly	13.32	32.56	43.78	51.60	69.49	79.60	84.91	91.64	3.18± 0.04^{b}
G/ENR/NR (6/3/1) +0% Gly	14.56	39.56	57.56	68.79	69.79	76.76	79.59	84.79	3.45± 0.05^{e}
G/ENR/NR (6/2/2) +0% Gly	14.68	40.57	55.79	65.38	66.90	74.68	77.47	83.96	3.48± 0.03^{c}
ENR-25	8.32	30.43	41.78	56.83	61.54	68.78	73.78	75.56	3.57± 0.03^{d}
NR	7.12	28.56	37.97	54.67	59.34	64.23	69.78	72.46	3.59± 0.05^{d}

[*]Mean ± SD (n=3). The difference superscripts in the same column indicate the significant differences ($p<0.05$).

Table 19. L*, a* and b* -values of films from gelatin, ENR-25, NR, G/ENR-25 blends and G/ENR-25/NR blends.

Film types	**L***	**a***	**b***
Gelatin (G)+25% Gly	90.30 ± 1.59^{c}	-1.16 ± 0.02^{c}	$1.58\pm0.09^{a,\#}$
G/ENR (6/4)+25% Gly	84.42 ± 1.16^{b}	-1.29 ± 0.02^{a}	1.58 ± 0.03^{a}
G/ENR (6/4)+0 % Gly	85.87 ± 1.65^{b}	-1.28 ± 0.02^{b}	1.61 ± 0.03^{a}
G/ENR/NR (6:3:1) +0 % Gly	84.46 ± 0.37^{b}	-1.27 ± 0.01^{b}	1.57 ± 0.03^{a}
G/ENR/NR (6:2:2) +0 % Gly	83.47 ± 0.43^{ab}	-1.29 ± 0.03^{ab}	1.65 ± 0.03^{b}
ENR-25	81.58 ± 0.17^{a}	-1.30 ± 0.17^{a}	2.24 ± 0.17^{d}
NR	82.17 ± 0.09^{a}	-1.27 ± 0.03^{b}	2.10 ± 0.03^{c}

[#] Mean ± SD (n=3). The difference superscripts in the same column indicate the significant differences ($p<0.05$)

6.4 Film solubility

Figure 36 shows solubility in water of the selected film samples (gelatin film, ENR-25 film, NR film, G/ENR-25 (6/4) blend film and G/ENR-25/NR (6/2/2) blend film without glycerol). Gelatin film was completely dissolved in water, due to the high hydrophilicity of gelatin molecules. Moreover, the matrix of gelatin film was stabilized only by weak bond (hydrogen bond), which is easily disrupted by water (Cuq *et al.*, 1997; Orliaac *et al.*, 2003; Shiku *et al.*, 2003). In contrast, rubber films, NR and ENR-25, had very low water solubility, due to the hydrophobic nature of rubber molecules. ENR-25 film had higher solubility than did the NR film, most likely due to the presence of epoxy group with make the ENR more polar than the NR. The blend films had much lower solubility than the gelatin film simply because of the lower amount of hydrophilic components (gelatin and glycerol) in the film. Besides, it might be due to the presence of interaction between gelatin and ENR molecules. Among the blend films, film of G/ENR/NR (6/2/2) blend exhibited lower solubility than did that of G/ENR (6/4) blend. This was due to the lower polarity or hydropilicity of the added NR on compared to the ENR-25. Moreover, gelatin possibly interacted with NR by the aid of ENR as compatibilizer, resulted in increased hydrophobicity of the blend film. As a consequence, the film matrix was less accessible by water and thus the leaching out of soluble component (gelatin) was impeded. As compared to the gelatin film, blend film of G/ENR (6/4) and that of G/ENR/NR (6/2/2) had decreased solubility by about 2.3 times and 2.8 times, respectively.

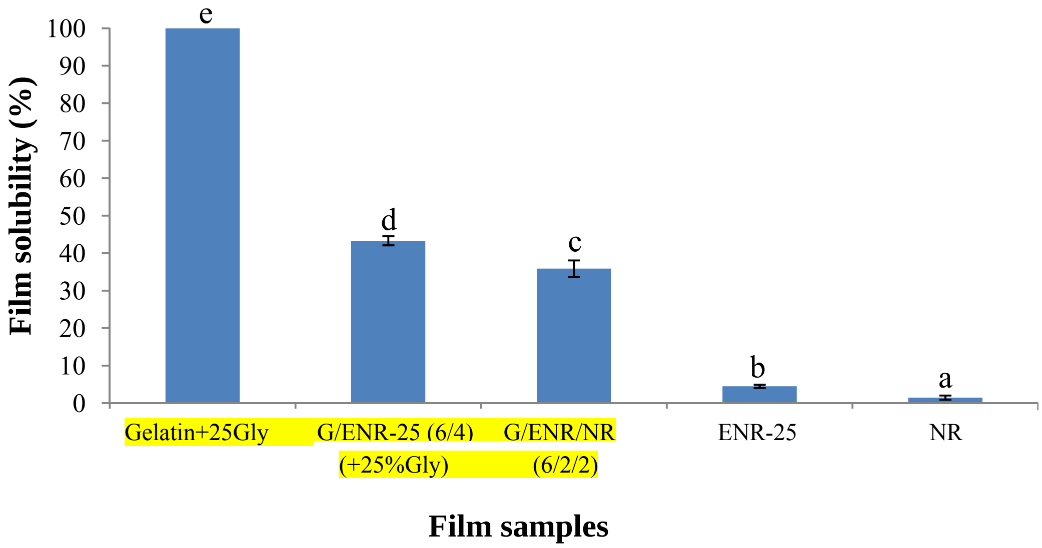


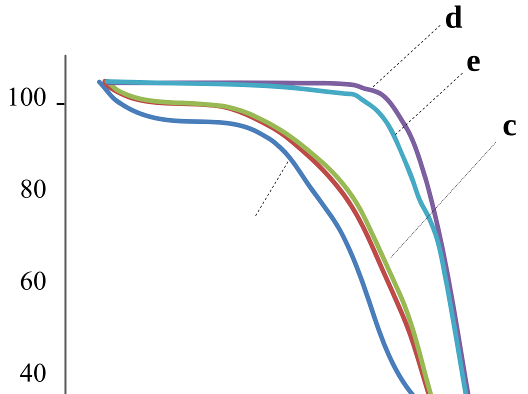
Figure 36. Solubility of films selected from gelatin (+25%Gly), ENR-25, NR, G/ENR-25 (6/4) blend with 25% glycerol and G/ENR-25/NR (6/2/2) blend without glycerol. Bars represent the standard deviation (n=3). Different letters indicate the significant differences ($p<0.05$).

6.5 Thermal property (TGA analysis)

Thermal degradation behavior of polymer can be measured by using thermogravimetric analyzer (TGA). Figure 37 shows TGA thermograms of the selected film samples and their corresponding degradation temperature (T_d) and weight loss (Δw) are shown in Table 20. ENR-25 and NR films showed similar degradation behavior as observed on TGA thermogram. Both rubber films (ENR-25 and NR) exhibited mainly one stage of weight loss observed at T_d = 318.5°C (Δw=92.50%) for ENR-25 film and T_d = 291.2°C (Δw=94.88%) for NR film. For gelatin-based films, they exhibited three stages of weight loss. The initial weight loss (Δw_1=4.52-8.39%) at onset temperature (Td_1) about 46.05-

52.23°C of all gelatin-based films was related to the loss of free water adsorbed in the films. This was in agreement with the reports of Langmaier *et al.* (2008) and Nuthong *et al.* (2009). The second weight loss (Δw_2=22.96-26.62%) observed at temperatures (T_{d2}) ranged from 214.21-249.79°C was most likely associated with the degradation of protein fraction of small molecular size as well as a plasticizer incorporated in the film matrix (gelatin film) and possibly some fractions of rubber added in the blend films. The third weight loss (Δw_3=48.92-61.49%) of the gelatin-based films appeared at temperatures (T_{d3}) ranged from 283.84°C to 313.80°C, which mainly associated with the degradation of major protein component in the blend films. The degradation of gelatin film was inconsistent with that of Barreto *et al.* (2003) who reported the initial temperature of degradation around 295-300°C of the pure protein films. The degradation involved the formation of CO_2, CO, NH_3 and other unsaturated compounds, suggesting that the reaction mechanism included at the same time the scission of the C-N, C(O)-NH, C(O)-NH_2, -NH_2 and C(O)-OH bonds of the proteins and the mechanism of reaction occurred by random scission of the protein chains (Schmidt *et al.*, 2005). From the result, it was noted that degradation of ENR-25 and NR films occurred at higher temperature than that of the gelatin film and the blend films.

When ENR-25 or Nr+ENR-25 was incorporated, the resulted blend films (G/ENR-25 and G/ENR-25/NR) exhibited the increased degradation temperatures (T_{d2} and T_{d3}) as compared to the gelatin film. This result suggested that the thermal stability of the blend films was increased, compared to that of the gelatin film. This improvement of thermal stability of the blend films most likely resulted from the characteristic of ENR and NR incorporated and also the presence of interaction between gelatin and ENR in the film matrix. Therefore, thermal property of gelatin, ENR-25 and blend films was varied depending on the differences in film compositions and molecular interactions which stabilized the film matrix. G/ENR-25 blend films with and without NR had improved thermal stability as compared to gelatin film.



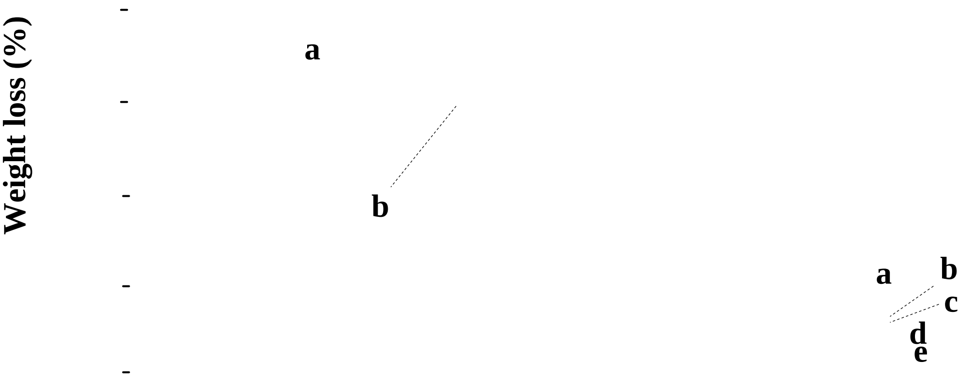


Figure 37. TGA Thermograms of films from gelatin (+25% glycerol) (a), G/ENR-25 (6/4) blend (b), G/ENR-25/NR (6/2/2) blend without glycerol (c), ENR-25 (d) and NR (e).

Table 20. Thermal degradation temperature (T_d, °C) and weight loss (Δw, %) of films from gelatin (+25% glycerol), G/ENR-25 (6/4) blend, G/ENR-25/NR (6/2/2) blend without glycerol, ENR-25 and NR.

Films	Δ_1*		Δ_2		Δ_3		Residual mass (%)
	T_{d1}	Δw_1	T_{d2}	Δw_2	T_{d3}	Δw_3	
Gelatin (25% Gly)	46.05	8.39	214.21	26.62	283.84	48.92	16.08
geatin/ENR-25 (6/4)	48.84	4.74	247.69	22.96	308.13	60.82	11.48
geatin/NR/ENR-25(6/2/2) (0% Gly)	52.23	4.52	249.79	25.36	313.80	61.49	9.63
ENR-25	318.51	92.50	-	-	-	-	7.50
NR	291.2	94.88	-	-	-	-	5.20

* Δ_1, Δ_2 and Δ_3 refer to the first, second and third stage weight loss, respectively, as observed in TGA thermogram.

6.6 Film morphology

Figure 38 illustrates the SEM micrographs of the surface and freeze-fractured cross-section of the selected films (gelatin film, ENR-25 film, NR film, G/ENR-25 (6/4) blend film and G/ENR-25/NR (6/2/2) blend film without glycerol). All pure films (gelatin, ENR-25 and NR films) had smooth and dense surface and cross-section. In contrast, G/ENR-25 (6/4) blend film exhibited slightly rougher surface and cross-section, which might resulted from difference in molecular alignment of gelatin and ENR molecules as well as the presence of partial phase separation. However, from the image of cross-sectional structure of the G/ENR-25 (6/4) blend film, it suggested that gelatin and ENR-25 was well mixed, due mainly to great interaction between their molecules. However, G/ENR-25/NR (6/2/2) blend film exhibited more rougher surface and irregular internal structure, due mostly to the presence of immiscible phase of NR incorporated in the blend. However, from the cross-sectional SEM image of this film, it suggested that the blend was compatible since the adhesion between phases seemed to be good with no distinct separation at the interface. The ENR-25, containing reactive epoxy group and a non-polar segment (unepoxidized isoprene unit), was presumably acted as a compatibilizer for gelatin and NR phases. The similar results were reported for tertiary blend of starch/ENR/NR (Nakason et *al.,* 2001). Norman *et al.,* (2009) also studied the effect of ENR-50 as a compatibilizer on morphology of SBR/NBR blend. As revealed from their SEM images, SBR/NBR blend added with ENR-50 had better adhesion among the blend components than did the SBR/NBR blend without ENR-50.

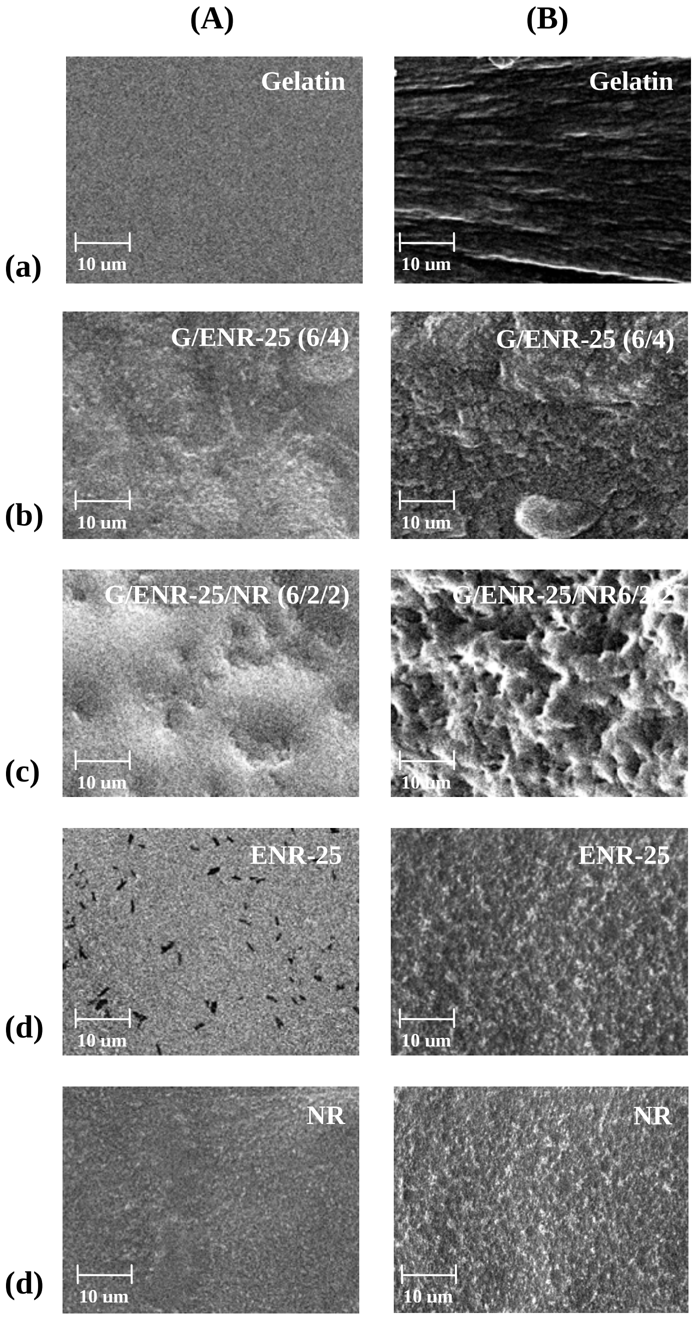


Figure 38. SEM micrographs (x2500) of the surface (A) and freeze-fractured cross section (B) of films from gelatin (+25% glycerol) (a), G/ENR-25 (6/4) blend (b), G/ENR-25/NR (6/2/2) blend without glycerol (c), ENR-25 (d) and NR (e).

CHAPTER 4

CONCLUSIONS

1. Glycerol, as a plasticizer, at an appropriate amount was required to be incorporated into the gelatin in order to produce sufficiently flexible gelatin film. Increasing glycerol content resulted in decreased TS and stiffness but increased EAB and WVP of the gelatin film.

2. Properties of bovine gelatin film could be modified by ENR incorporation. The addition of ENR with appropriate type (i.e., epoxy content) and amount could improve the flexibility and slightly decrease WVP of the gelatin film, mainly due to the intrinsic properties of added ENR and also to the compatibility of gelatin and ENR which resulted from the specific chemical interactions.

3. Addition of ENR together with the reduced amount of glycerol added could greatly improve the water-vapor barrier property of the gelatin film. Nevertheless, the obtained blend film exhibited much decreased flexibility.

4. Incorporation of NR for partial ENR substitution at appropriate level and exclusion of glycerol could markedly improve the strength, flexibility and water-vapor barrier property of the blend film based on gelatin, compared to the gelatin film. It also improved the water resistance and thermal stability of the gelatin film. The aforementioned property improvement more likely resulted from a uniform dispersion of NR in the film matrix by the aid of incorporated ENR acting as a compatibilizer.

5. Rubber blend of NR and ENR, incorporated in gelatin at an appropriate amount, could behave not only as a toughness modifier but also a water-vapor barrier promoter for gelatin film.

SUGGESTIONS

1. Additional molecular characterization of the blend films should be carried out.

2. The mechanism for property enhancement by NR and ENR in gelatin film should be elucidated.

3. The stability upon storage and applications of the blend films should be further studied.

4. The effect of molecular weight of ENR (i.e., low-molecular-weight ENR or liquid ENR) as compatibilizer in the blend films should be further investigated.

REFERENCES

Abdullah, I. and Ahmad, S., 1992. LNR as a compatibiliser in the blending of NR with PP. J. Mater. Forum**. 16**: 353–357.

Achet, D. and He, X.W. 1995. Determination of the renaturation level in films. Polymer. 36: 787–791.

Alexy, P., Bakos, D., Hanzelova, S., Kukolikova, L., Kupec, J., Charvatova, K., Chiellini, E. and Cinelli, P. 2003. Poly(vinyl alcohol)-collagen hydrolysate thermoplastic blends: I. Experimental design optimisation and biodegradation behaviour. J. Polym. Testing. 22: 801-809.

Albertson, A.C. and Karlsson, S. 1994. Chemistry and Biochemistry of Polymer Biodegradation. *In* Chemistry and Technology of Biodegradable Polymers (Griffin, G.J.L. ed.). p. 7–17. Blackie Press. London.

Akinlabi, A.K., Okieimen, F.E. and Aigbodion, A.I. 2005. Thermal aging properties and chemical resistance of blends of natural rubber and epoxidized natural rubber. Polym. Adv. Technol. 16: 76:88

AOAC. 1999. Official Method of Analysis. 14th ed. Association of Official Analytical Chemists, Washington, D.C. USA.

Arroyo, M., Lopez-Manchado, M.A. and Herrero, B. 2003. Organo-montmorillonite as substitute of carbon black in natural rubber compounds. Polymer. **44**: 2447-2454.

Arvanitoyannis, S. 2002. Formation and Properties of Collagen and Gelatin Films and Coatings. *In* Protein-Based Films and Coatings (Gennadios, A. ed.) p. 275–304. CRC Press. Boca Raton.

Avena-Bustillos, R.J., Avena-Bustillos, C.W., Olson, D.A., Olson, B., Chilou, E., and Yee, P.J. 2006. Water vapor permeability of mammalian and fish gelatin films. J. Food Sci. Technol. 71: 202–207.

Avena-Bustillos, R.J. and Krochta, J.M. 1993. Water vapor permeability of caseinate-based edible films as affected by pH, calcium crosslinking and lipid content. J. Food Sci. 58: 904-907.

Asgher, A and Henrickson, R.L. 1982. Chemical, Biochemical, Functional and Nutritional Characteristics of Collagen in Food System. *In* Advance in Food Research, Vol. 28. (Chichester, C.O., Marta, E.M. and Schweigert, B.S. eds.). p. 237-273. Academic Press. London.

ASTM. 1989. Annual Book of ASTM Standards. American Society for Testing and Materials. Philadelphia.

Audic, J.L. and Chaufer, B. 2005. Influence of plasticizers and crosslinking on the properties of biodegradable films made from sodium caseinate. Eur. Polym. J. **41**: 1934–1942.

Baker, C.S.L. and Gelling, I.R. 1987. Epoxidized Natural Rubber. *In* Developments in Rubber Technologym, Vol. IV. (A. Whelan and K.S. Lee eds.). p. 87-117. Elsevier Applied Science Poblishers Ltd. London.

Bailey, A.J. and Light, N.D. 1989. Connective Tissue in Meat and Meat Products. Elsev. Appl. Sci. New York. Elsevier Applied Science Poblishers Ltd. London.

Baker, C.S.L, Gelling, I. R. and Newell, R. 1 985. Modification of natural rubber. Rubber Chem. Technol. 58: 61-72.

Bamdad, F., Goli, A.H. and Kadivar, M. 2006. Preparation and characterization of proteinous film from lentil (*Lens culinaris*): edible film from lenil (*Lens culinaris*). Food Res. Int. 39: 106-111.

Barreto, P.L.M., Pires, A.T.N. and Soldi, V. 2003. Thermal degradation of edible films based on milk proteins and gelatin inert atmosphere. Polym. Degrad. Stabil. 79: 147-152.

Baudouin, C., Yolande S.K., Philippe M. and Dominique T. 2001. Molecular evolution of protein atomic composition. Science. 293: 297-300.

Belitz, H.D. and Grosch, W. 1999. Meat. *In* Food Chemistry. (Beliz, H.D. and Grosch, W. eds.). p. 540-547. Springer Berlin / Heidelberg Publisher. New York

Bergman, I. and R. Loxley. 1963. Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. J. Anal. Chem. 35: 1961-1965.

Bergo, P. and Sobral, P.J.A. 2007. Effects of plasticizer on physical properties of pigskin gelatin films. Food Hydrocol. 21: 1285-1289.

Bertan, L.C., Tanada-Palmu, P.S., Siani, A.C., and Grosso, C.R.F. 2005. Effect of fatty acids and "Brazilian elemi" on composite films based on gelatin. Food Hydrocol. 19: 73–82.

Bertuzzi, M.A., Castro-Vidaurre, E.F., Armada, M. and Gottifredi, J.C. 2006. Water vapor permeability of edible starch based films. J. Food Eng. 74: 211-216.

Białopiotrowicz, T. and Jańczuk, B. 2001. The changes of the surface free energy of the adsorptive gelatin films. Eur. Polym. J. 37:1047-1051.

Bigi, A., Bracci, B., Panzamolta. S., Rubini, K. and Roveri, N. 2001. Mechanical and thermal properties of gelatin film at different degree of glutaraldehdy crosslinkings. Biomaterial. 22: 763-768.

Bogdanovic, J., Halsey, N.A., Wood. R.A. and Hamilton, R.G. 2008. Bovine and porcine gelatin sensitivity in children sensitized to milk and meat. Allergy Clin. Immunol. 124: 1108-1110.

Bradbury, J.H. and Perera, M.C.S. 1985. Epoxidation of low-molecular-weight *Euphorbia lactiflua* natural rubber with "in situ" formed performic acid. J. Appl. Polym. Sci. 30: 3340-3350.

Bremner, H.A. 1992. Fish Flesh Structure and The Role of Collagen-Its Postmortem Aspects and Implications for Fish Processing. *In* Quality Assurance in the Fish Industry. (Hass, H.H, Jacobsen, M. and Liston, J. eds.). p. 39-62. Elsevier Amsterdam.

Brosse, J.C., Boccaccio, G. and Pautrat, R. 1981. Proceeding of a Symposium UNIDO & IRRDB May 14 – 15, Phuket, Thailand. p. 201 – 204.

Burfield, D.R., Lim, K.L. and Law, K.S. 1984. Epoxidation of natural rubber latices: methods of preparation and poperties of modified rubber. J. Appl. Polym. Sci. 29: 1661 – 1673.

Burjandze, T.V. 1979. Hydroxyproline content and location in relation to collagen thermal stability. Biopolymer. 18: 931-936.

Carvalho, A. J. F., Job, A.E., Alves, N., Curvelo, A.A.S. and Gandini A. 2002. Thermoplastic starch/natural rubber blends. Carbohydr. Polym. 53: 95- 102.

Carvalho, R.A. and Grosso, C.R.F. 2004. Characterization of gelatin based films modified with transglutaminase, glyoxal and formaldehyde. Food Hydrocol. 18: 717-726.

Carvalho, R.A., Maria, T.M.C., Moraes, I.C.F., Bergo, P.V.A., Kamimura, E.S., Habitante, A.M.Q.B. and Sobral, P.J.A. 2009. Study of some physical properties of biodegradable films based on blends of gelatin and poly(vinyl alcohol) using a response-surface methodology. Mat. Sci. Eng. C. 29: 485-491.

Challa, G., 1993. Polymer Chemistry: An Introduction. p. 144–171. Ellis Horwood. New York.

Chaudhry, A.N. and Billingham, N.C. 2001. Characterisation and oxidative degradation of a room-temperature vulcanised poly(dimethylsiloxane) rubber. J. Polym. Degrad. Stab. 73: 505-510.

Chen, H. 1995. Functional properties and applications of edible films made from milk proteins. Science. **78**: 2563–2583.

Cheng, L.H., Karim, A.A. and Seow, C.C. 2006. Effects of water–glycerol and water–sorbitol interactions on the physical properties of konjac glucomannan films. J. Food Sci. **71**: 62–67.

Cheremisinoffet, N.P. 1993. Epoxidized Rubber. *In* Elastomer Technology Handbook. p. 636 – 657. CRC Press. USA.

Chick, J. and Ustunol, Z. 1998. Mechanical and barrier properties of lactic acid and rennet-precipitated casein-based edible films. J. Food. Sci. **63**: 1024–1027.

Chin, P.S. Cheng, W.P. Lan, C.M. and Pong, K.S. 1974. Deproteinised Natural Rubber Latex. Proceeding Rubber Research Institute of Malaysia Planters Conference. p.252-262.

Chinnabhark, K., Benjakul, S. and Prodpran, T. 2007. Effect of pH on the properties of protein-based film from bigeye snapper (*Priacanthus tayenus*) surimi. J. Biores. Technol. 98: 221-225.

Cho, S.M., Gu, Y.S. and Kim, S.B. 2005. Extracting optimization and physical properties of yellowfin tuna (*Thunnus albacores*) skin gelatin compared to mammalinian gelatins. Food Hydrocol. 19: 221-229.

Choi, W.S. and Han, J.H., 2002. Film-forming mechanism and heat denaturation effects on the physical and chemical properties of pea-protein-isolate edible films. J. Food Sci. **67**: 1399–1406.

Cruz, A.G.B., Góes, J.C., Figueiró, S.D., Feitosa, J.P.A., Ricardo, N.M.P.S. and Sombra, A. S. B. 2002. On the piezoelectricity of collagen/natural rubber blend films. Polymer. 39: 1267-1272.

Cuq, B. 2002. Formation and Properties of Fish Myofibrillar Protein Films and Coating. *In* Protein-Based Film and Coating (Gennadios, A. Ed.). p. 224. CRC Press. New York.

Cuq, B., Aymamrd, C., Cuq, J.L. and Guilbert, S. 1995. Edible packaging films based on fish myofibrillar proteins: formulation and functional properties. J. Food Sci. 60: 1369-1374.

Cuq, B., Gontard, N., Cuq, J.L. and Guilbert, S. 1997. Selected functional properties of fish myofibrillar protein-based film as affected by hydrophilic plasticizers. Agric. Food Chem. 45: 622-626.

Dahlan, H.M., 1998. The roles of LNR in the compatibilisation of NR/PE blends. Ph.D. Thesis, Kebangsaan University.

Dahlan, H.M., Khairul-Zaman, M.D. and Ibrahim, A. 2002. Liquid natural rubber (LNR) as a compatibiliser in NR/LLDPE blends-II: the effects of electron–beam (EB) irradiation. Radiat. Phys. Chem. **64**: 429–436.

Davey, J.E. and Loadman, M.J.R. 1984. Chemical demon-stration of the randomness of epoxidation of natural rubber. Br. Polym. 16: 134-138.

Davis, G. 2003. Characterization and characteristics of degradable polymer sacks. Mater. Charact. 51: 147-157.

Eastoe, J.E. and Leach, A.A. 1977. Chemical Constitution of Gelatin. *In* The Science and Technology of gelatin. (Ward, A.G and Courts, A. eds.). p. 92-98. Academic Press. London.

Famá, L. Famá, M., Rojas, A.M., Goyanes, S. and Gerschenson, L. 2006. Mechanical properties of tapioca-starch edible films containing sorbates. Lebensm.-Wiss. Technol. **38**: 631–639.

Fernández-Diaz, M.D., Montero, P. and Gòmez-Guillèn, M.C. 2001. Gel properties of collagens from skins of cod (*Gadus morhua*) and hake (*Merluccius merluccius*) and their modification by coenhancers magnesium sulphate, glycerol and transglutaminase. J. Food Chem. **74**: 161–167.

Foegeding, E.A. and Lanier, T.C. 1996. Characteristics of edible muscle tissues. *In* Food Chemistry (Fennema, O.R. ed.). p. 879–942. Marcel Dekker. New York.

Garcia, F.T. and Sobral, P.J.A. 2005. Effect of the thermal treatment of the filmogenic solution on the mechanical properties, color and opacity of films based on muscle proteins of two varieties of tilapia. Swiss Soc. J. Food Sci. Technol. 38: 289-296.

Gelling, I.R. 1985. Modification of natural rubber latex. Rubber Chem. Technol. 58(1): 86-96.

Gelling, I.R.1991. Epoxidized natural rubber. J. Nat. Rubber Res. 6(3): 184-205

Gennadios, A. and Weller, C.L. 1990. Edible films and coating from wheat and corn protein. J. Food Technol. 44: 63-69.

Gennadios, A., Weller, C.L. and Testin, R.F. 1993. Modification of physical and barrier properties of edible wheat gluten-based films. J. Cereal Chem. 70: 426-429.

Gennadios, A., McHugh, T.H., Weller, C.L. and Krochta, J.M. 1994. Edible Coatings and Films Based on Protein, *In* Edible Coating and Films to Improve Food Quality. (Krochta, J.M., Baldin, E.A. and Nisperos-Carriedo, M. eds.). p. 210-278. Technomic Pub. Co., Inc. Lancaster.

Gennadios, A., Weller, C.L., Hanna, M.A. and Froning, G.W. 1996. Mechanical and barrier properties of egg albumen films. J. Food Sci. 61: 585-589.

Gennadios, A., Handa, A., Froning, G.W., Weller, C.L. and Hanna, M.A. 1998. Physical properties of egg white-dialdehyde starch films. J. Agric. Food Chem. 46: 1297-1302.

Ghanbarzadeh, B., Musavi, M., Oromiehie, A.R., Rezayi, K., Razmi Rad, E. and Milani, J. 2007. Effect of plasticizing sugars on water vapor permeability, surface energy and microstructure properties of zein films. J. Food Sci. Technol. 40: 1191-1197.

Gilseman, M. and Ross-Murphy, S.B. 2000. Rheological characterisation of gelatins from mammalian and marine sources. Food Hydrocol. 14: 191–195.

Giménez, B., Turnay, J., Gómez-Guillén, M.C, and Montero P. 2005. Use of lactic acid for extraction of fish skin gelatin. Food Hydrocol. 19: 941–950.

Gómez-Guillén, M.C., Sarabia, A.I. and Montero, P., 2001. Extraction of gelatin from megrim (Lepidorhombus boscii) skins with several organic acids. J. Food Sci. **66**: 213–216.

Gontard, N., Guilbert, S. and Cuq, J.L. 1993. Water and glycerol as plasticizers affect mechanical and water vapor barrier properties of an edible wheat gluten film. J. Food Sci. 58: 206-211.

Gounga, M.E., Xu, S.Y. and Wang, Z. 2007. Whey protein isolate-based edible films as effected by protein concentration, glycerol ratio and pullulan addition in film formation. J. Food Eng. 83: 521-530.

Griffin, G.J.L. 1994. The Chemistry and Technology of Biodegradable Polymers. *In* Chemistry and Technology of Biodegradable Polymers. (Blackie, A. ed.). p. 135–149. London.

Griffiths, P.C., Cheung, A.Y.F., Jenkins, R.L ., Howe, A.M., Pitt, A.R., and Heenan, R K, 2000. **The interaction between a partially fluorinated alkyl sulphate and gelatin in aqueous solutions**. Langmuir. 20: 1161-1167.

Grosso, C. 2006. Edible films produced with gelatin and casein cross-linked with transglutaminase. Food Res. Int**. 39**(4): 458–466.

Gunasekaran, S., Natarajan, R.K., Kala, A. 2006. FTIR spectra and mechanical strength analysis of some selected rubber derivatives. Spectroc. Acta Pt. A-Molec. Biomolec. Spectr. 68: 323-30.

Gustavson, K. 1956. The Chemistry and Relative of Collagen. Academic Press. New York.

Hamaguchi, P.Y., Yin, W.W. and Tanaka, M. 2007. Effect of pH on the formation of edible films made from the muscle proteins of blue marlin (*Makaira mazara*). J. Food Chem. 100: 914-920.

Han, J.H. and Floros, J.D. 1997. Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. J. Plastic Film Sheet. 13: 287-298.

Hang-Wan, V.M., Kim, M.S. and Lee, S.Y. 2005. Water vapor permeability and mechanical properties of soy protein isolate edible films composed of different plasticizer combinations. J. Food Sci. 70: 387-391.

Haug, I.J., Derget, K.I. and Smidrod, O. 2004. Physical and rheological properties of fish gelatin compared to mammalian gelatin. Food Hydrocol. 18: 203-213.

Herald, T.J., Gnanasambandam, R., Mcguire, B.H. and Hachmeister, K.A. 1995. Degradable wheat gluten films: preparation, properties and applications, J. Food Sci. 60: 1147-1331.

Hernandez-Munoz, P., Lopez-Rubio, A., Valle, V., Almenar, E. and Gavara, R. 2004. Mechanical and water barrier properties of glutenin films influenced by storage time. J. Agric. Food Chem. 52: 79-83.

Holzer, P. 1994. Calcitonin Gene-Related Peptide. *In* Gut Peptides, Biochemistry and Physiology. (Walsh, J.H. and Dockray, G.J. eds.). p.493−523. Raven Press. New York.

Hultin, H.O. 1976. Characteristics of Muscel Tissue. *In* Principles of Food Scince. Part I. Food Chemistry. (Fennema, O.R. ed.). p. 577-617. Marcel Dekker. New York.

Ibrahim, A. and Dahlan, M., 1998. Thermoplastic natural rubber blends. Prog. Polym. Sci. **23**: 665–706.

Ibrahim, A., Sahrim, A. and Che-Som, S., 1995. Blending of NR with LLDPE. J. Appl. Polym. Sci. 58: 1125–1133.

Irissin-Mangata, J., Bauduin, G., Boutevin, B. and Gontard, N. 2001. New plasticizers for wheat gluten films. Eur. Polym. J. 37: 1533-1541.

Ismail, H. and Poh, B.T. 2000. Cure and tear properties of ENR 25/ SMR L and ENR 50/SMR L blends. Eur. Polym. J. 36: 2403–2408.

Iwata, K., Ishizaki, S., Handa, A. and Tanaka, M. 2000. Preparation and characterization of edible films from fish water-soluble proteins. J. Fish. Aquat. Sci.. 66: 372 - 378.

Jangchud, A. and Chinan, M.S. 1999. Properties of peanut protein film: sorption isotherm and plasticizer effect. Lebensm-Wiss. U.-Technol. 32: 89-94.

Jones. R.T. 1987. Gelatin: Structure and Manufacture. *In* Hard Capsules Development and Technology. (Ridgway, K. ed.). p. 366-394. Academic Press. London.

Jongjareonrak, A., Benjakul, S., Visessanguan, W., Prodpran, T. and Tanaka, M. 2006. Characterization of edible films from skin gelatin of brownstripe red snapper and bigeye snapper. Food Hydrocol. **20**: 492–501.

Kim, S.J. and Ustunol, Z. 2001. Sensory attributes of whey protein isolate and candelilla wax emulsion edible films. J. Food Sci. **66**: 909–911.

Kester, J.J and Fennema, O.R. 1986. Edible film and coatings: a review. J. Food Technol. 40: 47-59.

Klinklai, W., Kawahara, S., Mizumo, T., Yoshizawa, M., Sakdapipanich, J.T. and Isono Y. J. 2003. Depolymerization and ionic conductivity of enzymatically deproteinized natural rubber having epoxy group. Polymer. **39**: 1707–1712.

Kittiphattanabawon, P. 2004. Extraction and characterization of collagen and gelatin from bigeye snapper (*Priacanthus tayenus*) skin and bone. Master of Science Thesis. Prince of Songkla University.

Kristo, E., Biliaderis, C.G. and Zampraka, 2007. A. Water vapour barrier and tensile properties of composite caseinate-pullulan films: biopolymer composition effects and impact of beeswax lamination. Food Chem. **101**: 753–764.

Krochta, J.M. and Mulder-Johnson, J. 1997. Edible and biodegradable polymer films: challenges and opportunities. Food Technol. 51: 61-74.

Krochta, J.M. 2002. Protein and Raw Material for Film and Coatings: Definitions, Current Stutus, and Opportunities. *In* Protein-Based Film and Coating. (Gennadios, A., ed.). p. 1-39. CRC Press. New York,

Langmaier, F., Mokrejs, P., Kolomaznik, K. and Mladek, M. 2008. Plasticizing collagen hydrolysate with glycerol and low-molecular weight poly(ethylene glycols). Thermochim. Acta. 469: 52-58.

Ledward , D. A. 1986. Gelation of Gelatin. *In* Functional properties of food macromolecules, (Mitchell, J.R. and Ledward, D.A. Eds.). p. 233–289.Elsevier Applied Science Publishers. London .

Li , B., Kennedy, J.F., Peng, J.L., Yie, X. and Xie, B.J. 2007. Preparation and performance evaluation of glucomannan–chitosan–nisin ternary antimicrobial blend film. Carbohydr. Polym. 65: 488-494.

Lim, L.T., Mine, Y. and Tung, M.A. 1998. Transglutaminase cross-linked egg white protein film: tensile properties and oxygen permeability. Agric. Food. Chem. 46: 4022-4029.

Limpan, N., Prodpran, T., Benjakul, S. and Prasarpran, S. 2010. Properties of biodegradable blend films based on fish myofibrillar protein and polyvinyl alcohol as influenced by blend composition and pH level. J. Food Eng. (In Press).

Livonnier, H. 1998. The first FRANCO–THAI Rubber Technology Symposium. Bangkok, Thailand. 15-17 April 1998. p. 57-87.

Marquie, C. 2001. Chemical reactions in cottonseed protein cross-linking by formaldehyde, glutaraldehyde, and glyoxal for the formation of protein films with enhanced mechanical properties. J. Agric. Food Chem. 49: 4676-4681.

McHugh, T. H., Aujard, J. F. and Krochta, J. M. 1994. Plasticized whey protein edible films: water vapor permeability properties. J. Food Sci. 59: 416-423.

Menegalli, F.C., Sobral, P.J.A., Roques M. and Laurent, S. 1999. Characteristics of gelatin bio-films in relation to drying process conditions near melting. Drying Technol. 17: 1697–1706.

Miller, K.S. and Krochta, J.M. 1997. Oxygen and aroma barrier properties of edible films: a review. Food Sci. Technol**. 8**: 228–237.

Moore, G.R.P., Martelli, S.M., Gandolfo, C., Sobral, P.J.A. and Laurindo, J.B. 2006. Influence of the glycerol concentration on some physical properties of feather keratin films. Food Hydrocol. 20: 975–982.

Muyonga, J.H., Cole, C.G.B., and Duodu, K.G., 2004. Characterization of acid soluble collagen from skins of young and adult Nile perch (*Lates nilotics*). Food Chem. 85: 81–89.

Nakason, C., Kaesaman, A. and Klinpituksa, P. 2001. Preparation, thermal and flow properties of epoxidized natural rubber. Sci. Technol. 23: 415 -424.

Nor, H.M. and Ebdon, J.R.. 1998. Telechelic liquid natural rubber: a review. Prog. Polym. Sci. 23: 143–177.

Noriman, N.Z., Ismail, H. and Rashid, A.A. 2009. Characterization of styrene butadiene rubber/recycled acrylonitrile-butadiene rubber (SBR/NBRr) blends: the effects of epoxidized natural rubber (ENR-50) as a compatibilizer. Polym. Testing. 29: 200-208.

Norland, R. E. 1990. Fish Gelatin. *In* Advance in Fisheries Technology and Biotechnology for Increased Profitability (Voight, M.N. and Botta, J.K. eds.). p. 325-333. Technomic Plublishing. Lancaster, PA.

Nuthong, P., Benjakul, S. and Prodpran, T. 2009. Characterization of porcine plasma protein-based films as affected by pretreatment and cross-linking agents. Int. J. Biol. Macromol. 44: 143-148.

Olabisi, O., Rubenson, L.M. and Shaw, M.T. 1979. Polymer-Polymer Miscibility. Academic Press, New York.

Orliac, O., Rouilly, A. Silvestre, F. and Rigal, L. 2003. Effects of various plasticizers on the mechanical properties, water resistance and aging of thermo-moulded films made from sunflower proteins. Ind. Crops Products. 18: 91-100.

Okwu, U.N. and Okieimen, F.E. 1998. Properties of formic acid crosslinked epoxidized rubber (FC-ENR) blends with dry natural rubber. Eur. polym. J. 35: 1855-1859.

Ou , H.Y., Chen, G.B., Choi, D.B., Cho, K.A., Okabe, M. and Cha, W.S. 2004. Cellulose production from gluconobacter oxydans TQ-B2. Biotechnol. Bioprocess Eng. 9: 166-170.

Park, H.J. and Chinnan, M.S. 1995. Gas and water vapor barrier properties of edible films from protein and cellulosic materials. J. Food Eng. 25: 497-507.

Pascholick, T.M., Garcia, F.T., Sobral, P.J. A. and Habitante, A.M.Q.B. 2003. Characterization of some functional properties of edible films based on muscle proteins of Nile Tilapia. Food Hydrocolloid. 17: 419-427.

Pautrat, B. and Marteau, J. 1976. Rheology and morphology of concentrated immiscible polymer blends. Rubber Chem. Technol. 49: 1060-1074.

Perez-Gago, M.B. and Krochta, J.M., 1999. Denaturation time and temperature effects on solubility, tensile properties, and oxygen permeability of whey protein edible films. J. Food Sci. **66**: 705–710.

Perez-Mateos, M., Montero, P. and Gomez-Guillen, M.C. 2009. Formulation and stability of biodegradable films made from cod gelatin and sunflower oil blends. Food Hydrocol. 23: 53-61.

Pierro, P., Chico, B., Villalonga, R., Mariniello, L., Damiao, A.E. and Masi, P. 2006. Chitosan-whey protein edible films produced in the absence or presence of transglutaminase: analysis of their mechanical and barrier properties, Biomacromolecules. 7: 44–749.

Poh, B.T., Ismail, H. and Tan, K.S. 2002. Effect of filler loading on tensile and tear properties of SMR L/ENR 25 and SMR L/SBR blends cured via a semi-efficient vulcanization system. Polym. Test. **21**: 801–806.

Qi, Q., Liang, G.H. and Zhang, L.Q.A. 2006. Strategy to prepare high performance starch/rubber composites: in-situ modification during latex compounding process. Carbohydr. Polym. 65: 109-113.

Ravindran, T., Gopinathan-Nayar, M. R., and Joseph-Francis, D. 1983. Production of hydroxyl-terminated liquid natural rubber: mechanism of photochemical depolymerization and hydroxylation. J. Appl. Polym. Sci. 35: 227-1239.

Rhim, J.W., Wu, Y., Weller, C.L. and Schnepf, M. 1999. Physical characteristics of a composite film of soy protein isolate and propyleneglycol algenate. J. Food Sci. 64: 149–152.

Rodríguez, F., Calvo, J.B., Quintana, E., Rubí, R. and Cela, R. 2006. Suitability of solid-phase microextraction for the determination of organophosphate flame retardants and plasticizers in water samples. J. Chromatogr. **1108**: 158-165.

Rodriguéz, M., Osés, J.,. Ziani, K and Maté, J.I. 2006. Combined effect of plasticizers and surfactants on the physical properties of starch based edible films. Food Res. Int. **39:** 840–846.

Rodriguéz J., Spearman M., Huzel N. and Butler, M. 2005. Enhanced production of monomeric interferon by CHO cells through the control of culture conditions. Biotechnol Prog 21: 22–30.

Röper, H., and Koch, V. 1990. The role of starch in biodegradable thermoplastic materials. Starch/Stärke. **42**: 123–130.

Rouilly, A., Rigal, L. and Gilbert, R.G. 2004. Synthesis and properties of composites of starch and chemically modified natural rubber. Polymer. **45**: 7813–7820.

Roy, S., Gennadios, A., Weller, C.L. and Testin, R.F. 2000. Water vapor transport parameters of a cast wheat gluten film. Ind. Crops Prod. 11: 43-50.

Roy, S., Gupta, R. and Dee, R. 1990. Elastomer Technology Handbook. p. 633-644

Roy, S., Namboodri, C.S.S., Maiti, B.R. and Gupta, B.R. 1993. Kinetic modeling of the epoxidation of natural rubber with in-situ-formed peracid. Polym. Eng. Sci. 33: 92-96.

Schmidt, V., Giacomelli, C., and Soldi, V. 2005. Thermal stability of film fromed by soy protein isolate-sodium dodecyl sulfate. Polym. Degrad. Stabil. 87: 25-31.

Shellhammer, T.H. and Krochta, J.M. 1997. Whey protein emulsion film performance as affected by lipid type and amount. J. Food Sci. 62: 390-394.

Shiku, Y., Hamaguchi, P.Y., Benjakul, S., Visessanguan, W. and Tanaka, M. 2004. Effect of surimi quality on properties of edible films based on Alaska Pollack. Food Chem. 86: 493-499.

Shiku, Y., Hamaguchi, P.Y. and Tanaka, M. 2003. Effect of pH on the preparation of edible films based on fish myofibrillar proteins. J. Fish. Aquat. Sci. 69: 1026-1032.

Shogren, R.L., Fanta, G. and Doane, W.M. 1993. Development of starch based plastics: a reexamination of selected polymer systems in historical perspective. Starch/Stärke. **45**: 276–280.

Sikorski, Z.E., Kolakowska, A. and Pan, B.S. 1990. The Nutritive Composition of the Major Groups of Marine Food Organism. *In* Seafood: Resources, Nutritional Composition, and Preservation. (Sikorski, Z.E., ed.). p. 29-54. CRC Press, Inc., Florida.

Simon-Lukasik, K.V. and Ludescher, R.D. 2003. Erythrosin B phosphorescence as probe of oxygen diffusion in amorphous gelatin films. Food Hydrocolloid. 15: 114-123.

Sobral, P.J.A., Menegalli, F.C., Hubinger, M.D. and Roques, M.A. 2001. Mechanical, water vapor barrier and thermal properties of gelatin based edible films. Food Hydrocol. **15**: 423–432.

Sorbal, P.J.A., Santos, J.S.D., Garcia, F.T. 2005. Effect of protein and plasticizer concentrations in film forming solutions on physical properties of edible films based on muscle proteins of a Thai tilapia. J. Food Eng. 70: 93-100.

Sothornvit, R. and Krochta, J.M. 2001. Plasticizer effect on mechanical properties of β-lactoglobulin films. Food Eng. 50: 149-155.

Stansby, G. 1987. Gelatin Gel. *In:* Advance in Meat Research. Vol. IV. (Pearson, A.M., Dutson, T.R. and Bailey, A.J. eds.). p. 209-222. Nostrand Reinhold. New York.

Steel, R.G.D. and Torrie, J.H. 1980. Principles and Procedures of Statistic: a Biometrical Approach. (2nd ed.). McGraw-Hill. New York.

Stuchell, Y.M. and Krochta, J.M. 1995. Edible coatings on frozen king salmon: effect of whey protein isolate and acetylated monoglycerides on moisture loss and lipid oxidation. J. Food Sci. 60: 28-31.

Takayuki, S., Warunee, K., and Seiichi, K. 2006. Characterization of epoxidized natural rubber by 2D NMR spectroscopy. Polymer 48: 750–757.

Talja, R.A., Helen, H., Roos, Y.H. and Jouppila, K. 2006. Effect of various polyols and polyol contents on physical and mechanical properties of potato starch-based films. Carbohydr. Polym. **67**: 288–295.

Tanaka, M., Iwata, K., Sanguandeekul, R., Handa, A. and Ishizaki, S. 2001. Influence of plasticizers on the properties of edible films prepared from fish water-soluble proteins. J. Fish. Aquat. Sci. 67: 346-351.

Tang, R., Du, Y., Zheng, H. and Fan, L. 2003. Preparation and characterization of soy protein isolate-carboxymethylated konjac glucomannan blend films. J. Appl. Polym. Sci. 88: 1095-1099.

Tangpakdee, J., Kawahara, S., and Tanaka, Y. 1998. Novel method for preparation of low molecular weight natural rubber latex. Rubb. Chem. Technol. 71:215-241.

Thomazine, M., Carvalho, R.A. and Sobral, P.J.A. 2005. Physical properties of gelatin films plasticized by blends of glycerol and sorbitol. Food Sci. **71**: 172–176.

Torres, J.A. 1994. Edible Films and Coatings from Proteins. *In*: Protein Functionality in Food Systems, (Hettiarachchy, N.S. and Ziegler, G.R. Eds.). p. 467–507. Marcel Dekker, New York.

Utracki, L.A. 2002. Compatibilization of polymer blends. J. Chem. Eng. **80**: 1008–1016.

Vanin, F.M., Sobral, P.J.A., Menegalli, F.C., Carvalho, R.A. and Habitante, A.M.Q.B. 2005. Effects of plasticizers and their concentrations on thermal and functional properties of gelatin-based films. Food Hydrocol. **19**: 899–907.

Wang, L., Auty, M.A.E., Rau, A., Kerry, J.F. and Kerry, J.P. 2009. Effect of pH and addition of corn oil on the properties of gelatin-based biopolymer films. Food Eng. 90: 11-19.

Wong, D.W.S. 1989. Mechanism and Theory in Food Chemistry. Van Nostrand Reinhold. New York.

Wool, R.P. 1995. Degradable Polymers. *In:* The science and engineering of polymer composite degradation. (Scott, G. and Gilead, D. Eds.). p. 138–152.Chapman & Hall, London

Yang, L. and Paulson, A.T. 2002. Mechanical and water vapour barrier properties of edible gellan films. Food Res. Int. **33**: 563–570.

Zhang, J. F. and Sun, X. 2004. Mechanical properties of poly(lactic acid)/starch composites compatibilizerd by maleic anhydride. Biomacromolecules. 5: 1446-1451.

APPENDIX

APPENDIX

A. ANALYTICAL METHODS

1. Determination of moisture content (AOAC, 2000)

Method

1. Dry the empty dish and lid in the oven at 105°C for 3 h and transfer to dessicator to cool. Weigh the empty dish and lid.
2. Weigh about 3 g of sample to the dish. Spread the sample to the uniformity.
3. Place the dish with sample in the oven. Dry for 3 h at 105°C.
4. After drying, transfer the dish with partially covered lid to the desiccator to cool. Reweigh the dish and its dried sample.

Calculation

$$\text{Moisture content (\%)} = \frac{(W1 - W2) \times 100}{W1}$$

where W1 = weight (g) of sample before drying

W2 = weight (g) of sample after drying

2. Determination of protein content (AOAC, 2000)

Reagents

1. Kjedahl catalyst: Mix 9 part of potassium sulphate (K_2SO_4) with 1 part of copper sulphate ($CuSO_4$)
2. Sulfuric acid (H_2SO_4)
3. 40% NaOH solution (w/v)
4. 0.2 N HCl solution
5. 4% H_3BO_3 solution (w/v)
6. Indicator solution: Mix 100 ml of 0.1% methyl red (in 95% ethanol) with 200 ml of 0.2% bromocresol green (in 95% ethanol)

Method

1. Place sample (0.5-1.0 g) in digestion flask.
2. Add 5 g Kjedahl catalyst and 20 ml of conc. H_2SO_4
3. Prepare a tube containing the above chemical except sample as blank. Place flasks in inclined position and heat gently until frothing ceases. Boil briskly until

solution clears.

4. Cool and add 60 ml distilled water cautiously.
5. Immediately connect flask to digestion bulb on condenser and with tip of condenser immersed in standard acid and 5-7 indicator in receiver. Rotate flask to mix content thoroughly; then heat until all NH_3 is distilled.
6. Remove receiver, wash tip of condenser and titrate excess standard acid distilled with standard NaOH solution.

Calculation

$$\text{Protein content (\%)} = \frac{(A-B) \times N \times 1.4007 \times 6.25}{W}$$

where A = volume (ml) of 0.2 N HCl used sample titration

B = volume (ml) of 0.2 N HCl used in blank titration

N = normality of HCl

W = weight (g) of sample

14.007 = atomic weight of nitrogen

6.25 = the protein-nitrogen conversion factor for fish and its by-products

3. Determination of ash content (AOAC, 2000)

Method

1. Place the crucible and lid in the furnace at 550°C overnight to ensure that impurities on the surface of crucible are burned off.
2. Cool the crucible in the desiccator (30 min).
3. Weigh the crucible and lid to 3 decimal places.
4. Weigh about 5 g sample into the crucible. Heat over low Bunsen flame with lid half covered. When fumes are no longer produced, place crucible and lid in furnace.
5. Heat at 550°C overnight. During heating, do not cover the lid. Place the lid after complete heating to prevent loss of fluffy ash. Cool down in the desiccator.
6. Weigh the ash with crucible and lid when the sample turns to gray. If not, return the crucible and lid to the furnace for the further ashing.

Calculation

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

4. Determination of fat content (AOAC, 2000)

Reagent

1. Petroleum ether

Method

1. Place the bottle and lid in the incubator at 105^{o}C overnight to ensure that weight of bottle is stable.
2. Weigh about 3-5 g of sample to paper filter and wrap.
3. Take the sample into extraction thimble and transfer into soxhlet.
4. Fill petroleum ether about 250 ml into the bottle and take it on the heating mantle.
5. Connect the soxhlet apparatus and turn on the water to cool them and then switch on the heating mantle.
6. Heat the sample about 14 h (heat rate of 150 drop/min).
7. Evaporate the solvent by using the vacuum condenser.
8. Incubate the bottle at 80-90^{o}C until solvent is completely evaporated and bottle is completely dried.
9. After drying, transfer the bottle with partially covered lid to the desiccator to cool. Reweigh the bottle and its dried content.

Calculation

$$\text{Fat content (\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

5. Hydroxyproline content (Bergman and Loxley, 1963)

Reagent

1. 6 N HCl
2. Oxidant solution (the mixture of 7% (w/v) chlororamine T and acetate/citrate buffer, pH 6 at a ratio of 1:4 (v/v))
3. Ehrich's reagent solution (the mixture of solution A (2 g of p-dymethyllamino benzaldehyde in 3 ml of 60% (v/v) perchloric acid (w/v))
4. Isopropanol
5. Hydroxyproline standard solution (400 ppm)

Method

Sample preparation

1. Weight about 0.1 – 2.0 g sample (depending on type of sample) into screw cap tube.
2. Add 6 N HCl into the sample at the ratio of 1:10 (solid/acid, w/v).
3. Heat at 110^{o}C for 24 h in oil bath.
4. Clarify the hydrolysate with activated carbon and filter through Whatman No.4 filter paper.
5. Neutralize the filtrate with 10 M NaOH and 1 M NaOH to obtain the pH 6.0-6.5.

Hydroxyproline determination

1. Transfer 0.1 ml of the neutralized sample into a test tube and add 0.2 ml of isopropanol then mix well.
2. Add 0.1 ml of oxidant solution and mix wall.
3. Add 1.3 ml of Ehrlich's reagent solution.
4. Heat the mixtures at 60^{o}C for 25 h in a water bath and then cool for 2-3 min in running water.
5. Add isopropanol at a ratio of 3.3 ml and mix well.
6. Read absorbance at 558 nm.
7. Plot the standard curves and calculate the unknown.

Table: Experimental set up for the hydroxyproline's assay

Tube number	Water (µL)	400 ppm Hydroxyproline	Effective Hydroxyproline

		(µL)	Concentration (mg/ml)
1	100	0	0
2	97.5	2.5	10
3	95.0	5.0	20
4	92.5	7.5	30
5	90.0	10.0	40
6	87.5	12.5	50
7	85.0	15.0	60
8	0	0	unknown

B. SUPPORTING DATA

Table B1: Thickness, mechanical properties, moisture content and water-vapor permeability of gelatin films, gelatin/ENR (G/ENR) blend films and ENR films.

Films	Thickness (µm)	TS (MPa)	EAB (%)	E ($\times10^{2}$MPa)	MC (%)	WVP ($\times10^{-11}$g.m/m^{2}.s.Pa)
Gelatin+0% Gly	29.76 ± 0.08^{a}	78.19 ± 0.32^{e}	4.64 ± 0.04^{a}	20.56 ± 0.04^{e}	3.95 ± 0.06^{a}	$6.43\pm0.04^{a*}$
Gelatin+15% Gly	29.24 ± 1.25^{a}	50.96 ± 0.52^{d}	5.77 ± 0.06^{b}	12.66 ± 0.32^{d}	5.33 ± 0.25^{b}	7.81 ± 0.06^{b}
Gelatin+20% Gly	29.08 ± 0.87^{a}	41.87 ± 0.16^{c}	9.0 ± 10.04^{c}	3.38 ± 0.17^{c}	9.52 ± 0.03^{c}	8.45 ± 0.04^{c}
Gelatin+25% Gly	29.68 ± 1.77^{a}	28.71 ± 0.37^{b}	24.64 ± 0.03^{d}	1.72 ± 0.05^{b}	11.1 ± 20.04^{d}	9.06 ± 0.03^{cd}
Gelatin+30% Gly	29.28 ± 1.14^{a}	17.22 ± 0.10^{a}	32.03 ± 0.03^{e}	1.07 ± 0.06^{a}	15.92 ± 0.26^{e}	9.50 ± 0.03^{d}

[*] Mean ± SD (n=3). The different superscripts in the same column indicate the significant differences ($p < 0.05$).

Table B2: Water vapor permeability (WVP) of gelatin films, gelatin/ENR (G/ENR) blend films and ENR films.

Films	Thickness (μm)	TS (MPa)	EAB (%)	E (x10^{2}MPa)	WVP (x10^{-11}g.m/m^{2}.s.Pa)
Gelatin+25% Gly	26.16±0.54^{d}	27.47±1.95^{g}	20.02±2.13^{a}	36.51±1.86^{i}	8.86±0.34^{h*}
G:ENR-10 (8/2)	26.03±0.37^{d}	19.15±1.06^{f}	37.15±2.75^{b}	37.19±1.14^{j}	6.55±0.19^{cde}
G:ENR-10 (6/4)	24.21±0.24^{c}	14.23±1.14^{e}	49.69±2.08^{c}	17.58±0.88^{h}	6.12±0.20^{c}
G:ENR-10 (5/5)	22.71±0.19^{b}	6.93±1.18^{bd}	62.20±3.29^{d}	6.47±1.10^{e}	6.25±0.18^{ef}
ENR-10	18.88±0.12^{a}	10.17±1.50^{d}	771.29±15.94^{e}	0.76±0.16^{c}	4.79±0.22^{a}
G:ENR-25 (8/2)	25.92±0.50^{d}	10.70±2.28^{d}	60.55±2.08^{d}	15.28±1.59^{g}	6.16±0.19^{cde}
G:ENR-25 (6/4)	24.07±0.20^{c}	9.86±1.13^{cd}	77.02±3.07^{f}	6.52±2.14^{e}	6.76±0.58^{cd}
G:ENR-25 (5/5)	22.57±0.12^{b}	7.17±1.61^{bc}	83.84±2.96^{f}	5.57±1.02^{de}	6.82±0.23^{cde}
ENR-25	18.63±0.19^{a}	7.94±1.30^{bc}	888.391±4.15^{g}	0.54±0.16^{b}	4.82±0.18^{a}
G:ENR-50 (8/2)	25.84±0.42^{d}	6.72±1.03^{ab}	68.39±1.57^{f}	7.87±1.23^{f}	6.32±0.25^{cdf}
G:ENR-50 (6/4)	23.93±0.27^{c}	5.35±0.75^{ab}	77.75±2.87^{f}	4.67±0.67^{d}	7.17±0.31^{fg}
G:ENR-50 (5/5)	22.1±30.22^{b}	5.22±1.20^{a}	86.6±73.90^{f}	3.7±60.75^{d}	7.32±0.07^{g}
ENR-50	18.54±0.11^{a}	6.77±0.47ab	975.3±519.42^{h}	0.42±0.04^{a}	5.03±0.23^{b}

[*] Mean ± SD (n=3). The different superscripts in the same column indicate the significant differences ($p < 0.05$).

Table B3: Water vapor permeability of films from gelatin and G/ENR-25 (6/4) blends containing different glycerol contents.

Films	WVP (x10^{-11}g.m/m^{2}.s.Pa)
Gelatin+0% Gly	6.32±0.21^{e*}
Gelatin+25% Gly	4.64±0.36^{d}
G:ENR-25(6/4)+0%Gly	6.32±0.46^{c}
G:ENR-25(6/4)+2.25%Gly	6.15±0.28^{bc}
G:ENR-25(6/4)+6.25%Gly	5.86±0.25^{b}
G:ENR-25(6/4)+25%Gly	4.64±0.36^{a}
ENR-25	6.13±0.17^{bc}

[*] Mean ± SD (n=3). The different superscripts in the same column indicate the significant differences ($p < 0.05$).

Table B4: Solubility of selected films from gelatin (+25%Gly), ENR-25, NR, G/ENR-25 (6/4) blend with 25% glycerol and G/ENR-25/NR (6/2/2) blend without glycerol.

Films	Film solubility (%)
Gelatin+25% Gly	100±0.00[e*]
G:ENR-25(6/4)+25%Gly	43.32±1.21[d]
G/NR/ENR-25 (6/2/2) +0%Gly	35.89±2.19[c]
ENR-25	4.47±0.45[b]
NR	1.48±0.55[a]

[*] Mean ± SD (n=3). The different superscripts in the same column indicate the significant differences ($p < 0.05$).

VITAE

Name	Miss Pornsawan Chamnanvatchakit
Student ID	5011020020

Educational Attainment

Degree	**Name of Institution**	**Year of Graduation**
Bachelor of Science (Polymer Science)	Prince of Songkla University	2006

Scholarship Awards during Enrolment

The Scholarship for Development of Agro-Industry to the Excellence from Faculty of Agro-Industry, Prince of Songkla University.

List of Publication and Proceedings

Chumnanvatchakit, P., Prodpran, T. and Benjakul, S. 2009. Use of epoxidized natural rubber (ENR) to modify the properties of bovine gelatin film: effect of gelatin/ENR ratio and epoxy content of ENR. The 47th Kasetsart University Annual Conference, Bangkok, Thailand. March 17-20.

Chumnanvatchkit, P., Prodpran, T. and Benjakul, S. 2008. Influence of glycerol concentration on mechanical, physical and water-vapor barrier properties of bovine gelatin film. The Sixth Regional IMT-GT Uninet conference 2008. Penang, Malaysia. August 28-30.