UNIVERSITI MALAYSIA PAHANG

BORANG PENGESAHAN STATUS TESIS

JUDUL : FABRICATION AND ANTIMICROBIAL ANALYSIS OF COMPOSITE BIODEGRADABLE FILM FROM BREADFRUIT

SESI PENGAJIAN : 2010/2011

Saya **SHAHAIDAH BT SHARIFF**
(HURUF BESAR)

mengaku membenarkan tesis (PSM/~~Sarjana~~/~~Doktor Falsafah~~)* ini disimpan di Perpustakaan Universiti Malaysia Pahang dengan syarat-syarat kegunaan seperti berikut :

1. Tesis adalah hakmilik Universiti Malaysia Pahang
2. Perpustakaan Universiti Malaysia Pahang dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. **Sila tandakan (√)

[ ]	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)
[ ]	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
[√]	TIDAK TERHAD	

Disahkan oleh

(TANDATANGAN PENULIS) (TANDATANGAN PENYELIA)

Alamat Tetap **215-1 Batu 4 ½**
Bertam Malim 75250,
Melaka

Pn. Norashikin bt Mat Zain
Nama Penyelia

Tarikh : 3 DISEMBER 2010

Tarikh:

CATATAN :
* Potong yang tidak berkenaan.
** Jika tesis ini **SULIT** atau **TERHAD**, sila lampirkan surat daripada pihak berkuasa/organisasiberkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai **SULIT** atau **TERHAD**.
♦ Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau Lapuran Projek Sarjana Muda (PSM).

" I hereby declare that I have read through this thesis and to my opinion this thesis is a adequate in term of scope and quality for the purpose of awarding the degree of Bachelor of Chemical Engineering"

Signature : ______________________________

Name of Supervisor : Mdm. NORASHIKIN BT MAT ZAIN

Date : 3th December 2010

FABRICATION AND ANTIMICROBIAL ANALYSIS OF COMPOSITE BIODEGRADABLE FILM FROM BREADFRUIT

SHAHAIDAH BT SHARIFF

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

Faculty of Chemical and Natural Resources Engineering
Universiti Malaysia Pahang

NOVEMBER 2010

DECLARATION

"I declare that this thesis entitled 'Fabrication and Antimicrobial Analysis of Composite Biodegradable Film from Breadfruit' 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."

Signature :
Name of Candidate : SHAHAIDAH BT SHARIFF
Date :

"I dedicated this work to my beloved lecturer, family, technical staf and friends for their support and encouragement."

ACKNOWLEDGEMENTS

Praise is to God for His help and guidance that I finally able to complete this undergraduate research project. Predominantly I would like to extend my sincerest gratitude to my supervisor, Madam Norashikin bt Mat Zain for her willingness in overseeing the progress of my thesis from its initial phases till the completion of it. I do believe that all her counsel and comments are for the benefit of producing the best research.

Secondly, I would like to extend my words of appreciation to my encouraging laboratory staff (Mr. Anuar, Mr. Arman, Mdm. Nor Hafizah, Mdm. Intan Syafinas, Mr. Razak, Mr. Hairul Nizam, Mr. Farid, Mr. Zulhabri) and all the laboratory staffs for the roles they had played in giving us guideline and valuable advices during my research.

The experiences and knowledge I gained throughout the process of completing this research will be the most valuable experience to better equip us for the challenges which lie ahead. In particular, my truthful thankful is also extends to my entire colleagues and others who have provided assistance without anticipate any return for it. Their views and tips are useful indeed. Unfortunately, it is not possible to list all of them in this limited space.

Last but definitely not least, to my beloved family members and my closest acquaintance, I cannot find the appropriate words that could express my appreciations for their loves, and support for me throughout my studies in Universiti Malaysia Pahang (UMP).

ABSTRACT

This study will be focusing on fabrication and antimicrobial analysis of composite biodegradable film from breadfruit starch-chitosan. The films were characterized in terms of physical, chemical and also the antimicrobial analysis. The antimicrobial effect of composite films was tested on *Bacillus substilis (B.substilis)* and *Eschericha coli (E.coli)* represent Gram-positive bacteria and Gram-negative bacteria respectively. The sample was prepared by cutting each sample into 2cm x 2cm and were placed into solid media that bacterial growth plate. Inhibition of bacterial growth was examined using zone inhibition method on solid media.The composite biodegradable film were characterized by fourier transform infrared spectroscopy (FTIR) analysis by preparing the sample with 1cm x 1 cm and revealed the spectrum of composite film containing chitosan and breadfruit starch at peak shifted from 3100 cm^{-1} to 3400 cm^{-1} due to hydroxyl stretching interactions. The amino acid group peak of chitosan at bands 1650.40 cm^{-1}. The composite biodegradable film also undergoes scanning electron microscopy (SEM) to characterize the morphology of the films with scan area of 0.3cm x 6cm. The cross section of the composite film shown distinctive film structure. Furthermore, water solubility also tested by cutting the each sample into 5cm x 5 cm and were dried at 70°C in a vacuum oven for 24 hours to constant weight. The sample films was immersed into 20ml of distilled water and placed into 50ml screw cap tubes and gently shaking for 24 hours . The result expose the decreasing percentage of water solubility by the addition of chitosan. Tensile strength (TS) and Elongation (*E*) break also tested and the result shows that TS inversely proportional to *E*. Degradation analysis also expose the film degrade under soil condition. Moreover, the characterization of breadfruit flour also characterized in terms of their structure.

ABSTRAK

Kajian ini berfokus pada fabrikasi dan analisis antimikrob terhadap filem komposit dari Sukun-kitosan pati. Filem komposit ini dikaji dari segi fizikal, kimia dan juga analisis antimikrob. Kesan antimikrob dari filem komposit diuji pada Bacillus Substilis(*B.substilis*) dan Eschericha coli (*E.coli*) yang masing-masing merupakan bakteria Gram-positif dan bakteria Gram-negatif. Sampel disediakan menjadi 2cm x 2cm dan ditempatkan ke dalam media pepejal yang mengalami pertumbuhan bakteria. Perencatan pertumbuhan bakteria telah diteliti dengan menggunakan kaedah zon perencatan pada filem komposit. Filem komposit juga diuji dengan spektroskopi inframerah (FTIR), sampel disediakan dengan 1cm x 1cm dan diuji untuk mengenalpasti kehadiran kumpulan berfungsi dalam filem komposit dan kumpulan hidroksil dikenalpasti pada puncak 3100 cm^{-1} hingga 3400 cm^{-1}. Kumpulan asid amino pada kitosan di puncak 1650,40 cm^{-1}. Morfologi permukaan juga dikaji melalui mikroskopi pengimbas elektron (SEM) dan sampel filem disediakan dengan kawasan 0.3cm x 6cm. Bahagian melintang filem komposit menunjukkan struktur filem yang berbeza. Selanjutnya, kelarutan air juga diuji dengan memotong setiap sampel ke cm 5cm x 5 dikeringkan pada 70 ° C dalam oven selama 24 jam untuk berat malar. Kemudian, sampel direndam dalam 20ml air suling dan ditempatkan ke dalam balang 50ml dan digoncang secara konsisten selama 24 jam. Peratus kelarutan dan hasilnya menunjukkan peratusan penurunan kelarutan dalam air dengan penambahan kitosan. Kekuatan tarik (TS) dan Pemulihan (*E*) istirahat juga diukur dengan menggunakan alat uji mekanik universal dan hasilnya menunjukkan TS berkadar songsang dengan *E*. Degradasi analisis juga menunjukkan filem komposit mangalami penyusutan berat selepas beberapa hari di bawah tanah. Selain itu, ciri-ciri tepung sukun juga dikaji dalam struktur tepung.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

PEG	-	Polyethylene glycol
AM film	-	AntiMicrobial film
O_2	-	Oxygen gas
HCl	-	Hydrochloric acid
NH_2	-	Amide
NaOH	-	Sodium hydroxide
OH	-	Hydroxyl group
CHO	-	Aldehyde group
NH_3^+	-	Ammonium
C=O	-	Carbonyl group
TS	-	Tensile strength
E	-	Elongation at break
FTIR	-	Fourier Transform Infrared
SEM	-	Scanning Electron Microscope

LIST OF SYMBOLS

%	-	Percentage
g	-	gram
cm	-	centimetre

CHAPTER 1

INTRODUCTION

1.1 Background to the study

The breadfruit tree, Artocarpus utilis there is some confusion regarding the origin of the Breadfruit tree. In immature fruits fructose was the main sugar, but this was replaced by glucose and sucrose as maturation proceeded. Levels of Fe, Na, P, Ca and K were thought to have some nutritional importance due to its starchy content attempts have been made to utilize breadfruit flour as a source of dough for Western diets, in Nigeria, Olatunji and Akinrele (1978) examined the qualities of wheat flour that had been diluted with flours from certain tropical plants including breadfruit. They found that levels of non-wheat flour in excess of 10% of the total mix gave unsatisfactory results. Different results were obtained by Arceley and Graham (1984) who showed that bread, cakes and puddings containing high levels (up to 75%) of breadfruit flour were well accepted by members of the tasting panel in Puerto Rico, indicating the usefulness of breadfruit flour as a supplementary dietary product.

Polymers have been used in agriculture and horticulture since the middle of the last century. In the past, plasticulture (that is the use of plastics in agriculture) was introduced primarily in developed countries, but is recently spreading to developing countries as well. Growth is particularly strong in areas with limited farmland such as in Europe, Japan and Korea (Briassoulis, 2007).

1.2 Identification of problem statement

A serious negative side effect associated with the steadily growing use of plastics in agriculture concerns the parallel growing disposal problem of thousands of tons of agricultural plastic wastes produced each year. Unfortunately, a large portion of these is left on the field or burnt uncontrollably by the farmers releasing harmful substances with the associated obvious negative consequences to the environment. Aesthetic pollution and landscape degradation of regions of natural beauty represent an additional negative environmental impact (Briassoulis 2007). The large production of non-biodegradable plastic-based waste has become a global problem in terms of both cost and ethics. Natural ingredients based on polysaccharides, proteins and lipids, extracted from plants, foods and animal tissues offer alternative packaging options that minimize environmental pollution at a relatively low cost. Applications of these ingredients in the food and pharmaceutical industries have been specified. Edible packaging could function as a barrier for water, oxygen and lipid transfer in food systems and therefore contribute to the extension in the shell-life (Wang *et al*. 2009).

In the food packaging sector, starch-based material has received great attention owing to its biodegradability, edible, wide availability as agricultural surplus raw material, abundant, can be produced at low cost and at large scale, nonallergic, easy to use and thermoprocessable. Several studies are concentrated on the development of starch-based materials for the above-mentioned reasons. Starches are polymers that naturally occur in a variety of botanical sources such as wheat, corn, potatoes and tapioca. It is a renewable resource widely available and can be obtained from different left over of harvesting and raw material industrialization. Besides, the preponderance of amylose in starch gives rise to stronger films. However, biodegradable products based on starch, possess many disadvantages, mainly attributed to the water solubility, brittle nature of starch films and poor mechanical properties. In order to improve mechanical properties and water resistance, starch can be modified by several methods such as blending with synthetic or natural polymers. One of the effective strategies to overcome the poor mechanical properties, while preserving the biodegradability of the materials, is to associate starch with chitosan (Salleh *et al*. 2007).

1.3 Research Objectives

In order to achieve the above objective, the following scopes have been identified:

a. To fabricate composite biodegradable film from breadfruit starch-chitosan.
b. To characterize composite biodegradable film in terms of morphology, physical and chemical properties.
c. To analyze the antimicrobial activity of the fabricated biodegradable film.

Packaging is one of the most promising active packaging systems. Increase demand in food safety, quality, convenience and environmental concerns associated with the handling of plastic waste has emphasized the importance in developing biodegradable and edible films from natural polymers, such as starch. Starch-based film is considered an economical material for antimicrobial packaging. Chitosan has a widely been used in antimicrobial films, to provide edible protective coating, dipping and spraying for the food products due to its antimicrobial properties. chitosan act as antimicrobial agent into starch-based film enhance antimicrobial, physical and mechanical properties of starch-based film. The starch-based film also having antimicrobial properties that can extend shelf-life of the food packed. The antimicrobial effect of composite films was tested on *Bacillus substilis (B.substilis)* and *Eschericha coli (E.coli)* represent Gram-positive bacteria and Gram-negative bacteria respectively. Inhibition of bacterial growth might be able examined using on solid media and liquid culture test (optical density measurements). The control (pure wheat starch) and AM film (incorporated with chitosan and lactic acid) were produced by casting method (Salleh *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).

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 (Shen *et al*. 2010).

1.4 Scope of study

The scope of study in this biodegradable films are:

a. To study the fabrication of composite biodegradable film from breadfruit starch-chitosan.
b. To study the characterization of composite biodegradable film using scanning electron microscopy (SEM), fourier transform infra-red (FTIR), water solubility of film, tensile strength (TS).
c. To study effect of antimicrobial activity of composite biodegradable film by agar diffusion method (zone inhibition assay).
d. To study the biodegradability of composite biodegradable film.
e. To study the characterization of composite biodegradable film.

1.5 Significance and benefit of study

The significance and benefit of this study are:

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

CHAPTER 2

LITERATURE REVIEW

2.1 Biodegradable film

Biodegradable is being able to be broken down by natural processes into more basic components and usually broken by bacteria, fungi, or other simple organism. It is also often associated with environmentally friendly product and it has been made from renewable and natural polymers such as starch (Bourtoom *et al.* 2008)

Biodegradable edible films and coatings based on protein resources physical properties and applications in food quality management (Arvanitoyannis, 2006).

Derived from natural sources, these biodegradable, biocompatible and non-toxic polymers meet the growing demands for environment-friendly products (Clarinval and Halleux, 2005).

In this study, biodegradable films and composites for agricultural applications (greenhouse, walk-in tunnel, low tunnel covers, and mulching) are in the focus. Petroleum-based plastics often remain undegraded after discard and a time-consuming and uneconomical recycling is unavoidable. The adsoption of biopolymers avoids the removal of residual materials from the growing environment after functional compliance (Espi *et al*., 2006 and Joo *et al*., 2005).

The film forming and thermoplastic properties due to their abilities to form weak intermolecular interactions, i.e. hydrogen, electrostatic and hydrophobic bonds make, i.e. sodium caseinate a promising raw material for the production of biodegradable films and coatings (Audic and Chaufer, 2005).

An enzymatic catalyzed cross-linking of proteins associated with a controlled additive crystallization appears to be a promising way to improve physicochemical properties of biodegradable films and coatings. The application of a biodegradable film as barrier between fruits and vegetables and their surroundings is becoming an increasingly important venture because consumers demand of hygienic and sanitary products (Koide *et al.*, 2007).

Although biodegradable films are more expensive than the petrochemical materials, they will biodegrade into CO2, water, and biomass under aerobic conditions, or methane andbiomass under anaerobic conditions (Avella *et al*., 2005).

The production of biodegradable and edible films from carbohydrates and proteins adds value to low-cost raw materials and can play an important role in food preservation (Ave´rous *et al*., 2001, Krochta and Miller, 1997).

Several studies reported the use of starches from different sources to prepare films and coatings with different properties, and have indicated that these carbohydrates are promising materials in this regard (Ave´rous *et al*., 2001, Larotonda *et al*., 2005, Mali *et al*., 2005).

2.2 Food Packaging

Food packaging is packaging for food to protect food from spoil. The materials most used for food packaging are the petrochemical-based polymers, due to their availability in large quantities at low cost and favourable functionality characteristics, such as, good tensile and tear strength, good barrier properties to O_2 and heat sealability (Alves *et al.,* 2006) .

Usually it also contain of non-biodegradable which leading to serious ecological problem and cause of that the biopolymers commonly used to produce biodegradable films. Antimicrobial packaging is a form of active packaging. Active packaging interacts with the product or the headspace between the package and the food system, to obtain a desired outcome (Labuza, Rooney and Brody).

Antimicrobial food packaging acts to reduce, inhibit or retard the growth of microorganisms that may be present in the packed food or packaging material itself (Appendini, *et al*., 2002).

The most successful commercial application of antimicrobial packaging has been sachets that are enclosed loose or attached to the interior of a package.

2.3 Composite

Composite made from two or more constituent materials with significantly different physical or chemical properties which remain separate and distinct on a macroscopic level within the finished structure. The surface morphology and conductivity of the PANI/SPSF composite film were characterized by environmental scanning electron microscopy and four-probe technique,respectively (Lu *et al*. 2008).

2.4 Chitosan

Chitosan is a biodegradable natural polymer with great potential for pharmaceutical applications due to its biocompatibility, high charge density, non-toxicity and mucoadhesion (Sinha *et al*. 2004).

A natural linear biopolyaminosaccharide is obtained by alkaline deacetylation of chitin, which is the second abundant polysaccharide next to cellulose (Muzzarelli, 1977 and Roberts, 1992).

It also a weak base and is insoluble in water and organic solvents, however, it is soluble in dilute aqueous acidic solution (pH < 6.5), which can convert the glucosamine units into a soluble form $R–NH3^+$ (Chandy and Sharma, 1990).

Chitosan is known to have good complexing ability; the $–NH_2$ groups on the chain are involved in specific interactions with metals. It has one primary amino and two free hydroxyl groups for each C_6 building unit. When the degree of deacetylation of chitin reaches about 50% (depending on the origin of the polymer), it becomes soluble in aqueous acidic media (Rinaudo, 2006).

Chitosan is a cationic polysaccharide and a copolymer of β (1→4) linked glucosamine and N-acetyl glucosamine. Since chitosan has reactive amino/hydroxyl groups, it can be subjected to chemical modification to obtain a derivative with the desired characteristics (Rekha *et al.*, 2009).

The biological properties of chitosan include biocompatibility, biodegradability, non-toxicity, hemostaticity, antitumoral and antiviral activity(Chandy *et al*, 1993).

Due to its biocompatibility, biodegradability and it sability to open intercellular tight junctions, chitosan may become a valuable excipient for oral drug delivery systems (Berradaa *et al*., 2005).

Chitosan is a non-toxic, biocompatible, biodegradable, natural polysaccharide derived from the exoskeletons of crustaceans and insects. Chitosan's biodegradability, immunological activity and high viscosity make it an excellent candidate as a depot/adjuvant for parenteral vaccination (Zaharoff *et al.*, 2007).

2.4.1 Degree of acetylation of chitosan

The characterization of a chitosan sample requires the determination of its average. Various techniques, in addition to potentiometric titration (Rusu-Balaita *et al*, 2003).

It also have been proposed, such as IR, elemental analysis, an enzymatic reaction or UV. The fraction of $–NH_2$ in the polymer can be obtained by dissolution of neutral chitosan in the presence of a small excess of HCl on the basis of stoichiometry followed by neutralization of the protonated $-NH_2$ groups by NaOH using pH or conductivity measurements (Rinaudo *et al*. 2006).

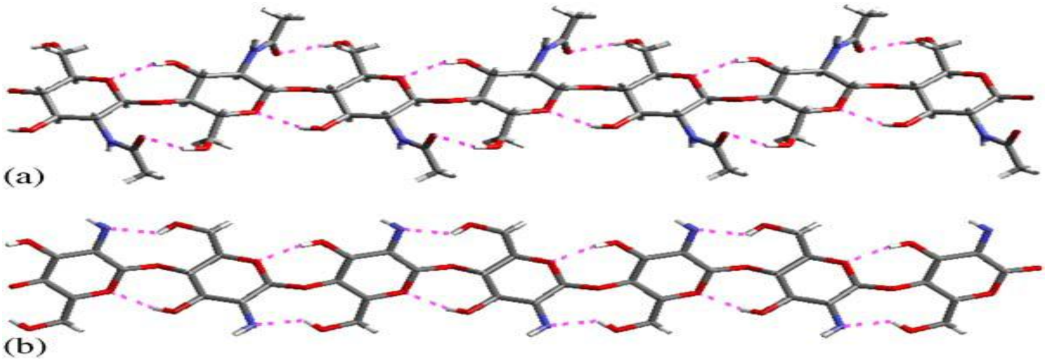


Figure 2.1 : Molecular modelling (a) of a chitin chain with two H—OH 3 and O 5, (2) between—OH 6 and O of CQO; and (b) of a chitosan chain with two H bonds (1) between—OH 3 and O 5, and (2) between—OH 6 and N.

2.5 Chitin

Chitin is the principal component of protective cuticles of crustaceans such as crabs, shrimps, prawns, lobsters and cell walls of some fungi such as aspergillus and mucor.. Chitin is a straight homopolymer composed of (1,4) linked N-acetyl-glucosamine units while chitosan comprises of copolymers of glucosamine and N-acetyl-glucosamine (Kas, 1997, Singla and Chawla, 2001, Kato *et al*., 2003).

Chitin, a homopolymer comprising β-(1-4)-linked N-acetyl- glucosamine residues is one of the most abundant, easily obtained and renewable natural polymers, second only to cellulose. It is commonly found in the exoskeletons or cuticles of many invertebrates and in the cell walls of most fungi because of its high crystallinity, chitin is insoluble in aqueous solutions and organic solvents (Lason *et al.,* 2000).

2.6 Carbohydrate

Carbohydrate interactions play crucial roles in numerous biological processes (Grün *et al.,* 2006). It is a part of protein interactions are involved in a range of biological mechanisms, starting with fertilization and extending to pathologies. It also mediate diverse cellular activities, including cell recognition and growth control. Carbohydrate also contains of sugars and starches, which provide energy for humans and animals, and cellulose which make up many plant structures. The classifications of carbohydrate has three types which are monosaccharide, polysaccharide and oligosaccharide. The heavy B-chain possess the carbohydrate binding site (specific for galactose and N-acetylgalactosamine) whereas the light A-chain is devoid of such sites. The B-chain can bind to cell surface glycolipids and glycoproteins having beta-1,4-linked galactose residues and facilitates the transport of the toxic protein (A-chain of ricin) into the cell (June *et al*. 2006)

2.6.1 Monosaccharide

Monosaccharides are a type of simple carbohydrate, or simple sugar. It is obviously differentiated from disaccharides and polysaccharides by the number of rings the chemical compound and it has a single ring carbohydrate and also classified as aldehyde or ketone depending on what type of carbonyl group is contained in the formula. An aldehyde has a carbon bonded between hydrogen and oxygen, while a ketone has a bond between carbon and oxygen. These are expressed in formulaic mode as aldehyde (-CHO) or ketone (-CO-). The interaction between saccharide (S) and electrolyte (E) in aqueous solution is of great importance in biochemistry and biophysics (Kelei, *et al.* 2000).

The density data for monosaccharide (d-xylose, d-arabinose, d-glucose and d-galactose) NaCl water systems, combined with data for the related binary systems, have been employed to calculate infinite dilution apparent molar volumes of the monosaccharides and NaCl and enables to obtain some information on the solvation of both electrolyte (ions) in aqueous monosaccharide and saccharide in aqueous NaCl solutions (Kelei., *et al.* 2000).

2.6.1 Polysaccharide

Polysaccharides are chains of sugar units that form in configurations from tens to thousands of units long. For example chitin is a polysaccharide that is a component of fungal cell walls, and forms the outer skeletons of insects and crustaceans. Regarding Pornsack., *et al.* (2006) statement two anionic polysaccharides, (sodium alginates and low methoxy pectins), that can react with divalent cations (e.g. calcium ions) to form cross-linked and water-insoluble gels.

2.6.2 Oligosaccharide

Oligosaccharides are a diverse and complex class of compounds that are broadly distributed in nature. Their biological significance ranges from defined roles as structural elements and energy sources, notably cellulose and starch, to less defined roles as constituents of glycoproteins and glycolipids on mammalian cell surfaces and microbial metabolites (Varki 1993). Vicinal glycosylation has been shown to markedly affect the conformational properties of a branched oligosaccharide compared with its parent disaccharides (Jesus *et al*., 1999).

2.7 Starch

Starch is one of themost commonly used rawmaterials to prepare biodegradable film, because it is a renewable source, widely available, relatively easy to handle, and inexpensive (Maizura *et al.*, 2007).

Starch biosynthesis is a complex process. Sucrose (derived from photosynthesis) is the starting point for alpha-glucan deposition. In the cell cytosol the sucrose is converted to uridine diphosphate glucose (UDP-glucose) and fructose by sucrose synthase, the UDP-glucose being subsequently converted to glucose-1-phosphate(G-1-P) in the presence of pyrophosphate (PP_i) by UDP-glucosepyrophosphorylase (Tester *et al*., 2004).

Starch has unique thermal properties and functionality that have permitted its wide use in food products and industrial applications (Wajira *et al.,* 2008).

Starch, as an abundant raw material with low cost, has been applied in the field of degradable plastics, and blend films containing starch are potential materials in the agriculture, medicine, and packaging industries (Xiong *et al.,* 2008).

Starch blended with synthetic polymer polyvinyl alcohol (PVA) has been studied as a potential biodegradable polymer (Follain *et al.,* 2005; Xiao and Yang, 2006, Zhai *et al.,* 2002).

Depending on the degree of biodegradability, it seems that PVA might provide a stable support medium for starch films (Jayasekara *et al*. 2004).

Starch molecules play an important role in the functional properties of the whole starch, therefore correlations established between the thermal and functional properties of starches aid in understanding the potential relationships between the structural features of the starch molecules and their effects on more than one kind of functional behavior (Amalia *et al.,* 2005).

2.7.1 Amylose and amylopectin fine structure

Amylose and amylopectin have different structures and properties which have been discussed and reviewed by many authors. Amylose is a relatively long, linear α-glucan containing around 99% (1→4)-α- and (1→6)-α- linkages and differs in size and structure depending on botanical origin and amylose has a molecular weight of approximately 1×10^5–1×10^6 (Tester *et al*. 2004).

Amylopectin is a much larger molecule than amylose with a molecular weight of 1×10^7–1×10^9 and a heavily branched structure built from about 95% (1→4)-α- and 5% (1→6)-α- linkages (Tester *et al*. 2004).

2.8 Antimicrobial

Antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoans. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). Disinfectants are antimicrobial substances used on non-living objects. Antimicrobial polymers can be used in several food related applications including packaging. One is to extend the shelf-life and promote safety by reducing the rate of growth of specific microorganisms by direct contact of the package with the surface of solid foods. Second, antimicrobial packaging materials could be self-sterilizing or sanitizing. Such antimicrobial packaging materials greatly reduce the potential for recontamination of processed products and simplify the treatment of materials in order to eliminate product contamination. Antimicrobial activity of silver is much higher than other metals, such as mercury, copper, lead, chromium and tin also the development of antibiotic resistant bacteria stains issue has resulted a new era for the revival of the long well-known antibacterial properties of silver and silver ions by the way the antibacterial mechanism of silver and its ions involves the interaction with the thiol groups of protein molecules present inside or outside the cell membrane and binds the bacterial cell membrane which inhibiting the replication capacity of DNA molecule, thereby affecting the cell viability (Vimala *et al*. 2010).

2.9 Polyethylene glycol (PEG)

Polyethylene glycol (PEG) is widely used as a covalent modifier of biological macromolecules and particulates as well as a carrier for low molecular weight **(**Samuel 1995). In addition Particular attention is paid to the comparative attributes of various reactive PEG derivatives, properties of the linkages formed, and possible side reactions **(**Samuel 1995).

Polyethylene glycols (PEGs) are high molecular weight polymers of ethylene oxide and also are designated by a number that roughly represents the average molecular weight of the polymers (Hermansky *et al.* 1995).

2.10 Acetic acid

Regarding Kyoko *et al.*, (2007) statement, acetic acid promoted water absorption of amylopectin in rice starch. As the concentration of acetic acid increased, the values of maximum, minimum, final viscosity, and consistency decreased, and that of breakdown increased (Kyoko *et al*,. 2007).

CHAPTER 3

METHODOLOGY

3.1 Materials

Breadfruit was obtained from a local farmer (Bertam Malim Melaka, Malaysia). Acetic acid that used to dissolve chitosan was purchased from Fluka Chemical (Malaysia). Chitosan and Polyethylene glycol (PEG 400) was purchased from Sigma-Aldrich (Malaysia).

3.2 Flour Preparation

The breadfruit was cleaning and peeling. After that, it was ground into slurry with the addition of water. Then, the slurry was filtered through filter cloth and the filtrate was settled over night without shaking to recover the sediment as starch. The starch was dried at 40˚C in oven around 4 hours. Finally, the breadfruit flour was produced.

3.3 Isolation of starch

To isolate the starch, 2g of breadfruit flour was taken and add with 2 ml of water. After that, the solution was heated and stirred by magnetic stirrer until the solution was gelatinized.

3.4 Chitosan solution preparation

The chitosan was weighed for 1g, 2g and 3g of three sample. 100 ml of 1% acetic acid was prepared and mix together with 1g of chitosan. Then, heat to 30˚C and stirred around 300 rpm constantly by using stirrer until the solution seem like gel mixture.

3.5 Film preparation

The starch and chitosan solution was mixed together. The mixture was heated and still stirred with 300 rpm. To prepare the antimicrobial film, chitosan was added at different concentrations (1,2 and 3 g/100 g starch) in one time during the mixing period. Then, Polyethylene glycol (PEG 400) was added into the mixture and stirrer until the mixture miscible. Before antimicrobial agents were added into starch paste. When chitosan was incorporated into film, the pH of the gel-like mixture was 4.0 because of acetic acid used in preparing chitosan solution. The warm mixture was casted on framed glass plates, and then dried at 50 ˚C for 4 hours.

3.6 Film Characterization

3.6.1 Antimicrobial activity

3.6.1.1 Culture preparation

Antimicrobial activity test was carried out using agar diffusion method. Indicator cultures were used *Eschericha coli (E.coli)* and *Bacillus substilis (B.substilis)* representing Gram-negative and Gram-positive bacteria respectively. Then, 100 µL of inoculum solution was added to 5 ml of the appropriate soft agar, which was overlaid onto hard agar plates.

3.6.1.2 Antimicrobial test

The antimicrobial test was carried out according to the method developed by Seydim and Sarikus (2006) with some modifications. The inhibitory zone test on solid medium was used for determination of the antimicrobial effects of films on *E. coli* and *B.substilis*. Composite biodegradable films were cut into square (2cm x 2cm) with a punch and square films were placed carefully on the bacterial lawns. The petri dishes were then incubated at 30 ˚C for 48 hour in the appropriate incubation chamber. The plates were examined to find the zone of inhibition of the films, and the diameter of the zone was measured at two cross sectional points and the average was taken as the inhibition zone.

3.6.2 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 Whatman qualifation circle 125mm Dia Cat No 1001 125 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

$$WS\ (\%) = ((Wo - W_f)/W_o) \times 100$$

where W_o is the initial weight of the film expressed as dry matter and W_f is the weight of the desiccated undissolved film.

3.6.3 Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) of film samples was obtained using a Carl Zeiss EVO 50 Oxford instruments scanning electron microscope. All samples were examined using an accelerating voltage of 12.00 kV. The composite biodegradable film undergoes scanning electron microscopy (SEM) to characterize the morphology of the films with 0.3cm x 6cm of films was dyed into liquid nitrogen and each sample was coated by platinum to increase the conductivity so that make easy to see the structure of each samples before SEM observation. Micrographs of drying exposed surface and fracture (cryofracture) of films 1g, 2g and 3g of chitosan were carried out.

3.6.4 Fourier Transform Infrared (FT-IR) spectra analysis.

The Fourier transform infrared (FT-IR) spectra of the films were performed at room temperature using an IR Spectrometer (Perkin–Elmer, model 2000, USA). The characteristic absorption bands of the composite films were detected at wavenumbers ranging from 500 to 4500 /cm.

3.6.5 Tensile strength (TS) analysis

Rectangular specimens of the composite films with dimensions of 40 mm×5 mm×0.06–0.10 mm were tested by a universal mechanical testing instrument (Instron-5567, Instron Corp., USA) at room temperature and relative humidity of 50%. The tensile strength and the elongation were evaluated at a displacement rate of 20mm/min with 20 mm gauge length. All results were the mean values of three specimens.

3.6.6 Degradation analysis

Three sample of rectangular films with dimensions of 5 cm x 5 cm x 5cm was prepared and weigh each of film sample whereby 0.6g for each. After that, plant into three sample of soil respectively. Each of sample was placed at outdoor of laboratory and the result was taken each three days by measure the film weight.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Antimicrobial assay

The antimicrobial activity of composite biodegradable film that contains breadfruit starch incorporated with chitosan against *E.coli* and *B.subtilis* is shown in table 4.1 and figure 4.1

Table 4.1 : Inhibition of *E.coli* and *B.substilis* on agar plates figure based on average zone diameter expressed as an area (cm) of inhibition zone

Film (chitosan : breadfruit starch), g	Inhibition Diameter (cm)	
	Eschericha coli	Bacillus subtilis
1 : 2	0.153	0.500
2 : 2	0.180	0.600
3 : 2	0.340	0.760

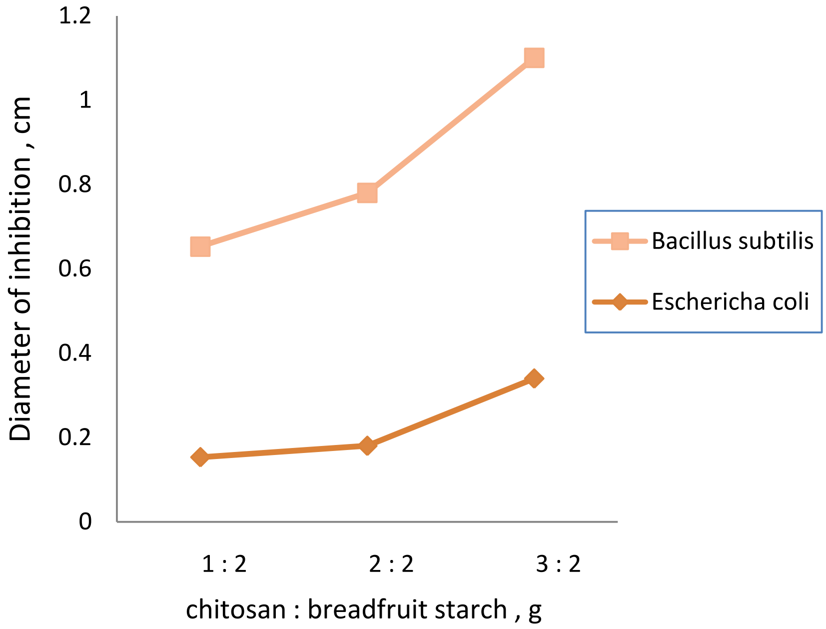
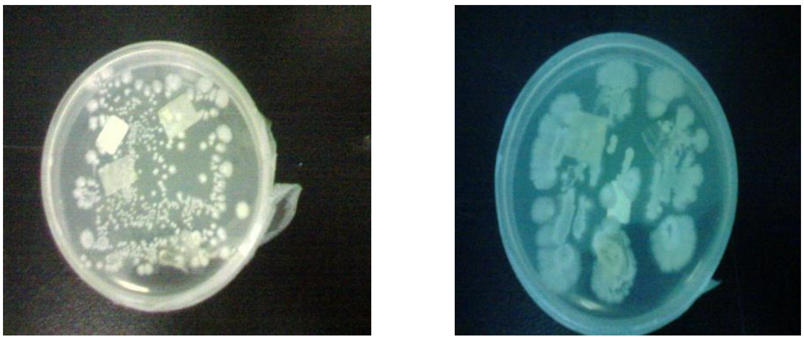


Figure 4.1: Inhibition of *E.coli* and *B.substilis* on agar plates figure based on average zone diameter expressed as an area (cm) of inhibition zone



(a) (b)

Figure 4.2 : Comparison of inhibition area of (a) *E.coli* and (b) *B.substilis*

According to the result shows that the diameter of clearing zone enlarged rapidly by increasing of chitosan content 1.0 gram to 3.0 gram in figure 4.1. The antimicrobial mechanism of chitosan is due to its NH_2 group can be protonated to NH_3^+ and readily form electrostatic interactions with anionic groups of microbial cell membranes leading to the leakage of proteinaceous and other intracellular constituents of the microorganism (Shen *et al.*, 2010). Table 4.1 showed that the inhibitory effect of film on *E.coli* and *B.substilis* differed greatly and the reason for this difference was related to the sensitivity of *E.coli* and *B.substilis* to chitosan. Regarding to Salleh *et al*. (2007) was reported chitosan can inhibit the growth of a wide variety of fungi, yeasts and bacteria. Means that, their characteristics posses immense potential as a packaging material owing to its biodegradability, biocompatibility and antimicrobial activity (Hirano *et al.* 1994). Like the inhibitory pattern of starch films incorporated with chitosan 1-3 gram on *E.coli* were found smoothly inhibit by the increasing of chitosan content. However, when the addition of chitosan,, the inhibitory pattern revealed the best inhibition on *B.substilis* . This phenomenon occur due to in terms of type of bacteria presence. According to the *E.coli* presence as bacteria Gram negative that has their own characteristics such as has their peptidoglycan layer is thin, single layer causes easy to inhibited with film presence, it difficult to defense themselves. Therefore, the inhibitory pattern like smoothly. Meanwhile, for *B.substilis* presence as bacteria Gram Positive also has their own characteristics whereby the peptidoglycan layer is thickness, multilayer. Commonly, *B.substilis* found in soil and it may contaminate food and causes food poisoning. Its spore can survive in the extreme heat during cooking because it has long-chain polysaccharides. Therefore, it was not easy inhibited by presence of film. In some cases, it were inhibited by the addition of chitosan content due to the antimicrobial activity of chitosan.

4.2 Water solubility (WS)

Table 4.2 : Effect of weigth loss of composite biodegradable films

Sample (chitosan: breadfruit starch)	Initial Weight of sample,W (g)	Final weight of sample,W (g)	Weight of filter paper before ,W (g)	Weight of filter paper after, W (g)	Percentage of water solubility, (%)
1 : 2	0.20	0.1004	1.1	1.2004	50
2 : 2	0.30	0.2010	1.1	1.3010	33
3 : 2	0.40	0.3100	1.1	1.4100	23

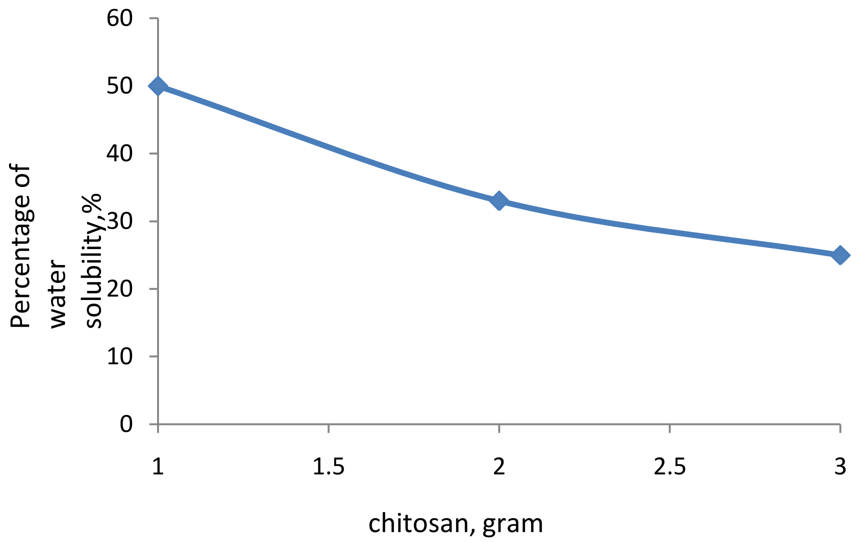
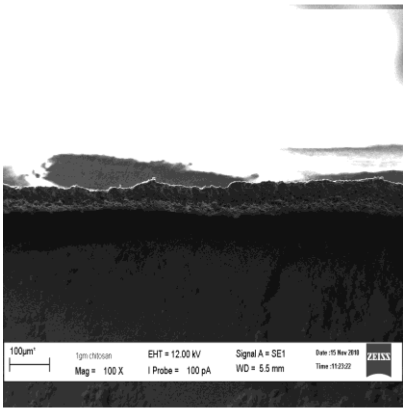
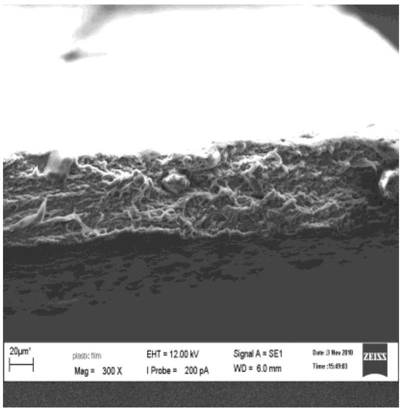


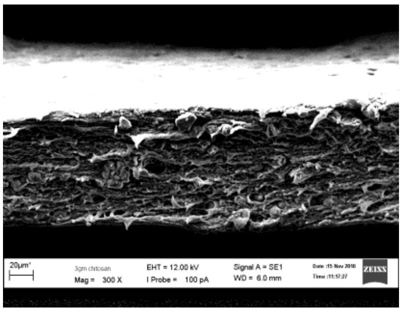
Figure 4.3 : Graph of percentage water solubility versus chitosan content

Regarding to the result, significant decrease in water solubility was observed in breadfruit starch film incorporated with greater of chitosan content. When 3 g of chitosan was added, its water solubility was decreased by 54% (from 50% to 23%). Actually, the biodegradable films did not dissolve totally even after 24 hours of incubation with gentle motion. Its shows the films maintain their integrity. This indicates that the films whether intramolecular or intermolecular network remained intact and only the monomers, small peptides, and non-protein material were soluble (Stuchella *et al.* 1994). Incorporation breadfruit starch with chitosan causes enhanced the water resistence of the films due to the strong hydrogen bonding interaction between chitosan and starch and low water solubility or relative hydrophobicity of chitosan itself (Shen *et al.* 2010). Water resistance is an importance property of biodegradable or edible films for application as food protection whereby water activity is high or when the film must be in contact with water during processing of the coated food(to avoid exudation of fresh or frozen products) (Bourtoom *et al.*, 2008). Therefore, higher solubility would indicate lower water resistance. Generally, potential applications may require water insolubility to enhance product integrity and water resistance (Shen *et al.* 2010). 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).

4.3 Scanning electron microscopy (SEM) analysis

(a) (b)



(c)

Figure 4.4 : cross section of scanning electron microscopy (SEM) of the composite film for (a)1g of chitosan, (b)2g of chitosan and (c)3g of chitosan

The cross section of scanning electron microscopy for composite films formed from breadfruit starch and chitosan are shown in figure 4.4. It is clear from the images that distinctive film structures were formed and were dependent on the type of content ingredients employed. For instance, figure 4.4. A shows cross section of film contain 1g of chitosan incorporate with 2g of breadfruit starch. The structure look likes smoothly rolling and thin layer while B shows cross section of film containing 2g of chitosan incorporate with 2g of breadfruit starch. The structure seems like more grosser than 1g of chitosan. C shows cross section of film containing 3g of chitosan incorporate with 2g of breadfruit starch whereby the structure obviously looks more roughness than others. In terms of morphology observation, it can be observed that the internal morphology dramatically changes as more chitosan were added to composite biodegradable film. Instead of that, we has found that the roughness of composite film increase by the increasing of chitosan content. Indirectly, it is shows the separation phase between starch and chitosan component. Instead of the characteristics separation phase it also might be suitable to capture any microb or foreign molecule that want enter to the film. Chitosan forms good films and membrane (Salleh *et., al* 2007). More addition, Nath *et al.* (2009) reported that the thin layers reduce the attraction forces of particles and help to disperse in aqua/or matrix media.

4.4 FT-IR analysis

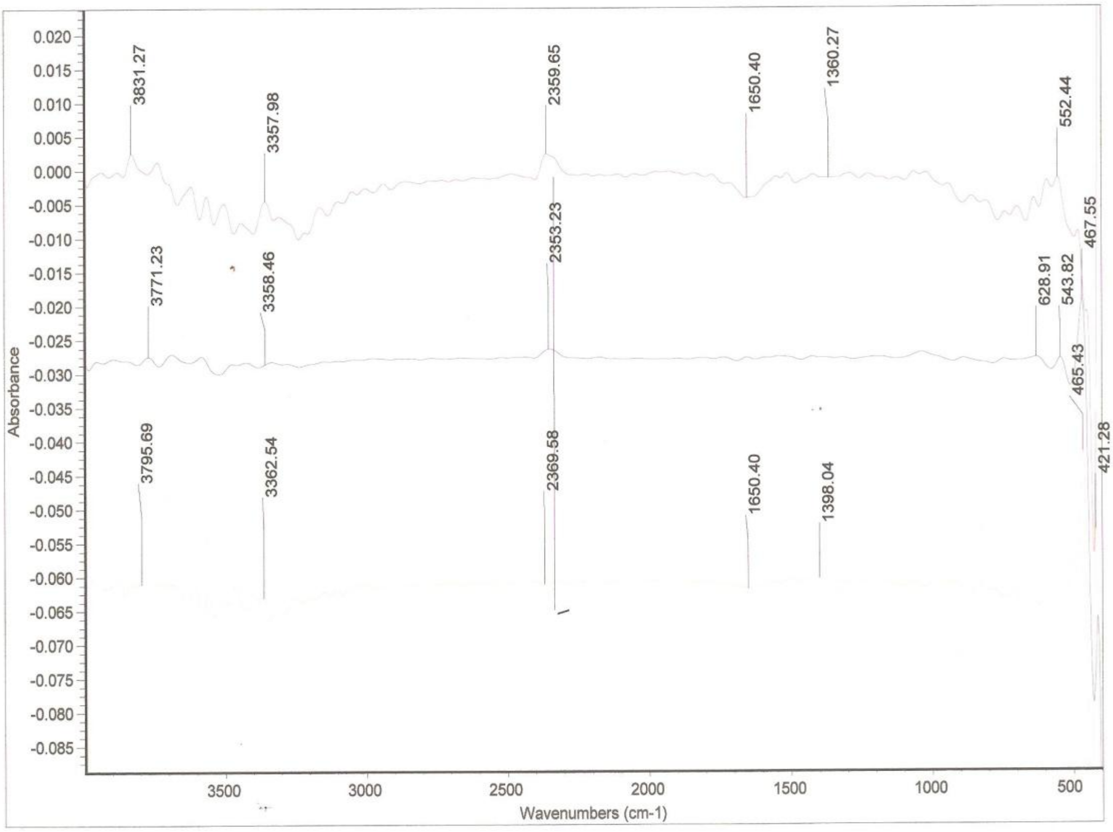


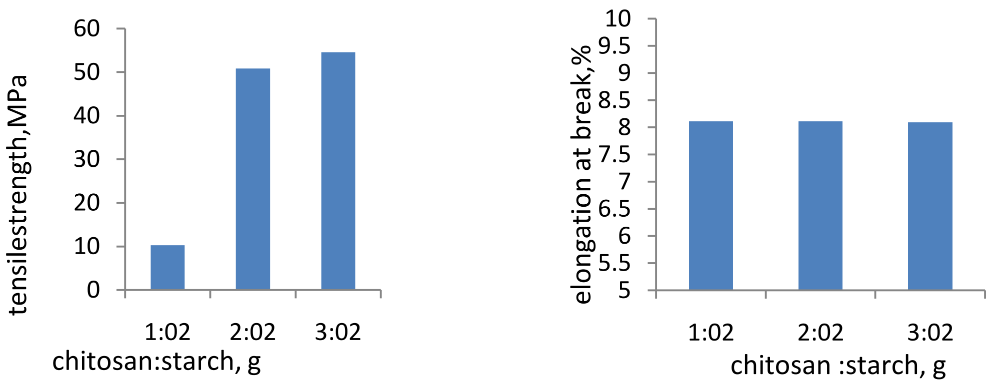
Figure 4.5 : molecular interaction between breadfruit starch and chitosan

FT-IR spectroscopy was used to identify the interactions between functional groups of breadfruit starch with chitosan. Regarding Osman *et al*. (2003) the carbonyl group, C=O stretching observed at 1650 cm^{-1}, NH_2 band at 1590 cm^{-1}, the ammonium band NH_3^+ appears as small shoulder at 1514 cm^{-1} probably due to interaction between NH_3^+ of chitosan and the –COO– of acetic acid to form the O=C–NHR band. In Xiong *et al*. (2008) reported that the spectrum of starch strong and broad absorption peak at 3403 cm^{-1} was assigned to the characteristic absorption peak of the stretching vibration of –OH. In this observation, the bands at 1650.40 cm^{-1} attributed to the stretching vibration of C=O. Meanwhile, the bands at range 1500 cm^{-1} shifted to 1600 cm^{-1} was attributed to the stretching vibration of N-H. When two or more substances are mixed, physical blends versus chemical interactions are reflected by changes in characteristics spectra peaks (Xu *et al*. 2005). According to Shen *et al*. (2010) was reported that 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. Regarding to the result obtained shows when the increasing of chitosan content, the peaks at 3100 cm^{-1} -3400 cm^{-1} also become smoothly narrower which revealed the hydrogen bonding. Instead of hydrogen bonding, it related to the hydroxyl group tendency to form hydrogen bond and it will increase hydrophilicity act as membrane filtration that use in biochemical field to filter a few elements such as bacteria. Therefore, its characteristics useful for antimicrobial activity.

4.5 Tensile strength (TS) analysis

Table 4.3 : Result of tensile strength (TS) and elongation at break (E)

Sample (chitosan : starch), g	Tensile strength, MPa	Elongation at break, %
1:2	10.28789	8.109000
2:2	50.83600	8.105297
3:2	54.57809	8.096507



(a) (b)

Figure 4.6 : Effect of chitosan ratios on (a) tensile strength (TS) and (b) elongation at break (*E*) of the composite biodegradable films.

The tensile strength (TS) of biodegradable blend films from breadfruit starch-chitosan with different chitosan ratios is shown in figure 4.6 (a). The TS of biodegradable films was affected by the chitosan ratios. The results demonstrated that the TS of biodegradable films increased with the addition of chitosan, and the maximum occurred at the chitosan and breadfruit starch ratio of 2:2 and 3:2. However, the ratios 1:2 shows significantly different compare to both ratio. This phenomenon probably occur due to the thickness of 1:2 films was different with others. The TS value of biodegradable films was increasing with ratio chitosan and breadfruit starch 1: 2 to 3:2 are attributable to a high formation of intermolecular hydrogen bonding between NH_3^+ of the chitosan backbone and OH^- of the breadfruit starch. The amino groups (NH_2) of chitosan were protonated to NH_3^+ in 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. (Bourtoom *et al*., 2008). Means that, the number of NH_3^+ groups increased with chitosan ratio increase.

Figure B shows the percentage value of elongation at the break (*E*) of films was affected by the presence of chitosan content. The average *E* values of biodegradable films behaved inversely to the TS value whereby decreasing from 8.10900% to 8.096507% eventhough the value of *E* decreasing was not significant different, it still shows the *E* was reduced in the presence of chitosan. This phenomenon occur probably due to the increased crystallinity of starch in the blend film (Bourtoom *et al*. 2008). Regarding to Xu *et al*. (2005) statement was observed that the crystalline structure of waxy starch in the composite film was apparent by the addition of chitosan.

4.6 Degradation analysis

Table 4.4 : Degradation of composite biodegradable film weight

	Sample (chitosan : breadfruit starch)		
	A (1 : 2)	B (1 : 1)	C (3 : 2)
Weight of polyethylene bag, g	9.003	9.400	9.600
Weight of soil, g	1700	1700	1700
Weight of initial film, g	0.6000	0.6000	0.6000
3 day	0.1983	0.2449	0.3690

6 day	0.1537	0.1954	0.3238
9 day	0.1223	0.1528	0.2931
12 day	0.0931	0.1173	0.2519
15 day	0.0302	0.0895	0.1639
18 day	0.0063	0.0475	0.1174
21 day	0.0000	0.0353	0.060

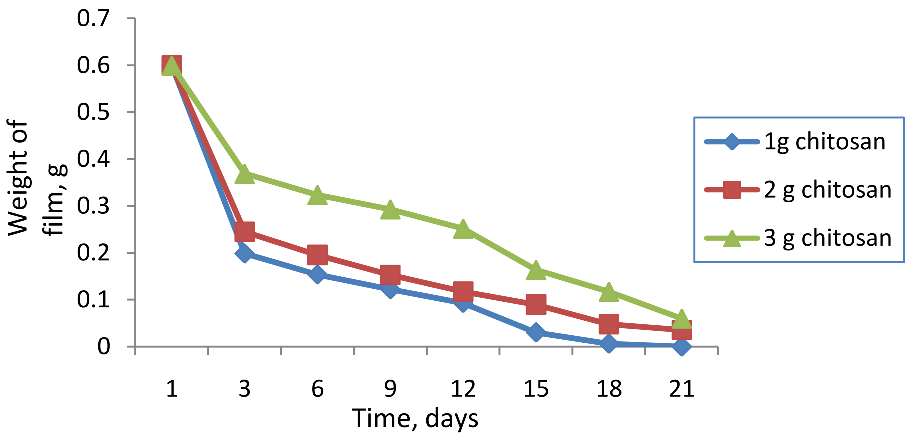


Figure 4.7 : graph of weight losses of composite biodegradable film versus time

The degradation analysis of composite film is proven that the film tendency to degrade under soil condition. The degradability of the composite biodegradable film was tested indirectly, through monitoring the loss of weight samples of the films in the soil under real field conditions. The data that prove is shown in the table 4.4. From the figure 4.7, it can be seen that the biodegradation rates of film 3g chitosan were slightly lower than film 1g of chitosan. In addition, the film 1g of chitosan good coherent totally degrade after 21 days. Regarding Xiong *et al. (2008)*, this phenomenon occur due to the chitosan made the miscibility and compatibility increase and form dense structure which reduce infiltration velocity of microorganisms. During stage 0-3 days relatively rapid degradation rate could be assigned to the degradation of chitosan and second stage at 3-21 days relatively slow degradation rate could be described to the erosion of the chitosan and starch component. This phenomenon related same with Rui *et al*., (2006) statement.

4.7 Breadfruit flour analysis

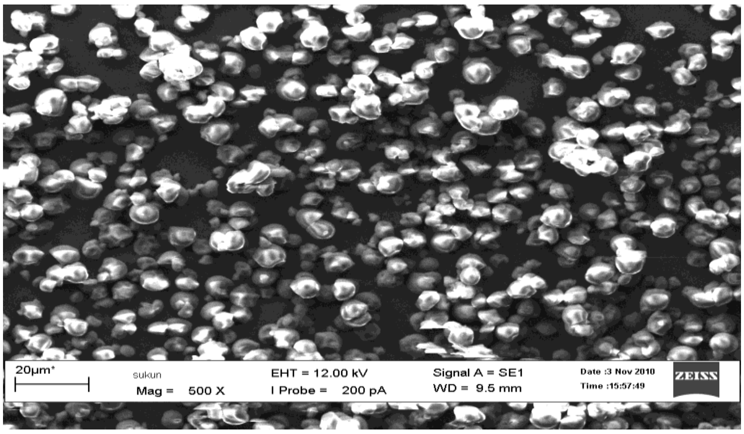


Figure 4.8 : Scanning electron microscopy of breadfruit flour structure

Regarding to the result analysis shows the structure of breadfruit flour was clearly observed under scanning electron microscopy (SEM). The structure has their granule and it will form starch when the flour undergoes gelatinization process. The size of structure looks like spherical shape that enhance the surface area of flour. According Kayode *et al*. (2004) reported partially attributed this increase in water absorption to the surface area of the starch phase and excessive dilution at high concentrations of starch of the continuous gluten phase. Therefore, it is also reasonable that the high water absorption capacity of the breadfruit starch. Regarding Kayode *et al*. (2004) statement, it has high carbohydrate content (76.7%) and has been used as an important source of energy. Functional properties of breadfruit as a component of composite flour have been already studied (Esuoso, *et al*. 1995). Therefore the high carbohydrate content of breadfruit makes it a valuable sources of starch. Regarding Akubor (1997) shows breadfruit flour has higher fat (11%), protein(17.06%) and caloric (415 kcal/kg). Regarding table 4.6 selected functional groups of water absorption capacity shows highest around 170.00% indirectly supported the Kayode *et al*., (2004) statement above.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Composite biodegradable film from breadfruit was fabricated successfully by casting technique. Composite biodegradable film also good antimicrobial film by presence of chitosan concentration due to the molecular hydrogen bonding between breadfruit starch and chitosan. Therefore the chitosan showed the qualities in the field of antimicrobial packaging due to antimicrobial activities and indicate that the film had synergistic antimicrobial effect when chitosan and breadfruit starch were combined. In terms of cross section morphology of the film were examined by SEM revealed that there was interaction and separation phase between starch and chitosan molecules. Meanwhile, in terms of physical characterization were examined by TS and *E* at break, TS of biodegradable film was increase by the addition of chitosan concentration. For *E* at break showed the *E* inversely proportional to TS. Regarding to chemical analysis by FT-IR revealed the interaction of hydrogen bonding between chitosan and breadfruit starch occur. In addition, in terms of water solubility of film , when the chitosan concentration increase the water solubility was decreasing and its shows the high water resistence and good for food packaging application.

5.2 RECOMMENDATION

Instead of study the composite biodegradable film, when the film had been keep it will cause volatile of the acetic acid. In my suggestion, it might be adjusted the pH of the film. In addition, my recommendation to study the time taken for the film totally undergoes degradation in terms of biodegradable films.

REFERENCES

Amalia T., Koushik S., Jeffrey D. K., Peter K., Pamela J. W., (2005). Functional properties of starch from normal and mutant corn genotypes. Carbohydrate polymer. 61 : 238-247.

Arvanitoyannis6, S.K. Mallapragada and B. Narasim, Editors, (2006). Handbook of Biodegradable Polymeric Materials and their Applications. 1 : 200–235.

A. Varki (1993). Biological roles of oligosaccharides. pp. 97–130.

Avella, M., De Vlieger, J. J., Errico, M. E., Fischer, S., Vacca, P., & Volpe, M. G. (2005). Biodegradable starch/clay nanocomposite films for food packaging applications. *Food Chemistry*. 93: 467-474.

Avérous, L. Avérous, C. Fringant and L. Moro (2001). Plasticized starch–cellulose interactions in polysaccharides composites, Polymer. 42: 6565–6572.

Bertuzzi, M. A., Armada, M., & Gottifredi, J. C. (2007). Physicochemical characterization of starch based films. Journal of food engineering, 82(1): 17-25.

B. Li, J.F. Kennedy, J.L. Peng, X. Yie and B.J. Xie, (2006). Preparation and performance evaluation of glucomannan–chitosan–nisin ternary antimicrobial blend film. *Carbohydrate Polymers* 65 : 488–494.

Clarinval and Halleux, A.-M. Clarinval and J. Halleux, (2005). Classification of biodegradable polymers. 3–29.

C.H. Grün, Anal (2006). Procedure to study carbohydrate–protein interactions Biochem. 354 : 54–63

D. Briassoulis (2007). Polymer Degradation and Stability. 92 : 1115-1132

Nath D. C. D, S. Bandyopadhyay, S. gupta, A. yu, D. Blackburn, C. White (2009). Surface-coated fly ash used as filler in biodegradable poly(vinyl alcohol) composite films: Part 1—The modification process

Espi E., A. Salmeron, A. Fontecha, Y. Garcia, A.I. Real (2006). Plastic films for agricultural applications, Journal of Plastic Film & Sheeting 22 : 85–102.

Esuoso, K.O. and Bamiro, F.O., (1995). Studies of the baking pproperties of non wheat flours breadfruit (Artocarpus artilis). Int. J. Food Sci. Nutr. 46 : 267-273

Flores, Fama, Rojas, Goyanes, and Gerschenson (2007).

Follain, Joly, Dole, & Bliard, 2005; Xiao & Yang, 2006; Zhai, Yoshii, Kume, & Hashim, 2002; Zhai, Yoshii, & Kume, 2002

F.D.S. Larotonda, K.N. Matsui, P.J.A. Sobral and J.B. Laurindo, (2005). Hygroscopicity and water vapor permeability of Kraft paper impregnated with starch acetate, *Journal of Food Engineering* 71 : 394–402

Han Guo Xiong, Shang W. T., Hua L. T, Peng Z. (2008). The structure and properties of a starch-based biodegradable film. *Carbohydrate Polymers.* 71: 263–268.

Hirano S., Seino H, Akiyama Y and Nonaka I (1994). Biotechnology and bioactive polymers.

Jesus J. B., Juan L. A., Francisco J. C., and Ana P., (1999). Free and protein-bound carbohydrate structure. 5: 549-555

June H. W., Tanuja S., Anthony H., Albert M. W., (2006). Carbohydrate recognition factors of the lectin domains present in the *Ricinus communis* toxic protein (ricin). *Biochemie.* 88 : 201-217

Jayasekara, Harding, Bowater, Christie, & Lonergan, (2004).

Kas, 1997; Singla and Chawla, 2001; Kato et al.,2003

K. Vimala, Y. Murali Mohan, K. Samba Sivudu, K. Varaprasad, S. Ravindra, N. Narayana Reddy, Y. Padma, B. Sreedha and K. (2010). Fabrication of porous chitosan films impregnated with silver nanoparticles: A facile approach for superior antibacterial application. *Biointerfaces* 76 :248–258

Kelei Z., jianji W., Yongkui Y., Hanqing W., (2000). Volumetric properties for the monosaccharide (d-xylose, d-arabinose, d-glucose, d-galactose)–NaCl–water systems at 298.15 K. *Carbohydrate research*. 328 : 383-391.

Kyoko O., Midori K., Atsuko S., Keiko H., (2007). Effects of acetic acid on the rice gelatinization and pasting properties of rice starch during cooking. *research International*. 40: 224-231.

K.S. Miller and J.M. Krochta, (1997). Oxygen and aroma barrier properties of edible films. *Trends in Food Science & Technology* 8 : 228–237.

Labuza & Breene,. T. Labuza and W. Breene, (1989). Applications of active packaging for improvement of shelf-life and nutritional quality of fresh and extended shelf-life foods. *Journal of Food Processing and Preservation* 13 : 1–89.

L. Rusu-Balaita, J. Desbrières and M. Rinaudo, (2003). Formation of a biocompatible polyelectrolyte complex: chitosan-hyaluronan complex stability, Polym Bull. 50 : 91–98

Maizura, M. Fazilah, A. Norziah, M. H Karim, A. A. (2007). Antibacterial activity and mechanical properties of partially hydrolyzed sago starch –alginate edible film containing lemongrass oil. *Journal of food science*. 72: 324-330

M. Berradaa, A. Serreqia, F. Dabbarha, A. Owusub, A. Gupta and S. Lehnert, (2005). A novel non-toxic camptothecin formulation for cancer chemotherapy. *Biomaterials*. 26 : 2115–2120

M.R. Rekha, C.P. Sharma, (2009). Synthesis and evaluation of lauryl succinyl chitosan particles towards oral insulin delivery and absorption. *Journal of Controlled Release.* 135 : 144–151

M. Rinaudo Prog. Polym. Sci. 31 (2006) 616–632

P. Appendini, J.H. Hotchkiss Innoatie, (2002). *Food Science & Emerging Technologies.* 3 :113-126

P.I. Akubor (1997). Proximate composition and selected functional properties of African breadfruit and sweet potato flour blends. *Plant Foods for Human Nutrition.* 51**:** 53–60

Pornsak S., Mark A. B., Ross A. K., (2006). Development of polysaccharide gel coated pellets for oral administration: 1. Physico-mechanical properties. *International Journal of pharmaceutics.* 326 : 80-88.

R.F. Tester et al. / Journal of Cereal Science 39 (2004) 151–165

Rui. D, Yun C., Pu C., Lina Z., Bing L., (2006). Properties and biodegradability of water resistence soy protein/poly-(caprolactone)/toluene-2,4-diisocyanate composite. *Polymer Degradation and Stability*. 91: 2189-2197

Salleh E., and I. I Muhamad (2007). Mechanical Properties and Antimicrobial Analysis of Antimicrobial Starchh-Based Film

Samuel Zalipsky (1995). Chemistry of polyethylene glycol conjugates with biologically active molecules. *Advance Drug Delivery Reviews.*16 : 157- 182.

S.B. Joo, M.N. Kim, S.S. Im and J.S. Yoon, (2005). Biodegradation of plastics in compost prepared at different composting conditions. *Macromolecular Symposia* **224** :355–365

S. Mali, L.S. Sakanaka, F. Yamashita and M.V.E. Grossmann, (2005). Water sorption and mechanical properties of cassava starch films and their relation to plasticizing effect. *Carbohydrate Polymers.* 60 : 283–289

S. Koide, J. Shi, (2007). Microbial and quality evaluation of green peppers stored in biodegradable film packaging. *Food Control* 18 : 1121–1125

S. J. Hermansky, D. A. Neptun, K. A. Loughran and H. W. Leung, (1995). Effects of Polyethylene Glycol 400 (PEG 400) Following 13 Weeks of Gavage Treatment in Fischer-344 Rats. Food and chemical toxicology. 33 : 139-149.

Stuchell, Y. M., & Krochta, J. M. (1994). Enzymatic treatments and thermal effects on edible soy protein films. *Journal of food science*. 59 : 1322-1337

T. Chandy and C.P. Sharma, (1993). Chitosan matrix for oral sustained delivery of ampicillin. *Biomaterials.* 14 : 939–944

T. Lason, Aggeliki Martinou, Dimitris Kafetzopoulos and Vassilis Bouriotis .Chitin deacetylases: new, versatile tools in biotechnology.

T. Bourtoom, M .S . Chinnan. (2008). Preparation and properties of rice starch-chitosan blend biodegradable film. *Food Science and Technology.* 41:1633-1641

Wajira S. Ratnayake and David S. Jackson (2008). Advances in Food and Nutrition Research . 55 : 221-268

Xiao Li, S., Jia, M. W., Yonghong, C., Guohua, Z. (2010). Antimicrobial and physical properties of sweet potato starch films incorporated with potassium sorbate or chitosan. *Food hydrocolloids*. 24: 285–290

Xu, X. Y., Kim, K. M., Hanna, M. A., & Nag, D. (2005). Chitosan-starch composite film: preparation and characterization. *Industrial crops and products*. *21* :185-192

V. Alves et al. / Desalination 199 (2006) 331

V.R. Sinha et al. / International Journal of Pharmaceutics 274 (2004). 1-33

Yan Lu, Li Wang, Baohui Zhao, Guangsen Xiao, Yi Ren, Xudong Wang, Chenxi Li, (2008). Fabrication of conducting polyaniline composite film using honeycomb ordered sulfonated polysulfone film as template Thin Solid Films. 516 : 6365–6370.

Zaharoff D. A., Rogers C. J., Hance K. W., Schlom J., Greiner J. W., Chitosan solution enhances both humoral and cell-mediated immune responses to subcutaneous vaccination.

Z. Osman, A. K. Arof (2003). FTIR studies of chitosan acetate based polymer electrolytes. 993-999.