Xylan-based Nicotiana Tabacum Stalks Derived Active Food Packaging

Ahmad Zaid Azri bin Ahmad Zakaria
F15A0271

A report submitted in fulfillment of the requirement for the degree of Bachelor of Applied Science Bioindustrial Technology with Honours

FACULTY OF BIOENGINEERING AND TECHNOLOGY
UNIVERSITI MALAYSIA KELANTAN

2019

DECLARATION

I hereby declare that the work embodied in this Report is result of original research except for citations and quotations. I also declare that it has not been previously submitted to any other degree of any institutions but Universiti Malaysia Kelantan.

____

Student

Name : Ahmad Zaid Azri bin Ahmad Zakaria

Matric No. : F15A0271

Date : 10 December 2018

I certify that the report of this final year project entitled "Xylan-Based Nicotiana Tabaccum Stalk Derived Active Food Packaging" by Ahmad Zaid Azri bin Ahmad Zakaria, matric number F15A0271 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Bioindustrial Technology) with Honors, Faculty of Bioengineering and Technology, Universiti Malaysia Kelantan.

____

Supervisor

Name : Dr. Siti Roshayu binti Hassan

Cop :

Date : 10 December 2018

ACKNOWLEDGEMENT

In completing my final year project, I received a lot of help and assistance from people around me. So with this opportunity, I would like to express an endless thanks to people who involved and gave support either directly or indirectly in order to finish this project.

First and foremost, I would like to thank Allah who gives His blessing upon me and also for giving me strength to finish this project. Next, to the one who always inspired me to finish the project, Dr. Siti Roshayu binti Hassan, I would like to express my deepest gratitude for she always finding time to help me. With her advices, encouragement, guidance and also endless support, I manage to finish this project successfully.

Furthermore, not forgetting the main reason why am I doing this study, I would like to give an eternal gratitude to my family who gave me countless of help and support for me to finish this study. Last but not least, thanks to all my friends who have always been there for me since the first day I picked this project as my final year project.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

°C	Degree celcius
g	gram
wt %	Weightage percentage
mL	Millilitre
mm	Millimetre
rpm	Rotation per minute

Xylan-Based Nicotiana Tabaccum Stalk Derived Active Food Packaging

ABSTRACT

This study is about production of active material food packaging from natural resources which is xylan. The xylan used were extracted from tobacco stalk using DMSO extraction producing water soluble xylan. The formulation of food packaging consist of tapioca starch, glycerol and xylan. The tapioca starch used to produce thin film, glycerol act as plasticizer while xylan used as film enhancement. The formulation of sample packaging was divided into five which are 0.0 wt%, 0.1, wt%, 0.3 wt%, 0.5 wt% and 1.0 wt%. From texture analysis, 0.3 wt% has the optimum formulation to produce food packaging from tobacco stalk. After obtaining optimum formulation, the formulation was added with 1.5 wt% of lemongrass essential oil for antifungal properties. Two samples were prepared to show the difference between the presence and absence of essential oil. Several test has been conducted to ensure that the food packaging is long lasting, has bio-degradable and antifungal ability and also to check the tensile of the food packaging. The end result show that 0.3 wt% with lemongrass essential oil can inhibit fungus growth compare to 0.3 wt% without lemongrass essential oil.

Tangkai Tembakau Berasaskan Xylan Sebagai Aplikasi Pembungkusan Makanan Aktif.

ABSTRAK

Kajian ini adalah tentang penghasilan bahan aktif pembungkus makanan dari sumber natural iaitu xylan. Xylan yang digunakan diekstrak dari batang tembakau menggunakan pengesktrakan DMSO menghasilkan xylan yang larut di dalam air. Formula bagi pembungkus makanan ini mengandungi kanji ubi, gliserol, dan juga xylan. Kanji ubi digunakan untuk menghasilkan filem nipis, gliserol pula digunakan sebagai pemplastik manakala xylan digunakan untuk menambahbaik filem. Formula bagi pembungkus makanan telah dibahagikan kepada lima iaitu 0.0 wt%, 0.1, wt%, 0.3 wt%, 0.5 wt% dan 1.0 wt%. Melalui analisa tekstur, 0.3 wt% mempunyai formula optimum untuk menghasilkan pembungkus makanan dari batang tembakau. Setelah formula optimum diperolehi, formula ini telah ditambah dengan pati minyak serai wangi yang mempunyai peratus berat sebanyak1.5 wt% untuk ciri-ciri anti-kulat. Dua sampel telah disiapkan untuk menunjukkan perbezaan antara kehadiran dan ketidakhadiran pati minyak. Beberapa ujian telah dilakukan untuk memastikan pembungkus makanan tahan lama, bersifat terbiodegradasi dan kebolehan anti-kulat serta memeriksa tensil pembungkus makanan. Keputusan akhir menunjukkan 0.3 wt% yang mempunyai pati minyak serai wangi boleh membantutkan pertumbuhan kulat berbanding 0.3 wt% tanpa pati minyak serai wangi.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Plastic are polymers with assemble of identical chemical subunits, called monomers (Judith, 2016). The monomers linked together to form chains and form structure. According to the IUPAC terminology, plastic is a polymetric material that may contain other substances to improve performance and reduce cost and can be molded into various shapes. They are usually formed by synthetic reaction and displays non-degradable and non-renewable properties. Therefore, the amount of plastic waste is increasing through over the year and cause pollution. For about 50 years more, global production and consumption of plastic have known to be continuously rising. Currently, production of petrochemical-based is about 99% in the market (Science Learning Hub, 2014). It is estimated that about 299 million tons of plastics were produced in 2013 and sharing enormous upward trend over the past years (Gaelle Gourmelon, 2015). Plastic is relatively inexpensive and have various functions but can lead to pollution to environment.

Bioplastic is types of plastic that produce from natural biopolymers include starches, cellulose derivatives, chitosan/chitin, gums, proteins (animal or plant-based) and lipids. Commercial plastics and bio-based plastic act similar in function but different in base raw material. Commercial plastic made of about 4% of oil that the world uses every year. Bioplastics predicted to increase in production between 10% to 30% in total plastic market by 2020 (Science Learning Hub, 2014).Bioplastics is made from biopolymer which is renewable and sustainable alternative from oil-based plastics (Chen, 2014). Bioplastic is biodegradable which mean it can be decomposed naturally and locally with the help of microbes in a suitable environment. Natural feedstock are commonly used to create bioplastic include corn, potaotes, rice, wheat and palm fibres. Bioplastic can transformed into plates, cutlery, bottles, bag, textiles, and packaging material especially food (Buildabroad, 2017). Bioplastic can save environment because it can be degrade.

There are many function of bioplastics especially in packaging includes food, automobile, appliances and product. The main objective of this study is focusing only on food packaging. Food packaging is majorly used in almost every food and beverages industries. The main advantage of bioplastic is it has lower cost needs in disposing it.

1.2 Problem Statement

Existing commercial petroleum-based plastics usage as food packaging is too excessive. Bioplastics has developed for years. Some researcher have used sago starch-alginate edible film (Maizura et al , 2007). Many researcher try to improve the stability and safety of food packaging (Cutter, 2006). But there are still some drawbacks to previous formulation of film. Bioplastic film that easily burst and rupture are not a good for food packaging

Material that involved in bioplastic film formulation is the main factors in producing good film. To solve the drawback of a bioplastic film, raw materials that were used must have good properties to enhance the film. For examples Huq et al. (2012) used alginate with cellulose, Vasconez et al (2009) used chitosan-tapioca starch film and Kanmani et al. (2014) used carrageenan/grapefruit seed. Some researcher produces bioplastic film from rare material which will cost higher price to obtain.

This study use xylan-tapioca starch to form bioplastic film as active food packaging application. Xylan was taken from tobacco plant. Tobacco plant has leave and stalk. The leaves will be taken as nicotine sources due to high content of it. The stalks usually were burn or disposed for further used. So this study takes a step further step to reduce waste of renewable material that contain beneficial component besides reduce pollution to environment. Xylan indeed has its own advantages but there is a drawback of xylan film in term of its high internal cohesion, which can result in fragmented and fragile films which cannot be used in food packaging application.

1.3 Objectives

The objectives of this study are:

- To determine the optimum xylan based on the physical and mechanical properties of starch-based bioplastic film

- To investigate the antimicrobial properties of bioplastic film with addition of lemon grass essential oil.

1.4 Scope of Study

The scope of this study includes the finding formulation and the optimum composition of xylan in the tapioca starch bioplastic film which use different percentage of xylan in each film. Characterization of each film will determine the good film by mechanical, physical and chemical properties. The antimicrobial properties determined by addition of lemon grass essential oil which lead to inhibition by bacteria and fungus.

1.5 Significance of Study

This study will lead to a new innovation of bioplastic film which more advances and have more good properties in future. This bioplastic film also gives new information about new raw material used and formulation. The film physical, mechanical and chemical characterization can be compared to others from past and future research. Good properties for a film can be enhance to produce more suitable and relevant for active food packaging.

CHAPTER 2

LITERATURE REVIEW

2.1 Food Packaging

Food packaging is a food wrapping in order to preserve freshness and quality of food for elevated period of time (Cutter, 2006). It is the best technique to preserve the food throughout the distribution chains. The distribution chain is from manufacturer to the dealer and then reach costumer. In other words, packaging as a barrier that protect food from environmental influences such as moisture, light, dust, pests, volatiles, both chemical and microbiological contamination. The packaging is primarily passive component that acts as the barrier between the foods and/or the atmosphere and the external environment (Bettina & Kvalv, 2018). For fresh food, its need highly gas permeable or perforated packaging material because it can prevent the gas exchange throughout packaging (Hussein et al., 2015). Without proper packaging, the food can be contaminated by impurities. Recent years shows that many researchers trying to develop a new model of food packaging with increased shelf life, safety and quality of food. Prolonged shelf life of a food before being consume benefits consumer (Robertson, 2010).

Due to evolution of the global food supply chains, packaging systems has to be enhanced. Packaging also prevents wasting food supply. Good food packaging elevated the shelf life of the food itself. So the food material can withstand longer time consuming until its usage. The decreasing in food waste will reduce the cost of unneeded waste management. According to the World Health Organization (WHO), the food waste in low developed countries is between 30% and 50% due to improper food preservation, protection, storage, and transportation. In developed countries, in contrary the food waste is only 2-3%. The low efficiency of waste management can lead to much greater financial loss than that the cost of food spoilt. Figure 1 shows the distribution chain of food from producer to the consumer (Coles & Kirwan, 2003).

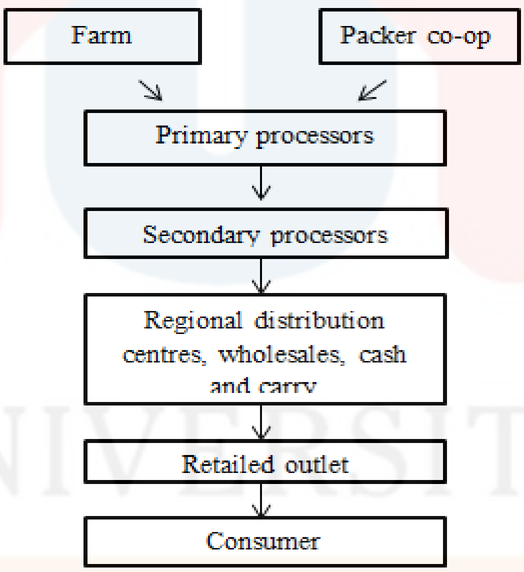


Figure 2.1: Food distribution systems (adapted from Paine & Paine, 1983)

Packaging that involve food science development, processing and preservation technique will lead to safety towards consumer. The successful of the food technology reflects by the fact that the billions of packs of food are being safe to be consumed every day (Coles & Kirwan, 2003).

In society aspect, good looking packaging will attract media publicity and increase the marketing region. The solid waste from food products also will lessen and promotes recycling food waste for animal feed and compost fertilizer. Besides that, the bulk purchase and distribution cost will be lower and efficient with less product damage during transportation. In addition, tampering and adulteration can be avoided due to secure packaging. Safe packaging increases the hygienic of the food (Coles & Kirwan, 2003).

The awareness of packaging is related to packaging litter and the volume of the packaging after becoming waste. The cost of disposal and recovery are one of the important factor of producing good packaging. In environment context, pollution is a major problem that needs to be handles. The packaging must not cause pollution or less impact pollution. The invention or new innovations of packaging is associated to less usage of environment sources.

Food packaging must have certain design and criteria that must be followed to produce a good packaging. The design of packaging must be compatible with a certain food and following the distribution standard. These includes the material used, physical factors, chemical factors, microbiological factors, sizing, durability, containment factor, shelf life, and impact to the surrounding and consumer (Coles & Kirwan, 2003).

2.2 Food Packaging Material

Materials used for food packaging is a vital factor in producing good and efficient food packaging. To improve some quality of food product, the material used has to be modified as well as the food product needs. The type of material used will determine the shelf life of the product (Betty & Marsh, 2007). The material used must be suitable for certain type of food that wanted to be wrapped. The aim of changing the material is a purpose to approach to good packaging design for safety food, ecological friendly and cost effective on the process and performance of the packaging system (Coles & Kirwan, 2003). Different material give different in term of its characteristics, features and performances (Yam, 2009). Materials that have been traditionally used in food packaging includes glass, metals paper and paperboards, and plastics (Betty & Marsh, 2007).

2.2.1 Active Food Packaging

There is an increasing trend to natural high-quality foods, which are not being processed or minimized and does not have any preservatives content. but have an acceptable shelf-life (Singh et al., 2011). Due to this trend, the function of food packaging has to be improvised and refined to develop a good packaging (Zhuang et al., 2014). Active packaging is one of the innovative method to maintain or prolong the shelf life of food product while ensuring their quality, safety and integrity (Bettina & Kvalv, 2018). Active packaging systems is the interaction the food and surrounding environment. (European Commission, 2009). Active packaging systems can be divided into two systems such as scavenging (absorbers) and active-releasing systems (emitters).

Usually active substances such as antimicrobials and antioxidants will prolong the food inside the packaging. The fact that any contaminations from microbial growth can occur on the surface of the food. Therefore, the addition of active substances in the active packaging is more efficient than added into a food (Bettina & Kvalv, 2018). Many variety of active packaging systems has been developed and numerous reviews have emphasized the potential of active packaging technologies to supply safer, healthier, higher-quality foods to the consumer (Kuorwel and others 2015).

2.2.2 Glass

Glass can be one of the material that makes food packaging. Glass containers used in food packaging are often surface-coated to provide lubrication in the production line and eliminate scratching or surface abrasion and line jams. Glass coatings also increase and preserve the strength of the bottle to reduce breakage from physical contact. Improved break resistance allows manufacturers to use thinner glass, which reduces weight and is better for disposal and transportation (McKown, 2000). Glass is odorless and chemically inert with virtually all food products. So glass has several advantages for food-packaging applications.

One of the advantages of glass is impermeable which completely isolated the product and the surrounding, so it maintains product freshness for a long period of time without disturbing the taste or flavor. Glass has ability to withstand high processing temperatures which makes glass has the ability for heat sterilization of both low- acid and high-acid foods. Glass is rigid, have good insulation, and can be produced in numerous different shapes. The transparency of glass allows visually let consumers to see the product, yet variations in glass color can protect light-sensitive contents. Finally, glass packaging benefits the environment because it is reusable and recyclable. Like any material, glass has some disadvantage. Despite efforts to use thinner glass, its heavy weight increases the ttransportation costs. Another concern is its brittleness and susceptibility to breakage from internal pressure, impact, or thermal shock (Betty & Marsh, 2007).

2.2.2 Metal

Metal is a most versatile packaging. It has a combination of excellent physical protection and barrier properties, formability and decorative potential, recyclability, and consumer acceptance. There are two metals which has major used in packaging are aluminium and steel. Aluminium commonly used to make cans, foil, and laminated paper or plastic packaging. Magnesium and manganese are often added to aluminium to improve its strength properties (Page and others 2003). Aluminium is highly resistant to most forms of corrosion and provides a highly effective barrier to the effects of air, temperature, moisture, and chemical impurities. Aluminium has good flexibility and surface resilience, excellent malleability and formability, and outstanding embossing potential.

Laminates and metallized films is another type of packaging involves aluminium. Lamination of packaging involves the binding of aluminium foil to paper or plastic film to improve barrier properties. Although lamination to plastic enables heat seal ability, the seal does not completely bar moisture and air. The facts that laminated aluminium is expensive, it is usually used to package high value foods such as dried soups, herbs, and spices. A less expensive alternative to laminated packaging is metallized film. Metallized films are plastics embedded with thin layer of aluminium metal (Fellows and Axtell 2002). These films have improved packaging barrier properties to moisture, oils, air, and odours, and the highly reflective surface of the aluminium is attractive to consumers. Metallized film is more flexible.

2.2.4 Paper and Paperboard

Paper and paperboard are sheet materials made from cellulose fibres derived from wood. Paper and paperboards are commonly used in corrugated boxes, milk cartons, folding cartons, bags and sacks, and wrapping paper. Toilet paper, paper plates, and cups are other examples of paper and paperboard products. Plain paper is not used to protect foods for long periods of time because it has poor barrier properties and is not heat sealable. When used as food packaging, paper is almost always treated, coated, laminated, or impregnated with materials such as waxes, resins, or lacquers to improve functional and protective properties. There are many different types of paper used in food packaging which are kraft paper, sulfite paper, greaseproof paper, glassine and parchment paper. Paperboard is thicker than paper and made by multiple layers. Used commonly for shipping such boxes, cartons and trays. The type of paperboard are white board, solid board, chipboard fibreboard (Betty & Marsh, 2007).

2.2.5 Plastics

Plastic is a material that has the greatest production in food packaging because of low production cost. Common material that meets these criteria are polyethylene- and co-polymer that is used as a based material that is flexible, safe, low cost and also versatile (Tice, 2003). There are some advantages of using plastic as food packaging. Fluid and mouldable, plastics can be made into sheets, shapes, and structures for flexibility. They are chemically resistant. In fact, many plastics are heat sealable, easy to print, and can be integrated into production processes where the package is formed, filled, and sealed in the same production line. The major disadvantage of plastics is their variable permeability to light, gases, vapours, and low molecular weight molecules.

There are two major categories of plastics which are thermosets and thermoplastics. Thermosets are polymers that solidify or has irreversibly shape when heated and cannot be remoulded. They are strong, durable and they tend to be used primarily in automobiles and construction applications such as adhesives and coatings, not in food packaging applications. On the other hand, thermoplastics are polymers that soften upon exposure to heat and return to their original condition at room temperature. Thermoplastics can easily be shaped and molded into various products such as bottles, jugs, and plastic films, so they are ideal for food packaging. Moreover, thermoplastics are recyclable which can be melted and reused as raw materials for production of new products. But it faces health concerns regarding residual monomer and components in plastics, including stabilizers, plasticizers and condensation components such as bisphenol A. To ensure public safety, the food organization have to carefully use the good packaging for their product.

Despite these safety concerns, the use of plastics in food packaging has continued to increase due to the low cost of materials and functional advantages such as thermosealability, microwavability, optical properties, and unlimited sizes and shapes over traditional materials (Lopez-Rubio and others 2004). Many types of plastics are being used as materials for packaging food, including, polyester, polystyrene, polyamide, and ethylene vinyl alcohol. There are more than 30 types of plastics have been used as packaging materials (Lau and Wong 2000).

Polyolefin is a term for polyethylene and polypropylene, these 2 most widely used plastics in food packaging. Polyethylene and polypropylene possess a successful combination of properties, including flexibility, strength, lightness, stability, moisture and chemical resistance, and easy in production process, and are suitable for recycling and reuse. The simplest and cheapest plastic made by addition polymerization of ethylene is polyethylene. It used as film applications where heat sealing is necessary such as bread and frozen food bags, flexible lids, and squeezable food bottles. Polyethylene bags are sometimes reused for grocery and non-grocery purpose. Milk bottles are especially recycled.

Next is polyesters. Polyethylene terephthalate (PET or PETE), polycarbonate, and polyethylene naphthalate (PEN) are polyesters. The most commonly used polyester in food packaging is Polyethylene terephthalate (PETE). PETE has a good barrier to gases oxygen, carbon dioxide and moisture. It also has good resistance to heat, mineral oils, solvents, and acids, but not to bases. PETE has become the major choice for many food products, particularly beverages and mineral waters packaging. The use of PETE to make plastic bottles for carbonated drinks is increasing steadily (van Willige and others 2002). The main factor of its popularity are it is glass-like transparency, adequate gas barrier for retention of carbonation, light weight, and shatter resistance. The 3 major packaging applications of PETE are containers such as bottles, jars, and tubs, semi rigid sheets for thermoforming, and thin-oriented films. PETE exists both as an amorphous which is transparent and a semi crystalline thermoplastic material. Amorphous PETE has better ductility but less stiffness and hardness than semi crystalline PETE, which has good strength, ductility, stiffness, and hardness. Recycled PETE from soda bottles is used as fibers, insulation, and other nonfood packaging applications (Betty & Marsh, 2007).

a) Bioplastics

Bioplastic is one of the initiative towards green technology of plastic usage that is made up of natural resources such as biopolymers include starches, cellulose derivatives, chitosan/chitin, gums, proteins from animal or plant and lipids. A good quality of bioplastic must have a long shelf life, antimicrobial properties, high in term of durability including have flexibility, printability, transparency, barrier, heat resistance, gloss and many more (Bioplastics, Retreived on April 10, 2018). Bio-based plastics can help to reduce the dependency on limited fossil resources, which are expected to become significantly more expensive in the future because it uses several method to isolate natural compound. It is also degradable and renewable (Bioplastics, 2014)

Application of edible film (bioplastic) as food packaging has 2 types which are direct and indirect method. Direct application is a number of method that been employed, including foaming, dipping, spraying, casting, brushing, wrapping, or rolling (Cutter, 2006). In indirect applications, casting technologies may be employed. In this process, the film-forming solution must be poured down onto a smooth, flat and level surface with or without a mold to contain the solution and allowed to dry and set. Applying film with gel or coating should exhibit number of functional properties, such as moisture barrier ability, water or lipid solubility, color, transparency, desired mechanical or rheological characteristics and non-toxic. These can be influenced by addition of compound, plasticizers, cross-linking agent, antimicrobial, antioxidant, or textural additives. Film must also resist to abrasion and breakage (Arzu, 2015).

One of the common main composition of bioplastics is starch. Starch are composed of amylose and amylopectin. Most of starch derived from cereal grains, potatoes, tapioca, or arrowroot. Starch-based film exhibit physical characteristics similar to plastic film which is odorless, tasteless, colorless, and non-toxic, biologically absorbable, semi- permeable to gases. Higher content of amylose will result in flexible, oxygen impermeable, oil resistant, heat-sealable, and water soluble.

Edible films form from natural source are unable to inhibit the growth of microbial on the surface of the food and fruits products. Addition several types of antimicrobial agent extend the shelf life of the food products in improving the functionality of biocomposite film (Atef et al., 2015). Preferences for this study is to use the natural instead of synthetic antimicrobial such as plant extract for food preservation. High content of phenolic compound are mostly attributed in herbs and spice essential oil extract which can enhance the inhibition.

To improve the edible film qualities, combination of various formulation of edible material has recommended. Two or more edible material needs for combination of a new composite that can improve in gas exchange, adhesiveness to coat products, have moisture vapor permeability properties and also antimicrobial and antifungal properties. Plasticizers is an important component to modify mechanical properties of composite film such as glycerol.

b) Properties of Bioplastic

One of the properties of bioplastic is it can be biodegradable with using renewable sources (Science Learning Hub, 2014). Biodegradable can happen to bioplastics when it is under certain environment condition. Bioplastic also must have a high durability to many purposes. Bio-based, durable plastics, such as bio-based polyethylene (PE) or bio-based polyethylene terephthalate (PET) have properties that are identical to the conventional versions (European Bioplastics, 2016). These bioplastic is equivalent to their fossil counterparts which can reduce a product's carbon footprint. Additionally, they can be mechanically recycled according to existing recycling system. Many more enhancement have been made by many researcher to increase the properties such as flexibility, printability, transparency, barrier, heat resistance and gloss (European Bioplastics, 2016).

CHAPTER 3

METHODOLOGY

3.1 Research Flow Chart

This study involve the raw material preparation, lemongrass essential oil extraction, xylan extraction, culturing bacteria strains, bioplastic film formation and characterization. Figure 3.1 shows the overall process of this study.

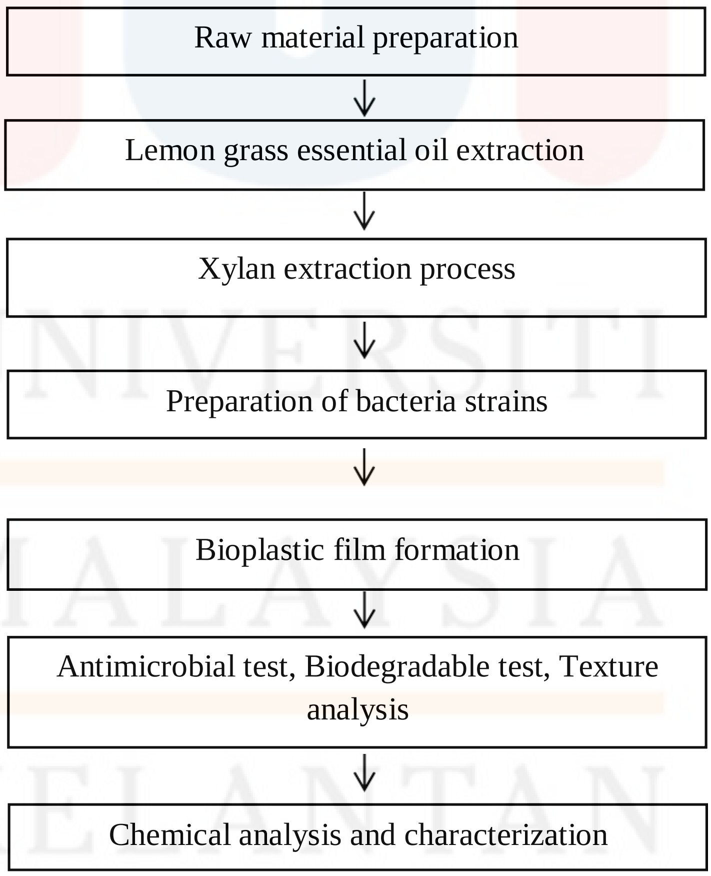


Figure 3.1: Flow chart overall process.

3.2 Raw Material Preparation

Stalks of *Nicotiana tabacum* were collected from Bachok, Kelantan. The tobacco stalks were cut into small pieces and were grind into fine powder before undergo drying process until the tobacco is completely dried. Tapioca starch were purchased from local store in Jeli, Kelantan. Lemon grass were obtained by using steam distillation process.

3.3 Xylan Extraction Process

Extraction of xylan was done by dimethyl sulphoxide (DMSO) extraction which were then produced the water-soluble hemicellulose. Tobacco stalks powder were mixed with DMSO solution with approximately 14mL/g ratio. The extraction process then was left overnight at room temperature for 18 hours. The extract was centrifuged at 5000 rpm for 20 minutes followed by neutralization (pH 5.50) with glacial acetic acid. Then crystallization process taken place by addition of 95% of ethanol at double volume. The solution was then centrifuged at 8000 rpm for 10 minutes at 4 ^{o}C. The xylan was obtained after the samples were dried in drying oven until a constant weight obtained. Pure xylan then stored until further used.

3.4 Preparation of Lemongrass Essential Oil

Lemon grass essential oils extracted by using steam distillation method (Attokaran, 2011). The apparatus for steam distillation set up with 100 g of lemongrass powder. The extraction process was run until there is no more essential oils were evaporated. It uses reflux technique. The collected distillate obtained organic solvent, essential oil and water. The organic solvent was then removed by liquid-liquid extraction using dichloromethane (DCM) same value as the distillate. Sample were then added with 5 g of sodium sulphate to remove water.

3.5 Preparation of Bacterial Strains

Bacterial strain which is *Eschericia coli* were obtained from the culture stock from Universiti Malaysia Kelantan. The stock culture then was grow with nutrient agar (NA) in petri dish to maintain its availability for this research. The strains were reserved in incubator with the supplement of 30% glycerol and are stored at -80 ⁰C until used.

3.6 Xylan –based Bioplastic Film Formation

First formulation consists of 10 g of gelatinized tapioca starch with 50mL distilled water, 3 g xylan, 5 mL glycerol. All component was mixed and heated at 90°C with controlled stirring for 30 min. The film then casted on the aluminum foils. The film dried in room temperature for two days (Kanmani, 2014).

Second formulation use 5 sample that were prepared by casting. Formulation of the film consists of 5 wt.% tapioca starch, 1.5 wt.% of glycerol which act as plasticizer in order to have better stabilizing and durable packaging (Rhim & Ng, 2007), 93.5 wt.% of distilled water. Percentage of xylan added by replacing the percentage of distilled water. All components were mixed and homogenized at 25°C and with controlled stirring. The homogenized mixture was heated up to 96°C for 40 minutes to fully gelatinize the starch (Medina et al, 2017). Bioplastic mixture was then casted on a petri dish as moulding medium. 5 sample of different formulation have as shown in Table 3.1.

Table 3.1: Formulation of 5 samples of film

Sample	Tapioca starch (wt%)	Glycerol (wt%)	Distilled water (wt%)	Tobacco Xylan (wt%)
Sample A	5	1.5	93.5	0.0
Sample B	5	1.5	93.4	0.1
Sample C	5	1.5	93.2	0.3
Sample D	5	1.5	93.0	0.5
Sample E	5	1.5	92.5	1.0

3.7 Xylan-based Bioplastic Film Formation with Essential Oil

The optimum bioplastic film formulation was added with lemon grass essential oil. Similar sample preparation was done with addition 1.5% lemon grass essential oil to the film mixture and was homogenized for 2 minutes. Then bioplastic sample was casted on petri dish.

3.8 Texture Analysis

The samples were analyzed in context of surface, flexibility, and color. Analysis of the texture were done using CT texture analyzer to measure the hardness, deformation at hardness, load at target, deformation at target, and fracturability.

3.9 Biodegradable Analysis

The best bioplastic formulation undergoes biodegradable analysis. Four samples were prepared which were 0.3% of xylan with lemon grass essential oil, and without essential oil also 0.0% of xylan which was control with essential oil and without essential oil. Biodegradation process were run according to soil burial test. Samples were buried for five days with similar depth and conditions. Each day, the samples replicates were taken out and the biodegradation rate were calculated. The rate of biodegradation calculated by mass reduction calculation. (Dinesh, 2016).

$$Biodegradability\ rate(\%) = \frac{initial\ mass - final\ mass\ (g)}{initial\ mass}\ x\ 100 \qquad (3.1)$$

3.10 Tensile Strength Test

Bioplastic samples with 0.0% and 0.3% of xylan were tested for their tensile strength. Both is added with lemon grass oil. The samples were cut in 5cm x 1cm and manually being pulled on a ruler. The first rupture stopped the process and the elongation distance was recorded.

3.11 Antimicrobial Test

100 µL of each bacterial strains (*Escherechia coli*) streaked on the nutrient agar by cotton swab. Nutrient agar plate divided into 6 region include control, essential oil and sample selected. The antimicrobial test plate was incubated for 24 hours for culture bacteria. Result was taken the next day as presence or absence of inhibition zone.

3.12 Chemical Analysis

3.12.1 Fourier-transform Infrared Spectroscopy (FTIR)

FTIR were used to determine the composition of functional group of xylan, lemon grass and bioplastic films.

3.12.2 Scanning Electron Microscopy (SEM)

SEM was used to determine the physical condition under several magnifications. Xylan and sample with 0.3% of xylan without lemon grass essential oil were tested before and on day 3. Additionally, sample day 5 0.3% xylan were tested also for comparison.

3.12.3 Thermogravimetric Analysis (TGA)

TGA used to measures the weight changes associated with thermal event. TGA were used to determine the compositional analysis and thermal stability. It also predicted the short-term and long-term thermal stability. 4 samples were analysed by TGA which is 0.3% xylan with and without lemongrass oil also 0.0% xylan (control) with and without lemongrass oil.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Xylan-based Bioplastic Film Formulation

There were 2 formulations that had been suggested in this study. Both of the formulation were shown and discussed.

4.1.1 First formulation

The result from first formulation show that the bioplastic films were too sticky because due to high content of glycerol in the composition. The bioplastic films also do not dried after 2 day and extend until 4 days. Yet, the film still not completely dried. This happened because of the high viscosity and less content of starch. Figure 4.1 shows the bioplastic film. The bioplastic film was clear and has a shine surface.

Figure 4.1: Bioplastic film of first formulation

4.1 (b) Second Formulation

The result from second formulation show the bioplastic film was well dried. The film was clear and can be easily peeled off from the mold which was petri dish. The bioplastic film was thin and suitable for active food packaging purposes. The film has some adhesiveness due to glycerol content and well stick to its film. Film sample C (see Table4.1) shows the best film compared to others in physical aspect. Figure 4.2 shows the five sample of different formulation.

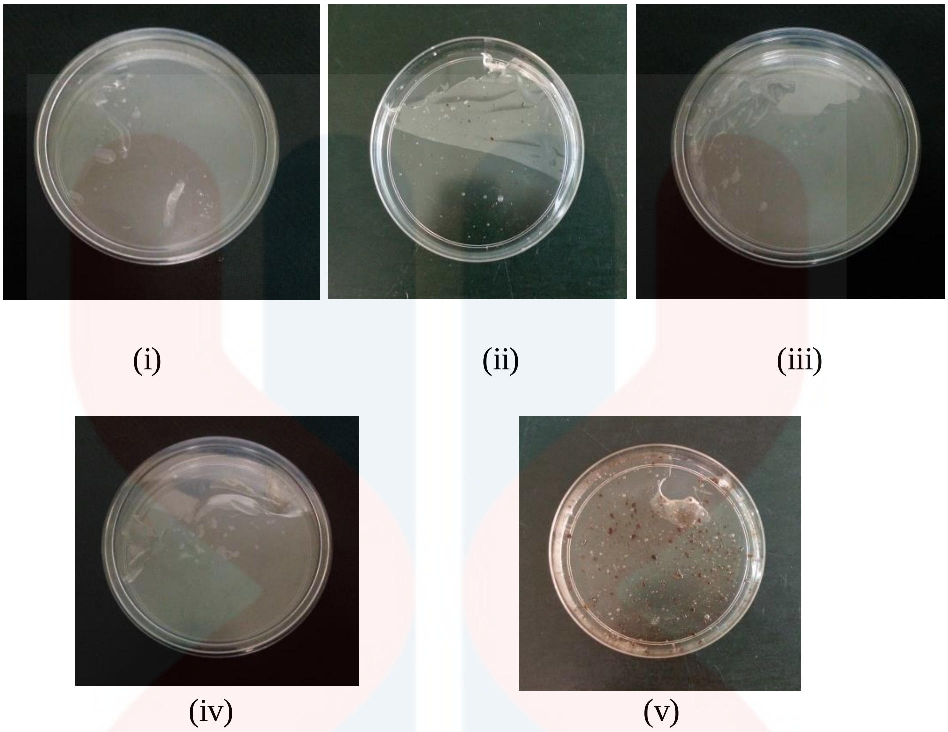


Figure 4.2: 5 samples of different xylan composition; (i) 0.0%, (ii) 0.1%, (iii) 0.3%, (iv) 0.5%, (v) 1.0%

Table 4.1 indicates the texture analysis. Result show that sample A (0.0% xylan) has dull surface and has less flexibility because of no other composite beside starch. So no modification takes place. Sample B shows slightly dull because it contains 0.1% xylan. One of the function of xylan is xylan can create a fine thin film. So in proportion of 0.1% the surface of the sample can be slightly dull. Sample C has slight less dull than sample B due to composition of xylan is 0.3% which is higher with a significant flexibility. Overall sample C is a good film because the film shines and clear. Sample D has slightly shiny but still has dull surface. Sample E has dull surface with undissolved xylan. It is because of the formulation used in this study be a limiting factor and it already exceeded. So this formulation cannot support the excessive xylan result of undissolved xylan in the mixture. This shows that the supported percentage of xylan composition is only at 0.5% in this study. Overall of these samples, there are no change in color but for sample E, it is slight yellow in color because of the undissolved xylan. It stains color of xylan color which is yellowish color. The other samples do not stain color because of xylan completely dissolved in the mixture with addition of fine film properties as advantage.

Table 4.1: Texture properties

Sample	Xylan (%)	Surface	Flexibility	Color
Sample A	0.0	Dull	Less flexible	No color
Sample B	0.1	Slight dull	Less flexible	No color
Sample C	0.3	Slight dull	Flexible	No color
Sample D	0.5	Slight dull	More flexible	No color
Sample E	1.0	Dull with undissolved xylan	More flexible	Slight yellow

Table 4.2 shows the texture analysis by CT Analyzer for determination of durability, flexibility and strength of 5 different formulations. From the tabulated data, sample E has the lowest hardness among other samples which is 666 g. Hardness of the sample will be higher if the sample is strong. If the hardness is lower, it means the sample has lower strength. So sample E indicates that the sample is weak in strength while the highest hardness is sample C which is 1931 g. The deformation at hardness for sample C is second highest among the other samples which is 9.71 mm means that it is easily deform its shape. So sample C is strong indicates by hardness and flexible indicates by deformation at hardness. It is a good film. Sample B is the highest deformation at hardness which is 10.93 mm, has highest flexibility but in slight low of hardness which 1541 g. Sample B is still a good film. The load at target indicates the

load that act on the surface of the sample. The highest number of load that need to be done is sample C which is 396 g. 369 g need to be done to a single target but easily deform at the same target. This show that sample C has high durability and at the same time has highest deformation. The gap between the number among other samples is too far off with low value. For fracturability value, sample C also has the highest value. Sample C start to fracture is at 97.30g. So the sample will only break when it is reaching the number. Other samples have lower value of fracturability, so it will break faster than sample C. Sample C has the best physical properties and morphological properties. The best formulation composition is sample C.

Table 4.2: Texture Analysis

Sample	**Xylan (%)**	**Hardness (g)**	**Deformation at hardness (mm)**	**Load at target (g)**	**Deformation at target (mm)**	**Fracturability (g)**
A	0.0	951.00	6.12	46	12.40	25.90
B	0.1	1541.00	10.93	42	12.49	89.30
C	0.3	1931.00	9.71	396	12.92	97.30
D	0.5	1264.00	7.62	2	12.48	44.00
E	1.0	666.00	7.54	11	12.49	23.70

4.2 Xylan-based Bioplastic Film Incorporated with Lemongrass Essential Oil

0.3% xylan film is the good film compared to the other 4 sample. So the experiment continued to produce the film incorporated with lemongrass essential oil (eo). 4 samples were prepared including control for each with and without essential oil for 0.3% and 0.0% xylan.

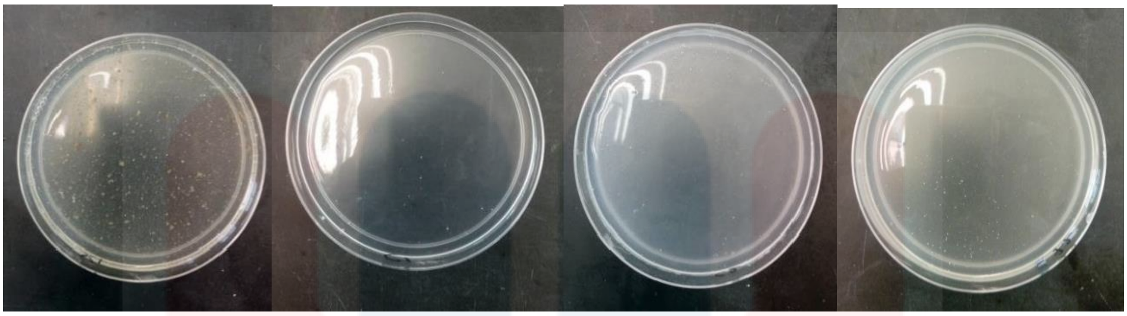


Figure 4.3: Xylan film incorporated with lemongrass essential oil (i) 0.3%, (ii) 0.0%, (iii) 0.0% eo, (iv) 0.3% eo

4.4 Biodegradation Analysis

Biodegradation test were done to analyze the biodegradability of 4 types of film. Significant result was obtained from the biodegradability test. Based on Table 4.3, the rate percent of the biodegradation are increasing over time. These indicate soil is a good biodegradation medium. Two groups of samples differentiated with the presence or absence of lemon grass oil. A group consists of two samples with different composition of the xylan. The four samples were being test in five days buried in soil with the size of the 2 cm x 1 cm for each of the sample as shown in Figure 4.4(i). Figure 4.4(ii) shows the degradation happen on day 1. Day 1 has already shown a significant in biodegradation. This indicates the biodegradation happened.

Table 4.3 shows both sample with the lemon grass oil remained up to day 5 shown in Figure 4.4(iii) and 4.4(iv). Both sample of without lemon grass were remained only until day 3. This shows that the difference in time between these two types is 2 days. The depletion of bioplastic film in day 3 for both samples without lemon grass oil show that the samples are fully degraded in the soil. The samples with lemon grass oil degrade slower than samples without lemon grass oil. This is due to the presence of the antimicrobial properties by lemon grass which has the ability of inhibit growth of microbe. Thus, the process of degradation slows. Microbial agent is the major factors that affect the biodegradation process. The less microbial agent at surround the sample in the soil, the slower the process of the biodegradation will occur. Both plastic without lemon grass oil fully degrade in the same day.

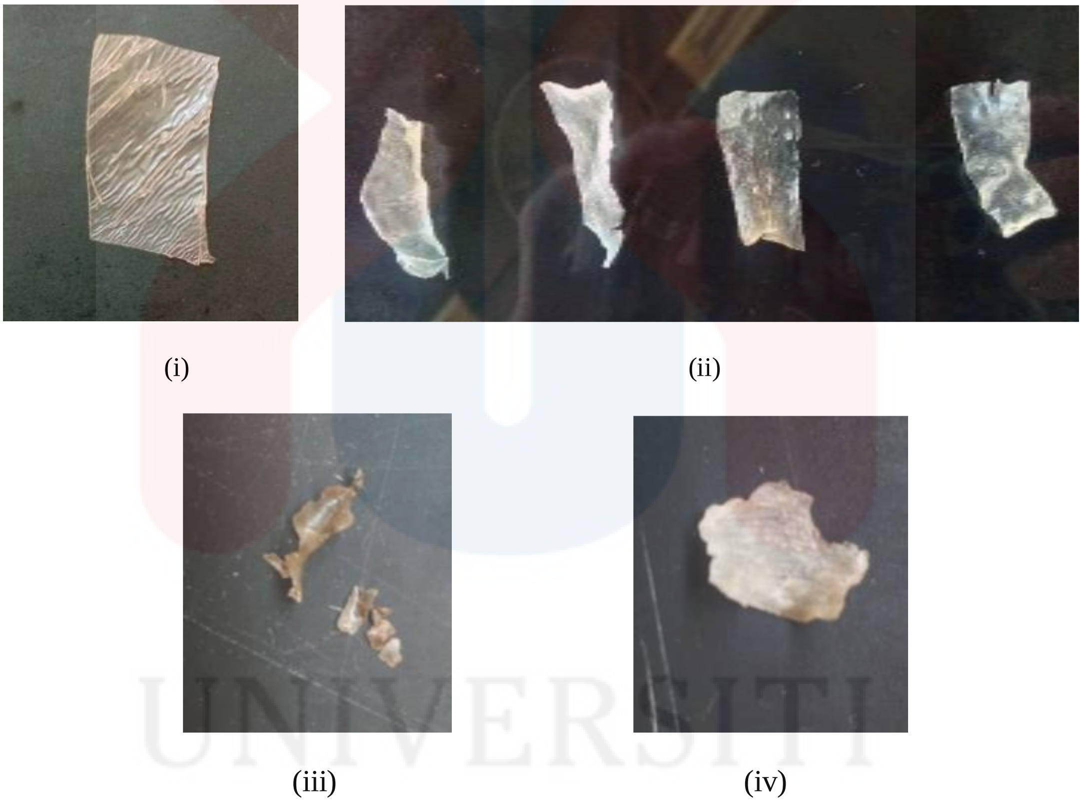


Figure 4.4: (i) days 0, (ii) day1, (iii) day 5 (eo), (iv) day 5 of biodegradability analysis

Figure 4.5: Biodegradable process

Table 4.3: Biodegradability test result

Sample	Xylan	Initial weight(g)	% Biodegradability				
			Day 1	Day 2	Day 3	Day 4	Day 5
With lemon grass (eo)	0.0	0.042	Present	Present	Present	Present	Present
			9.52%	30.95%	66.67%	85.71	97.62%
	0.3	0.048	Present	Present	Present	Present	Present
			20.83%	37.50%	39.58%	58.33%	79.17%
Without lemon grass (eo)	0.0	0.041	Present	Present	Present	Absent	Absent
			26.83%	48.78%	75.61%	100.00%	100.00%
	0.3	0.041	Present	Present	Present	Absent	Absent
			21.95%	30.85%	51.22%	100.00%	100.00%

Figure 4.6 shows the bar graph of the biodegradation process rate. Overall the rate is higher for all samples but slightly slower for film with lemon grass essential oil. The faster rate of biodegradability is film with 0.0% of xylan (control) without lemon

grass oil but to form a good active food packaging, the rate of biodegradation cannot be too fast or it can decompose during food wrapping in certain surrounding condition that allows to. While the slower rate of biodegradability is film with 0.3% xylan with addition of lemon grass oil. 0.3% xylan is has the good ability of biodegradation because of it took a longer time to biodegrade, so it can withstand a longer time of wrapping. The rate biodegradation shows the significance in biodegradability of these films in soil.

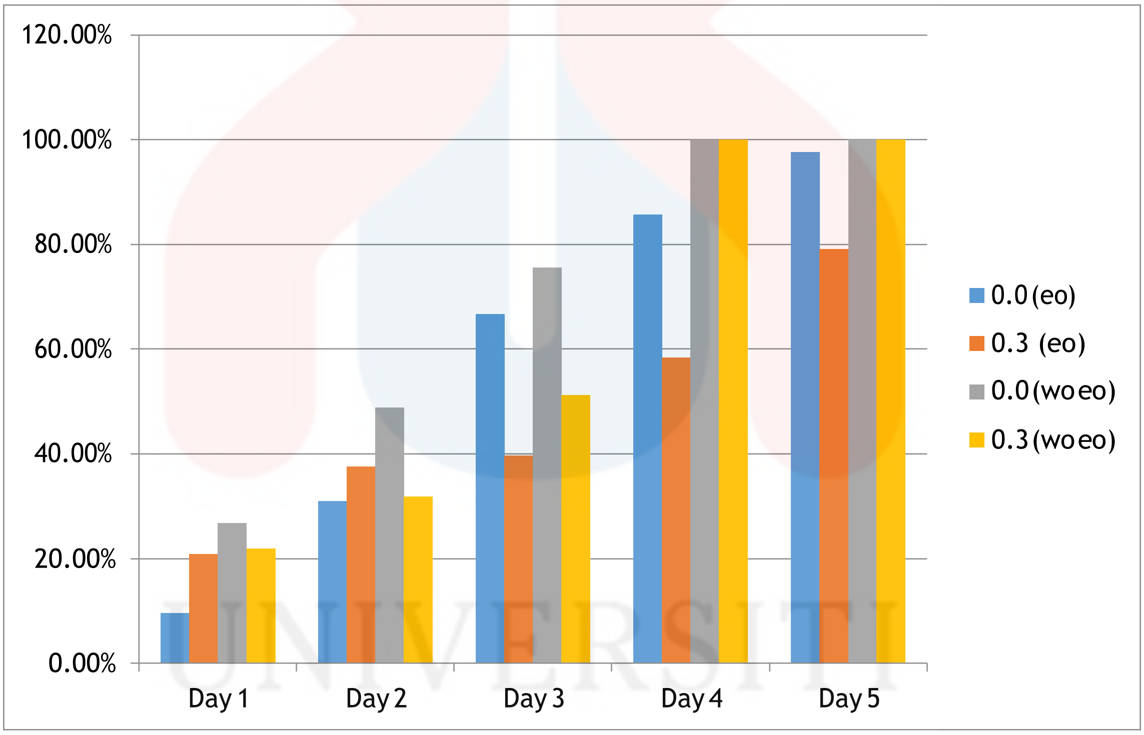
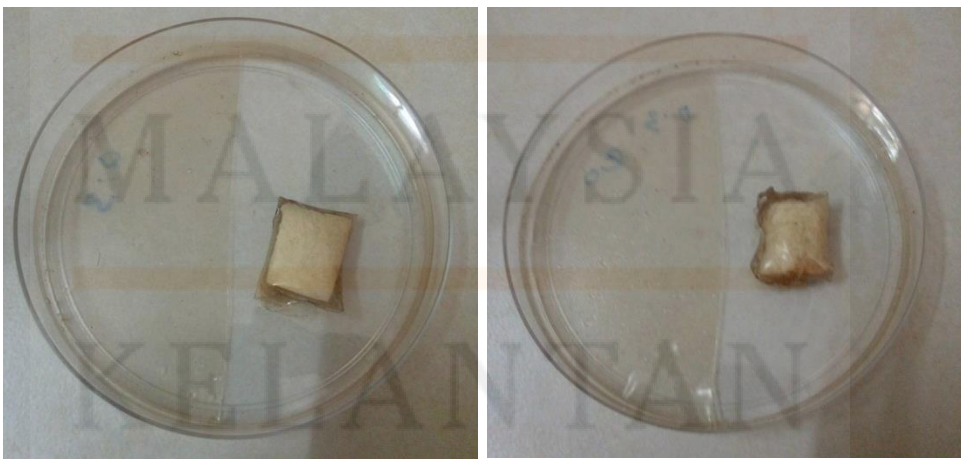


Figure 4.6: Biodegradability rate of 4 different plastic

4.5 Antimicrobial Analysis

Antimicrobial analysis was conducted to determine antimicrobial properties by the inhibition of microbe due to incorporated lemongrass essential oil. Agar plate that were used is nutrient agar (NA) were divided into six region to observe differences in inhibition zone that produced. *Escherichia coli (E.coli)* were streak and a cut sample from 0.3% xylan with essential oil was duplicated used. One cut of 0.3% xylan without essential oil, one control and one sample from pure essential oil. Based on Figure 4.6, the antimicrobial test tried twice but no inhibition zone was seen on the plate. Bhoj et al. (2018) have studied that *E.coli* cannot be inhibited by *Cymbopogon citratus* which is lemongrass. So, in Figure 4.6, this study continued with antifungal analysis by wrapping a piece of bread with 2 samples which 0.3% xylan film with essential oil and without. Figure 4.6 shows that the film, whether it has antimicrobial properties or not, still inhibit the growth of microbe. It is due to the film is absorb moisture, the internal of the wrapping has low humidity. Thus inhibit the microbe growth. Film with essential oil may inhibit a stronger effect towards the microbe growth. The wrapped bread sustained with no fungus grow until day 11.

(i)



(ii)

Figure 4.7: Antimicrobial analysis (i) agar plate, (ii) food wrapping

4.6 Tensile Strength Analysis

Tensile strength analysis was done manually and measure with a ruler for 0.0% xylan film with lemongrass compared with 0.3% xylan film with lemongrass. Table 4.4 show the elongation by each sample recorded with initial and final reading. Sample with 0.3% xylan has the furthest elongation compared to the 0.0% xylan. It is due to the existence of xylan that strengthens; increase its durability and flexibility to the film. Based on the table 5, the reading before for both is 5.0cm. Final reading for 0.0% xylan is 5.5cm which means the elongation only elongates 0.5cm while for 0.3% xylan elongates 1.1cm. This shows that the 0.3% xylan has stronger in tensile properties. 0.0% of xylan film has weak tensile strength properties. This test proved that the existence of xylan can improved the flexibility and exhibit the strength of the film. The drawback of the xylan which is xylan is fragmented and easily fracture has already optimized by starch and glycerol in the formula composition.

Table 4.4: Tensile strength test

Sample (% xylan)	Initial reading (cm)	Final reading (cm)	Elongation (cm)
0.0	5.0	5.5	0.5
0.3	5.0	6.1	1.1

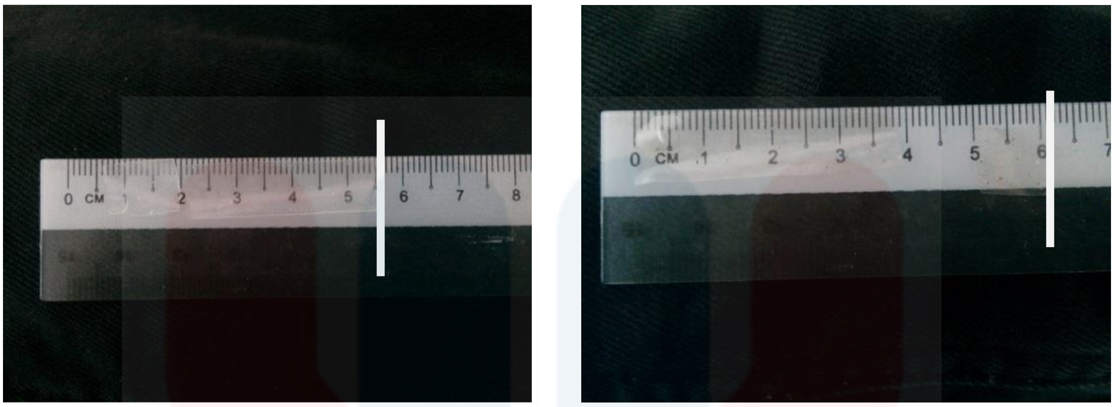


Figure 4.8: Comparison between the 0.0% and 0.3% of xylan film in tensile

4.7 Chemical Analysis

4.7.1 FTIR Analysis

FTIR analysis were carried and Figure 4.9 shows the FTIR peak for xylan. The five samples of different types of xylan percent shown in Figure 4.10.

a) Xylan

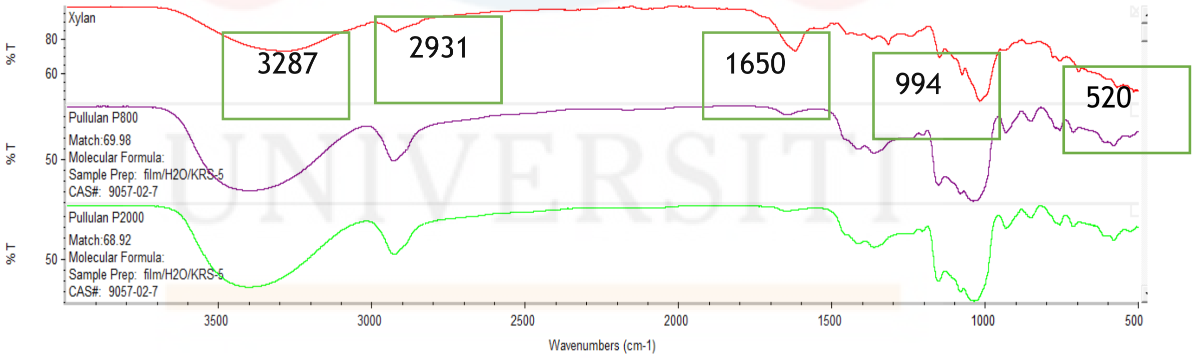


Figure 4.9: FTIR analysis of xylan

Pullulan is a polysaccharide polymer consisting of maltose unit. It is produce from starch by the fungus *Aureobasidium pullulans.* Pullulan matching percent in pure xylan is 69.98%. xylan is a heterogeneous polysaccharide. It is high match percentage of the polysaccharides (pullulan) in the FTIR analysis (Figure 4.9). This shows that the xylan contains high concentration of polysaccharides. It is proved that the xylan significant chain of saccharides. The significant in absorbance at 3287 cm^{-1}, 2391 cm^{-1}, 1650 cm^{-1}, 994 cm^{-1}, 520 cm^{-1} spectrums associated with xylan. Significance peak at 994 cm^{-1} which is the steep gradient. Based on the figure 4.10, the peak on the same point change but almost 1000 cm^{-1}. This indicate that the differences among these samples were the percentage of xylan content.

b) Bioplastic Film Sample Analysis

Bioplastic film sample were analyzed by their spectrum with FTIR. Figure 4.10 shows the 5 samples were compared to the pullulan P500 and P2000.

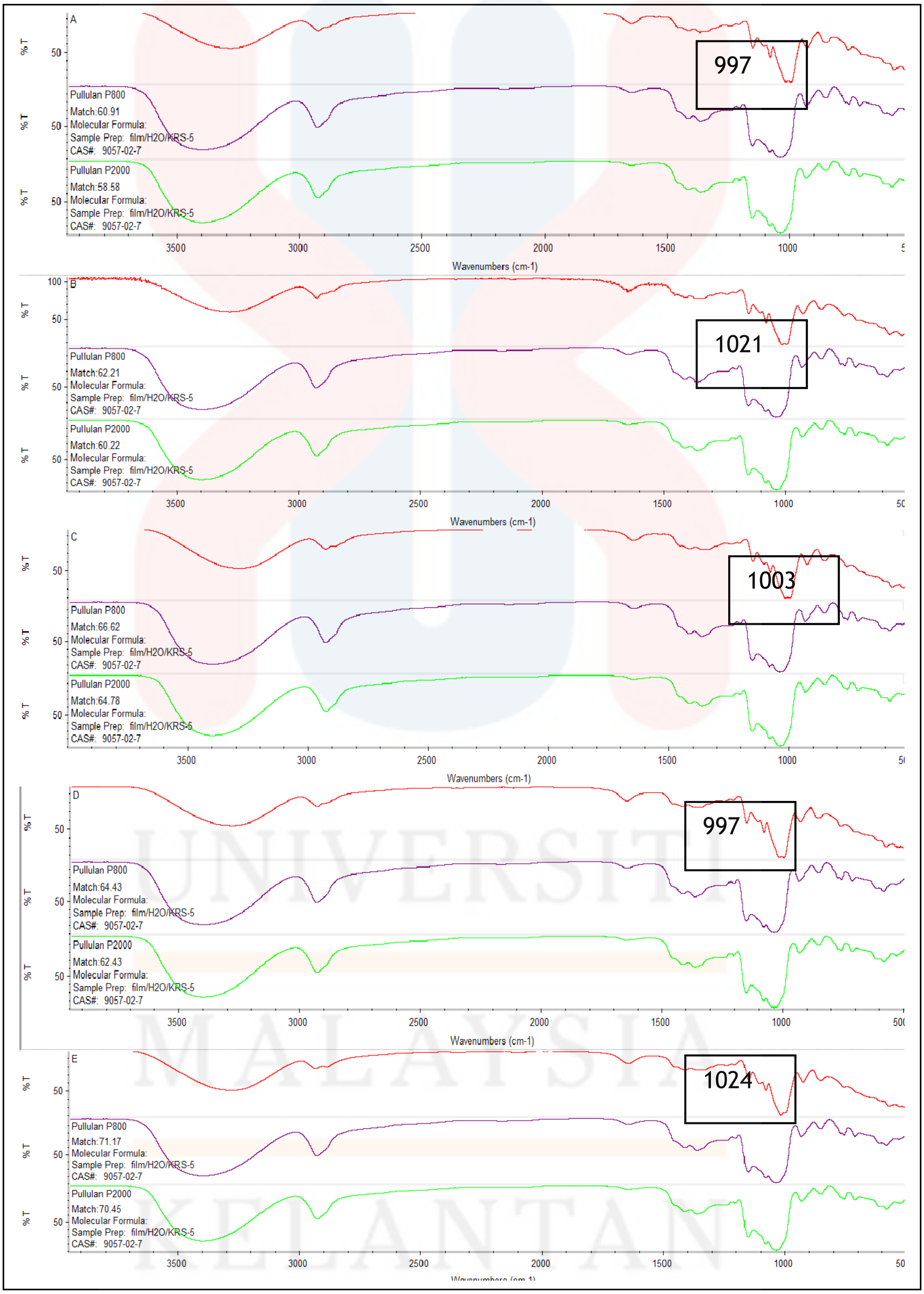


Figure 4.10: FTIR analysis of 5 sample different (A,B,C,D,E)

4.7.2 SEM Analysis

SEM analysis were run for xylan, 0.3% xylan film with essential oil and without essential oil on day 0 and day 5.

a) Xylan

Figure 4.11 shows SEM analysis of xylan from tobacco were compared with xylan from corn cobs (Elquio et al, 2010). The surface of the xylan is a sticky-like structure due to adhesiveness properties of xylan. The bigger particle is the biomass of the xylan that was not separated during separation. To make a fine film, xylan must be separated from its biomass.

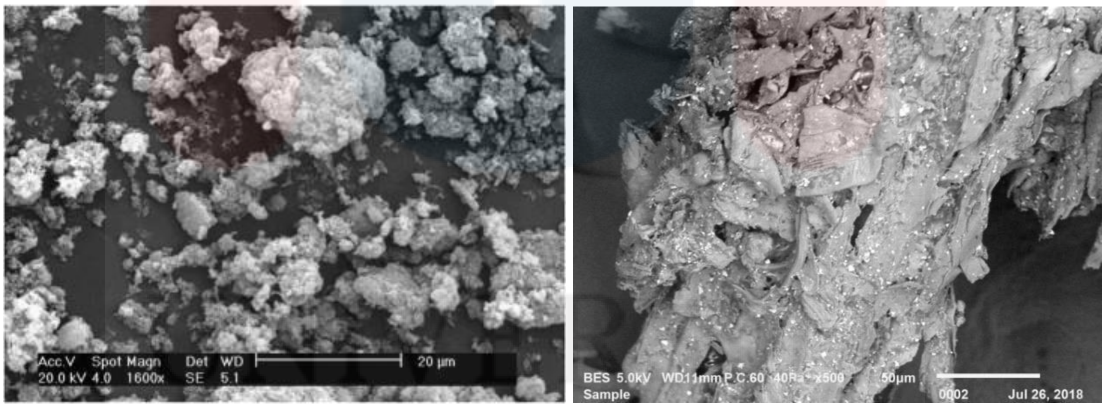


Figure 4.11: SEM analysis of xylan from corn cobs (left) and xylan from tobacco (x500) (right)

Source: Elquio et al (2010)

b) Biodegradable Sample SEM

Figure 4.12 shows biodegradable sample for day 0 and day 3 for 0.3% xylan film without essential oil were compared. On day 0, the film surface is smooth and no crack was seen under magnification of 1600x. Under same magnification on day 3, SEM analysis shows many crack and film rupture due to biodegradation occurred. Significance of this phenomenon showing that biodegradation is absolutely occurred to the film under soil buried condition with several factors such as microbe and moisture in the soil. Figure 4.13 shows the image of SEM analysis of 0.3% xylan film with lemongrass on day 5. There were cracks seen, burst and rupture of the film under effect of biodegradability.

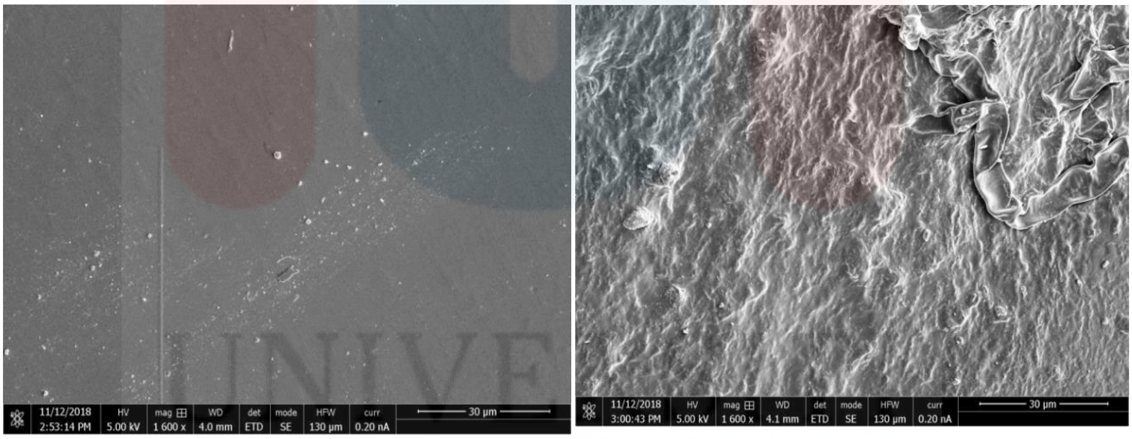


Figure 4.12: SEM analysis day 0 (left) and day 3 (right) of 0.3% xylan film under 1600xmagnification

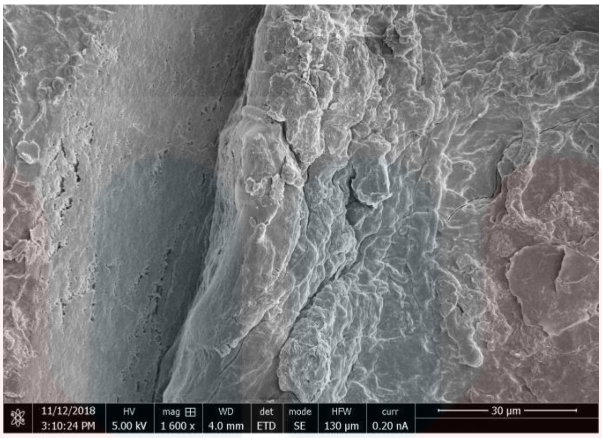
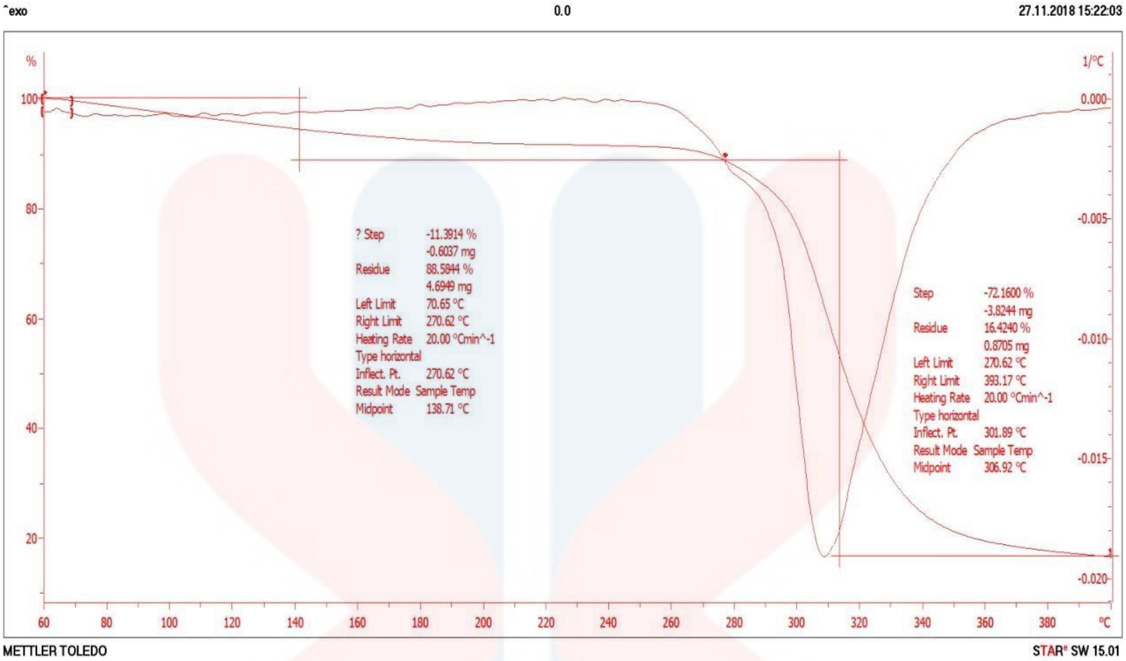


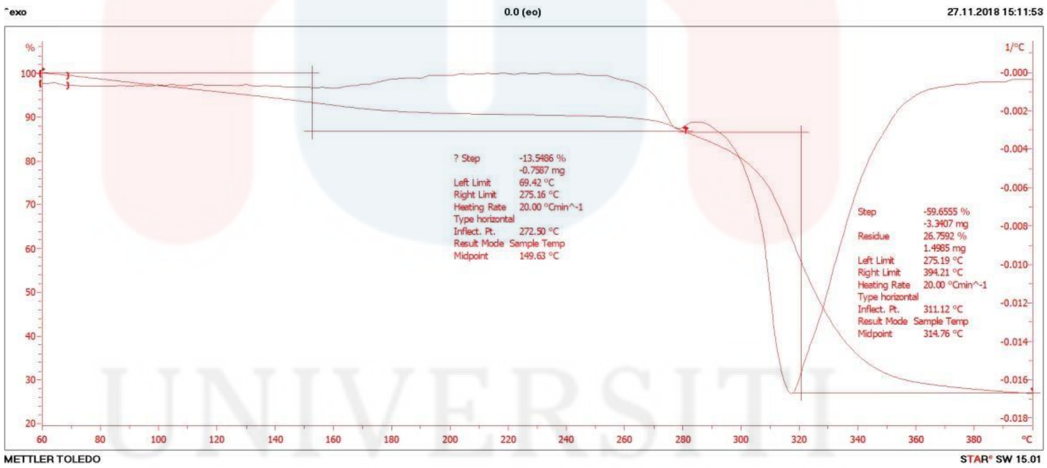
Figure 4.13: SEM analysis on day 5 of 0.3% xylan ffilm with lemongrass(eo) 1600xmagnification

4.7.3 Thermal Properties Using Thermal gravimetric Analysis (TGA)

Thermal gravimetric analysis (TGA) is one of the method to analyse mass of sample measured over time depending on temperature change. In this analysis, nitrogen atmosphere was used as it has less reactive reaction in atmosphere. In this study, the initial temperature was 60°C and increased by 20°C within one minute to reach 400°C. Four samples were used in the TGA which are 0.0 wt%, 0.0 wt% with essential oil, 0.3 wt% and 0.3 wt% with essential oil. These four samples were used to differentiate samples with and without essential oil. Figure 4.14, Figure 4.15, Figure 4.16 and Figure 4.17, shows the result of TGA using STARe Evaluation software.

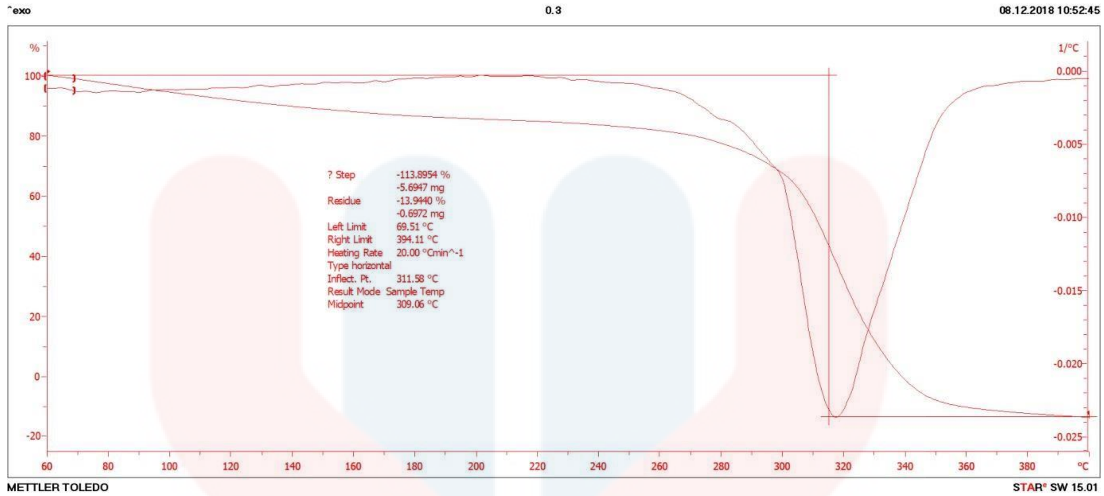


(a)



(b)

Figure 4.14(a) and Figure 4.14(b) shows the result TGA 0.0 wt% with and without essential. These samples were used as control to be differentiated with 0.3 wt%. Based on the figures, it shows that at 270 °C, the degradation occurs. This can be seen in both of the figure which shows the same result.



(c)

In Figure 4.14(c), the degradation starts to happen rapidly when it reached 280°C, this might happen because of the formulation of the plastic that differ to formula of 0.0 wt%.

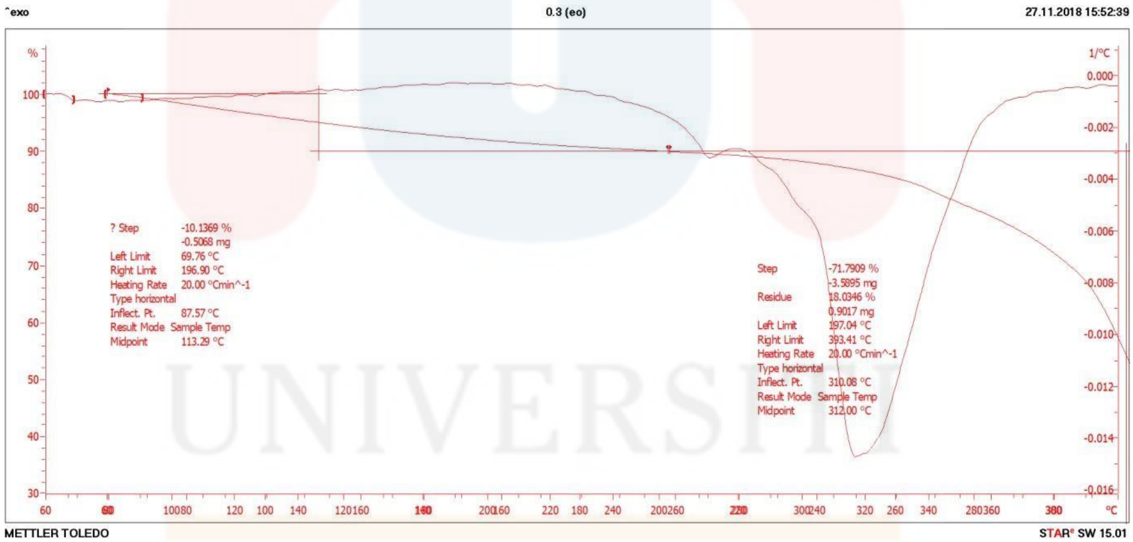


Figure 4.14: a) 0.0% xylan with essential oil, b) 0.0% xylan without essential oil, c) 0.3% xylan with essential oil, d) 0.3 % xylan without essential oil

In Figure 4.14(d), the result show 0.3 wt% with essential oil. It shows that the degradation starts to happen rapidly when it reached 340°C. This shows that 0.3 wt% with essential oil shows greater thermal decomposition compared to 0.3 wt% without essential oil.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In conclusion, this study successful developed an optimum xylan-tapioca bioplastic film incorporated with lemongrass essential oil as antimicrobial and antifungal properties investigated. The extraction used is by DMSO extraction and the formulation consist of xylan, tapioca starch, glycerol and distilled water. The film with best formulation is 0.3 wt% xylan with essential oil. It has very good properties and can be used as active food packaging. It has high durability, good tensile properties, good surface and high flexibility.

This study surely can replace petrochemical-based plastic and a step ahead taken to reduce pollution that cause by petrochemical-based plastic in future. Besides that, this study can lead to reduce waste disposal and more sustainable future.

5.2 Recommendations

To obtained more accurate and good result from this experiment, one of the recommendation that being suggest is the extraction process needs to be more efficient and more purity. It is because the extraction is the first step of the bioplastic film making. Highest purity of xylan can lead to highest performance of the xylan as became a film. Xylan also must undergo downstream process well to produce a maximum reaction of xylan. One of the example is extracted xylan must be freeze dried to produce xylan powder.

For the antimicrobial properties, should be tested with bacteria that inhibit by lemongrass essential oil. Significant inhibition zone will appear well on the plate. *E.coli* cannot be inhibited by lemongrass essential oil. The casting method has to be cast correctly with precise thickness to uniformly casted by using casting machine. Characterization can be upgrade to more advance and precise test to obtain more accurate result from the film.

References

Atef, M., Rezaei, M., & Behrooz, R. (2015). Characterization of physical, mechanical, and antibacterial properties of agar-cellulose bionanocomposite films incorporated with savory essential oil. *Food Hydrocolloids*, *45*, 150–157.

Attokaran, M. (2011). Methods of Extraction of Essential Oils. *Natural Food Flavors and Colorants*, 25–28.

Bettina, R., & Kvalv, M. (2018). Active Packaging Applications for Food, *17*, 165–199.

Betty, B., & Marsh, K. (2007). Food Packaging—Roles, Materials, and Environmental Issues. Journal of Food Science, 72(3).

Bioplastic: A Sustainable Material Revolution. (n.d.). Retrieved April 2, 2018, from https://buildabroad.org/2017/01/05/bioplastic/

Chen, Y. J. (2014). Bioplastics and their role in achieving global sustainability. *Journal of Chemical and Pharmaceutical Research*, *6*(1), 226–231.

Coles, R., & Kirwan, M. J. (2003). *Food Packaging Technology*. CRC Press.

Cutter, C. N. (2006). Opportunities for bio-based packaging technologies to improve the quality and safety of fresh and further processed muscle foods. *Meat Science*, *74*(1), 131–142.

European Commission. 2009. EU Guidance to the Commission Regulation (EC) No 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into the contact with food (version 1.0). Available from: https://ec.europa.eu/food/safety/chemical_safety/food_contact_materials_en. Retrieved on 5 June 2018

Fellows P, Axtell B. 2002. Packaging materials. In: Fellows P, Axtell B, editors. *Appropriate*

Food Packaging: Materials And Methods For Small Businesses. Essex, U.K.: ITDG Publishing.25–77

Gaelle Gourmelon. (2015). Global Plastic Production Rises, Recycling Lags | Worldwatch Institute. Retrieved April 2, 2018, from http://www.worldwatch.org/global-plastic-production-rises-recycling-lags-0

Hussein, Z., Caleb, O. J., & Linus, U. (2015). Perforation-mediated modi fi ed atmosphere packaging of fresh and minimally processed produce — A review. *Food Packaging and Shelf Life*, *6*, 7–20.

Kuorwel KK, Cran MJ, Orbell JD, Buddhadasa S, Bigger SW. 2015. Review of mechanical properties, migration, and potential applications in active food packaging systems containing nanoclays and nanosilver. *Comp Rev Food Sci Food Safety* 14(4):411–30.

Lau OW, Wong SK. 2000. Contamination in food from packaging materials. J Chrom.

A 882(1–2):255–70.

Lopez-Rubio A, Almenar E, Hernandez-Munoz P, Lagaron JM, Catala R, Gavara R.

2004. Overview of active polymer-based packaging technologies for food application.
Food Rev Int 20(4):357–87.

McKown C. 2000. Containers. In: Coatings on glass—technology roadmap workshop. 2000 Jan 18–19. Livermore, *Calif: Sandia National Laboratories Report*. 8–10. 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*(6).

Materials – European Bioplastics e.V. (n.d.). Retrieved April 10, 2018, from https://www.european-bioplastics.org/bioplastics/materials/

Medina-jaramillo, C., Ochoa-yepes, O., Bernal, C., & Famá, L. (2017). Active and smart biodegradable packaging based on starch and natural extracts. *Carbohydrate Polymers*, *176*(August), 187–194.

Page B, Edwards M, May N. 2003. Metal cans. In: Coles R, McDowell D, Kirwan MJ,

editors. *Food Packaging Technology*. London, U.K.:Blackwell Publishing, CRCPress.

121–51.

Rhim, J. W., & Ng, P. K. W. (2007). Natural biopolymer-based nanocomposite films for packaging applications. *Critical Reviews in Food Science and Nutrition*, *47*(4), 411–433. https://doi.org/10.1080/10408390600846366

Robertson G. L. (2010). *Food Packaging and Shelf Life - A Practical Guide*. CRC Press.

Singh P, Abas Wani A, Saengerlaub S. 2011. Active packaging of food products: recent trends. *J Nutr Food Sci* 41(4):249–60

Tice, P. (2003). Packaging Materials: 4. Polyethylene for Food Packaging Applications. *ILSI Europe Report Series*.

Van Willige RWG, Linssen JPH, Meinders MBJ, van der Steger HJ, Voragen AGJ.2002

Influence of flavor absorption on oxygen permeation through LDPE, PP, PC and PET plastics food packaging. *Food Addit Contam* 19(3):303–13

What are the advantages of bioplastic products? – European Bioplastics e.V. (n.d.). Retrieved April 10, 2018, from https://www.european-bioplastics.org/faq-items/what-are-the-advantages-of-bioplastic-products/

Yam, K. L. (2009). Encyclopedia Of Packaging 3rd Edn, (January), 659– 674.

Zhuang H, Barth MM, Cisneros-Zevallos L. 2014. Modified atmosphere packaging for fresh fruits and vegetables. In: Han JH, editor. *Innovations in food packaging*. 2nd ed. San Diego, U.S.A.: Academic Press.445–73.

Appendices