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JUDUL : FABRICATION AND ANTIMICROBIAL ANANLYSIS OF COMPOSITE BIODEGRADABLE FILM FROM YAM STARCH.

SESI PENGAJIAN : 2010/2011

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FABRICATION AND ANTIMICROBIAL ANALYSIS OF COMPOSITE BIODEGRADABLE FILM FROM YAM STARCH

SITI NUR FARAHANA BINTI ISMAIL

**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
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NOVEMBER 2010

‘I declare that this thesis entitled “Fabrication and Analysis of Antimicrobial Composite Biodegradable Film from Yam Starch” 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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DEDICATION

To my beloved mother and father
My family members,
My friends, my fellow colleague
and my supervisor.

For all your care, support and love me

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Bismillahirrahmanirrahim

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ABSTRACT

The purpose of this study is to produce biocomposite packaging film which is environmental friendly in nature and have potential to be used in the food packaging industry application. The objectives of this study are to fabricate different types of composite biodegradable film from yam starch and analyse antimicrobial activities of the films in resistance to *Escherichia coli* and *Bacillus subtilis*. Other than that, the films were characterized in term of physical and chemical properties. In this study, the biodegradable film will be fabricated with addition of antimicrobial agent to lead complete degradation process. Chitosan is used ad antimicrobial agent. The film preparation is by mix the yam flour with 1% acetic acid, chitosan, PEG 400 and water. Then, from the solution produced, the film is casted on the glass plate and left at ambient temperature until it has become dry. The film was then peeled from the glass plate after the drying is complete. The film is characterized by using fourier transform infrared (FT-IR) spectra, scanning electron microscope (SEM), antimicrobial activity, water solubility and viscosity. From the result, Fourier Transform Infrared (FT-IR) spectra analysis revealed starch crystallinity and hydrogen bonds were formed between chitosan and starch at wavelength 400-4000cm^{-1}. For water solubility, the film containing 3% chitosan only have 34.9578 % water solubility compared to film that containing 1% chitosan have 59.2309 % water solubility. Films containing 3% chitosan is also showed it has the highest viscosity value, which is 1413 cP at 20 rpm. From antimicrobial test, the result showed that, the more chitosan is used, the lower the colony to breed.

ABSTRAK

Tujuan kajian ini adalah untuk menghasilkan filem pembungkusan biokomposit yang mesra alam dan mempunyai potensi untuk digunakan dalam aplikasi industri bungkusan makanan. Tujuan kajian ini adalah untuk menhasilkan komposit filem yang boleh terurai dari pati ubi serta menganalisa filem dalam merencat aktiviti mikrob Escherichia coli dan Bacillus subtilis. Selain itu, objektif kajian adalah untuk mengkaji filem dari segi fizikal dan kimia. Dalam kajian ini, filem boleh urai akan dibuat dengan penambahan agen antimikrob dalam meneruskan proses penguraian. Kitosan digunakan sebagai agen antimikrob. Penghasilan filem adalah dengan mencampurkan tepung ubi dengan asid asetik 1%, kitosan, PEG 400 dan air. Kemudian, dari larutan yang dihasilkan, filem ini dituang pada plat kaca dan dibiarkan pada suhu bilik sehingga kering. Filem ini kemudian dikupas dari plat kaca selepas proses pengeringan berlaku. Filem ini kemudian dikaji dengan menggunakan 'Fourier Transform Infrared (FT-IR) spectra', scanning electron microscope (SEM), ujian aktiviti antimikrob, kelarutan air dan kelikatan. Hasilnya, analisis spekta Fourier Transform Infrared (FT-IR) menunjukkan kanji terhablur dan ikatan hidrogen antara kitosan dan kanji terbentuk pada gelombang 400-4000 cm^{-1}. Untuk kelarutan dalam air, filem yang mengandungi kitosan 3% mempunyai kelarutan dalam air hanya 34.9578 % jika dibandingkan dengan filem yang mengandungi kitosan 1% yang mempunyai kelarutan dalam air 59.2309 % Filem yang mengandungi kitosan 3% juga menunjukkan nilai kelikatan tertinggi, iaitu 1413 cP pada 20 rpm. Dari ujian antimikrob, hasil menunjukkan lebih banyak kitosan digunakan, semakin kurang koloni yang dapat dibiakkan.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

FTIR	Fourier Transform Infrared
Mw	Weight Average Molecular Weight
PEG	Poly (ethylene glycol)
%v/v	volume percentage for chemical per basis
WS	Water Solubility
CS	Chitosan
E.coli	*Escherichia coli*
B.subtilis	*Bacillus subtilis*
TS	Tensile Strength
E	Elongation at break
SEM	Scanning Electron Microscopy

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Research Background

Increased use of synthetic packaging films has led to serious ecological problems due to their total non-biodegradability. Continuous awareness by one and all towards environmental pollution by the latter and as a result the need for a safe, eco-friendly atmosphere has led to a paradigm shift on the use of biodegradable materials, especially from renewable agriculture feedstock and food processing industry (Tharanathan., 2003). Most widely used polymeric materials for packaging purposes, developed in the past 50–60 years, are durable and inert in the presence of microorganisms, leading to a long-term performance. However, in view of the current emphasis on environmental pollution problems and the shortage of land for solid waste management, the need for environmentally degradable polymers has increased (Mali *et al.*, 2002). This development has received widespread government support in developing country. Several studies have been performed to analyze the properties of starch based biodegradable films.

Yams, the tubers of Dioscorea sp., are important staple food in many tropical countries. In Malaysia and Singapore, yam is also known 'taro'. Interestingly, yam is also been used as health food and herbal medicinal ingredients in traditional medicine (Hsu *et al.*, 2003). In Japanese island, yams and yams products are regarded as a folk medicine for the treatment of importance, possibly because of the vegetable's high vitamin E content. Yam products generally have a lower glycemic index than potato products, which means that they will provide a more sustained form of energy, and give better protection against obesity and diabetes. Yam (Dioscorea sp) could be a good source of starch for the production of edible films and coatings, since its starch contains about 30% of amylose, and amylose is responsible for the film forming capacity of starches (Durango et al., 2006). 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 (Shen *et al*., 2010).

Antimicrobial films and coatings have innovate the concept of active packaging and have been developed to reduce, inhibit or delay the growth of microorganisms on the surface of foods in contact with the packaged product (Durango et al., 2006). Although starch films have been widely study, but research on antimicrobial starch films is relatively rare and reported on yam. The addition of antimicrobial films or coatings can be more efficient than adding antimicrobial agents directly to the food. Antimicrobial films and coatings have innovate the concept of active packaging and have been developed to reduce, inhibit or delay the growth of microorganisms on the surface of foods in contact with the packaged product (Durango et al., 2006). 89% of outbreaks caused by food contamination by food workers, pathogens were transferred to food by workers' hands (Shen *et al*., 2010). Microbial contamination can controlled by antimicrobial substances incorporated into packaging materials by reducing the growth rate and maximum growth population and extending the lag-phase of the target microorganism or by inactivating microorganism by contact. Chitosan owning a broad spectrum of antimicrobial activity exhibits differing inhibitory efficiency against different fungi, Gram-positive and Gram-negative bacteria. Chitosan exerts an antifungi effect by suppressing sporulation and spore germination. In this study, the microorganism is used were *Escherichia coli* (E.*coli*) and *Basillus subtilis* (B.*subtilis*). This is because of to differentiate microbial activity based on Gram positive bacteria (B.*subtilis*) and Gram negative bacteria (E.*coli*). Furthermore, E.coli is the most common bacteria from human body, whereas B.*subtilis* is the most common bacteria that was found in the food.

Although there are many studies on petrochemical-based film that have been done, the demand for biodegradable polymer-based edible film and environmentally friendly has spurred interest, and chitosan is ideally positioned in this regard. Chitosan [poly-(b-1/4)-2-amino-2-deoxy-D-glucopyranose] is a collective name for a group of partially and fully deacetylated chitin compounds due to its unique biological characteristics, including biodegradability and nontoxicity, many applications have been found either alone or blended with other natural polymers (starch, gelatin, alginates) in the food, pharmaceutical, textile, agriculture, water treatment and cosmetics industries (Kong *et al*., 2010).

The physical and biodegradable properties of a starch based biodegradable film are characterized by Fourier Transform Infrared (FT-IR) spectra, Tensile strength and elongation, Scanning Electron Microscopy (SEM). Then, to analyze the antimicrobial activity, the film will undergo antimicrobial test for antimicrobial analysis. The objectives of this study are to fabricate difference types of composite biodegradable films from yam starch, to evaluate antimicrobial activities of chitosan-incorporated yam starch films in resistance of *E.coli* and B.*subtilis* and to characterize the difference types of composite biodegradable film from yam starch.

1.2 Identification of Problem

Plastics are durable and their degradation process is very slow, possibly up to hundreds of year, the disposal of plastics contributes significantly to their environmental impact. With more and more plastics products, particularly plastics packaging, being disposed of soon after their purchase, the landfill spaced required by plastics waste in growing concern. The difficulties in collecting, identifying, transporting, cleaning and re-processing of plastics packaging materials often render the attempt of recycling non economical making disposal to landfill to more convenient alternative. The statistics showed over 67 millions tones of packaging waste are generated annually in certain developing country. In developed countries, food packaging represents 60% of all packaging (Davis and Song., 2006). Several studies have been performed to analyze the properties of starch based biodegradable films, but research on antimicrobial starch films is relatively rare.

1.3 Objectives

The main of this research are:

a) To fabricate difference types of composite biodegradable films from yam starch.
b) To evaluate antimicrobial activities of chitosan-incorporated yam starch films in resistance of *E.coli* and B.*subtilis*
c) To characterize the difference types of composite biodegradable film from yam starch.

1.4 Scope of Study

In general, the scopes of research are as following:

a) To study about the yam flour making process
b) To fabricate the composite biodegradable film from yam starch using fabrication process.
c) To study the physical properties of the film based on FT-IR, SEM and Tensile Strength (TS) & Elongation at break (E).
d) To study the antimicrobial activity of the films.

1.5 Significance of Study

The significance of this research are:

a) New alternative to replace nondegradable plastic.
b) Application in wide filed such as industry and medical
c) To save the environment
d) To reduce the landfill space.

CHAPTER 2

LITERATURE REVIEW

2.1 Yam

Yam (Dioscorea sp.) is widely cultivated in the tropical and subtropical regions of the world and is known for its high carbohydrate and medicinal value (Riley *et al*., 2008) In Taiwan, the fresh tuber slices are widely used as functional foods, and the dried slices are used as traditional Chinese herbal medicines. Yams (the tubers of the Dioscorea spp.), consumed and regarded as medicinal food in traditional Chinese herbal medicine, are seasonal foods and easily deteriorate during storage. It is of great importance to prolong the storage of yams for supplying in the off-season and without losing nutritional functionality (Hsu *et al*., 2003). The extracts of yam provide significant antioxidative activity and modified serum lipid levels in humans

Figure 2.1: Yam (Dioscorea sp.)

Yam (Dioscorea sp) could be a good source of starch for the production of edible films and coatings, since its starch contains about 30% of amylose, and amylose is responsible for the film forming capacity of starches (Durango *et al*., 2006).

On dry basis, the chemical composition of yam starch was: ash (0.17 ± 0.01%), protein (0.20 ± 0.01%), lipids (0.27 ± 0.02%) and starch (98.30 ± 0.05%). The amylase and amylopectin contents of native yam starch were 30 and 70%, respectively. This amylose content is relevant for the film forming capacity of the starch (Mali *et al*., 2002)

2.2 Starch

Starch is one of the major polysaccharides in plants and is in the form of granules that exist naturally within the plant cells (Mali *et al*., 2002) and 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 (Shen *et al*., 2010). Starch is a one of polysaccharide that have physical combination of branched and linear polymers (amylopectin and amylose, respectively), but it contains only a single type of carbohydrate, glucose.

Starch films possess low permeability and are thus attractive materials for food packaging. Starch is also useful for making agricultural mulch films because it degrades into harmless products when placed in contact with soil microorganisms (Chandra and Rustgi., 1998).

Starch based films and coatings exhibit different properties, attributed to the amylose content in the starch (Durango *et al*., 2006) and could contribute to new solutions in reducing the amount of plastic wastes and these polymers are obtained from renewable sources unlike synthetic polymers (Mali *et al*., 2004).

Starch is the main carbohydrate reserve in yam tubers, accounting for up to 85% of the dry weight matter. Yam starch occurs as granules, consisting of amylose (10 – 30%) and amylopectin (70 – 90%) molecules. (Riley *et al*., 2008) In Brazil, yam (Dioscorea alata) is being studied as an alternative source because of several desirable properties of its starch, such as, stability to high temperature and low pH.

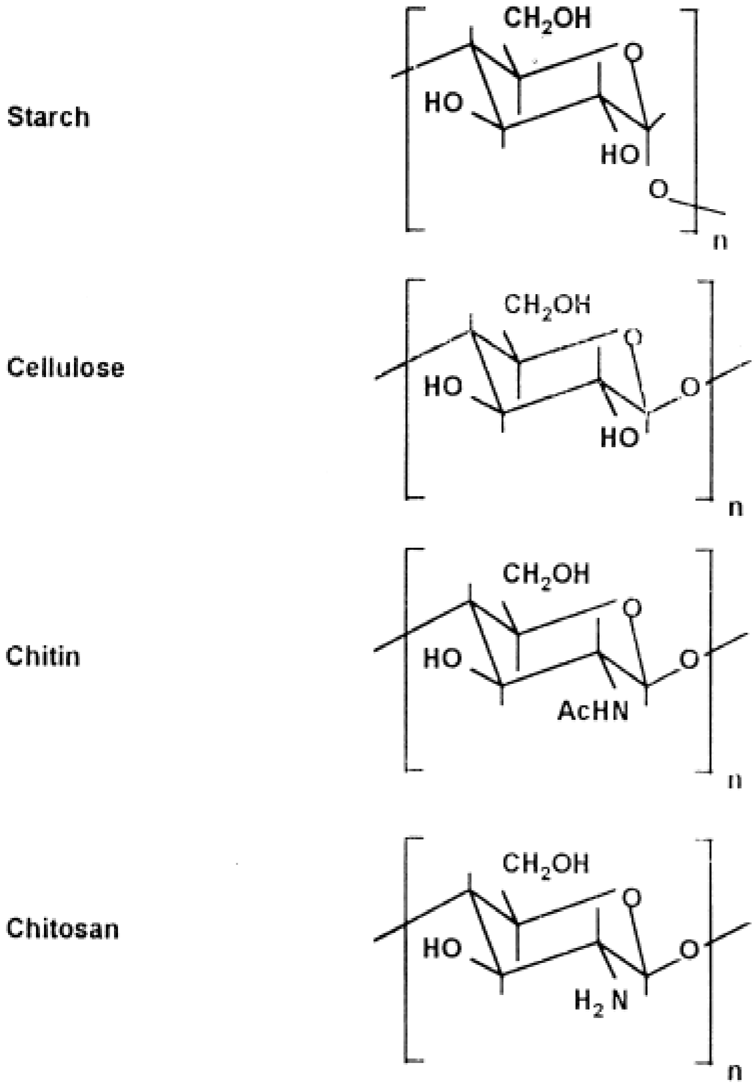


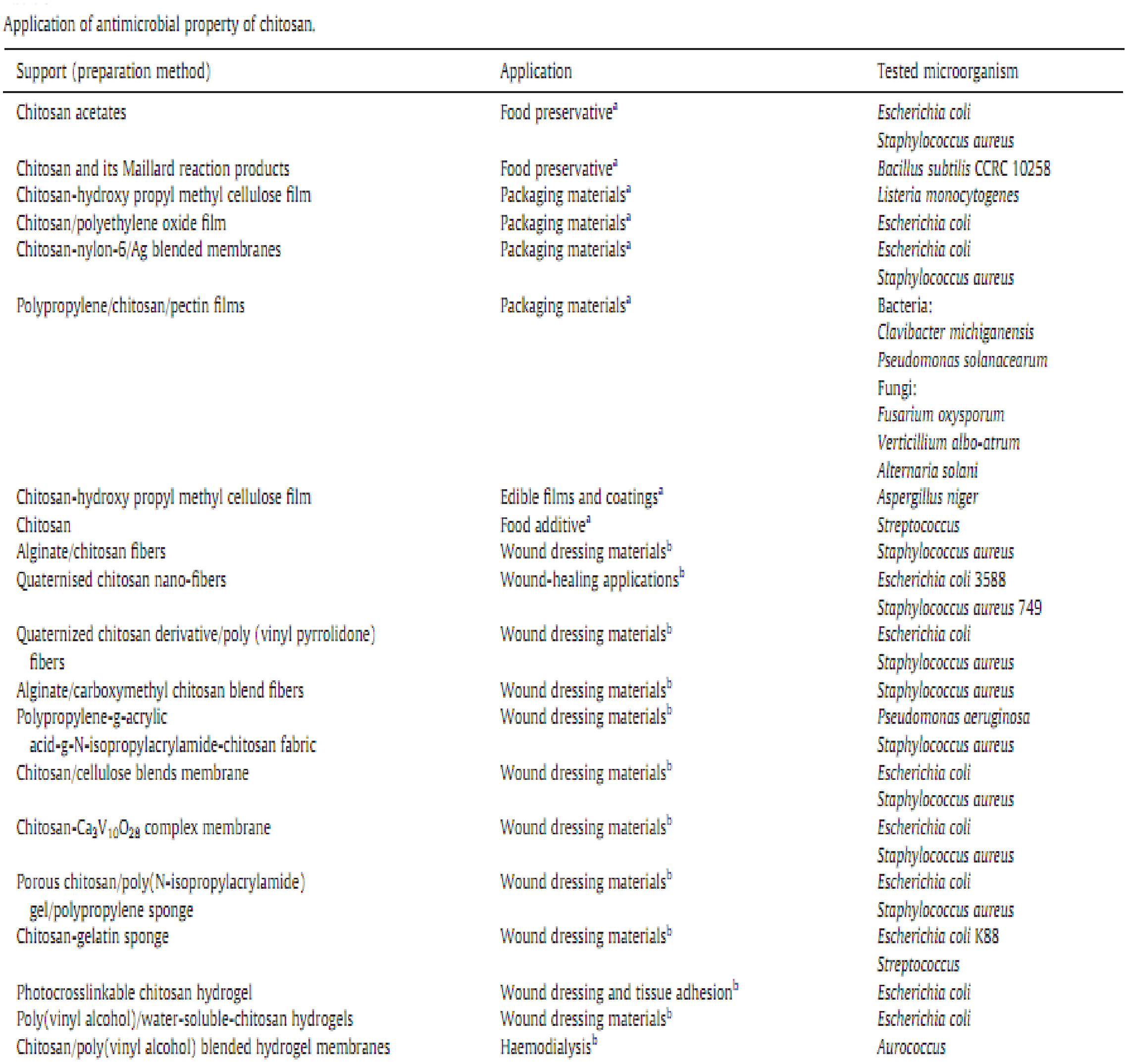
Figure 2.2: Structure of polysaccharides

Yam starch is a promising polymer for biofilm production due to their homogeneous matrix and stable structure at ambient conditions and relatively low water barrier properties (Mali *et al.*, 2004) and is widely study because it exhibits several desirable properties, such as stability at high temperatures and a low pH, high gelatinization temperature ranges (71.9 – 73.0–76.9 ^oC), high amylose contents (27 g/100 g), and restricted swelling (Fu *et al.*, 2005).

2.3 Chitosan

Chitin is consists of 2-acetamido-2-deoxy-b-D-glucose. Chitosan is the N-deacetylated derivate of chitin. Chitin and chitosan have a range of current and potential applications in photography, cosmetics, artificial skin, dressing, food and nutrition, ophthalmology, water engineering, metal capture from wastewater, paper finishing, solid-state batteries, drug delivery system, biotechnology, cell-stimulating materials (Shih *et al.*,2009) pharmaceutical, textile, agriculture, water treatment and cosmetics industries (Kong *et al.*, 2010). The table below is showed the application of chitosan.

Table 2.3: Table of chitosan application



Application of antimicrobial property of chitosan.

Support (preparation method)	Application	Tested microorganism
Chitosan acetates	Food preservative[a]	*Escherichia coli* *Staphylococcus aureus*
Chitosan and its Maillard reaction products	Food preservative[a]	*Bacillus subtilis* CCRC 10258
Chitosan-hydroxy propyl methyl cellulose film	Packaging materials[a]	*Listeria monocytogenes*
Chitosan/polyethylene oxide film	Packaging materials[a]	*Escherichia coli*
Chitosan-nylon-6/Ag blended membranes	Packaging materials[a]	*Escherichia coli* *Staphylococcus aureus*
Polypropylene/chitosan/pectin films	Packaging materials[a]	Bacteria: *Clavibacter michiganensis* *Pseudomonas solanacearum* Fungi: *Fusarium oxysporum* *Verticillium albo-atrum* *Alternaria solani*
Chitosan-hydroxy propyl methyl cellulose film	Edible films and coatings[a]	*Aspergillus niger*
Chitosan	Food additive[a]	*Streptococcus*
Alginate/chitosan fibers	Wound dressing materials[b]	*Staphylococcus aureus*
Quaternised chitosan nano-fibers	Wound-healing applications[b]	*Escherichia coli* 3588 *Staphylococcus aureus* 749
Quaternized chitosan derivative/poly (vinyl pyrrolidone) fibers	Wound dressing materials[b]	*Escherichia coli* *Staphylococcus aureus*
Alginate/carboxymethyl chitosan blend fibers	Wound dressing materials[b]	*Staphylococcus aureus*
Polypropylene-g-acrylic acid-g-N-isopropylacrylamide-chitosan fabric	Wound dressing materials[b]	*Pseudomonas aeruginosa* *Staphylococcus aureus*
Chitosan/cellulose blends membrane	Wound dressing materials[b]	*Escherichia coli* *Staphylococcus aureus*
Chitosan-$Ca_3V_{10}O_{28}$ complex membrane	Wound dressing materials[b]	*Escherichia coli* *Staphylococcus aureus*
Porous chitosan/poly(N-isopropylacrylamide) gel/polypropylene sponge	Wound dressing materials[b]	*Escherichia coli* *Staphylococcus aureus*
Chitosan-gelatin sponge	Wound dressing materials[b]	*Escherichia coli* K88 *Streptococcus*
Photocrosslinkable chitosan hydrogel	Wound dressing and tissue adhesion[b]	*Escherichia coli*
Poly(vinyl alcohol)/water-soluble-chitosan hydrogels	Wound dressing materials[b]	*Escherichia coli*
Chitosan/poly(vinyl alcohol) blended hydrogel membranes	Haemodialysis[b]	*Aurococcus*

The ideal antimicrobial polymer should possess the following characteristics: (1) easily and inexpensively synthesized, (2) stable in long-term usage and storage at the temperature of its intended application, (3) not soluble in water for a water-disinfection application, (4) does not decompose to and/or emit toxic products, (5) should not be toxic or irritating to those who are handling it, (6) can be regenerated upon loss of activity, and (7) biocidal to a broad spectrum of pathogenic microorganisms in brief times of contact (Kong *et al.*, 2010).

Chitosan films are biodegradable, biocompatible, flexible, durable, strong, tough and hard to break, have moderate values of water and oxygen permeability, decrease the respiratory rate of food and also inhibit the microbial growth (Agullo *et al.*, 2003). Chitosan obtained from chitin (of shrimp waste silage) has low molecular weight compared to the commercial chitosan. The low molecular weight of chitosan obtained can be attributed to greater susceptibility to degradation of chitin and/or depolymerization during the removal of proteins and minerals in the silage, and in the purification procedures and subsequent deacetylation (Camacho *et al.*, 2010).

Due to its property of inhibiting the growth of many pathogenic bacteria and fungi, chitosan has widely been used in antimicrobial films and coatings. In some fungi, by interaction with the strongly electronegative microbial surface leading to changes in permeability, metabolic disturbances, and eventually death, chitosan can produce alterations of membrane functions. Chitosan antimicrobial activity against bacteria could be due to the polycationic nature of its molecule, which allows interaction and formation of polyelectrolyte complexes with acid polymers produced at the bacteria cell surface (lipopolysaccharides, teichoic and teichuronic acids or capsular polysaccharides (Durango *et al.*, 2006).

Antimicrobial activity of chitosan has been demonstrated against many bacteria, filamentous fungi and yeasts. Chitosan has wide spectrum of activity and high killing rate against Gram-positive and Gram-negative bacteria, however, it is low toxicity toward mammalian cells. In many food applications, low water solubility forces chitosan to be dissolved in dilute acid solution, such as acetic or lactic acid solution. This acidic ambience can adversely affect chitosan molecules via hydrolysis and chain depolymerization. The development of modified water-soluble derivative is an efficient approach in practice and the chitosan structure is chemically more stable compared with the acidic solution. It was observed that, chitosan acetate inhibited the growth of two main waterborne food pathogens, E. *coli* and S. *aureus* (Kong *et al.*, 2010).

Most of the mechanical properties of chitosan films are comparable to those of commercial polymers of medium strength such as cellulose. The mechanic and permeable properties of chitosan films can be controlled by selecting molecular weight, a suitable solvent system and the addition of plasticizer agents, dispersants and compatibilizers, among however, the presence of such compounds can affect the antimicrobial activity of chitosan films (Camacho *et al.*, 2010).

2.4 Petroleum-Based Film

The majority of plastic products are made from petroleum-based synthetic polymers that do not degrade in a landfill or in compost like environment. High molecular weight synthetic polymers containing largely C-C bond are generally resistant to biodegradation because microbial enzymes are not accessible to them due to their hydrophobic nature (Muthukumar *et al*., 2010).

2.5 Biodegradable Film

Biodegradable is a polymer blends and composites containing natural polymers as biodegradable additives (such as starch, cellulose and their derivatives) (Alain and Sylvie., 2008)

Biopolymer films and coatings from polysaccharides, proteins and lipids, formulated either with one or more components have the potential to control mass transfer and thus extend food shelf life and this study is also shows starch films are excellent oxygen barriers, due to their tightly packed, ordered hydrogen-bonded network structure and low solubility (Mali *et al*., 2004).

Film based food preservative materials are safer and more widely applied presently, especially as food package. Various kinds of chitosan-based packaging films modified with new polymeric material have been developed. The process endows these cooperating films with antimicrobial property as well as advantageous mechanical characteristics as showed in table 2.3. Beside of the biodegradability, environmental attribute is an important functional attribute. Thus, the concept of biodegradability enjoys both user-friendly and eco-friendly attributes, and the raw materials are essentially derived

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 (Bourtoom and Chinnan., 2008).

An additional advantage of biodegradable packaging materials is that on biodegradation or disintegration and composting they may act as fertilizer and soil conditioner, facilitating better yield of the crops (Tharanathan., 2003)

2.5 Plasticizer

The addition of plasticizers overcomes starch film brittleness and improves flexibility and extensibility. Plasticizers must be compatible with the film forming polymer. They reduce intermolecular forces and increase the mobility of polymer chains. Hydrophilic compounds such as polyols (glycerol, sorbitol and polyethylene glycol) are commonly used as plasticizers in hydrophilic film formulations (Mali *et al.*, 2004). The mechanical properties of chitosan film were improved by blending with PEG oligomer as plasticizer (Suyatma et al., 2005). Hydrophilic compounds such as polyols (glycerol, sorbitol and polyethylene glycol) are commonly used as plasticizers in hydrophilic film formulations and avoid cracking of the film during handling and storage and affect gas, water vapor and solute permeabilities (Mali *et al.*, 2004).

Poly(ethyleneglycol) (PEG) is fascinating synthetic polymer. It shows biodegradability, biocompatibility, less toxicity and hydrophilicity and has been used in many kinds of applications (Roberts *et al.*, 2002). The more the content of PEG increased in the film forming solution, the more the water solubility decreased (Sebastien *et al.*, 2006). PEG concentration also influent to the flexibleity of the film. The higher concentration PEG was used, the more flexible they were.

When incorporated into human body, PEG with molecular weight lower than 20 kDa but higher than 400 Da is cleared immediately without structural change in the urea.Whereas lowmolecular-weight oligomers of PEG of about 400 Da or less is degraded in vivo by alcohol dehydrogenase to toxic metabolites, but PEG with molecular weight above 1000 Da is safety, and so, non-toxic (Tanuma et al., 2010).

2.6 Film characterization

2.6.1 Tensile strength and Elongation at break

Encountered during its application and subsequent shipping or handling of the food, biodegradable film must endure the normal stress to maintain its integrity and barrier properties. Tensile strength is the maximum tensile stress sustained by the sample during the tension test. If maximum tensile stress occurs at either the yield point or the breaking point, it is designated tensile strength at yield or at break, respectively. (Bourtoom and Chinnan., 2008),

Elongation at the break (E) is defined as an indication of the films' flexibility and stretch ability (extensibility). Elongation of film is determined at the point when the films breaks under tensile testing and is expressed as the percentage of change of the original length of the specimen between the grips of a films to stretch.

2.6.2 Water Solubility

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) (Bourtoom and Chinnan., 2008).

Normally, higher solubility would indicate lower water resistance, but in some case, high solubility may be an advantage for some applications such as 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.

2.6.3 Fourier Transform Infrared (FT-IR) spectra

FTIR spectroscopy was used to determine the interactions between yam starch and chitosan (Bourtoom and Chinnan., 2008). When two components are mixed, the physical blends versus chemical interactions are affected by changes in the characteristic spectra peaks (Shen *et al*., 2010).

2.6.4 Degradation factor

There are three environment effects on the degradation of starch and pro-oxidant blended polyolefins film, which are marine environment, soil burial and open sunlight. Marine environment is found to be one of the major sites for dumping of waste especially plastics, and there is a need to develop strategies to degrade them faster than what is observed from current studies. Burying plastic in soil is not a good idea since it may persist longer than the other two strategies. Open sunlight appears to be a good way to subject polyolefins to abiotic degradation/deterioration (Muthukumar *et al., 2010*).

2.6.5 Soil Burial

Aerobic soil environments generally contain consortia of several different types of degrading bacteria and fungi which operate cooperatively. This is the process of degradation cellulose to glucose and cellodextrins by primary microorganisms, and secondary microorganism. The final products from aerobic biodegradation are ultimately CO2 and water. Increase in carbonyl index at the end of 150 days in sample exposed to UV and soil clearly reveal that the oxidation has taken place (Muthukumar *et al., 2010*).

Bacteria present in soil are important agents for material degradation. Particularly affected are cellulosic plant life, wood products, and textiles subject to cellulytic degradation Chandra and Rustgi., 1998).

Starch is normally the main site for biodegradative attack in starch-containing biocomposites.(Vilaplana *et al*., 2010)

CHAPTER 3

METHODOLOGY

3.1 Introduction

In this chapter, the mothod that used in this study is described extensively. In general, the method can be divided into two phases. The first phase is to fabricate film from yam starch, and the second phase is to characterize the film.

3.2 Materials

Yam was obtained from a local farmer in Kuantan. All chemicals like acetic acid glacial, polyethylene glycol 400, Chitosan is provided from University Malaysia Pahang (UMP) laboratory. E.*coli* and B.*subtilis* were cultured and maintained in the microbiology laboratory of UMP.

3.3 Equipments and Apparatus

Apparatus that involved in this study are domestic blender, sieve (shaker), mortar and pestle, polyethylene bags, glass plates, biker, stirrer and petri dish. Meanwhile for the equipment, the film is characterized by using fourie transform infrared, EVO 50 EDX scanning electron microscope, tensile INSTRON Instrument and viscosity meter.

3.4 Film Fabrication

3.4.1 Production of Yam Flour and Starch Isolation

Yam tubers were rinsed, peeled, diced and then, disintegrated in a domestic blender with 100 ml distilled water. The mixture was sieved with filter cloth and the solids retained were exhaustively rinsed on the sieve with the distilled water again. The filtrate was allowed to stand at room temperature for overnight and decant was discarded. Starch precipitate will be produced at the bottom of the solution that was filtered overnight. Solution at the top is removed while the starch precipitate at the bottom will be filtered by using filter paper. The filtered starch will leave under room temperature for 24 hours. Then, on the next day, the filtered starch will be dried in a convection oven at 40 C until 12% moisture. Lastly, the starch was ground with a mortar and pestle to pass a 0.105 mm sieve and then stored in powder jar.

Figure 3.4.1: Yam starch

3.4.2 Solution Preparation and Film Fabrication

4g of starch was dispersed in 100ml distilled water was stirred for 1 hour at room temperature, and heated to 100^{o}C. After gelatinization, 5ml polyethylene glycol 400 was added as a plasticizer and the resulting dispersion was subjected to further mixing for 30 minutes. To prepare for the second solution, which is chitosan solution, chitosan was mixed at different concentration (1%, 2% and 3%) in the 100ml 1% acetic acid aqueous solution. after that mixed both of solution together for 1 hour. To prepare the antimicrobial film, the solution that produced will degassed under vacuum for 1 hour. Next, the warm mixture was casted on the framed glass plates and left at ambient temperature until it has become dry. The film was then peeled off the glass plate after the drying is complete.

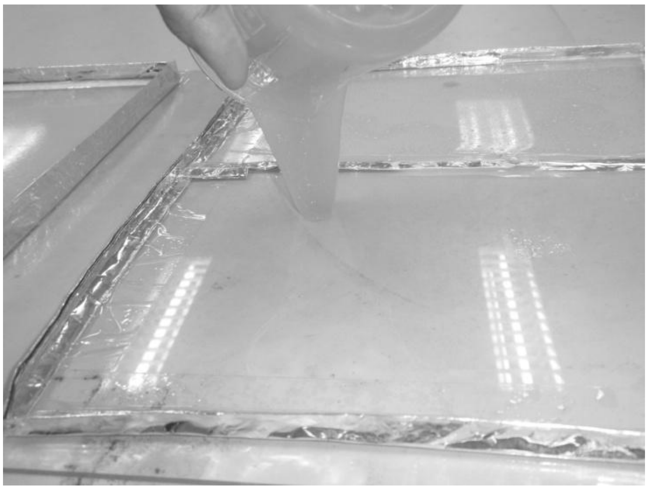


Figure 3.4.2: Process of film fabrication on glass plate

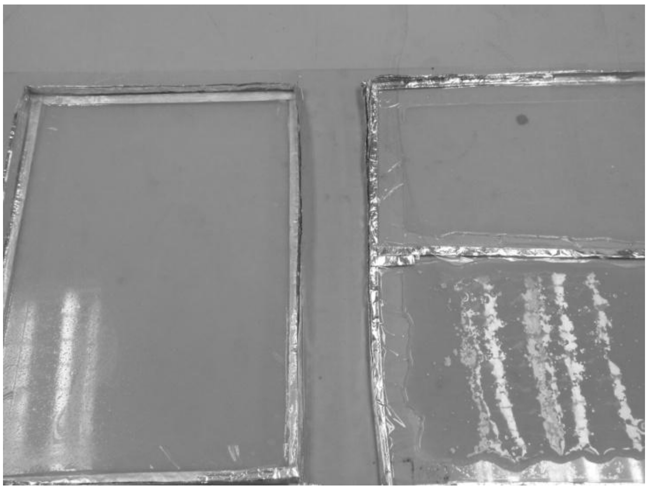


Figure 3.4.3: The film is left to be dried at ambient temperature.

3.5 Film characterization

3.5.1 FT-IR spectra analysis

Method from (Shen *et al.*, 2010) was used for the Fourier transform infrared analysis. To perform FT-IR measurement, 2.5 cm x 2.5 cm film was placed on the Gc plate of FT-IR. The Fourier transform infrared (FT-IR) spectra of the films were recorded in an IR Spectrometer in the wavelength range 400-4000 cm^{-1}. When two or more substances are mixed, physical blends versus chemical interactions are reflected by changes in characteristic spectra peaks.

3.5.2 SEM

The chitosan-based films were mounted on the specimen holder with aluminium tape and then sputtered with gold in BAL-TEC SCD 005 sputter coater (BAL-TEC AG, Balzers, Liechtenstein). All the specimens were examined with EVO 50 EDX spectrometer scanning electron microscope under high vacuum condition and at an accelerating voltage of 20.0 kV. Samples were photographed at tilt angles of 60–90^{o} to the electron beam for the views in the cross section.

3.5.3 Tensile strength and Elongation at break

Tensile strength was measured with INSTRON Instrument. All three samples, 2.54 cm x 12 cm, were cut from each film. Initial grip separation and crosshead speed were set at 50 mm and 50 mm/min, respectively.

3.5.4 Water Solubility

A modified method from (Rhim *et al.*, 2007) was used to measure film solubility. WS of each film was determined as the percentage of film dry matter solubilized after 24 h immersion in distilled water. Three randomly selected 2x2cm samples from each type of film were first dried at 105 ^{o}C for 24 h to determine the weight of the initial dry matter. An additional three pieces of weighed film were placed in a 50ml beaker containing 30ml of distilled water. Beakers were covered with aluminum foil and stored for overnight. Undissolved dry matter was determined by removing the film pieces from the beakers, gently rinsing them with distilled water, and then oven drying the rinsed films (105 ^{o}C, 24 h). Total soluble matter was calculated from the initial gross mass and the final dry mass using the following equation:

%FS = [(film mass before test - film mass after test)/ film mass before test] x 100%

3.5.5 Viscosity

Viscosity of starch-chitosan was tested after the solution is produced. This test is done by using the viscosity meter at a different speed at which the 5, 10, 20, 30 and 40 rpm. The value obtained will be recorded and will be analyzed using graph analysis.

3.5.6 Antimicrobial test

The antimicrobial test was carried out according to the method developed by (Shen *et al*., 2010). The inhibitory zone test on solid medium or semisolid medium was used for determination of the antimicrobial effects of films on E.*coli* and B.*subtilis*. Yam starch films were cut into a disc (diameter ¼ 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 petri dishes were then incubated at 30 ^{o}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 subtracted from the film disc area and the difference in area was reported as the ‘zone of inhibition’.

CHAPTER 4

RESULT AND DISCUSSION

4.1 FT-IR spectra analysis

When two components are mixed, the physical blends versus chemical interactions are affected by changes in the characteristic spectra peaks (Bourtoom and Chinnan., 2008). This chitosan film spectrum was very similar to the result in the study of (Shen *et al.*, 2010). FT-IR spectra of yam starch films incorporated with varying levels of chitosan were depicted in Fig. 4.1. The spectra were used to identify possible interactions between functional groups of starch with chitosan. All spectra showed with the similar patern of spectra wavelength. From Fig. 4.1, it can be concluded that all spectra show similar patterns with the peaks at 3427.92 cm^{-1}, 1654.48 cm^{-1} and around 1012.94 cm^{-1} broadened. Absorption in this area reveals O–H and N–H bond stretching at about 3427.92 cm^{-1}, C–H symmetrical deformation at 1654.48 cm^{-1} and C–O bond stretching centered at 1012.94 cm^{-1}. There is no C=O bond stretching (amide I) in the spectra length. Besides, with addition of chitosan, the peaks at 3300 cm^{-1} - 3400 cm^{-1} become weaker and narrower, which revealed the hydrogen bonding interaction between starch and chitosan.

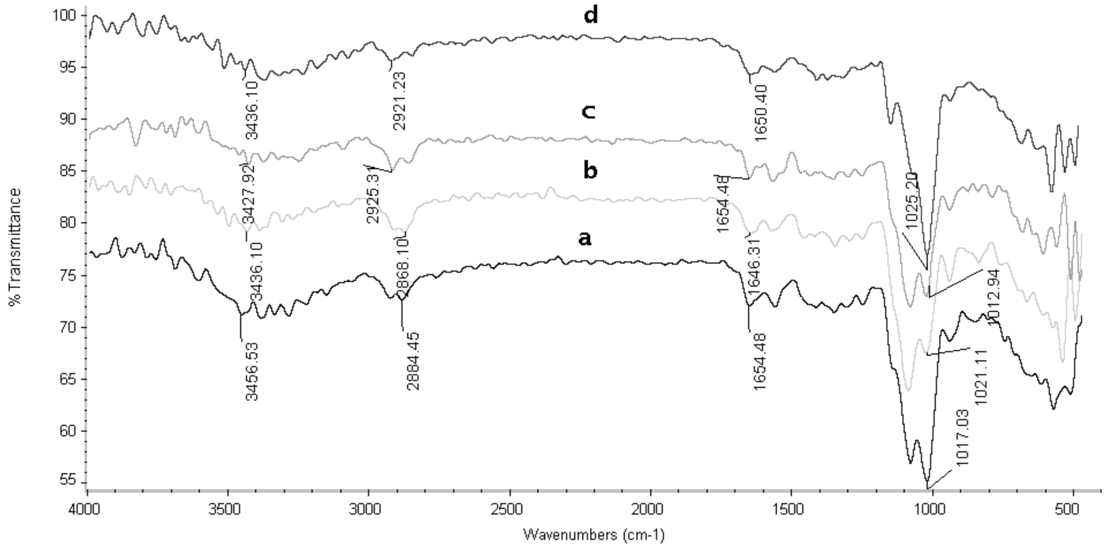


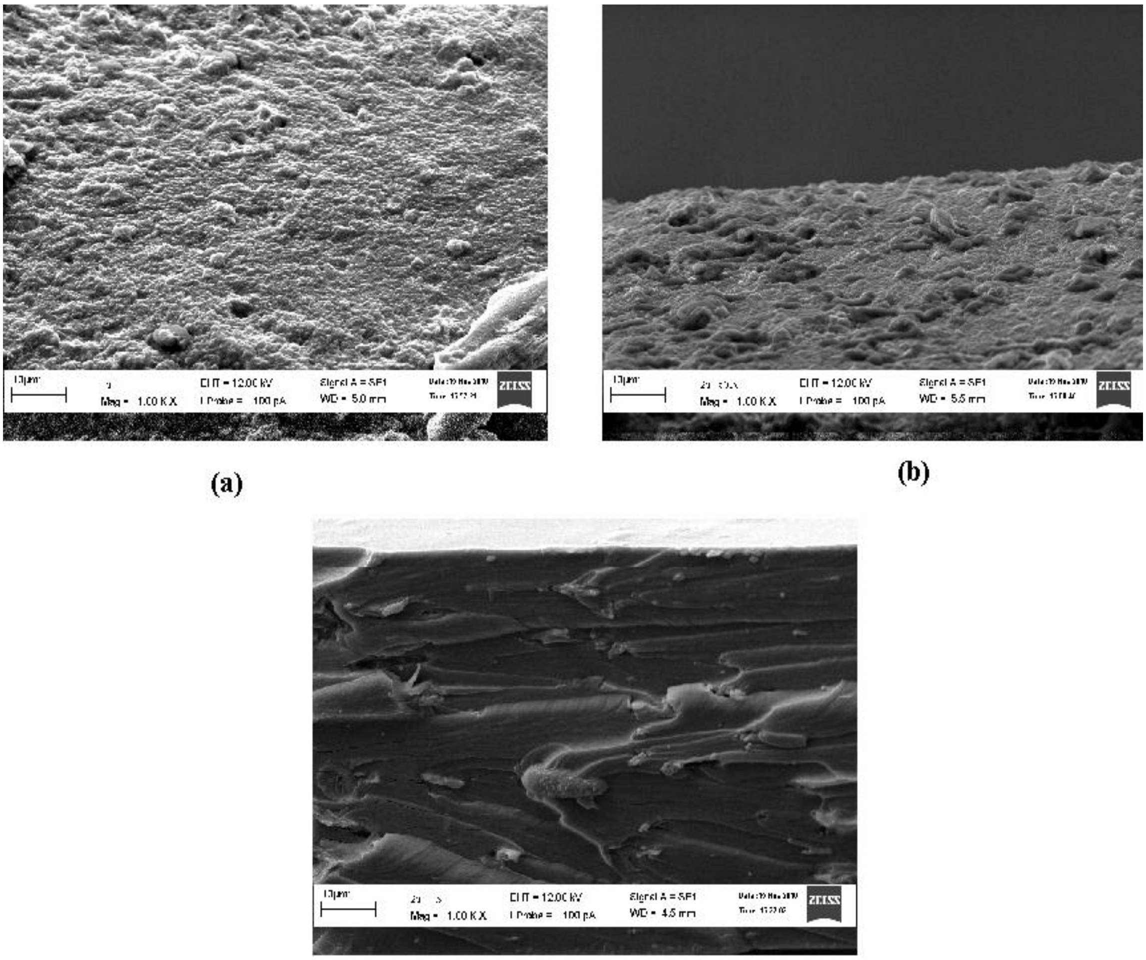
Fig. 4.1: FT-IR spectra of yam starch films incorporated with 1% chitosan (a), 2% of chitosan (b), 3% of chitosan (c), and spectra of chitosan film only (d)

4.2 SEM

Figure 4.2.1 (a, b and c) shows the SEM cross section images that magnify the samples 1000 times, while Fig.4.2.2 (a,b and c) shows the surface of film images. Fig. 4.2.1 (a) is image of cross section for film with 1% chitosan, fig. 4.2.1 (b) is image of cross section for film with 2% chitosan and fig. 4.2.1 (c) is image of cross section for film with 3%. Meanwhile, fig. 4.2.2 (a) is image of surface for film with 1% chitosan, fig. 4.2.2 (b) is image of surface for film with 2% chitosan and fig. 4.2.2 (c) is image of surface for film with 3%.

Morphology structure of fig. 4.2.1 (a) is too rough and cavernous. Fig. 4.2.1 (b) morphology is become smoother and less cavernous, while fig. 4.2.1 (c) showed the film morphology structure is smooth and less cavernous also. If films are produced by the high-pressure method, the water molecules in the solvent will vaporize immediately after the pressure is relieved and a cavernous structure forms on the 1% surface. When chitosan content is increases, their surface tends to become smoother. Molecular weight of chitosan and molecular weight of cellulose give effect to the viscosity. The change of viscosity of viscose films caused by chitosan cannot be overlooked. When the viscosity of viscose films increases, it will suppress the water molecules from vaporizing, causing film that contain 2% and 3% of chitosan lose the cavernous structure and their surface to become smoother.

For fig. 4.2.2 (a,b and c), scanning electron microscope (SEM) indicated that when the chitosan content in the blend increased up to 3% the surface structure became smoother.

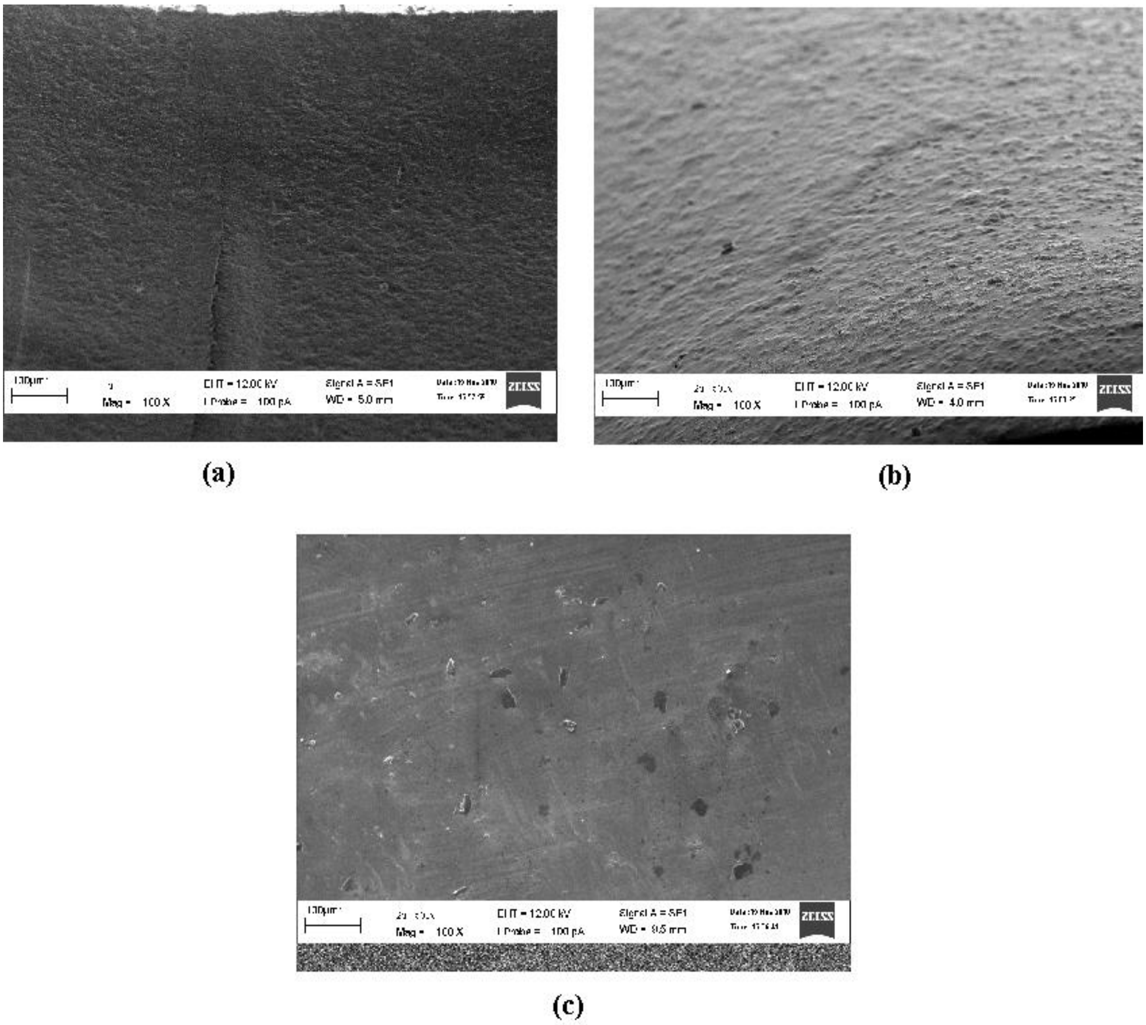


(a)

(b)

(c)

Fig. 4.2.1: Scanning electronic microscopic images of cross section (a) film with 1% of chitosan, (b) film with 2% of chitosan, (c) film with 3% of chitosan



(a) (b)

(c)

Fig. 4.2.2: Scanning electronic microscopic images of surface (a) film with 1% of chitosan, (b) film with 2% of chitosan, (c) film with 3% of chitosan

4.3 Tensile strength and Elongation at break

The Tensile Strength of biodegradable blend films from yam starch-chitosan with different chitosan ratios is shown in table 4.3 and fig. 4.3. The TS of biodegradable blend films was affected by the chitosan ratios. The results demonstrated that the time to break and Tensile Strength of biodegradable blend films were increased with the addition of chitosan, film sample with 1% concentration of chitosan take short time to break which is at 11.78 seconds at tensile strength 62.75362 MPa, followed by film sample with 2% concentration of chitosan at 45.46 seconds at 76.71713 MPa tensile strength and the maximum occurred at sample film with 3% of chitosan which is take 45.01 seconds to break and at 88.41336 MPa tensile strength.

The increasing Tensile Strength values of the biodegradable blend films, with the increase of yam starch and chitosan ratios from 2:3 to 2:1, are attributable to a high formation of intermolecular hydrogen bonding between NH_3^+ of the chitosan backbone and OH^- of the yam starch. The amino groups (NH2) of chitosan were protonated to NH_3^+ in the acetic acid solution, whereas the ordered crystalline structures of starch molecules were destroyed with the gelatinization process, resulting in the OH^- groups being exposed to readily form hydrogen bonds with NH_3^+ of the chitosan. The number of NH_3^+ groups increased with increased chitosan ratios in the film forming solution. However, the Tensile Strength of biodegradable blend film prepared at the starch to chitosan ratio of 2:2 (2%) and 2:3 (3%) was not significantly different. This phenomenon indicated the critical ratios of the greatest miscibility of the two main film forming components.

Table 4.3: Table of tensile stress and time to break

Sample	Tensile Stress (MPa)	Time to break (sec)
Control	267.0186	3.55
Film with 1% of chitosan	62.75362	11.78
Film with 2% of chitosan	76.71713	45.46
Film with 3% of chitosan	88.41336	45.01

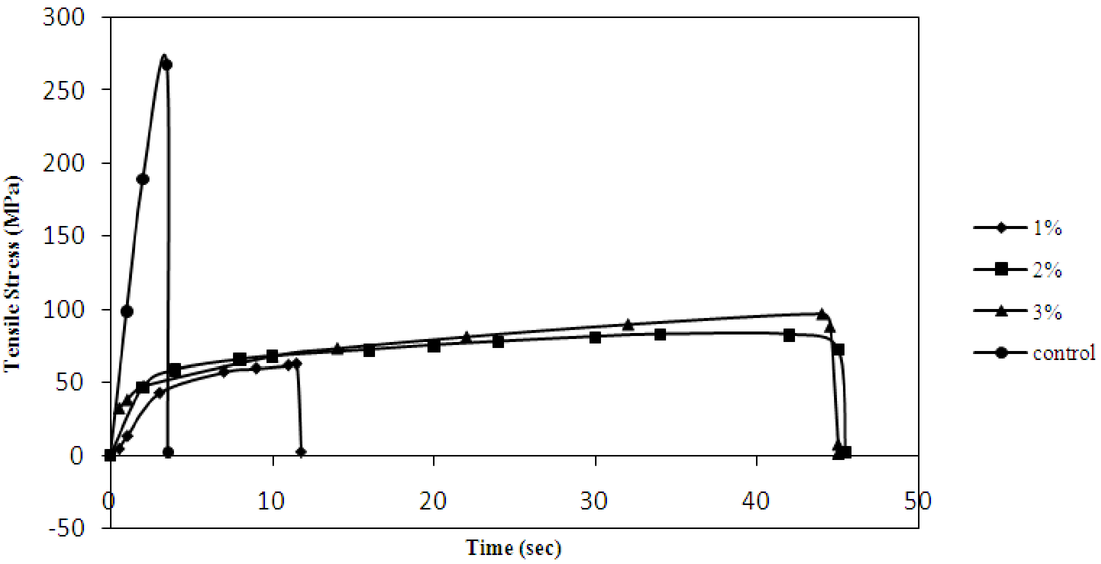


Fig. 4.3: Graph of tensile stress versus time to break

4.4 Water Solubility

The results demonstrated in the table 4.4 showed that water solubility of yam starch films decreased with the addition of chitosan. The film containing 3% chitosan have 34.9578 % water solubility, followed by film containing 2% of chitosan, 35.7758 % water solubility and the maximum water solubility is in films that containing 1% chitosan, which is 59.2309 % water solubility. It is because of the strong hydrogen bonding interaction between chitosan and starch and the low water solubility or also known as relative hydrophobicity of chitosan. These results could arise from the fact that higher chitosan content (3%) induced a yam starch and chitosan interaction and resulted in a decrease in the water solubility of film. Hydrophilic characteristics of chitosan profoundly determine water solubility. The use of chitosan is limited by the compound's poor solubility in water. Chitosan and its derivatives showed the hydrophilic–lipophilic variation influences the antimicrobial properties (Kong *et al.*, 2010).

Table 4.4: Water solubility of film

Sample	Weight of Sample (g)		Percentage of Solubility (%)
	W_i	W_f	
1	0.0288	0.0117	59.23%
2	0.1032	0.0663	35.78%
3	0.1012	0.0658	34.96%

pH is also an important parameter. For soluble chitosan, pH is a crucial factor relating to solubility, and can further alter antimicrobial activity. Another pH effect is protonation of chitosan and its derivatives. Antimicrobial activity of chitosan and its derivatives is exhibited only when the pH is below the respective pKa, which is the molecule's pKa is 6.3–6.5, (Lim and Hudson., 2004) the value at which the soluble molecule could be disassociated as ions in solution. This mechanism is not restricted to soluble chitosan, but extends to solid chitosan.

Biodegradable blend film pieces produced from yam and chitosan maintain their integrity like did not dissolve or break apart even after 24 h of incubation. This indicates that the yam starch and chitosan intra and/or intermolecular network remained intact and only the monomers, small peptides, and non-protein material were soluble.

4.5 Viscosity

For viscosity, the results in the table 4.5 and fig.4.5 demonstrated that viscosity of yam starch-chitosan solution increase with the addition of chitosan. At speed 40 rpm, the solution containing 1% chitosan showed 31.5 Cp of viscosity, followed by solution containing 2% of chitosan, 63 cP and the maximum viscosity is sample with containing 3% of chitosan which is 75 cP.

Film sample with lack of starch (control) also showed high viscosity because of it only produced by chitosan and acetic acid without mix with starch solution. So, the viscosity of this sample is also high.

Table 4.5: Table of viscosity

Starch (gm)	Chitosan (gm)	Acetic Acid (mL)	PEG (mL)	Speed (rpm)	Viscosity (Cp)
0	1	100	5	5	108.8
				10	923.8
				20	788.8
				30	752.8
				40	698.9
2	1	100	5	5	2
				10	6
				20	27
				30	31
				40	31.5
2	2	100	5	5	18
				10	25
				20	39
				30	57
				40	63
2	3	100	5	5	22
				10	36
				20	48
				30	70
				40	75

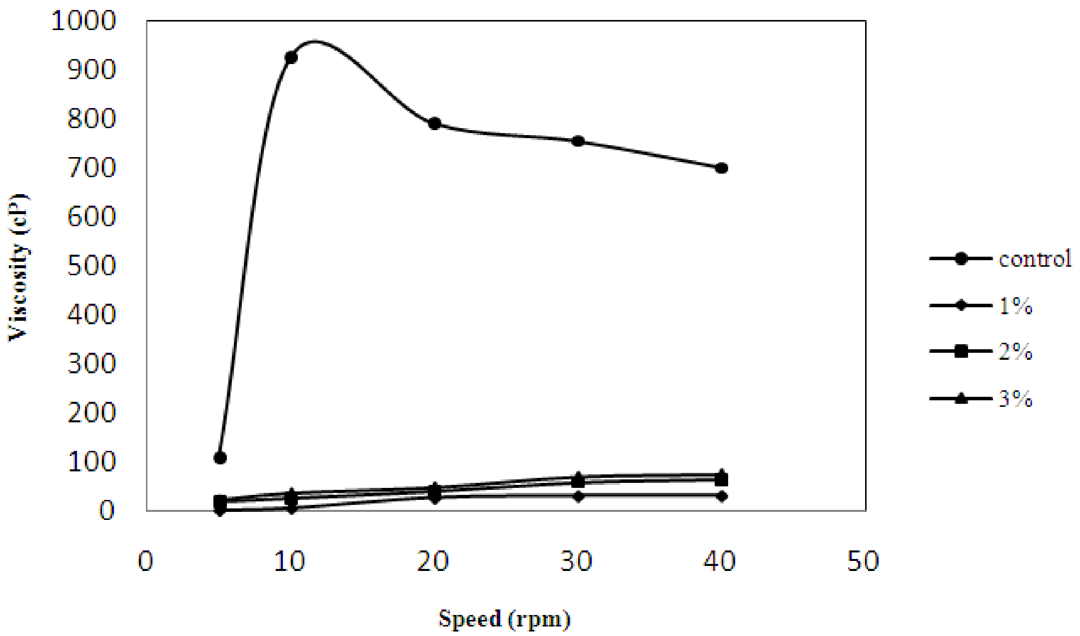
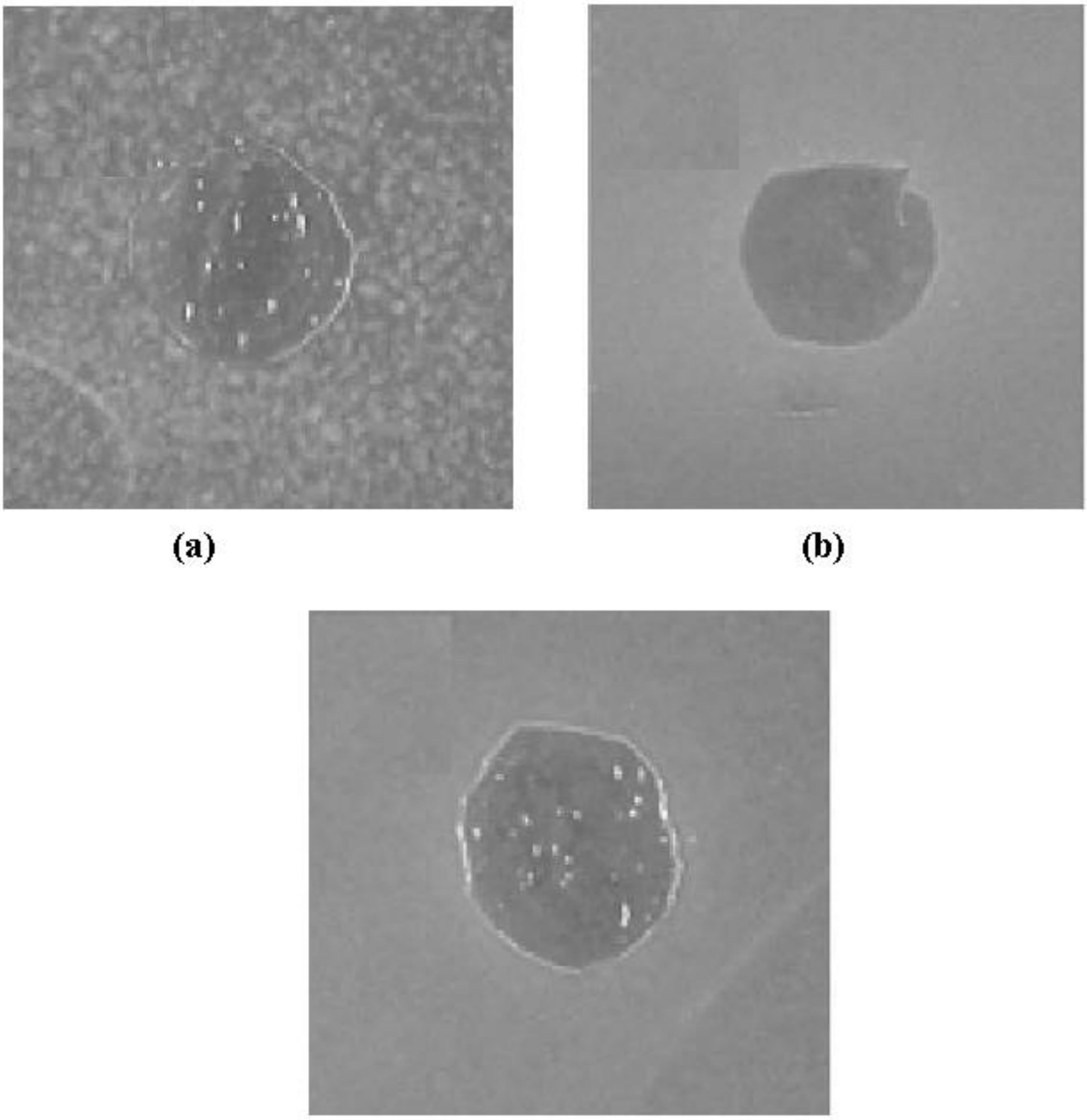


Fig: 4.5: Graph of speed versus viscosity of solution

4.6 Antimicrobial test

Fig 4.6.1 is showed about the inhibitory effect of film on B.*subtilis* whereas fig 4.6.2 is showed about the inhibitory effect of film on E.*coli*. The film with highest concentration of chitosan (3%) had greater inhibition on both B.*subtilis* and E.*coli* than other solution in agar plate. A wide clear zone on solid media was observed for E.*coli* growth inhibition whereas inhibition for B.*subtilis* was not as effective as E.*coli*. That means, inhibitory effect of the film on B. *subtilis* is low compared to the E.*coli.*

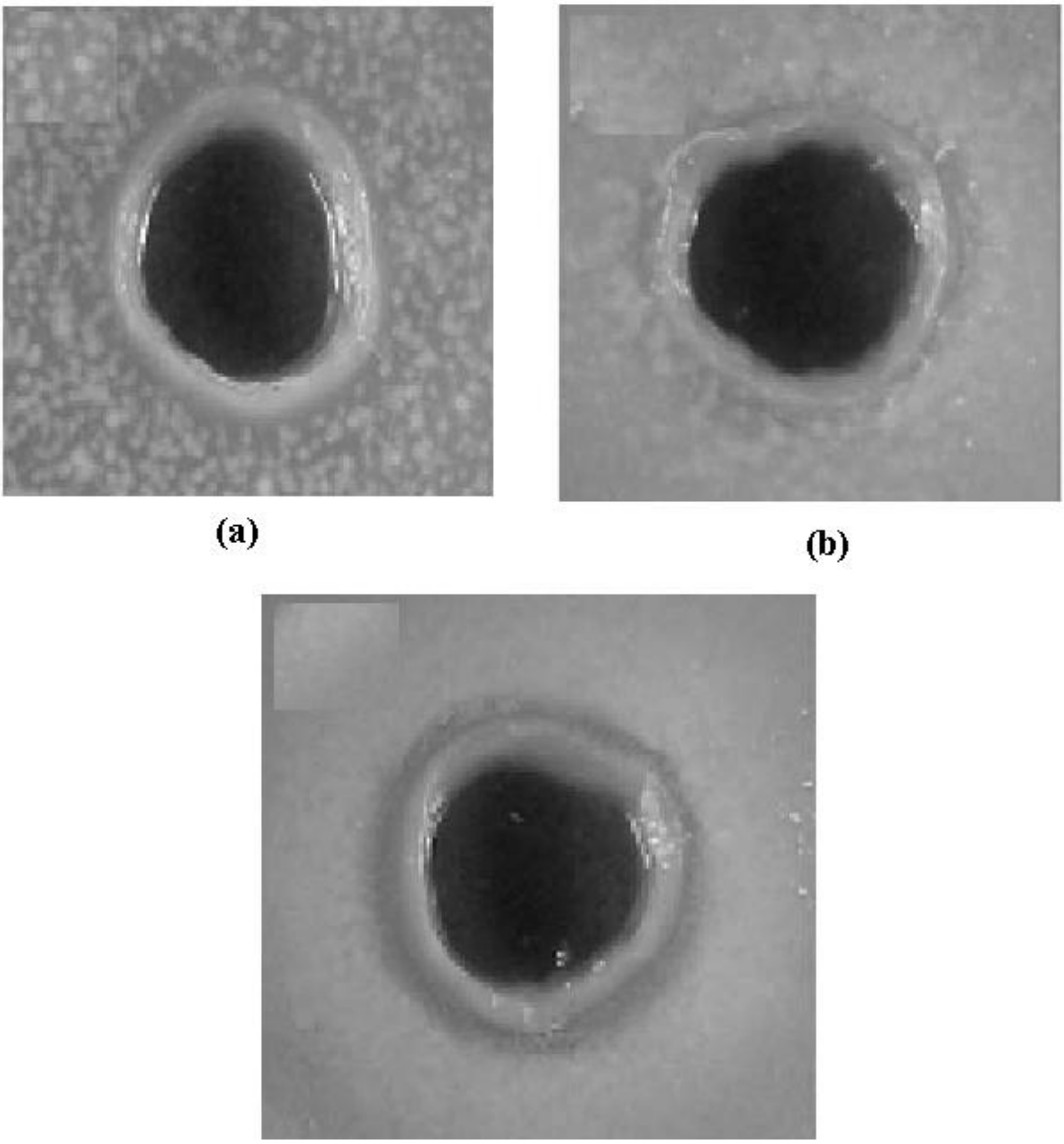


(a)

(b)

(c)

Fig. 4.6.1: Inhibitory zone effect of yam starch film with (a) 1% of chitosan (b) 2% of chiotsan and (c) 3% of chitosan on B.*subtilis*



(a) (b)

(c)

Fig. 4.6.2: Inhibitory zone effect of yam starch film with (a) 1% of chitosan (b) 2% of chiotsan and (c) 3% of chitosan on E.*coli*

Table 4.6 is showed about the antimicrobial activity of yam starch films incorporated with chitosan. Based on the inhibitory zone area in the table, the film with highest concentration of chitosan (3%) had greater inhibition on both B.*subtilis* and E.*coli* than 2% and 1% of chitosan in both medium. However, the inhibition zone area for B.*subtilis* was not as effective as E.*coli*.

Table 4.6: Antimicrobial activity of yam starch films incorporated with chitosan

Yam starch (g)	Chitosan (%)	Inhibitory zone on E.coli medium (mm2)	Inhibitory zone on B.subtilis medium (mm2)
2	1	0.02	0.01
2	2	0.03	0.02
2	3	0.05	0.025

The antimicrobial activity of chitosan contribution of the polymers to each elementary process in the lethal action of the cationic biocide considered as follows: (1) adsorption onto the bacterial cell surface, (2) diffusion through the cell wall, (3) adsorption onto the cytoplasmic membrane, (4) disruption of the cytoplasmic membrane, (5) leakage of the cytoplasmic constituents, and (6) death of the cell (Kong *et al*., 2010).

Minimal inhibitory concentration (MIC) of chitosan is significantly decreased against all tested bacteria. The characteristic of chitosan that owing asufficient touch with solution, soluble chitosan are readily affected by outer environmental factors as well as many intrinsic factors.

CHAPTER 5

CONCLUSSION AND RECOMMENDATION

5.1 Conclussion

Yam starch-chitosan biodegradable blend films were prepared successfully by casting on leveled trays. FTIR analyses of starch-chitosan blend films indicated that introduction of chitosan increased the crystalline peak structure of starch film and showed that chitosan–starch hydrogen bonding interaction is the intrinsic factor which determines the mechanical and physical properties of the films. It also was indicated from both SEM and tensile strength test that the surface of blend films tended to become smoother and the strength of films was enhanced with the increase of chitosan content in blend films.

The film with high concentration of chitosan showed the best performance in physical and chemical properties. From the result of FTIR, SEM and tensile strength and elongation at break, film with 3% of chitosan demonstrated a good physical characteristic. Their morphology showed the surface of film tends to become smoother with increasing of chitosan concentration. Other than that, time to break and Tensile Strength of this film is more longer compare to other film. Encountered its application in handling of the food, this film is suitable to endure the normal. Meanwhile, in response on antimicrobial activity, this film is surely can be a good antimicrobial film. With 3% of concentration of chitosan, this film cans inhibit the colony to breed.

The functional properties of chitosan films can be improved when chitosan films are combined with other film- forming materials. Inherent antibacterial properties and film-forming ability of chitosan make it an ideal choice for use as a biodegradable antimicrobial packaging material that can be used to improve the storability of perishable foods. It has convincingly been proved that chitosan films exhibit good antimicrobial activity which can help extend the food shelf life (Kong *et al*., 2010).

5.2 Recommendations

Antimicrobial packaging is gaining interest from researchers and industry due to its potential to provide quality and safety benefits. Currently, development is limited due to availability of antimicrobials and new polymer materials, regulatory concerns, and appropriate testing methods. With the advent of new materials and more information, this may change. Therefore, research on the other substance that never explore as an alternative to make biocomposite film need to be more extended

In order to improve production of film with better water solubility and stronger antimicrobial activity, the use of chitosan must be substitute with quaternary ammonium chitosan. Quaternary ammonium chitosan is another major example to improve solubility of chitosan by introducing hydrophilic groups into molecule. After quarternization, derivates exhibited better water solubility and stronger antibacterial activity as compared to chitosan (Kong *et al*., 2010).

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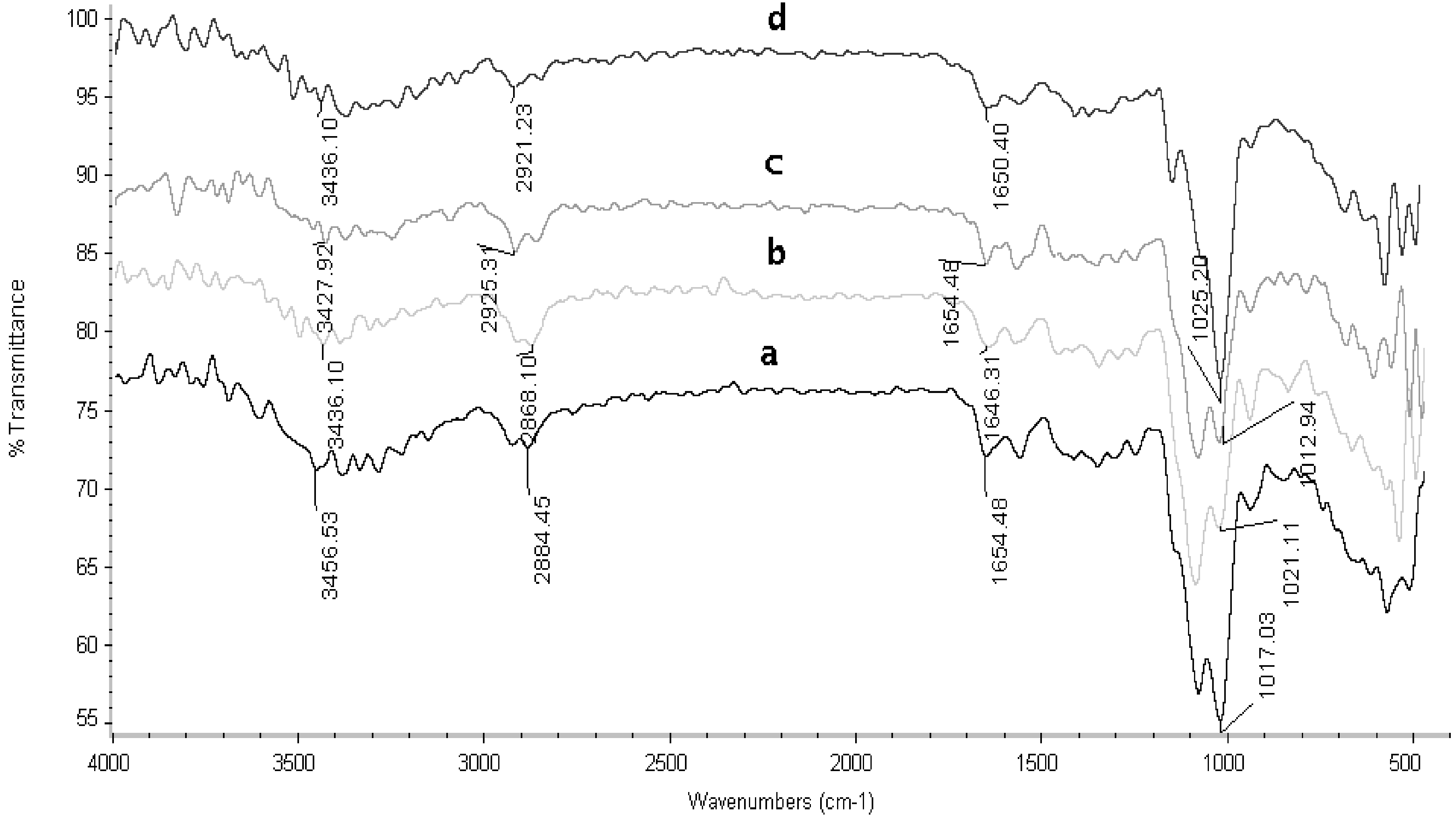
APPENDIX A



d
c
b
a
3436.10
2921.23
1650.40
1025.20
3427.92
2925.31
1654.48
1012.94
3436.10
2868.10
1646.31
1021.11
3456.53
2884.45
1654.48
1017.03
% Transmittance
100
95
90
85
80
75
70
65
60
55
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)

APPENDIX B

VISCOSITY RESULT

Starch	**Chitosan**	**Acetic Acid**	**PEG**	**Speed**	**Viscosity**
(gm)	**(gm)**	**(mL)**	**(mL)**	**(rpm)**	**(Cp)**
0	1	100	5	5	108.8
				10	923.8
				20	788.8
				30	752.8
				40	698.9
2	1	100	5	5	2
				10	6
				20	27
				30	31
				40	31.5
2	2	100	5	5	18
				10	25
				20	39
				30	57
				40	63
2	3	100	5	5	22
				10	36
				20	48
				30	70
				40	75