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.