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DEVELOPMENT OF BACTERIAL CELLULOSE/ALGINATE/GELATIN FILM FOR FOOD PRESERVATIVE

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ในบรรจุภัณฑ์สำหรับอาหาร คอมโพสิตฟิล์มที่บรรจุสารต้านจุลินทรีย์เป็นสิ่งที่น่าดึงดูดและน่าสนใจมาก เนื่องจากมันสามารถเพิ่มคุณภาพและป้องกันอาหารจากจุลินทรีย์ก่อโรคระหว่างการเก็บได้ การประยุกต์ใช้คอมโพสิตฟิล์มจากพอลิเมอร์ธรรมชาติที่มีสมบัติต้านจุลินทรีย์จึงเป็นทางเลือกหนึ่งที่มีประสิทธิภาพสำหรับยืดอายุของอาหาร รวมทั้งการมีสุขภาพที่ดีและปลอดภัยต่อสิ่งแวดล้อมมากขึ้นด้วย ในการศึกษานี้แบคทีเรียเซลลูโลส (BC)-โซเดียมอัลจิเนต (A)-เจลาติน (G) หรือคอมโพสิตฟิล์ม BAG ถูกสร้างขึ้นด้วยวิธีการหล่อและอบฟิล์มที่อุณหภูมิห้อง โดยมีการเติมกลีเซอรอล กรดแทนนิกและสารสกัดเปลือกมังคุดที่ผ่านการสกัดด้วยเอทานอลเพื่อปรับปรุงสมบัติของฟิล์ม ซึ่งกลีเซอรอลทำหน้าที่เป็นพลาสติไซเซอร์และถูกเติมเพื่อปรับปรุงความสามารถในการเปลี่ยนแปลงรูปร่างและป้องกันการหดตัวของฟิล์มหลังกระบวนการทำให้แห้ง กรดแทนนิกทำหน้าที่เป็นสารเชื่อมโยงที่ได้จากธรรมชาติสำหรับการเชื่อมโยงเจลาตินและมีสมบัติต้านจุลินทรีย์อีกด้วย ส่วนสารสกัดเปลือกมังคุดถูกใช้เป็นสารต้านจุลินทรีย์ โดยฟิล์มที่ผ่านการปรับปรุงจะถูกนำไปศึกษาคุณลักษณะทางกายภาพ ทางเคมี เชิงกลและสมบัติต้านจุลินทรีย์ ในแง่ของสมบัติทางกายภาพและเชิงกลสามารถแสดงองค์ประกอบที่เหมาะสมของฟิล์ม BAG ที่อัตราส่วน เท่ากับ 60/20/20 โดยน้ำหนัก อัตราส่วนที่เหมาะสมของกลีเซอรอลต่อสารละลายเจลาติน คือ 2:10 โดยน้ำหนัก ซึ่งฟิล์ม BAG ที่มีการเติมกลีเซอรอล (MBAG) พบว่า มีความยืดหยุ่น การยืดตัว ณ จุดขาดและความคงตัวทางเคมีที่ดีกว่า นอกจากนี้อัตราส่วนที่เหมาะสมของกรดแทนนิกต่อสารละลายเจลาติน คือ 10 มิลลิกรัม ต่อกรัมของสารละลายเจลาติน ซึ่งพบว่า ฟิล์ม BAG ที่มีการเติมกรดแทนนิก (MBAGT) มีโครงสร้างเป็นแผ่นชิดกันมากขึ้น ส่งผลให้ประสิทธิภาพการดูดซึมน้ำและอัตราการซึมผ่านออกซิเจนลดลง นอกจากนี้กรดแทนนิกยังแสดงหน้าที่เป็นพลาสติไซเซอร์ต่อฟิล์ม MBAGTM ในสภาวะฟิล์มเปียก โดยเข้าไปเพิ่มการยืดตัว ณ จุดขาด ของฟิล์มที่บวมน้ำอีกครั้ง ในส่วนของฟิล์มที่มีสารสกัดเปลือกมังคุด (MBAGTM) จะได้ฟิล์มที่มีลักษณะผิวเรียบกว่าและหนากว่า เนื่องจากการเติมสารสกัดจะไปเพิ่มความหนาของฟิล์มและเพิ่มช่องว่างระหว่างชั้นของแผ่นฟิล์มด้วย นอกจากนี้ สมบัติการต้านจุลินทรีย์จะแสดงเป็นความเข้มข้นต่ำสุดที่สามารถยับยั้งการเจริญเติบโตของจุลินทรีย์ (MIC) ซึ่งพบว่า สารสกัดเปลือกมังคุดและกรดแทนนิกสามารถยับยั้งแบคทีเรียก่อโรคในอาหารได้ ประกอบด้วย *Escherichia coli, Salmonella typhimurium, Listeria monocytogenes* และ *Staphylococcus aureus* ยิ่งไปกว่านั้นสารสกัดจากเปลือกมังคุดเป็นสารที่มีฤทธิ์ยับยั้งเชื้อจุลินทรีย์ได้สูงกว่ากรดแทนนิก ดังนั้นผลที่ได้แสดงให้เห็นว่าฟิล์ม MBAGTM มีศักยภาพที่ดีและสามารถนำมาใช้เป็นบรรจุภัณฑ์สำหรับอาหารได้

ภาควิชา วิศวกรรมเคมี ลายมือชื่อนิสิต.....

สาขาวิชา วิศวกรรมเคมี ลายมือชื่อ อ. ที่ปรึกษาวิทยานิพนธ์หลัก.....

ปีการศึกษา 2555

5370509321: MAJOR CHEMICAL ENGINEERING
KEYWORDS: BACTERIAL CELLULOSE / SODIUM ALGINATE / GELATIN / COMPOSITE FILM / ANTIMICROBIAL/ FOOD PACKAGING

SUTASINEE SEETABHAWANG: DEVELOPMENT OF BACTERIAL CELLULOSE/ ALGINATE/GELATIN FILM FOR FOOD PRESERVATIVE.
ADVISOR: ASSOC. PROF. MUENDUEN PHISALAPHONG, Ph.D., 141 pp.

In food packaging, composite films containing antimicrobial agents have attracted much interest because it could enhance food quality and prevent food from foodborne pathogens during storage. The application of biopolymer composites with natural antimicrobial properties for food packaging could be an efficient way for extending shelf life as well as increasing health and environmental safety. In this study, the bacterial cellulose (BC)-sodium alginate (A)-gelatin (G) or BAG composite films was fabricated by casting and curing at room temperature. Three compounds, glycerol, tannic acid and mangosteen ethanolic extract were added to improve the film properties. Glycerol as a plasticizer was added to improve the plasticity and prevent shrinkage of films after drying process. Tannic acid was used as an eco-friendly cross linking agent with anti-microbial properties. Mangosteen ethanolic extract was used as an antimicrobial agent. The modified films were characterized for physical, chemical, mechanical and antimicrobial properties. Based on physical and mechanical properties, the optimal composition of the BAG film was at the ratio of BC/A/G equal 60/20/20 (by weight). The optimal weight ratio of glycerol to gelatin solution was at 2:10. The BAG plasticized with glycerol (MBAG) has superior flexibility, elongation at break and chemical stability. The optimal weight ratio for tannic acid to gelatin solution was at 10 mg per gram gelatin solution. The film of MBAG containing tannic acid (MBAGT) has a denser packed sheet structure, resulting in reduced water absorption capacity and oxygen permeability. The addition of Tannic acid had positive effect on plasticizer properties of MBAGT films in wet state and helped to increase EB of the re-swollen films. The mangosteen ethanolic extract incorporated in the composite films provided the MBAGTM films with a smoother surface. The addition of the extract enhanced the thickness as well as the inter-space of the sheet layers of the films. The results of MIC showed that mangosteen ethanolic extracted and tannic acid could inhibit bacteria in food; *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes* and *Staphylococcus aureus* and the mangosteen ethanolic extract had higher antibacterial activities compared to tannic acid. The results revealed that the MBAGTM film has good potential to be used in food packaging.

Department: ……Chemical Engineering…. Student's Signature ……..……
Field of Study: ……Chemical Engineering…. Advisor's Signature ……..……..
Academic Year: ……2012……...

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CHAPTER I
INTRODUCTION

1.1 Introduction

To enhance quality and food safety, researches on antimicrobial food packaging have been developed in order to prevent food from foodborne pathogens during storage. For exportation, extending shelf-life for food products is very important. Currently, the application of natural antimicrobials for food packaging to inhibit microbial growth in food while maintaining quality, freshness and safety has been an area of interest. To reduce impact of non-biodegradable materials on environment, biopolymer films are attractive for food packaging applications so as to reduce pollution of traditional plastic films. Biopolymer films could be prepared from proteins (gelatin, casein etc.), polysaccharides (cellulose, starch, alginate etc.) or their combination.

Bacterial cellulose (BC) is a linear polysaccharide of glucose units, which can be biosynthesized by *Acetobacter xylinum* using glucose as a substrate (Mühlethaler, 1949). BC has many advantage properties such as high water holding capacity, hydrophilicity, superior water resistance and high mechanical strength. It is composed of ultrafine-fibers stacked in a stratified structure with high purity, high biocompatibility and non-toxicity. However, it exhibits poor rehydration ability after drying due to high crystallinity (Lin et al., 2009).

Sodium alginate is a linear polysaccharide copolymer of (1-4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) monomers, which can be isolated from algae and seaweed. It forms well-characterized hydrogel with water resistance by adding divalent cations as crosslinked agents such as $CaCl_2$ under physiological conditions (Nwe et al., 2010). Calcium cross-linked sodium alginate hydrogels have been used in both biomedical and food applications. It is non-toxic, safety and high biocompatibility. Moreover, sodium alginate is also used in the physical form of a hydrogel with small pores in nanometer size scale.

Gelatin is a water soluble protein, which is produced by hydrolysis of animal collagen. It is unique among hydrocolloids in forming thermo-reversible structure. However, it exhibits poor mechanical properties such as brittleness and fragility. Due to its hydrophilic nature, gelatin has poor water resistance when exposes to wet and humid conditions (Sobral et al., 2001). Gelatin could be added to provide the necessary workability to composite packaging film.

The dehydration of these gelified structures produces strong cohesive films that require plasticizers. The addition of plasticizer leads to a decrease in intermolecular forces along polymer chains which improves the flexibility and chain mobility. Plasticizers could reduce intermolecular hydrogen bonding while increasing intermolecular spacing (Audic and Chaufer, 2005). Therefore, it can increase film flexibility, decrease brittleness and avoid shrinking during handling and storage. With the addition of plasticizers, films are easier to be peeled from the support during manufacture (Guilbert et al., 1996). The selection of plasticizer for specified system normally depends on the compatibility and permanence of the plasticizer (Cheng et al., 2006). The more commonly used plasticizers in edible carbohydrate based films are polyols, mainly glycerol and sorbitol (Yang and Paulson, 2000). Glycerol and sorbitol were the most used gelatin plasticizers (Thomazine et al., 2005). Plasticizing effect of glycerol on alginate-based film was reported (Da Silva et al., 2009).

Glycerol (syn. Glycerine) is a simple polyol compound. It is a colorless, odorless, viscous liquid that is widely used in pharmaceutical formulations. Glycerol has three hydroxyl groups that are responsible for its solubility in water and its hygroscopic nature. The glycerol backbone is central to all lipids known as triglycerides.

Sorbitol is a sugar alcohol, which the human body metabolizes slowly. It is found in apples, pears, peaches and prunes or is synthesized by sorbitol-6-phosphate dehydrogenase and then converted to fructose by succinate dehydrogenase and sorbitol dehydrogenase.

Chemical and physical treatments can be applied to modify the polymer network through cross-linking of proteins to introduce stable covalent bonds between protein segments (Gerrard, 2002). The cross-linking agents include aldehydes, genipin, some enzyme and phenolic compounds. Aldehydes have toxicity which may

not be tolerable in many fields and both genipin and some enzyme have high cost limits. A potential alternative cross-linker is phenolic compound (Bigi et al., 2002; De Carvalho et al., 2004).

Tannic acid (syn. Tannin) is one of hydrolysable tannin of phenolic compounds. It is soluble in water. Tannic acid can be found in different plant species. It can be extracted from nutgall (chestnut), which contains a glucose linking through ester bonds to an average of nine to ten molecules of gallic acid. Tannic acid could interactive or reactive with proteins, resulting in improvement in gel or film properties of gelatin-based materials (Wu et al., 2001; Chatterjee et al., 2000; Strauss et al., 2004). Due to enormous reducing power of free hydroxyl groups, it has antioxidant property. Owning to protein binding capacity, it causes inhibition of microbial growth and has antimicrobial property. Tannic acid exhibited antimicrobial activity against foodborne pathogens such as *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus* (Akiyama et al., 2001; Taguri et al., 2004).

Mangosteen extract is isolated from all parts of mangosteen (syn. *Garcinia mangostana* Linn. or *G. mangostana*), especially the fruit rind of *G. mangostana.* Mangosteen is a very popular fruit, commonly known as the "Queen of fruits" in Thailand. It contains many complex phenolic compounds such as xanthones, flavonoids, tannins, and other bioactive substances (Phothitirat et al., 2009). α-, β- and γ-mangostins, garcinone E, 8-deoxygartanin and gartanin are the most studied xanthones. α-mangostin is a major compound in xanthone and is usual specified for the quality of mangosteen extract. The mangosteen extract also exhibits antioxidant, antitumoral, antiinflammatory, anti-allergic, antibacterial and antifungal activities (Pedraza-Chaverri et al., 2008).

This work aims to develop the novel films from the blend of bacterial cellulose, sodium alginate and gelatin (BAG). For further application as food packaging materials, the physical and functional antimicrobial properties of the films were improved by incorporating glycerol/ sorbitol as plasticizer, tannic acid as cross-linking and mangosteen ethanolic extract as antimicrobial agent.

1.2 Objectives

The overall objective of this study is to develop the novel films from the blend of bacterial cellulose, sodium alginate and gelatin (BAG) and to improve the functional antimicrobial and physical properties of the films by adding mangosteen extract as antimicrobial agent, tannic acid as crosslinking agent and glycerol/ sorbitol as plasticizer. Specifically, the study aimed:

1. To develop bacterial cellulose/sodium alginate/gelatin (BAG) films containing ethanolic extracts of *G. mangostana*
2. To investigate the effects of the blend compositions, type of plasticizers, crosslinking agent content and antimicrobial agent content on the film characteristics

1.3 Research scopes

1. Fabricate bacterial cellulose/sodium alginate/gelatin (BAG) composite films by casting and curing at room temperature.
2. Study effect of plasticizer content (glycerol/ sorbitol) on BAG films.
3. Study effect of crosslinking agent content (tannic acid) on BAG films.
4. Study effect of antimicrobial agent (ethanolic extracts of *G. mangostana*) on BAG films.
5. Examine release characteristics of ethanolic extracts of *G. mangostana* from BAG films.
6. Characterize physical properties of BAG films by
    a. Scanning Electron Micrographs (SEM) for preliminary investigation of morphology
    b. Universal testing machine for determination of mechanical properties of films
    c. Oxygen permeation tester for measuring oxygen transmission rate (OTR)
    d. Water vapor permeation tester for measuring water vapor transmission rate (WVTR)

e. Water absorption capacity (WAC)

7. Characterize the chemical properties of BAG film by
   a. Fourier transform infrared (FT-IR) spectrometer for identifying chemical structure
   b. X-ray diffraction (XRD) for determination of crystallinity (%)
8. Characterize the biological properties of BAG film by
   a. Antibacterial activity
   b. Antifungal activity

1.4 Overview

This thesis is organized as the list below. Chapter II shows all background, theory and literature reviews relating to this study. First, cellulose, bacterial cellulose (BC), alginate, gelatin, glycerol, D-sorbitol, tannic acid and mangosteen ethanolic extract are described in terms of properties, sources, and applications. Then packaging usage for food, in term of definition, and characteristics are reviewed. Finally, the previous modification of BC and comparison of its properties with those of the native BC are reviewed.

Chapter III presents the experimental design in term of materials and methods. First, Chemical and Equipment lists are shown. Then the preparation of BAG composite films and the modification were explained. Finally, the methods for characterization are revealed.

Chapter IV presents the characteristics of bacterial cellulose/alginate/gelatin composite films (BAG) and all of modified BAG films.

Chapter V presents the conclusion and recommendations.

CHAPTER II
THEORY AND LITERATURE REVIEW

2.1 Bacterial cellulose

Cellulose is the major biopolymer of earth and tremendous economic importance globally. It forms the basic structure matrix of cell walls of all plants, many fungi and some algae.

Apart from plants, certain bacteria, algae and fungi produce cellulose as well. Among the cellulose-forming bacteria, *Acetobacter* strains (reclassified as the genus *Gluconacetobacter*) are especially suitable for the formation of cellulose. They are not pathogenic and can procure glucose, sugar, glycerol, or other organic substrates to convert them into pure cellulose.

Acetobacter xylinum (*A. xylinum*) is a simple Gram-negative bacterium which has an ability to synthesize a large amount of excellent quality of cellulose formed as twisting ribbons of microfibrillar bundles (Czaja et al., 2006). A single cell of *Acetobacter* has a linear row of pores from which glucan chain polymer aggregates are spun as shown in Figure 2.1.

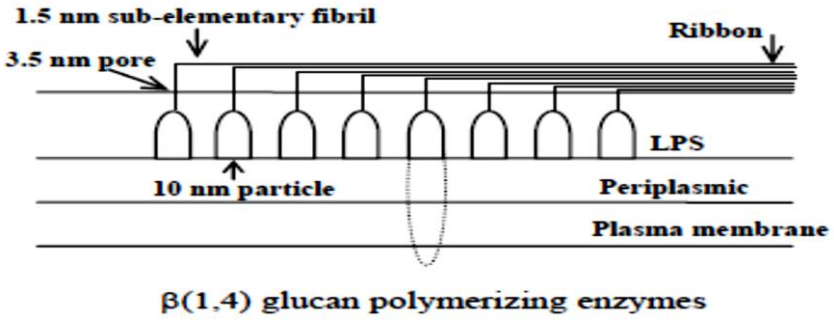


Figure 2.1 Scheme for the formation of bacterial cellulose (Jonas and Farah, 1998).

Bacterial cellulose is an organic compound with the formula $(C_6 H_{10}O_5)_n$ as shown in Figure 2.2**,** which is the repeating unit of D-glucose joined by β-1,4-glycosidic linkages (glucan) as polysaccharides as carbohydrate. Bacterial cellulose is of particular importance owing to its unique structure which is quite different from the common synthetic polymers. Formed by repeated connection of glucose building blocks, the highly functionalized, linear stiff-chain homopolymer is characterized by its hydrophilicity, chirality, biodegradability, and broad chemical-modifying capacity (Klemm et al., 2005). Therefore, it is an insoluble structure and most organic solvents.

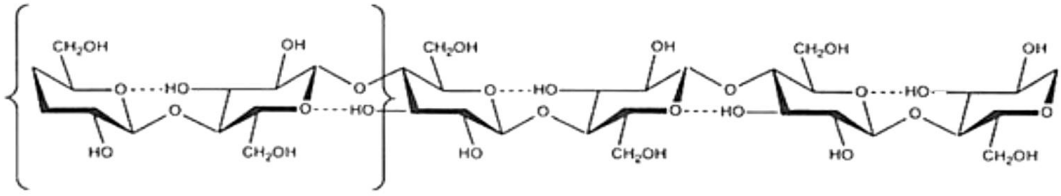


Figure 2.2 Structure of bacterial cellulose (Brown, 1996).

When cellulose molecule is completely extended, its chain resembles a flat ribbon with hydroxyl groups extending laterally from the edges. This molecular structure is also the basis for extensive inter-and intra-molecular hydrogen bond networks forming semi-crystalline fiber morphologies. The hydrogen atoms oriented above and below the plane of the ribbon and are thus hydrophilic. This structure allows for Van der Waals interactions between the hydrogen atoms as shown in Figure 2.3. The properties of cellulose are determined by the supra-molecular order and specific assembling, these again being controlled by the origin and treatment of the cellulose.

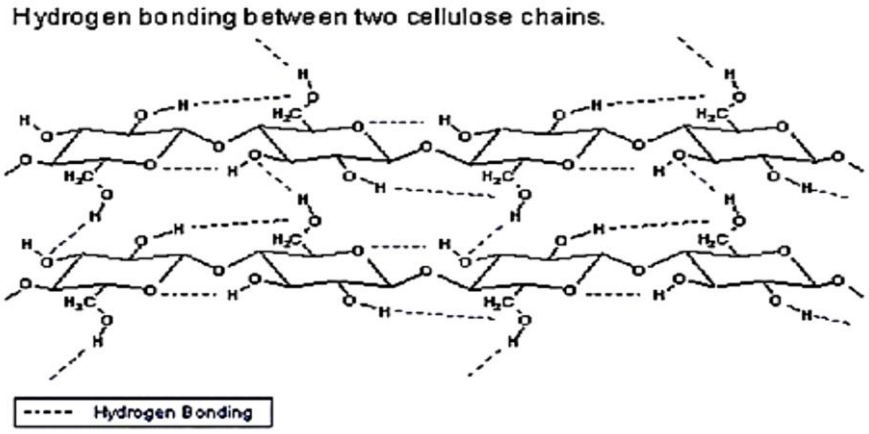


Figure 2.3 Hydrogen bond system of the cellulose

Moreover, macroscopic morphology of BC strictly depends on culture conditions. In static conditions, bacteria accumulate cellulose mats on the surface of nutrient broth at the oxygen-rich air-liquid interface. The subfibrils of cellulose are continuously extruded from linearly ordered pores at the surface of the bacterial cell, crystallized into microfibrils and forced bons to forming parallel but disorganized planes.

2.1.1 Properties of cellulose nanofibrils produced by bacteria

The cellulose nanofibrils produced by bacteria are 3-8 nm in diameters and the entangled mesh of these fibrils produces a white gelatinous membrane known as a pellicle. This membrane of pure cellulose and cells entrapped within it could be cleaned and dried and the product used for many exciting new applications.

In terms of the molecular formula, BC is identical to cellulose of plant origin apart from alien groups such as carbonyl and carboxyl units in the latter as a result of the plant cellulose processing. But important structural features and properties significant for practical application of BC are quite different from wood cellulose:

- BC is high purity
- BC is high degree of polymerization (up to 8000)
- BC is high crystallinity (of 70 to 80%)

- BC is high water content (to 99%)
- BC is high mechanical stability

Due to these reasons, BC provides significant advantages over plant cellulose including:

- BC has finer and more complex structure.
- BC is composed of pure cellulose, no need to remove hemicellulose or lignin.
- BC has longer fiber length so it is much stronger.
- BC can be grown to virtually any shape.
- BC can be produced on a variety of substrates.

One of the unique features of this pure cellulose membrane is that it is very strong in the never dried state and it could hold hundreds of times its weight in water. This great absorptivity and strength constitute two of the many novel features of microbial cellulose.

In addition, the size of BC fibrils is about 100 times smaller than that of plant cellulose as shown in Figure 2.4.

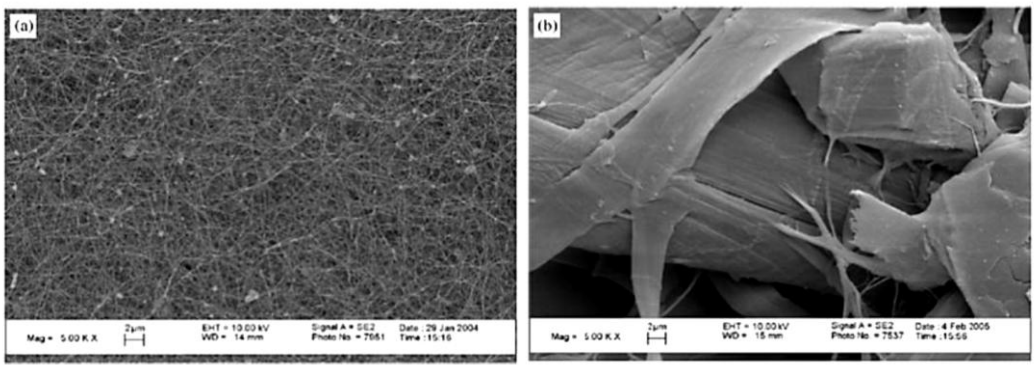


Figure 2.4 A comparison of microfibrillar organization between BC (a) and wood pulp (b) (Czaja et al., 2006).

2.1.2 Application of BC as film packaging

Among available packaging materials, cellulose-based products have attracted increasing interest due to their edibility, biodegradability and potential. Moreover, bacterial cellulose can be altered to suit many potential commercial applications due to physical properties such as hydrophilicity, tensile strength and high purity.

The specific application of bacterial cellulose as a dialysis membrane was examined by Shibazaki et al. (1993). Bacterial cellulose film showed a significantly higher permeation rate and a greater molecular weight cut-off when compared to a commercial dialysis membrane (regenerated cellulose membrane, RBC). Similar observations were reported by Shanshan et al. (2012) prepared cellulose films from solution of bacterial cellulose in NMMO (regenerated BC films). It found that RBC films had better mechanical and barrier properties, and the thermal stability was similar to that of the native BC

Therefore, the additional benefit of the bacterial cellulose film compared to the regenerated cellulose membrane was that the added mechanical property allowed the use of a thinner material.

Currently, BC based materials have been modified for applications as antimicrobial film. For example, BC films were developed to control *L. monocytogenes* and other bacteria on the surface of frankfurters as models for higher value meat products by containing nisin (Nguyen et al., 2008) and to control release of sorbic acid (Jipa et al., 2012a), vanillin (Stroescu et al., 2013) and potassium sorbate (Jipa et al., 2012b) as antimicrobial agent for antimicrobial food packaging material. Biodegradable packaging and edible films also gain much more interesting worldwide. Biodegradable food packaging materials from poly (vinyl alcohol) and BC was investigated in order to use them as food packaging materials and also irradiated to avoid microbial recontamination (Stoica-Guzun et al., 2013).

2.2 Sodium alginate

Sodium alginate is water soluble sodium salt of alginic acid. It is a linear polysaccharide copolymer of (1-4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) monomers with the formula $(C_6H_7O_6Na)_n$ as shown in Figure 2.5. It can be isolated from the cell wall of brown algae and brown seaweed, and its form as gum. Sodium alginate is widely used in food and pharmaceutical industries because of non-toxic polysaccharide.

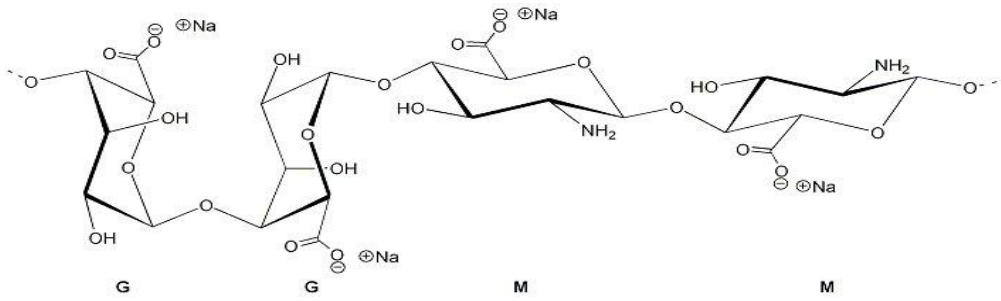


Figure 2.5 Structure of sodium alginate

2.2.1 Properties of sodium alginate

Sodium alginate forms well-characterized hydrogel with water resistance. It is recognized as a cold gelling agent that needs no heat to gel by adding divalent cations as crosslinked agents. The gelation and crosslinking of the polymers are mainly achieved by the exchange of sodium ions from the guluronic acids with the divalent cations, and the stacking of these guluronic groups to form the characteristic 'egg-box structure' (Gombotz and Wee, 1998).

Generally, alginate that coordinated to sodium is a very flexible chain. When sodium is replaced by calcium ion which is circle dots in the image below coordinates to two alginate chains, linking them together. The flexible chains become less flexible and form a huge network–a gel. Mechanism of sodium alginate and calcium ions (which holds a charge of +2) knocks away two sodium ions (each holding a charge of

+1). The alginate molecule contains loads of hydroxyl groups (-OH) that can be coordinated to cations-that is ions with a positive charge such as sodium and calcium (Draget et al., 2005) as shown in Figure 2.6.

Alginate gel is of interest since alginate is able to use for viscosity tuning. The gel can be formed without any heating and the gel network still remains through freeze and thaw cycles. Its gel is thermally stable and therefore continues to provide functionality even when food is heated. It is also effective at both highly acidic and neutral pH levels and it is no thermo-reversible as gelatin.

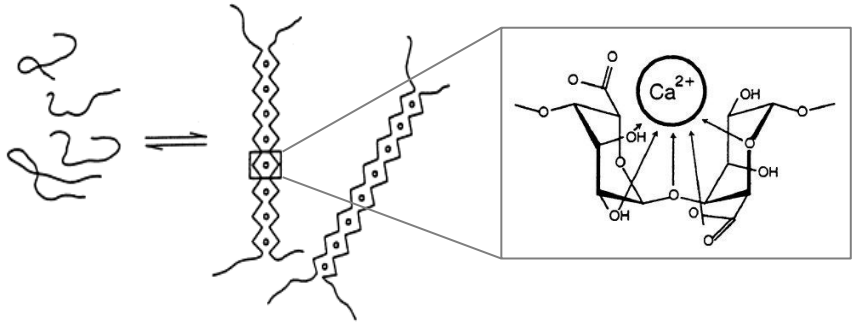


Figure 2.6 Schematic representation of the egg-box association of the poly-L-guluronate sequences of alginate crosslinked by calcium ions. The figure shows conversion of random coils to buckled ribbon like structures which contain arrays of Ca^{2+} ions. The magnitude figure shows the proposed stereochemistry of Ca^{2+} ion complexation. The oxygen atoms involved in the coordination sphere are shown as filled circles (Rees, 1981, modified).

Sodium alginate crosslinking will reduce significantly its swelling in the presence of the solvent, resulting generally in a reduction of the permeability of different solutes. Moreover, alginate films exhibit poor moisture barriers due to hydrophilic property, but incorporation of calcium can reduce the water vapor permeability of these films, making them water insoluble (Rhim, 2004).

As with other edible films, behavior of alginate films will depend upon the surrounding relative humidity (RH) as well as the plasticizer. Therefore, analyzing the properties of alginate films under different RH conditions would use different

plasticizers. It is important in determining whether these films are suitable for a specific food.

2.2.2 Application of sodium alginate as film packaging

Alginate is of interest as a potential biopolymer film or coating component because of its unique colloidal properties, which include thickening, stabilizing, suspending, film forming, gel producing, and emulsion stabilizing. Though edible films prepared from hydrocolloids like alginate form strong films, they exhibit poor water resistance because of their hydrophilic nature. The ability of alginate to make strong and insoluble gels with calcium ions can be utilized to improve such properties of alginate films. However, gel formation of alginate with calcium ions is so instantaneous that it might prevent casting to make films in some cases (Rhim, 2004).

Sodium alginate was used for edible films, biodegradable, antimicrobial film or combined them by blending with other compound such as pectin (Galus and Lenart, 2013; Da Silva et al., 2009) and natamycin as antimicrobial agent (Bierhalz et al., 2012).

Carbohydrate based films occurred strong cohesive films in step dehydration. The addition of plasticizer leads to a decrease in intermolecular forces along polymer chains which improves the flexibility and chain mobility. The plasticizers commonly used are polyols, mainly glycerol and sorbitol (Yang and Paulson, 2000).

2.3 Gelatin

Gelatin is a water soluble protein with formula $C_{102}H_{151}N_{31}O_{39}$ as shown in Figure 2.7, which is produced by a controlled hydrolysis of the fibrous insoluble collagen, which is a protein widely found in nature and is the major constituent of skin, bones and connective tissue. The main of fibrous protein gelatins consist of cartilages and skins. Therefore, the source, age of the animal, and type of collagen, are all intrinsic factors influencing the properties of the gelatins.

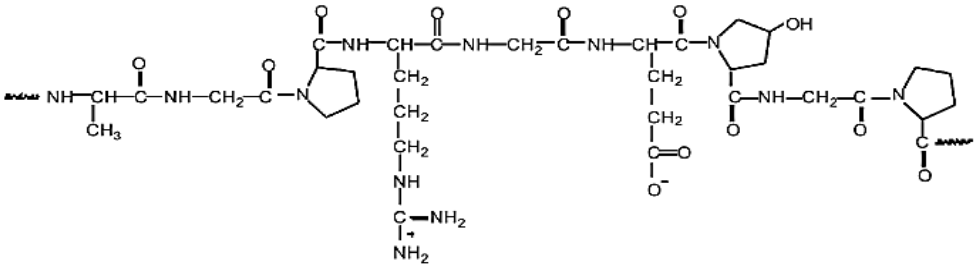


Figure 2.7 Structure of gelatin (Peña et al., 2010)

Gelatin could be divided two type, depending on the pre-treatment procedure and are known commercially as type-A gelatin (isoelectric point at pH 7-9) and type-B gelatin (isoelectric point at pH 4.8-5.2) obtained under acid and alkaline pre-treatment conditions, respectively. It was generally agreed that alkaline processing is more effective than the acid-extraction method.

According to the total gelatin production in 2007, the most abundant sources of gelatin are pig skin (46%), bovine hide (29.4%), pork and cattle bones (23.1%) and fish gelatin was accounted for less than 1.5% (Gómez-Guillén et al., 2009). Most commercial gelatins are made from pork or non-religiously slaughtered beef. It was generally agreed that beef sources carry more of a risk than those from pork and bones carry a higher risk than skins. The main drawback of fish gelatins is that gels based on them tend to be less stable, lower gelling and poorer mechanical properties, and have worse rheological properties than gelatins from land mammals (Fernandez-Diaz et al., 2001).

All of gelatin is unique among hydrocolloids in forming thermo-reversible with a melting point close to body temperature and it is excellent biodegradability and biocompatibility, which is particularly significant in edible, pharmaceutical applications. Moreover, it also could be added to provide the necessary workability to composite packaging film.

Gelatin is particularly attractive for forming hydrogel packaging because it is inexpensive and biodegradable. In addition, its structure facilitates multiple combinations of molecular interactions. Extensive covalent cross-linking during the cooling of set gelation may cause an almost complete loss of thermo-reversibility in the resultant gelatin gel, depending on whether covalent crosslinking occurs predominantly before or after formation of the hydrogen-bonded triple-helix junction zones (Babin and Dickinson, 2001).

Normally, gelatin gel became stiffer when left at room temperature for a few minutes. This phenomenon of gelation can be explained by chemical crosslinking of gelatin molecules which when cooled to lower temperatures will reinforce the physical crosslinks such as ionic and hydrogen bonding interactions in particular. At the optimum pH conditions used for the crosslinking reaction, it could be increased number of carboxylate ions from both the protein and phenolic.

2.3.1 Properties of gelatin

Besides their basic hydration properties, such as swelling and solubility, the most important properties of gelatin can be divided into two groups (Schrieber and Gareis, 2007):

i) Properties associated with their gelling behavior, i.e. gel formation, texturizing, thickening and water binding capacity
ii) Properties related to their surface behavior, which include emulsion and foam formation and stabilization, adhesion and cohesion, protective colloid function, and film-forming capacity

The major properties involving packaging films are below:

2.3.1.1 Gelling properties

Gel formation, viscosity and texture are closely related properties, which vary mainly by the structure, molecular size and temperature of the system. The basic mechanism of gelatin is related to the reverse coil-to-helix transition triggered by cooling solutions below 30 °C. The gelation process for gelatin is thermo-reversible; gelatin gels melt by raising the temperature.

2.3.1.2 Water binding properties

Gelatin chains in solution may be covalently crosslinked to form matrix capable of swelling in the aqueous forming solutions, which are commonly known as gelatin hydrogels. Hydrogels are characterized by hydrophilicity and insolubility in water and swelling to an equilibrium volume while preserving their shape. The chemical cross-linkers are small bifunctional molecules or polyfunctional macromolecules, for instance polyphenolic compound such as tannic acid (Cao et al., 2007; Zhang et al., 2010; Peña et al., 2010; Frazier et al., 2010).

2.3.1.3 Surface properties

Gelatin surface properties are based on the presence of charged groups in the protein side chains and on certain parts of the collagen sequence containing either hydrophilic or hydrophobic amino acids. Both hydrophobic and hydrophilic parts tend to migrate towards surfaces. Hence, reducing the surface tension of aqueous systems and forming the required identically charged film around the components of the dispersed phase which could be additionally strengthened by gel formation.

2.3.1.4 Film-forming properties

Gelatin-based biodegradable materials for food packaging or biomedical applications is focused on developing films with improved mechanical and water resistance properties by combining gelatin with biopolymers with different characteristics, such as polysaccharides as bacterial cellulose (Nakayama et al., 2004; Lin et al., 2009), chitosan (Arvanitoyannis et al., 1998), new hydrophobic or hydrophilic plasticizers (Andreuccetti et al., 2009; Cao et al., 2009), as well as cross-linking agents such as phenolic compound as tannic acid (Zhang et al., 2010; Peña et al., 2010; Deaville et al., 2007).

The conformational state of dehydrated gelatin films obtained by casting method differs when the solvent is evaporated at room temperature or lower or at temperatures above 35 °C (cold- and hot-cast films, respectively). At room temperature, a helical structure is obtained. At temperatures above 35 °C, the conformation of a statistical coil is obtained and films are typically more brittle than cold-cast films and do not shows the helix-coil transition temperature (Fakirov and Bhattacharyya, 2007). The tightly bounds (hydrogen bonds and hydrophobic interactions) presented in gelatin structure and the polar groups of amino acids result in brittle materials in dry state with high moisture absorption (Karnnet et al., 2005).

2.3.2 Application of gelatin as film packaging

Gelatin films or Protein-based films were reported to have better oxygen barrier properties but poor water barrier due to their hydrophilic nature with other types of films. Gelatin films may serve as gas and solute barriers, thereby improving the quality and shelf life of muscle foods. Despite these successes, gelatin lacks strength and requires a drying step to form more durable films. Moreover, gelatin was widely used to prepare edible and biodegradable films for food packaging with plasticizer addition. For improved properties, gelatin was combined with polysaccharide compounds such as sodium alginate (Dong et al., 2006) and starch (Veiga-Santos et al., 2007).

In drying processes, it was found that plasticizers affected the quality of the formed films because dehydration may produce brittle films. Thus, plasticizers must be added to reduce inter-chain interactions improving film flexibility.

Currently, Meat industry uses collagen films during the processing of meat products. When heated, intact collagen films can form a “skin” or edible film that becomes an integral part of the meat product (Cutter, 2006). These commercially available collagen films have been purported to reduce shrink loss, increase permeability of smoke to the meat product, increase juiciness, allow for easy removal of nets after cooking or smoking, and absorb fluid exudates. Protein coatings derived from collagen also have been used to reduce transport of gas and moisture in meats (Baker et al., 1994; Gennadios, 2002).

Additional studies have demonstrated that gelatin could be used to carry antioxidants to reduce oxidation, enhance color stability, to retain flavor, taste and aroma of foods during refrigerated or frozen storage.

2.4 Plasticizers

Polyols are the most used of plasticizers that are widely used in hydrocolloid-based films or protein-based materials due to their ability to reduce intermolecular hydrogen bonding while increasing intermolecular spacing (Audic and Chaufer, 2005). The composition, size and shape of a plasticizer as well as its compatibility could affect the interactions between the plasticizer and the polymer, including its ability to attract water to the plasticized protein films (Sothornvit and Krochta, 2001). Moreover, plasticizers with characteristics such as small size, high polarity, more polar groups per molecule, and greater distance between polar groups within a molecule generally impart greater plasticizing effects on a polymeric system.

The selection of a plasticizer for a specified system is normally based on the compatibility between plasticizer and protein, permanence of the plasticizer in the film, the amount necessary for plasticization, and the desired physical properties of the films (Cheng et al., 2006). Sorbitol, glycerol or the combination of sorbitol and glycerol are generally used as plasticizer for gelatin-based films (Thomazine et al., 2005; Jongjareonrak et al., 2006).

2.4.1 Glycerol

Glycerol (*syn.* glycerine or glycerin) is a simple polyol compound with formula $C_3H_8O_3$ as shown in Figure 2.8. It can be obtained by the saponification of natural fats and oils or by the fermentation of glucose. Glycerol consists of a propane molecule attached to three hydroxyl (OH) groups that are responsible for its solubility in water and its hygroscopic nature. The glycerol backbone is central to all lipids known as triglycerides. Glycerol is a colorless, odorless and viscous liquid with sweet-tasting and low toxicity. It is widely used in food industry and pharmaceutical formulations.

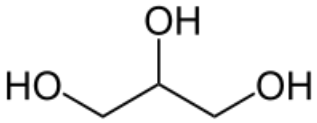


Figure 2.8 Structure of glycerol

2.4.2 Sorbitol

Sorbitol is a polyol (sugar alcohol) with formula $C_6H_{14}O_6$ as shown in Figure 2.9. It is found naturally in a number of fruits, including apples, pears, peaches, and prunes. It can be obtained by reduction of glucose, changing the aldehyde group to a hydroxyl group or synthesized by sorbitol-6-phosphate dehydrogenase, and converted to fructose by succinate dehydrogenase and sorbitol dehydrogenase. Sorbitol can be metabolized slowly in human body.

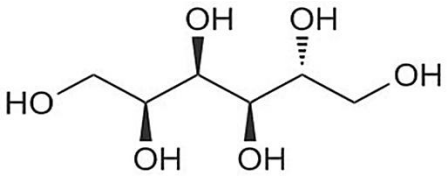


Figure 2.9 Structure of sorbitol

2.5 Tannic acid

Tannic acid (*syn*. Tannin) is a phenolic compound with the formula $(C_{76}H_{52}O_{46})_n$ as shown in Figure 2.10. Tannic acid extracted from nutgall (chestnut) or bark contains a glucose linking through ester bonds to an average of nine to ten molecules of gallic acid.

Tannic acid is a part of hydrolysable tannin, which is a diverse group of polyphenols that are formed as secondary metabolites in plants. It includes a wide range of oligomeric and polymeric polyphenols. It is usually classified in hydrolysable tannin (*syn*. gallotannin) and condensed tannin (*syn*. proanthocyanidins) (Hagerman and Butler, 1991). Moreover, tannic acid is high water-soluble phenolic compounds, relatively rigid and spherical, and high molecular weights between 500 and 3000 Da (Taguri et al., 2004). Thermal processing could breakdown the ester bonds of a polygalloyls and could enhance hydrolysis of tannic acid.

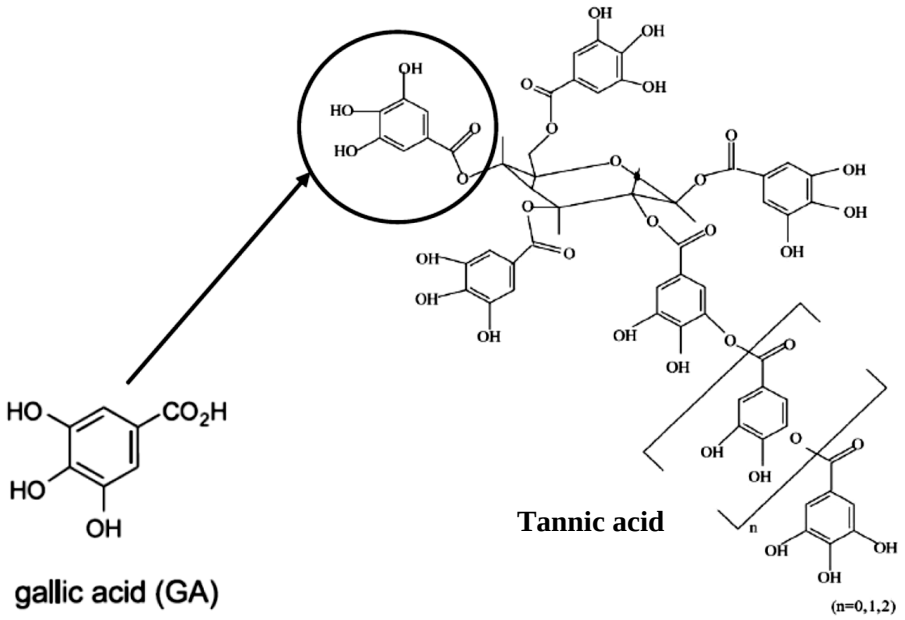


Figure 2.10 The chemical structures of tannic acid and gallic acid

Tannic acid acts like mild acid on the basis of many phenolic-OH groups. High quality tannin contains 65-76% tannic acid. It is present in a variety of plants and fruits and considered as a 'generally recognized as safe' (GRAS) food additive (Akiyama et al., 2001). In addition, tannic acid has several feature properties including antioxidant capacity, astringency properties and anti-allergenic, antiinflammatory, antimicrobial, cardioprotective and anti-thrombotic activities (Balasaundram et al., 2006). Hence, it is a very interesting raw material for the development of green polymeric materials for food packaging and medical applications.

2.5.1 Properties of tannic acid

2.5.1.1 The physical properties of tannic acid

Gelatin-tannic acid films turned from light yellow to brownish as tannic content increased. The transparency of these films was reduced with increasing tannic acid content. Sodium hydroxide (NaOH) also has effect on the transparency of these films, since NaOH can oxidize tannic acid, causing intense yellow colour (Peña et al., 2010).

2.5.1.2 Tannic acid as protein-binding agents

Tannic acid tends to have a strong affinity for proline-rich protein as gelatin (Taylor et al., 2007). Moreover, the conformational flexibility of tannic acid is necessary for strong binding, which is unlikely to be bound to specific ligand binding sites on the protein (Deaville et al., 2007). Tannic acid-protein interactions were suggested as a surface phenomenon in which tannic molecules effectively coat the surface of the protein (Spencer et al., 1988).

The combination of polyphenol-protein was the duple functions of hydrogen bonds and hydrophobic interaction. The mechanism of tannic acid-gelatin (protien) precipitation or tannic acid combining with protein was suggested as following:

In the first stage, tannic acid which contained hydrophobic groups such as galloyl group entered into hydrophobic district of gelatin by hydrophobic reaction to form stable soluble tannic acid-gelatin complexes (Yi et al., 2006; Hagerman, 1992). These were confirmed by absorption peak of tannic at 280 nm shifts toward longer wavelength from the UV-visible absorption spectra (Cao et al., 2007). Moreover, the phenolic hydroxyl groups of the tannic acid molecules were able to enter the hydrophobic areas of the gelatin resulting in a strong hydrophobic interaction.

In the second stage, phenolic hydroxyl groups of tannic acid combined with polar groups of gelatin (amide carbonyl of the peptide backbone) under alkaline conditions to form covalent C-N bonds and generated cross-linked networks by hydrogen bonds. When the degree of combination was suffice, the complex precipitated from the solution. This stage was dominated by less specific binding and aggregation (Frazier *et al*., 2003).

The mechanism could be expressed as follows:

Tannin acid solution + protein solution

↔ [Tannin acid m · protein] solution ↔ [Tannin acid n · protein] precipitate

The reaction was reversible and alkali could make the complex reversed to polyphenol and protein. Continuously increased protein could result in more cross-linking between tannic acid and protein molecules (CaO et al., 2007).

Hydrophobic interaction was the driving influence in the reaction between tannic acid and gelatin. Hydrophobic interactions between tannic acid and protein contribute to the formation of complexes but are considered far weaker than hydrogen bonding. Moreover, larger molecular weight tannic acid is better protein precipitants than smaller tannic acid and the ratio of tannic acid to protein also affects precipitation in vitro (Hagerman and Klucher, 1986).

In the other hand, the polyphenolic structure of tannic acid enabled it to interact by hydrogen bonding with the polar groups of the gelatin, such as peptide, carbonyl, and guanidine groups. Hydrophobic areas were formed by tannic acid combining with many sites of the protein molecules by hydrogen bonding and then

the complexes of tannic and gelatin began to precipitate (Baxter *et al*., 1997). The formation of covalent cross-links between gelatin and tannic acid is also possible but only under oxidizing conditions in which quinones react with side chain amino groups of peptides (Strauss and Gibson, 2004).

2.5.1.3 Effect of pH to tannic acid binding protein

Tannic acid binds proteins in acidic to mildly basic aqueous solutions, so the pH played an important role in tannic acid-gelatin systems. When the pH varied from 5 to 7, the system was stable and the diameters of the nanospheres were distributed evenly. When the pH was below 4, the solution appeared turbid and began to show flocculent deposits. If the pH was low enough, the tannic acid was easy to condense, leading to an increased molecular weight of the tannic acid, resulting in a stronger ability of gelatin to be precipitated (Yi et al., 2006). When the pH was above 8, tannic would be subject to oxidative hydrolysis.

Thus, hydrogen bonding is greatly decreased and protein precipitation does not readily occur at high pH. Besides molecular size and shape of tannic acid, factors that affect tannic acid-protein interactions have been studied in vitro (Martin et al., 1985).

2.5.1.4 Antimicrobial properties

Numerous studies have suggested that tannic acid has antimicrobial activity against foodborne pathogens. Tannic acid inhibited growth of *Escherichia coli* (Pyla et al., 2010), *Listeria monocytogenes* (Rhode et al., 2006), *Staphylococcus aureus* (Akiyama et al., 2001) and *Clostridium perfringens* (Fernandez-Miyakawa, 2010) etc. The antimicrobial activity of the water fraction was further enhanced when this fraction was thermally processed and it was shown that the thermal treatment of pure tannic acid enhanced the antimicrobial activity by inducing the partial hydrolysis of this compound at an ester linkage between two gallic acids or between gallic acid and polyol (Kim et al., 2010).

2.6 Mangosteen ethanolic extracts

Mangosteen is very famous fruit and has been known as the “Queen of fruits” or “super fruits” in Thailand. It has been interested to be used as active constituents in functional products.

Mangosteen extract is isolated from all parts of mangosteen (syn. *Garcinia mangostana* Linn. or *G. mangostana*). The fruit rind of *G. mangostana*, especially contains many complex phenolic compounds such as xanthones, flavonoids, tannins, and other bioactive substances (Phothitirat et al., 2009).

Mangostins are one of derivative of xanthone obtained by boiling the rind in water. After tannic is removed by exhausting by boiling in alcohol and evaporating, the obtained product is mangostin and resin. The resin is precipitated by re-dissolving it in alcohol and water, and then evaporating the water. It occurs in small yellow scales, tasteless neutral, insoluble in water, but readily soluble in alcohol and ether. Mangostins included α-, β- and γ-mangostins. Mangostin in α-form is a major compound in mangosteen, so α-mangostin could specify quality of mangosteen extract.

The fruit shell contains 7-13% tannin and the seeds contain 3% oil. The rind of the fruit contains tannin, a resin and a bitter principle called mangostins as shown in Figure 2.11. The rind contains 5.5% of tannin, and a resin as well as a yellow crystalline bitter principle. Mangostins contain three compounds including α-mangostin, β-mangostin and γ-mangostin that α-mangostin is a major compound in xanthone and is usual specified for the quality of mangosteen extract.

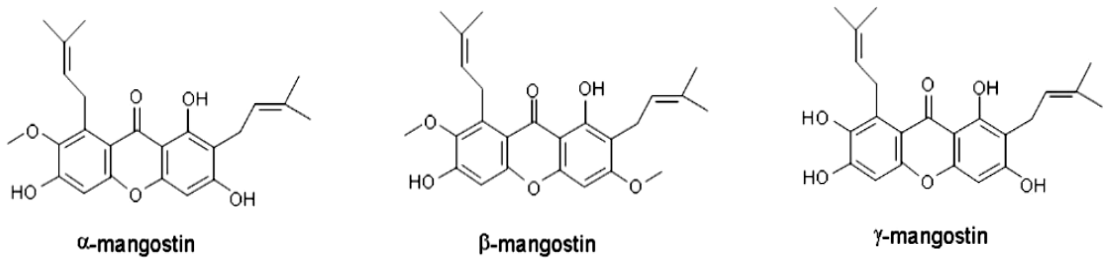


Figure 2.11 Mangostins structure (Chaverri et al., 2008)

2.6.1 Xanthones

The chemical structure of xanthones consists of two benzene rings connected by carbonyl group (C=O) and oxygen. Each ring is conjugated in a fused formation not allowing free rotation on the carbon-carbon bonds. The xanthones backbone is attached to distinct functional groups at benzene ring in various positions. The difference in functional groups and positions affect to specific functionalities or properties of xanthones (Jantaravinid, 2009) as shown in Figure 2.12.

2.6.2 Alpha-Mangostin (α-mangostin)

α-Mangostin, yellow-colored, is the major extracted derivative of xanthones which has demonstrated active antimicrobial activities against gram-positive bacteria including *Staphylococcus aureus* and methicillin-sensitive *Staphylococcus aureus* (MRSA) (Sundaram et al., 1983; Mahabusarakam et al., 1986).

The chemical IUPAC name of α-mangostin is 1, 3, 6-Trihydroxy-7-methoxy-2, 8-diprenylxanthone with formula $(C_{24}H_{26}O_6)_n$ and molecular weight of 410.47 g/mole as shown in Figure 2.11. Its boiling point is about 180-181 ºC. The solubility of α-mangostin in water is very poor, whereas it solutes clearly in organic solvent, for example, ethanol, chloroform and methanol, etc (Phadungkarn et al., 2009).

In addition, Kaomongkolgit et al. have reported that α-mangostin had no cytotoxic effects on human gingival fibroblasts up to 4000 μg/mL (2009). Antibacterial action of α-mangostin was first studied by Nguyen and Marquis. They reported that the antimicrobial action of α-mangostin was from targeting cytoplasmic enzymes (2011). However, the action of enzymetargeted antimicrobials would be expected to require considerable time (Grohs et al., 2003), which seems in contrast to the reported rapid bactericidal action of α-mangostin. The rapid antimicrobial action is suggestive of the antimicrobial action of the natural antimicrobial peptides or peptidomimetics which act on bacterial membrane (Bai et al., 2009; Isaksson et al., 2011).

The stability of α-mangostin from dichloromethanic extraction was studied by Yodhnu et al. (2009). It was found that α-mangostin was stable in these condition:

storage at 80 ºC for 3 h, storage under UV-light at 254 or 366 nm of wavelength for 6 h and under 3 N NaOH solution supplementation followed by heating at 80 ºC for 3 h.

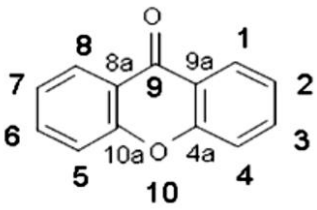
2.6.3 Properties of xanthone involving with antimicrobial including antifungal and antibacterial in food and medicine

2.6.3.1 Antifungal activity

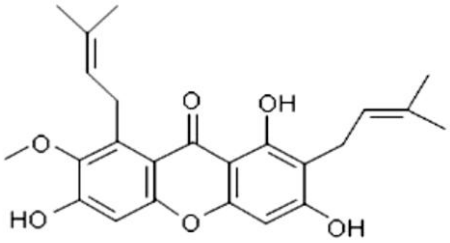
The antifungal activity of several xanthones isolated from fruit hulls of *G. mangostana* and α-mangostin-derivatives could against three phytopathogenic fungi including *Fusarium oxysporum vasinfectum*, *Alternaria tenuis* (*A. alternate*) and *Drechslera oryzae* (*Cochliobolus miyabeanus*) (Gopalakrishnan et al., 1997).

Epidermophyton floccosum, *Alternaria solani*, *Mucor* sp., *Rhizupus* sp. and *Cunninghamella echinulata* were highly susceptible to xanthones, whereas *Trichophyton mentagrophytes*, *Microsporum canis*, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* sp., *Fusarium roseum* and *Curvularia lunata* were only moderately susceptible to them (Sundaram et al., 1983).

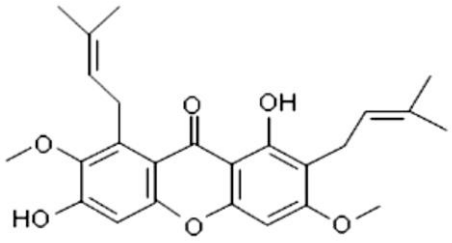
In addition, the activities of mangostin, gartanin and γ-mangostin against *Candida albicans*, *Cryptococcus neoformans*, *T. mentagrophytes* and *Microsporum gypseum* were tested. All of the components showed moderate activities against *T. mentagrophytes* and *M. gypseum* but exhibited no activity against *C. albicans* and *C. neoformans* (Mahabusarakam et al., 1986).



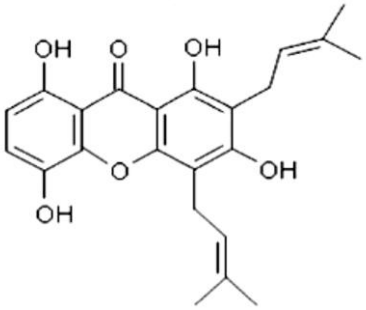
Xanthone nucleus



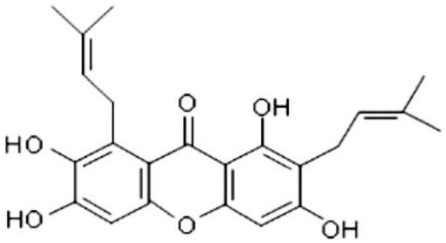
α-mangostin



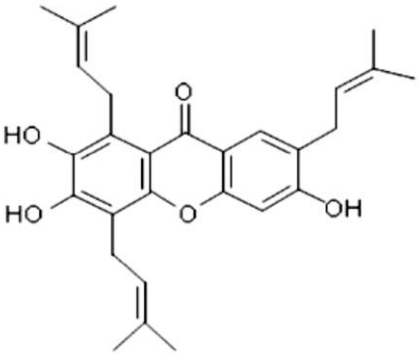
β-mangostin



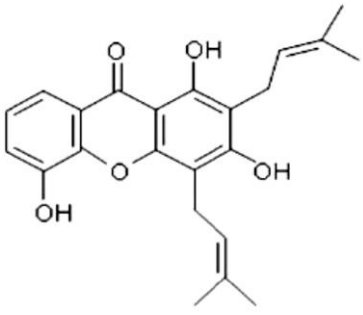
Gartanine



γ-mangostin



Garsinone E



8-deoxygartanine

Figure 2.12 Xanthone nucleuses with IUPAC numbers of carbons and chemical structure of the most studied xanthones (Chaverri et al., 2008)

2.6.3.2 Antibacterial activity

α-Mangostin was reported to have activity against normal, methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* (MRSA) (Mahabusarakam et al., 1986; Iinuma et al., 1996) and *S. aureus, P. aeruginosa, Salmonella typhimurium* and *Bacillus subtilis* were highly susceptible to xanthones, whereas *Proteus sp., Klebsiella sp.* and *Escherichia coli* were only moderately susceptible to them (Sundaram et al., 1983).

The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. MIC is important to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. A lower MIC is an indication of a better antimicrobial agent. Another, the minimum bactericidal concentration (MBC) is the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on to antibiotic-free media.

The order of the efficacy determined by the MIC (mg/ml) showed that α-mangostin and most of xanthone derivatives could have activities against bacterial strain.

2.7 Gelatin-Bacterial cellulose Blends

Gelatin is rarely used alone because of its low intensity and high brittleness and being often used after modification through several methods. Blending of polymer is one of the simplest methods to obtain a variety of physical and chemical properties from the constituent polymer at a molecular level. The gain in newer properties depends on the degree of compatibility or miscibility of the polymer.

The efficacy of cross-linked gelatin-based sponges composed of gelatin and BC (polysaccharides) for food material and food packaging was reported. BC nano-composites films by using gelatin and its enzymatically modified form (EMG) could enhance the rehydration abilities properties of BC. Due to the polar functional groups

of gelatin and EMG as well as BC's porous networks with lower level of crystallinity contributed to the rehydration ability of composites (Lin et al., 2009) and gelatin crosslinking disrupted the crystallization formed from the hydrogen bonds between cellulose molecules. Crosslinking can also enhance the rehydration ratio (Nakayama et al., 2004).

Moreover, alkaline treated BC/gelatin composites (ATBC/G) crosslinked with EDC (ATBC/G/E) could improve the mechanical strength and hydrophilic property of BC composites. While increased gelatin concentrations in addition to the EDC treatment decreased crystallinity in the composites. The FT-IR spectrograph of the ATBC/G composites revealed that the OH groups of the composites tended to increase (Chang et al., 2012).

2.8 Gelatin cross-linked by tannic acid

The crosslinking methods can be classified as physical crosslinking and chemical crosslinking. Physical treatments such as UV-and -radiation could induce crosslinked gelatin gel with weak interaction, whereas chemical agents such as aldehydes could cause crosslinking between the amino acid chains of gelatin with strong interaction. However, these agents have high toxicity and might contaminate the product. Therefore, the natural crosslinking agent such as genipin, ferulic and tannic acid has been used as crosslinking agent for gelatin (Bigi et al., 2002; Cao et al., 2007).

Tannic acid could be a suitable crosslinking agent for gelatin to be used in packing as edible films. Gelatin-tannic acid (GT) film has decreased swelling ratio and solubility, since the presence of tannic reduced the water affinity of gelatin (Peña et al., 2010). However, no obvious effect was detected on water vapor permeability of the film. Mechanical and thermal behaviors varied as a function of the tannic acid content. Moreover, it was found that the properties of the films treated by tannic acid became better after being stored for more than 90 days (Cao et al., 2007). In addition, hydrogen and/or hydrophobic interactions between gelatin and tannic molecules induce changes in the formation of triple helix in gelatin and surely reduced the

mobility of the side chains, as reflected in an increment of the glass transition temperature of GT films.

Cross-linking reactions between gelatin and tannic acid also involved with tannic acid (TA) content. At a low TA content, the crosslinking effect was predominant and the cross-linked structure was stable even under boiling. Both the rigidity of the protein matrix increased and the mechanical properties of the GT films were enhanced. At higher TA content, grafting and branching reactions between gelatin and TA were enhanced, whereas some amount of TA molecules not involved in cross-linking (Zhang et al., 2010). Therefore, the small amount of TA is sufficient to crosslink gelatin in GT film. Addition of TA in powder form is better than TA solution.

2.9 Tween-80 (Polysorbate 80)

Tween-80 is a nonionic surfactant and emulsifier derived from polyethoxylated sorbitan and oleic acid. It is a viscous, water-soluble yellow liquid. The hydrophilic groups in this compound are polyethers, also known as polyoxyethylene groups which are polymers of ethylene oxide.

Tween-80 is often used in food and other products as an emulsifier, and it was added to prevention of phase separation (Brandelero et al., 2012).

2.10 The ethanol extracts

Many solvents were used to extract compound from plants such as DMSO, ethanol, methanol and water. In this work, mangosteen that was extracted by ethanol was applied as natural antimicrobial. Crude mangosteens from the ethanol extracts have better antimicrobial activity than those from water extract and were safety to apply for food packaging. Moreover, mangosteens from ethanol extract was potent in inhibiting bacterial growth of both gram-negative and gram positive bacteria (Mishra et al., 2009).

2.11 The pH and microbial growth

The most of foodborne microorganism suitably grows at approximate pH 7 (6.6-7.5) but some microorganism can grow below pH 4. Generally, bacteria, yeast and fungi widely grow at pH range of 6.0-8.0, 4.5-6.0 and 3.5-4.0, respectively. When pH in a food is reduced below the lower limit for growth of microbial species, the cells not only stop growing but also lose viability. The minimum pH value for the growth of some foodborne bacteria is shown in Table 2.1 and 2.2.

Table 2.1 Reported minimum pH values for the growth of some foodborne bacteria (James et al., 2000, modified)

Foodborne Bacteria	pH
Escherichia coli 0157:H7	4.5
Listeria monocytogenes	4.1
Salmonella spp.	4.5
Staphylococcus aureus	4.0

Table 2.2 Characteristic and survival/growth parameters of pathogenic microorganisms commonly associated and processed meat products (Tarté, 2009, modified)

Organism	pH range	Associated meat products
Gram positive		
Listeria monocytogenes	4.4-9.0	Delicatessen meats, frankfurters, seafood
Staphylococcus aureus	4.4-10.0	Delicatessen meats, meat salads
Gram negative		
Escherichia coli *Salmonella typhimurium*	4.4-9.0	Fresh meat
Molds		
Aspergillus niger	2.0-8.5	Low pH meat

The pH in food can vary to a great extent, depending on types. Foods can be divided into 2 groups as shown in Table 2.3 and 2.4:

1) high-acid foods (pH below 4.6) such as fruits, fruit juices, fermented foods and salad dressings

2) low-acid foods (pH 4.6 and above) such as vegetable, meat, fish, milk and soups

Table 2.3 Approximate pH values of some fresh fruits and vegetables (James et al., 2000, modified)

Product	pH	Product	pH
Vegetables		**Fruits**	
Asparagus (buds and stalks)	5.7–6.1	Apples	2.9–3.3
Beans (string and Lima)	4.6–6.5	Apple cider	
Beets (sugar)	4.2–4.4	Apple juice	3.6–3.8
Broccoli	6.5	Bananas	3.3–4.1
Cabbage (green)	5.4–6.0	Figs	4.5–4.7
Carrots	4.9–5.2; 6.0	Grapefruit (juice)	4.6
Cauliflower	5.6	Grapes	3.0
Celery	5.7–6.0	Limes	3.4–4.5
Corn (sweet)	7.3	Melons (honeydew)	1.8–2.0
Cucumbers	3.8	Oranges (juice)	6.3–6.7
Eggplant	4.5	Plums	3.6–4.3
Lettuce	6.0	Watermelons	2.8–4.6
Onions (red)	5.3–5.8		5.2–5.6
Parsley	5.7–6.0		
Potatoes (tubers and sweet)	5.3–5.6		
Pumpkin	4.8–5.2		
Spinach	5.5–6.0		
Squash	5.0–5.4		
Tomatoes (whole)	4.2–4.3		

Table 2.4 Approximate pH values of dairy, meat, poultry, and fish products (James et al., 2000, modified)

Product	pH	Product	pH
Dairy products		**Fish and shellfish**	
Butter	6.1–6.4	Fish (most species)*	6.6–6.8
Buttermilk	4.5	Clams	6.5
Milk	6.3–6.5	Crabs	7.0
Cream	6.5	Oysters	4.8–6.3
Cheese (American mild and cheddar)	4.9; 5.9	Tuna fish	5.2–6.1
		Shrimp	6.8–7.0
Meat and poultry		Salmon	6.1–6.3
Beef (ground)	5.1–6.2	White fish	5.5
Ham	5.9–6.1		
Veal	6.0		
Chicken	6.2–6.4		
Liver	6.0–6.4		

*Just after death.

From Table 2.1, 2.2, 2.3 and 2.4, it demonstrates that pH of vegetables is more than pH of fruits. Therefore, typically, fresh fruits are subjects to spoilage by yeast and fungi, whereas fresh vegetables are subject to infection by bacterial soft rots. Fish rot faster than meat, because the pH of meat after rigor mortis is approximately 5.6 (pork: 5.3-6.9, beef: 5.1-6.2 and mutton: 5.4-6.7), which are below the pH of fish (6.2-6.5).

CHAPTER III
MATERIALS AND METHODS

3.1 Materials and chemicals

Table 3.1 Main materials

Material	Supplier (country)
Bacterial cellulose pellicles (size 1 cm × 1 cm × 1 cm)	The laboratory of Pramote Thammarate at the Institute of Food Research and Product Development, Kasetsart University, Bangkok
Sodium alginate	Acros, Belgium
Gelatin from porcine skin type B (pI 5.2, 180 g bloom)	Fluka, Norway
Tannic acid (food grade)	Sigma-Aldrich, USA
Mangosteen ethanolic extract with tween-80	Bungon Kietthanakorn at Thai-China Flavours and Fragrances Co., Ltd., Phra Nakhon Si Ayutthaya, Thailand.
Glycerol (99.5% v/v)	Ajax Fine Chem Pty Ltd., Australia
D-sorbitol powder	Sigma-Aldrich, USA

Table 3.2 Minor chemicals

Chemical	Supplier (country)
Calcium chloride dehydrate	Ajax Fine Chem Pty Ltd., Australia
Absolute ethanol	QRec, New Zealand
Sodium hydroxide	Ranken, India
α-mangostin	ChromaDex, USA
Gallic acid	Sigma-Aldrich, China
Folin-Ciocalteu reagent	Sigma-Aldrich, Switzerland

Sodium acetate	Ajax Fine Chem Pty Ltd., Australia
Glacial acetic acid	QRec, New Zealand
Sodium acetate trihydrate	Ajax Fine Chem Pty Ltd., Australia
Sodium chloride	Ajax Fine Chem Pty Ltd., Australia
Potassium chloride	Ajax Fine Chem Pty Ltd., Australia
Di-sodium hydrogen phosphate	Ajax Fine Chem Pty Ltd., Australia
Potassium di-hydrogen phosphate	Ajax Fine Chem Pty Ltd., Australia
Tween-80	Ranken, India

3.2 Equipment

- Petri plate and mold
- Homogenizer (Phillip Cusina)
- Micrometer thickness gage (Mitutoyo, Japan)
- Fourier transform infrared (FT-IR) spectrometer (Perkin Elmer Spectrum One Massachusetts, USA)
- Universal testing machine (Hounsfield H 10 KM, Redhill, England)
- Scanning electron microscopy, SEM (JOEL JSM-5410LV, Tokyo, Japan)
- UV-visible spectrophotometer (Shimadzu UV-2550, Tokyo, Japan)
- Oxygen permeation tester (Illinois Instruments, Model 8000, Johnsburg, IL)
- Water vapor permeation tester (Lyssy L80-4000)
- X-ray diffraction, XRD (Bruker AXS Model D8 Discover,USA)
- Autoclave (Model Tomy Autoclave SS-325, Nerima-ku, Tokyo, Japan)

3.3 Preparation of BC slurry

The BC pellicles was treated with 1.0% (w/v) NaOH at room temperature (about 30 °C) for 24 h to remove bacterial cells and rinsed with deionized water (DI) until the pH was 7.0. Then the purified BC pellicles were crushed and homogenized to form BC slurry by using a homogenizer at ambient temperature.

3.4 Preparation of sodium alginate solution

Sodium alginate of 2% (w/v) was dissolved in distilled water with constant stirring at room temperature to form gel-like solution.

3.5 Preparation of gelatin solution

Gelatin powder from porcine skin (15 g) was hydrated with distilled water at room temperature and heated up to 50±5 °C with constant stirring until completely dissolved to obtain 15% (w/v) gelatin solution.

3.6 **Antimicrobial agent:** Mangosteens from the crude ethanol extract of *G. mangostana* in form of solution containing Tween-80

3.6.1 Quantitative determination of bioactive compounds in the mangosteen ethanolic extract

3.6.1.1 Determination of content of total phenolic compounds

The total phenolic compounds content was evaluated using modified Folin-Ciocalteu procedure (Pothitirat et al., 2009). A standard solution was prepared by dissolving 12.50 mg of gallic acid in 100 ml of absolute ethanol in volumetric flask. Various concentrations of the standard solution were provided at the final concentrations of 0.24, 0.49, 0.98, 1.95, 3.91, 7.81, 15.63, 31.25, 62.50 and 125.00

µg/ml. The mangosteen ethanolic extract sample or the standard solution of 0.4 mL was mixed with 1.6 mL of sodium bicarbonate solution (7.5% w/v) and then was added with 1.0 mL of the Folin-Ciocalteu reagent (previously diluted at 1:10 with deionized water). The mixture was shaken for well-mixing and incubated at room temperature for 30 minutes. The content of total phenolic compounds was calculated and the mean values (n=3) were recorded in grams of gallic acid equivalents (GAE)/100g of the extract by using a UV–visible spectrophotometer (Shimadzu UV-2550, Tokyo, Japan). The absorbance of the mixture and standard solution were measured at 765 nm wavelength.

3.6.1.2 Determination of content of mangostin content

The analytical condition for the determination of mangostin content was modified from the method previously reported by Pothitirat and Gritsanapan (2008). A stock solution of α-mangostin standard was prepared by dissolving 1.88 mg of α-mangostin in 100 ml of absolute ethanol in a volumetric flask. Afterwards, various concentrations of the standard solution were prepared to provide the final concentrations at 14.69, 29.38, 58.75, 117.50, 235.00, 470.00, 940.00 and 1880.00 µg/ml. The absorbance of all samples and the standard solutions were measured at 320 nm by UV–visible spectrophotometer (Shimadzu UV-2550, Tokyo, Japan). The mangostin content was calculated and the mean values (n=3) were recorded in grams of α-mangostin equivalents (AME)/100g of the extract.

3.6.2 Testing of antimicrobial activity of tannic acid and mangosteen ethanolic extract

3.6.2.1 Determination of the minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the extract to inhibit the growth of microorganism (no visible growth). The microorganisms used in this test were as follow: gram negative bacteria

including *Escherichia coli* (*E. coli*) and *Salmonella typhimurium* (*S. typhimurium*), gram positive bacteria including *Staphylococcus aureus* (*S. aureus*) and *Listeria monocytogenes* (*L. monocytogenes*), and fungi as *Aspergillus niger* (*A. niger*).

3.6.2.1.1 The minimum inhibitory concentration (MIC) of tannic acid

The minimum inhibitory concentration (MIC) values for the bacteria were determined by twofold serial micro-dilution assay (Wikler, 2006; Pothitirat et al., 2009). The aqueous tannic acid extract was dissolved in sterile DI water at the concentration of 30.00 mg/ml. The solutions were prepared by the serial doubling dilution with the final concentrations of 30.00, 15.00, 7.50, 3.75, 1.88, 0.94, 0.47, 0.23 and 0.12 mg/ml, respectively using medium as solvent. Then, 1 ml of the diluted extract solutions was transferred to the test tubes containing 1 ml of $1x10^8$ colony forming units (CFU) of tested microorganism. In addition, the control tube contained only microorganism and the medium. After 24 h incubation at 37 °C, the lowest concentration of the extract that showed no visible growth (turbidity = 0) was recorded as MIC. Each experiment was done three duplicate.

3.6.2.1.2 The minimum inhibitory concentration (MIC) of the mangosteen ethanolic extract

Mangosteens from the crude ethanol extract were testified for the antimicrobial activities in the similar procedure. The mangosteen solution containing tween-80 was initially prepared at 5.84 mg/ml DMSO. The diluted solutions were prepared by the serial doubling dilution using medium so as to reduce the effect of DMSO on viability of the microorganism. Then the prepared solutions were tested following the same procedure in Topic 3.6.2.1.1.

3.6.2.2 Determination of the minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) was the lowest concentration of the extract required to kill a particular bacterium. The broth from inhibitory concentration was sub-cultured to agar media without antibiotic and incubated for 24 h at 37 °C. The lowest concentration of the extract that showed no viable cell in the agar media was recorded as MBC. Each experiment was done three duplicate.

3.7 Preparation of BAG films

3.7.1 Preparation of BAG films with/without plasticizers

The BC slurry was mixed with the alginate and gelatin solutions at different ratios to form bacterial cellulose-alginate-gelatin (BAG) blend mixtures. Glycerol and/or sorbitol were added as a plasticizer at a certain concentration ratio (0, 1, 2, 3 g per 10 g gelatin solution). MBAG refers to BAG containing plasticizer. The mixture was thoroughly stirred at 50±5 °C until the homogeneous mixture was formed and subsequently it was poured into polystyrene petri plates and incubated at room temperature for 1 day to form films with an average thickness of 50±10 μm. After that it was cross-linked by an aqueous solution of 1% (w/v) $CaCl_2$ for 1 h and rinsed with distilled water to remove the excess chlorides. The cross-linked gel was air-dried at room temperature and stored in plastic before use.

3.7.2 Tannic acid cross-linked MBAG films

The selected MBAG films were modified by supplement of tannic acid as crosslinking agent for gelatin. The prepared BC slurry was mixed with the alginate and gelatin solutions. Then glycerol at suitable content was added to the mixture to form the blend mixture for the fabrication of MBAG. The mixture was thoroughly

stirred at 50±5 °C until the homogeneous mixture was formed, and subsequently tannic acid was added at a certain amount ratio (0, 5, 10, 15, 20 mg per g gelatin solution). During the tannic acid addition, the pH of the blend mixture was adjusted to 7.0 with 1 N NaOH solution in order to prevent the formation of gel. The film forming solution was maintained at 50±5 °C until the homogeneous mixture was formed, and afterward it was poured into polystyrene petri plates and incubated at room temperature for 1 day to form films with an average thickness of 50±10 µm. After that it was cross-linked by an aqueous solution of 1% (w/v) $CaCl_2$ for 1 h and rinsed with distilled water to remove the excess chlorides and tannic acid. The cross-linked film was air-dried at room temperature and stored in plastic before use. MBAGT refers to MBAG containing tannic acid.

3.7.3 Supplement of mangosteen ethanolic extract as antimicrobial agent

The functional antimicrobial properties of the selected MBAGT film was improved by incorporating manosteen extract. Mangosteen extract was obtained from the crude ethanolic extract of *G. mangostana* in form of solution containing tween-80. The blend mixture for the fabrication of MBAGT was added with the mangosteen extract at concentration ratio of 0, 1, 5, 10% v/v (0, 5.93, 11.72 and 22.80 mg/ml). The mixture was thoroughly stirred at 50±5 °C until the homogeneous mixture was formed, and then it was poured into polystyrene petri plates and incubated at room temperature for 1.5 days to form films with an average thickness of 50±10 µm. After that it was cross-linked by an aqueous solution of 1% (w/v) $CaCl_2$ for 1 h and rinsed with distilled water to remove the excess chlorides and tannic acid. The cross-linked film was air-dried at room temperature and stored in plastic. MBAGTM refers to MBAGT containing the mangosteen extract.

3.8 Characterization of the films

The films were characterized by micrometer thickness gage for investigating thickness, by Fourier transform infrared (FT-IR) spectrometer for identifying the chemical structure, by Swelling tester for estimating water absorption capacity, by universal testing machine for determining Tensile strength and elongation at break, by Scanning electron micrographs (SEM) for investigating morphology, by Oxygen permeation tester for estimating oxygen gas transmission rate (OTR), by Water vapor permeation tester for estimating water vapor transmission rate (WVTR), and by X-ray diffraction(XRD) for estimating %crystallinity.

Moreover, biological characteristic of the films were investigated by antimicrobial activity: antibacterial assay and antifungal assay (disc diffusion method), and testing of amount bacteria in chicken sample.

3.8.1 Film thickness

Film thickness was measured with a micrometer thickness gage (Mitutoyo, Japan) MYL 314 with a sensitivity of 0.01 mm. The film thickness was the average value determined from 10 measurements of each sample.

3.8.2 Transparency

The transparency of the films was determined as previously described by Han and Floros (1997). The film samples were cut into rectangles and placed on the internal side of cuvette. The transmittance of films was determined at 600 nm using a UV–visible spectrophotometer (Shimadzu UV-2550, Tokyo, Japan). The transparency of the films was calculated as follows:

$$Transparency = log\,(T_{600}/x) \quad \textbf{(1)}$$

Where T_{600} is the transmittance at 600 nm and x is the film thickness (mm)

3.8.3 Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectroscopy was used to identify the chemical structure of the films which it is primary used to identify the chemical structure of sample. The FT-IR spectra of the films were measured at wave numbers ranging from 4000 to 400 cm^{-1} by a Perkin Elmer (Spectrum One, Massachusetts, USA) at Scientific and technological research equipment centre, Chulalongkorn University.

3.8.4 Water absorption capacity

Water absorption capacity (WAC) was determined by immersing the pre-weighted of dried BAG films in distilled water at room temperature until equilibration. After that the films were removed from the water and excess water at the surface of the films was blotted out with soft paper. The weights of the re-swollen films were measured. The procedure was repeated until no further weight change was observed. The water content was calculated using the following equation:

$$\%WAC = \frac{(W_s - W_d)}{W_d} \times 100 \quad \textbf{(2)}$$

Where W_s is the weight of re-swollen samples (g); W_d is the weight of dry samples (g). The WAC was the average value determined from 3 measurements.

3.8.5 Mechanical properties

All the films under the study in dry and re-swollen forms were tested for tensile strength and elongation at break. The film samples were cut into strip-shaped specimens of 20 mm width and 10 cm long (70 mm between the grips). The maximum tensile strength and break strain of the film samples were determined with a Hounsfield (London, UK) H10KM universal testing machine. The test conditions

followed ASTM D 882. The tensile strength and break strain were the average values determined from 5 specimens.

3.8.6 Scanning electron microscopy

Scanning electron microscopy (SEM) was examined the surface properties of the films by JOEL JSM-5410LV (Tokyo, Japan) at Scientific and technological research equipment centre, Chulalongkorn University. The BAG films in wet and re-swollen form films were frozen in liquid nitrogen, immediately snapped, vacuum-dried and then sputtered with gold and photographed in a Balzers-SCD 040 sputter coater (Balzers, Liechtenstein). The coated specimens were kept in dry place before experiment. The accelerating voltage was adjusted to 10 kV which is considered to be a suitable condition since too high energy can be burn the samples. The specimens were examined at magnification 200×, 10,000X and 3,500X for overview surface morphology, surface morphology and cross sectional morphology, respectively.

3.8.7 Oxygen permeability

Oxygen gas transmission rate (OTR) of the films with diameter over 13 cm was determined with the oxygen permeation tester: Illinois Instruments (Johnsburg, IL) Model 8000 at Thai packaging centre, Thailand Institute of Scientific and Technological Research. The test condition followed ASTM D 3985-05 oxygen gas transmission rate through plastic film and sheeting using a Coulometric Sensor. The determination of OTR was done at 23 °C and 0% relative humidity (%RH). The films were held in such a manner that it separate two side of test chamber. One side was exposed to a nitrogen atmosphere. Testing was completed when the concentration of oxygen in the nitrogen side was constant.

3.8.8 Water vapor permeability

Water vapor transmission rate (WVTR) of the films with diameter over 13 cm was determined with the water vapor permeation tester: Lyssy L80-4000 at Thai packaging centre, Thailand Institute of Scientific and Technological Research. The test conditions followed ASTM E 398-03 (Reapproved 2009) water vapor transmission rate of sheet materials using relative humidity measurement. The determination of WVTR was done at 38 °C and 90% relative humidity (%RH). The test specimen was sealed to the open mount of test dish containing a desiccant, and the assembly placed in a controlled atmosphere. Periodic weighting was performed to determine the rate of water vapor movement through the specimen into the desiccant.

3.8.9 X-ray diffraction

X-ray diffraction patterns of the BAG films were determined with X-ray diffractometer (Bruker AXS Model D8 Discover, USA) at Scientific and technological research equipment centre, Chulalongkorn University. The operation conditions were as follows: Cu Target, 40 kV Voltage, 40 mA Current, 5-40 degree angle, 0.02 degree increment and scan speed of 0.5 sec/step with VÅNTEC-1 Detector (Super Speed Detector). The degree of crystallinity (%) was calculated by Topas program using the following equation:

$$\textbf{Degree of crystallinity (\%)} = \frac{Crystalline\ area \times 100}{Total\ area\ (Crystalline\ area + Amorphous\ area)} \quad (3)$$

3.8.10 Antibacterial assay

The antibacterial test of the MBAGTM films were examined against gram negative bacteria including *Escherichia coli* (*E. coli*) and *Salmonella typhimurium* (*S. typhimurium*) and gram positive bacteria including *Listeria monocytogenes* (*L. monocytogenes*) and *Staphylococcus aureus* (*S. aureus*) by disc diffusion method at

Microbiology Laboratory, Department of Microbiology, Faculty of Sciences, Chulalongkorn University.

The film samples were cut into 38 mm diameter discs and then placed on agar plates. These samples were sterilized by using UV irradiation for 20 min and were seeded with 0.1 ml of inoculums containing approximately 10^5-10^6 CFU/ml of tested bacteria. The plates were then incubated at 37 °C for 48 h. under aerobic conditions. Observations of the diameter of the inhibitory zone surrounding film discs were made. Experiments were done in triplicate.

3.8.11 Antifungal assay

The antifungal test of the films was examined against *Aspergillus niger* (*A. niger*) by disc diffusion method at Microbiology Laboratory, Department of Microbiology, Faculty of Sciences, Chulalongkorn University. The film samples were punch into round-shaped sample of 38 mm diameter according to the method described by AATCC TM 39-1989 (Anti-bacterial Activity Assessment of Textile Materials: parallel Streak Method). The samples used for the antifungal assay were sterilized by using UV irradiation for 20 min in each side. The test of *A. niger* was performed in the AGAR plate for a week of incubation at 30 °C.

3.9 Release of bioactive compounds from the MBAGTM films

3.9.1 Preparation of acetate buffer

Acetate buffer pH 5.6 was used to simulate the suitable pH condition for common meat just after death that pH range about 5.8-5.4 (Tarté, 2009) and human skin that pH about 5.5 (Suwantong et al., 2007). For 100 ml of acetate buffer solution preparation, 0.1 M acetic acid (5.8 ml made to 1000 ml) and 0.1 M sodium acetate trihydrate (13.6 g/l) were mixed in the proportion during 4.8 ml of 0.1 M acetic acid solution and 45.2 ml of 0.1 M sodium acetate. Then the solution was adjusted the final volume to 100 ml with deionized water and adjusted the final pH using a sensitive pH meter to obtain acetate buffer at pH 5.6.

3.9.2 Preparation of phosphate buffer saline (PBS)

Phosphate buffer saline pH 7.4 was used to simulate the suitable pH condition for growing of bacteria (Tarté, 2009). For 1000 ml of phosphate buffer solution preparation, 2.7 mM potassium chloride (0.20 g), 10 mM di-sodium hydrogen phosphate (1.42 g), 1.76 mM potassium di-hydrogen phosphate (0.24 g) were dissolved in 1 liter of deionized water. Then the solution was adjusted the pH before use with 137 mM sodium chloride (8.0145 g) to obtain PBS buffer at pH 7.4.

3.9.3 Actual bioactive compound content

Actual amount of bioactive compound content in the MBAGTM films were determined. Each specimen (circular disc at 3.0 cm in diameter) was immersed in the absolute ethanol 10 ml at room temperature (30 °C) and stirred at 100 rpm for 72 h. After that, the solution was collected and the actual amount of bioactive compound content was measured by Shimadzu UV-2550 UV-visible spectrophotometer at the wavelength of 765 nm for phenolic compound and 320 nm for mangostins following the analytical methods which were previously described in Topic 3.8.

3.9.4 Bioactive compounds release assay

3.9.4.1 Release in acetate buffer

The release characteristics in acetate buffer at pH 5.6 of bioactive compounds from the MBAGTM films were investigated by total immersion (Suwantong et al., 2007). The releasing medium is composed of 96.5%v/v acetate buffer with 0.5%v/v Tween 80 and 3%v/v ethanol. Each specimen (circular disc at 3.0 cm in diameter) was immersed in 30 ml of the medium at the room temperature (≈ 30 °C). At a specified immersion period ranging from 0 to 72 h (4320 min), either 0.7 ml of a sample solution was withdrawn from medium. The amounts of bioactive compounds in the sample solutions were determined using the UV-visible spectrophotometer at the wavelength of 765 nm for phenolic compounds and at wavelength of 320 nm for mangostins.

3.9.4.2 Release in phosphate buffer saline (PBS)

The release characteristics in PBS at pH 7.4 of bioactive compounds from the MBAGTM films were investigated by total immersion (Suwantong et al., 2007). The releasing medium is composed of 96.5%v/v PBS with 0.5%v/v Tween 80 and 3%v/v ethanol. Each specimen (circular disc at 3.0 cm in diameter) was immersed in 30 ml of the medium at the room temperature approximate 30 °C. At a specified immersion period ranging from 0 to 72 h (4320 min), either 0.7 ml of a sample solution was withdrawn from medium. The amounts of bioactive compounds in the sample solutions were determined using the UV-visible spectrophotometer at the wavelength of 765 nm for phenolic compounds and at wavelength of 320 nm for mangostins.

CHAPTER IV
RESULTS AND DISCUSSION

BC has been used for various applications including food applications. This work aims to develop the novel films from the blend of BC, sodium alginate and gelatin (BAG) for further application as food packaging materials. In order to improve the physical and functional antimicrobial properties of the film, it was incorporated with glycerol/ sorbitol as plasticizer, tannic acid as cross-linking and antimicrobial agent and mangosteen ethanolic extract as antimicrobial agent. The effects and the optimal content of the supplement agents were investigated. The developed films were characterized for the changes of physical, chemical, mechanical and biological properties comparing with the unmodified BC film. MBAG refers to BAG containing plasticizer; MBAGT refers to MBAG containing tannic acid and MBAGTM refers to MBAGT containing the mangosteen ethanolic extract.

4.1 Bioactive compounds in mangosteen ethanolic extract

In this study, mangosteen ethanolic extract was applied as natural antimicrobial agent. The crude extracts were analyzed by UV-Vis spectroscopic technique for determining the contents of bioactive compounds consisting in those extracts. Phenolic compounds were expressed by gallic acid equivalents (GAE) and mangostins were revealed by α-mangostin equivalents (AME). The contents of bioactive compounds in mangosteen ethanolic extract were shown in Table 4.1. The crude mangosteen ethanolic extract was composed of phenolic compound and mangostins at 0.45±0.02 mg GAE/ mL extract and 366.69±0.04 mg AME/ mL extract, respectively. The mangostins, which are the major compounds in mangosteen ethanolic extract, are typically hydrophobic components (Nguyen and Marquis, 2011).

Table 4.1 The contents of bioactive compounds in mangosteen ethanolic extract

Mangosteen Ethanolic Extract (ME)	Bioactive compounds	Amount of bioactive compounds (mg) in mangosteen ethanolic (per 1 mL) extracts
Conc. ME	Phenolic compound	0.44
	Mangostins	366.69
1% v/v of ME	Phenolic compound	N/A
	Mangostins	5.93
5% v/v of ME	Phenolic compound	N/A
	Mangostins	11.72
10% v/v of ME	Phenolic compound	N/A
	Mangostins	22.80

4.2 Characterization of BAG films and modified BAG films by plasticizer (MBAG films)

4.2.1 Transparency

In Figure 4.1, the alphabets beneath the films show the transparency of the films. The film thickness was around 0.05 mm. Films of BC, sodium alginate and BC/A at a ratio of 60/40 was slightly opaque, whereas a film of gelatin was more transparent. Transparency of the films was increased with increasing gelatin content. It was found that the BAG film at a ratio of 60/10/30 was a highly transparent film. The surface roughness and film thickness might also affect transparency of films (Park et al., 2008). However, no significant difference in the degree of transparency was observed between the films with and without the addition of glycerol, sorbitol and the mixture of glycerol and sorbitol as shown in Figure 4.2.

Figure 4.1 Transparency of films: bacterial cellulose (A), sodium alginate (B), gelatin (C) and BC/A at a ratio of 60/40 (D)

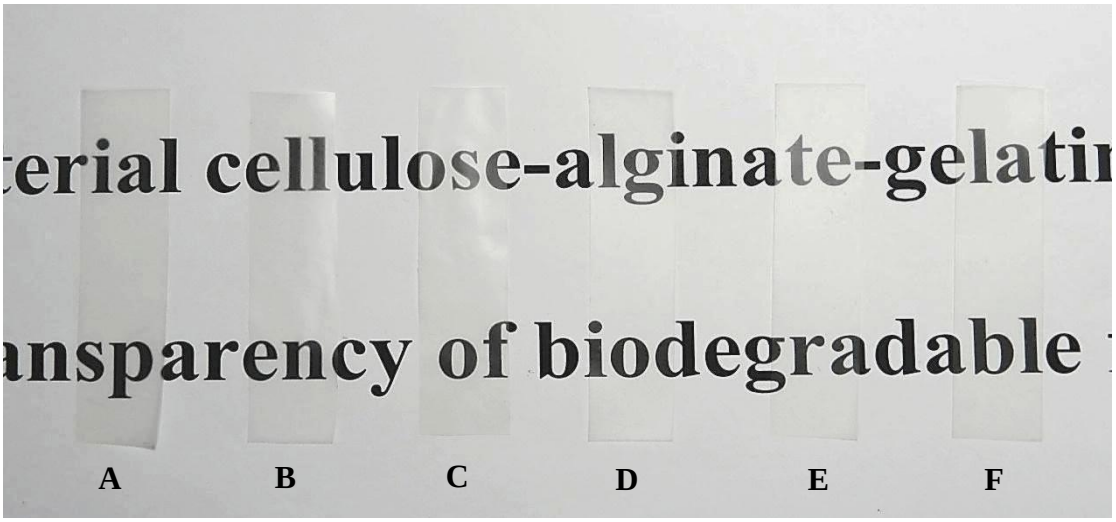


Figure 4.2 Transparency of films: the BAG films at a ratio of 60/10/30 (A), 60/20/20 (B) and 60/30/10 (C), and the MBAG films at a ratio of 60/20/20 with glycerol (D), sorbitol (E), the mixture of glycerol and sorbitol (F)

4.2.2 Fourier transforms infrared spectroscopy (FT-IR)

The FT-IR spectra of BC, sodium alginate, gelatin, BC/A and the BAG films without and with plasticizer adding at a ratio of 60/10/30, 60/20/20 and 60/30/10 were determined with the wavenumber ranging from 4000 to 400 cm^{-1} as shown in Figure 4.3 and 4.4.

Figure 4.3 exhibits the main functional groups of each pure key component. The three main peaks of BC were C-O-C and O-H stretching vibration at 1061and 3392 cm^{-1}, and H-O-H bending vibration of absorbed water molecules consisting in the structure of BC at 1647 cm^{-1}. The five main peaks of alginate were C-O-C at 1035 cm^{-1}, overlapping O-H and N-H at 3411 cm^{-1}, the ester group (-COO) stretching vibration at 1423 cm^{-1} and H-O-H bending vibration of absorbed water molecules consisting in the structure of sodium alginate at 1607 cm^{-1} which it also can be assigned to C=O stretching (Lojewska et al., 2005). Additionally, the characteristic absorption bands of sodium alginate appeared around 820 cm^{-1} (Xiao et al., 2001). The four main peaks of gelatin were the N-H stretching of amide at 3408 cm^{-1} due to the extension of the group. The intense bands of the peptide groups at 1643 and 1543 cm^{-1}as bands of amide I (extension of C=O) and amide II (extension of C–N and angular deformation of the N–H ligation), respectively. Amide III, with bands at 1239 cm^{-1}, represents components of the extension of C-N and N-H on the flexion surface of amide bonds and absorptions as a result of the vibrations of groups $C–H_2$ of the glycine and proline (Sionkowska et al., 2004; Andreuccetti et al., 2009). The last one, the BC/A at a ratio of 60/40 shows main five peaks as the C-O-C and the O-H stretching vibration at 1026 and 3392 cm^{-1}, the H-O-H bending vibration of absorbed water molecules consisting in the structure of BC, at 1611 cm^{-1}, the ester group (-COO) at 1424 cm^{-1} and the characteristic absorption bands of sodium alginate and 819 cm^{-1}.

The FT-IR spectra of BAG films at ratios of 60/10/30, 60/20/20 and 60/30/10 without plasticizers and at ratios of 60/20/20 with plasticizers exhibit the characteristic absorption bands with no appearance of new peaks as shown in Figure 4.4 A to F. The BAG films at ratio of 60/10/30, 60/20/20 and 60/30/10 appeared peaks of main functional groups. The C-O-C stretching vibration of BC and alginate

is observed at about 1026-1059 cm^{-1}, the O-H and N-H stretching vibration at about 3400-3404 cm^{-1}, the H-O-H bending vibration of absorbed water molecules consisting in the structure of BC appeared at about 1613-1650 cm^{-1} which it also can be assigned to the C=O stretching. The ester group stretching vibration (-COO) of alginate appeared at 1423-1424 cm^{-1}. The symmetric of N-H bending vibration in amide of gelatin appeared at 1535 cm^{-1}, and the C-N stretching bands appeared at about 1241-1316 cm^{-1} of gelatin.

For the films at ratios of 60/20/20 with glycerol, sorbitol and the mixture of glycerol and sorbitol (GS) adding, multiple shifted bands were observed as follows. The C-O-C stretching bands were shifted to 1060, 1035 and 1059 cm^{-1}, and the O-H and the N-H stretching bands were shifted to 3401, 3400 and 3393 cm^{-1}, respectively. The H-O-H bending peaks were shifted to 1650, 1635 and 1649 cm^{-1} and the -COO stretching peaks were shifted to 1431, 1424 and 1431 cm^{-1}, respectively. The symmetric of N-H bending peaks were shifted to 1535, no peak and 1541 cm^{-1} and the C-N stretching peaks were shifted to 1243, 1250 and 1243 cm^{-1}, respectively.

It was shown that the MBAG films with glycerol adding tended to increase in amplitudes of the gelatin characteristic absorption band and free water peaks (Bergo and Sobral, 2007). The C=O stretching was shifted to higher frequency at 1650 cm^{-1} and appeared N-H bending vibration of at 1535 cm^{-1}. The FT-IR peaks of the MBAG films with sorbitol adding tended to increase in amplitudes of the sodium alginate characteristic absorption band. These shifts could be attributed to intermolecular interactions between the hydroxyl group of cellulose, the carboxyl group of sodium alginate and amide group of gelatin.

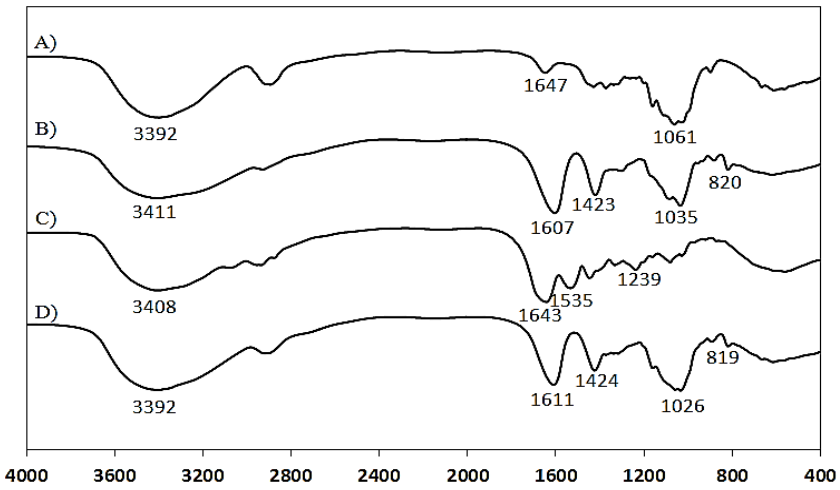


Figure 4.3 FT-IR of films at a ratio of BC (A), Sodium alginate (B), Gelatin (C) and BC/A film (D)

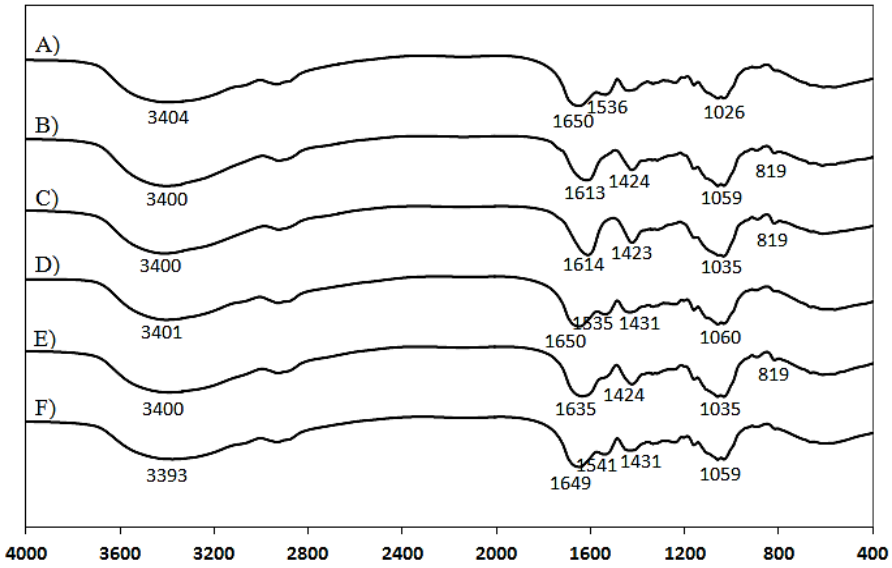


Figure 4.4 FT-IR of BAG films at a ratio of 60/10/30 (A), 60/20/20 (B), 60/30/10 (C) and films at a ratio of 60/20/20 with glycerol (D), sorbitol (E) and the mixture of glycerol and sorbitol (F) addition, respectively.

4.2.3 Water absorption capacity (WAC)

The WAC of the films in DI water is shown in Figure 4.5 and 4.6. The results indicated that the WAC values were correlated with the gelatin content. The WAC of BC, sodium alginate and BC/A was 254.4, 296.8 and 208.7%, respectively, whereas the BAG films at a ratio of 60/10/30, 60/20/20 and 60/30/10 was 411.9, 344.5 and 232.5%, respectively. The increased WAC of the BAG films at a ratio of 60/10/30 was due to very hydrophilic nature and more flexible structure of gelatin. Similar observations were previously reported in modified BC by BC/gelatin composites via cross-linking (Chang et al., 2012).

The addition of plasticizer into the BAG films up to certain amount enhanced WAC of the BAG films. The results indicated that WAC increased with the increase of plasticizer content. However, excess of plasticizer at a ratio of plasticizer to gelatin solution more than 2:10 (w/w) caused the decrease of the WAC, which should be due to the migration of plasticizer from the re-swollen film surface. Similar observations were previously reported in DCMC crosslinked gelatin edible film (Mu et al., 2012). The plasticized films at a ratio of plasticizer to gelatin solution at 2:10 (w/w) showed better rehydration ability and water absorption capacity.

For the effect of type of plasticizers, WAC of the films plasticized with sorbitol (S) had a greater WAC than that plasticized with the mixture of glycerol and sorbitol (GS) and glycerol (Gly), respectively. Because sorbitol is polyol containing multiple hydroxyl groups in the structure, it could attract with -OH of water molecules.

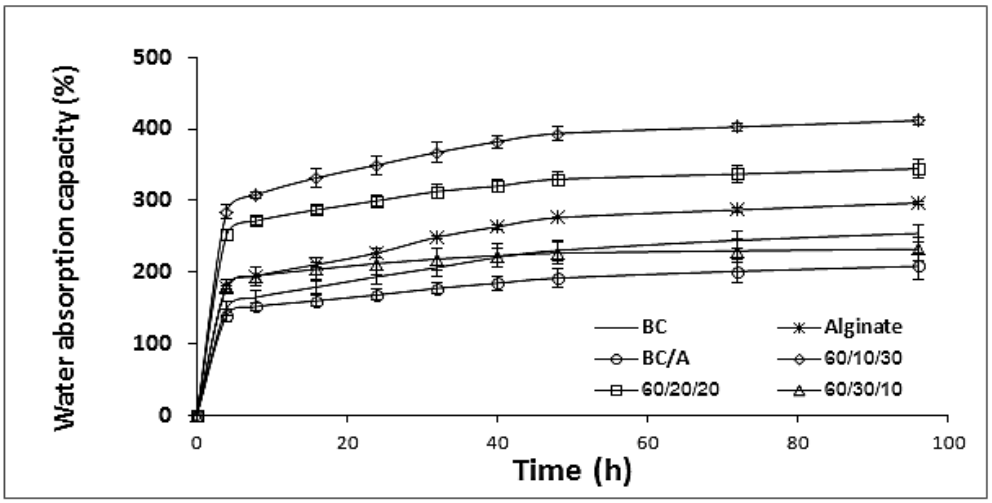


Figure 4.5 Water absorption capacity (%) of the composite films: BC (—); Alginate (—✳—); BC/A (—○—); BAG films at a ratio of 60/10/30 (—◇—), 60/20/20 (—□—) and 60/30/10 (—△—) without plasticizer, respectively.

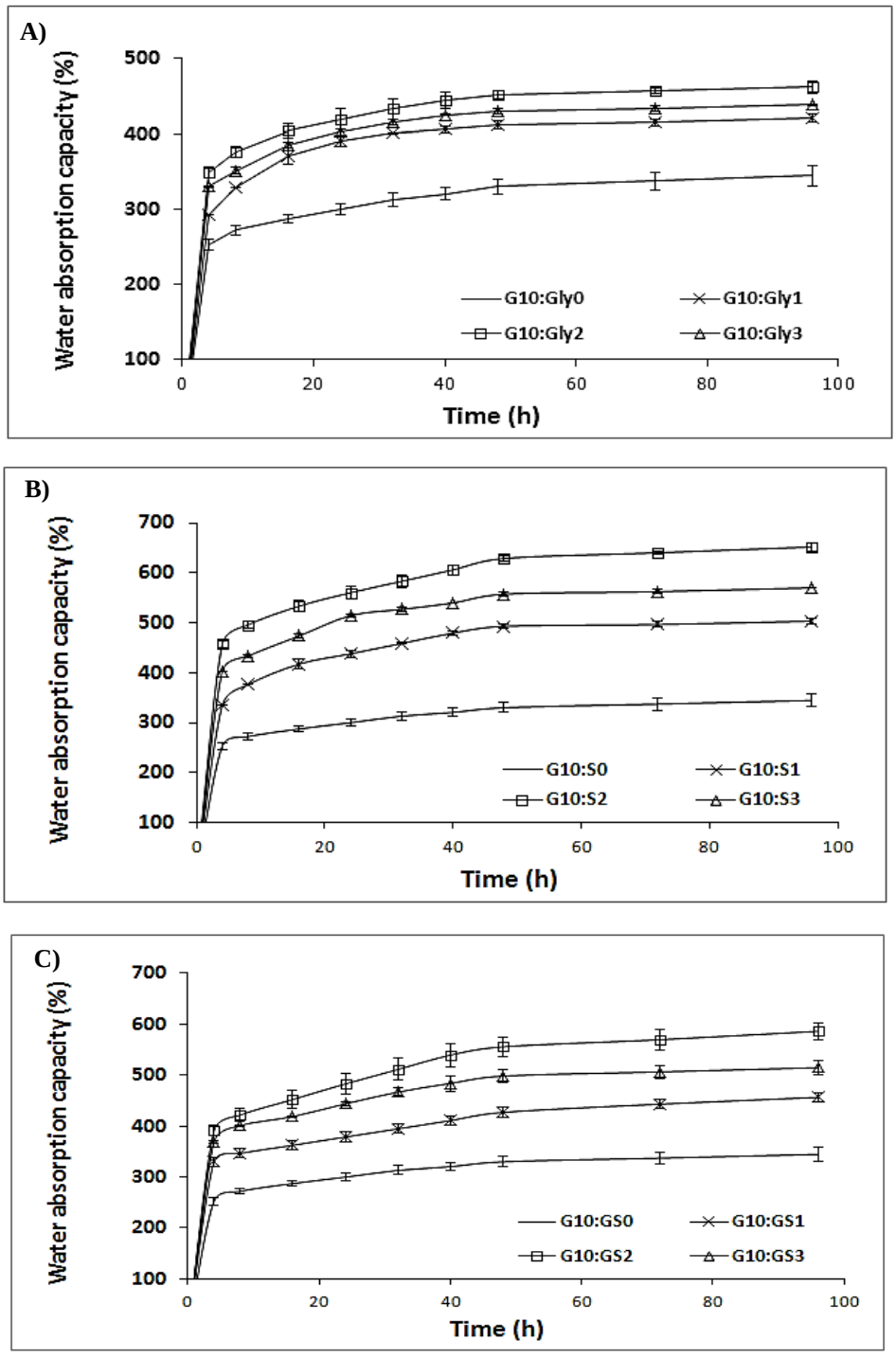


Figure 4.6 Water absorption capacity (%) of the composite films: films at a ratio of 60/20/20 with glycerol (A), sorbitol (B) and the mixture of glycerol and sorbitol (C); G:Gly, G:S and G:GS refer to a ratio of gelatin to glycerol, sorbitol and the mixture of glycerol and sorbitol (w/w), respectively.

4.2.4 Mechanical properties

Tensile strength (TS) and elongation at break (EB) of the homogenized BC, sodium alginate and BAG films in dry state were shown in Figure 4.7 A and B. The TS and EB of the BC film were 57.5 MPa and 1.0%, whereas those of the BC/A films were 147.9 MP and 2.1%, respectively. For TS and EB of the BAG films without plasticizer at ratios of 60/10/30, 60/20/20 and 60/30/10 were 166.5, 181.8 and 177.5 MPa, and 2.0, 2.9 and 2.0%, respectively. And the TS and EB of BAG films in dry state in different ratios of each plasticizer were shown in Figure 4.8 A and B.

Overall, the films at a ratio of 60/20/20 (w/w) showed superior mechanical properties in dry state; the TS and EB were at 181.8 and 2.9%, respectively. Moreover, the results showed that there was no significant difference between the mechanical properties of the dried films with and without addition of glycerol as plasticizer. The function of plasticizer is to enhance the flexibility and plasticity to the films but it might not affect some functional properties (Vanin et al., 2005).

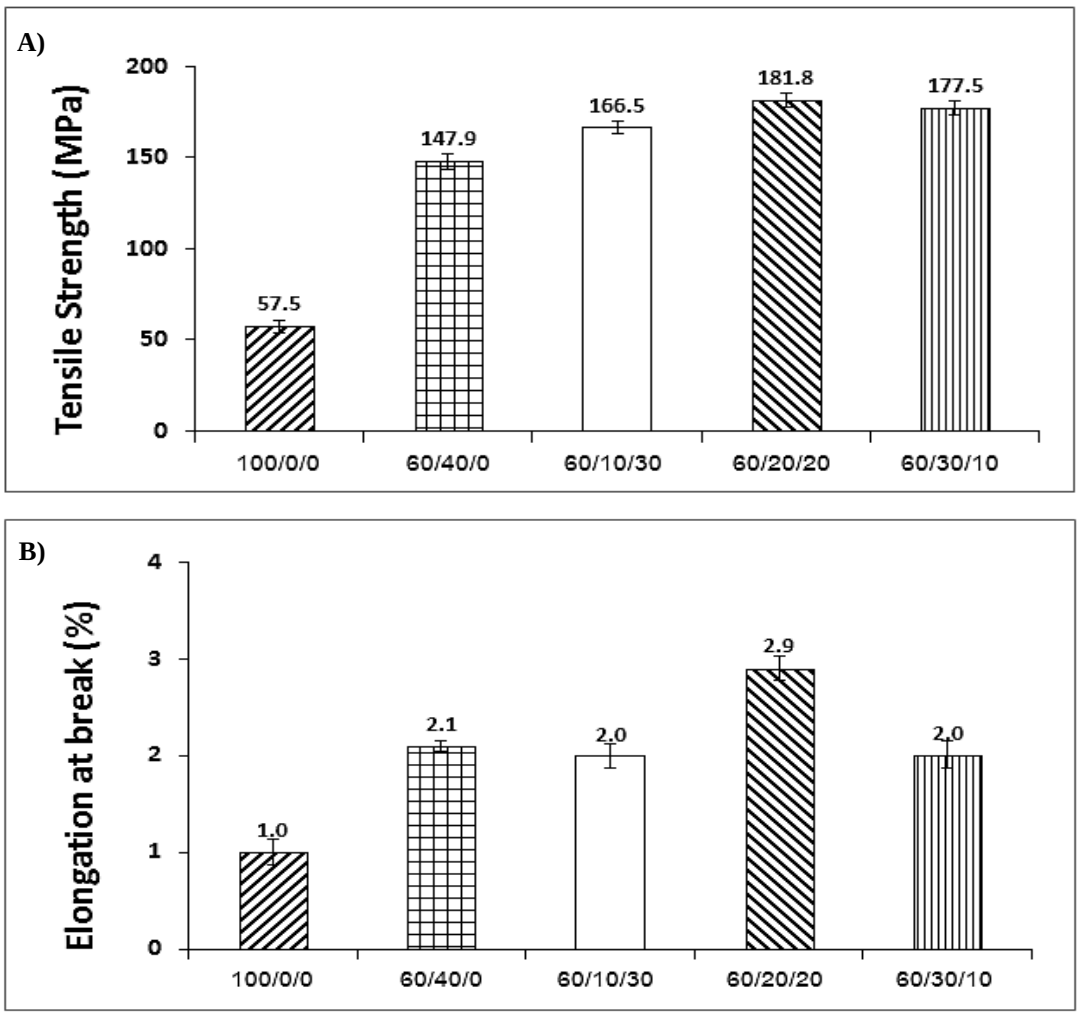


Figure 4.7 Mechanical properties of BAG films in dry state without plasticizer addition: Tensile strength (A) and Elongation at break (B).

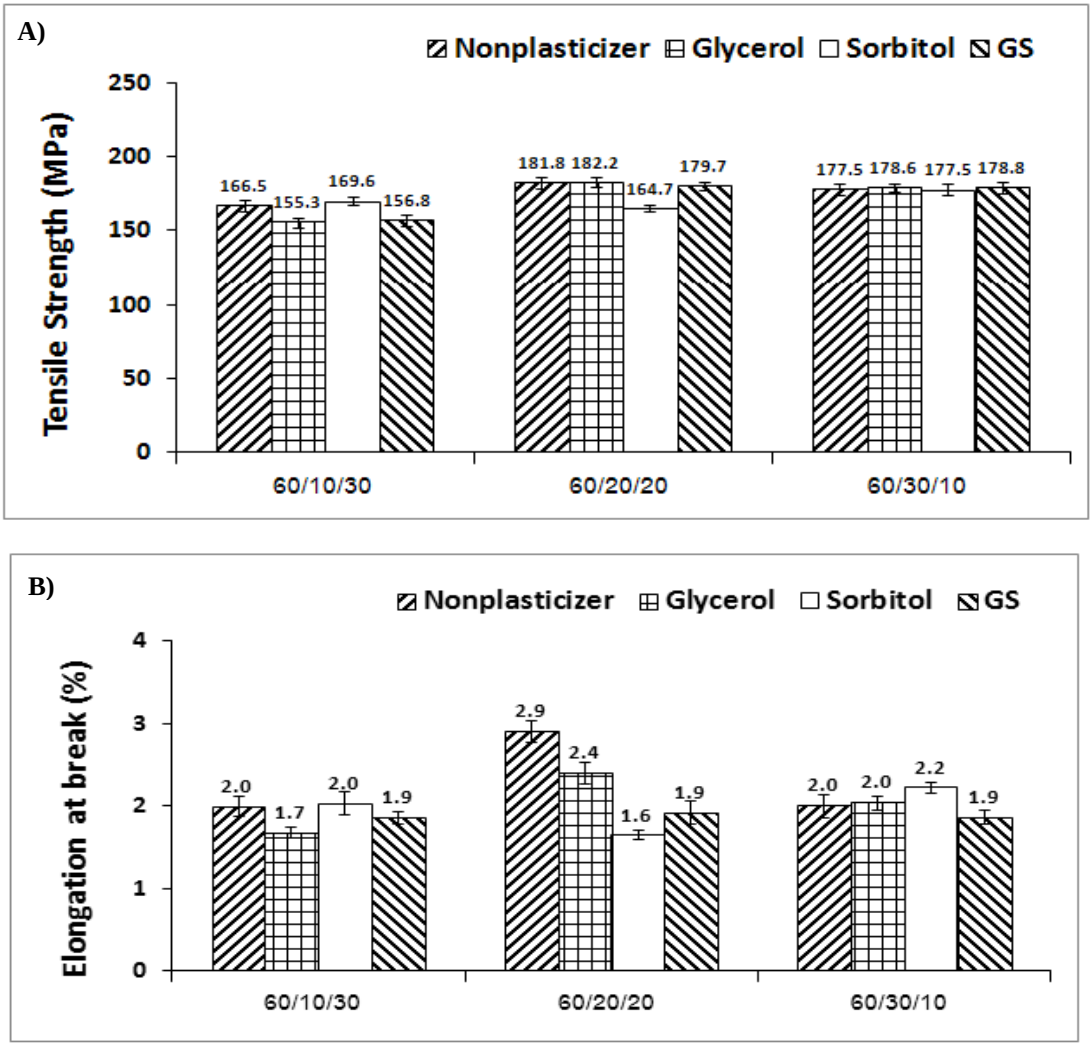


Figure 4.8 Mechanical properties of BAG films in dry state without and with plasticizer addition: Tensile strength (A) and Elongation at break (B).

Tensile strength (TS) and elongation at break (EB) of the homogenized BC, alginate and BAG films in wet state were shown in Figure 4.9 A and B. The TS and EB of the BC film were 2.2 MPa and 1.9%, whereas those of the BC/A films were 15.3 MP and 18.0%, respectively. For TS and EB of the BAG films without plasticizer at ratios of 60/10/30, 60/20/20 and 60/30/10 were 2.3, 9.6 and 11.3 MPa, and 14.8, 30.3 and 28.0%, respectively. And the TS and EB of BAG films in wet state in different ratio of each plasticizer were shown in Figure 4.10 A and B.

Overall, the films at a ratio of 60/30/10 (w/w) showed superior mechanical properties in wet state; the TS and EB were 9.6 MPa and 30.3%, respectively. It was shown that TS and EB of the MBAG film with glycerol were more stable with higher TS and EB than the others. Since both gelatin, glycerol and sorbitol are hydrophilic and high flexible components, significantly improved EB of the BAG films in re-swollen form with the addition of glycerol, sorbitol and the mixture of glycerol and sorbitol (GS) as plasticizer was observed. For the MBAG film at ratios of 60/10/30, 60/20/20 and 60/30/10 with glycerol adding as plasticizer, the EB increased to 22.9, 44.6 and 45.7%, respectively.

It was found that TS of the re-swollen BAG films was reduced with the addition of plasticizer. In the wet state, the TS of the BAG films plasticized with glycerol was more than that plasticized with GS and S, respectively. Overall, gelatin and glycerol helped to improve high flexibility and lowered TS of the BAG films in the wet state. It was previously reported that the addition of glycerol to gellan films increased extensibility, but reduced the mechanical strength (Yang and Paulson, 2000) and the softness and flexibility of gelatin-based films could be improved by the addition of plasticizers.

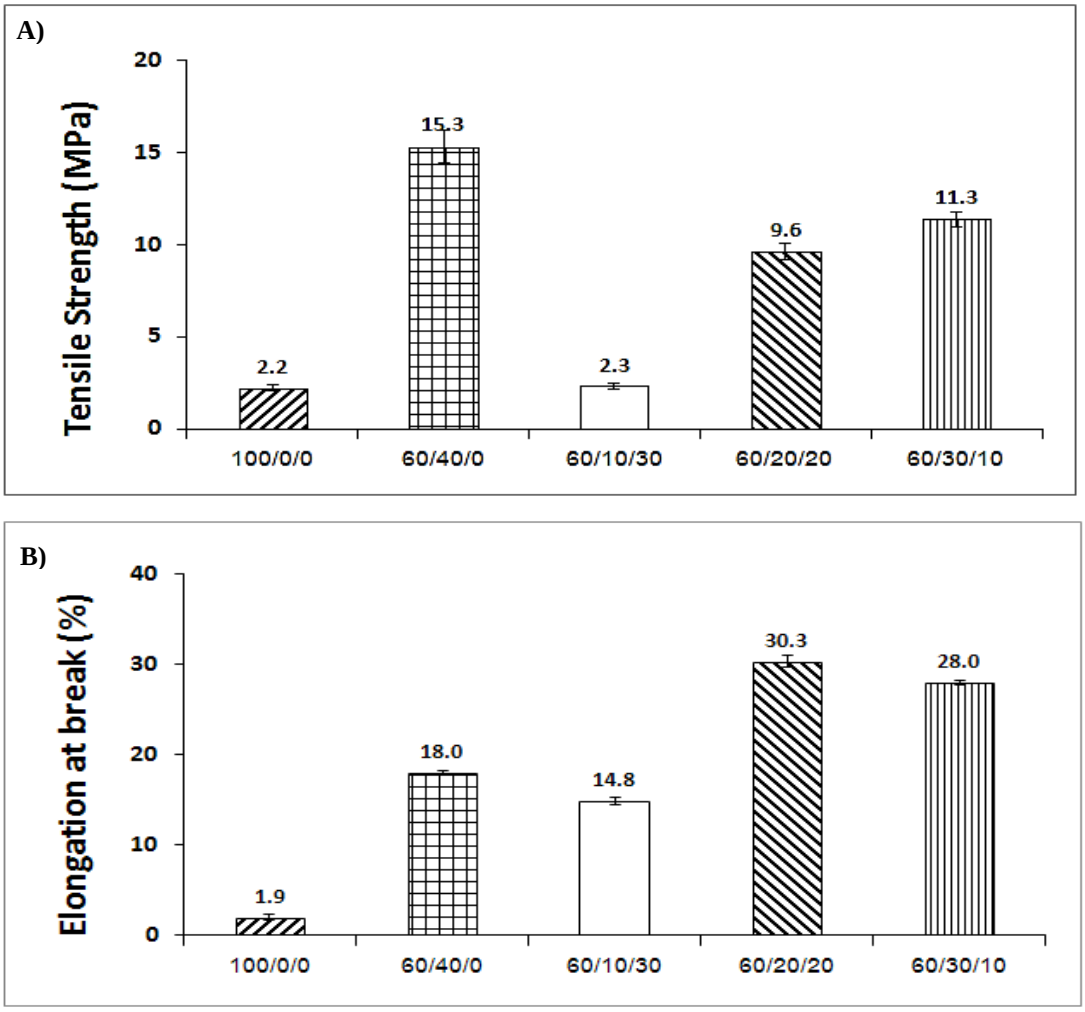


Figure 4.9 Mechanical properties of BAG films in wet state without plasticizer addition: Tensile strength (A) and Elongation at break (B).

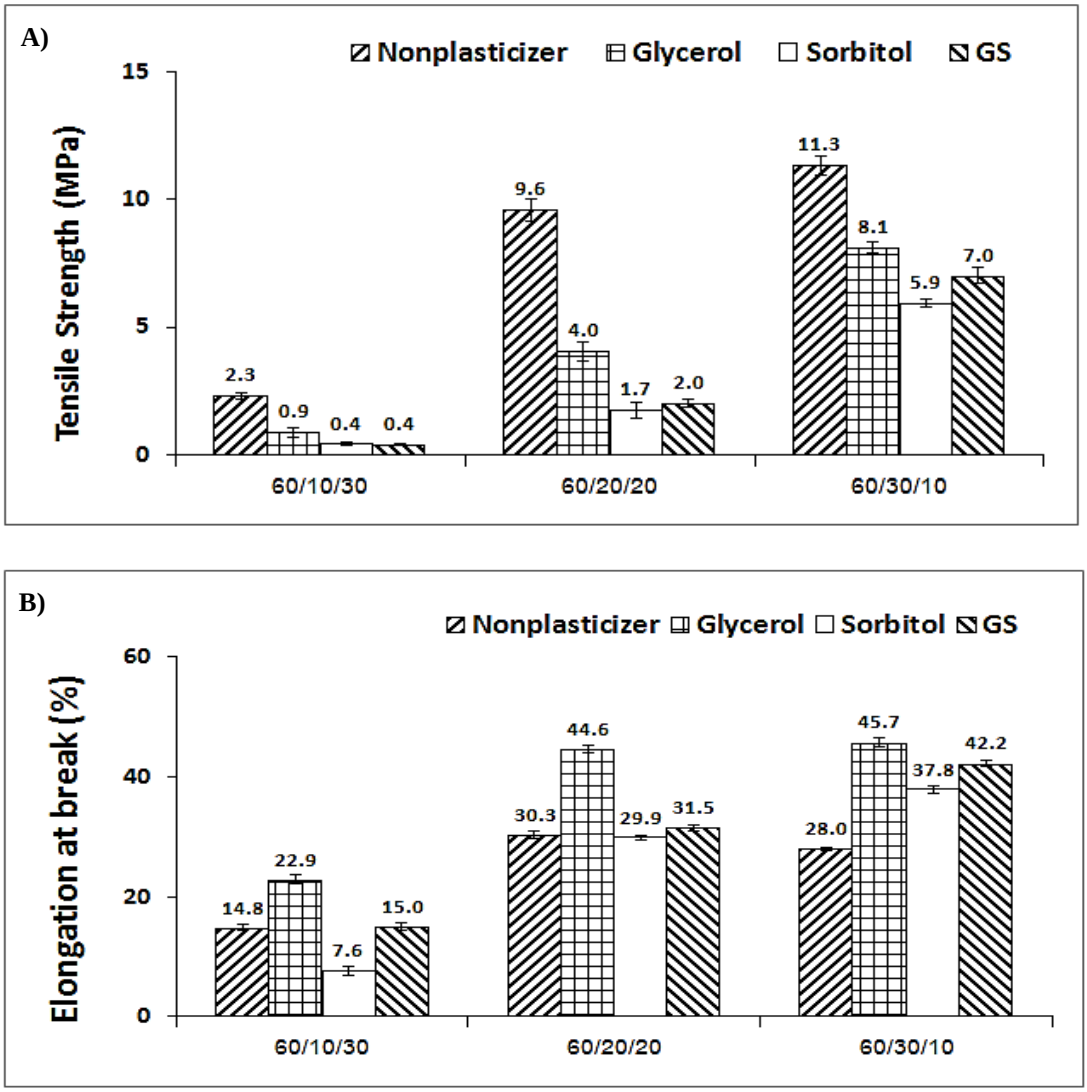


Figure 4.10 Mechanical properties of BAG films in wet state without and with plasticizer addition: Tensile strength (A) and Elongation at break (B).

4.2.5 Scanning electron microscope (SEM)

SEM images of overview surface morphology at 200 magnifications of the films in dry state were shown in Figure 4.11 (A-H). The surface roughness of BC film has been shown in Figure 4.11 (A) due to cellulose fibrils. Sodium alginate and gelatin films have smooth surfaces. Under the cross linking with $CaCl_2$, the alginate film formed wavy surface pattern. BC/A and BAG films also exhibited surface roughness from cellulose fibrils.

SEM photographs revealed surface morphology at 10,000 magnifications of the BAG films at different ratio are shown in Figure 4.12 (A-F) and the MBAG films in dry and wet state at a ratio of 60/20/20 plasticized with glycerol in Figure 4.12 (G and H). From the SEM photograph exhibited that sodium alginate and gelatin lodged in BAG networks and wrapped up parts of the BC fibril network. Sodium alginate-gelatin-embedded BC formed a film on which only several enlarged cellulose ribbons were observed. Moreover, sodium alginate and gelatin became the continuous phase and formed a more massive film when BC was blended with both ones. A substantial amount of sodium alginate and gelatin not only filled the network but also embedded completely on cellulose ribbons. However, a few bigger holes still existed in the composites.

Compared to the dried films, the re-swollen films exhibited a looser fibril network structure according to the high water absorption of the films. The BAG film at a ratio of 60/10/30 found that it was looser structure than the film at other ratio and the film at a ratio of 60/30/10 found that it was not clearly observed of cellulose ribbon because a lot of sodium alginate content increased thickener in the film affect well wrap cellulose ribbon and the structure of the film became less uniform with noticeable excessive alginate on the film surfaces. For the film at a ratio of 60/20/20 displayed good incorporation of sodium alginate and gelatin into the BC fibril network because the structure its ordered cellulose ribbon rather.

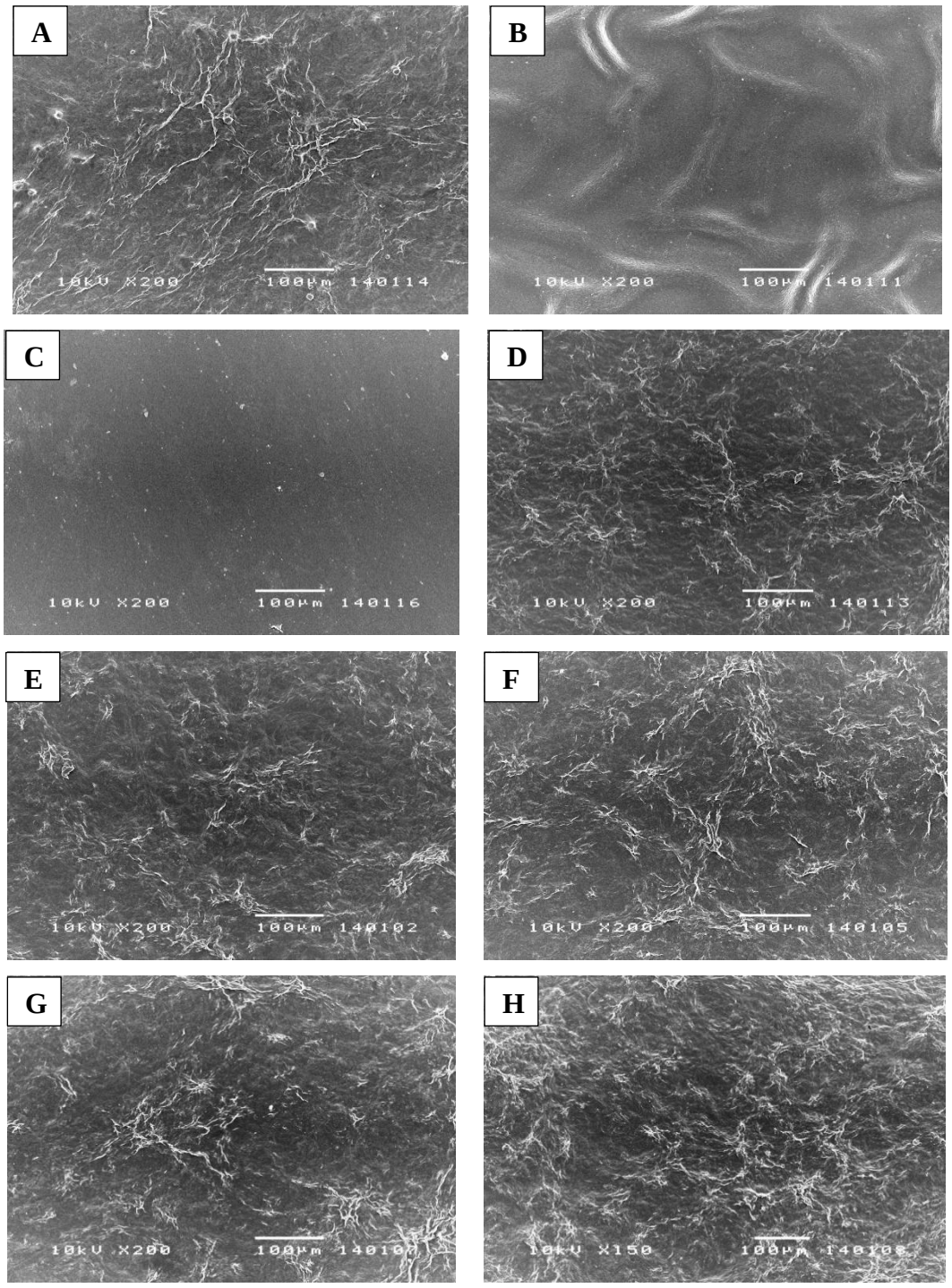


Figure 4.11 SEM images of overview surface morphology of the films in dry state: BC (A); Sodium alginate (B); Gelatin (C); BC/A (D); BAG films at a ratio of 60/10/30 (E), 60/20/20 (F), 60/30/10 (G) and MBAG film at a ratio of 60/20/20 plasticized with glycerol (H).

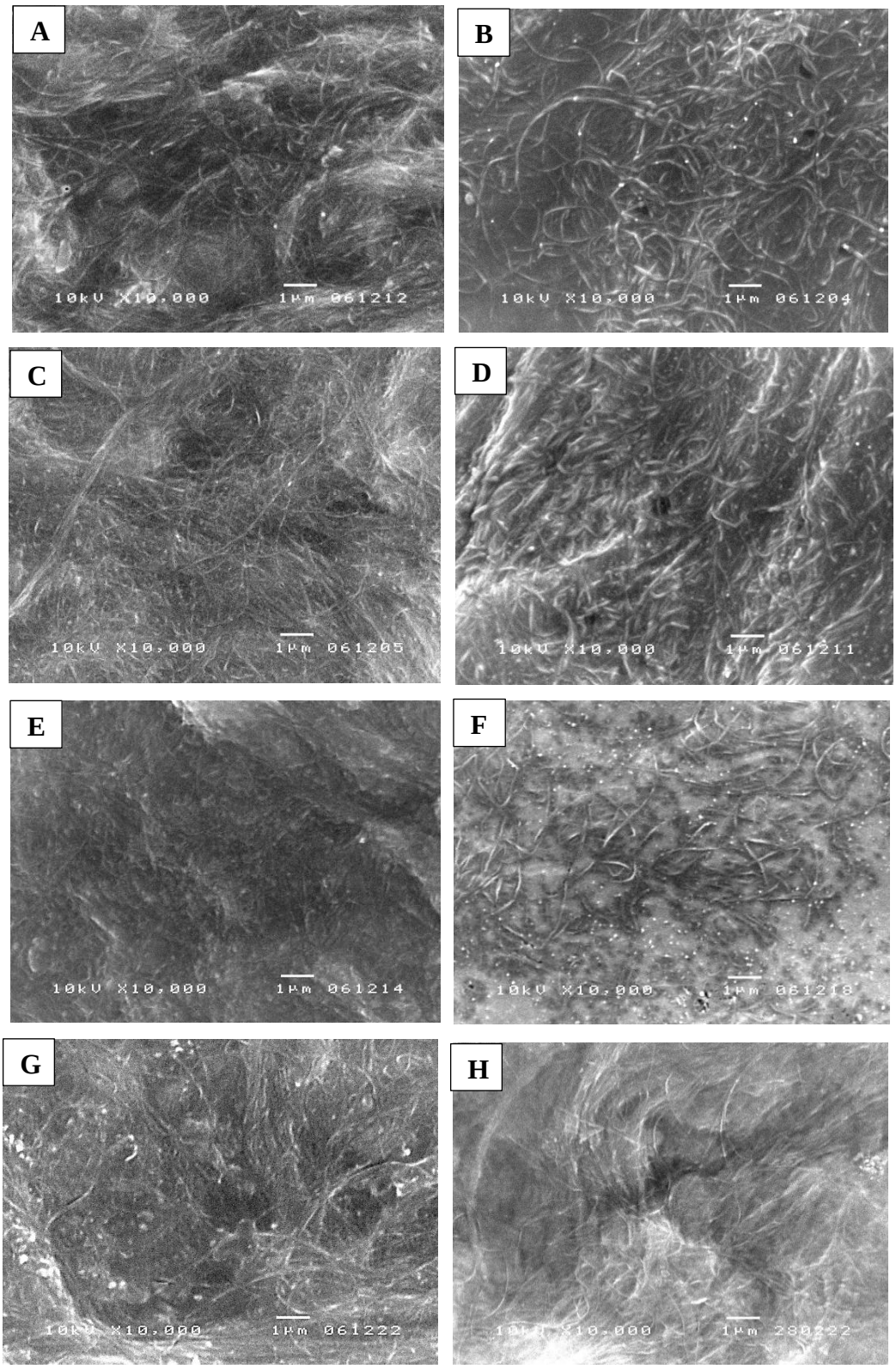


Figure 4.12 SEM images of surface morphology of BAG films in dry (left) and re-swollen (right) forms at a ratio of 60/10/30 (A and B), 60/20/20 (C and D), 60/30/10 (E and F) and MBAG film at a ratio of 60/20/20 plasticized with glycerol (G and H)

Figure 4.13 expressed the cross sectional morphologies of the BAG films at different ratio without glycerol (Figure 4.13 A-C) and the BAG film at a ratio of 60/20/20 with glycerol (Figure 4.13 D) at 3,500 magnifications. The images showed that the thickness of layer in the BAG films increased with the increase of sodium alginate content. However, the interlayer space between sheets was still observed. Moreover, gelatin could also penetrate into the fiber networks and partially filling in empty space between BC fibrils (Chang et al., 2012).

Moreover, in comparison with the native films, the addition of glycerol generated the looser BC fibril network as shown in Figure 4.12 (G, H) and Figure 4.13 (D). The result demonstrated that glycerol addition significantly increased void fraction of inter-chain in the BC fibril networks.

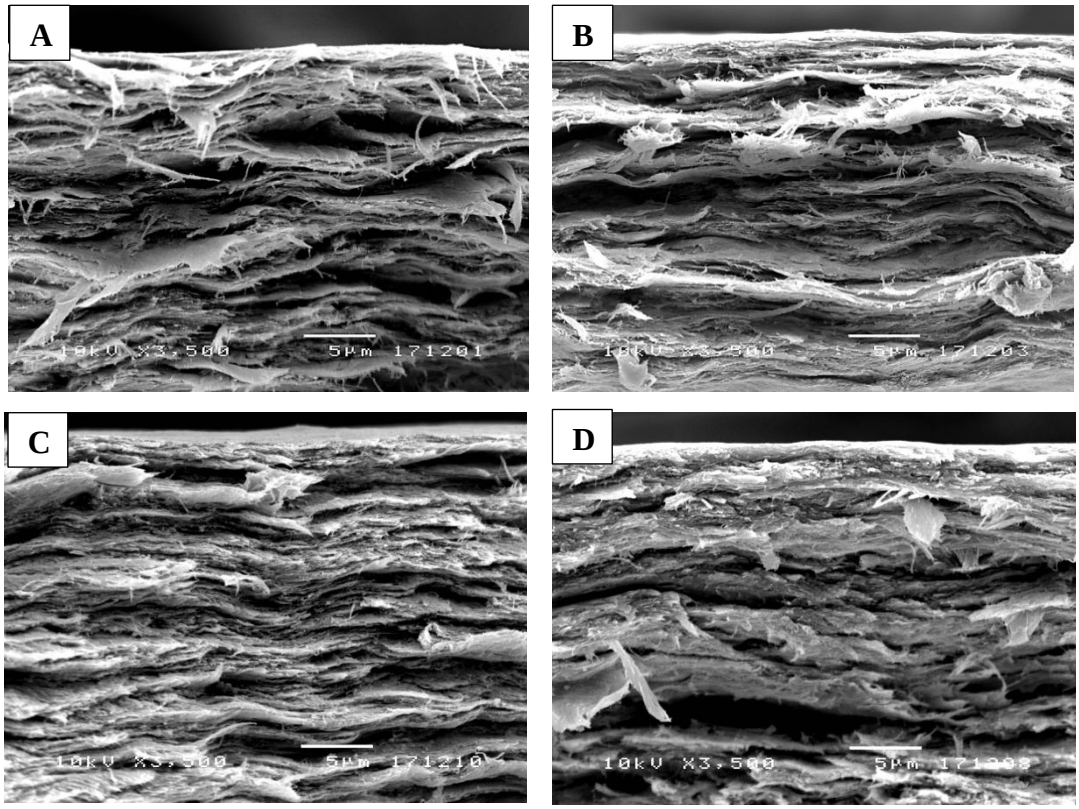


Figure 4.13 SEM images of cross-sectional morphology: BAG films in dry form at a ratio of 60/10/30 (A), 60/20/20 (B), 60/30/10 (C) and MBAG film at 60/20/20 plasticized with glycerol (D)

4.3 Characterization of MBAG films modified by tannic acid (MBAGT films)

4.3.1 Transparency

In Figure 4.14, the alphabets beneath the films show the transparency of the films. The film thickness was around 0.05 mm (50±10 μm). Transparencies of the films were relatively constant, not depending on tannic acid content. However, the tannic acid binding gelatin affected the color of the films. Since tannic acid could react with gelatin that is proteins, it caused the precipitation of tannic-proteins, which was responsible for the brownish color of the film.

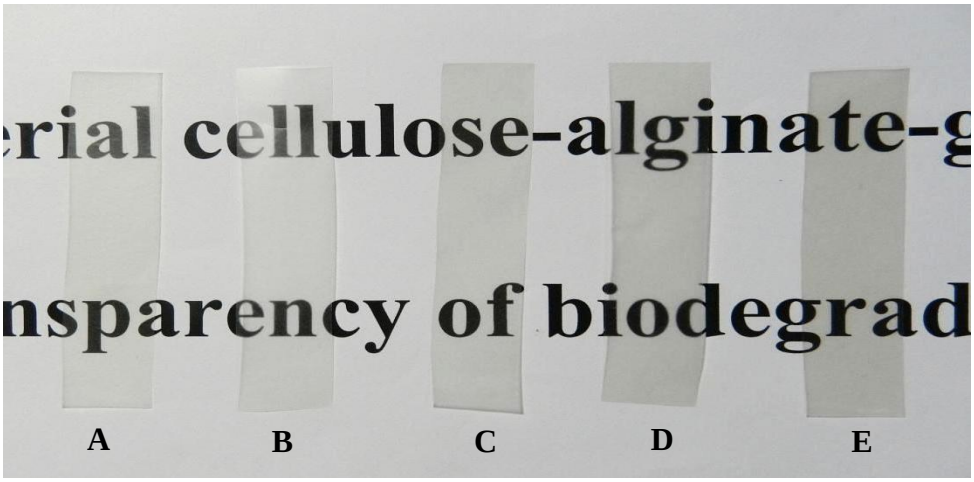


Figure 4.14 Transparency of films: the BAG films at a ratio of 60/20/20 without plasticizer (A), with glycerol (B), with glycerol and adding tannic acid at a ratio of 5 mg (C), 10 mg (D) and 15 mg (E) tannic acid per g gelatin solution, respectively

4.3.2 Fourier transforms infrared spectroscopy (FT-IR)

The FT-IR spectra of tannic acid, the BAG, MBAG, MBAGT films at a ratio of mg tannic acid to g gelatin as 5 mg, 10 mg and 15 mg are shown in Figure 4.15 A, B, C, D, E and F, respectively.

Generally, two broader peaks at around 1319 cm^{-1} and between 1290 and 1150 cm^{-1} (1200 cm^{-1}) can be observed in the spectra of gallic acid, tannic acid (gallotannin), and ellagitannin. Both peaks can be assigned to the combination of C-O stretching and -OH deformation vibrations (Edelmann and Lendl, 2002). The characteristic bands at 3408, 1615, and 1710 cm^{-1} represent phenolic hydroxyl group (-OH), double bond (C=C) in aromatic ring, and ester groups of phenolic compounds, respectively.

The BAG, MBAG and MBAGT exhibited the characteristic absorption bands with no appearance of new peaks. The BAG and MBAG films showed multiple bands that previously explained in Topic 4.1.2. For the MBAGT at the ratio of 5, 10 and 15 mg tannic acid to g gelatin solution, it was found that the C-O-C stretching peaks were shifted to 1060, 1059 and 1059 cm^{-1} and the O-H and the N-H stretching bands were shifted to 3413, 3412 and 3408 cm^{-1}, respectively. The H-O-H bending peaks were shifted to 1646, 1644 and 1650 cm^{-1}. The C=O stretching and the -COO stretching peaks were shifted to 1436, 1433 and 1443 cm^{-1}, respectively. The N-H bending peaks were shifted to 1541, 1536 and 1536 cm^{-1} and the C-N stretching peaks were shifted to 1236, 1236 and 1234 cm^{-1}, respectively.

Related to the content of tannic acid on MBAGT films, the results indicated that FT-IR peaks of the MBAGT films tended to increase in amplitudes of the H-O-H bending peaks or free water peaks at about 1646-1650 nm^{-1} and these changes were indicative of greater disorder in gelatin and were probably associated with the loss of triple helix state (Muyonga et al., 2004) Because tannic acid is high molecular weight hydrophilic highly compound, hydroxyl groups of tannin could either form hydrogen-bonded side-chain interactions or bind with carbonyl groups of gelatin molecules as indicated by additional the wavelength of -COO stretching peaks and H-O-H bending peaks group that were part of gallic acid unit in tannic found in MBAGT films.

Previously, these shifts could be attributed to the bending of the O-H bonds and the stretching of the C-O bonds in secondary and tertiary alcohols (Aewsiri et al., 2010).

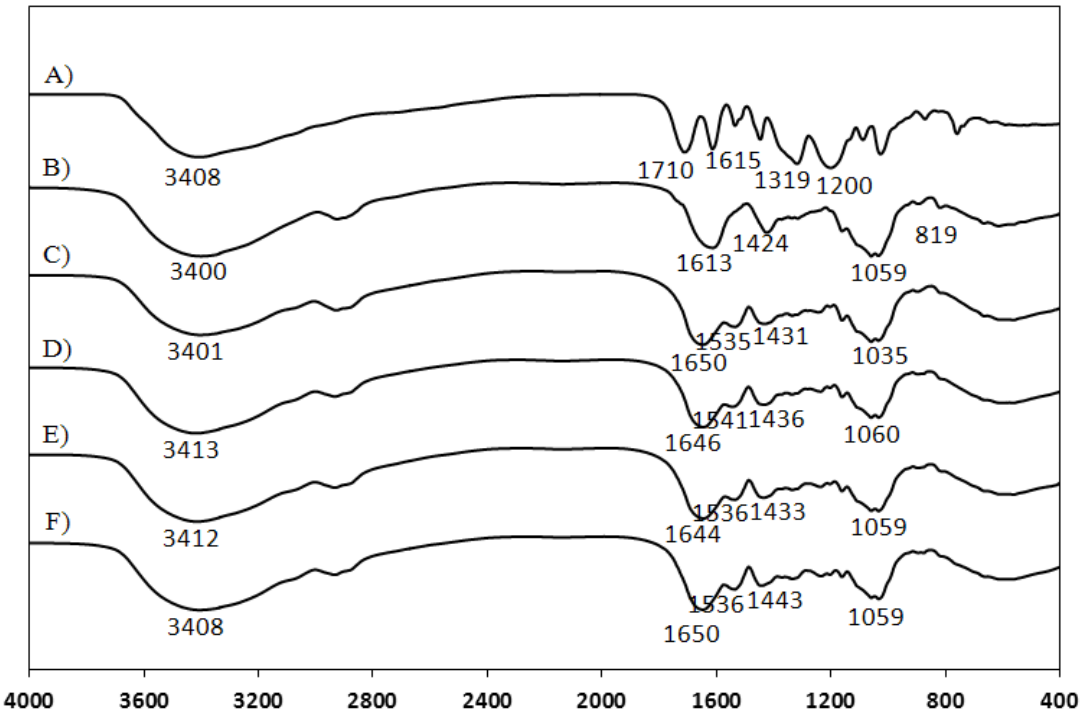


Figure 4.15 FT-IR of tannic acid (A), the BAG (B), MBAG (C), MBAGT at a ratio of mg tannic acid to g gelatin solution at 5.0 (D), 10.0 (E) and 15.0 (F) films.

4.3.3 Water absorption capacity (WAC)

The WAC of the MBAGT films in DI water is shown in Figure 4.16. The results indicated that the WAC values were inversely related with the tannic acid content. The WAC of the BAG film at a ratio of 60/20/20 and the MBAG film plasticized with glycerol adding (MBAG) was 333.6 and 462.2%. The WAC of the MBAG with tannic acid adding (MBAGT) at a ratio of 5, 10 and 15 mg tannic acid to g gelatin solution was 458.2, 389.9 and 345.6%, respectively. Similar observations were previously reported in modified gelatin film by tannin addition that it could repel enhancing water (Peña et al., 2010). Film-forming solution of gelatin cross-liked by tannic acid had effected to swelling ratio (Cao et al., 2007; Zhang et al., 2010).

For the addition of tannic acid as a crosslinking agent for gelatin, it was proposed that the increase of cross-linking degree resulted in the decrease of gelatin combination with water. Thus, the reduction in WAC values with increasing tannic content suggested the presence of interactions between tannic and gelatin. The rate of WAC is influenced by hydrophobic and/or hydrogen interactions between gelatin and tannic groups. The WAC rate decreased with the tannic acid content. In general, cross-linking and/or hydrogen interactions with other components decrease water uptake by proteins since polar-side-chain groups less exposed to bind water (Peña et al., 2010; Cao et al., 2007; Kim et al., 2005) and the more hydrophobic property from cross-linking with tannic acid, leading less water vapor absorption. In this work, the MBAGT film at a ratio of mg tannic acid to g gelatin solution at 10:1 was selected as it had high WAC and did not shrink or shrunk only slightly under the developed process.

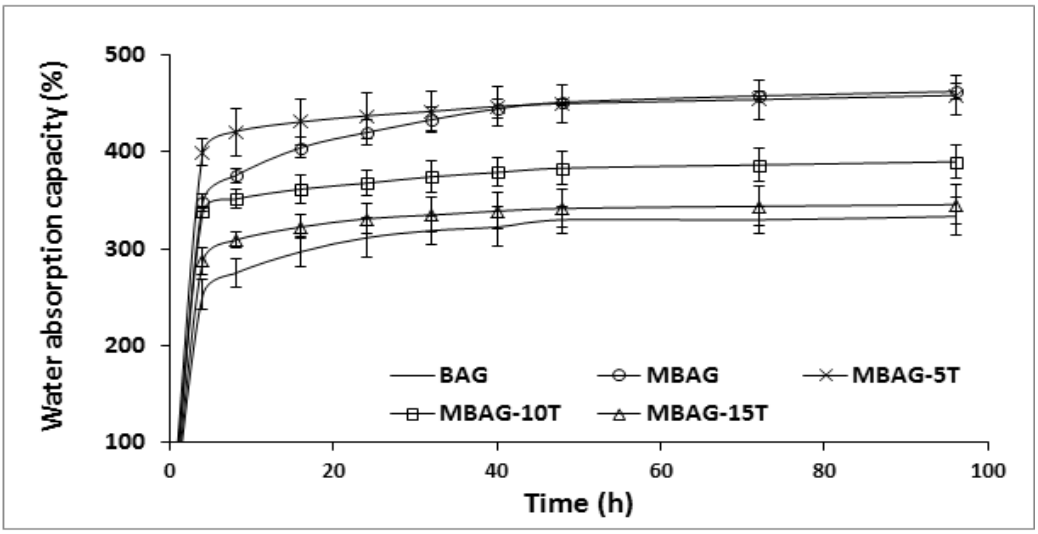


Figure 4.16 Water absorption capacity (%) of the composite films: BAG (—); MBAG (—○—); MBAG-5T (—×—); MBAG-10T (—□—) and MBAG-15T (—△—), respectively.

4.3.4 Mechanical properties

Tensile strength (TS) and elongation at break (EB) of the films of BAG, MBAG and MBAGT at a ratio of 5, 10 and 15 mg tannic acid to g gelatin solution in dry state are shown in Figure 4.17 A and B. The MBAGT films at a ratio of 5, 10 and 15 mg tannic acid to g gelatin solution exhibited slightly decreased mechanical properties from MBAG and BAG film to 174.8, 177.5 and 181.3 MPa, respectively. It could probably cause by some interfering of hydroxyl groups of tannic acid to the hydrogen bonding of cellulose. On the other hand, the hydrophobic interactions between tannic acid and gelatin contributed to the formation of complexes are considered far weaker than hydrogen bonding. However, at higher tannic acid content, the TS increased since the covalent cross-links between gelatin and tannin formed under high tannic acid concentration was more stable (Cao et al., 2007). Similar observations were previously reported in protein cross-linking with phenolic (Prodpran et al., 2012). According to the previous report (Zhang et al., 2010), tannic acid displayed a dual role as both a cross-linker (improving tensile strength) and plasticizer (improving elongation). However, no significant effect of tannic acid addition on plasticizer properties of the MBAGT films in dry state was observed. It was found that the EB of the MBAGT film slightly increased with the addition of 10 mg tannic acid/ g gelatin solution, but decreased with the addition of tannic acid at as 5 and 15 mg.

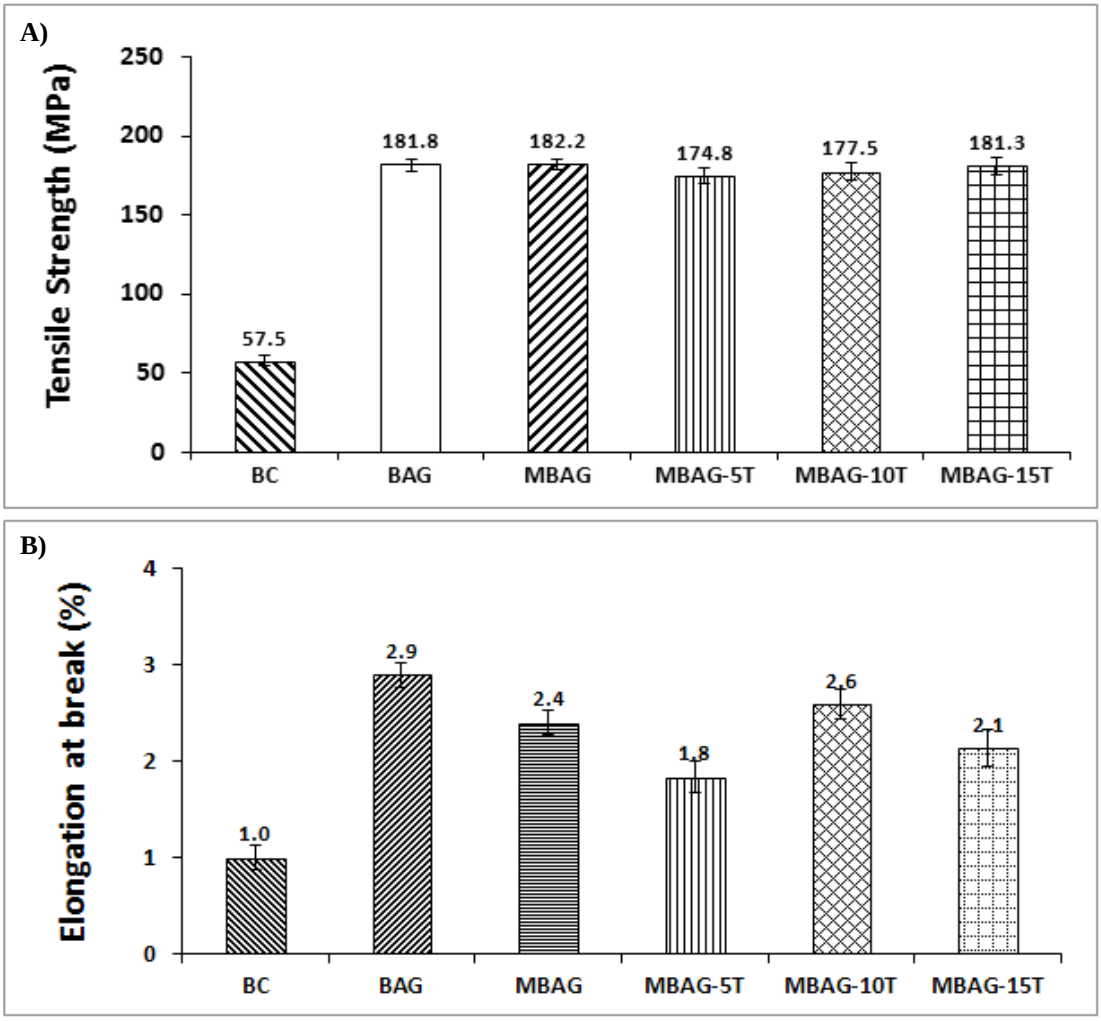


Figure 4.17 Mechanical properties of BAG films in dry state at a ratio of 5, 10 and 15 mg tannic acid to g gelatin solution (w/w): Tensile strength (A) and Elongation at break (B).

TS and EB of the films in wet state were shown in Figure 4.18 A and B. In wet state, the TS tended to decrease whereas EB tended to increase with the addition of tannic acid. The observations of the mechanical properties of the re-swollen films implied that the reactions between tannic acid and gelatin might be reversible.

Therefore, the interactions between tannic acid and gelatin in the MBAGT film should be hydrophobic interactions rather than strong hydrogen bonding. The reaction could reverse from tannic acid-gelatin precipitate to tannic acid-gelatin solution which similarly observation reported in tannin-protein interaction (Cao et al., 2007) as following:

Tannic acid solution + gelatin solution

↔ [Tannic acid m · gelatin] solution ↔ [Tannic acid n · gelatin] precipitate

Although the tannic acid-gelatin interaction was not strong, it had positive effect on plasticizer properties of MBAGT films in wet state and helped to increase EB of the re-swollen films.

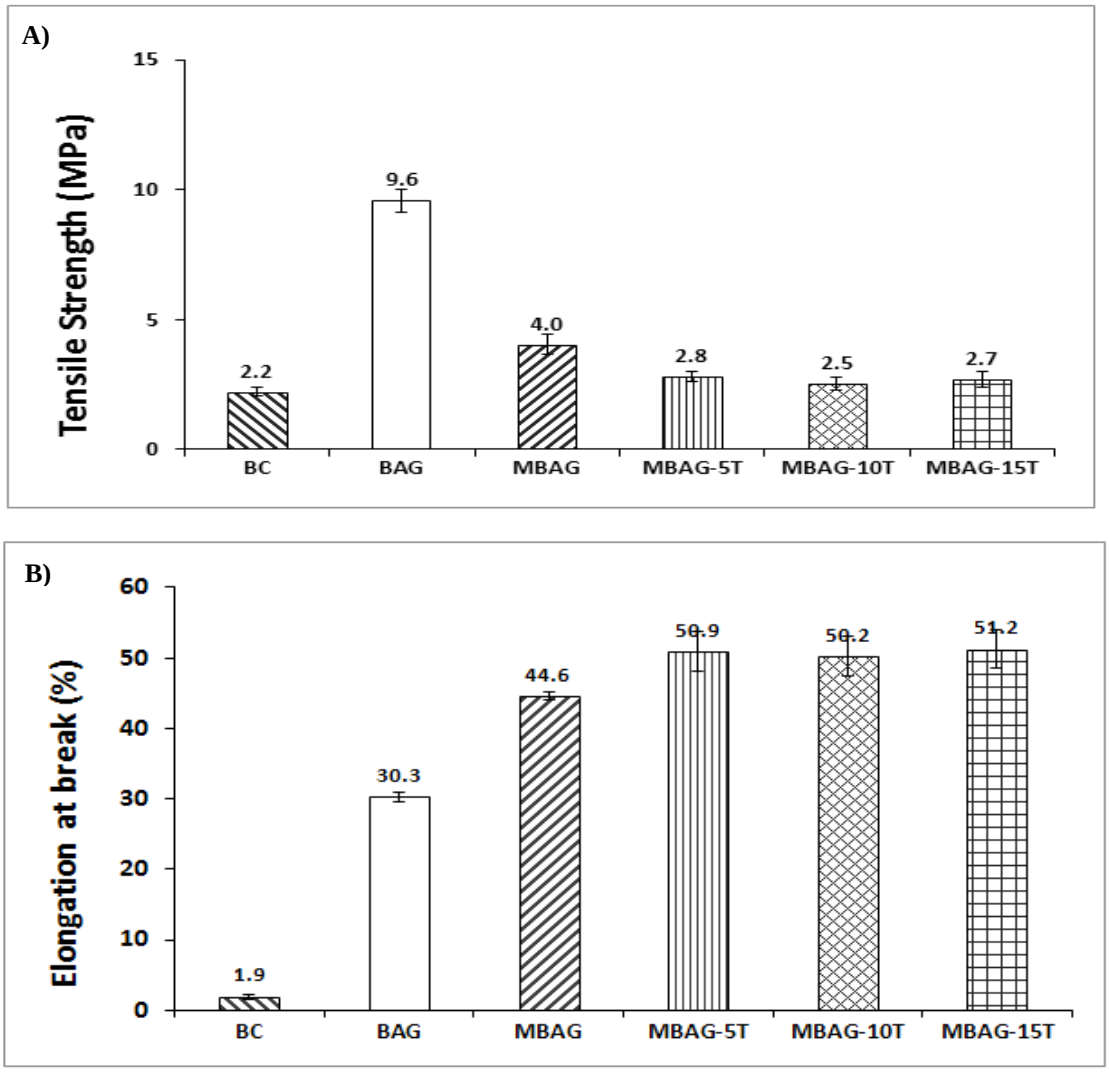


Figure 4.18 Mechanical properties of BAG films in wet state at a ratio of 5, 10 and 15 mg tannic acid to g gelatin solution (w/w): Tensile strength (A) and Elongation at break (B).

4.3.5 Scanning electron microscope (SEM)

SEM images of overview surface morphology of MBAGT films in dry state at 200 magnifications are shown in Figure 4.19 A, B and C. The surface roughness of MBAGT films due to cellulose fibrils distribution on surface was observed. It was noticed that the MBAGT film at a ratio of 10 mg and 15 mg tannic acid to g gelatin solution showed a smoother surface than that of 5 mg tannic acid content. It could probably suggest that the better tannic acid-gelatin interactions at higher tannic acid content might cause more precipitates covering the surface.

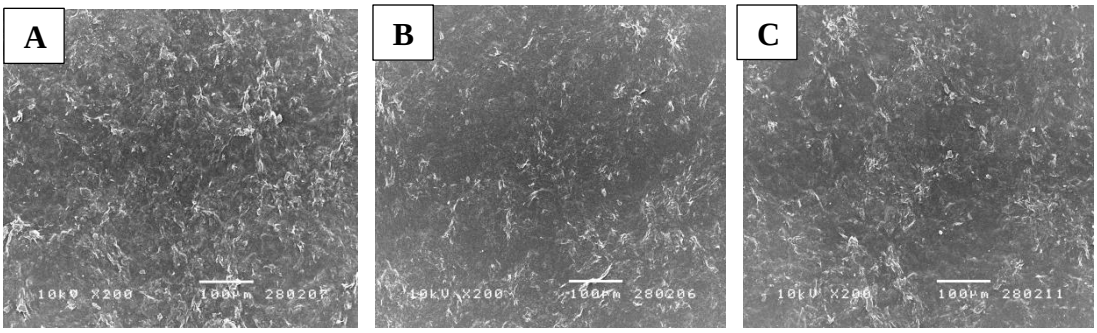


Figure 4.19 SEM images of overview surface morphology of MBAGT films in dry form at ratio of mg tannic acid to g gelatin solution (w/w): 5 mg (A); 10 mg (B); 15 mg (C).

SEM photographs revealed the surface morphology of the MBAGT films at 10,000 magnifications at a ratio of 5, 10 and 15 mg tannic acid to g gelatin solution are shown in Figure 4.20 (A-F), both in dry and wet states, respectively. The surface MBAGT films showed the formation of fibrils in gel and no significant difference in surface morphology was observed with the change of tannic acid content. The MBAGT films both in dry and re-swollen forms exhibited a dense structure without meso- and macropores. With the tannic acid cross-linked gelatin gels covering BC fibrils, the MBAGT films displayed better gel dispersion on cellulose fibrils as compared to MBAG films.

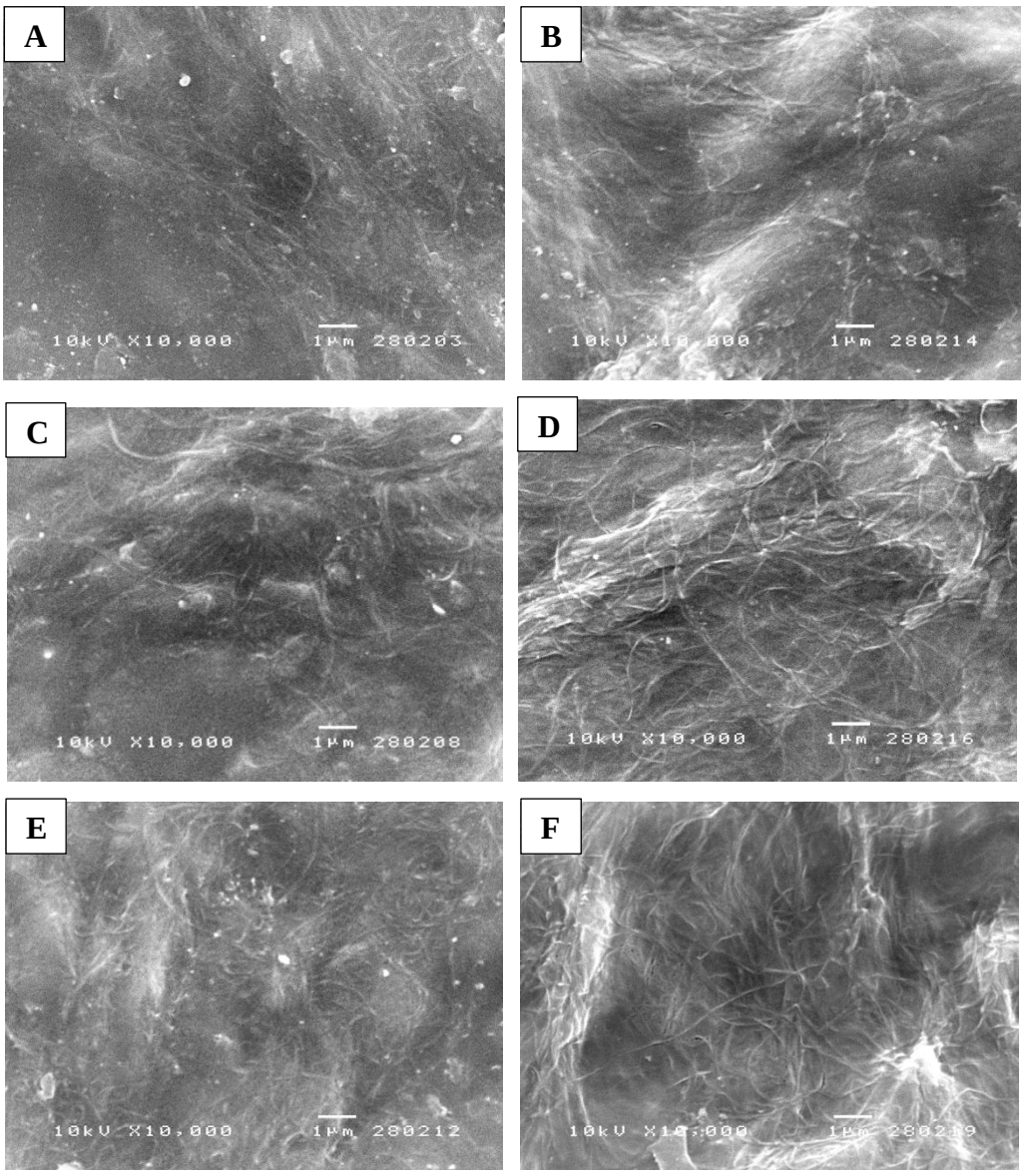


Figure 4.20 SEM images of surface morphology of MBAGT films in dry (left) and re-swollen (right) forms at a ratio of mg tannic acid to gram gelatin solution at 5 mg (A and B), 10 mg (C and D) and 15 mg (E and F).

Figure 4.21 expressed the cross sectional morphologies of the MBAGT films at different tannic acid content. The MBAGT films showed dense-packed sheet structure. However, no significant difference was observed with the change in tannic acid content. Compared to the MBAG films, the MBAGT films showed an apparent decrease in free volume of the packed sheet. Therefore, the cross-linking of gelatin by

tannic acid could reduce forced orientation relaxation. It was previously reported that with the use of tannic acid as a gelatin cross-linking agent, the alignment of the gelatin strand along the direction of deformation increased and the thickness of the layers, as well as the interlayer space decreased (De Carvalho and Grosso, 2004; Cao et al., 2007).

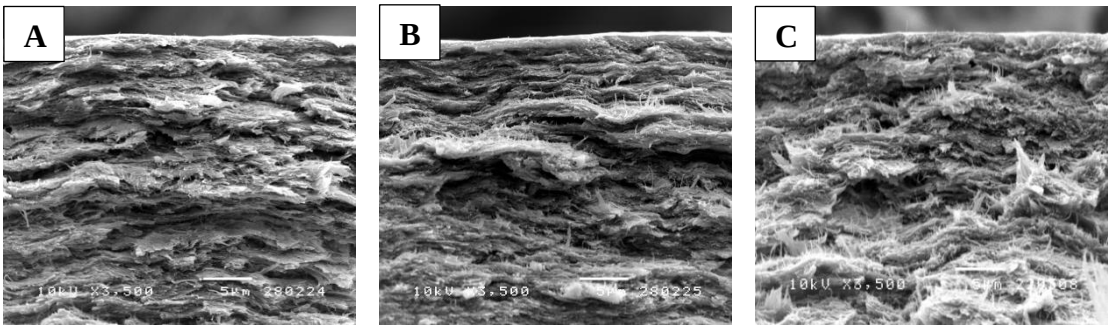


Figure 4.21 SEM images of cross sectional morphology: the MBAGT films in dry form at a ratio of 5 mg (A), 10 mg (B) and 15 mg (C) tannic acid to g gelatin solution.

4.4 Characterization of MBAGT films modified by mangosteen ethanolic extracts (MBAGTM films)

4.4.1 Transparency

In Figure 4.22, the alphabets beneath the films show the transparency of MBAGTM films. The film thickness was around 0.05 mm (50±10 μm). The MBAGTM film refers to the MBAGT film at a ratio of 60/20/20 which was modified for antimicrobial activities by adding the mangosteen extract. The MBAGTM films were more opaque as compared with the BAG, MBAG and MBAGT films. The transparency of the films decreased with the increase of mangosteen ethanolic extract from 1% to 5%. However, the increase of the mangosteen extract to 10% showed the relatively improved of film transparency compared to those with the lower mangosteen extract content. The color of the MBAGTM films changed from

brownish to yellowish brown color because the mangosteen ethanolic extract with tween 80 was yellowish brown in color.

The determination of the film transparency by using UV–visible spectrophotometer was shown in Figure 4.23. The result showed that the film transparency from high to low was in the following order: BAG > MBAG > MBAGT > MBAGTM10% > MBAGTM1% > MBAGTM5%.

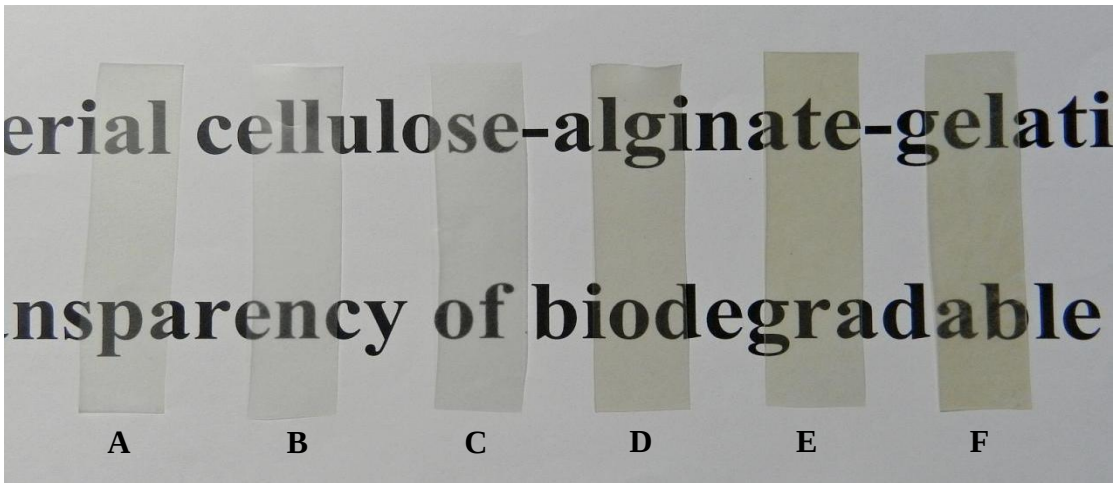


Figure 4.22 Transparency of films: the BAG films at a ratio of 60/20/20 without plasticizer (A), with glycerol (B), with glycerol and adding tannic acid at a ratio of 10 mg (C) tannic acid per g gelatin solution, with glycerol, tannic acid and the mangosteen ethanolic extract at concentration of 1% (D), 5% (E) and 10% (F) v/v, respectively.

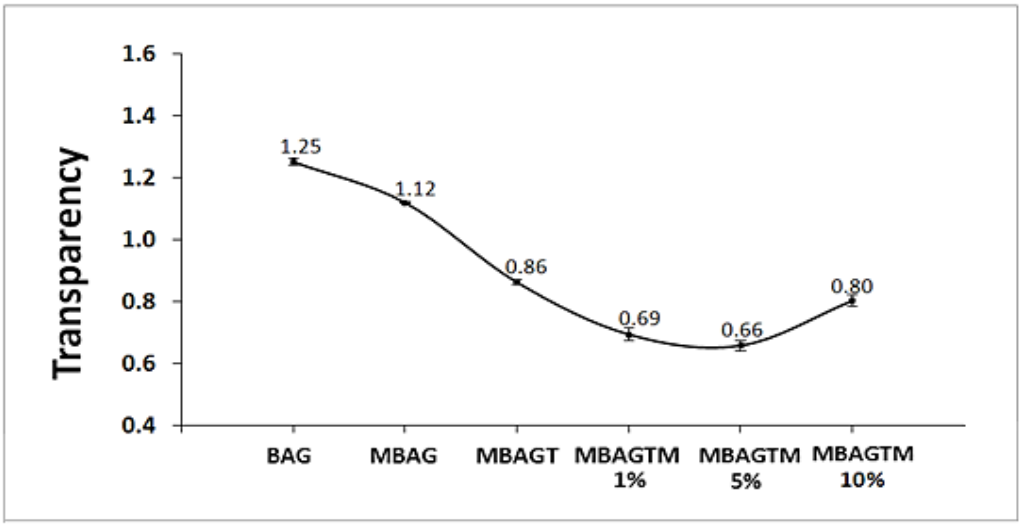


Figure 4.23 Transparency of films by using UV-visible spectrophotometer

4.4.2 Fourier transforms infrared spectroscopy (FT-IR)

The FT-IR spectra of mangosteen ethanolic extract, the BAG, MBAG, MBAGT and MBAGTM films at concentration of 1, 5 and 10% v/v mangosteen ethanolic extract are shown in Figure 4.24 A, B, C, D, E, F and G, respectively.

The characteristic absorptions of mangosteen extract are known to be associated with the stretching vibrations of C-H group stretching in aromatic ring of both aromatic and alkene at 2923 cm^{-1}, ether groups (C-O-C) and C-O (-C-OH) at 1108 cm^{-1}, ketone group (C=O) as chelate carbonyl group in the structure of xanthone backbone at 1736 cm^{-1} and phenolic hydroxyl group at 3370 cm^{-1}. The chelating of carbonyl group in the backbone of xanthone is shown in Figure 4.25. In particular, the band at 1108 cm^{-1} arises most probably from the C-O of aromatic -OH group.

The BAG, MBAG, MBAGT and MBAGTM exhibited the characteristic absorption bands with no appearance of new peaks. The BAG and MBAG films showed multiple bands that previously explained in topic 4.1.2 and the MBAGT film showed multiple bands that previously explained in topic 4.2.2. The changes in absorption spectrum of the MBAGTM films containing 1, 5 and 10% v/v mangosteen ethanolic extract were as follows. The C-O-C stretching peak were shifted to 1059, 1060 and 1060 cm^{-1} and the O-H and the N-H stretching bands were shifted to 3400,

3352 and 3352 cm^{-1}, respectively. The H-O-H bending peaks were shifted to 1646, 1648 and 1657 cm^{-1} which it also can be assigned to the C=O stretching and the -COO stretching peaks were shifted to 1437, 1442 and 1443 cm^{-1}, respectively. The symmetric of N-H bending peaks were shifted to 1535, 1536 and 1535 cm^{-1} and the C-N stretching peaks were shifted to 1236, 1237 and 1237 cm^{-1}, respectively. The arising of stretching vibrations of C-H group stretching in aromatic ring of both aromatic and alkene at around 2926-2932 cm^{-1} showed that the MBAGTM films contained mangosteen extract. The amplitude of this peaks increased with increasing of the extract content. The shift of this peak could imply weakly interactions between the functional groups of the film and cyclic alkenes in mangosteen extract.

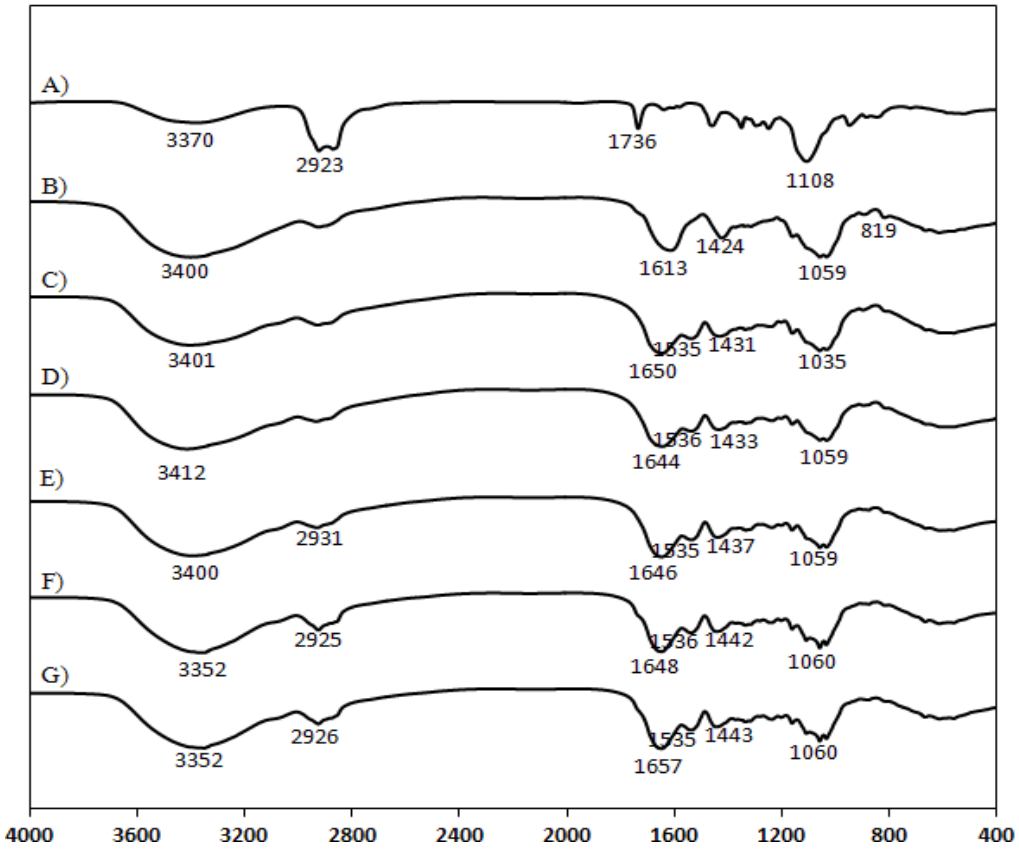


Figure 4.24 FT-IR of mangosteen ethanolic extract (A), the BAG (B), MBAG (C), MBAGT-10 mg (D) and MBAGTM at 1% (E), 5% (F) and 10% (G) v/v concentration of mangosteen ethanolic extract, respectively.

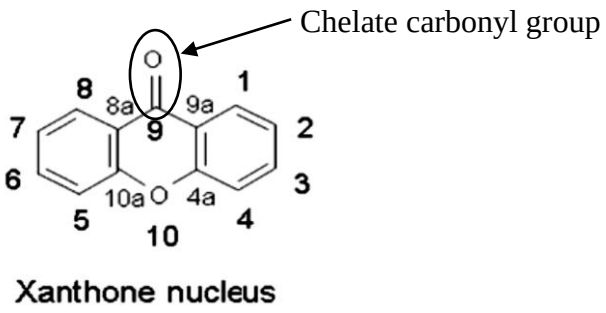


Figure 4.25 Chelating of carbonyl group in the backbone of xanthone (Chaverri et al., 2008)

4.4.3 Water absorption capacity (WAC)

The WAC of the MBAGTM films in DI water is shown in Figure 4.26. The WAC values decreased with the increase of mangosteen ethanolic extract content. The WACs of the BAG, MBAG and MBAGT films were 333.6, 462.2 and 389.9%, respectively, whereas the WACs of the MBAGTM films containing the mangosteen extract at 1, 5 and 10% v/v were 321.9, 282.2 and 269.6% respectively. The ethanolic extract contained hydrophobic compounds which were less hydrophilic than BC, alginate and gelatin and the extract compounds could filled in the micropores of the films, reducing the void volume. Therefore the supplement of mangosteen ethanolic extract reduced the WAC of the films.

It was previously reported that the hydrophobic nature of cellulose could be generated with the presence of hydrophobic substances in cellulose domains (Yamane et al., 2006). For the same reason, with the supplement of hydrophobic compounds from the mangosteen ethanolic extract, the hydrophobic nature of the modified films was enhanced.

However, the WACS of the MBAGTM films were only slightly decreased with the increase of the mangosteen extract from 5% to 10% v/v. Therefore, the addition of the extract more than 5 % might not further lower void volume of the film. On the other hand, the high supplement of the mangosteen ethanolic extract might also have interfering effects on the chemical bonds of the films, resulting in a looser

film structure, which could be a reason for almost equal WAC of the MBAGTM film with the increase of the mangosteen extract from 5 to 10 % (v/v).

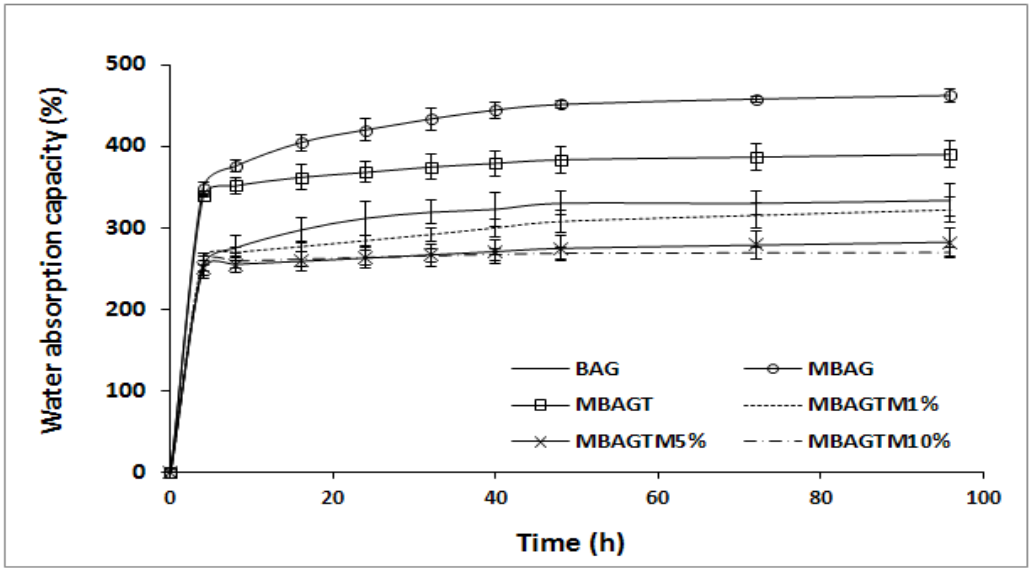


Figure 4.26 Water absorption capacity (%) of the composite films: BAG (——); MBAG (—○—); MBAGT (—□—); MBAGTM1% (·····); MBAGTM5% ( ) and MBAGTM10% (- - -), respectively.

4.4.4 Mechanical properties

Tensile strength (TS) and elongation at break (EB) of the films in dry state of BAG, MBAG, MBAGT and MBAGTM containing the mangosteen extract at 1, 5 and 10% v/v were shown in Figure 4.27 A and B.

Compared to the MBAGT film, the MBAGTM films with 1% v/v of the extract exhibited slightly increased TS and EB to 188.9 MPa and 3.0%, respectively, which could be owing to the effect of filling pores or coating fibrils of the extract compounds on interconnections of network BAG fibrils. Nevertheless, the MBAGTM films with 5 and 10% v/v of the extract exhibited the significant decrease in TS and the increase in EB, which could again be a result of the loosen structure at the high supplement of the mangosteen ethanolic extract as discussed previously in Topic

4.4.3.

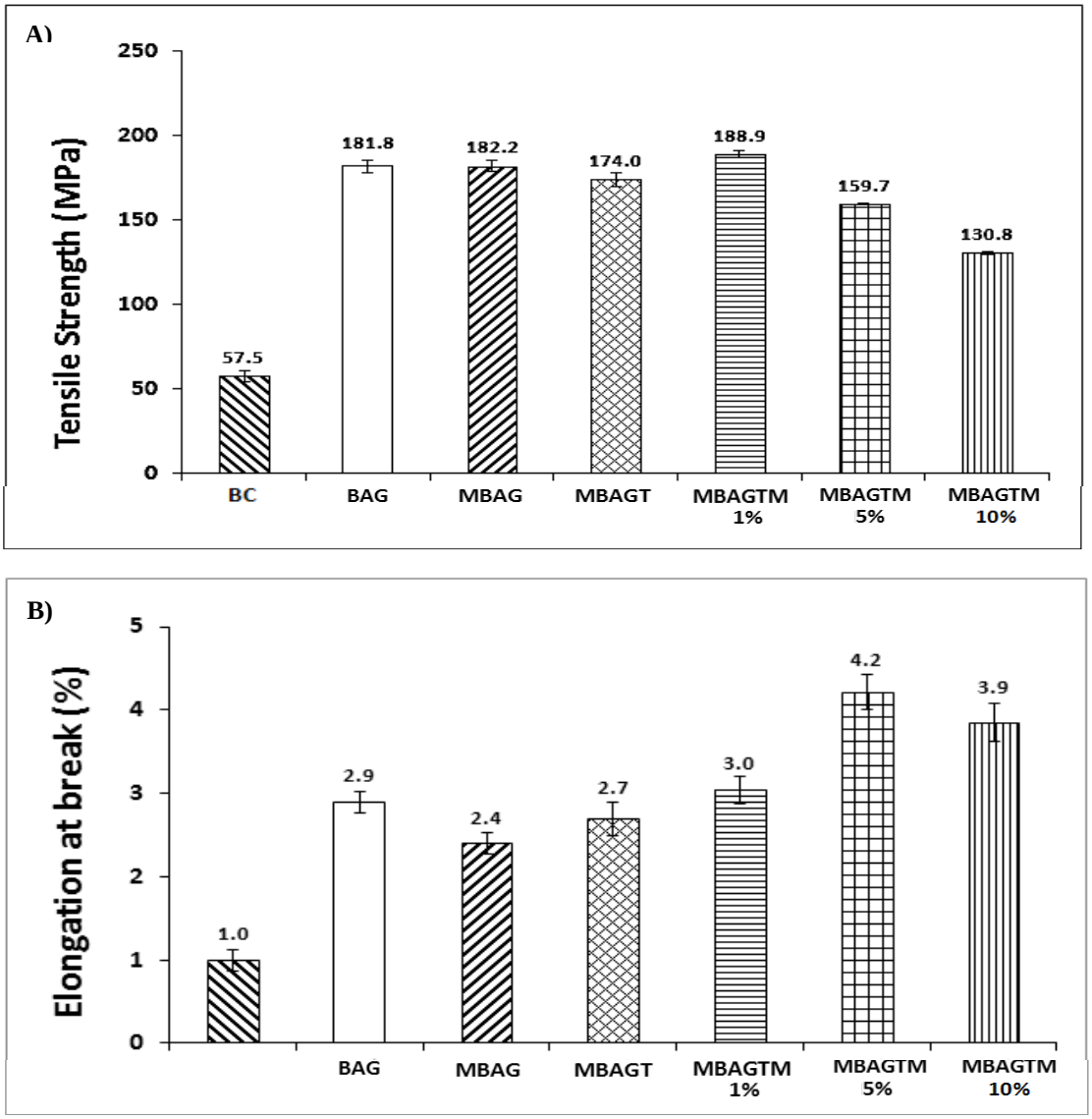


Figure 4.27 Mechanical properties of MBAGTM films in dry state at 1%, 5% and 10% v/v concentration of mangosteen ethanolic extract: Tensile strength (A) and Elongation at break (B).

Tensile strength (TS) and elongation at break (EB) of the films in wet state were shown in Figure 4.28 A and B. In wet state, the TS of the MBAGTM films which contained the mangosteen extract at 1, 5 and 10% v/v was slightly lower than

those of the MBAGT films. The EB of the MBAGTM film at 1% v/v of the mangosteen extract increased to 63.0% but the EB of the films decreased to 46.7 and 47.2% with the further adding the mangosteen extract to 5 and 10% v/v. The TS of the re-swollen was only about 0.11-0.15 of the dry films, whereas the EB of the re-swollen films was about 10-20 folds greater than the dry films. Overall, these results should be a consequence of a very relaxed network structure of the films in wet state.

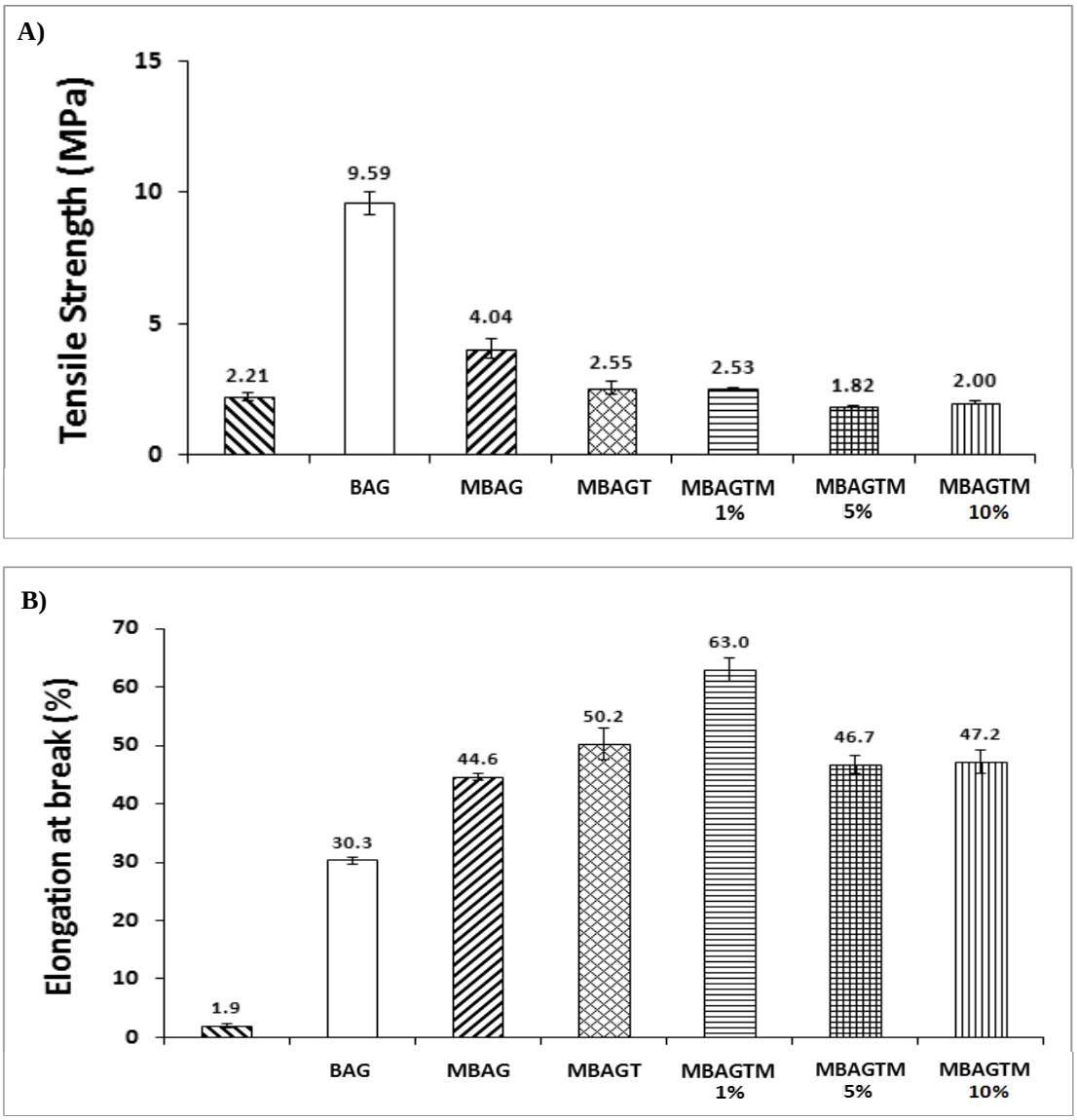


Figure 4.28 Mechanical properties of MBAGTM films in wet state at 1%, 5% and 10% v/v concentration of mangosteen ethanolic extract: Tensile strength (A) and Elongation at break (B).

4.4.5 Scanning electron microscope (SEM)

The SEM photographs of surface morphology of films in dry state at 200 magnifications were showed in Figure 4.29 A, B and C. The surface roughness of nanocellulose fibers of MBAGTM films was still observed but not as much as being observed on the MBAGT films.

SEM photographs in Figure 4.30 (A-F) reveal the surface morphology at 10,000 magnifications of the MBAGTM films in dry and wet states. After adding mangosteen ethanolic extract (1, 5 and 10% v/v) into the mixture slurry, the extract could penetrate into empty space in microporus structures of BC fibrils, alginate and tannic acid cross-liked gelatin. The extract incorporated in the composite films resulting in a smoother surface. Especially the films in dry state, nanocellulose fibrils could barely be observed on the film surface and the thickness of the films increased with noticeable excess extract compounds on the film surfaces.

Compared to the dry films, the re-swollen films exhibited a loose fibril network. The addition of mangosteen ethanolic extract might affect cellulose nanofibril orientation and enhance the free volume for motion of cellulose fibril. Moreover, the MBAGTM, especially a 10% v/v mangosteen ethanolic extract supplement showed small cracks or hairline fractures distributed along some part of BAG fibrils as shown in Figure 4.30 (B, D and F).

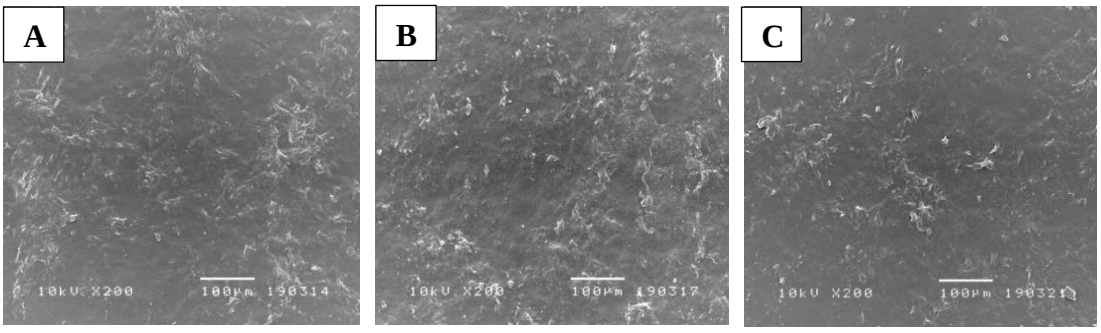


Figure 4.29 SEM images of overview surface morphology at magnification 10 kV 200X of MBAGTM at concentration of 1%, 5% and 10% v/v mangosteen ethanolic extract in dry state.

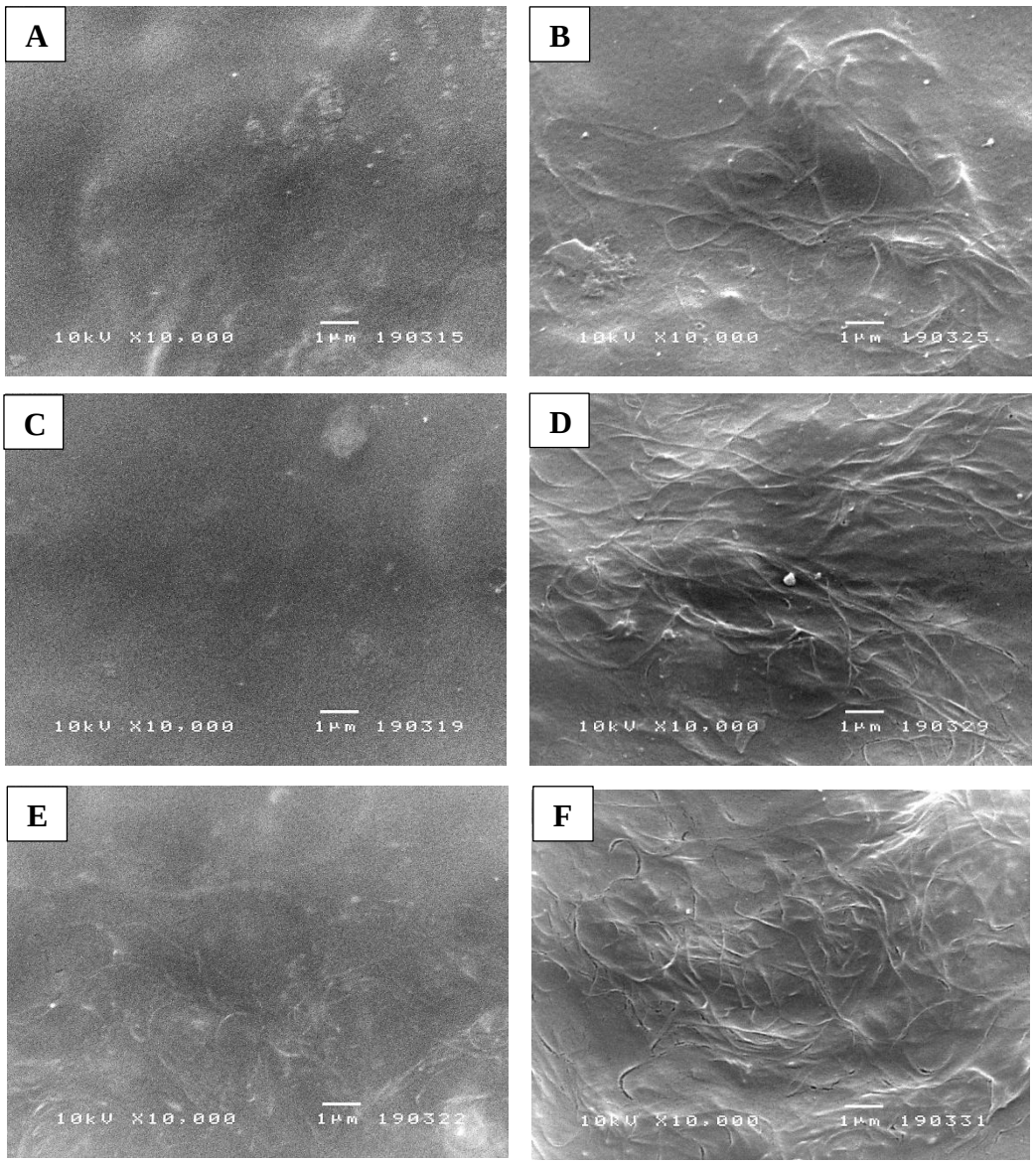


Figure 4.30 SEM images of surface morphology of MBAGTM films in dry (left) and re-swollen (right) forms at concentration of 1% (A and B), 5% (C and D) and 10% (E and F) v/v mangosteen ethanolic extract.

Figure 4.31 illustrates the cross sectional morphologies of the MBAGTM films. As compared to the MBAGT films, each layer thickness of the film sheet of MBAGTM was enhanced, along with the increases of void fraction or free volume and disorder of orientation. Since the compounds in the extract could penetrate into the film sheet and coated on the surface of the sheet, the addition of the mangosteen

extract might increase heterogeneous and hydrophobic characters in the films. The ethanolic extract contained hydrophobic compounds, whereas main components of the BAG film were hydrophilic compounds. Therefore the supplement of the ethanolic extract promoted the heterogeneous compatibility or solubility in the films. It might lead to noticeable increase in free volume space of the modified films (MBAGT films) as shown in the SEM images of film cross-sections (Figure 4.31). Therefore, the addition of the extract enhanced the thickness as well as the inter-space of the sheet layers of the MBAGTM films.

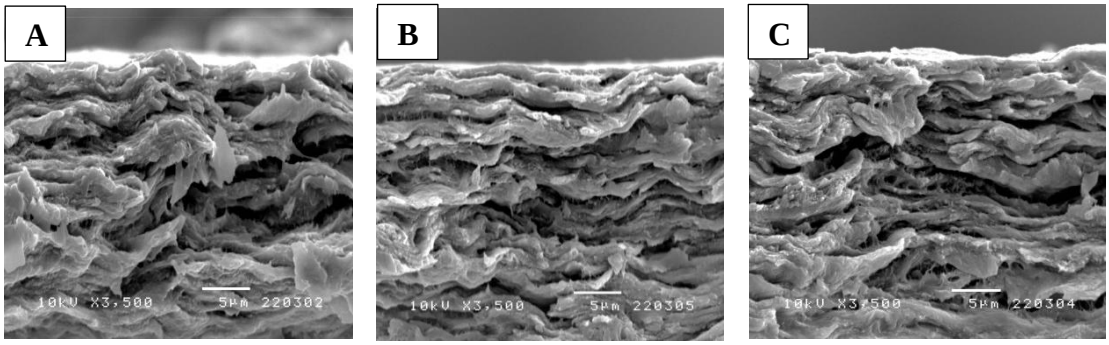


Figure 4.31 SEM images of cross sectional morphology: the MBAGT films in dry state at concentration of 1%, 5% and 10% v/v mangosteen ethanolic extract

4.4.6 X-ray diffraction

The X-ray diffraction (XRD) was used to determine the crystallinity of the films. The XRD patterns and crystallinity (%) of BC, BAG, MBAG, MBAGT and MBAGTM films containing mangosteen ethanolic extract at 1, 5 and 10% v/v were shown in Figure 4.32. The pattern of BC exhibits three main peaks at 2θ of 14.54˚, 16.87˚ and 22.68˚ (related to more crystalline order), which are corresponding the (1 $\bar{1}$ 1), (1 1 0) and (2 0 0) reflexion planes of cellulose I, respectively, as already reported by Cai and Kim (2010), Retegi et al. (2010) and Phisalaphong et al. (2008). Both gelatin and alginate are amorphous compounds; therefore their crystallinities are extremely low.

The reflexion planes: reflective-angle and d-spacing values of BC, BAG, MBAG, MBAGT and the MBAGTM films are shown in Table 4.2. Except for the three main peaks of BC, all of modified film by sodium alginate, gelatin, tannic acid and mangosteen ethanolic extract showed no other characteristics peaks implying amorphous nature of the other polymers.

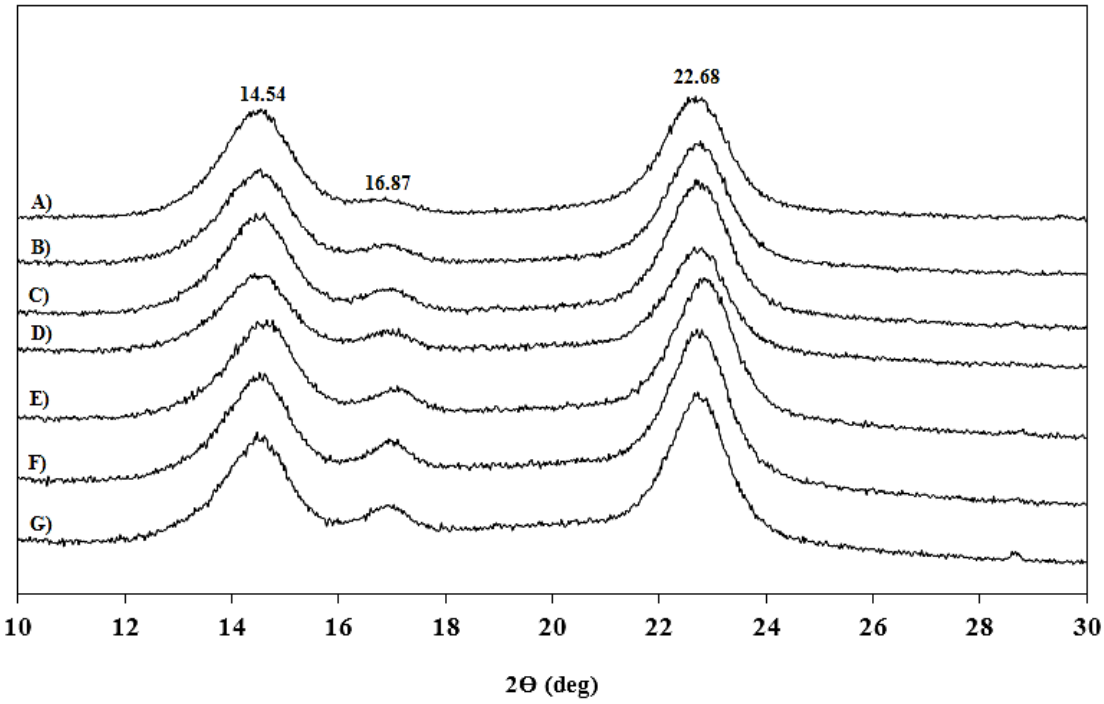


Figure 4.32 The X-ray diffraction (XRD) patterns of BC (A), BAG (B), MBAG (C), MBAGT (D) and MBAGTM films at 1% (E), 5% (F) and 10% (G) v/v concentration of mangosteen ethanolic extract

According to Table 4.3, the degree of crystallinity (%) of BC, BAG, MBAG, MBAGT and MBAGTM at 1, 5 and 10% v/v of the extract was 68.82, 67.68, 71.24, 66.21, 65.93, 59.43 and 59.21 %, respectively. The addition of glycerol as a plasticizer into BAG film (referred as MBAG film) supported the molecular movements and could promote crystallization; therefore, an elevated degree of crystallinity to 71.24 % of the composite MBAG film was obtained. However, the results exhibited that the crystallinity of the films tended to decrease with adding

tannic acid and mangosteen ethanolic extract into the MBAG film. It can be explained by the strong interaction between BC, sodium alginate and gelatin which has destroyed the close packing of the BC molecules for the formation of regular crystallites. Similar observations were previously reported in alginate/gelatin blend films for drug controlled release (Dong et al., 2006). Furthermore, the addition of tannic acid decreased crystallinity of the film due to the enlarged intermolecular interactions between hydroxyl groups in tannic acid and NH_2 side-chain groups in gelatin, which might limit the molecular movements, thus preventing crystallization (Peña et al., 2010).

Table 4.2 The reflexion planes: reflective-angle and d-spacing values of BC, BAG, MBAG, MBAGT and the MBAGTM films

Film sample	**2θ [d(1 $\bar{1}$ 1)]**	**2θ [d(1 1 0)]**	**2θ [d(2 0 0)]**
BC	14.54˚ (6.09)	16.87˚ (5.25)	22.68˚ (3.92)
BAG	14.54˚ (6.09)	16.89˚ (5.24)	22.77˚ (3.90)
MBAG	14.61˚ (6.06)	16.96˚ (5.23)	22.68˚ (3.92)
MBAGT	14.36˚ (6.09)	17.12˚ (5.18))	22.66˚ (3.90)
MBAGTM1%	14.65˚ (6.04)	17.10˚ (5.18)	22.84˚ (3.89)
MBAGTM5%	14.52˚ (6.10)	16.94˚ (5.23)	22.70˚ (3.91)
MBAGTM10%	14.47˚ (6.12)	17.03˚ (5.21)	22.73˚ (3.91)

Table 4.3 Degree of crystallinity (%) of BC (A), MBAG (B), MBAGT (C) and MBAGTM films at concentration of 1% (D), 5% (E) and 10% (F) v/v mangosteen ethanolic extract

Film	**BC**	**BAG**	**MBAG**	**MBAGT**	**MBAGTM 1%**	**MBAGTM 5%**	**MBAGTM 10%**
Crystalline area	9646	9657	10489	8312	11356	12002	11597
Amorphous area	4359	4611	4235	4243	5868	8192	7988
Crystallinity (%)	68.82	67.68	71.24	66.21	65.93	59.43	59.21

4.4.7 Oxygen permeability

Table 4.4 showed the results of the oxygen transmission rate (OTR) of the BAG, MBAG, MBAGT and MBAGTM films (mean values from the duplicate testing) comparing with the commercial film packaging. Due to the extremely small pore diameter of the films in dry state, the corresponding values of OTR were considerably low. The OTR of BAG, MBAG, MBAGT and MBAGTM10% was 0.887, 1.080, 0.764 and 1.100 cc/m^2/day, respectively.

The OTR of films in dry state decreased from the general BC film because of the reduction of pore diameter into nanometer-sized pores, whereas the approximate diameter of oxygen molecule is around 0.36 nm (Kanjanamosit et al., 2009) or 11 Å (Hambleton et al., 2009), so that oxygen could slightly flow through the films.

Generally, the film of sodium alginate is impervious to oils and fats but it is poor moisture barriers and has high oxygen permeability coefficient. On the other hand, hydrogen bonds in gelatin films can be used as the barrier to oxygen. Thus, gelatin adding offers a lower chance of protein oxidation. Gelatin films do not allow oxygen-protein interactions (Krochta and Johnson; 1997) and can significantly prevent lipid oxidation. As shown in the previous SEM images, sodium alginate and gelatin could fill empty space between BC fibrils leading to the decreased pore diameter and consequently, causing the reduction in oxygen permeability of the films. In addition, according to the interpenetrating effects, polymer chains are confined to a smaller volume through strong interactions (electrostatic attractions and intermolecular hydrogen bonding); this could also lead to the reduction of free volume for the interfacial polymers, which takes longer for oxygen molecules to travel through and causes them to have more interactions (Svagan et al., 2012; Gu et al., 2013). Moreover, it was previously suggested that due to the hydrophilic nature of proteins of gelatin, it can limit the resistance to water vapor transmission, but provide excellent oxygen barriers at low relative humidity (Wang et al., 2010).

The addition of plasticizers as glycerol increased the oxygen permeability since glycerol can induce a structural change of the BAG film. Similar observations were previously reported that the addition of plasticizers such as glycerol increased oxygen permeability for polysaccharide films (Sothornvit and Pitak, 2007) such as

HPMC composite films (Imran et al., 2010) and gelatin films (Herring et al., 2010). However, the OTR increased only slightly by adding glycerol to form the MBAG films.

The addition of tannic acid reduced the oxygen permeability because tannic acid could crosslink gelatin, resulted in decreasing free volume space as well as pore diameter of the MBAGT film in dry state. Further addition of the mangosteen ethanolic extract into the films slightly improved the OTR of the films because of the increased free volume as previously discussed in topic 4.4.5.

When compared with the commercial packaging films such LDPE, HDPE, polypropylene, polyester, PET, polyvinyl chloride (plasticized) and polystyrene (PS) as shown in Table 4.4, the OTR of all developed films in this study were within the range of those of commercial packaging films. This result indicated that the BAG and modified BAG films have good potential to be used as packaging films to prevent food oxidation.

Table 4.4 The oxygen transmission rate of the BAG film at a ratio of 60/20/20, the MBAG film, the MBAGT and MBAGTM film (Mean value from duplicate testing) and commercial film packaging (*Modern plastics encyclopedia, 1979-1980)

Material	**OTR±S.D. (cc/m^2.day)**
BAG	0.887±0.026
MBAG	1.080±0.042
MBAGT	0.764±0.042
MBAGTM10%	1.100±0.000
Low density polyethylene (LDPE)*	500
High density polyethylene (HDPE)*	185
Polypropylene*	150-240
Polyester, PET*	3.0-4.0
Polyvinyl chloride (plasticized)*	2-400
Polystyrene (PS)*	250-350

4.4.8 Water vapor permeability

In the food industry, the difficulty with composite films is the relatively high water vapor permeability. Permeability in packaging is controlled by the diffusivity and solubility of water within the film matrix. Thus implementing food nanotechnology, new organization of firmly linked three dimensional networks can be fabricated to prevent diffusion of water in foodstuffs (De Moura et al., 2009; Imran et al., 2012).

In general, WVTR values for composite films produced from protein and polysaccharide mixtures were lower in comparison to the WVTR values of those films formed from protein alone (Wang et al., 2010). All films were analyzed for water vapor transmission property by following the ASTM E-96. Table 5.10 showed the results of the water vapor transmission rate (WVTR) of the BAG, MBAG, MBAGT and MBAGTM films (using mean values from duplicate testing) comparing with the commercial film packaging. The water vapor transmission rate (WVTR) of BAG, MBAG, MBAGT and MBAGTM10% was 769.00, 793.00, 976.32 and 1402.08 $g/m^2/day$, respectively.

BC is high hydrophilic with a uniform porous structure. Therefore, water vapor could be easier to diffuse though the film resulted in high WVTR.

The WVTR of the BAG film should reduce from BC film because sodium alginate and gelatin wrapped between BC fibril which the ionic crosslinking in sodium alginate reduced polymer segmental mobility as resulting in hard diffusing though the film. It has been previously explained that calcium ions are located in cavities linking two L-guluronic acid chains of alginate; the ability of L-guluronic acid to form an insoluble net makes alginate films a good barrier to water vapor (Olivas and Barbosa-Cànovas, 2008; Hableton et al., 2009). The chemical interaction between water and film materials that resulted in a volume expansion and the ultimately increase of free volume was found to have marked effects on water vapor permeation (Kääriäinen et al., 2011).

With the addition of glycerol as plasticize, the WVTR of the MBAG film was slightly higher than the unplasticized film. The incorporation of glycerol provoked a reorganization of the BAG network, which became less dense with a larger free

volume, facilitating greater mobility and as a consequence, greater WVTR was obtained. The approximate diameter of water molecule is 2.4 Å (Hambleton et al., 2009). Glycerol plasticized film had given significantly higher permeability, which may be caused by the higher number of available polar (-OH) groups in MBAG composite films. In general, the addition of a plasticizer modifies the properties of the film by reducing the intermolecular bonds between the polymer chains, thus increasing the WVTP of the films. The water also plays the role of plasticizer in hydrophilic coatings and the density or local viscosity decrease promoting the diffusing molecules mobility (Guilbert and Biquet, 1996).

For the MBAGT film, the addition of tannic acid to crosslinked gelatin showed a slightly positive impact on the WVTR. It could probably be assumed that polar groups that were many hydroxy groups of tannic acid compounds incorporated with water, so that the apparent WVTR did not change or slightly changed with adding tannic acid. Similar observations were previously reported that tannic acid slightly increased WVTP of gelatin films (Cao et al., 2007) and addition of tannic acid, caffeic acid and ferulic acid at all levels increased WVTP of resulting films (Nuthong et al., 2009). Furthermore, with adding mangosteen ethanolic extract into the films, the WVTR increased. Because the extract is hydrophobic compound and the main components of the film are hydrophilic compounds, therefore the heterogeneous compatibility or solubility occurred, which could lead to increased free volume space of MBAGTM film as previously discussed in Topic 4.4.5. Moreover, the excess mangosteen ethanolic extract caused small crack or fracture on surface films, which might have small effect on WVTR and OTR of the films. Mangosteen ethanolic extract is recognized to improve the water barrier properties of biopolymer films due to its hydrophobic nature and reduce the water sorption capacity of the films. However, it was found that the increase of free volume had marked effects on WVTR of the MBAGTM films; as a result the WVTR of the modified film significantly enhanced.

Table 4.5 The water vapor permeability measurement of the BAG film at a ratio of 60/20/20, modified the films and BAGM film (Mean value from duplicate testing) and commercial film packaging (*Modern plastics encyclopedia, 1979-1980)

Material	**WVTR±S.D. (g/m^2.day)**
BAG	769.00±1.41
MBAG	793.00±1.41
MBAGT	976.32±13.58
MBAGTM10%	1402.08±20.36
Low density polyethylene (LDPE)*	1.0-1.5
High density polyethylene (HDPE)*	0.3
Polypropylene*	0.7
Polyester, PET*	1.0-1.3
Polyvinyl chloride (plasticized)*	5-30
Polystyrene (PS)*	7-10

4.5 Antimicrobial activities

4.5.1 The minimum inhibitory concentration test (MIC) and the minimum bactericidal concentration test (MBC)

The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of mangosteen ethanolic extracted from *G. mangostana* fruit pericarp (in ethanol solution with tween-80) and tannic acid was shown in Table 4.6.

The antimicrobial activity of mangosteen ethanolic extract at concentrations of 5.84, 2.96, 1.46, 0.73, 0.365, 0.1825, 0.09125, 0.0456, 0.0228 and 0.0114 mg/ml was determined on four selected strains of pathogenic bacteria using a broth microdilution method. To compare the antimicrobial potential of different samples, the results of the lowest concentration of extract required to completely inhibit the growth of the microorganisms at concentrations of 1×10^8 CFU/mL (MIC).

The results of MIC exhibited that mangosteen ethanolic extracted and tannic acid could inhibit bacteria in food; *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes* and *Staphylococcus aureus*. The MIC for mangosteen ethanolic extract possessed at 1.46 mg/ml concentration for *Escherichia coli* and *S. aureus*, and at 0.73 mg/ml concentration for *S. typhimurium* and *L. monocytogenes*. Tannic acid possessed at 30 mg/ml concentration for *E. coli*, *S. typhimurium* and *S. aureus*, and at 15 mg/ml concentration for *L. monocytogenes*.

The MBC for mangosteen ethanolic extract possessed at 1.46 mg/ml concentration for all bacteria, whereas tannic acid possessed at 30 mg/ml concentration for *E. coli*, *S. typhimurium* and *S. aureus*, and at 15 mg/ml concentration for *L. monocytogenes*. Antimicrobial agents with low activity against an organism had a high MIC while a highly active antimicrobial agent gave a low MIC. These results indicated that the mangosteen ethanolic extract exhibited more effective antibacterial activity against the bacteria than tannic acid.

Table 4.6 The minimum inhibitory and minimum bactericidal concentrations (MIC and MBC) of mangosteen ethanolic extract and tannic acid

Microorganisms	Mangosteen ethanolic extract		Tannic acid	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
E. coli (−)	1.46	1.46	30	30
S. typhimurium (−)	0.73	1.46	30	30
L. monocytogenes (+)	0.73	1.46	15	15
S. aureus (+)	1.46	1.46	30	30

4.5.2 Antimicrobial assay

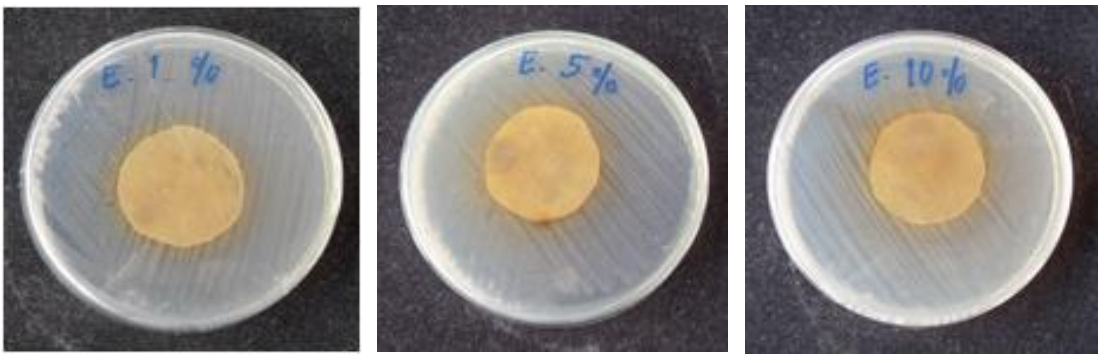
The study on antimicrobial activities of MBAGTM films containing mangosteen ethanolic extract against bacteria consisted of *E. coli*, *S. typhimurium* and *S. aureus* and fungi as *A. niger* was evaluated by Disc diffusion method (Voravuthikunchai et al,. 2004). The films containing mangosteen ethanolic extract did not show clear inhibition zones surrounding the film discs in the agar for all microbial tests as shown in Table 4.7, 4.8 and Figure 4.33, 4.34.

Although the disc diffusion method is a common test for antimicrobial activities, the disc diffusion assay has no meaning or provides very poor results for some certain components. According to this method, in order to determine antimicrobial activity, it is required diffusion of antimicrobial agent at certain distance in the agar (or medium). Because diffusion is influenced by molecular weight and other properties such as polarities of the test components and the medium, the result from the evaluation can be very poor for components with non-or low diffusion in the agar. Non polar compound, very high molecular weight compound or compound entrapped in the film does not (or slightly) diffuse in agar, thus it does not produce clear zones. These results indicated that the mangosteen ethanolic extract might not or

only slightly released from the MBAGTM films and barely diffused in the agar. It was probably encapsulated and restrained in the films.

Table 4.7 Antimicrobial activities of MBAGTM films against *E. coli*, *S. typhimurium* and *S. aureus*

Microorganisms	Sample	Clear zone (mm)
E. coli	MBAGTM1%	0
	MBAGTM5%	0
	MBAGTM10%	0
S. typhimurium	MBAGTM1%	0
	MBAGTM5%	0
	MBAGTM10%	0
S. aureus	MBAGTM1%	0
	MBAGTM5%	0
	MBAGTM10%	0



E. coli

S. typhimurium

S. Aureus

Figure 4.33 Inhibitory effect of the MBAGTM samples at 1%, 5% and 10% v/v concentration of mangosteen ethanolic extract on the growth of bacteria for 24 h incubated at 37 °C

Table 4.8 Antimicrobial activities of MBAGTM films against *Aspergillus niger*

Microorganisms	Sample	Observed growth
		Grade
A. niger	MBAGTM1%	5
	MBAGTM5%	5
	MBAGTM10%	5

* Grade was used as a measurement of fungal growth: 0 = none, 1 = only apparent under microscope, 2 = trace (<10%), 3 = light growth (10-30%), 4 = medium growth (30-60%) and 5 = heavy growth (> 60%)

Figure 4.34 The growth of *A. niger* on the MBAGTM specimens, at 30°C at the end of the incubation 7 days

4.6 Absorption and release of bioactive compounds from MBAGTM films

4.6.1 Actual amount of bioactive compounds (phenolic compound and mangostins ethanolic extract)

The summaries of the actual amount of bioactive compounds that were added into MBAGTM films are shown in Table 4.9. The total amount of bioactive compounds in the MBAGTM film were determined to evaluate the holding capacity of the extract in the MBAGTM film and use for analysis of the release characteristics of the mangosteen extract. The MBAGTM at 1, 5 and 10% v/v concentrations of mangosteen ethanolic extract contained phenolic compounds (gallic acid equivalents) at 1.065±0.015, 62.414±0.314 and 102.005±0.635 mg/cm^3 of MBAGTM film and mangostins as 18.149±0.272, 127.256±0.356 and 237.466±0.393 mg/cm^3 of MBAGTM film, respectively. These results exhibited that phenolic compounds and mangostins increased with increasing the extract concentration.

Table 4.9 Actual amount of bioactive compounds in the films

Films	Bioactive compounds	Actual amount in MBAGTM film (mg/cm^3)
MBAGTM1%	Phenolic compounds (GAE)	1.065±0.015
MBAGTM5%		62.414±0.314
MBAGTM10%		102.005±0.635
MBAGTM1%	Mangostins (AME)	18.149±0.272
MBAGTM5%		127.256±0.356
MBAGTM10%		237.466±0.393

4.6.2 Release of bioactive compounds (phenolic compound and mangostins ethanolic extract)

In this study, the cumulative release profiles of bioactive compounds from MBAGTM films containing mangosteen ethanolic extracts were reported as the percentage of the weight of bioactive compounds (phenolic compounds expressed as gallic acid equivalents (GAE) and mangostins expressed as α-mangostin equivalents (AME)) released divided by the actual weight of bioactive compounds containing in MBAGTM films. Acetate buffer at pH 5.6 and Phosphate buffer saline (PBS) at pH 7.4 were used as the solutions.

The cumulative releases of phenolic compounds from MBAGTM films into acetate buffer and PBS are shown in Figure 4.35 A and B. The MBAGTM films modified by supplementation of mangosteen ethanolic extracts with various concentrations of 1, 5 and 10% v/v were investigated for the total amount of released phenolic compounds after the end of total immersion (at 72 h). The results showed that the percentage of cumulative release of phenolic compound from the MBAGTM films at 1, 5 and 10% (v/v) of mangosteen ethanolic extracts were 4.98, 0.09, and 0.05% in the acetate buffer solution at pH 5.6 and 4.89, 0.09, and 0.05% in the PBS buffer solution at pH 7.4, respectively. The percentage of cumulative release of mangostins from the MBAGTM films at 1, 5 and 10% v/v (v/v) of mangosteen ethanolic extracts were 0.53, 0.12, and 0.06% in the acetate buffer solution at pH 5.6 and 1.25, 0.27, and 0.07% in the PBS buffer solution at pH 7.4, respectively (Figure 4.36 A and B). Overall, the releases of phenolic compounds and mangosins from MBAGTM films mainly occurred within 6 h of the immersion. No significant difference of the release of phenolic compounds in the buffers pH 5.6 and pH 7.4, whereas mangosteens released more in the buffer pH 7.4. The releases of phenolic compounds and mangosins from the MBAGTM were very low, especially from the films with high content (5-10%v/v) of mangosteen ethanolic extracts. It was suggested that the accumulation of extract compounds at high concentrations might form agglomerated granules within the cross-linked films and prevented the release of the components.

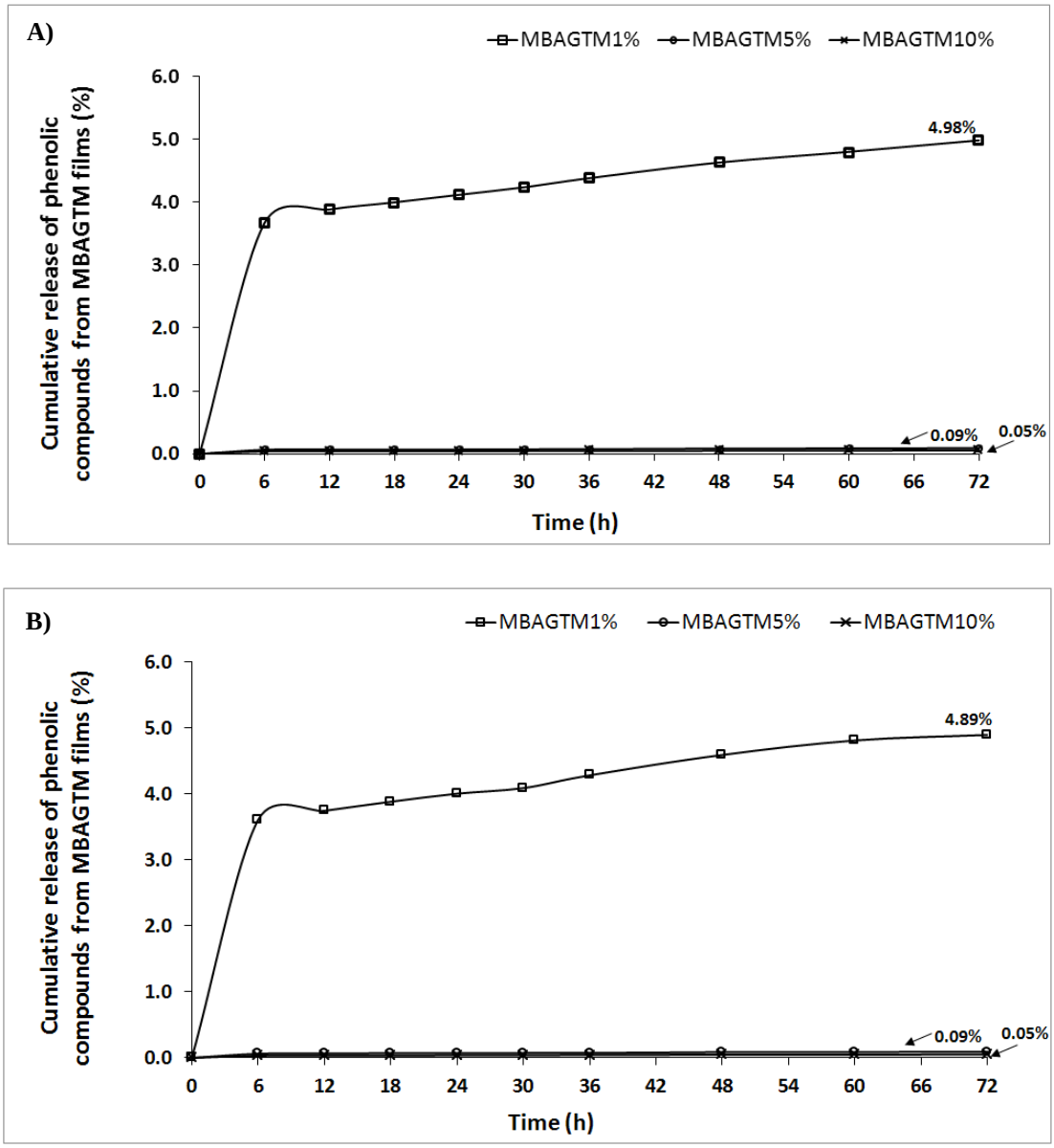


Figure 4.35 Cumulative release profile of phenolic compound from MBAGTM films in acetate buffer at pH 5.6 (A) and PBS at pH 7.4 (B) reported as the percentage of the weight of phenolic compound (GAE) released divided by the actual amount of phenolic compound; MBAGTM at 1% (⊣□⊢), 5% (⊣○⊢) and 10% (⊣×⊢) v/v, respectively

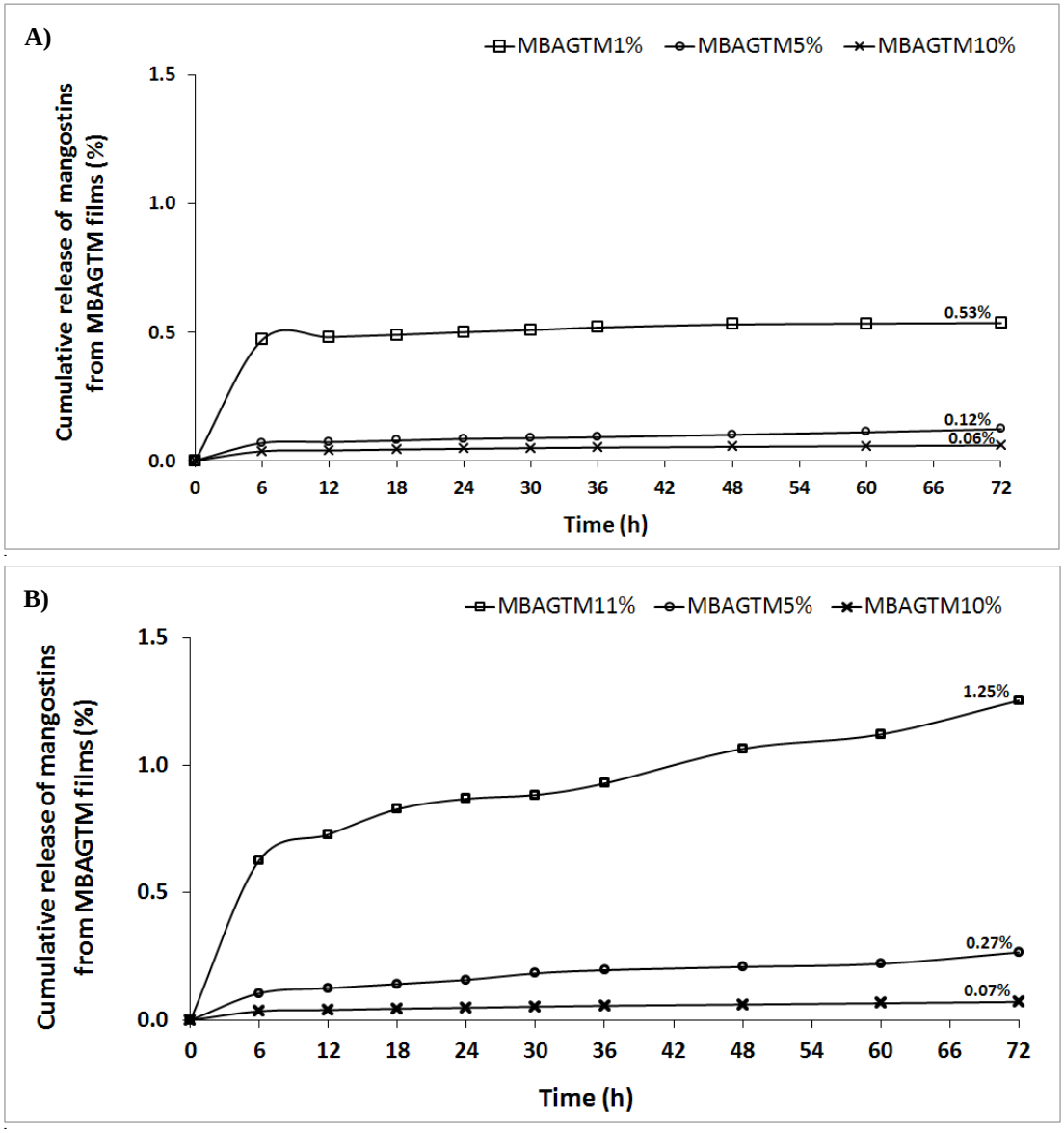


Figure 4.36 Cumulative release profile of mangosins from MBAGTM films in acetate buffer at pH 5.6 (A) and PBS at pH 7.4 (B) reported as the percentage of the weight of mangosins (AME) released divided by the actual amount of mangosins; MBAGTM at 1% (—□—), 5% (—○—) and 10% (—×—) v/v, respectively

CHAPTER V
CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The bacterial cellulose/ sodium alginate/ gelatin (BAG) films and modified BAG films were successful prepared by casting technique. Generally, the composite films shrink in dry state so that plasticizers are used to improve flexibility and resistance to cracking of the films. The composite film with BC/A/G at a ratio of 60/20/20 has superior mechanical properties for both dry and wet states. The SEM images show that sodium alginate and gelatin could penetrate into the nanocellulose fibril networks, fill up of the spaces and coat on the fibril surfaces. The BAG film at a ratio of 60/20/20 displayed good incorporation of sodium alginate and gelatin into the BC fibril network, which forms ribbon-like structure of cellulose fibrils containing gels. Furthermore, the FT-IR revealed the shifts attributed to intermolecular interactions between the hydroxyl group of cellulose, the carboxyl group of sodium alginate and amide group of gelatin. The results indicated that glycerol is a suitable plasticizer for the BAG films. Glycerol is a hydrophilic plasticizer so that it enhanced water absorption capacity and improved elongation at break of the re-swollen films. However, excess glycerol content could attribute to the migration of glycerol. The optimal ratio of glycerol to gelatin was at 2:10 (w/w).

The MBAG film was further modified by the supplement of tannic acid as crosslnking and antimicrobial agent. Currently, the most used crosslinking agents are phenolic compounds due to their safety and moderate cost. The FT-IR revealed hydroxyl groups of tannic acid could form hydrogen bonds with amino group of gelatin as well as increasing amplitudes of free water peaks. These changes were indicative of greater disorder. The interactions between tannic acid and gelatin should be hydrophobic interactions rather than strong hydrogen bonding. Although the tannic acid-gelatin interaction was not strong, it had positive effect on plasticizer properties of the MBAGT films in wet state and helped to increase EB of the re-swollen films. The optimal weight ratio of mg tannic acid to gram gelatin solution was at 10:1.

In order to enhance food quality, prevent food from foodborne pathogens during storage and delay food spoilage, new food packaging films with antimicrobial functions have been developed. The mangosteen ethanolic extract was selected as antimicrobial agent for inhibition growth of foodborne pathogens. The FT-IR spectra of the modified films revealed weakly interactions between the functional groups of the MBAGT film and the mangosteen ethanolic extract compounds. The ethanolic extract contained hydrophobic compounds could filled in the micropores of the films and also coated on cellulose fibrils, resulting in smoother surface of the films. However, the supplement of the mangosteen ethanolic extract might also have interfering effects on the chemical bonds of the films, resulting in a looser film structure. Therefore, the addition of the extract enhanced the thickness as well as the inter-space of the sheet layers of the MBAGTM films. As a result, the WVTR and the OTR of the modified films increased to some extent.

The results of MIC showed that mangosteen ethanolic extracted and tannic acid could inhibit bacteria in food. Mangosteen ethanolic extract possessed at 1.46 mg/ml concentration for *E. coli* and *S. aureus*, and at 0.73 mg/ml concentration for *S. typhimurium* and *L. monocytogenes*. On the other hand, tannic acid possessed at 30 mg/ml concentration for *E. coli*, *S. typhimurium* and *S. aureus*, and at 15 mg/ml concentration for *L. monocytogenes*. The MBC for mangosteen ethanolic extract possessed at 1.46 mg/ml concentration for all bacteria, whereas tannic acid possessed at 30 mg/ml concentration for *E. coli*, *S. typhimurium* and *S. aureus*, and at 15 mg/ml concentration for *L. monocytogenes*. The result of the study on antimicrobial activities of MBAGTM films containing mangosteen ethanolic extract by Disc diffusion method and the study of cumulative release of active compounds, mangosins and phenolic componds from the films revealed that the mangosteen ethanolic extract might not or only slightly released from the MBAGTM films. It was suggested that the accumulation of extract compounds at high concentrations might form agglomerated granules within the cross-linked films and prevented the release of the components.

5.2 Recommendations for future studies

Based on this study, further works for the improvement of the biopolymer composite films are recommended as following.

1. Development of procedure to adjust the film structure in order to control the release rate of the applied active components at optimal level.

2. Application the film for encapsulation of mangosteen ethanolic extract in medicine in order to use as drug carrier or wound dressing.

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APPENDICES

Appendix A
Standard calibration curve

1. Preparation of standard for phenolic compound determination

Chemicals

1. Gallic acid powder
2. Sodium bicarbonate solution (7.5% w/v)
3. Folin-Ciocalteu reagent (diluted at 1:10 with deionized water)
4. Water

Folin-Ciocalteu precedure

A standard solution was prepared by dissolving 12.50 mg of gallic acid in 100 ml of absolute ethanol in volumetric flask. Standard solution 0.4 ml was mixed with 1.6 mL of sodium bicarbonate solution (7.5% w/v) and then was added with 1.0 mL of the Folin-Ciocalteu reagent (previously diluted at 1:10 with deionized water). The mixture was shaken for well-mixing and incubated at room temperature for 30 minutes. Various concentrations of the standard solution were provided the final concentrations of 0.24, 0.49, 0.98, 1.95, 3.91, 7.81, 15.63, 31.25, 62.50 and 125.00 µg/ml.

TableA1 Standard calibration curve of phenolic compounds (gallic acid equivalents) analyzed by UV-Vis spectrophotometer

Concentration (mg/mL)	Absorbance (765 nm)			Average	S.D.
	1	2	3		
0.24	0.001	0.001	0.001	0.001	0.000
0.49	0.004	0.003	0.003	0.003	0.001
0.98	0.009	0.008	0.007	0.008	0.001
1.95	0.011	0.010	0.010	0.010	0.001
3.91	0.031	0.030	0.030	0.030	0.001
7.81	0.057	0.056	0.056	0.056	0.001
15.63	0.132	0.131	0.131	0.131	0.001
31.25	0.254	0.253	0.253	0.253	0.001
62.50	0.492	0.490	0.491	0.491	0.001
125.00	1.015	1.014	1.014	1.014	0.001

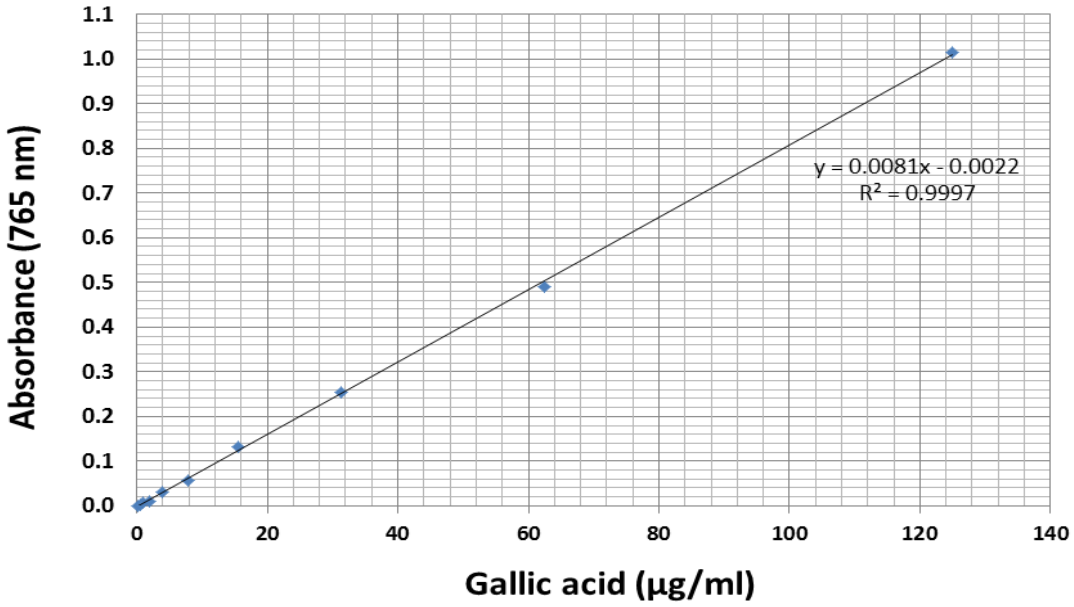

Figure A1 Standard calibration curve of phenolic compound for quantitative determination of bioactive compounds content

TableA2 Standard calibration curve of phenolic compounds (gallic acid equivalents) for release analysis in acetate buffer at pH 5.6 analyzed by UV-Vis spectrophotometer

Concentration (mg/mL)	Absorbance (765 nm)			Average	S.D.
	1	2	3		
15.63	0.013	0.012	0.012	0.012	0.001
31.25	0.022	0.025	0.024	0.008	0.002
62.50	0.089	0.088	0.087	0.088	0.001
125.00	0.299	0.298	0.301	0.299	0.002
250.00	0.841	0.845	0.850	0.845	0.005
500.00	1.748	1.751	1.752	1.750	0.002

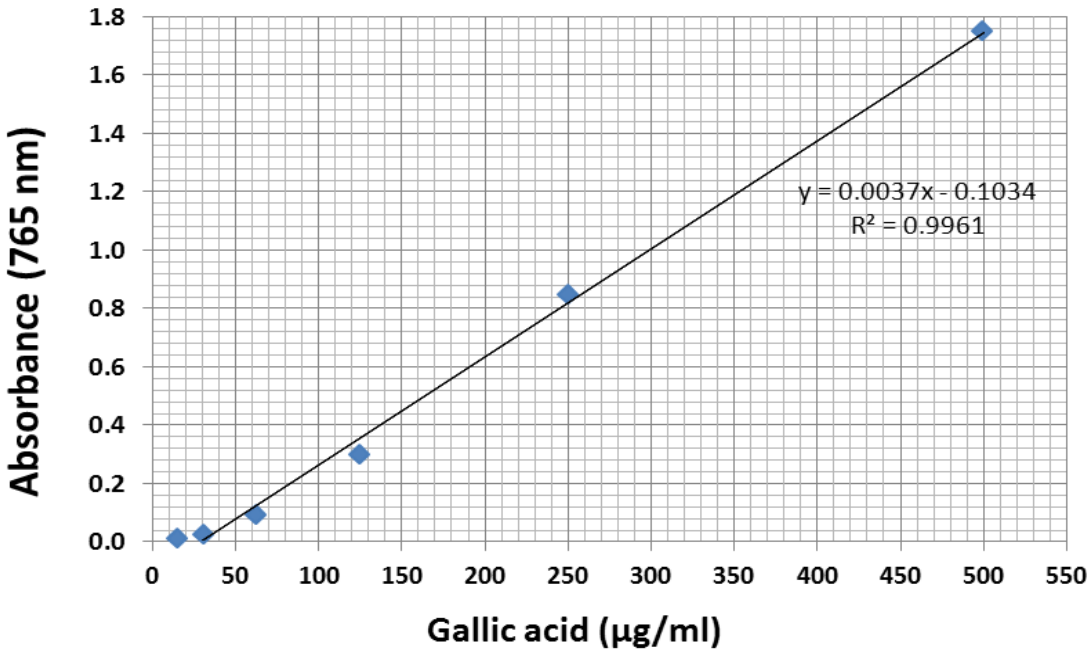

Figure A2 Standard calibration curve of phenolic acid for release analysis in acetate buffer at pH 5.6

TableA3 Standard calibration curve of phenolic compounds (gallic acid equivalents) for release analysis in release analysis in PBS at pH 7.4 analyzed by UV-Vis spectrophotometer

Concentration (mg/mL)	Absorbance (765 nm)			Average	S.D.
	1	2	3		
31.25	0.012	0.013	0.012	0.008	0.002
62.50	0.055	0.055	0.056	0.088	0.001
125.00	0.262	0.265	0.269	0.299	0.002
250.00	0.856	0.826	0.810	0.845	0.005
500.00	1.715	1.725	1.709	1.750	0.002

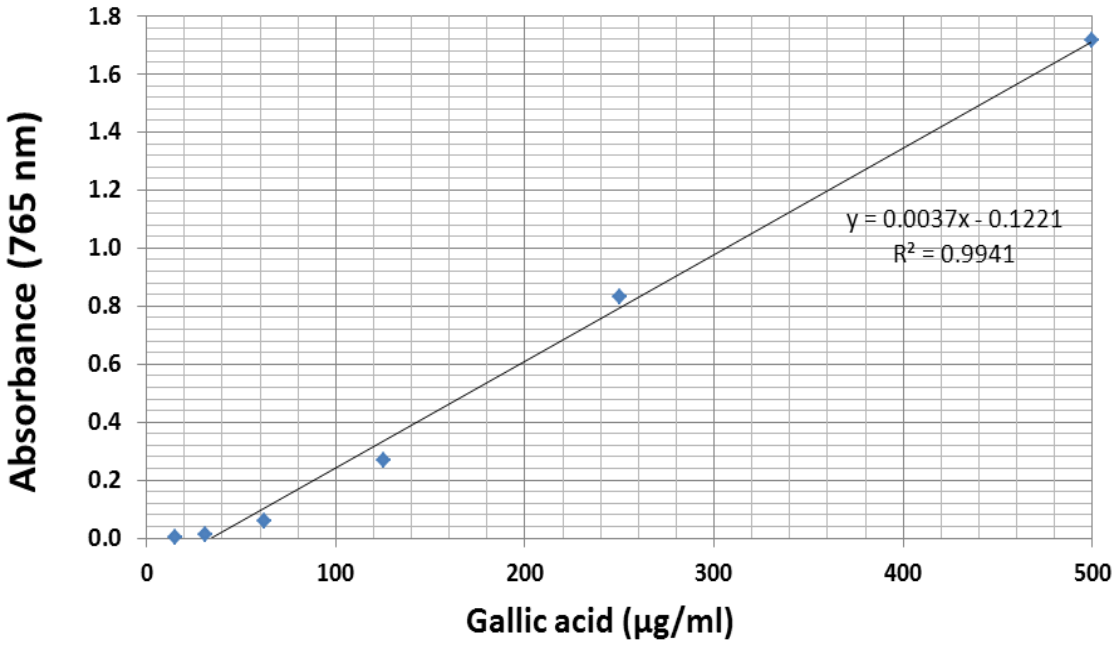

Figure A3 Standard calibration curve of phenolic acid for release analysis in PBS at pH 7.4

A.2 Preparation of standard for mangostins content determination

Chemicals

1. α-mangostin powder
2. Absolute ethanol

Determination of α-mangostin

A stock solution of α-mangostin reference standard was prepared by dissolving 1.88 mg of α-mangostin in 100 ml of ethanol in a volumetric flask. Standard solution 1 ml was mixed with 1 mL of absolute ethanol. The mixture was shaken for well-mixing and incubated at room temperature for 30 minutes. Afterwards, various concentrations of the standard solution were prepared to provide final concentrations at 14.69, 29.38, 58.75, 117.50, 235.00, 470.00, 940.00 and 1880.00 µg/ml.

Table A4 Standard calibration curve of mangostins data (alpha-mangostin equivalents) analyzed by UV-Vis spectrophotometer

Concentration (mg/mL)	Absorbance (320 nm)			Average	S.D.
	1	2	3		
14.69	0.000	0.000	0.000	0.000	0.000
29.38	0.014	0.012	0.012	0.012	0.013
58.75	0.041	0.042	0.041	0.041	0.001
117.50	0.105	0.103	0.106	0.105	0.002
235.00	0.226	0.225	0.225	0.225	0.001
470.00	0.465	0.468	0.466	0.466	0.002
940.00	0.945	0.924	0.925	0.931	0.012
1880.00	1.871	1.868	1.870	1.870	0.002

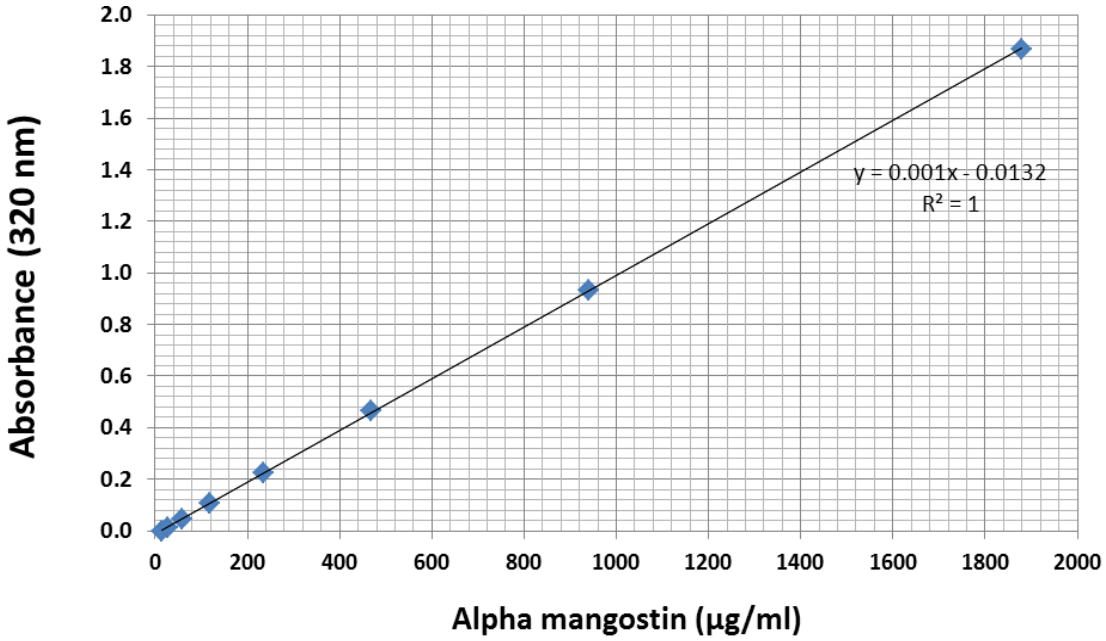

Figure A4 Standard calibration curve of mangostins for quantitative determination of bioactive compounds content

TableA5 Standard calibration curve of mangostins data (alpha-mangostin equivalents) for release analysis in acetate buffer at pH 5.6 analyzed by UV-Vis spectrophotometer

Concentration (mg/mL)	**Absorbance (765 nm)**			**Average**	**S.D.**
	1	**2**	**3**		
11.72	0.002	0.003	0.002	0.002	0.001
23.44	0.047	0.047	0.053	0.049	0.003
46.88	0.149	0.145	0.151	0.148	0.003
93.75	0.330	0.330	0.330	0.330	0.000
187.50	0.659	0.652	0.672	0.661	0.010
375.00	1.249	1.224	1.269	1.247	0.023
750.00	2.354	2.323	2.372	2.350	0.025

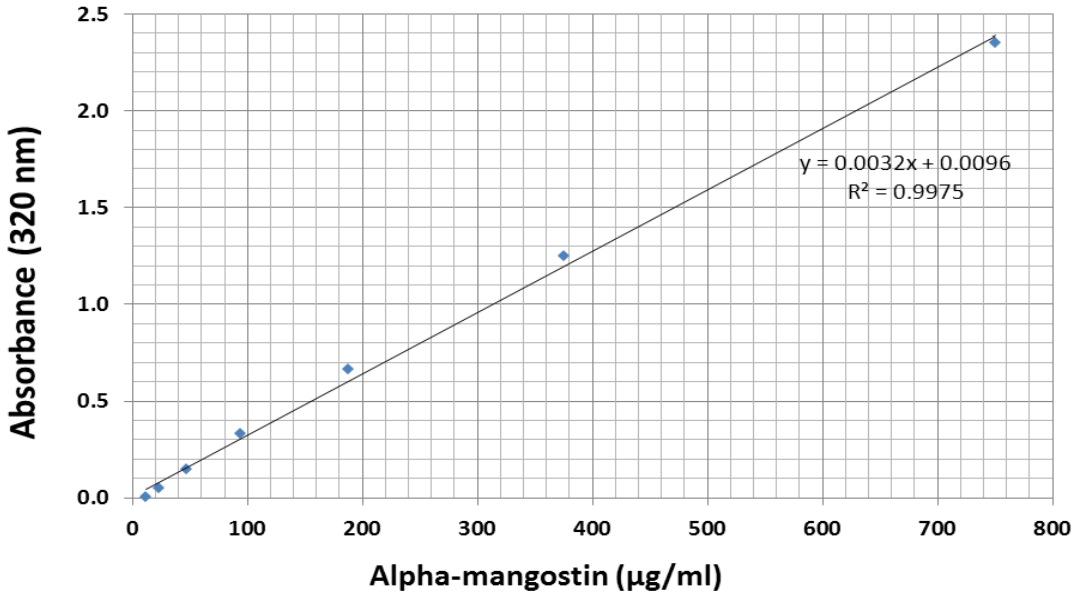

Figure A5 Standard calibration curve of mangostins for release analysis in acetate buffer at pH 5.6

TableA6 Standard calibration curve of mangostins data (alpha-mangostin equivalents) for release analysis in release analysis in PBS at pH 7.4 analyzed by UV-Vis spectrophotometer

Concentration (mg/mL)	**Absorbance (765 nm)**			**Average**	**S.D.**
	1	**2**	**3**		
1.47	0.001	0.001	0.001	0.001	0.000
2.93	0.008	0.012	0.010	0.010	0.002
5.86	0.016	0.017	0.017	0.017	0.001
11.72	0.038	0.038	0.038	0.038	0.000
23.44	0.078	0.079	0.078	0.078	0.001
46.88	0.155	0.156	0.157	0.156	0.001
93.75	0.303	0.304	0.303	0.303	0.001
187.50	0.597	0.594	0.596	0.596	0.002
375.00	1.134	1.132	1.131	1.132	0.002
750.00	2.154	2.145	2.208	2.169	0.034

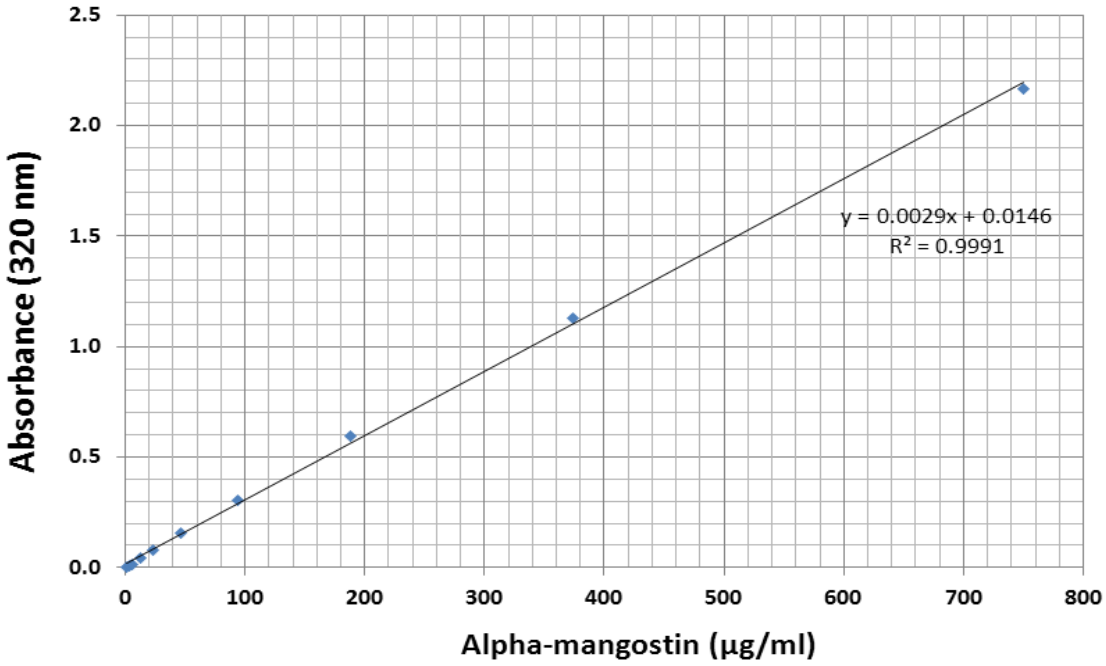

Figure A6 Standard calibration curve of mangostins for release analysis in PBS at pH 7.4

Appendix B

Data of Experiments

Table B1 Data of Table 4.1

Mangosteen Ethanolic Extract (ME)	**Bioactive compounds**	**Amount of bioactive compounds (mg) in mangosteen ethanolic (per 1 mL) extracts**				
		1	**2**	**3**	**Average**	**S.D.**
Conc. ME	Phenolic compound	0.46	0.42	0.45	0.44	0.02
	Mangostins	392.81	349.80	357.48	366.69	0.04
1% v/v of ME	Phenolic compound	-	-	-	-	-
	Mangostins	5.93	5.89	5.97	5.93	0.04
5% v/v of ME	Phenolic compound	-	-	-	-	-
	Mangostins	11.75	11.70	11.73	11.72	0.03
10% v/v of ME	Phenolic compound	-	-	-	-	-
	Mangostins	22.80	22.87	22.75	22.80	0.06

Table B2 Data of Figure 4.5

Sample	**Water absorption capacity (%)**				
	1	**2**	**3**	**Average**	**S.D.**
BC	265.00	257.14	240.91	254.35	12.29
Alginate	300.00	295.24	295.24	296.83	2.75
BC/A	227.27	208.70	190.00	208.66	18.64
60/10/30	408.77	408.93	417.86	411.85	5.20
60/20/20	325.53	356.52	341.86	341.30	15.50
60/30/10	216.67	245.45	228.33	230.15	14.48

Table B3 Data of Figure 4.6

Part	Sample	Water absorption capacity (%)				
		1	**2**	**3**	**Average**	**S.D.**
Glycerol (Gly)	G10:Gly0	325.53	356.52	341.86	341.30	15.50
	G10:Gly1	417.86	426.79	417.86	420.83	5.15
	G10:Gly2	466.47	466.47	453.33	462.22	7.70
	G10:Gly3	438.46	437.97	438.46	438.30	0.28
Sorbitol (S)	G10:S0	325.53	356.52	341.86	341.30	15.50
	G10:S1	504.17	504.17	502.04	503.46	1.23
	G10:S2	661.36	644.19	650.00	651.85	8.74
	G10:S3	576.92	566.67	566.67	570.09	5.92
The mixture of Gly and S	G10:GS0	325.53	356.52	341.86	341.30	15.50
	G10:GS1	448.39	464.52	456.45	456.45	8.06
	G10:GS2	591.18	600.00	566.67	585.95	17.27
	G10:GS3	517.28	526.67	500.00	514.65	13.53

Table B4 Data of Figure 4.7

Sample	Tensile strength (MPa)						
	1	**2**	**3**	**4**	**5**	**Average**	**S.D.**
100/0/0 (BC)	54.6	60.6	61.2	57.2	54.1	57.5	3.3
60/40/0 (BC/A)	151.5	146.9	152.8	144.6	143.9	147.9	3.6
60/10/30	169.3	164.7	170.6	165.3	162.8	166.5	3.3
60/20/20	182.0	186.5	181.3	178.2	181.1	181.8	3.0
60/30/10	181.7	174.8	175.9	180.1	174.9	177.5	3.7
Sample	**Elongation at break (%)**						
	1	**2**	**3**	**4**	**5**	**Average**	**S.D.**
100/0/0 (BC)	1.16	0.94	0.85	1.06	0.98	1.00	0.12
60/40/0 (BC/A)	2.11	2.14	2.10	2.01	2.00	2.07	0.06
60/10/30	2.06	1.91	2.01	1.86	2.11	1.99	0.10
60/20/20	2.71	2.84	3.06	3.01	2.94	2.91	0.14
60/30/10	1.88	2.05	1.89	2.19	1.97	2.00	0.13

Table B5 Data of Figure 4.8

Sample		Tensile strength (MPa)						
		1	**2**	**3**	**4**	**5**	**Average**	**S.D.**
60/10/30	No plasticizer	169.3	164.7	170.6	165.3	162.8	166.5	3.3
	Glycerol	154.8	158.4	157.8	154.6	150.7	155.3	3.1
	Sorbitol	168.7	172.3	170.8	166.3	169.7	169.6	2.3
	Mixture of GS	156.6	159.3	159.6	152.6	155.9	156.8	3.2
60/20/20	No plasticizer	182.0	186.5	181.3	178.2	181.1	181.8	3.0
	Glycerol	178.2	181.4	183.7	185.8	182.1	182.2	2.8
	Sorbitol	162.7	168.6	165.1	164.1	163.2	164.7	2.3
	Mixture of GS	179.9	183.7	177.1	178.2	179.4	179.7	2.5
60/30/10	No plasticizer	181.7	174.8	175.9	180.1	174.9	177.5	3.7
	Glycerol	177.7	181.1	180.5	175.8	177.7	187.6	2.2
	Sorbitol	175.9	173.9	182.2	178.1	177.2	177.5	3.1
	Mixture of GS	182.8	180.8	175.7	176.4	178.2	178.8	3.0
Sample		**Elongation at break (%)**						
		1	**2**	**3**	**4**	**5**	**Average**	**S.D.**
60/10/30	No plasticizer	2.06	1.91	2.01	1.86	2.11	1.99	0.10
	Glycerol	1.77	1.64	1.63	1.65	1.75	1.69	0.07
	Sorbitol	1.89	2.07	2.20	1.97	2.10	2.05	0.12
	Mixture of GS	1.76	1.91	1.93	1.89	1.79	1.86	0.08
60/20/20	No plasticizer	2.71	2.84	3.06	3.01	2.94	2.91	0.14
	Glycerol	2.41	2.33	2.53	2.57	2.32	2.43	0.11
	Sorbitol	1.59	1.64	1.67	1.60	1.73	1.65	0.06
	Mixture of GS	1.86	2.07	1.99	1.79	1.89	1.92	0.11
60/30/10	No plasticizer	1.88	2.05	1.89	2.19	1.97	2.00	0.13
	Glycerol	1.96	2.07	1.99	2.14	2.08	2.05	0.08
	Sorbitol	2.29	2.14	2.20	2.26	2.36	2.25	0.08
	Mixture of GS	1.82	1.99	1.81	1.84	1.88	1.87	0.07

Table B6 Data of Figure 4.9

Sample	Tensile strength (MPa)						
	1	**2**	**3**	**4**	**5**	**Average**	**S.D.**
100/0/0 (BC)	2.22	2.37	1.98	2.26	2.18	2.20	0.14
60/40/0 (BC/A)	16.20	14.21	15.60	15.04	15.46	15.30	0.74
60/10/30	2.44	2.36	2.28	2.11	2.30	2.30	0.12
60/20/20	9.38	9.57	10.06	9.51	9.42	9.59	0.27
60/30/10	11.68	11.25	10.86	11.55	11.42	11.35	0.32
Sample	**Elongation at break (%)**						
	1	**2**	**3**	**4**	**5**	**Average**	**S.D.**
100/0/0 (BC)	1.54	2.23	2.14	1.81	1.75	1.89	0.29
60/40/0 (BC/A)	18.00	18.15	17.72	18.12	17.89	17.98	0.18
60/10/30	14.18	15.26	14.86	15.12	14.55	14.79	0.44
60/20/20	29.97	31.21	29.89	30.29	30.10	30.29	0.53
60/30/10	11.71	11.11	10.86	11.55	11.42	27.97	0.24

Table B7 Data of Figure 4.10

Sample		Tensile strength (MPa)						
		1	**2**	**3**	**4**	**5**	**Average**	**S.D.**
60/10/30	No plasticizer	2.44	2.36	2.28	2.11	2.30	2.30	0.12
	Glycerol	0.73	0.78	0.87	1.12	0.92	0.88	0.15
	Sorbitol	0.47	0.42	0.34	0.37	0.41	0.40	0.05
	Mixture of GS	0.38	0.42	0.36	0.45	0.41	0.40	0.03
60/20/20	No plasticizer	9.38	9.57	10.06	9.51	9.42	9.59	0.27
	Glycerol	4.39	3.76	3.75	4.35	3.95	4.04	0.31
	Sorbitol	1.58	1.53	2.05	1.95	1.67	1.75	0.23
	Mixture of GS	1.84	2.16	2.05	1.93	2.04	2.00	0.12
60/30/10	No plasticizer	11.68	11.25	10.86	11.55	11.42	11.35	0.32
	Glycerol	7.91	8.35	7.98	8.19	8.12	8.11	0.17
	Sorbitol	5.83	5.81	6.03	6.08	5.89	5.93	0.12
	Mixture of GS	7.30	6.75	6.86	7.24	6.92	7.01	0.24

Sample		Elongation at break (%)						
		1	**2**	**3**	**4**	**5**	**Average**	**S.D.**
60/10/30	No plasticizer	14.18	15.26	14.86	15.12	14.55	14.79	0.44
	Glycerol	21.91	23.57	22.67	23.29	22.85	22.86	0.64
	Sorbitol	8.15	7.71	7.73	6.57	7.81	7.59	0.60
	Mixture of GS	15.51	14.90	14.32	15.58	14.87	15.04	0.52
60/20/20	No plasticizer	29.97	31.21	29.89	30.29	30.10	30.29	0.53
	Glycerol	43.71	44.84	45.12	44.54	45.01	44.64	0.57
	Sorbitol	30.36	29.70	29.86	29.94	29.68	29.91	0.27
	Mixture of GS	32.27	31.43	31.27	31.14	31.56	31.53	0.44
60/30/10	No plasticizer	11.71	11.11	10.86	11.55	11.42	27.97	0.24
	Glycerol	46.74	45.43	44.99	45.56	45.74	45.69	0.65
	Sorbitol	38.01	37.19	37.94	38.29	37.76	37.84	0.41
	Mixture of GS	42.29	42.60	41.54	42.59	41.87	42.18	0.46

Table B8 Data of Figure 4.16

Sample	Water absorption capacity (%)				
	1	**2**	**3**	**Average**	**S.D.**
BAG	325.53	356.52	341.86	341.30	15.50
MBAG	466.47	466.47	453.33	462.22	7.70
MBAG-5T	482.50	445.45	446.77	458.24	21.02
MBAG-10T	375.41	386.21	408.06	389.89	16.64
MBAG-15T	356.45	322.58	357.89	345.64	19.99

Table B9 Data of Figure 4.17

Sample	Tensile strength (MPa)						
	1	**2**	**3**	**4**	**5**	**Average**	**S.D.**
BC	54.6	60.6	61.2	57.2	54.1	57.5	3.3
BAG	182.0	186.5	181.3	178.2	181.1	181.8	3.0

MBAG	178.2	181.4	183.7	185.8	182.1	182.2	2.8
MBAG-5T	168.2	180.0	174.5	178.6	172.6	174.8	4.7
MBAG-10T	182.1	174.5	171.2	181.4	178.4	177.5	4.6
MBAG-15T	187.3	176.8	179.8	188.6	184.2	181.3	4.3
Sample		**Elongation at break (%)**					
	1	**2**	**3**	**4**	**5**	**Average**	**S.D.**
BC	1.16	0.94	0.85	1.06	0.98	1.00	0.12
BAG	2.71	2.84	3.06	3.01	2.94	2.91	0.14
MBAG	2.41	2.33	2.53	2.57	2.32	2.43	0.11
MBAG-5T	1.79	1.71	2.01	1.86	1.81	1.84	0.11
MBAG-10T	2.68	2.36	2.74	2.66	2.52	2.59	0.15
MBAG-15T	1.95	2.36	2.00	2.04	2.34	2.14	0.20

Table B10 Data of Figure 4.18

Sample	**Tensile strength (MPa)**						
	1	**2**	**3**	**4**	**5**	**Average**	**S.D.**
BC	2.22	2.37	1.98	2.26	2.18	2.20	0.14
BAG	9.38	9.57	10.06	9.51	9.42	9.59	0.27
MBAG	4.39	3.76	3.75	4.35	3.95	4.04	0.31
MBAG-5T	2.95	2.57	3.06	2.64	2.75	2.79	0.21
MBAG-10T	2.51	2.46	2.36	2.72	2.63	2.55	0.25
MBAG-15T	3.00	2.36	2.58	3.04	2.57	2.71	0.30
Sample		**Elongation at break (%)**					
	1	**2**	**3**	**4**	**5**	**Average**	**S.D.**
BC	1.54	2.23	2.14	1.81	1.75	1.89	0.29
BAG	29.97	31.21	29.89	30.29	30.10	30.29	0.53
MBAG	43.71	44.84	45.12	44.54	45.01	44.64	0.57
MBAG-5T	52.24	55.16	48.80	48.60	49.80	50.92	2.78
MBAG-10T	48.40	53.80	50.60	51.80	46.60	50.24	2.82
MBAG-15T	55.80	51.04	49.80	50.80	48.80	51.25	2.70

Table B11 Data of Figure 4.23

Sample	Transparency						
	1	**2**	**3**	**4**	**5**	**Average**	**S.D.**
BAG	1.24	1.25	1.25	1.26	1.26	1.25	0.01
MBAG	1.12	1.12	1.12	1.12	1.12	1.12	0.00
MBAG-10T	0.86	0.87	0.86	0.86	0.86	0.86	0.01
MBAGTM1%	0.68	0.68	0.71	0.71	0.69	0.69	0.02
MBAGTM5%	0.64	0.65	0.67	0.68	0.67	0.66	0.02
MBAGTM10%	0.82	0.79	0.80	0.79	0.83	0.80	0.02

Table B12 Data of Figure 4.26

Sample	Water absorption capacity (%)				
	1	**2**	**3**	**Average**	**S.D.**
BAG	325.53	356.52	341.86	341.30	15.50
MBAG	466.47	466.47	453.33	462.22	7.70
MBAG-10T	375.41	386.21	408.06	389.89	16.64
MBAGTM1%	306.45	337.78	321.43	321.89	15.67
MBAGTM5%	294.25	262.82	289.41	282.16	16.92
MBAGTM10%	263.24	268.75	276.92	269.64	6.89

Table B13 Data of Figure 4.27

Sample	Tensile strength (MPa)						
	1	**2**	**3**	**4**	**5**	**Average**	**S.D.**
BC	54.6	60.6	61.2	57.2	54.1	57.5	3.3
BAG	182.0	186.5	181.3	178.2	181.1	181.8	3.0
MBAG	178.2	181.4	183.7	185.8	182.1	182.2	2.8
MBAGT	182.1	174.5	171.2	181.4	178.4	177.5	4.6
MBAGTM1%	183.9	172.4	192.8	196.1	199.1	188.9	10.8
MBAGTM5%	163.2	154.7	168.0	155.3	157.4	159.7	5.7
MBAGTM10%	127.2	137.1	141.1	126.7	121.9	130.8	8.0

Sample		Elongation at break (%)					
	1	2	3	4	5	Average	S.D.
BC	1.16	0.94	0.85	1.06	0.98	1.00	0.12
BAG	2.71	2.84	3.06	3.01	2.94	2.91	0.14
MBAG	2.41	2.33	2.53	2.57	2.32	2.43	0.11
MBAGT	2.68	2.36	2.74	2.66	2.52	2.59	0.15
MBAGTM1%	3.20	2.81	2.02	2.98	3.20	3.04	0.16
MBAGTM5%	4.00	3.98	4.46	4.32	4.32	4.22	0.21
MBAGTM10%	3.54	4.08	4.02	3.89	3.72	3.85	0.22

Table B14 Data of Figure 4.28

Sample	Tensile strength (MPa)						
	1	2	3	4	5	Average	S.D.
BC	2.22	2.37	1.98	2.26	2.18	2.20	0.14
BAG	9.38	9.57	10.06	9.51	9.42	9.59	0.27
MBAG	4.39	3.76	3.75	4.35	3.95	4.04	0.31
MBAGT	2.51	2.46	2.36	2.72	2.63	2.55	0.25
MBAGTM1%	2.39	2.86	2.79	2.36	2.24	2.53	0.28
MBAGTM5%	1.60	1.61	1.96	1.88	2.01	1.82	0.18
MBAGTM10%	1.81	2.30	2.10	2.00	1.79	2.00	0.21
Sample		**Elongation at break (%)**					
	1	**2**	**3**	**4**	**5**	**Average**	**S.D.**
BC	1.54	2.23	2.14	1.81	1.75	1.89	0.29
BAG	29.97	31.21	29.89	30.29	30.10	30.29	0.53
MBAG	43.71	44.84	45.12	44.54	45.01	44.64	0.57
MBAGT	48.40	53.80	50.60	51.80	46.60	50.24	2.82
MBAGTM1%	60.80	63.60	63.60	61.40	65.80	63.04	2.00
MBAGTM5%	46.00	44.50	46.70	48.20	48.20	46.72	1.57
MBAGTM10%	48.20	50.00	46.20	46.40	45.20	47.20	1.90

Table B15 Data of Table 4.4

Sample	OTR (cc/m^2.day)			
	1	**2**	**Average**	**S.D.**
BAG	0.869	0.906	0.887	0.026
MBAG	1.050	1.110	1.080	0.042
MBAG-10T	0.794	0.734	0.764	0.042
MBAGTM10%	1.10	1.10	1.100	0.000

Table B16 Data of Table 4.5

Sample	WVTR (g/m^2.day)			
	1	**2**	**Average**	**S.D.**
BAG	770.00	768.00	769.00	1.41
MBAG	792.00	794.00	793.00	0.042
MBAG-10T	985.92	966.72	976.32	13.58
MBAGTM10%	1416.48	1387.68	1402.08	20.36

Table B17 Data of Table 4.6

	Tube	Final concentration	1% V/V ME		3% TA	
			MIC	**MBC**	**MIC**	**MBC**
Staphylococcus aureus	1	1:1	-	-	-	-
	2	1:2	-	-	+	+
	3	1:4	-	-	+	+
	4	1:8	+	+	+	+
	5	1:16	+	+	+	+
	6	1:32	+	+	+	+
	7	1:64	+	+	+	+
	8	1:128	+	+	+	+
	9	1:256	+	+	+	+
	10	1:512	+	+	+	+
	11	Inoculum	+	+	+	+

	Tube	Final concentration	1% V/V ME		3% TA	
			MIC	MBC	MIC	MBC
Escherichia coli	1	1:1	-	-	-	-
	2	1:2	-	-	+	+
	3	1:4	-	-	+	+
	4	1:8	+	+	+	+
	5	1:16	+	+	+	+
	6	1:32	+	+	+	+
	7	1:64	+	+	+	+
	8	1:128	+	+	+	+
	9	1:256	+	+	+	+
	10	1:512	+	+	+	+
	11	Inoculum	+	+	+	+

	Tube	Final concentration	1% V/V ME		3% TA	
			MIC	MBC	MIC	MBC
Listeria monocytogenes	1	1:1	-	-	-	-
	2	1:2	-	-	-	-
	3	1:4	-	-	+	+
	4	1:8	-	+	+	+
	5	1:16	+	+	+	+
	6	1:32	+	+	+	+
	7	1:64	+	+	+	+
	8	1:128	+	+	+	+
	9	1:256	+	+	+	+
	10	1:512	+	+	+	+
	11	Inoculum	+	+	+	+

	Tube	Final concentration	1% V/V ME		3% TA	
			MIC	MBC	MIC	MBC
Salmonella typhimurium	1	1:1	-	-	-	-
	2	1:2	-	-	+	+
	3	1:4	-	-	+	+
	4	1:8	-	+	+	+
	5	1:16	+	+	+	+
	6	1:32	+	+	+	+
	7	1:64	+	+	+	+
	8	1:128	+	+	+	+
	9	1:256	+	+	+	+
	10	1:512	+	+	+	+
	11	Inoculum	+	+	+	+

Table B18 Data of Table 4.7

Microorganisms	Mangosteen ethanolic extract supplementation (%v/v)	Clear zone (mm)				
		1	2	3	Average	S.D.
E. coli	MBAGTM-1%	0.00	0.00	0.00	0.00	0.00
	MBAGTM-5%	0.00	0.00	0.00	0.00	0.00
	MBAGTM-10%	0.00	0.00	0.00	0.00	0.00
S. typhimurium	MBAGTM-1%	0.00	0.00	0.00	0.00	0.00
	MBAGTM-5%	0.00	0.00	0.00	0.00	0.00
	MBAGTM-10%	0.00	0.00	0.00	0.00	0.00
S. Aureus	MBAGTM-1%	0.00	0.00	0.00	0.00	0.00
	MBAGTM-5%	0.00	0.00	0.00	0.00	0.00
	MBAGTM-10%	0.00	0.00	0.00	0.00	0.00

Table B19 Data of Table 4.9

Films	Bioactive compounds	Actual amount in modified BC film (mg/cm^3)				
		1	2	3	Average	S.D.
First time						
MBAGTM1%	Phenolic compounds (GAE)	0.431	0.450	0.470	0.450	0.019
MBAGTM5%		50.134	49.710	49.427	49.757	0.356
MBAGTM10%		82.507	83.407	82.249	82.721	0.608
MBAGTM1%	Mangostins (AME)	10.569	10.411	10.569	10.561	0.091
MBAGTM5%		114.678	115.244	114.253	114.725	0.497
MBAGTM10%		198.211	198.469	198.983	198.555	0.393
Second time						
MBAGTM1%	Phenolic compounds (GAE)	0.606	0.625	0.606	0.612	0.011
MBAGTM5%		12.909	12.626	12.626	12.720	0.163
MBAGTM10%		19.456	19.198	19.327	19.327	0.129
MBAGTM1%	Mangostins (AME)	7.738	7.895	7.266	7.633	0.327
MBAGTM5%		12.626	12.343	12.626	12.531	0.163
MBAGTM10%		39.169	38.654	38.911	38.911	0.257
Summary of Actual content						
MBAGTM1%	Phenolic compounds (GAE)	1.056	1.056	1.082	1.065	0.015
MBAGTM5%		62.760	62.335	62.147	62.414	0.314
MBAGTM10%		101.705	102.734	101.576	102.005	0.635
MBAGTM1%	Mangostins (AME)	18.306	18.306	17.834	18.149	0.272
MBAGTM5%		127.304	127.587	127.879	127.256	0.356
MBAGTM10%		237.380	237.123	237.895	237.466	0.393

Table B20 Data of Figure 4.35

		Cumulative release of phenolic compounds (%)					
		Acetate buffer at pH 5.6					
	Films	MBAGTM 1%	S.D.	MBAGTM 5%	S.D.	MBAGTM 10%	S.D.
Time (h)	0.0	0.000	0.000	0.000	0.000	0.000	0.000
	6.0	3.672	0.000	0.064	0.000	0.040	0.000
	12.0	3.884	0.000	0.067	0.000	0.041	0.000
	18.0	3.998	0.000	0.069	0.000	0.043	0.000
	24.0	4.118	0.000	0.071	0.000	0.043	0.000
	30.0	4.238	0.000	0.074	0.000	0.045	0.000
	36.0	4.382	0.000	0.077	0.000	0.046	0.000
	48.0	4.630	0.000	0.084	0.000	0.049	0.000
	60.0	4.801	0.000	0.087	0.000	0.052	0.000
	72.0	4.983	0.000	0.092	0.000	0.055	0.000
		PBS at pH 7.4					
	Films	MBAGTM1 %	S.D.	MBAGTM5 %	S.D.	MBAGTM 10%	S.D.
Time (h)	0.0	0.000	0.000	0.000	0.000	0.000	0.000
	6.0	3.605	0.000	0.063	0.000	0.034	0.000
	12.0	3.742	0.000	0.067	0.000	0.035	0.000
	18.0	3.881	0.000	0.069	0.000	0.038	0.000
	24.0	4.003	0.000	0.071	0.000	0.040	0.000
	30.0	4.087	0.000	0.074	0.000	0.041	0.000
	36.0	4.281	0.000	0.078	0.000	0.044	0.000
	48.0	4.590	0.000	0.087	0.000	0.048	0.000
	60.0	4.808	0.000	0.090	0.000	0.048	0.000
	72.0	4.893	0.000	0.092	0.000	0.052	0.000

Table B21 Data of Figure 4.36

		Cumulative release of mangostins (%)					
		Acetate buffer at pH 5.6					
	Films	MBAGTM 1%	S.D.	MBAGTM 5%	S.D.	MBAGTM 10%	S.D.
Time (h)	0.0	0.000	0.000	0.000	0.000	0.000	0.000
	6.0	0.469	0.000	0.069	0.000	0.037	0.000
	12.0	0.480	0.000	0.072	0.000	0.039	0.000
	18.0	0.488	0.000	0.078	0.000	0.043	0.000
	24.0	0.499	0.000	0.084	0.000	0.046	0.000
	30.0	0.507	0.000	0.087	0.000	0.049	0.000
	36.0	0.517	0.000	0.091	0.000	0.051	0.000
	48.0	0.529	0.000	0.100	0.000	0.054	0.000
	60.0	0.532	0.000	0.110	0.000	0.056	0.000
	72.0	0.535	0.000	0.123	0.000	0.059	0.000
		PBS at pH 7.4					
	Films	MBAGTM 1%	S.D.	MBAGTM 5%	S.D.	MBAGTM 10%	S.D.
Time (h)	0.0	0.000	0.000	0.000	0.000	0.000	0.000
	6.0	0.628	0.000	0.106	0.000	0.036	0.000
	12.0	0.729	0.000	0.126	0.000	0.041	0.000
	18.0	0.829	0.000	0.143	0.000	0.046	0.000
	24.0	0.869	0.000	0.159	0.000	0.049	0.000
	30.0	0.884	0.000	0.184	0.000	0.054	0.000
	36.0	0.929	0.000	0.197	0.000	0.057	0.000
	48.0	1.065	0.000	0.209	0.000	0.062	0.000
	60.0	1.122	0.000	0.222	0.000	0.067	0.000
	72.0	1.254	0.000	0.267	0.000	0.072	0.000

VITAE

Miss Sutasinee Seetabhawang was born on May 7th, 1988 in Narathiwat, Thailand. She finished high school from Princess Chulabhorn's College Nakhon Sri Thammarat, Nakhon Sri Thammarat in March, 2006. She received her Bacheler's Degree in Science (Industrial Chemistry) from King Mongkut's Institute of technology Ladkrabung, Bangkok in April, 2010. She continued Master degree in chemical engineering at Chulalongkorn University in June, 2010. Her academic publications are as follows:

1. EFFECT OF PLASTICIZERS ON CHARACTERISTICS OF BACTERIAL CELLULOSE /ALGINATE/GELATIN COMPOSITE FILMS in the 5th Pure and Applied Chemistry International Conference 2013 (PACCON2013) on January 23-25, 2013 at the Tide Resort, Bangsaen Beach, Chon Buri, Thailand.

2. BIOSYNTHESIS AND CHARACTERIZATION OF NANOCELLULOSE-GELATIN FILMS in Journal of Materials 6 (2013) 782-794.