FABRICATION AND ANTIMICROBIAL ANALYSIS OF COMPOSITE BIODEGRADABLE FILM FROM CHEMPEDAK SEEDS

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Signature : ……….……..……………………

Name of Supervisor : Madam Norashikin binti Mat Zain

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SYAMSUTAJRI BINTI SYAMSOL BAHRI

**A thesis submitted in fulfillment
of the requirements for the award of the Degree of
Bachelor of Chemical Engineering (Biotechnology)**

**Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang**

NOVEMBER 2010

I declare that this thesis entitled ""Fabrication and Antimicrobial Analysis of Composite Biodegradable Film from Chempedak Seeds" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree."

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Name : Syamsutajri binti Syamsol Bahri

Date : ………………………………

To my beloved mother,
Madam Hasnah bt Abdul Rahim,
and father,
Mr Syamsol Bahri bin Husin.

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ABSTRACT

Chempedak seed flour is a starchy material with low-cost. Films carrying antimicrobial agent has been the most potential type of packaging in industry. The most suitable additives have not yet discovered but starch films have been widely researched. Chempedak seed starch film has been the best source as it has organic fibers to enhance the film. The aim of this study were to develop antimicrobial biodegradable films based on chempedak seed flour, and to characterize their morphology, physical and mechanical properties. Films were prepared by casting method. The preparation of films include of the preparation of chempedak seed flour, isolation of the starch and preparation of solution at temperature range of 80°C to 100°C. Fourier transform infra-red (FT-IR) spectra analysis revealed that starch crytallinity was retarded with chitosan incorporation and that hydrogen bonds were formed between chitosan and starch at wavelength 3400-3600 cm^{-1}. Films incorporated with 2% chitosan have lower water solubility which was 27.76% and higher viscocity (428.9 cP) at 10 rpm while SEM micrograph of the film obviously showed insoluble starch granule. The antimicrobial activities of films were examined against *E. coli* and *B. subtilis*. Stronger bacterial effects showed with *B. subtilis* than *E. coli* in the presence of 2% chitosan. TS were increase ith the increasing of chitosan incorporation. As a conclusion, the case studies on the analysis of antimicrobial activity of chempedak seed biodegradable film incorporated with chitosan could be used as an alternative to starch for biodegradable film. Furthermore, the films exhibit good antimicrobial activity which can help extend the food shelf life.

ABSTRAK

Tepung biji chempedak merupakan bahan berkaji dan boleh didapati dengan kos yang rendah. Filem dengan agen antimikrob telah menjadi sumber yang berpotensi dalam industri pembungkusan. Filem daripada chempedak menjadi sumber terbaik kerana mempunyai serat organik untuk meningkatkan filem. Tujuan kajian ini adalah untuk menganalisis filem bio-urai antimikrob berdasarkan tepung biji chempedak, dan untuk mengkarakterisasikan morfologi, sifat fizikal dan mekanikal. Filem dibuat dengan proses *casting*. Penyusunan filem meliputi penyusunan tepung biji chempedak, isolasi kanji dan persiapan larutan pada suhu antara 80°C hingga 100°C. Transformasi fourier infra-merah (FT-IR) analisis spektrum menunjukkan bahawa filem *crytallinity* menurun dengan peningkatan *chitosan* dan ikatan hidrogen terbentuk antara *chitosan* dan kanji pada panjang gelombang 3400 cm^{-1}-3600 cm^{-1}. *Chitosan* 2% mempunyai kelarutan dalam air yang lebih rendah iaitu sebanyak 27.76% dan kelikatan lebih tinggi (428,9 cP) pada 10 rpm, sementara filem mikrograf SEM menunjukkan dengan jelas granul kanji. Filem menunjukkan kesan bakteria kuat dengan bakteria *B. subtilis* pada kepekatan *chitosan* 2%. Kekuatan tarikan lebih tinggi dengan peningkatan *chitosan* pada filem. Sebagai kesimpulan, kajian kes pada analisis aktiviti antimikrob filem daripada chempedak boleh urai digabungkan dengan *chitosan* boleh digunakan sebagai alternatif untuk kanji. Selain itu filem menunjukkan aktiviti antimikrob yang baik yang boleh membantu memanjangkan masa simpanan makanan.

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LIST OF ABBREVIATIONS

AFM	-	Atomic Force Microscopy
DSC	-	Differential Scanning Calorimetry
FTIR	-	Fourier Transform Infrared
Mw	-	Weight Average Molecular Weight
PEG	-	Poly (ethylene glycol)
TGA	-	Thermo Gravimetric Analysis
WVP	-	Water Vapor Permeability
%v/v	-	volume percentage for chemical per basis
WS	-	Water Solubility
CS	-	Chitosan
CSF	-	Chempedak Seed Flour
E.coli	-	*Escherichia coli*
B.subtilis	-	*Bacillus subtilis*
S.areus	-	*Staphylococcus aureus*
EPIC	-	Environment and Plastics Industry Council
TS	-	Tensile Strength

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Starch is one of the most commonly used raw materials to prepare biodegradable film because it is a renewable source, widely available, relatively easy to handle, and inexpensive (Maizura *et al.,* 2007). Films carrying food additives, such as antioxidants, antimicrobial agents, will be the developing tendency of functional food packaging in the future (Li *et al.*, 2006). As usual, antimicrobial films are allowed to contain higher concentrations of antimicrobial agents than that is permitted in food. Therefore, when food is packaged with antimicrobial film, the antimicrobial agents in the film are gradually released to the food surface and will remain there in a high concentration, which extends food shelf life and decreases the actual concentration of antimicrobial agents in the whole food (Pranoto *et al.,* 2005).

The possibility of adding these ingredients in biodegradable packaging based on raw materials would reduce packaging disposal, made from conventional polymers, that promotes damage to the environment (Parra *et al.,* 2004; Seydim and Sarikus, 2006; Pelissari *et al.,* 2009).

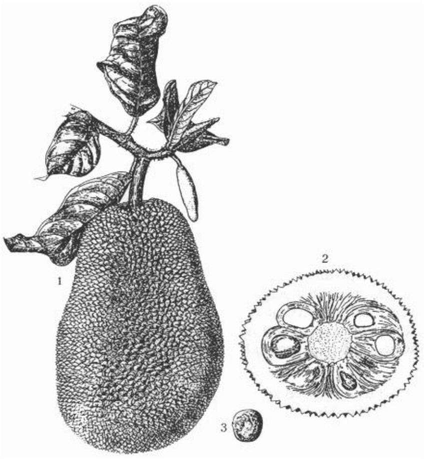


Figure 1.1: Chempedak *(Artocarpus integer Merr.)*

Chempedak *(Artocarpus integer Merr.)* is found widely in Peninsular Thailand, Borneo and Peninsular Malaysia, particularly in Perak and Kedah (Mardiana and Noor Aziah, 2009). Chempedak flesh is usually eaten fresh or cooked, while the seeds are considered as waste. The preparation of chempedak seed starch-based film is a good way to maximize the use of the waste.

A number of studies on the antimicrobial characteristics of films made from chitosan have been carried out earlier (Chen *et al.*, 1996; Coma *et al.*, 2002; Ouattara *et al.,* 2000a, 2000b). Among other polymers, chitosan has received a significant attention as antimicrobial film-forming agent for food preservation to the researchers due to its biodegradability, biocompatibility, cytotoxicity, and antimicrobial activity. Chitosan films are easily prepared by evaporation of its dilute acid solutions (Park *et al.*, 2002). Chitosan provides unique functional, nutritional, and biomedical properties, and its present and potential uses range from dietary fiber to a functional ingredient and processing aid. Some of the well known applications of chitosan include its use for prevention of water pollution, medicine against hypertension, antimicrobial and hypocholesterolemic activity, flavor encapsulation, seed coating, film-forming, and controlled release of food ingredients and drugs (Dunn *et al.,* 1997; Muzzarelli, 1985, 1996; Muzzarelli and De Vincenzi, 1997; Onishi, Nagai *et al.*, 1997; Struszczyk and Pospieszny, 1997).

Dutta *et al.*, 2009 stated that binding of antimicrobials to polymeric surfaces has been achieved by different means, ranging from simply spreading antimicrobial solutions onto the polymer surface or by more sophisticated means such as combining the antimicrobials with binders. They also state that these binders can be of a cellulosic, or an acrylic co-polymer nature.

1.2 Problem Statement

At recent years, we are constantly striving to meet the growing demands within the packaging industry. There is an increase in public awareness of environmental issues, particularly surrounding the disposal of packaging. Increased use of synthetic packaging films had led to serious ecological problems due to their non-biodegradability. As stated by Tharanathan (2003), the materials most used for food packaging are the petrochemical based polymers, due to their availability in large quantities at low cost and favorable functionality characteristics, such as, good tensile and tear strength, good barrier properties to O_2, and heat seability. Petroleum-based plastics often remain undegraded after discard and a time-consuming and uneconomical recycling is unavoidable (Frohberg *et al.,* 2010). The adoption of biopolymers avoids the removal of residuel materials from the growing environment after functional compliance (Espi *et al.,* 2006, Joo *et al.*, 2005). Several studies have been performed to analyze the properties of starch-based biodegradable films (Arvanitoyannis and Biliaderis, 1998; Garcia *et al.,* 1999; Lourdin *et al.,* 1995; Mali and Grossmann, 2003). The use of a biopolymer such as starch can be an interesting solution because this polymer is relatively inexpensive, abundant, biodegradable, and edible (Mali and Grossmann, 2003).

The development and application of these technologies is limited due to two main factors. First, there is a lack of knowledge about the effectiveness of most systems, consumer resistance, and economic impact of this technology. Second, there are no specific regulations for active packaging up to now. As a result, a more exhaustive study of the chemical, microbiological, and physiological effects of the applied technologies must be carried out. A careful environmental impact study must also be performed before commercial implementation of these packages (Lopez-Rubio *et al.,* 2004).

Although starch films have been widely researched, research on antimicrobial starch films is relatively scarce and reported on tapioca (Flores *et al.,* 2007a) and yam (Durango *et al.,* 2006). Therefore, it’s essential to research on antimicrobial chempedak seed starch film. Among the food-borne bacteria, *Escherichia coli* and *Staphylococcus aureus* are currently observed in a wide range of food products. Furthermore, they are the human pathogens that cause the most economically important food-borne diseases throughout the world (Elizaquı´vel and Aznar, 2008). *E. coli* is the most common bacteria from human feces, and *S. aureus* is one of the indigenous microbiota on human skin (Fujimoto *et al.,* 2006). Guzewich and Ross (1999) found that in 89% of outbreaks caused by food contamination by food workers, pathogens were transferred to food by workers’ hands. Therefore, *E. coli* and *S. aureus* are two of important pathogens that should be control in the food industry.

1.3 Significance of Study

The demand for natural antimicrobial ingredients has grown because consumers are more conscious about the potential health risks associated with the consumption of synthetic components, despite their efficiency (Moreira *et al.,* 2005; Nielsen and Rios, 2000; Ozdemir and Floros, 2004; Suppakul *et al.,* 2003).

The development of complementary methods to inhibit the growth of pathogenic bacteria such as packaging material-associated antimicrobial agents is an active area of research. There has been increasing interest in antimicrobial edible packaging materials. Among other polymers, chitosan has received a significant attention as antimicrobial film-forming agent for food preservation to the researchers due to its biodegradability, biocompatibility, cytotoxicity, and antimicrobial activity.

1.4 Objectives

The main objectives of this study are:

a. To fabricate difference types of antimicrobial biodegradable films from chempedak seeds.
b. To analyze antimicrobial activities of chempedak seeds starch film incorporated with chitosan.
c. To characterize the difference types of antimicrobial biodegradable film from chempedak seeds.

1.5 Scope of Study

In general, the scopes of study of this research are:

a. To study about the chempedak-seeds flour making process.
b. To fabricate the antimicrobial biodegradable film from chempedak seeds using fabrication process.
c. To study the antimicrobial activity of the films in resistance of *E. coli* and *B. subtilis.*
d. To study the physical characterization of the film.

CHAPTER 2

LITERATURE REVIEW

2.1 Biodegradable Film

2.1.1 Introduction

Biodegradable film starch based known as biodegradable polymer is now commercially for conversion into many applications in which conventional plastics are used. Polymer materials are solid, non-metallic compounds of high molecular weights (Callister, 2000). They are comprised of repeating macromolecules, and have varying characteristics depending upon their composition. Items made from bio-film will fully biodegrade when purposely or accidentally disposed of into a biologically active environment like a compost heap, the soil, a lake, the sea or a sewage treatment plant. In the United States, currently less than 10% of plastic products are recycled at the end of their useful life (Chiellini *et al.,* 2001). Recycling must be recognized as a disposal technique, not a final goal for material development.

Guan and Hanna (2002) documented how biodegradable loose-fill packaging materials may be developed from renewable biopolymers such as starch. The starch material is treated by an acetylation process, chemical treatments, and post-extrusion steaming. Mechanical properties of the material are adequate, and true biodegradability is achieved. Biodegradable film has similar properties to conventional thermoplastics and is processed using the same technologies. However, being starch based, all bio-film grades are completely biodegradable to carbon dioxide, water and carbon based humus. Unlike some systems which use additives, in conventional biodegradation of bio-film there is no polymer or toxic residue. Biodegradation of bio-film occurs when it is attacked by micro-organisms which exist in a biological activity in the environment. In a healthy compost heap, a bag made from bio-film will biodegrade in about 30 to 40 days with disintegration starting much sooner. Other grades when molded into a thicker item can take longer to biodegrade completely. Some of the important properties of a biodegradable biomaterial can be summarized as follows (LIoyd, 2002);

a. The material should not evoke a sustained inflammatory or toxic response upon implantation in the body.
b. The material should have acceptable shelf life.
c. The degradation time of the material should match the healing or regeneration process.
d. The material should have appropriate mechanical properties for the indicated application and the variation in mechanical properties withdegradation should be compatible with the healing or regeneration process.
e. The degradation products should be non-toxic, and able to get metabolized and cleared from the body.
f. The material should have appropriate permeability and processibility for the intended application.

2.1.2 Historical Perpectives

Biodegradable films and coatings were used hundreds of years ago. For example, wax has been applied to citrus fruits to delay their hydration since the twelfth and thirteen century in China (Debeaufort *et al.,* 1998), a protein film was used to preserve the appearance of some foodstuffs in Asia in the fifteenth century (Debeaufort *et al.*, 1998; Han and Gennadios, 2005), fats were used to coat meat cuts to prevent shrinkage. Later, in the nine teeth century, gelatin films were used to cover meat stuffs and also sucrose was chosen as an edible protective coating on nuts, almonds and hazelnuts to prevent oxidation and racidness (Debeaufort *et al.,* 1998) In the last 30 years, petrochemical polymers, commonly called plastic, have been widely used materials for packaging because of their high performance and low cost (Callegarin *et al*., 1997) but environmental problems occur due to their non-biodegradability. Thus, edible or biodegradable packaging made from various biological resources and their applications have recently been investigated. Shellac and wax coatings on fruits and vegetables, zein coatings on candies and sugar coatings on nuts are the most commercial examples of edible coatings (Han and Gennadios, 2005).

Research on biodegradable plastics based on starch began in the 1970s and continues today at various labs all over the world. Starch or amylum is a polysaccharide carbohydrate consisting of a large number of glucose units joined together by glycosidic bonds. Starch is produced by all green plants as an energy store. It is the most important carbohydrate in the human diet and is contained in such staple foods as potatoes, wheat, maize (corn), rice, and cassava.

2.1.3 Biopolymer

From the book of “Polymer Nanocomposites” edited by Yiu Wing Mai and Zhong-Zhen Yu, biodegradable polymers are defined as those undergo microbially induced chain scission leading to the mineralization. Specific conditions in terms of pH, humidity, oxygenation and the presence of some metals are required to ensure the biodegradation of such polymers. Biodegradable polymers may be made from bio-sources like corn, wood cellulose, etc. or can also synthesized by bacteria from small molecules like butyric acid or valeric acid. Other biodegradable polymers can be derived from the petroleum sources or may be obtained from mixed sources of biomass and petroleum. Biopolymer materials are currently incorporated into adhesives, paints, engine lubricants, and construction materials (Fomin *et al.,* 2001).

A review of biodegradable polymers stated by Environment and Plastics Industry Council (EPIC), biodegradable polymers may be naturally occurring or they may be synthesized by chemicals means. There are many polymers produced from feedstocks derived from petrochemical or biological resources that are biodegradable. The feedstocks used to produce the polymers may come from the processing of crops grown for the purpose or the by products of others crops (so called renewable resources) or they may come from petrochemical feedstocks (so called non-renewable resources). The natural polymers fall into four broad groups:

a. Polysaccharides – Starch, Cellulose
b. Proteins – Gelatin, Casein, Silk, Wool
c. Polyesters – Polyhydroxyalkanoates
d. Others – Lignin, Shellac, Natural Rubbers

In the review, they were stated also that it cannot be automatically assumed that natural polymers are good for environmental degradation. The rate of degradation and the formation of the ultimate metabolites depend very much on the structural complexity of the material and the material conditions selected for the degradation trial.

The use of biopolymers can be an important tool in environmentally- friendly management because of the large amount of polymers used in many applications. Most biodegradable polymers have excellent properties comparable to many petroleum-based plastics, are readily biodegradable, and may soon be competing with commodity plastics. So, biodegradable polymers have great commercial potential for bio-plastics, but some of the properties such as brittleness, low heat distortion temperature, low gas permeability, low melt viscosity for further processing, etc, restrict their use in a wide range o applications. Many reports paint a more optimistic picture for the economic promise of biopolymers. As Salmoral *et al.,* (2000) reported, a number of major chemical companies are gaining interest in developing biopolymer technologies used to manufacture products from renewable resources. Tharanathan (2003) reported that synthetic plastics will never be totally replaced by biodegradable materials. However, he believes that in niche markets where the development is feasible, there exists an opportunity for manufacturers to find a large profit.

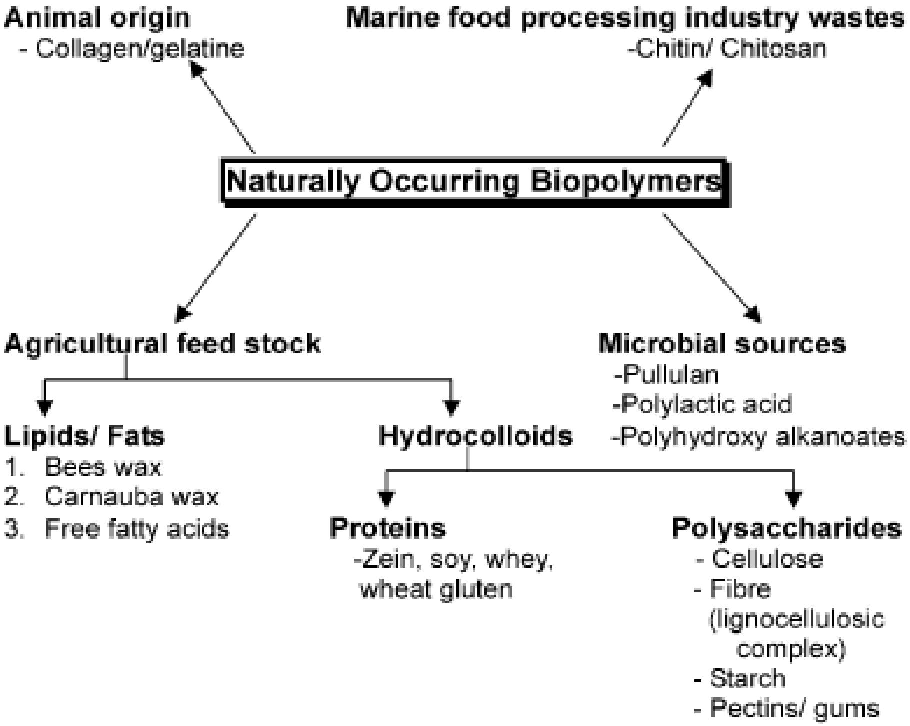


Figure 2.1: Naturally occurring biopolymers of use in biodegradable packaging films and composites.

There are a number of other biological materials that have been examined and manipulated by biopolymer researchers. Wheat contains starch and gluten, both of which are employed by the biopolymer industry. Canola derivatives have potential as both polymers and plasticizers (Crawford 2001). The biopolymer industry has a positive future, driven mainly by the environmental of using renewable resource feedstock sources. The ultimate goal for those working in development is to find a material with optimum technical performance, and full biodegradability.

2.1.4 Composite Biodegradable Film

According to Tharanathan (2003) two types of biomolecules, hollocolloids and lipid are generally used in combination for the preparation of biodegradable packaging films or composites. Individually they lack structural integrity and characteristics functionality. For example, hydrocolloids compensated by adding lipids, which are very good moisture barriers. Composite films are in fact a mixture of these and other ingredients in varying proportions, which determine their barrier (to hydrogen, oxygen, carbon dioxide and aroma compounds) and other mechanical properties. Sometimes a composite film formulation can be tailor made to suit the needs of a specific commodity or farm produce.

Use of plasticizers such as glycerin, ethylene glycol, sorbitol, etc in the film formation or composites is advantageous to impart pliability and flexibility, which improves handling (Garcia *et al.*, 2000). Use of plasticizers also reduces the brittleness of the film by interfering with the hydrogen bonding between the lipid and hydrocolloid molecules.

2.2 Starch

Starch is another raw material in abundance, especially in corn, having thermoplastic properties upon disruption of its molecular structure (Tharanathan, 1995; Tharanathan and Saroja, 2001). Preponderance of amylose (> 70%) in amylomaize starches gives stronger, more flexible films, Branched structure of amylopectin generally leads to films with poor mechanical properties (decrease tensile strength and elongation) (Tharanathan, 2003). Substitution of the hydroxyl groups in the molecule weaken the hydrogen bonding ability and thereby improve freezethaw stability and solution clarity (Tharanathan, 2003). Starch is composed of two types of molecules, amylose and amylopectin, which are arranged in a relatively waterinsoluble granule of a particular size.

Some other definitions stated that starch as a molecule composed of long chains of a-glucose molecules linked together (repeating unit $C_{12}H_{16}O_5$). These linkages occur in chains of a-1,4 linkages with branches formed as a result of a-1,6 linkages (see below). This polysaccharide is widely distributed in the vegetable kingdom and is stored in all grains and tubers. A not-so-obvious consequence of the a-linkages in starch is that this polymer is highly amorphous, making it more readily attacked by human and animal enzyme systems and broken down into glucose.

Some cultivated plant varieties have pure amylopectin starch without amylose, known as waxy starches. The most used is waxy maize, others are glutinous rice, waxy potato starch. Waxy starches have less retrogradation, resulting in a more stable paste. High amylose starch, amylomaize, is cultivated for the use of its gel strength.

Resistant starch is starch that escapes digestion in the small intestine of healthy individuals. In order to increase the digestibility, starch is cooked. Hence, before humans started using fire, eating grains was not a very useful way to get energy.

Starch is one of the extensively studied biopolymers derived from renewable crops grown in surplus in the world, and is naturally biodegradable. It is also one of the most abundant and versatile among natural polymers, and has been extensively researched as a raw material for the development o biodegradable hybrid composites and blends (Griffin, 1971; Otey *et al.*, 1976, 1987; Doane *et al.*, 1998). The starch polymer is composed of two major components, amylose and amylopectin. The amylose is mostly composed of linear α-D-(1-4)-glucan whereas, amylopectin is a highly branched α-D-(1-4)-glucan with α-D-(1-6) linkages at the branch points. The linear amylose molecules constitute about 30% of common cornstarch and have molecular weights of 200 000-700 000, while the branched amylopectin molecules have molecular weights as high as 100-200 million.

Commercially important starch is obtained from corn, wheat, rice, potatoes, tapioca and peas. Starch is a polysaccharide that is produced in almost all plants by photosynthesis (Tester *et al.*, 2004). Most recently, starch-based films for food packaging have received increasing attention from food scientists.

Starch alone is hardly useable as a packaging material due mainly to its poor mechanical properties (e.g. brittleness) and its hydrophilic nature. They are often modified mechanically, physically or chemically and/or combined with plasticizer or polymeric additives. The boundary between starch biopolymer and biodegradable polymer here can thus become diffused (Davis and Song, 2006)

2.2.1 Properties

Pure starch is a white, tasteless and odorless powder that is insoluble in cold water or alcohol. It consists of two types of molecules: the linear and helical amylose and the branched amylopectin. Depending on the plant, starch generally contains 20 to 25% amylose and 75 to 80% amylopectin. Glycogen, the glucose store of animals, is a more branched version of amylopectin. Starch can be used as a thickening, stiffening or gluing agent when dissolved in warm water, giving wheatpaste.

2.2.2 Carbohydrate Unit

A carbohydrate is an organic compound with general formula $C_m(H_2O)_n$, that is, consisting only of carbon, hydrogen and oxygen, the last two in the 2:1 atom ratio. Carbohydrates are divided into four chemical groupings: monosaccharide, disaccharide, oligosaccharide, and polysaccharide. In general, the monosaccharide and disaccharides, which are smaller (lower molecular weight) carbohydrates, are commonly referred to as sugars. Carbohydrates perform numerous roles in living things. Polysaccharides serve for the storage of energy (e.g., starch and glycogen) and as structural components (e.g., cellulose in plants and chitin in arthropods). In food science and in many informal contexts, the term carbohydrate often means any food that is particularly rich in starch (such as cereals, bread and pasta) or sugar (such as candy, jams and desserts).

2.2.2.1 Polysaccharide

Polysaccharides are known for their structural complexity and functional diversity (Tharanathan, 2003). They are the complex carbohydrates and made up of chains of monosaccharides (the sugars) which are linked together by glycosidic bonds, which are formed by the condensation reaction. The linkage of monosaccharides into chains creates chains of greatly varying length, ranging from chains of just two monosaccharides, which makes a disaccharide to the polysaccharides, which consists of many thousands of the sugars.

Polysaccharides also are macromolecules formed from many monosaccharide units joined together by glycosidic linkages. Polysaccharides gain renewed interest as biomaterials due to the growing body of literature pointing to their unique biological functions ranging from cell signaling to immune recognition. This combined with new synthetic routes currently available to modify polysaccharides or synthesize oligosaccharide moieties, biodegradability and ability to fabricate appropriate structures, make them one of the most important and extensively investigated natural biomaterials. (Lakshmi *et al.*, 2007).

Polysaccharides used in edible or biodegradable films and coatings include cellulose, starch, pectin and algal gum. The properties of edible films depend on the type of film-forming materials and especially on their structural cohesion. Additives-such as plasticizers, cross-linking agents, anti microbial agents, anti-oxidants and texture agents-are used to alter the functional properties of the films. Among the natural polymers, starch has been considered as one of the most promising candidates for future materials because of the attractive combination of price, availability and thermoplasticity (Mali *et al.*, 2005).

2.2.3 Structure

Linear structure of some of these polysaccharides, for example, cellulose (1,4-b-d-glucan), amylose (a component of starch 1,4-a-d-glucan), chitosan(1,4-b-d-glucosamine polymer), renders their films tough, flexible and transparent. Their films are resistant to fats and oils. Cross-linking, for example, of chitosan with aldehydes make the film much more tough, water insoluble (or swellable) and highly resistant (Tharanathan, 2003).

Polysaccharides represent an important class of biological polymers. Their function in living organisms is usually either structure- or storage-related. Starch (a polymer of glucose) is used as a storage polysaccharide in plants, being found in the form of both amylose and the branched amylopectin. In animals, the structurally-similar glucose polymer is the more densely-branched glycogen, sometimes called 'animal starch'. Glycogen's properties allow it to be metabolized more quickly, which suits the active lives of moving animals.

Cellulose and chitin are examples of structural polysaccharides. Cellulose is used in the cell walls of plants and other organisms, and is claimed to be the most abundant organic molecule on earth. It has many uses such as a significant role in the paper and textile industries, and is used as a feedstock for the production of rayon (via the viscose process), cellulose acetate, celluloid, and nitrocellulose. Chitin has a similar structure, but has nitrogen-containing side branches, increasing its strength. It is found in arthropod exoskeletons and in the cell walls of some fungi. It also has multiple uses, including surgical threads.

The starch granules can be seen in the cell through a light microscope. The starch granules are made up of two polysaccharides, amylose and amylopectin. Amylose is an unbranched molecule made up of several thousand glucose units, coiled helically into a more compact shape. Amylopectin is also compact but has a branched structure and is made up of twice as many glucose units as amylose. Some reports claim that the amylose concentration is higher in the large granules of bimodal starches (Li *et al.*, 2001).

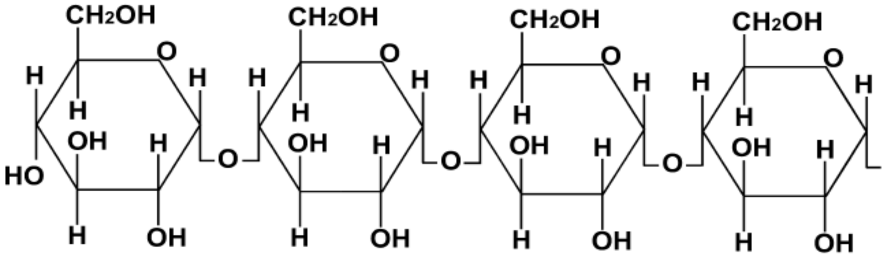


Figure 2.2: Amylose structure

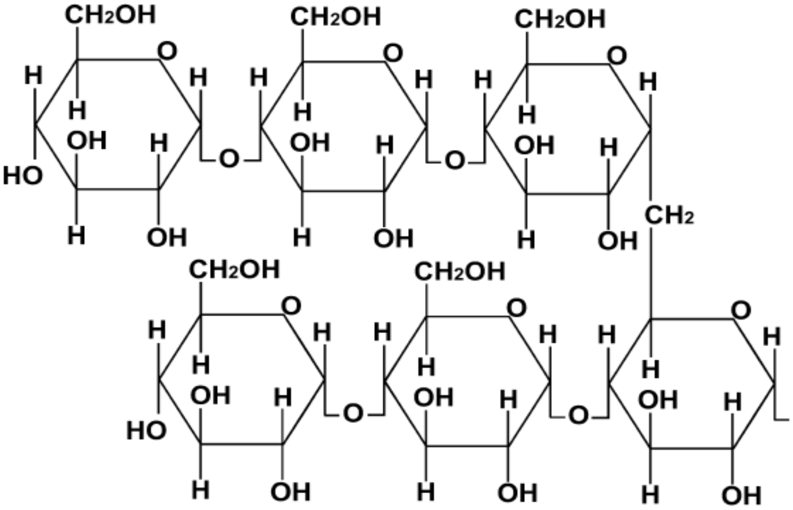


Figure 2.3: Amylopectin structure

2.3 Chitosan

Chitosan is a chitin derived polysaccharide and is one of the more abundant natural polymers, largely widespread in living organisms such as shellfish, insects, and mushrooms. Se´bastien *et al.,* (2006). It is a multi-purpose material that has found a wide range of applications ranging from dietary regime constituents, food packaging material, drug release components and for environmental pollutants (Agullo *et al.,* 2003; Arvanitoyannis, 1999; Ravi Kumar *et al.,* 2004).

Chitosan was thus used as a polymeric matrix to produce films from renewable resources which exhibit potential antifungal properties on mycotoxinogen strains

(Fusarium, Aspergillus, etc), because of its good film-forming properties and its recognized antimicrobial activity (Begin and Van Calsteren, 1999; Shahidi *et al.,* 1999). Chitosan is produced commercially by deacetylation of chitin , which is the structural element in the exoskeleton of crustaceans (crabs, shrimp, etc.) and cell walls of fungi.

Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). It has a number of commercial and possible biomedical uses. The potential of chitosan to act as food preservative of natural origin has been widely reported on the basis of in vitro trails as well as through direct application on real complex matrix foods (Coma *et al.,* 2001).

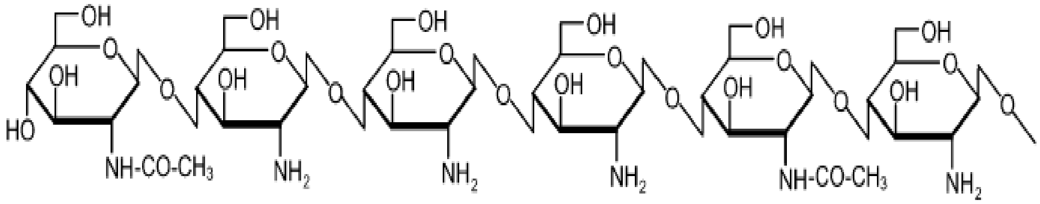


Figure 2.4: Chitosan structure

2.3.1 Properties

The amino group in chitosan has a pKa value of ~6.5, which leads to a protonation in acidic to neutral solution with a charge density dependent on pH and the %DA-value. This makes chitosan water soluble and a bioadhesive which readily binds to negatively charged surfaces such as mucosal membranes. Chitosan enhances the transport of polar drugs across epithelial surfaces, and is biocompatible and biodegradable. Purified qualities of chitosans are available for biomedical applications.

Chitosans are nontoxic, biocompatible, and biodegradable and have been widely used for pharmaceutical purposes and for other purposes such as clarification of waste water, in food products, in feed ingredients, and as a wet strength additive in the paper industry (Paul, 2000).

Their properties include solubility in various media, solution, viscocity, polyelectrolyte behavior, polyoxysalt formation, ability to form films, metal chelations, optical, and structural characteristics (Peter *et al*., 2000). As stated by Pradip *et al.*, 2004, the chemical and biological properties of chitosan are as follows:

a. Chemical properties: linear polymine, reactive amino groups, reactive hydroxyl groups available, chelates many transitional metal ions.
b. Biological properties of chitosan: biocompatible: natural polymer, biodegradable to normal body constituents, safe and non-toxic, binds to mammalian and microbial cells aggressively, regenerative effect on connective gum tissue, accelerates the formation of osteoblast responsible for bone formation, hemostatic, fungistatic, spermicidal, antitumor, anticholesteremic, accelerates bone formation, central nervous system depressant, immunoadjuvant.

2.4 Chempedak

2.4.1 Background of Chempedak

Chempedak (*Artocarpus integer (Thunb.) Merr*., *Artocarpus integrifolia L. f.*, *Artocarpus polyphema Persoon*, *Artocarpus champeden (Lour.) Stokes*) belongs to the Moraceae family, the same family as jackfruit (*Artocapus heterophyllus Lam*.) and breadfruit (*Artocarpusaltilis*). Chempedak (English), cempedak (Malay), bankong (wild), sonekadat (Myanmar) or champada (Thailand) is native to South East Asia and is widely distributed and cultivated in Burma, Indonesia, Peninsular Thailand and Peninsular Malaysia particularly in Perak and Kedah (Nakasone and Paull, 1998; Jansen, 1991).

It is strictly tropical in growing requirements and is always restricted to regions without a distinct dry season (Jansen, 1991). Chempedak is an evergreen monoecious tree and commonly found in abundant in primary lowland rain forest in its area of natural occurrence (Jansen, 1991). However, chempedak is usually grown in home gardens and sometimes in mixed orchard. The tree can grow up to 20 metres tall and is seldom buttressed. Chempedak is more seasonal than jackfruit as some flowers may be found at any time of the year. In Peninsular Malaysia, the flowers tend to bloom concentratively around the months of February to April and/or August to October while the fruits are harvested in between June and August. Meanwhile, in Sarawak, chempedak fruits ripen towards the end of the year in most years (Nakasone and Paull, 1998; Jansen, 1991). Since the crop is restricted to wet regions, chempedak is not widespread as jackfruit. The demands for chempedak fruit are rather small and often regarded as a locally orientated fruit. However, chempedak is a promising new tropical fruit outside of its current area of distribution. In Malaysia, a number of chempedak selections have been cloned such as CH29 cultivar, which produces attractive orange flesh while other cultivars, CH26, CH27 and CH28 are high-yielding cultivars. Jackfruit and chempedak occasionally hybridized and a clone has been selected in Malaysia called ‘Nangka-Chempedak CH/NA’ (Jansen, 1991). Chempedak plantation area, production and production value in states of Malaysia on 2003 is presented in Table 2.1.

Table 2.1: Plantation area, production value of chempedak in states of Malaysia

Source : Jabatan Pertanian Malaysia (2006)

State	Chempedak			
	Planted area (Ha)	Producing area (Ha)	Production (Mt)	Value of production (RM)
Johor	1,093.7	758.4	4,186.3	8,373
Kedah	1,472.9	1,007.5	6,920.6	13,841
Kelantan	489.4	92.2	340.5	681
Malacca	372.7	240.7	1,925.2	3,850
Negeri Sembilan	269.9	151.6	756.9	1,514
Pahang	939.0	287.3	1,244.8	2,490
Perak	1,113.4	340.7	1,629.6	3,259
Perlis	25.7	19.1	86.6	173
Penang	296.0	207.2	1,243.2	2,486
Selangor	677.4	623.6	3,693.1	7,386
Terengganu	707.0	105.2	287.3	575
Peninsular Malaysia	7,457.1	3,833.4	22,314.1	44,628
Sabah	1,229.3	645.5	3,810.8	7,622
Sarawak	2831.0	1,539.9	9,239.4	18,479
W. P. Labuan	138.0	100.0	323.0	676
Malaysia	11,655.4	6,118.8	35,687.3	71,375

2.4.2 Composition of Chempedak

Chempedak fruit weigh from 600 gram to 3500 gram and is generally smaller than jackfruit. The total edible portion (perianths + seeds) amounted for 25-50% of fresh fruit weight with total weight of all perianths in fresh fruit varies from 100-1200 gram. Chempedak composition is very similar to jackfruit. According to Nakasone and Paull (1998), the fruit is a good source of carbohydrate and vitamin A and a fair source of protein. The chemical composition of the edible portion of chempedak is shown in Table 2.2.

The total weight of chempedak seeds per fruit range from 65 gram to 880 gram, with weight of each seed ranging from 1 gram to 12 gram. The chemical composition of the seed based on dry weight is approximately: protein 10.0-13.0%, fat 0.5-1.5%, fibre 4.0-6.0%, ash 3.0-4.0% and the moisture content is 46.0-78.0% (Jansen, 1991). The comparison of chemical composition in chempedak seed is shown in Table (Mardiana, 2010). Most of the chemical compositions for chempedak and jackfruit seeds are comparable. Thus, these fruits may be exploited in the development of value-added food products.

Table 2.2: Composition of chempedak seed

Chemical composition (% dry weight)	**Chempedak seed**
Moisture	57.2
Protein	6.6
Crude fat	0.6
Crude fibre	1.4
Ash	1.4
Carbohydrate	32.8

2.5 Starch Film

Starch is one of the most commonly used raw materials to prepare biodegradable film, because it is a renewable source, widely available, relatively easy to handle, and inexpensive (Maizura *et al.*, 2007). Starch granules contain ordered regions, which are semi-crystalline and show birefringence. The overall crystallinity is about 2045%. Amylose and the residues around the branch points of amylopectin form the amorphous regions in the starch granule. The crystallinity arises mainly from ordered linear segments of amylopectin **(**Keetels *et al.*, 1996). Starch is an agricultural feedstock hydrocolloid biopolymer found in a variety of plants including (but not limited to) wheat, corn, rice, beans, and potatoes (Salmoral *et al.*, 2000, Martin *et al.*, 2001). In order to improve the physical and functional properties of starch films, blending with other biopolymers, hydrophobic substances and/ or antimicrobial compounds has been proposed (Ayranci and Tunc, 2003)

2.5.1 Starch Film-Forming Mechanism

2.5.1.1 Gelatinization

Starch becomes soluble in water when heated. The granules swell and burst, the semi-crystalline structure is lost and the smaller amylose molecules start leaching out of the granule, forming a network that holds water and increasing the mixture's viscosity. This process is called starch gelatinization. During cooking the starch becomes a paste and increases further in viscosity. During cooling or prolonged storage of the paste, the semi-crystalline structure partially recovers and the starch paste thickens, expelling water. This is mainly caused by the retrogradation of the amylose. This process is responsible for the hardening of bread or staling, and for the water layer on top of a starch gel (syneresis).

The gelatinization properties of starch are related to a variety of factors including the size, proportion and kind of crystalline organization, and ultra-structure of the starch granule. Goering and DeHaas, 1972 reported that small granule starch had, in general, a lower pasting temperature than large granule starch. However, small granule size is not necessarily associated with a low pasting temperature, as dasheen starch has a pasting temperature 207C higher than that of most other small granule starches. The low amylose content of dasheen and the fact that it is a tuber starch made this gelatinization temperature totally unexpected (Goering and DeHaas, 1972).

2.6 Film Characterization

2.6.1 Scanning Electron Microscopy (SEM)

The scanning electron microscope (SEM) is a type of electron microscope that images the sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition and other properties such as electrical conductivity.

The types of signals produced by an SEM include secondary electrons, back-scattered electrons (BSE), characteristic X-rays, light (cathodoluminescence), specimen current and transmitted electrons. Secondary electron detectors are common in all SEMs, but it is rare that a single machine would have detectors for all possible signals. The signals result from interactions of the electron beam with atoms at or near the surface of the sample. In the most common or standard detection mode, secondary electron imaging or SEI, the SEM can produce very high-resolution images of a sample surface, revealing details about less than 1 to 5 nm in size. Due to the very narrow electron beam, SEM micrographs have a large depth of field yielding a characteristic three-dimensional appearance useful for understanding the surface structure of a sample. This is exemplified by the micrograph of pollen shown to the right. A wide range of magnifications is possible, from about 10 times (about equivalent to that of a powerful hand-lens) to more than 500,000 times, about 250 times the magnification limit of the best light microscopes. Back-scattered electrons (BSE) are beam electrons that are reflected from the sample by elastic scattering. BSE are often used in analytical SEM along with the spectra made from the characteristic X-rays. Because the intensity of the BSE signal is strongly related to the atomic number (Z) of the specimen, BSE images can provide information about the distribution of different elements in the sample. For the same reason, BSE imaging can image colloidal gold immuno-labels of 5 or 10 nm diameter which would otherwise be difficult or impossible to detect in secondary electron images in biological specimens. Characteristic X-rays are emitted when the electron beam removes an inner shell electron from the sample, causing a higher energy electron to fill the shell and release energy. These characteristic X-rays are used to identify the composition and measure the abundance of elements in the sample.

2.6.1.1 Scanning Process and Image Formation

In a typical SEM, an electron beam is thermionically emitted from an electron gun fitted with a tungsten filament cathode. Tungsten is normally used in thermionic electron guns because it has the highest melting point and lowest vapour pressure of all metals, thereby allowing it to be heated for electron emission, and because of its low cost. Other types of electron emitters include lanthanum hexaboride (LaB_6) cathodes, which can be used in a standard tungsten filament SEM if the vacuum system is upgraded and field emission guns (FEG), which may be of the cold-cathode type using tungsten single crystal emitters or the thermally-assisted Schottky type, using emitters of zirconium oxide.

When the primary electron beam interacts with the sample, the electrons lose energy by repeated random scattering and absorption within a teardrop-shaped volume of the specimen known as the interaction volume, which extends from less than 100 nm to around 5 µm into the surface. The size of the interaction volume depends on the electron's landing energy, the atomic number of the specimen and the specimen's density. The energy exchange between the electron beam and the sample results in the reflection of high-energy electrons by elastic scattering, emission of secondary electrons by inelastic scattering and the emission of electromagnetic radiation, each of which can be detected by specialized detectors. The beam current absorbed by the specimen can also be detected and used to create images of the distribution of specimen current. Electronic amplifiers of various types are used to amplify the signals which are displayed as variations in brightness on a cathode ray tube. The raster scanning of the CRT display is synchronised with that of the beam on the specimen in the microscope, and the resulting image is therefore a distribution map of the intensity of the signal being emitted from the scanned area of the specimen. The image may be captured by photography from a high resolution cathode ray tube, but in modern machines is digitally captured and displayed on a computer monitor and saved to a computer's hard disk.

2.6.2 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry or DSC is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. Generally, the temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time. The reference sample should have a well-defined heat capacity over the range of temperatures to be scanned.

2.6.2.1 Basic Principle

The basic principle underlying this technique is that, when the sample undergoes a physical transformation such as phase transitions, more or less heat will need to flow to it than the reference to maintain both at the same temperature. Whether less or more heat must flow to the sample depends on whether the process is exothermic or endothermic. Likewise, as the sample undergoes exothermic processes (such as crystallization) less heat is required to raise the sample temperature. By observing the difference in heat flow between the sample and reference, differential scanning calorimeters are able to measure the amount of heat absorbed or released during such transitions. DSC may also be used to observe more subtle phase changes, such as glass transitions. It is widely used in industrial settings as a quality control instrument due to its applicability in evaluating sample purity and for studying polymer curing.

2.6.2.2 Polymer

DSC is used widely for examining polymers to check their composition. Melting points and glass transition temperatures for most polymers are available from standard compilations, and the method can show up possible polymer degradation by the lowering of the expected melting point, T_m, for example. T_m depends on the molecular weight of the polymer, so lower grades will have lower melting points than expected. The percentage crystallinity of a polymer can also be found using DSC. It can be found from the crystallisation peak from the DSC graph since the heat of fusion can be calculated from the area under an absorption peak. Impurities in polymers can be determined by examining thermograms for anomalous peaks, and plasticisers can be detected at their characteristic boiling points.

2.6.3 Fourier Transform Infrared (FTIR)

Fourier transform infrared spectroscopy (FTIR) is a technique which is used to obtain an infrared spectrum of absorption, emission, photoconductivity or Raman scattering of a solid, liquid or gas. An FTIR spectrometer simultaneously collects spectral data in a wide spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time. FTIR technique has made dispersive infrared spectrometers all but obsolete (except sometimes in the near infrared) and opened up new applications of infrared spectroscopy.

2.6.3.1 The Concept

The goal of any absorption spectroscopy (FTIR, ultraviolet-visible ("UV-Vis") spectroscopy, etc.) is to measure how well a sample absorbs light at each wavelength. The most straightforward way to do this, the "dispersive spectroscopy" technique, is to shine a monochromatic light beam at a sample, measure how much of the light is absorbed, and repeat for each different wavelength.

Fourier transform spectroscopy is a less intuitive way to obtain the same information. Rather than shining a monochromatic beam of light at the sample, this technique shines a beam containing many different frequencies of light at once, and measures how much of that beam is absorbed by the sample. Next, the beam is modified to contain a different combination of frequencies, giving a second data point. This process is repeated many times. Afterwards, a computer takes all this data and works backwards to infer what the absorption is at each wavelength.

As mentioned, computer processing is required to turn the raw data (light absorption for each mirror position) into the desired result (light absorption for each wavelength). The processing required turns out to be a common algorithm called the Fourier transform (hence the name, "Fourier transform spectroscopy"). The raw data is sometimes called an "interferogram".

2.6.4 Thermal Gravimetric Analysis

Thermogravimetric analysis or thermal gravimetric analysis (TGA) is a type of testing that is performed on samples to determine changes in weight in relation to change in temperature. Such analysis relies on a high degree of precision in three measurements: weight, temperature, and temperature change. As many weight loss curves look similar, the weight loss curve may require transformation before results may be interpreted. A derivative weight loss curve can be used to tell the point at which weight loss is most apparent. Again, interpretation is limited without further modifications and deconvolution of the overlapping peaks may be required.

TGA is commonly employed in research and testing to determine characteristics of materials such as polymers, to determine degradation temperatures, absorbed moisture content of materials, the level of inorganic and organic components in materials, decomposition points of explosives, and solvent residues. It is also often used to estimate the corrosion kinetics in high temperature oxidation.

Simultaneous TGA-DTA/DSC measures both heat flow and weight changes (TGA) in a material as a function of temperature or time in a controlled atmosphere. Simultaneous measurement of these two material properties not only improves productivity but also simplifies interpretation of the results. The complementary information obtained allows differentiation between endothermic and exothermic events which have no associated weight loss (e.g., melting and crystallization) and those which involve a weight loss (e.g., degradation).

2.7 Application

2.7.1 Food Packaging

As stated by Davis and Song (2006), a wide range of materials are used for packaging applications including metal, glass, wood, paper or pulp-based materials, plastics or combination of more than one materials as composites. They are applied in three broad categories of packaging:

a. Primary packaging, which is normally in contact with the goods and taken home by consumers.
b. Secondary packaging, which covers the larger packaging such as boxes, used to carry quantities of primary packaged goods.
c. Tertiary packaging, which refers to the packaging that is used to assist transport of large quantities of goods, such as wooden pallets and plastic wrapping.

Primary packaging materials is the most common types of material used for primary packaging are paper or pulp-based materials, glass, metals (e.g. aluminum and steels) and plastics. Secondary and tertiary packaging materials are normally in larger quantities and have less material variation and thus are relatively easier to collect and sort by wholesalers or retailers for recycling or reuse purposes. Primary packaging materials are not only more dispersed into households, they are also largely mixed, contaminated and often damaged and thus pose problems in recycling or reuse of the materials. The objectives in the development of biodegradable packaging are two-fold:

a. to utilize renewable and potentially more sustainable sources of raw materials (crops instead of crude oil);
b. to facilitate integrated waste management approaches so as to reduce landfill.

Table 2.3: Packaging films commonly used

Film Type	**Monomeric unit**	**Characteristics**
Polyethylene	Ethylene	Desirable mechanical properties, heat sealable
Polyvinylidene	Vinylidene	Desirable H2O/O2 barrier, not very strong, heat sealable
Polyester	Ethyleneglycol + terephthalic acid	Desirable mechanical properties, poor H2O/O2 barrier, not heat sealable
Polyamide (Nylon)	Diamine + various acids	Desirable strength, heat sealable, poor H2O/O2 barrier
Cellophane	Glucose (cellulose)	Desirable strength, good H2O/O2 barrier, not heat sealable.

2.7.2 Wound Dressing

Chitosan has excellent biological properties such as biodegradability, biocompatibility, antibacterial and wound healing activity (Khor and Lim, 2003; No *et al.*, 2002 and Ueno *et al.*, 2001). Specifically, Chitosan (CS) could achieve homeostasis, and allows the promotion of normal tissue regeneration (William and Herbert, 1985). CS also provides bacteriostatic and fungistatic activities (Mi *et al.*, 2001). For these reasons, CS has been one of the most important biomaterials for wound management. But the low mechanical strength of CS limits its further use as a wound dressing materials Wu *et al.*, (2004). The best biomaterials for wound dressing should be biocompatible and promote the growth of dermis and epidermis layers (Xiaodan *et al.*, 2008).

Chitosan has received great attention for medical and pharmaceutical applications due to its beneficial intrinsic properties. It is one of the natural polymers that have a high potential on wound healing application. However, pure chitosan films have a poor tensile strength and elasticity due to their brittleness. Hence, addition of other polymers is necessary to achieve films with improved strength and elasticity. Unlike conventional wound dressing which passively provides wound protection, effective wound dressing nowadays requires not only protection of the wound from surrounding environments but also to effectively promote the healing process by providing an optimum microenvironment for healing, to remove excess of wound exudate, and allowing continuous tissue reconstruction processes (Mi *et al.*, 2001). The ideal wound dressing, therefore, should have the following properties:

a. able to protect the wound from secondary infection,
b. provide a moisturized wound healing environment,
c. provide thermal insulation,
d. removable without causing trauma to the wound,
e. remove drainage and debris,
f. free from particulate and toxic products, and
g. promote tissue reconstruction processes.

CHAPTER 3

METHODOLOGY

3.1 Material

Chempedak seeds were obtained from a local farmer (Kuantan, Pahang). The seeds were removed and the flesh was cut manually. The seeds were cut into small pieces and then boiled at about 30 minutes. Next, the seeds were put into oven (60°C). After dried, the seeds were blended (dry blend). To isolate the starch, blended chempedak seeds were firstly ground into slurry with the addition of water, and then the slurry was filtered through filter cloth. The filtrate was settled over night without shaking to recover the sediment as starch. The starch was rinsed 3 times with water and dried at 40°C. Acetic acid glacial and chitosan, with a molecular weight lower than 2000 Da and a deacetylation degree higher than 85%, were obtain from the FKKSA, UMP laboratory store. *E. coli* and *B. subtilis* were maintained in the microbiology laboratory of Chemical Engineering and Natural Resources of University Malaysia Pahang.

3.2 Film Fabrication

3.2.1 Preparation of Flour

Chempedak seeds were obtained from a local farmer (Kedah and Negeri Sembilan). Chempedak seeds flour; CSF was prepared according to the modified of Tulyathan *et al*., (2002). °C First of all, chempedak seeds were cleaned and the seed coats were peeled off. The seeds were boiled for 30 minutes, chopped into small pieces and air-dried for 24 hour at 60°C and ground to 0.25 µm diameter (Universal Bench Top Grinder, Germany). The flour was then stored in an airtight plastic container at 5 °C prior to use.

3.2.2 Isolation of Starch

To isolate the starch, fresh chempedak seeds flour was firstly ground into slurry with the addition of water, and then the slurry was filtered through filter cloth. The filtrate was settled over night without shaking to recover the sediment as starch. The starch was rinsed 3 times with water and dried at 40 °C. Chitosan were purchased from alldrich-sigma. *E. coli* and *B. subtilis* were maintained in the microbiology laboratory of FKKSA, UMP.

3.2.3 Preparation of Solution

Film preparation was prepared according to Xiou *et al.,* (2010) method. 4 g of chempedak seed starch was dispersed in 100 ml, H_2O, moderately stirred for 20 min at room temperature, and then heated to 100°C for over 30 minutes. After gelatinization, the resulting dispersion was subjected to further mixing for 5 minutes.

3.2.4 Preparation of Film

To prepare the antimicrobial film, chitosan was first dissolved in 20 ml 1% acetic acid aqueous solution. Then the chitosan was added at different concentration with the starch solution in one time during the mixing period. When chitosan was incorporated into film, the pH of the gel-like mixture was 4.0 (acidic) because of acetic acid used in preparing chitosan solution. After being degassed under vacuum, the warm mixture was casted on framed glass plates, and then dried at 60°C for 4 hour. Starch film, without antimicrobial agents, was also prepared in the same way and used as a control.

3.3 Film Characterization

3.3.1 Fourier Transform Infrared (FTIR)

According to Xiou *et al.*, 2010, the fourier transform infrared (FT-IR) spectra of the films were recorded in an IR Spectrometer (Perkin-Elmer, model 2000, USA) in the wavelength range 400-4000 cm^{-1}. To perform FT-IR measurement, 5 mg completely dried film was first ground into powder and then dispersed in 200 mg KBr (pellet procedure). Signal averages were obtained at a resolution of 4 $cm^{-1.}$

3.3.2 Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) of film samples was obtained using a Philips XL-30 scanning electron microscope. The samples were coated with a fine gold layer before obtaining the micrographs. All samples were examined using an accelerating voltage of 10 kV. Micrographs of drying exposed surface and cross sectional of films were carried out.

3.3.3 Water Solubility (WS)

The water solubility was determined in triplicate according to a slightly modified method of Flores *et al.,* (2007). Before determination, the specimens of the films (4 x 4 cm) were dried to constant weight at 105 °C. Then portions of the films were placed into triangular vessels (50 mL) with 20 mL distilled water and subjected to occasionally gentle shaking for 24 h at room temperature (25 °C). Then, it was filtered through a filter paper to obtain the undissolved film, and the dry weight of the undissolved film was determined by desiccation at 105 °C for 24 hour. The water solubility of the film was calculated according to the equation:

$$WS\ (\%) = \frac{Wo - Wf}{Wo} \text{ x } 100$$

Where Wo is the initial weight of the film expressed as dry matter and Wf is the weight of the desiccated undissolved film.

3.3.4 Tensile Strength

The mechanical properties were determined according to our previously reported method with some modifications (Guohua *et al.,* 2006). All of the tested film sheets were cut into strips (70 mm x 25 mm). The dimensions of film samples used in tests were 70 mm x 25 mm, cut with sharp scissors. Tensile strength (TS) were measured with a crosshead speed of 50 mm/min and an initial gauge length of 100 mm. Film thickness was measured five times with a micrometer caliper. Mean values of TS and E were calculated from the five tests. Samples were clamped between grips and force and deformation were recorded during extension at 0.8 mm/s, with an initial distance between the grips of 50 mm.

3.3.5 Antimicrobial Activity

The antimicrobial test was carried out according to the method developed by Seydim and Sarikus (2006) and also Xiao *et al.,* (2010) with some modifications. The inhibitory zone test on solid medium or semisolid medium was used for determination of the antimicrobial effects of the films on *E. coli* and *B. subtilis*. Chempedak seeds starch films were cut into a disc (diameter = 5.1 mm) with a punch. During tests, 3 discs were placed carefully into each petri dish containing solid medium, where 0.1 mL seeding culture had been spread. The concentrations of the *E. coli* and *S. aureus* seeding culture were 5 x 10^9 CFU/mL, respectively. The petri dishes were then incubated at 30°C for 24 h in the appropriate incubation chamber. The plates were examined to find the ‘zone of inhibition’ of the film discs, and the diameter of the zone was measured with a sliding caliper in triplicate. The area of the whole zone was calculated then substrated from the film disc area and the difference in area was reported as the ‘zone of inhibition’. Finally, the units of colony formation in each agar plate were calculated to examine the antibacterial ability of each chitosan membrane.

3.3.6 Viscocity

The viscocity of the samples were measured using a viscometer in FKKSA laboratory. The solutions were put into the specific viscometer’s container and then the viscocity (cP), torque (%), shear rate (1/sec), shear stress (D/cm^2) were measured in 5 rpm, 10 rpm, 20 rpm, 30 rpm and 40 rpm.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Film Formation

The chempedak seed starch was gelatinized and mixed with antimicrobial agent before dried into films. All films formed self-supporting, slightly rough, clear and translucent films. The films formed can be seen in figure 4.1. The films formed at increasing concentration of chitosan were more homogeneous and showed more light yellowish, which may be the result of thickness of the chitosan. Both kinds of films had some fragments of granules present in the outer layer.

The film forming solution was obtained by dispersing chitosan in a 1% (v/v) aqueous acid solution. The preparation was filtered and degassed under reduced pressure. Film forming solutions were then poured in uniform layer onto a glass plate. The optimum conditions for casting were at ambient temperature for 2 days. The dried films were then peeled from the plate and samples were conditioned at 2 days before taking property measurements. In addition they were easy to handle when dried off. After drying, the film facing the plate was shiny and the side facing the room was duller for the films to which chitosan was added.

Figure 4.1: Chempedak Seed Film

4.2 Fourier Transform Infrared (FTIR)

The Fourier transform infrared (FT-IR) spectra of the films were recorded in an IR Spectrometer in the wavelength range 400–4000 cm^{-1}. To perform FT-IR measurement, 5 mg completely dried film was first ground into powder and then dispersed in 200 mg KBr (pellet procedure). Signal averages were obtained at a resolution of 4 cm^{-1}. Behavior of starch films is affected by the presence of crystalline phases. Fama *et al.*, 2005 verified the fact that the control films showed a higher degree of crystallinity, which contributed to the better mechanical behavior of the control starch film. This was also proved by FT-IR spectra analysis in our present work. FT-IR spectra of chempedak seed starch incorporate with chitosan films incorporated with varying levels of potassium sorbate or chitosan were depicted in figure 4.2. The spectra were used to identify possible interactions between functional groups of starch with chitosan. When two or more substances are mixed, physical blends versus chemical interactions are reflected by changes in characteristic spectra peaks (Xu *et al.*, 2005). From figure 4.2, it can be concluded that all spectra show similar patterns with the peaks at 3400 cm^{-1}- 3600 cm^{-1}, 2900 cm^{-1}- 3000 cm^{-1}, 1540 cm^{-1}- 1560 $cm^{-1}$and 1004 cm^{-1}- 1030 cm^{-1}. Absorption in this area reveals O–H and N–H bond stretching at about 3500 cm^{-1}, C–H symmetrical deformation at 1550 $cm^{-1}$and C–O bond stretching centered at 1022 cm^{-1}. In the spectra of chempedak seed starch films incorporated with chitosan, a change in band at 3400 cm^{-1} – 3500 cm^{-1}is obvious. With addition of chitosan, the peaks at 3400 cm^{-1}– 3500cm^{-1} and 1004 cm^{-1}- 1020 cm^{-1} become weaker and narrower; this revealed the hydrogen bonding interaction between starch and chitosan. In addition, the only difference between C0 and K0 existed in pH. The peak of K0 at 2900 cm^{-1}– 2930 cm^{-1} is slightly stronger than that of C0, and this difference resulted from the addition of acid. Meanwhile, the peak at 1559 cm^{-1} appeared and was enhanced with the addition of chitosan, a similar phenomenon can be found in the research done by Xu *et al.*, (2005). The infrared spectral data supported the mechanical and permeable properties data of sweet potato starch films incorporated with potassium sorbate or chitosan. Thus, it is absolutely concluded that the addition of antimicrobial agents disturbed the crystalline of starch and the crystallinity of starch is the most vital factor to determine the mechanical and permeable properties of sweet potato starch films.

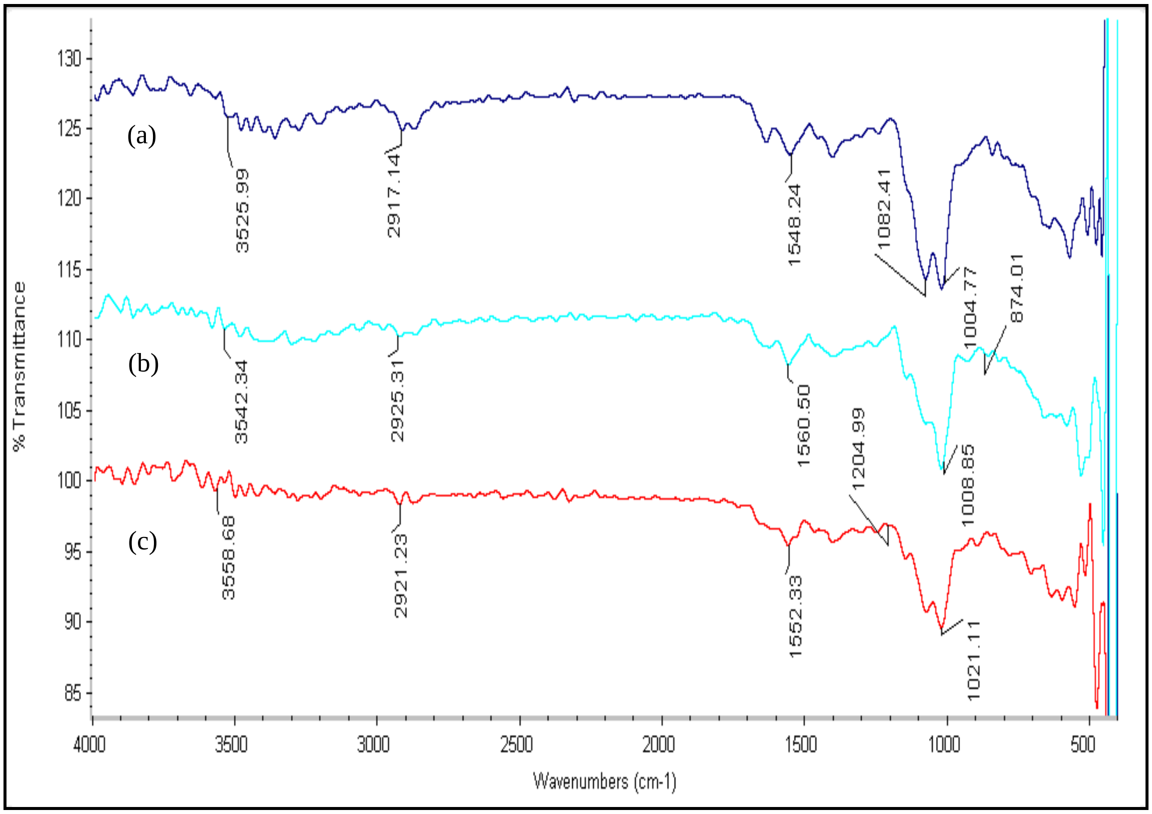
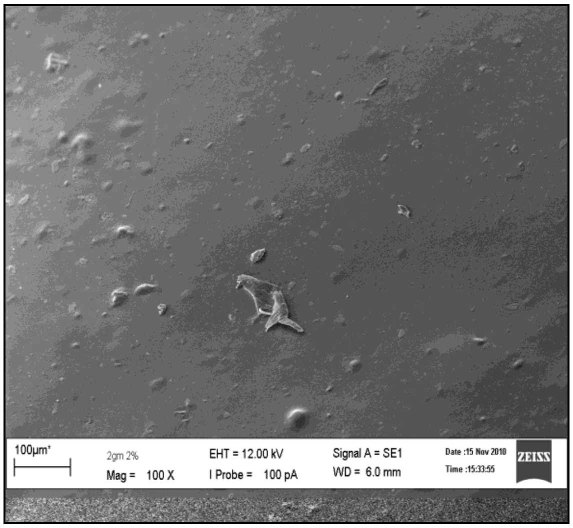


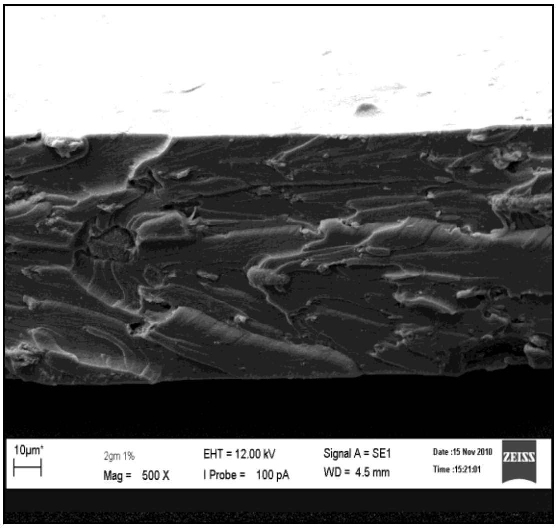
Figure 4.2: The FTIR Result of (a) Sample 1 (control) (b) Sample 2 (1% chitosan incorporation) (c) Sample 3 (2% chitosan incorporation).

4.3 Scanning Electron Microscopy (SEM)

Scanning electron microscopy surfaces and fractures of film samples are presented in figure 4.3. Micrographs of the film surfaces, at a magnification of 100x, did not show differences between the films. For starch base films, the presence of insoluble particles was observed. It is obvious that the starch granule were present in the surface of the films. The irregularities in the surface of starch flour films may be related to the presence of more than one macromolecule in the polymeric matrix (starch, protein and lipid). Fracture of micrographs figure (a), (c), and (e) showed homogeneity of chempedak seed starch films and chitosan. The pure starch film as stated by Eraricar *et al.,* 2009 exhibit characteristic patterns on the film surface. These patterns represent the withered ghost granules of starch. The intensity of films reduced with the decreasing concentration of starch as revealed from figure (a) to (f). Chitosan microdomains were dispersed within the starch matrix in the blend films with relatively good interfacial adhesion between the two components and were similar to the surface blends. Considering that chempedak seeds films are composed of starch, protein and lipid in different amounts, starch being the major component, it can be concluded that the dense areas of the polymeric matrix are composed of starch. The homogeneous matrix of these films is a good indicator of their structural integrity, and consequently good mechanical properties would be expected (Mali *et al.,* 2002).


100µm*
2gm 2%
Mag = 100 X
EHT = 12.00 kV
I Probe = 100 pA
Signal A = SE1
WD = 6.0 mm
Date :15 Nov 2010
Time :15:33:55
ZEISS

(a)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2gm 1%
Mag = 500 X
EHT = 12.00 kV
I Probe = 100 pA
Signal A = SE1
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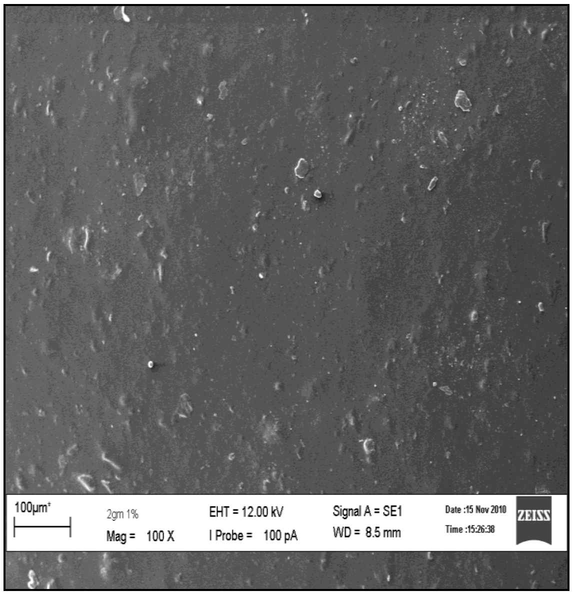
ZEISS

(b)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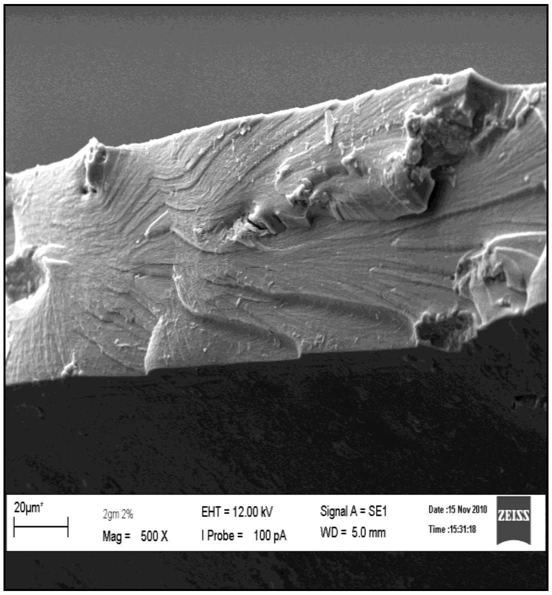
(c)


20µm*
2gm 2%
Mag = 500 X
EHT = 12.00 kV
I Probe = 100 pA
Signal A = SE1
WD = 5.0 mm
Date :15 Nov 2010
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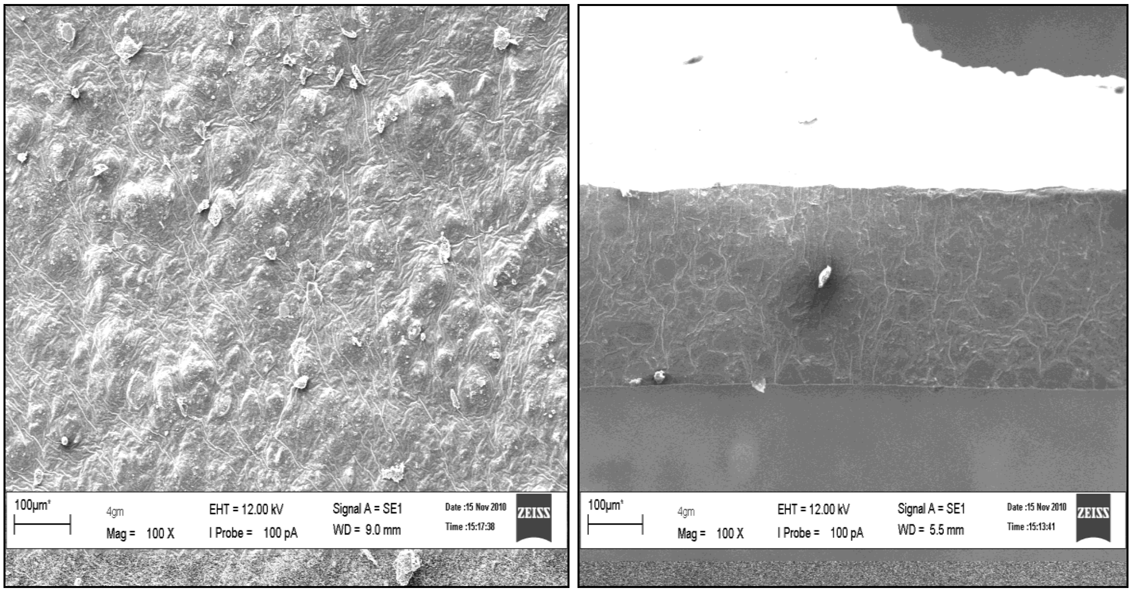
(d)



(e) (f)

Figure 4.3 (a) Surface at 100x (1% chitosan + 2 gram CSF) (b) cross sectional area at 500x (1% chitosan + 2 gram CSF) (c) surface at 100x (2% chitosan + 2 gram CSF) (d) cross sectional area at 500x (2% chitosan + 2 gram CSF) (e) surface at 100x (2% chitosan + 4 gram CSF) (f) cross sectional area at 100 x (2% chitosan + 4 gram CSF)

4.4 Water Solubility

Solubility in water is an important property of starch-based films. Potential applications may require water insolubility to enhance product integrity and water resistance. However, in other cases, water solubility of the film before product consumption might be useful as in encapsulation of food or additives (Bertuzzi *et al.,* 2007). WS of chempedak seeds starch films decreased with the addition of chitosan (table 4.1). Less formation of crystallites in the films render it to swell inwater easily and to disintegrate. Therefore, film solubility in water will be higher (Maizura *et al.,* 2007). This phenomenon could be seen in film with 1% chitosan incorporation which WS was higher than film with 2% chitosan incorporation. A significant decrease in WS was observed in chempedak seeds starch film incorporated with greater concentrations of chitosan (table 4.1). The incorporation of chitosan enhanced the water resistance of the chempedak seeds starch films. This is the result of the strong hydrogen bonding interaction between chitosan and starch and the low water solubility or relative hydrophobicity of chitosan itself.

Water resistance is an important property of biodegradable or edible films for applications as food protection where water activity is high, or when the film must be in contact with water during processing of the coated food (e.g. to avoid exudation of fresh or frozen products) (Gontard *et al.,* 1992). Generally, higher solubility would indicate lower water resistance. However, a high solubility may be an advantage for some applications. Film solubility is advantageous in situations when the films will be consumed with a product that is heated prior to consumption and may also be an important factor that determines biodegradability of films when used as packaging wrap. In the case of thermal gelatinization, addition of plasticizer increased water solubility, although the values were all lower than that of the films prepared with cold gelatinization.

Table 4.1: Water solubility of samples (CSF incorporated with chitosan)

Samples	**Water Solubility (%)**
Sample 1 (control)	7.6
Sample 2 (1% chitosan)	34.67
Sample 3 (2% chitosan)	27.76

4.5 Tensile Strength

The effects of chitosan incorporation on tensile strength (TS) and elongation at break (E) of chempedak seed starch films incorporated with chitosan are shown in figure 4.4 (a), (b) and (c). We can observe that TS of the films was increased when the addition of chitosan. The TS of films with 1% chitosan was lower than those of films with 2% chitosan. The mechanical properties of starch films with a chitosan incorporation resulted from inter-molecular hydrogen bonding interaction between NH_3 of the chitosan backbone and –OH groups of starch (Xu *et al.,* 2005).

This result can be explained by both the existence of irregularities at the microstructure level, and the presence of lipids in the flour, because lipids are unable to form a cohesive and continuous matrix, according Pe´ roval *et al.,* (2002).

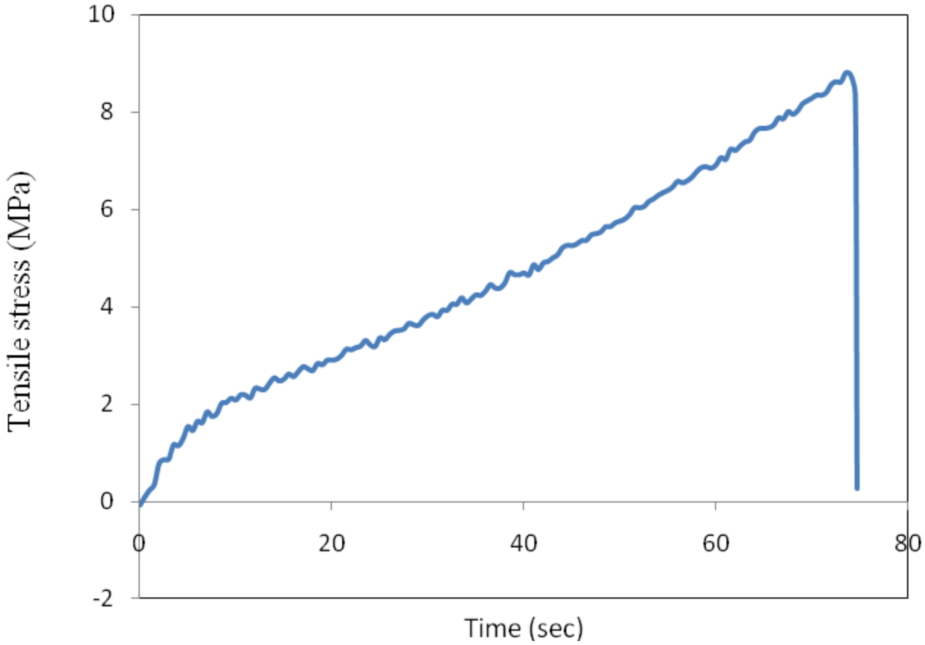


Figure 4.4: (a) The result of tensile strength for sample 1% chitosan incorporation

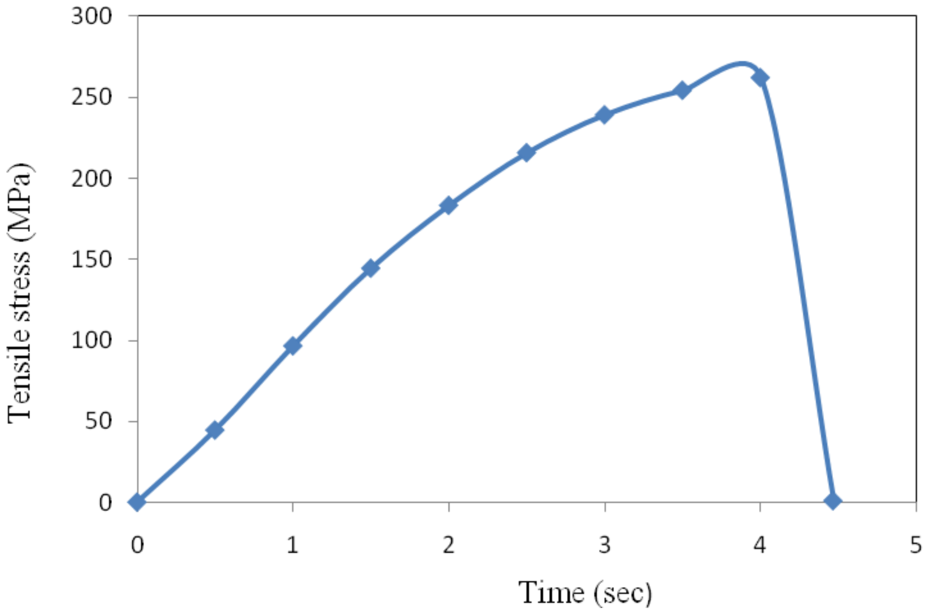


Figure 4.4: (b) The result of tensile strength for sample 2% chitosan incorporation

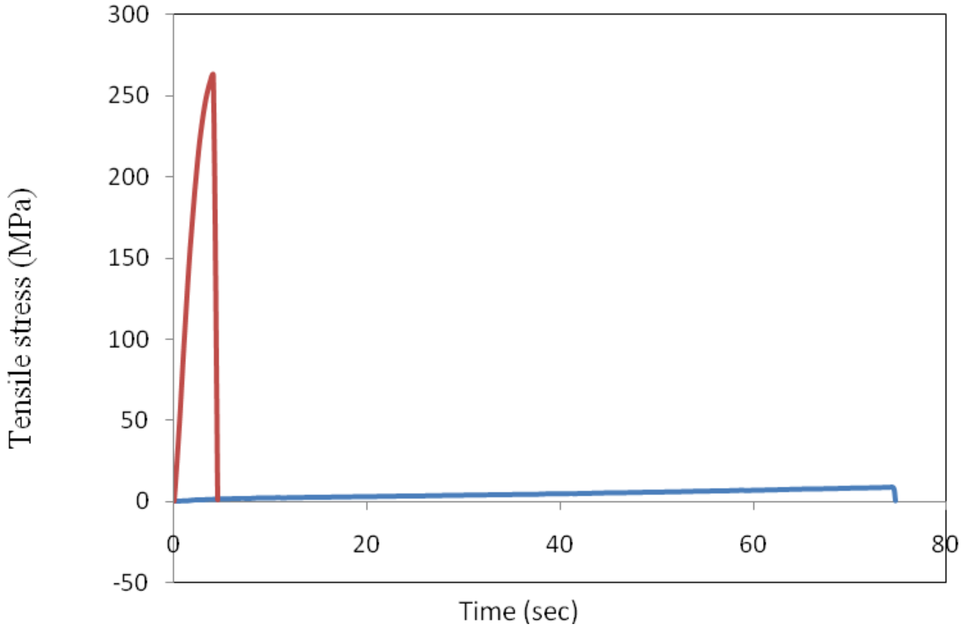


Figure 4.4: (c) The comparison between 1% and 2% chitosan incorporation

4.6 Antimicrobial Activity

The chempedak seeds starch film incorporation with chitosan has good antimicrobial properties. The expected results is based on analysis conducted by Xiao *et al.*, (2010) where when the addition of chitosan into the film increased from 1% to 2%, the diameter of the clearing zone enlarged rapidly (refer to figure 4.5). The antimicrobial activity of chempedak seeds starch films incorporated with chitosan against *E. coli* and *B. subtilis* is shown in table 4.2 and figure 4.5. The control chempedak seed film did not show any inhibitory effect on *E. coli* or *B. subtilis*. In addition, contamination could be obviously seen in these plates that no antimicrobial effect was observed for film. This contamination was brought about by the film specimen that is not completely sterilized before antimicrobial test. Moreover, no contamination was founded in those plates that strong antimicrobial effect was observed for the tested film. When the addition of chitosan increased from 1% to 2%, the diameter of the clearing zone slightly enlarged. The antimicrobial mechanism of chitosan is due to its NH_2 group can be protonated to NH þ and readily form electrostatic interactions with anionic groups of microbial cell membranes, leading to the leakage of proteinaceous and other intracellular constituents of the microorganisms (Pranoto *et al.,* 2005; Xu *et al.*, 2005). Films placed in plate containing *B. subtilis* (gram positive bacteria) shows stronger bacterial effect than in the plate containing *E. coli* (gram negative bacteria). Thus the inhibition area is lower. The main reason was because gram-positive cell walls typically lack the outer membrane found in gram-negative bacteria. So, the defense of cell wall to bacterial effect was reduced thus inhition area became less.

Table 4.2: Antimicrobial activity of CSF incorporated with chitosan

Concentration of CSF	Concentration of Chitosan	Inhibitory zone (mm^2)	Inhibitory zone (mm^2)
2 gram	1 gram	2.1 ± 1.40	1.80 ± 2.4
2 gram	2 gram	8.2 ± 3.2	7.3 ± 2.4


(i) (ii)

Figure 4.5: (a) Inhibition Zone of Antimicrobial Activity (i) *E. coli* (2% chitosan + CSF) (ii) *B. subtilis* (2% chitosan + CSF)

4.7 Viscocity

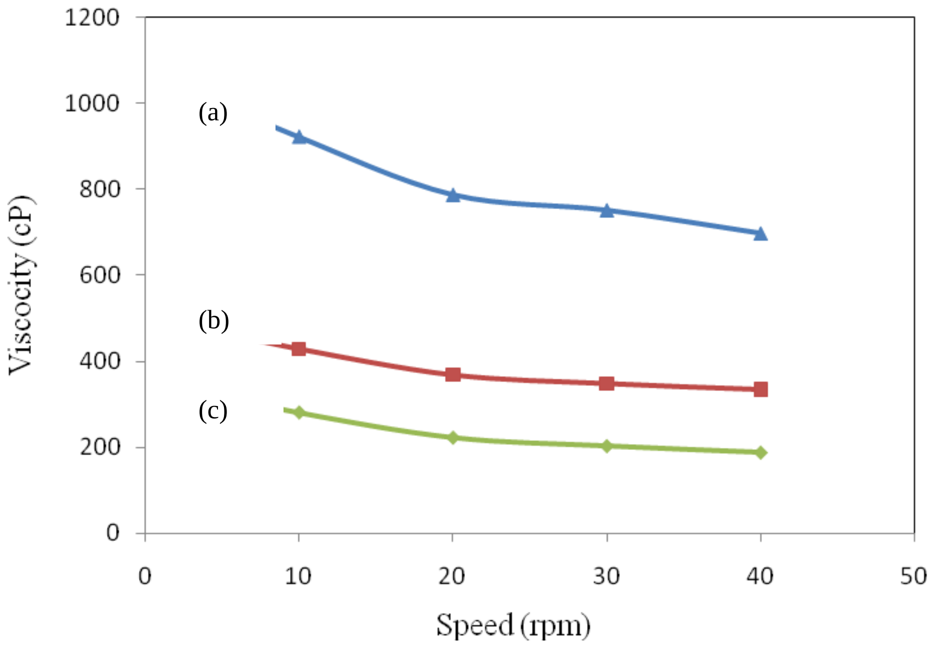


Figure 4.6: Comparison of viscocity solution for (a) control (b) 2% chitosan incorporation (c) 1% chitosan incorporation

A rheological model was used in the study. The coupled effects of the rheological model parameters on the velocity profile, the stress profiles, and the film tension are found to be related to the elongational viscosity curve. The stress profiles are related to both the magnitude and shape of the elongational viscosity curve. At high deformation rates, fluids with higher chain extensibility have higher elongational viscosity and require higher film tension; fluids with larger mobility factor have lower elongational viscosity and require lower film tension. Furthermore, this test is to clarify the behavior in film casting and their applicability. Based on the graph above (figure 4.5), viscocity increased with the increasing of chitosan incorporation.

Table 4.3: List of viscocity of solutions

Samples	rpm	Viscocity (cP)	S.STR (D/cm^2)	S.RATE (1/sec)	Torque (%)
Control	5	1000.8	16.8	1.7	16.8
	10	923.8	31.4	3.4	30.8
	20	788.8	53.9	6.8	51.5
	30	752.8	76.8	10.2	75
	40	698.9	95.1	13.6	93.2
1 % chitosan incorporation	5	467.9	7.85	1.7	7.8
	10	428.9	14.6	3.4	14.3
	20	368.9	25.1	6.8	24.5
	30	348.9	35.6	10.2	34.9
	40	335.2	45.6	13.6	44.7
2 % chitosan incorporation	5	323.9	5.71	1.7	5.6
	10	281.9	9.69	3.4	9.6
	20	223.5	15.2	6.8	14.8
	30	204	20.7	10.2	20.3
	40	189	25.7	13.6	25.2

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

It has convincingly been approved that chitosan films exhibit good antimicrobial activity which can help extend the food shelf life (Dutta *et al.*, 2009). A new alternatives of starch from chempedak seeds also successfully produced which is sometimes much cheaper than commercial starches. In conclusion chempedak seed starch based film incorporated with chitosan showed several interesting characteristics. The biodegradable chempedak seed starch-based films incorporated with chitosan showed an improvement in increasing chitosan concentration for antimicrobial activity and addition of starch content provided transparent films but the yellowness of the film attributed to the higher chitosan content.

Obtaining films with good antimicrobial would be an indication of the potential use of these antimicrobial packaging as a natural packaging and an alternative packaging to the synthetic polymer to protect food from oxidation reactions. Antimicrobial starch-based films exhibited good film forming property due to the presence of high density of amino groups and hydroxyl groups and inter and intra molecular hydrogen bonding.

Chempedak seed starch based films incorporated with chitosan can be used for dried products and may have potential to be commercial as food packaging. Since the sources of the raw material consider less, it can also be used in wound dressing application as the new alternative but further improvement of the films need to be done.

5.2 Recommendation

The chempedak seed antimicrobial biodegradable film can be improve by incorporate the film with plasticizers for instances polyethylene glycol (PEG), glycerol and also other composites. New studies are necessary to better understand how proteins, lipids and starch interact in the film polymeric matrix and to find correct procedures to prepare more homogeneous films. The use of reinforcing fibers is a good alternative to improve film mechanical properties and water sensibility, but will be the subject of a further paper.

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