CHARACTERIZATION AND ANTIMICROBIAL ANALYSIS OF RICE STARCH-CHITOSAN BLEND BIODEGRADABLE FILM WITH ADDITION OF CINNAMON ESSENTIAL OIL

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ABSTRACT

This study will be focusing on fabrication of composite biodegradable film from rice starch-chitosan blends with addition of cinnamon oil. The film was characterized in term of morphology, physical, chemical and also the antimicrobial analysis. For solution preparation, rice starch was dissolved in distilled water at concentrations of 2g/100 ml by heating the mixtures on hot plates and stirred until it gelatinized at 85°C for 5 minute. The mixtures were then cooled to 27°C. The chitosan solution was prepared by dispersing 2 g of chitosan in 100 ml of 1% v/v acetic acid.1 ml of cinnamon essential oil was added to the mixture. Polyethylene glycol (PEG) 400 was added as a plasticizer. The solution was cast onto flat glass plate. The thickness of the film was adjusted using a casting knife. The film was left to dry at room temperature before peeled off. The fabricated film was analyzed and characterized using Scanning electron microscopes (SEM), Fourier transform infrared Spectroscopy (FTIR), Thermogravimetric analyzer (TGA) and Differential scanning calorimeter (DSC). The antimicrobial analyses were performed using Zone inhibition assay and Liquid culture test. As a conclusion, the rice starch-chitosan blend biodegradable film with addition of cinnamon essential oil shows the highest thermal resistant compared to control film. Other than that, the film also shows the greatest antimicrobial activity towards *E.coli* and *B.subtilis.*

ABSTRAK

Kajian ini memfokuskan pemfabrikasian filem boleh biodegradasi daripada campuran antara chitosan dengan kanji beras dan juga minyak pati kulit kayu manis. Karakter filem ini dikenal pasti daripada segi morfologi, fizikal, kimia dan juga daripada segi analisis anti-mikrob. Dalam menyediakan campuran untuk membentuk filem tersebut, kanji beras dilarutkan di dalam air suling dalam kepekatan 2mg/100ml dan dipanaskan sehingga melikat pada suhu 85°C selama 5 minit. Larutan tersebut kemudian disejukkan sehingga suhu 27^0C. Larutan chitosan disediakan dengan melarutkan 2g chitosan ke dalam 100 ml acid asetik yang berkepekatan 1% v/v. 1 ml minyak pati kulit kayu manis dicampurkan ke dalam larutan. *Polyethylene glycol* (PEG) 400 ditambah ke dalam larutan sebagai ejen memplastik. Larutan dituangkan ke atas plat gelas yang rata. Ketebalan lapisan larutan dikawal menggunakan *casting knife*. Filem tersebut dibiarkan kering sebelum dicabut. Filem tersebut dikarakter menggunakan *Scanning electron microspoce* (SEM), *Fourier transform infrared spectroscopy* (FTIR), *Thermogravimetric analyzer* (TGA) dan juga *Differential scanning calorimeter* (DSC). Analisis anti-mikrob filem tersebut dijalankan dengan mengunakan teknik *Zone inhibition assay* dan juga *Liquid culture test.* Sebagai kesimpulan, filem boleh biodegradasi daripada campuran chitosan dengan kanji beras dan juga minyak pati kulit kayu manis menunjukkan ketahanan haba yang paling tinggi berbanding filem kontrol. Selain itu, filem tersebut menunjukkan aktiviti anti-mikrob paling tinggi terhadap *E.coli* dan *B. subtilis.*

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LIST OF ABBREVIATIONS

PVC	-	Polyvinyl chloride
PEO	-	Poly ethylene oxide
PEG	-	Poly ethylene glycol
SEM	-	Scanning electron microscopy
FTIR	-	Fourier transform infrared
TGA	-	Thermo gravimetric analyzer
DSC	-	Differential scanning calorimeter
Mr	-	Molecular weight
% v/v	-	Volume percentage for chemical per basis
%w/w	-	Weight percentage for chemical per basis
NS	-	Without sample
E. coli	-	*Escherichia coli*
B. subtilis	-	*Bacillus subtilis*

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Nowadays there are many research related on biodegradable film had been performed in order to help the environment. The majority of engineered plastic materials used today are made from synthetic polymers. Usage of conventional petroleum-based polymer products creates many potential problems due to their non-renewable nature and ultimate disposal (Mali and Grossmann, 2003). Cellulose and its derivatives, for example from the rice starch when used in such applications, offer advantages with respect to sustainability, limited environmental impact and simplified end-of-life disposal issues and this clearly helps to preserve the environment (Mali and Grossmann, 2003). Biodegradable polymer films are not meant to totally replace synthetic packaging films, but to limit moisture, aroma, and lipid migration between food components where traditional packaging cannot function (Xu *et al.*, 2005).

This study is to see how rice starch can work out with chitosan to improve the quality of biodegradable film produced since both of the sources were proven worthy in producing biodegradable plastics which will helps in preserving the quality of living for mankind in nowadays environment. Furthermore this study will improve the quality of the biodegradable film by which antimicrobial agent will also added in order to magnificent and increase the variability of its usage. As we all know nowadays plastic not only used in packaging material and stuff but also used in many other industries such as food industries, plantation and even in clinical and pharmaceutical side. Only few of them in the market contain antimicrobial or antiseptic agent (Xu *et al.*, 2005).

In certain industries such as food packaging and pharmaceutical, hygiene become priority since some of the bacteria and microorganisms such as *E.coli sp.* and *S. typhi sp.* bring harm and unwanted reaction which will cause problems. Thus, if only we can replace the non-biodegradable plastic which will bring pollution to our world with biodegradable plastic which also providing antimicrobial properties, this will bring huge advantages to our environment and many sectors (Lee *et al.*, 2003). For the antimicrobial agent, usage from natural sources seems to be more practical. Since the antimicrobial agent from natural sources bring less harm and side effect to human compared to chemical based antimicrobial agent for example ethanol, it become major choice by consumers.

Realizing this situation, Cinnamon Essential Oil will be used as the antimicrobial agent. Essential oils are well-known antimicrobial agents that could be used to control food spoilage and food borne pathogenic bacteria (Burt, 2004). It has been demonstrated that several spices, herbs and fruits containing essential oil effectively inhibit microbial growth, although different results are observed depending on test conditions, microorganisms, and the source of the antimicrobial compound (Roller, 2003).

1.2 Problem statement

The majority of engineered plastic materials used today are made from synthetic polymers. The use of conventional petroleum-based polymer products creates many potential problems due to their non-renewable nature and ultimate disposal. In Malaysia, plastic became the third largest waste volume in Malaysian municipal solid waste (MSW). For the record plastic manufacturing became an important role in many sectors such as food industries, pharmaceutical, clinical and last but not least municipal daily usage (Lee *et al.*, 2005).

Average person use about 350 plastic bags per year. The conventional petroleum-based polymer took around 400 to 500 years to biodegrade by itself. When incinerated, plastic produce dangerous gases that pollute the environment. Even though we recycle plastic to minimize its quantity in order to avoid pollution, the process used during the recycling process still releases heavy, toxic metals into the air and environment. Thus the best solution is to use biodegradable plastic (Bert *et al.*, 2002).

Much research had been done to produce biodegradable polymer. Biopolymer-based materials originated from naturally renewable resources such as polysaccharides, proteins, and lipids have become the best candidates to form this biodegradable film in recent years since such biopolymers have their environmental advantages of recyclability and reutilization compared to other conventional petroleum-based synthetic polymer (Lee *et al.*, 2007). Biopolymer films can also become barriers of gas and solute barriers and complement other types of packaging by reducing food quality deterioration and extending the life of foods (Debeaufort *et al.*, 1998).

The quality of its characterization was still became major problem in much research. For example, biodegradable film which produced from chitosan shows high water permeability, and low tensile strength compared to conventional petroleum-based film (Xu *et al.*, 2005). Some of the weakness of this natural biopolymer films also as they possess poor mechanical and water vapor barrier properties. But its most critical disadvantage is its barrier against water vapor due to its hydrophilic nature (Lee *et al.*, 2007).

As we speak, chitosan nowadays has high potential in making biodegradable film. Chitosan principally derived from chitin by deacetylation with an alkali which is the natural and low-cost biopolymer. Chitosan possess many excellent properties such as biocompatibility, biodegradability and non-toxicity (Wan *et al.*, 2006).The only flaw of this material is that its hygroscopic properties of the bio-packaging containing polysaccharides are responsible for their weak moisture barrier and thus have little or no influence on the dehydration or rehydration phenomena of the foodstuffs (Sebastien *et al.*, 2006).

Rice is the most widely consumed basic food in the world. Each year over 500 million tons of rice is harvested, providing sustenance to many countries and people throughout the world. The unique properties of rice starches are found in its many varieties. Due to different climates, soil characteristics and cultures, over 240,000 registered varieties of rice exist in the world. These varieties lead to a wide range of rice starches with many different characteristics including: different starting gelatinization temperatures, textures, processing stabilities and viscosities (Thawien *et al.*, 2007). Several studies have been performed to analyze the properties of starch-based biodegradable films. The use of a biopolymer such as starch can be an interesting solution because this polymer is relatively inexpensive, abundant, biodegradable, and edible but the main flaw by using starch is it possesses low quality of characterization compared to others biodegradable polymer (Mali and Grossmann, 2003).

Furthermore nowadays biodegradable film mainly does not possess antimicrobial properties which important in certain uses such as food packaging, clinical usage and pharmaceutical usage. The physicochemical properties, composition and antimicrobial activity of cinnamon essential oil (*Cinnamomum zeylanicum*) were studied thoroughly as we speak. Cinnamon essential oil is highly anti-microbial and anti-bacterial for a great diversity of infectious bacteria which lead to many interest of using it as antimicrobial agent in many research. Studies have shown the strength of cinnamon bark oil to eliminate many forms of pathogenic organisms (Fuselli *et al.*, 2005).

1.3 Objective

a. To fabricate composite film from rice starch-chitosan blends with combination of cinnamon oil.
b. To characterize composite biodegradable film in term of morphology, physical and chemical.
c. To analyze the antimicrobial activity of the fabricated biodegradable film.

1.4 Scope of study

a. Fabrication of composite biodegradable film from rice starch-chitosan with combination of cinnamon essential oil.
b. The characterization of the composite biodegradable film using various analysis method:
   i. Scanning electron microscope (SEM)
   ii. Fourier transform infrared (FTIR) spectroscopy
   iii. Differential scanning calorimetry (DSC)
   iv. Thermogravimetric analysis (TGA)
c. Antimicrobial analysis of biodegradable film:
   i. Agar diffusion method (zone inhibition assay)
   ii. Liquid culture test (optical density measurement)

1.5 Benefit and Significant of Research

a. Help to preserve the environment by producing competence biodegradable film.
b. Help to preserve food in food industries by its antimicrobial properties.
c. Help to store and preserve clinical equipment and maintain hygiene by its antimicrobial properties.
d. Helps to preserve medicine and maintain hygiene in pharmaceutical sector by its antimicrobial properties.

CHAPTER 2

LITERATURE REVIEW

2.1 Biodegradable film

The majority of engineered plastic materials used today are made from synthetic polymers. The use of conventional petroleum-based polymer products creates many potential problems due to their non-renewable nature and ultimate disposal. Cellulose and its derivatives, when used in such applications, offers advantages with respect to sustainability, limited environmental impact and simplified end-of-life disposal issues (Petersson and Oksman, 2006)

Early studies examined the application of chitosan, starch and cellulose derivatives which were shown have film forming properties (Krochta *et al.*, 1994). There is a considerable interest in biodegradable films made from renewable and natural polymers, such as starch (Lawton, 1996). Biodegradable polymer films are not meant to totally replace synthetic packaging films, but to limit moisture, aroma, and lipid migration between food components where traditional packaging cannot function.

For instance, biodegradable and edible films can be used for versatile food products to reduce loss of moisture, to restrict absorption of oxygen, to lessen migration of lipids, to improve mechanical handling properties, to provide physical protection, or to offer an alternative to the commercial packaging materials (Nelson and Fennema, 1998).

2.2 Chitosan

2.2.1 Overview

A linear β-1, 4-D-glucosamine, it is a biocompatible, nontoxic compound mainly obtained by deacetylation of chitin, a natural structural component present for instance in crustaceans such as crabs shells (Moller *et al.*, 2004). In other word, Chitosan is the N-deacetylated product of chitin, a linear polymer composing primarily of glucosamine and a natural polymer that can be extracted from outer shells of crusastaceans (Feng and Huang, 1996).

This biopolymer presents interesting properties such as excellent film-forming capacity and gas and aroma barrier properties at dry conditions, which makes it a suitable material for designing food coatings and packaging structures (Park *et al.*, 2002).

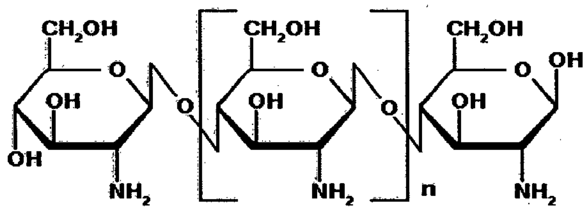

Figure 2.1: Chemical Formula of Chitosan in Haworth's Projection.

2.2.2 History

The origin of chitosan was first discovered by Braconnot in 1811. Braconnot was conducting research on mushrooms, he isolated what was later to be called chitin. The name chitin is derived from Greek, meaning tunic or envelope.. Twenty years later, there was a man who wrote an article on insects in which he noted that similar substance was present in the structure of insects as well as the structure of plants. He then called this astounding substance as chitin (Coma *et al.*, 2003). The name chitin is derived from Greek, meaning tunic or envelope.

The discovery of chitin leads to emerging of chitosan. It was first discovered by Rouget while experimenting with chitin. Rouget observed that the compound of chitin could be manipulated through chemical and temperature treatments for it to become soluble. In 1878, Ledderhose identified chitin to be made of glucosamine and acetic acid. It was not actually until 1894 that Hoppe-Seyler named the tailored chitin, chitosan.

2.2.3. Advantages of Chitosan

Chitosan is relatively low cost. They were widespread availability from a stable renewable source, that is, shellfish waste of the sea food industry, along with chitosan's ability to form a good film, are primary reasons to seek new applications of this polymer (Bangyekan *et al.,* 2005).

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 (Muzzarelli and Vincenzi, 1997).

Chitosan had several of special function including enhancing immune, persisting moist, broad spectrum antimicrobial, promoting regeneration of the epithelium and rehabilitation of the tissue and had abroad applicable value in increasing repair of oral tissue, treatments of periodontal or periapical disease and oral ulcer (Hong *et al.*, 2007).

Chitosan prepared by deacetylation of chitin which mainly obtained from crab and shrimp shell and is an aminopolysaccharide that is useful in chemical modification as a result of its reactive amino and hydroxyl groups. Chitin and chitosan can be considered to be an extremely low cost, nonhazardous, and environmentally biopolymer (Hong *et al.*, 2007).

2.3 Rich Starch

2.3.1 Overview

Rice is the most widely consumed basic food in the world. Each year over 500 million tons of rice is harvested, providing sustenance to many countries and people throughout the world. The unique properties of rice starches are found in its many varieties (Thawien and Chinnan, 2007). The use of rice starch as biopolymer be an interesting solution because this polymer is relatively inexpensive, abundant, biodegradable, and edible (Mali and Grossmann, 2003).

Rice starch and its major components, amylose and amylopectin, are biopolymers, which are attractive raw materials for use as barriers in packaging materials. They have been used to produce biodegradable films to partially or entirely replace plastic polymers because of its low cost and renewability, as well as possessing good mechanical properties (Xu *et al.*, 2005). However, wide application of starch film is limited by its mechanical properties and efficient barrier against low polarity compounds (Azeredo and Faria, 2000). This constraint has led to the development of the improved properties of rice-based films by modifying its starch properties and/or incorporating other materials.

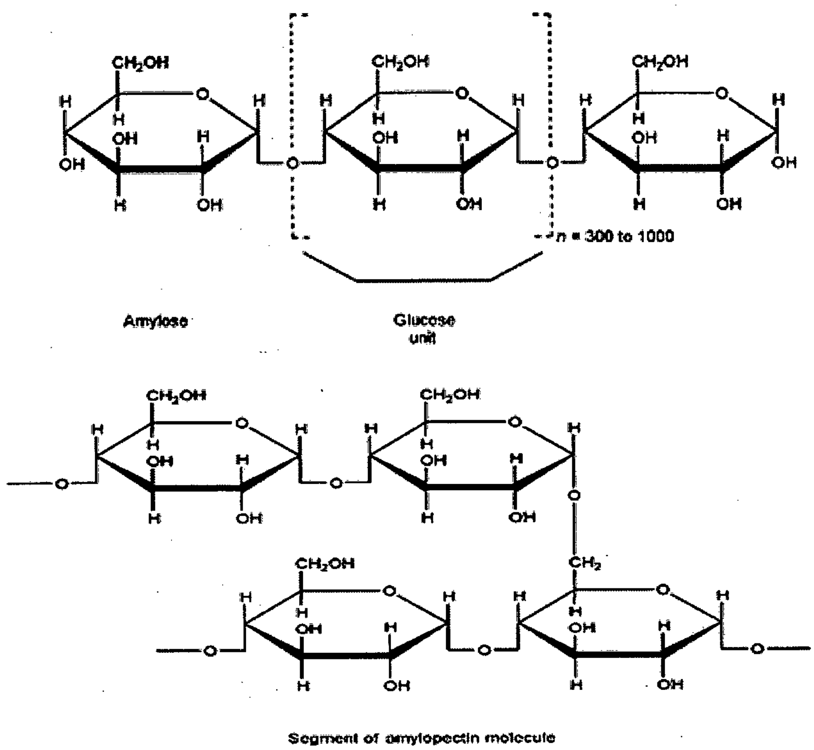

Figure 2.2: Amylose and amylopectin in Haworth's projection

2.3.2 Rice Starch Composition

2.3.2.1 Amylose

Starch consists mainly of amylose and amylopectin. Amylose is an essentially linear polymer of a-(1- 4) - linked D glucopyranosyl units with few (0.1% according to Ball *et al.*, 1996) a-(1-6) linkages. It has a number average degree of polymerisation (DPn) of 800 to 4920, average chain lengths (CL) of 250 to 670 and b-amylolysis limits of 73 to 95% (Morrison and Karkalas, 1990). Rice starch amyloses have DPn values of 920 to 1110 (with little variety difference between Indica and Japonica amylose), CL of 250 to 370 and b-amylolysis limits of 73 to 84%. They are slightly branched with 2–5 chains on average (Takeda *et al.*, 1996).

Wheat (DPn 1300) and maize (DPn 930) amyloses have a similar DPn, whereas potato (DPn 4920) and tapioca (DPn 2600) amyloses have higher DPn values (Champagne, 1996; Takeda *et al.*, 1986). Takeda, Tomooka, and Hizukuri

(1993) found ratios of branched to linear rice amylose molecules of 0.22:0.78 by mole and 0.32:0.68 by weight with DPn values of 1180 and 740, respectively. The branched amylose molecule has been suggested to have a structure intermediate between that of linear amylose and amylopectin and is consequently often referred to as intermediate material (Takeda *et al.*, 1993).

Typical levels of amyloses in starches are 15 to 25% (Manners, 1999). However, waxy (wx) rice (Sano, 1996) and, e.g. wx maize (Shure *et al.*, 1997) and wx wheat (Nakamura *et al.*, 1995) starches are virtually amylose free. On the contrary, mutants with high levels of amylose are also known. Amylose extender (ae) mutants of maize (Boyer and Preiss, 1998) have amylose contents in a range of 50 to 85%. In rice, amylose contents are classified as waxy (0 to 2% amylose), very low (5 to 12%), low (12-20%), intermediate (20 to 25%) or high (25 to 33%) (Juliano *et al.*, 1998).

The ae mutants of rice have amylose contents in a range of 35 to 40% (Juliano, 1998). In determining amylase contents, the existence of both lipid complexed amylase (LAM) and free amylose (FAM) (major fraction) must be taken into account (Morrison *et al.*, 2000). LAM may be present in the native, but is possibly also formed during hydrothermal treatment or gelatinisation of the starch (Maurice, 2001). Furthermore, amylose forms complexes with such as iodine and alcohols.

2.3.2.2 Amylopectin

Amylopectin consists of a-(1 - 4) linked D-glucosyl chains and is highly branched with 5 to 6% a (1 - 6)-bonds (Bule´on *et al.*, 1998). It has a DPn of 4700 to 12,800, CL values of 17 to 24 and b-amylolysis limits of 55 to 60% (Morrison and Karkalas, 1990). The individual chains may vary between 10 and 100 glucose units (Manners, 1999). Rice starch amylopectins have a DPn of 8200 to 12,800, CL of 19 to 23, b-amylolysis limits between 49 to 59% (Takeda *et al.*, 1997), average external chain lengths (ECL) of 11.3 to 15.8 and average internal chain lengths (ICL) of 3.2 to 5.7 (Lu *et al.*, 1997).

Compared to amylopectins of Japonica rice starch, waxy and non-waxy amylopectins of Indica rice starch have lower DPn values (Indica: DPn 4700 to 5800, Japonica: DPn 8200 to 12,800), but higher CL (Indica: CL 21 to 22, Japonica: CL 19 to 20) (Takeda *et al.*, 1997), higher ECL (Indica: ECL 13.2 to 15.8, Japonica: ECL 11.8–12.6) and higher ICL values 246 G.E. Vandeputte, J.A. Delcour / Carbohydrate Polymers 58 (2004) 245–266 (Indica: ICL 4.8 to 5.7, Japonica: ICL 3.2 to 4.6) (Lu *et al.*, 1997).

Waxy Japonica rice starches have the lowest CL (17 to 19) (Morrison and Karkalas, 1998). For wheat and maize, for example DPn values of 5000 to 9400 and 10,200, CL values of 19 to 20 and 22 and b-amylolysis limits of 56 to 59 and 60% have been reported (Shibanuma *et al.*, 1998). Recently, Takeda, Shibahara and Hanashiro (2003) reported the DPn of amylopectins from starches of different botanical orgins to be in the range of 9600 to 15,900.

Moreover, they revealed the presence of large (DPn 13,400 to 26,500), medium (DPn 4400 to 8400) and small (DPn 700 to 1200) species. Amylopectin is generally defined in terms of a cluster model (Nikuni *et al.*, 1998) with polymodal chain length distribution and a non-random nature of branching (Thompson, 2000).

2.3.2.3 Minor Components

The main minor components in starch, which are either at the surface or inside the starch granules, are lipids and proteins. Cereal starches contain up to, 1% lipids and, 0.25% proteins (Baldwin, 2001). Non-waxy rice starches (12.2 to 28.6% amylose) contain 0.9 to 1.3% lipids comprising 29 to 45% fatty acids and 48% lysophospholipids (Azudin and Morrison, 1996). Waxy rice starches (1.0 to 2.3% amylose) contain negligible amounts of lipids (Azudin and Morrison, 1996). Starch proteins are mostly either storage proteins or biosynthetic or degradative enzymes