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JUDUL : FABRICATION AND CHARACTERIZATION OF BIODEGRADABLE COMPOSITE FILM FROM BANANA STEM

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Saya **LIM RWI HAU**

(HURUF BESAR)

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FABRICATION AND CHARACTERIZATION OF BIODEGRADABLE COMPOSITE FILM FROM BANANA STEM

LIM RWI HAU

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

Faculty of Chemical and Natural Resources Engineering
Universiti Malaysia Pahang

APRIL 2009

I declare that this thesis entitled "*Fabrication and characterization of biodegradable composite film from banana stem*" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature	:	.....
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Date	:	April 16, 2009

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ABSTRACT

The diverse utilization of packaging films from bio-based compounds has received so much attention lately due to the fact that they are readily biodegradable. Banana stem fiber was subjected to acid hydrolysis and three types of film samples, banana stem fiber-chitosan, cassava starch-chitosan and banana stem fiber-cassava starch-chitosan were fabricated with the addition of PEG400. The film samples were later characterized in terms of their morphological and physical properties through FTIR, TGA, DSC and AFM. Analytical results showed that the three compounds used were almost identical in structure and therefore the miscibility between them was of considerable degree. Results also showed that the thermal stability of the three films was significantly noteworthy to be used as a packaging material. The addition of bio-fibers also affected the thermal and mechanical properties of the film samples. Thus, this study gave a new in-depth look into the usage of biofibers as reinforcing agents of biodegradable films of low thermal and mechanical properties.

ABSTRAK

Penggunaan filem pembungkusan mudah terbiodegradasi yang diperbuat daripada bahan biologi telah menerima perhatian yang meluas baru-baru ini. Serat batang pisang dihidrolisis melalui asid hidrolisis dan tiga jenis sampel filem dihasilkan iaitu serat batang pisang-chitosan, tepung ubi kayu-chitosan dan serat batang pisang-tepung ubi kayu-chitosan dengan campuran PEG400. Sampel filem tersebut kemudiannya dianalisis morfologi dan kualiti fizikal mereka melalui FTIR, TGA, DSC dan AFM. Keputusan analitikal menunjukkan ketiga-tiga bahan yang digunakan mempunyai struktur yang sangat identikal maka kebolehlarutan di antara ketiga-tiga bahan tersebut adalah agak tinggi. Keputusan juga menunjukkan kestabilan haba ketiga-tiga sampel filem tersebut adalah sesuai dengan penggunaan mereka sebagai filem pembungkusan. Penambahan serat tumbuhan juga memberi impak kepada kualiti haba dan mekanikal sampel-sampel filem tersebut. Oleh yang demikian, kajian ini memberikan satu pendedahan baru kepada penggunaan serat tumbuhan sebagai agen penguat untuk biofilem yang mempunyai kualiti haba dan mekanikal yang rendah.

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LIST OF ABBREVIATIONS/SYMBOLS

%	-	percentage
<	-	less than
>	-	more than
°C	-	degree celcius
µm	-	micrometer
10^{-5}	-	0.00001
10^{-7}	-	0.0000001
ABO	-	blood group system
AFM	-	Atomic Force Microscopy
alpha-Gal	-	alpha-Galactosidase A
ATR	-	attenuated total reflectance
cm	-	centimeter
CO_2	-	carbon dioxide
DA	-	degree of N-acetylation
DD	-	degree of deacetylation
DDA	-	degree of deacetylation
DNA	-	deoxyribonucleic acid
DRR	-	disease resistance response
DSC	-	Differential Scanning Calorimtry
et al.	-	et alii/and others
etc.	-	etcetera
FDA	-	Food and Drug Administration of the USA
FTIR	-	Fourier Transform Infrared
g	-	gram
H^+	-	hydrogen ion

H_2O	-	water
H_m	-	heat of melting
Hz	-	hertz
i.e.	-	*id est*/that is
IR	-	infrared
$J.g^{-1}$	-	joule per gram/unit for energy
kHz	-	kilohertz
LCD	-	liquid crystal display
M	-	molar
mg	-	milligram
mL	-	milliliter
mL/min	-	milliliter per minute
N/m	-	newton per meter
NCMC	-	N-carboxy-methylchitosan-N,O-sulfate
nm	-	nanometer
O_2	-	oxygen
O-GlcNAc	-	O-linked N-acetylglucosamine
pH	-	negative logarithm for hydrogen ion concentration
PoP	-	point-on-purchase
PR	-	pathogenesis-related gene
R	-	replicate gene
RH	-	relative humidity
rms	-	root-mean-square
RPM	-	revolution per minute
T_c	-	conclusion temperature
T_c	-	conclusion temperature
T_g	-	glass transition temperature
TGA	-	Thermogravimetric Analysis
T_m	-	melt transition temperature
T_o	-	onset temperature
T_o	-	oxidation temperature
T_{onset}	-	onset temperature
T_p	-	peak temperature

T_p	-	peak temperature
v/v	-	volume per volume
w/w	-	weight per weight
α	-	Alpha – glycoside link
β	-	Beta – glycoside link
ΔH	-	enthalpy

CHAPTER 1

INTRODUCTION

1.1 Research background

Almost the entire available consumer products have been dispensed through packaging system. This system is greatly utilized to fulfill at least one of the listed functions below (Davis and Song, 2005):

a) to provide product protection from physical damage, contamination and deterioration;
b) to give a product the sales appeal;
c) to ensure that the product identity is easily recognizable;
d) to give information about the product
e) to optimize distribution and storage costs;
f) to provide consumers with the convenience and safety.

Food packaging preserves and protects all types of foods and their raw materials (Tharanathan, 2003) with which their traceability, convenience, and tamper indication are secondary functions recognizably of increasing importance (Marsh and Bugusu, 2007). These protective films and suitable packaging by the food industry have become an ongoing topic of monumental interest because of their packaging potentiality attributed to the ability in increasing the shelf life of many food products (Sorrentino *et al.*, 2007). By means of the correct selection of materials and packaging technologies, it is able to keep the product's quality and freshness during the time required for its commercialization and most importantly, its consumption (Stewart *et al.*, 2002).

In recent years, bio-based, materials such as carbohydrates and proteins have, gradually if not extensively, been tested and experimented to develop biodegradable films which had been proven to have more and more versatile properties (Perez-Mateos *et al.*, 2009). Also, natural fibers present important advantages such as low density, appropriate stiffness and mechanical properties and high disposability and renewability. Moreover, they are recyclable and biodegradable. There has been lot of research on use of natural fibers in reinforcements (Mukhopadhyay *et al*., 2008). Natural fibres are getting the attention as a reinforcing agent in both thermoplastic and thermosett matrices (Pothan *et al*., 2006).This has indefinitely set off the diverse utilization of food packaging films made of bio-based materials.

1.2 Identification of problems

Global production of packaging materials is estimated at more than 180 million tons per year, spurred by the fact that both growth and demand are increasing annually. Within the plastic packaging market, food packaging is the largest growing sector (Cutter, 2006). For the last 20 years, petrochemical polymers, commonly called "plastics," have been booming and are by far the most widely used polymers for packaging due in part to their high performance, low cost (Callegarin *et al*., 1997), availability in large quantities at low cost and favorable functionality characteristics, such as good tensile and tear strength, good barrier properties to oxygen and heat-sealing capabilities (Alves *et al.*, 2006).

Indefinitely, plastics have indeed gained a unique position in food packaging technology for a number of quite different reasons including (Psomidaou *et al.*, 1997) :

a) higher strength, elongation and barrier properties against waterborne organisms responsible for food spoilage,
b) lower cost and higher energy effectiveness,
c) lightness and water resistance.

They are also incredibly durable and inert even in the presence of microorganisms, leading to a sustainable long-term performance (Mali *et al.*, 2002; Arvanitoyannis *et al.*, 1998). Until as recent as today, the largest part of all materials used in the packaging industries is derived from fossil fuels and practically non-biodegradable (Sorrentino *et al.*, 2007; Ban *et al.*, 2006). These traditional packaging materials also encourage the migration of harmful additives (Lopez-Rubio *et al*., 2006) into food products.

As the amount of plastic waste increases every year, the exact time needed for its biodegradation is unknown (Reis *et al*., 2008). Approximately 40 million metric tons of such films are consumed annually on a global basis (Ban *et al*., 2006). The world is also running out of landfill space as degradation of plastics requires a long time and most of them end up overburdening on landfill (Xu *et al*., 2005).

Waste is not confined only to plastic materials. According to Abdul Khalil *et al*. (2006), Malaysia has a large area of plantation of oil palm (3.87 million hectars), coir (147 thousand hectares), banana (34 thousand hectares), and pineapple (15 thousand hectares). Large quantities of cellulosic and non-cellulosic raw material are generated during harvesting (Abdul Khalil *et al*., 2006). The explosive expansion of these plantations in Malaysia has generated enormous amounts of plant wastes, creating problems in replanting operations and tremendous environmental concerns.

Packaging materials, especially for food products or produce, like any other short-term storage packaging materials, therefore represent a serious global environmental problem (Kirwan and Strawbridge, 2003) if no concerted actions are adopted to address and prevent it.

1.3 Significance of study

A big effort to extend the shelf life and enhance food quality while reducing packaging waste has encouraged the exploration of new bio-based packaging

materials, such as edible and biodegradable films from renewable resources (Tharanathan, 2003) for the goal of food packaging is to contain food in a cost-effective way that satisfies industry requirements and consumer desires, maintains food safety, and minimizes environmental impact (Marsh and Bugusu, 2007). Since the depletion of oil, societal and environmental pressures continue to prompt efforts to develop renewable, cost-effective, and environmentally friendly materials for the manufacture of a number of products, including these films (Ban *et al*., 2006).

Hence, at present, one of the major trends in the food packaging field is the development and use of polymeric materials of biodegradable and/or edible nature that decompose naturally causing no environmental problems when discarded as waste and can also be considered an alternative to traditional plastics obtained from petrochemical industry (Muratore *et al.*, 2005). This notable growth of interest in developing packaging materials based on biopolymers has been witnessed as early as the last decade (Mendieta-Taboada *et al.*, 2008).

Biological Degradation	- Fungi, Bacteria, Insects, Termites
Enzymatic Reaction	- Oxidation, Hydrolysis, Reduction
Chemical Reactions	- Oxidation, Hydrolysis, Reduction
Mechanical	- Chewing
Thermal Degradation	- Lightning, Sun, Man
Pyrolysis Reactions	- Dehydration, Hydrolysis, Oxidation
Water Degradation	- Rain, Sea, Ice, Due
Water Interactions	- Swelling, Shrinking, Freezing, Cracking, Cyclic Wetting and Drying
Weather Degradation	- Ultraviolet radiation, Water, Heat, Wind
Chemical Reactions	- Oxidation, Hydrolysis
Mechanical	- Erosion
Chemical Degradation	- Acids, Bases, Salts, Metals
Chemical Reactions	- Oxidation, Reduction, Dehydration, Hydrolysis
Mechanical Degradation	- Dust, Wind, Hail, Snow, Sand
	- Stress, Cracks, Fracture, Abrasion

Figure 1.1: Degradation reactions which occur when bio-based resources are exposed to nature (Rowell, 1998).

The search for biologically active compounds from natural sources has taken the center stage in recent years for their low or absent toxicity, their complete biodegradability, their avalailability from renewable sources, and, their low-cost if

compared with those compounds obtained by total chemical synthesis (Tringali, 2001). Also, the abundance of natural fibres combined with the ease of their processability is an attractive feature (Pothan *et al*., 2006). The incorporation of these plant fibers which are mostly residues of agriculture and agro-industries, allows a valorization of these wastes and a limitation of environmental damages. It had been demonstrated that natural fibers can reinforce concrete and exhibit the same performance behavior as that of conventional fiber reinforced concrete produced from steel and other inorganic/synthetic fibers (Bilba *et al.,* 2007).

Starch is the commonly used agricultural raw material, since it is a renewable source (Zhai *et al*., 2004). In the food packaging sector, starch-based material has received great attention owing to its biodegradability, wide availability and low cost (Avella *et al.*, 2005). Starch owes much of its functionality to the two major high-molecular-weight carbohydrate components, amylose and amylopectin, as well as to the physical organization of these macromolecules into the granular structure (Romero-Bastida *et al.*, 2005).

Chitosan is recognized for its antimicrobial activity and film-forming properties (Sebastien *et al.*, 2006) besides its biocide effects (Fernandez *et al.*, 2008). In addition, chitosan also possesses useful properties such as biodegradability, biocompatibility (Sashiwa *et al.*, 2003), and non-toxicity leading to extensively use over a wide range of applications (Bangyekan *et al.*, 2006).

The scope of films made with starch combined with other polysaccharides was widened to include chitosan for several reasons. First, chitosan is a biopolymer, obtained by N-deacetylation of chitin, which is the second most abundant polysaccharide on the earth after cellulose (Bangyekan *et al*., 2006). It is commercially available from a stable renewable source, that is, shellfish waste (shrimp and crab shells) of the sea-food industry. Second, chitosan forms good films and membranes (Vandamme *et al*., 2002). Since the use of synthetic polymers is dependent on the use of crude oil, nature has been touted as another possible resource for structural polymers (Jansson and Thuvander, 2004).

1.4 Objectives

The objectives of this study are:

a) To fabricate different types of biodegradable composite films from banana stem fiber.

b) To characterize different types of biodegradable composite films from banana stem fiber.

1.5 Scopes of study

The scopes of this study are:

a) Film preparation:
   i. Banana stem fiber-chitosan film
   ii. Cassava starch-chitosan film
   iii. Banana stem fiber-cassava starch-chitosan film

b) Film characterization:
   i. Morphological properties using AFM (Atomic Force Microscopy)
   ii. Physical properties tests using FTIR (Fourier Transform Infrared) spectroscopy, TGA (Thermal Gravimetric Analysis), and DSC (Differential Scanning Calorimetry)

CHAPTER 2

LITERATURE REVIEW

2.1 Carbohydrates

Carbohydrates are naturally occurring compounds of carbon, hydrogen and oxygen. Many carbohydrates have the empirical formula CH_2O (Fessenden *et al.*, 1998). One distinct example is the molecular formula for glucose which is $C_6H_{12}O$ and exactly 6 times CH_2O. Carbohydrates, commonly, referred to as sugars and starches, are polyhydroxy aldehydes and ketones, or compounds that can be hydrolyzed to them (Smith, 2008). They are the storehouses of chemical energy.

Carbohydrates are synthesized in green plants and algae by photosynthesis, a process that uses energy from the sun to convert carbon dioxide and water into glucose and oxygen (Smith, 2008). All carbohydrates vary dramatically in their properties such as the case of sucrose, also known as table sugar, and cotton that are both carbohydrates but displaying distinguishable characteristics (Fessenden *et al.*, 1998).

2.1.1 Carbohydrate units

One of the principal differences between various types of carbohydrates is the size of the molecules (Fessenden *et al*., 2008). One way to classify carbohydrates is by their chemical make-up.

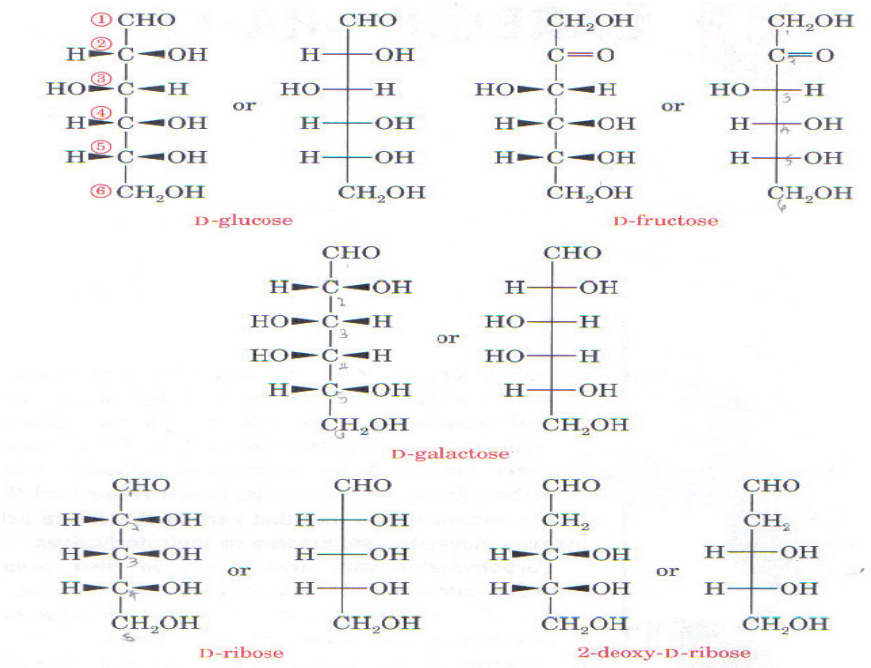


Figure 2.1: Some important carbohydrates (Fessenden *et al.*, 1998).

2.1.1.1 Monosaccharide

The monosaccharides, often called simple sugars, are the simplest carbohydrate units as they cannot be further hydrolyzed to smaller carbohydrate molecules (Fessenden *et al.*, 1998). The three most common sugars in this group are glucose (or dextrose), the most frequently seen sugar in fruits and vegetables (and, in digestion, the form of carbohydrate to which all others are eventually converted); fructose, associated with glucose in honey and in many fruits and vegetables; and galactose, derived from the more complex milk sugar, lactose (Smith, 2008). Each of these simple but nutritionally important sugars is a hexose, which means it contains six carbon atoms, 12 hydrogen atoms, and six oxygen atoms. All three require virtually no digestion but are readily absorbed into the bloodstream from the intestine.

Table 2.1: Classification of monosaccharides (Cui, 2005).

Number of carbon atoms	***Kind of Carbonyl Group***	
	Aldehyde	***Ketone***
3	Aldotriose	Triulose
4	Aldotetrose	Tetrulose
5	Aldopentose	Pentulose
6	Aldohexose	Hexulose
7	Aldoheptose	Heptulose
8	Aldooctose	Octulose
9	Aldononose	Nonulose

2.1.1.2 Disaccharide

Monosaccharides are also understood to be able to bond together to form dimers, trimers, etc. and ultimately, polymers. The dimers are always called disaccharides and sucrose is one of the many disaccharides that can be hydrolyzed to one unit of glucose plus one unit of fructose. The monosaccharides and disaccharides are soluble in water and are generally sweet tasting (Fessenden *et al.*, 1998).

$$\text{1 sucrose} \xrightarrow[\text{Heat}]{H_2O,\ H^+} \text{1 glucose} + \text{1 fructose}$$

2.1.1.3 Oligosaccharide and polysaccharide

Carbohydrates that are composed of two to eight units of monosaccharide are referred to as oligosaccharides (Greek oligo-, “a few”). Oligosaccharides and polysaccharides are composed of longer chains of monosaccharide units bound

together by glycosidic bonds (Fessenden *et al*., 1998). Oligosaccharides are found as a common form of protein post-translational modification. Such posttranslational modifications include the Lewis and ABO oligosaccharides responsible for blood group classifications and so of tissue incompatibilities, the alpha-Gal epitope responsible for hyperacute rejection in xenotransplantation, and O-GlcNAc modifications (Smith, 2008).

If more than eight units of monosaccharide result from the hydrolysis process, the carbohydrate is a polysaccharide (Fessenden *et al.*, 1998). Common examples of polysaccharides are starch found in rice, flour and corn-starch, cellulose, a fibrous constituent of plants and the principal component of cotton and chitin which can be found mostly in arthropods like crabs and insects.

2.2 Starch

Starch is the second most abundant polysaccharide. The word starch is derived from a cognate form of the Old High German ‘sterken’ (to stiffen). The Latin word ‘amylum’ is derived from the Greek ‘amylon’ (non-milled) from which the widely used words amylose, amylopectin, amylase and amylolysis originate (Vandamme *et al*., 2002). Starch can be found as a reserve polysaccharide present in the endosperm of the grain of corn, banana pulp, yucca (Romero-Bastida *et al.*, 2005) and many other grain-based foods. Starches from different origins, such as potato, corn, wheat, rice, and cassava, both natural and modified, have been utilized, mainly in the manufacture of edible films (Tapia-Blacido *et al*., 2005).

Readily known, starch is the main source of carbohydrate in the human diet, providing the human body with a large proportion of its energy (Vandamme *et al*., 2002) and contributing as a valuable ingredient to the food industry where it is being widely used as a thickener, gelling agent, bulking agent and water retention agent and in most cases, natural starch is partially crystalline in its native granule form (Iida *et al.*, 2008).

Starch is a natural polymer that can readily be cast into films. The matrix of starch-based films is normally formed during the drying of a gelatinized dispersion, as hydrogen bonds form between hydroxyl groups (Li *et al*., 2008). As these interactions are weak, the mechanical properties of starch-based films are of poor quality (Tapia-Blacido *et al*., 2005; Li *et al*., 2008). Unless genetically or chemically modified, starch cannot form films with adequate mechanical properties such as high percentage elongation, tensile and flexural strength (Arvanitoyannis and Biliaderis, 1999; Li *et al*., 2008).

2.2.1 Composition of starch

Starch can be separated into two principal fractions based upon solubility when triturated, meaning literally pulverized, with hot water. About 20% of starch is amylose which is soluble and the remaining 80% is amylopectin which is widely insoluble (Fessenden *et al.*, 1998; Smith, 2008). The two starch components have different properties and are not suitable for the same applications (Stawski, 2008; Paes *et al.*, 2008).

The amount of both starch components has been influential on many various properties of these materials such as swelling capacity, water solubility, water-binding capacity (Sandhu *et al*., 2005), barrier and mechanical properties of starch films (Rindlav-Westling *et al.*, 1998) and microscopic properties.

2.2.1.1 Amylose

The amylose fraction is essentially linear (Fessenden *et* al., 1998) and is the constituent responsible for the film-forming capacity of starches (Romero-Bastida *et al.*, 2005). Complete hydrolysis of amylose yields only D-glucose; partial hydrolysis yields maltose as the only disaccharide. Amylose is a linear polymer of 1, 4-linked α-D-glucose. The difference between amylose and cellulose is the glycoside link: β in

cellulose, α in amylose. This difference is responsible for the different properties of these polysaccharides (Fessenden *et al.*, 1998).

There are 250 or more glucose units per amylose molecule and the exact number depends upon the species of animal or plant. In the measurement of chain length, it is complicated by the fact that natural amylose degrades into smaller chains upon separation and purification. Amylose molecules form helices or coils around I_2 molecules. A deep blue color arises from electronic interactions between the two. This color is the basis of the iodine test for starch, in which a solution of iodine is added to an unknown as a test for the presence of starch (Fessenden *et al.*, 1998).

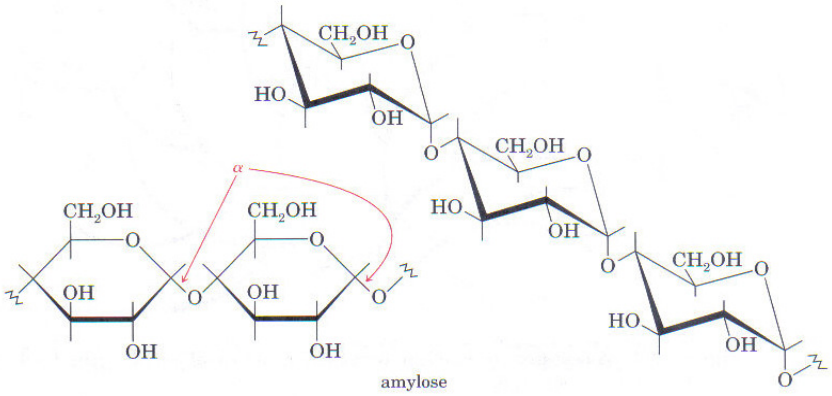


Figure 2.2: A structural formula of amylose (Fessenden *et al.*, 1998).

2.2.1.2 Amylopectin

Amylopectin's structure is highly branched (Fessenden *et al.*, 1998). The ability of amylopectin to form branched polymers is a unique feature of carbohydrates. It consists of a backbone of glucose units joined in α-glycosidic bonds. It also contains considerable branching along the chain. The linear linkages of amylopectin are formed by 1, 4-glycoside bonds, similar to amylose. The branches are linked to the chain with α-1, 6-glycosidic linkages (Smith, 2008).

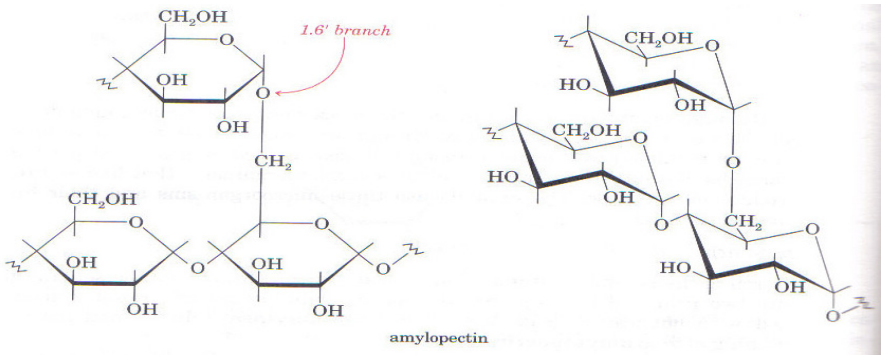


Figure 2.3: A structural formula of amylopectin (Fessenden *et al.*, 1998).

A much larger polysaccharide than amylose, it contains 1000 or more glucose units per molecule. Complete hydrolysis of amylopectin yields only D-glucose. However, incomplete hydrolysis yields a mixture of the disaccharides maltose and isomaltose, the latter arising from the 1, 6-branching. The oligosaccharide mixture obtained from the partial hydrolysis of amylopectin, referred to as dextrins, is used to make glue, paste, and fabric sizing (Fessenden *et al.*, 1998).

2.3 Crystalline structure

Linear and branched polymers do not form crystalline solids because their chains prevent efficient packing in a crystal lattice. Most polymer chains have a crystalline regions and amorphous chains (Smith, 2008):

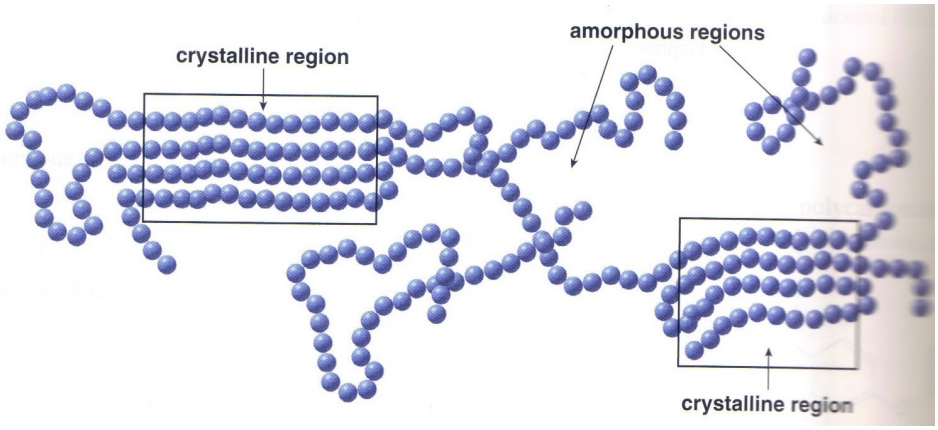


Figure 2.4: Crystalline and amorphous regions of a polymer (Smith, 2008).

a) Ordered crystalline regions, called crystallites, are places where sections of the polymer chain lie in close proximity and are held together by intermolecular interactions.
b) Amorphous regions are places where the polymer chains are randomly arranged, resulting in weak intermolecular interactions.

Crystalline regions impart toughness to a polymer, while amorphous regions impart flexibility. The greater the crystallinity of a polymer, which also means the larger the percentage of ordered regions, the harder the polymer. Branched polymers are generally more amorphous and, since branching prevents chains from packing closely, they are softer too (Smith, 2008). Two temperatures, T_g and T_m, often characterize a polymer's behavior on heating (Smith, 2008):

a) T_g, the glass transition temperature, is the temperature at which a hard amorphous polymer becomes soft.
b) T_m, the melt transition temperature, is the temperature at which the crystalline regions of the polymer melt to become amorphous. More ordered polymers have higher T_m values.

2.3.1 Plasticizer

Sometimes a polymer is too stiff and brittle to be useful in many applications. A low molecular weight compound called a plasticizer is therefore added to soften the polymer and give it flexibility (Smith, 2008). Plasticizers are generally small molecules such as polyols like sorbitol, glycerol and polyethylene glycol (PEG) that intersperse and intercalate among and between polymer chains, disrupting hydrogen bonding and spreading the chains apart, which not only increases flexibility, but also water vapor and gas permeabilities (Bourtoom, 2008). Plasticizer interacts with the polymer chains, replacing some of the intermolecular interactions between the polymer chains. This lowers the crystallinity of the polymer, making it more amorphous and softer (Smith, 2008). The most used plasticizers for starch-based films are sorbitol and glycerol (Muller *et al.*, 2008). Since plasticizers are more

volatile than the high molecular weight polymers, they slowly evaporate with time, making the polymer brittle and easily cracked (Smith, 2008).

2.4 Gelatinization of starch

Many starch-based ingredients that have been developed are principally used to take up water and to produce viscous fluids or gels to impart the desired textural quality to food products. In addition, water immobilization in foods prevents growth of micro-organisms, thereby contributing to a longer shelf life (Ritota *et al.*, 2008). Very often, the functional properties of starch are acquired by gelatinization, which comprises of heating a suspension of granules above a characteristic temperature, causing the starch granule to swell irreversibly (Marques *et al.*, 2006).

During swelling, the linear amylose molecules diffuse out of the swollen granules and are preferentially solubilized. The final state can be viewed as a continuous phase of amylose in which more or less overlapped swollen granules, enriched with amylopectin, are suspended. All of the current understandings of gelatinization are based on heating starch granules by using conduction heating modes. Under these conditions, gelatinization is understood as the cumulative irreversible changes that occur to a starch granule in the presence of moisture and heat (Palav and Seetharaman, 2006). These changes again as discussed beforehand and markedly pointed out, include granule swelling due to absorption of moisture in the amorphous regions of the granule, leaching of small molecular weight polymers including amylose, loss of the crystalline order and the consequent loss of birefringence, leaching of larger molecular weight polymers from the granule including fragments of amylopectin and finally starch solubilization (Sakonidou *et al.*, 2003).

On heating starch in excess water conditions, the granules are also found to swell in which the starch polymers are partially solubilized and leached from the granules, and finally the starch granules disintegrate (Bilbao-Sainz *et al.*, 2008) while

at low water contents, starch gives two distinct endotherms during gelatinization. It was reported that these endotherms were due to phase transitions, which were governed by the degree to which ordered regions within granules were hydrated. The high temperature transition is due to melting of crystallites without adequate moisture (Ratnayake and Jackson, 2007).

When excess water is present, the high temperature transition disappears. Further reports suggest that, at high water contents, the amorphous regions of the granules imbibed water and swelled, resulting in stripping or separation of starch chains from portions of these crystallites. When all crystals are stripped at high moisture levels, there would not be any crystallites remaining to be melted at high temperature (Ratnayake and Jackson, 2007). The uniqueness of gelatinization parameters for each starch can be determined and specifically the onset (T_o), peak (T_p), and conclusion (T_c) temperatures of the endothermic event with the associated enthalpy (ΔH). In excess water, T_o is typically 45-55ºC for most waxy and normal starches, T_p 55-65ºC and T_c 65-75ºC. For high-amylose starches, the gelatinization endotherm is very flat and ranges from approximately 70-105ºC, although this may incorporate (if not subtracted) the amylsoe-lipid dissociation endotherm between 95-105ºC. The gelatinization enthalpy is highly variable, ranging from about 10-20 J g^{-1} for most starches (Vandamme *et al*., 2002).

2.5 Chitin

The name ‘chitin’ came from the Greek word ‘chiton’, meaning a coat of mail, and was apparently, first used by Bradconnot in 1811 (Shahidi *et al*., 1999). Chitin, a homopolymer of b-(1 → 4)-linked N-acetyl-D-glucosamine, is also one of the most abundant, easily obtainable, renewable natural polymers, second only to cellulose (Tolaimate *et al.*, 2000). The principal structural polysaccharide of the arthropods like crabs, shrimps and insects is chitin. It occurs as an important constituent of the exoskeleton of many organisms, particularly crustaceans, insects and molluscs and in the walls of most fungi and some algae (Poirier and Charlet, 2002). Ubiquitous in fungi, it varies in its crystallinity, degree of covalent bonding to

other wall components, mainly glucans and degree of acetylation (Jolles and Muzzarelli, 1999).

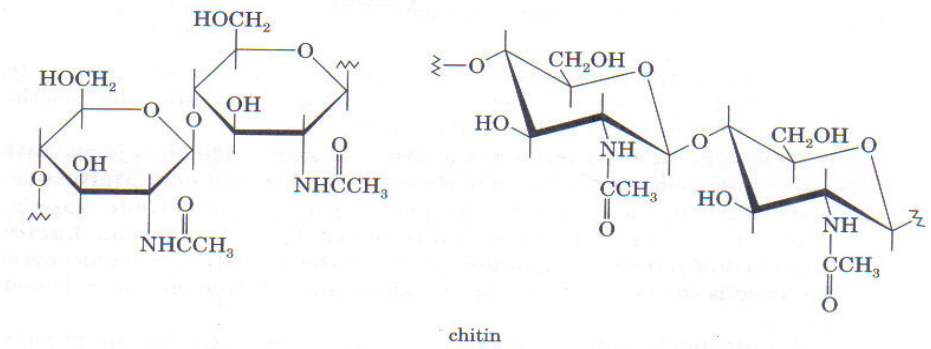


Figure 2.5: Structural formula of chitin (Fessenden *et al.*, 1998).

It has been estimated that 10 gigatons of chitin are biosynthesized each year (Jolles and Muzzarelli, 1999). As chitin is a linear polysaccharide consisting of β-linked *N*-acetyl-D-glucosamine, upon hydrolysis, chitin yields 2-amino-2-deoxy-D-glucose in which the acetyl group is lost in the hydrolysis step (Fessenden *et al.*, 1998). In nature, chitins are bonded to non-polysaccharide materials such as proteins and lipids (Fessenden *et al.*, 1998). Amazingly, chitin is chemically identical to cellulose, except that the secondary hydroxyl group on the alpha carbon atom of the cellulose molecule is substituted with acetoamide groups (Figure 2.6). The solubility of chitin is remarkably poorer than that of cellulose because of the high crystallinity of chitin, supported by hydrogen bonds mainly through the acetamido group (Jolles and Muzzarelli, 1999). A sharp nomenclature with respect to the degree of N-deacetylation has not been well-defined and specifically drawn between chitin and chitosan.

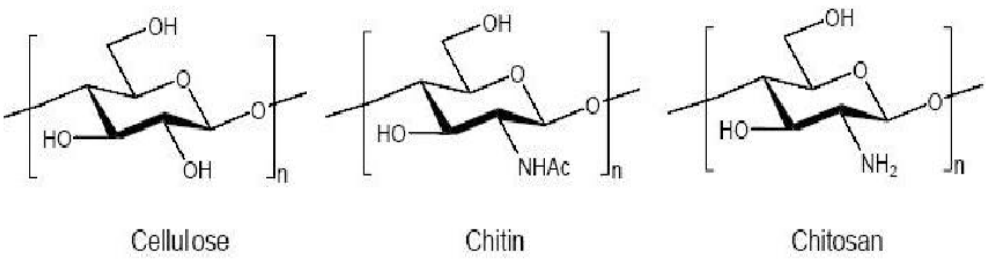


Figure 2.6: The chemical structures of cellulose, chitin and chitosan.

2.6 Chitosan

Chitosan (Figure 2.6) is a modified natural carbohydrate polymer derived from chitin, which is the second most abundant polysaccharide on earth next to cellulose and is available from waste products in the shellfish industry (Bangyekan *et al.*, 2006). A major component of the shells of crustacea such as crab, shrimp, and crawfish, it has received considerable attention for its commercial applications in biomedical, food, and chemical industries (Hong *et al.*, 2002). It can also be obtained from fungi, easily cultured on simple nutrients. Chitosan is present in the cell wall of *Mucorales* and can be isolated from the accompanying glucans by extraction with either acetic acid or alkali, the latter being preferred when glucans are to be dissolved (Jolles and Muzzarelli, 1999). Chitosan is produced by thermochemical alkaline deacetylation of chitin. It is a biopolymer with unique properties favorable for a broad variety of industrial and biomedical applications. It can also be prepared from suitable chitinous raw materials by a sequence of deproteinization and demineralization (Vandamme *et al*., 2002). Chitosan is characterized by its degree of N-acetylation (DA) and this degree of N-acetylation affects not only its physicochemical characteristics but also its biodegradability and immunological activity. It is worth noting that chitosan derived from b-chitin shows higher reactivity than that derived from a-chitin in N-phthaloylation (Tolaimate *et al.*, 2000).

Table 2.2: The sources of chitin and chitosan.

Chitin	***Chitosan***
Crustacea: Squid pens, prawns, shellfish (Borderias, *et al*., 2005), crabs, shrimps, prawns, lobsters.	Crustacea: Crabs, shrimps, prawns, lobsters (Qin *et al*., 2006).
Microorganism: *Aspergillus niger*, *Mucor rouxii*, *Penicillium notatum* (Roller and Covill, 1999).	Microorganism: *Aspergillus niger*, *Mucor rouxii*, *Penicillium notatum* (Roller and Covill, 1999).
Algae (Poirier and Charlet, 2002).	Algae
Insects: Arthropods (Jolles and Muzzarelli, 1999).	Insects: Arthropods
Molluscs	Molluscs (Borderias *et al*., 2005)

Table 2.3: Applications of chitin and chitosan

Chitin	***Chitosan***
Pharmaceutical: Wound healing, artificial membrane, and sutures (Prashanth and Tharanathan, 2007).	Waste treatment: Chelation of harmful metal ions such as lead, mercury and uranium out of industrial wastewaters (Roller and Covill, 1999).
Food and nutrition: Prebiotics and food preservation, immobilization matrix, dietary supplements, functional foods hypocholesterolemic, antioxidant, water purification (Prashanth and Tharanathan, 2007).	Waste treatment: The removal of suspended solids from food processing wastes (Roller and Covill, 1999).
Material science: Hydrocolloid, biosensors, cosmetics (moisturizer, skin care products), and packaging films/composite coating formulations (Prashanth and Tharanathan, 2007).	Food and nutrition: Used in foods such as biscuits, meat products and fish muscle and derivative products such as fish patties and sausages (Borderias *et al*., 2005).
Medical science: Anticoagulant, wound healing, wound dressing, suture threads, and contact lens (Prashanth and Tharanathan, 2007).	Medical science: Wound healing applications (Jolles and Muzzarelli, 1999).
	Food and nutrition: Food supplements (Tikhonov *et al*., 2006).

2.6.1 Composition of chitosan

Chitosan possesses repeating units of 1, 4 linked 2-deoxy-2-aminoglucose. The amino group NH_2 can be protonated to NH_3^+ and readily form electrostatic interactions with anionic groups in an acid environment. This property has been applied on edible films (Xu *et al.*, 2005). In general, chitosans have nitrogen content

higher than 7% and a degree of acetylation lower than 0.40 (Jolles and Muzzarelli, 1999).

2.6.2 Degree of acetylation of chitosan

There are many terms, sometimes rather confusing, referring to the degree of N-acetylation of chitosans such as degree of acetylation (DA), degree of deactylation (DD or DDA), or residual degree of acetyl groups (Vandamme *et al*., 2002) with all referring to the same meaning. The degree of acetylation (DA) represents the proportion of N-acetyl-D-glucosamine units with respect to the total number of units (Guinesi and Cavalheiro, 2006). It allows us to define the two terms chitin and chitosan. Thus, in the case of chitosan, its DA is considered to be below 50%. This value also determines the solubility limit of the polymer in dilute acidic solutions ($2 < pH < 6$) (Chatelet *et al*., 2001). A low degree of acetylation (DA) means a highly charged polyion in acidic solution (Berth and Dautzenberg, 2002). When the DA value is at 50% or lower, the polymer becomes water-soluble due to the protonation of the NH2 groups of the glucosamine unit (Liu *et al*., 2006).

While chitin refers to high DA polymers, ideally 100%, chitosan refers to low, ideally 0% DA (Liu *et al*., 2006). The degree of N-acetylation (DA) of the biopolymer that serves as an important parameter provides not only its physical–chemical properties, but also its biological, biomedical and food applications, among others (Chatelet *et al*., 2001; Guinesi and Cavalheiro, 2006). Commercial chitosans may contain insoluble highly acetylated fractions that come from the core of the granules submitted to heterogeneous deactylation (Jolles and Muzzarelli, 1999).

2.6.3 Biocide properties of chitosan

A special property of chitosan is its bioactivity, which determines the medical and veterinary application of this polymer (Rivero *et al*., 2008).

2.6.3.1 Antimicrobial agent

An antimicrobial agent is a natural or synthentic chemical that kills or inhibits the growth of microorganisms. Agents that kill organism are often called cidal agents, with a prefix indicating the kind of organism killed. Thus, there are bacteriocidal, fungicidal, and viricidal agents (Madigan *et al*., 2003). A bacteriocidal agent kills bacteria. It may or may not kill other kinds of microorganisms. Agents that do not kill but only inhibit growth are called static agents, and we can speak of bacteriostatic, fungistatic, and viristatic agents (Madigan *et al*., 2003).

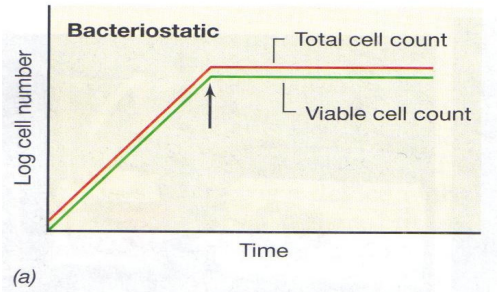


Figure 2.7: Bacteriostatic antimicrobial activity (Madigan *et al*., 2003).

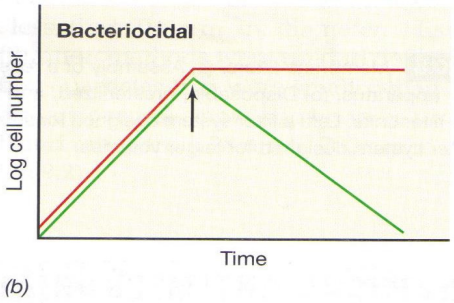


Figure 2.8: Bacteriocidal antimicrobial activity (Madigan *et al*., 2003).

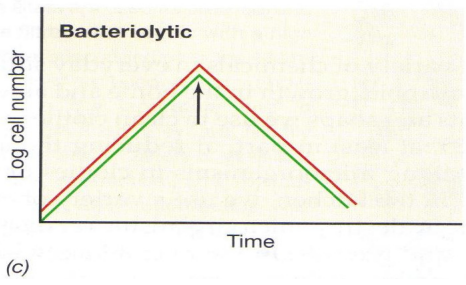


Figure 2.9: Bacteriolytic antimicrobial activity (Madigan *et al*., 2003).

2.6.3.2 Elicitation of defense responses by chitosan in plants

Plant's defense response against pathogens can be elicited by numerous external signals. Plant pathogens, known to be incompatible on a given plant species can elicit strong disease resistance responses, whereas an adapted compatible pathogen generates a weaker response and thus can more readily infect the plant tissue (Jolles and Muzzarelli, 1999).

The plant's response can be manipulated genetically by the transfer of 'R' genes, the genes controlling a qualitative potential for resistance against pathogens, or by treatment with elicitors such as chitosan (Jolles and Muzzarelli, 1999). Both of these manipulations can result in the rapid activation of a subset of genes called PR (pathogenesis-related) genes, generally regarded as the genes that functionally develop disease resistance. There was an early indication that chitosan had potential dual role in inducing a defense response as well as directly inhibiting fungi. There appear to be multiple modes by which chitosan can increase PR gene function, including activating cell surface or membrane receptors and internal effects on the plant's DNA conformation that in turn influence gene transcription (Jolles and Muzzarelli, 1999). Since chitosan confronts plant cell walls, cell membranes, the cytosol and the nucleus, all of which contain some negatively charged compounds, it concludes that chitosan may have multiple cellular targets.

Chitosan's targets within the membrane enable it to alter membrane function. Organelles of the cytosol capable of replicating such as mitochondria, chloroplasts, and nucleus possess polyanionic DNA (Jolles and Muzzarelli, 1999). At least two modes of action had been proposed for chitosan in living cells. First, in suspension-cultured plants and protoplasts, plant responses such as callose synthesis can be triggered by the polycationic chitosan as it localizes to the bound surface of protoplasts. Second, in intact pea endocarp and/or tobacco leaf tissue, chitosan can induce a set of genes known as disease resistance response (DRR) or pathogenesis-related (PR) genes and/or their promoters (Jolles and Muzzarelli, 1999). The current knowledge is that chitosan reaches the nucleus, is associated with limited DNA degradation and induces defense gene promoters in many plant species. Chitosan is a

poor mutagen, thus the DNA alteration is probably both weak and reparable (Jolles and Muzzarelli, 1999).

Chitin and its derivatives, primarily chitosan, constitute part of the natural signaling in the host-pathogen interactions. Chitosan can induce immunity in the plant to its true pathogens and all or portions of the non-host disease resistance response. Undoubtedly, chitosan is a versatile compound endowed with antimicrobial activity affecting growth and physiology of most microorganism, including algae, fungi, bacteria, protozoa, and viruses (Jolles and Muzzarelli, 1999).

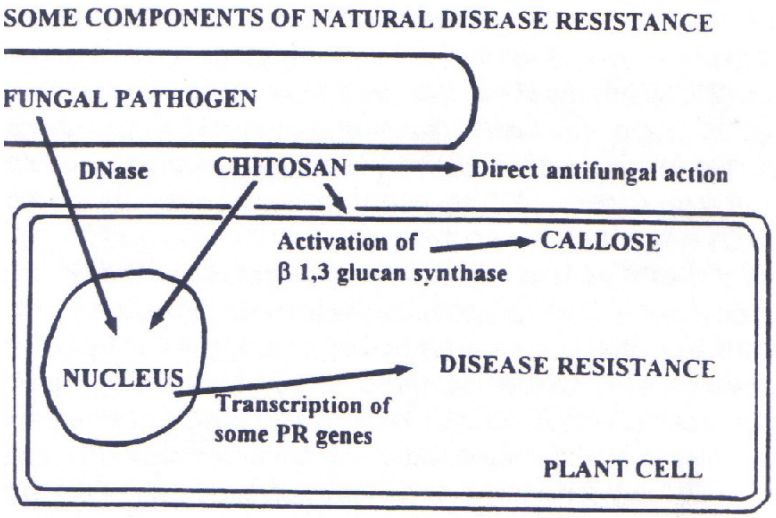


Figure 2.10: Some components of natural disease resistance (Jolles and Muzzarelli, 1999).

2.6.3.3 Economic applications of chitosan as microbial inhibitors

Chitosan has been approved by the Food and Drug Administration of the USA (FDA) as a wheat seed treatment. Chitosan has already been approved as a food additive in some countries, for instance in Japan and Korea (Fernandez-Saiz *et al*., 2008). Applications of chitosan to wheat seeds have been shown to influence the level lignin in nearly mature wheat plants, suggesting that the benefits of chitosan applied to seeds can be translocated to other parts of the developing plant (Jolles and

Muzzarelli, 1999). The applications of chitosan and its derivatives are widespread; they are used in agriculture, medicine, environment, food, etc. Apart from its antimicrobial effect, chitosan is also used in food as (Devlieghere *et al*., 2004):

a) clarifying agent in apple juice
b) antioxidant in sausages (Xie *et al.,* 2001)
c) enzymatic browning inhibitor in apple and pear juices and in potatoes.

2.6.3.4 Potential antimicrobial activity of chitosan-incorporated films

Chitosan shows antimicrobial activity along with the ability to resist environmental conditions and act as a source of nutrients (Jolles and Muzzarelli, 1999). It has been used to control growth of algae, and to inhibit viral multiplication in plants and *in vitro* (Koide, 1998). A potential application of chitosan resides in its ability to activate defense gene promoters (Jolles and Muzzarelli, 1999) since chitosan activates several defense processes in the host tissues, acts as a water-binding agent and inhibits various enzymes (Devlieghere *et al*., 2004) and microbial growth. The action of chitosan on inhibiting the germination and growth of plant pathogenic fungi was first reported on an array of fungi that did not include those with a predominance of chitosan as a natural component of the fungal cell wall. More recently, additional plant pathogenic fungi have been added to the chitosan-inhibited list (Jolles and Muzzarelli, 1999).

Several existing works have demonstrated the inherent biocide properties of this natural carbohydrate polymer against a wide range of microorganisms such as filamentous fungi, yeast and bacteria (Fernandez-Saiz *et al*., 2008). Chitosan has a wide inhibition spectrum for not only Gram-positive and Gram-negative bacteria and yeasts, but also moulds (Liu *et al*., 2004). As a new, natural antimicrobial agent, the possible uses of chitosan as a food preservative (Liu *et al*., 2004) and for antimicrobial packaging films (Ouattara *et al*., 2000) have been extensively studied. The nature of chitinous material, plain or derivative has a profound effect on its antimicrobial efficacy. The chemical structure differences between the plain and derivative chitosan also has effects on their functional differences (Jolles and

Muzzarelli, 1999). The antimicrobial activity of chitosan also depends on several factors such as the kind of chitosan (deacetylation degree, molecular weight) used, the pH of the medium, the temperature, the presence of several food components, etc (Devlieghere *et al*., 2004).

2.6.3.5 Effect of the nature of chitosan on antimicrobial activity

Plain chitosan seems to have a better molecular compatibility with the plant host and/or grain and fruit substrates than derivative chitosans such as N-carboxy-methylchitosan-N,O-sulfate (NCMC), thus inducing resistance to microbial attack. Chitosan antimicrobial action is more immediate on fungi and algae, followed by bacteria. Use of liquid chitosan as an antimicrobial agent is more effective than solid form. Liquid chitosan is readily or immediately uptaken by microbial and plant cells as compared with slow uptake of the solid (Jolles and Muzzarelli, 1999). Crustacean chitosan seems to exhibit more diverse antimicrobial mechanisms, including induction of chitosanase, phenolic compounds and blocking nutrient availability of the microbial cells, as compared with microbial chitosan, whose main antimicrobial mechanism seem to be induction of chitosanase only (Jolles and Muzzarelli, 1999). The antimicrobial activity of chitosan has been correlated with its degree of deacetylation and polymerization. Exogenous chitosan treatment with lower deacetylation exhibited better inhibition of toxigenic *A. flavus* growth and peanut amd maize seeds as compared with chitosan with higher deacetylation. Also, exogenous chitosan with lower deacetylation induced better antifungal activity and chitosanase production in bacteria (Jolles and Muzzarelli, 1999).

2.6.3.6 Biodegradabilty of chitosan

Biodegradable and non-toxic materials that are capable of activating host defenses to prevent infection are highly desirable. Lysozyme N-acetyl-D-glucosaminidase and lipases degrade chitins and chitosans. Nitrogen monoxide may

also play a role in the chemoenzymatic degradation process (Jolles and Muzzarelli, 1999). Jolles and Muzzarelli (1999) reported that chitin sutures developed in the early 1980s undergo relatively rapid biodegradation *in vivo*: the resistance to traction of their knots falls to 74% after 5 days and 19% after 15days. Increased knot resistance is claimed for N-acyl chitosans, particularly N-pentanoyl chitosan (degree of substitution <0.20) that is expected to last for 2 years or longer.

2.7 Microorganisms

Microorganisms are a large and diverse group of microscopic organisms that exist as single cell or cell clusters. This includes viruses, which are microscopic but not cellular. Microbial cells are therefore distinct from the cells of animals and plants, which are unable to live alone in nature and can exist only as parts of multicellular organisms (Madigan *et al*., 2003).

In contrast to macroorganisms, microorganisms are generally able to carry out their life processes of growth, energy-generation, and reproduction independently of other cells, either of the same kind or of a different kind (Madigan *et al*., 2003). Pathogens are disease-causing microorganisms. The presence of a particular type of microorganisms in a part of the body where it is not normally found is called an infection – and may lead to disease. Infection is the invasion or colonization of the body by pathogenic microorganisms. Disease is an abnormal state in which part or all of the body is not properly adjusted or incapable of performing its normal functions (Tortora *et al*., 1998).

2.8 Miscibility of starch and chitosan

The X-ray diffractogram of chitosan-coated film (Figure 2.11) shows the change in reflection characteristics of starch crystalline, indicating the chitosan-

starch interaction occurring at the molecular level which leads to the strong adhesion between starch base film and chitosan coating layer.

This fact is attributed to the hydrogen-bonding interaction between chitosan and starch molecules, leading to structural reorientation and subsequent starch crystallinity (Bangyekan *et al*., 2006).

When two components are mixed together, the physical blends versus chemical interactions are affected by changes in the characteristic infrared spectra (Bourtoom and Chinnan, 2008). In the spectrum of rice starch-chitosan biodegradable blend film, the amino group peak of chitosan shifted from 1541.15 to 1621.96 cm^{-1} (Figure 2.12). This phenomenon pointed out that interactions are present between the hydroxyl group of rice starch and the amino group of chitosan (Bourtoom and Chinnan, 2008; Xu *et al*., 2005).

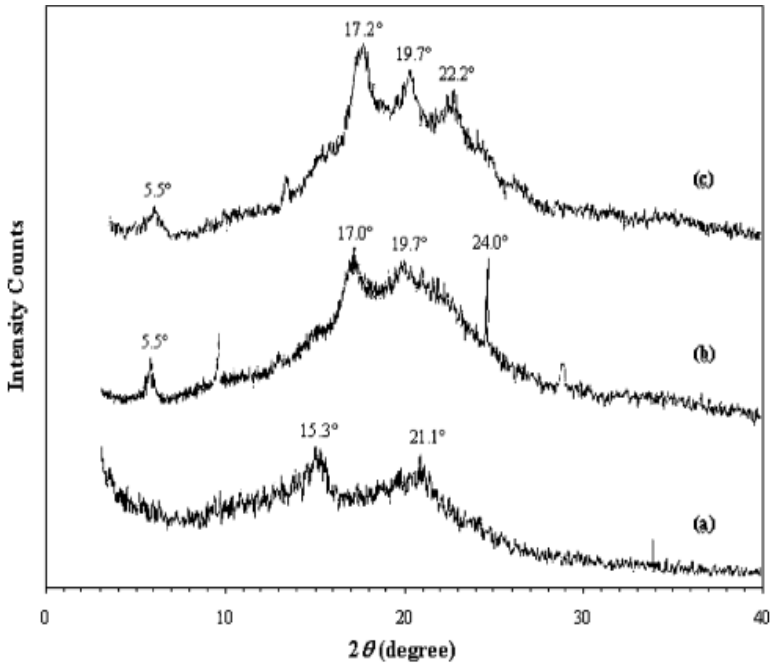


Figure 2.11: X-Ray diffractograms of: (a) free chitosan film, (b) glycerol-plasticized starch films, and (c) chitosan coated starch film (Bangyekan *et al*., 2006).

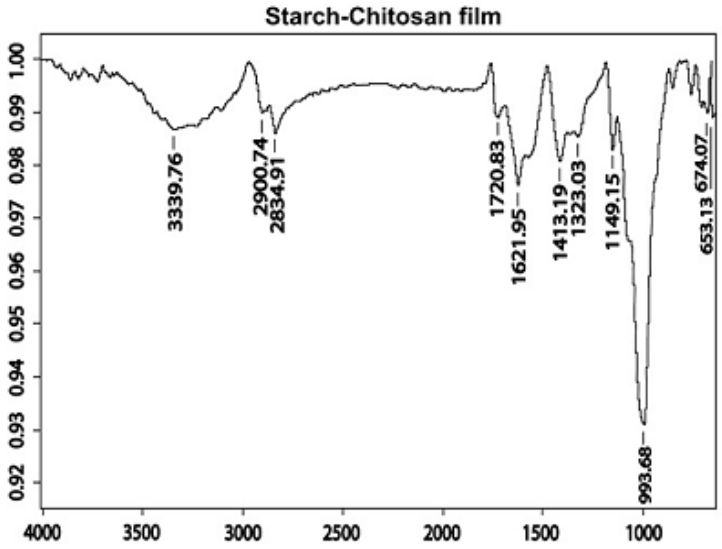


Figure 2.12: Attenuated total reflection (ATR) spectra of rice starch-chitosan biodegradable film with the ratio of rice starch to chitosan 1:1 (Bourtoom and Chinnan, 2008).

2.9 Packaging films

The commonly used packaging films are presented in Table 2.4. Although a total replacement of synthetic plastics by the biodegradable materials is just impossible, at least for some specific applications such a replacement seems obvious and useful. Towards this end, there exists a huge business opportunity. Nevertheless, such a replacement by biodegradable materials would also allow us preserve or extend our expensive, dwindling petroleum resources, and helps us save on our foreign exchange. Essential prerequisites of a good packaging film (Tharanathan, 2003) are:

a) allow for a slow but controlled respiration (reduced oxygen absorption) of the commodity, allow for a selective barrier to gases (carbon dioxide) and water vapor;
b) creation of a modified atmosphere with respect to internal gas composition, thus regulating the ripening process and leading to shelf-life extension;
c) lessening the migration of lipids—of use in con confectionery industry;

d) maintain structural integrity (delay loss of chlorophyll) and improve mechanical handling;
e) serve as a vehicle to incorporate food additives (flavors, colors, antioxidants, antimicrobial agents) and
f) prevent (or reduce) microbial spoilage during extended storage.

Table 2.4: Packaging films commonly used (Tharanathan, 2003).

Film type	***Monomeric Unit***	**Characteristics**
Polyethylene	Ethylene	Desirable mechanical properties, heat sealable.
Polyvinylidene	Vinylidene	Desirable H_2O/O_2 barrier, not very strong, heat sealable.
Polyester	Ethyleneglycol + terepththalic acid	Desirable mechanical properties, poor H_2O/O_2 barrier, not heat sealable.
Polyamide (Nylon)	Diamine + various acids	Desirable strength, heat sealable, poor H_2O/O_2 barrier.
Cellophane	Glucose (cellulose)	Desirable strength, good H_2O/O_2 barrier, not heat sealable.

2.10 Biopolymers

Bio-based polymers may be divided into three main categories based on their origin and production (Petersen *et al*., 1999):

a) Category 1

   Polymers directly extracted/removed from biomass. Examples are polysaccharides such as starch and cellulose and proteins like casein and gluten.

b) Category 2

Polymers produced by classical chemical synthesis using renewable bio-based monomers. A good example is polylactic acid, a bio-polyester polymerised from lactic acid monomers. The monomers themselves may be produced via fermentation of carbohydrate feedstock.

c) Category 3

Polymers produced by microorganisms or genetically modified bacteria. To date, this group of bio-based polymers consists mainly of the polyhydroxyalkonoates, but developments with bacterial cellulose are in progress.

The most common bio-based polymers, materials and packaging are presented in Figure 2.13 (Petersen *et al.*, 1999)

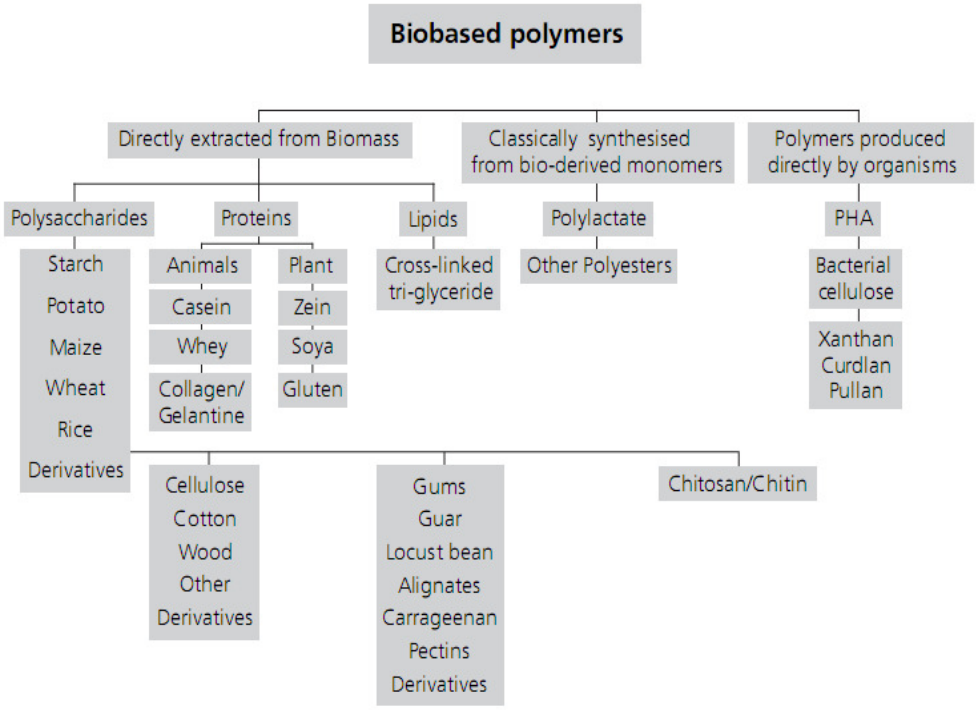


Figure 2.13: Schematic presentation of bio-based polymers based on their origin and method of production (Petersen *et al*., 1999).

2.11 Biofibers

Natural fibers are subdivided based on their origins, coming from plants, animals or minerals. All plant fibers are composed of cellulose while animal fibres consist of proteins (hair, silk, and wool). Plant fibers include bast (or stem or soft sclerenchyma) fibers, leaf or hard fibers, seed, fruit, wood, cereal straw, and other grass fibers (John and Thomas, 2008). The reinforcing efficiency of natural fiber is related to the nature of cellulose and its crystallinity. The main components of natural fibres are cellulose (α-cellulose), hemicellulose, lignin, pectins, and waxes (John and Thomas, 2008).

Table 2.5: List of important biofibers (John and Thomas, 2008)

Fiber source	***Species***	***Origin***
Abaca	*Musa textilis*	Leaf
Bagasse	-	Grass
Bamboo	(>1250 species)	Grass
Banana	*Musa indica*	Leaf
Oil Palm	*Elaeis guineensis*	Fruit
Pineapple	Ananus comosus	Leaf

2.12 Biodegradable polymers/films

Biodegradable polymers are defined as polymers that are degraded and catabolized eventually to carbon dioxide and water by microorganism (bacteria, fungi, etc.) under natural environment (Okada, 2002). These polymers, when they are degraded, should not generate any substances that are harmful to the natural environment (Okada, 2002). Biodegradable polymers are broken into three major categories namely polyesters produced from microorganisms, natural

polysaccharides and other biopolymers, and synthetic polymers, particularly aliphatic polyesters (Okada, 2002).

Table 2.6: Types of biodegradable polymers and their examples (Okada, 2002).

Types of biodegradable polymer	***Examples***
Polyesters from microorganisms	Derived from bio-organic sources
Natural polysaccharides and other bio-polymers	Starch
Synthetic polymers	Poly(L-lactide), poly(butylene succinate)

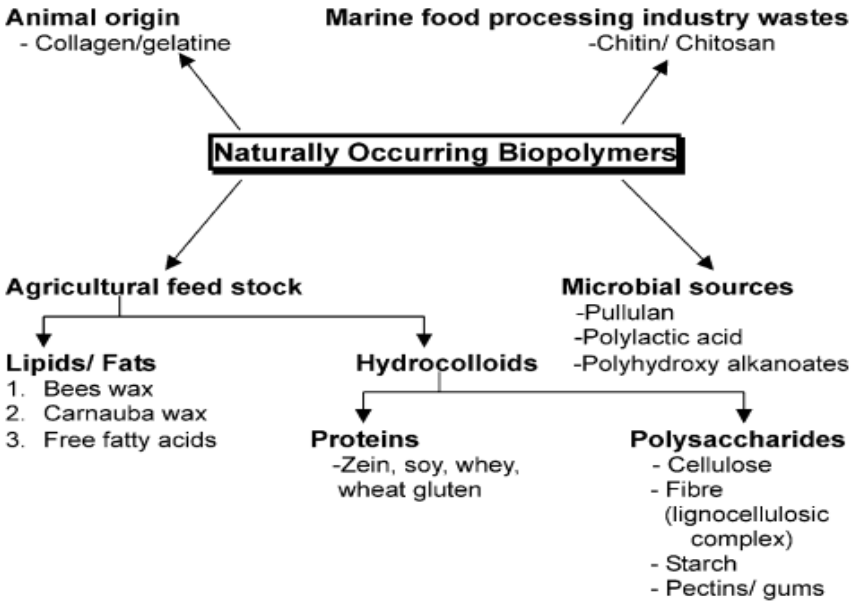


Figure 2.14: Naturally occurring biopolymers of use in biodegradable packaging films and composites (Tharanathan, 2003).

The various naturally occurring biopolymeric materials of use in composite film making and coating formulations are shown in Figure 2.14. Coating composites based on such biomolecules have brought a surge of new types of packaging materials into use. These biomolecules are compatible amongst themselves and with other hydrocolloids, surfactants and additives, and their aqueous solutions are usually stable at acidic and neutral pH. The composite solutions are preserved for

extended/repeated use by adding benzoic acid, sorbic acid or their sodium salts (Tharanathan, 2003).

2.13 Composite biodegradable films

When it comes to improvements in edible film technologies, most research has addressed film formulations using various combinations of edible materials. Two or more materials can be combined to improve gas exchange, adherence to coated products, or moisture vapor permeability properties (Cutter, 2006).

Polymer composites are mixtures of polymers with inorganic or organic additives having certain geometries (fibers, flakes, spheres, particulates) (Sorrentino *et al*., 2007). Composites are engineered materials made from two or more constituents with significantly different physical or chemical properties from their components, which remain separate and distinct within the finished structure (Simkovic, 2008). There are two types of constituent materials, which are known as matrix and reinforcement components.

Composite edible films and coatings can be formulated to combine the advantages of each component. Whereas biopolymers, such as proteins and polysaccharides, provide the supporting matrix, lipids provide a good barrier to water vapor (Rivero *et al*., 2008). The many types of composite films researched on and appeared in published journals are tabulated in Table 2.7.

Composite films are in fact a mixture of these and other ingredients in varying proportions, which determine their barrier (to H_2O, O_2, CO_2 and aroma compounds) and other mechanical properties. Sometimes a composite film formulation can be tailor made to suit to the needs of a specific commodity or farm produce. For example, oranges having a thick peel are prone to anaerobic conditions, which lead to an early senescence and spoilage if the composite film is rich in lipids (Tharanathan, 2003).

Table 2.7: The types of composite films, materials used, year, and reference.

Type of composite film	***Materials used***	***Year***	***Reference***
Emulsion/Bilayer film	Lipids and a mixture of proteins or polysaccharides.	2006	Cutter.
Chitosan-coated cassava film	Chitosan and cassava starch or tapioca.	2006	Bangyekan *et al*.
LDPE/Starch blends	1. Low density polyethylene, wheat starch, and soluble starch.	1997	Psomiadiaou *et al*.
		1998	
	2. Low density polyethylene, rice and potato starch		Arvanitoyannis *et al*.
Chitosan/PLA	Chitosan and PLA	2006	Sebastien *et al*.
Polyhydroxybutyrate-hydroxyvalerate (PHB-HV)/maize starch blends	Polyhydroxybutyrate-hydroxyvalerate (PHB-HV) with maize starch	2008	Reis *et al*.
Cod gelatin/sunflower oil blends	Sunflower oil and cod gelatin	2009	Perez-Mateos *et al*.
Starch/chitosan blend	1. Corn starch and chitosan.	2004	Zhai *et al*.
	2. Rice starch and chitosan.	2008	Bourtoom and Chinnan
Starch/clay nanocomposite films	Potato starch and purified clay.	2005	Avella *et al*.
Gelatin/poly(vinyl alcohol) blends	Pigskin gelatin and PVA	2008	Mendieta-Taboada *et al*.
Carageenan-pectin blend film	k-carageenan and pectin from citrus fruits	2006	Alves *et al*.
Bioactive composite film	Combination of vegetable oils, glycerin, citric acid, and antioxidants.	2006	Cutter.

2.14 Factors that affect the performance of biodegradable films

2.14.1 Concentration of starch

Starch consists primarily of branched and linear chains of glucose molecules, named as amylopectin and amylose respectively. Amylose is essentially a linear molecule with a few branches, whereas amylopectin is a highly branched molecule. Preponderance of amylose in starches gives stronger films (Mathew and Abraham, 2008). Branched structure of amylopectin generally leads to films with different mechanical properties, such as decreased tensile stress (Alves *et al*., 2007).

2.14.2 Concentration of chitosan

Chitosan, a de-N-acetylated analog of chitin, is a hetero-polysaccharide consisting of linear b-1,4-linked GlcN and GlcNAc units. Both the content and sequence of these units will determine the physico-chemical and the biological properties of the polymer. It is known that heterogeneous conditions during deacetylation provide a block-wise distribution whereas under homogeneous conditions a random distribution of acetyl groups appears in chitosan (Prashanth and Tharanathan, 2007).

Due to its rigid and specific crystalline structures, possible through intra- and intermolecular hydrogen bonding, chitosan has the ability to exist in nature in different polymorphic forms, whose properties vary considerably (Prashanth and Tharanathan, 2007).

The strength of starch-based films can be tremendously enhanced by the incorporation of either cellulosic fibers or chitosan or both. Film tensile showed a linear increase with an increase in fiber and chitosan content. A higher fiber and chitosan content resulted in a decrease in film stretch (Ban *et al*., 2006).

Chitosan inhibits the growth of a wide variety of bacterial and fungi showing broad spectra of antimicrobial activity, high-killing rate and low-toxicity toward mammalian cells (Sun, *et al*., 2006). Because the antimicrobial activity of chitosan is very limited, various efforts have been taken to improve it. Some researchers studied the effect of the molecular weight, degree of deacetylation, solvent, pH, etc. on the antimicrobial activity of chitosan, so as to enhance the activity by adjusting these factors (Sun, *et al*., 2006).

Some desirable properties of chitosan are that it forms films without the addition of additives, exhibits good oxygen and carbon dioxide permeability, as well as excellent mechanical properties (Cutter, 2007). Linear structure of some of these polysaccharides, for example, chitosan (1, 4-b-d-glucosamine polymer), renders their films tough, flexible and transparent (Tharanathan, 2003).

2.14.3 Concentration of plasticizer

Film characteristics are dependent on the cohesion of the polymeric matrix, which in turn is dependent on the structure of the polymer chains, the film obtainment process and the presence of plasticizer agents (Muller *et al*., 2008). The incorporation of plasticizers is necessary to reduce polymer intermolecular forces, increasing the mobility of the polymeric chains, and improving the mechanical characteristics of the film, such as the film extensibility (Muller *et al*., 2008). Plasticizers also affect the water barrier property of these films for they have a great affinity for water.

Water content and interactions in starch films are dependent not only on plasticizer concentration, but also on the relative humidity (RH) to which these materials are exposed. It has been reported that the interactions between the components of the system, formed by starch, plasticizer and water, vary according to the relative quantities of these components. It is possible that, under some conditions, phase separation occurs (Muller *et al*., 2008).

2.14.4 Amount of water

Water plays a significant role for the properties of starch. Water is compatible with starch and is an effective plasticizer. With increasing content of water starch shows both an increasing strain at break and stress at break (Jansson and Thuvander, 2004). Jansson and Thuvander (2004) also pointed out that material with a higher amount of water crystallizes more rapidly than material with higher overall plasticizer content. The change in water content and crystallinity are affecting the stress–strain properties.

Starch and chitosan are hydrophilic and retain considerable amount of water. The amount depends on the relative humidity, but in most cases is above 10%, in contrast to the small amount (about 1%) observed for most common synthetic polymers (Roff & Scott, 1971). Thus, the infrared spectra of starch and chitosan are greatly affected by the presence of absorption bands related to water (Rueda *et al*., 1999).

2.14.5 Thickness of film

Variation in degree of molecule stretch is a possible explanation for the variation seen in stiffness with thickness. As water evaporates, the volume decreases and the film shrinks, consequently the film will deform. The rate of deformation will depend on the thickness of the film as the evaporation of water becomes controlled by diffusion for the thicker specimens (Jansson and Thuvander, 2004). When thin films are made the water evaporates fast, at room temperature the rate of molecular movement is limited and the molecules in the film do not have time to respond to the shrinking of the film (Jansson and Thuvander, 2004). For the thick films, the water evaporates slowly and the molecules have enough time for relaxation. Since, the thicker films are exposed to a higher degree of water for a longer time the crystallinity in these films are expected to be higher than in the thin films (Jansson and Thuvander, 2004).

2.14.6 Poly(lactic acid) (PLA)

PLA belongs to the family of aliphatic polyester commonly made from lactic acid, which can be produced from renewable resources such as starch via fermentation processes (Sebastien *et al*., 2006). Due to its relatively hydrophobic nature the use of this polyester could reduce the hydrophobilic nature of chitosan-based films and consequently improve their moisture barrier properties and decrease overall the water/matrix interactions (Sebastien *et al*., 2006).

2.15 Innovations in food packaging

The food industry uses a lot of packaging materials, and thus even a small reduction in the amount of materials for each package would result in a significant cost reduction, and may improve solid waste problems. Food packaging has evolved from simple preservation methods to convenience, point-on-purchase (POP) marketing, material reduction, safety, tamper-proofing and environmental issues. Since the World Trade Center tragedy in 2001, food technologists have focused their attention on revising packaging systems and package designs to increase food safety and security. The level of concern regarding the use of food and water supplies as a form of bioterrorism has increased. Therefore, many applications of active packaging will be commercially developed for the security and safety enhancement of food products.

However, the functional, organoleptic, nutritional and mechanical properties of an edible film can be modified by the addition of various chemicals in minor amounts (Romero-Bastida *et al.*, 2005). Native and modified starches (from traditional and alternative sources) have been used since ancient times as a raw material to prepare different products. They are employed in foodstuffs because of their good thickening and gelling properties. Actually, they are used for the formation of starch-based edible films and coatings (Jansson and Thuvander, 2004; Mali *et al.*, 2005). However, the current tendency is to look for alternative sources for obtaining starch with better physicochemical and functional characteristics.

2.15.1 Active packaging

Active packaging primarily deals with maintaining or increasing quality and safety of packaged foods, i.e. shelf-life of packaged food products (Lopez-Rubio *et al.*, 2006).

2.15.2 Bioactive packaging

Bioactive packaging has a direct impact on the health of the consumer by generating healthier packaged foods (Lopez-Rubio *et al.*, 2006). It is envisaged that the development of these innovative concepts can be carried out by (Lopez-Rubio *et al.*, 2006):

a) integration and controlled release of bioactive components or nano-components from biodegradable and/or sustainable packaging systems,
b) micro- and nano-encapsulation of these active substances either in the packaging and/or within foods and
c) packaging provided with enzymatic activity exerting a health-promoting benefit through transformation of specific food-borne components

2.16 Atomic force microscopy (AFM)

Another form of microscopy useful for three-dimensional imaging of biological structures is the atomic force microscopy (AFM) (Madigan *et al*., 2003). AFM is a relatively new technique, which permits high resolution without the need of coating the sample. A very fine-tipped probe scans the surface of the specimen, generating a three-dimensional image (Vandamme *et al*., 2002). A tiny stylus is positioned extremely close to the specimen such that weak repulsive atomic forces are established between the probe and the specimen. As the specimen is scanned in both the horizontal and vertical directions, the stylus rides up and down the hills and valleys, constantly recording its interactions with the surface (Madigan *et al*., 2003).

This pattern is monitored by a series of detectors that feed the digital information into a computer that generates an image. Although the images obtained from an atomic force microscope appear similar to those from the scanning electron microscope. The AFM has the big advantage that specimen preparation is similar to that for light microscopy, that is, no fixatives or coatings are required (Madigan *et al*., 2003). The AFM also allows living and hydrated specimens to be viewed, something that is generally not possible with the electron microscopes (Madigan *et al*., 2003).

Figure 2.15: An atomic force microscope.

2.17 Fourier transform infrared (FTIR)

Fourier-transform infrared (FTIR) spectrometers offer the advantages of unusually high sensitivity, resolution, and speed of data acquisition (data for an entire spectrum can be obtained n 1 second or less). In the early days of FTIR, instruments were large, intricate, expensive devices controlled by expensive laboratory computers (Skoog *et al*., 2004). As the instrumentation evolved and the price of computers dropped dramatically while the power, speed, and ease of use improved by orders of magnitude, FTIR spectrometers have become a commonplace in the laboratory (Skoog *et al*., 2004). Fourier-transform instruments contain no

dispersing element, and all wavelengths are detected and measured simultaneously using a Michelson interferometer. To separate wavelengths, the source signal is modulated and passed through the sample in such a way that it can be recorded as an interferogram (Skoog *et al*., 2004).

The interferogram is subsequently decoded by a Fourier transformation, a mathematical operation conveniently carried out by the computer, which is now an integral part of nearly all spectrometers (Skoog *et al*., 2004).

Figure 2.16: Photo of a basic student-grade benchtop FTIR spectrometer. Spectra are recorded in a few seconds and displayed on the LCD panel for viewing and interpretation (Skoog *et al*., 2004).

Radiation of all frequencies from the IR source is reflected into the spectrometer where it is modulated by the moving mirror on the left. The modulated radiation is then reflected from the two mirrors on the right through the sample in the compartment at the bottom. After passing through the sample, the radiation falls on the detector. A data acquisition system attached to the detector records the signal and stores it in the memory of a computer as an interferogram (Figure 22) (Skoog *et al*., 2004).

The Attenuated Total Reflectance (ATR) technique is used to obtain the spectra of solids, liquids, semisolids, and thin films. ATR is performed using an

accessory that mounts in the sample compartment of an FTIR. At the heart of the accessory is a crystal of infrared transparent material of high refractive index. Typical materials used are zinc selenide, KRS-5(thallium iodide/thallium bromide) and germanium (Smith, 1996).

2.18 Thermal gravimetric analysis (TGA)

It is important to carry out studies on the thermal properties and thermal stability of blend films for their application in food and pharmaceutical industry as the edible or biodegradable films may be subjected to heat processes or heat decomposition during their preparation, processing or consumption (Mathew and Abraham, 2008).

Decomposition can be looked upon as the reverse reaction of synthesis. Polymers with a ceiling temperature can simply show a reverse of the polymerization reaction. Figure 2.20 represents typical thermogravimetric traces for the decomposition of poly(methyl methacrylate), PMMA, and polyttetrafluoroethylene, PTFE. This process is nothing else but the sublimation or evaporation, common when heating small molecules or rigid molecules (Wunderlich, 2005).

The reversal of polymerization, however, is not the norm for polymer decomposition. Most polymers show decomposition to multiple products. Especially in the presence of oxygen is there always a chance of oxidation, or even self-sustaining burning. Such reactions are better called pyrolysis than decomposition. The thermogravimetric is for this reason usually done in an inert atmosphere, such as nitrogen, unless oxidation is to be studied (Wunderlich, 2005). Decomposition traces normally show three stages of weight loss due to:

a) the evaporation of water and PEG400,
b) the dehydration of the saccharide rings, depolymerization and the decomposition of the acetylated and deacetylated units of polymer (Mathew and Abraham, 2008).

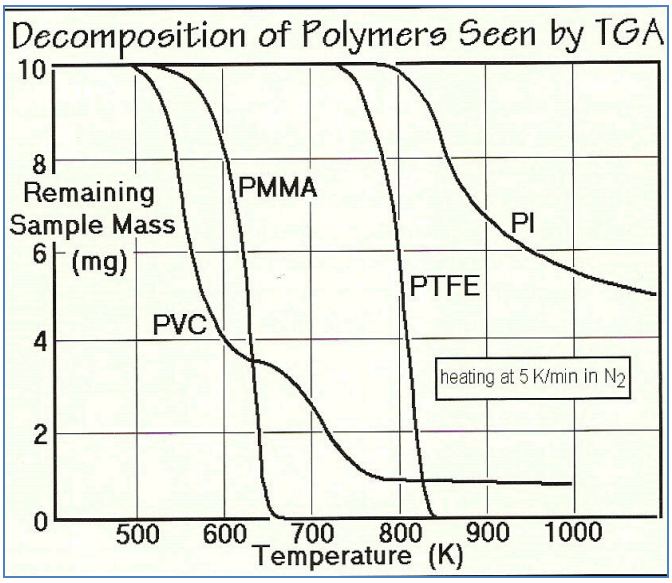


Figure 2.17: The typical decomposition trends of various polymers (Wunderlisch, 2005).

2.19 Differential scanning calorimetry (DSC)

Differential scanning calorimetry, DSC, is a technique which combines the ease of measurement of heating and cooling curves with the quantitative features of calorimetry. Temperature is measured continuously, and a differential technique is used to assess the heat flow into the sample and to equalize incidental heat gains and losses between reference and sample (Wunderlisch, 2005).

One of the important measurements obtained through DSC is the determination of heats of transition. The sample undergoing the transition remains at the transition temperature by absorbing or evolving the heat of transition (latent heat) (Wunderlisch, 2005). The temperature range for the DSC analysis from 25°C to 300°C was selected for two reasons (Rueda *et al*., 1999):

a) to avoid endothermic signals related to the melting of frozen water around 0°C,
b) to limit possible sample degradation since TGA analysis showed that the film samples were only thermally stable up to about 250°C.

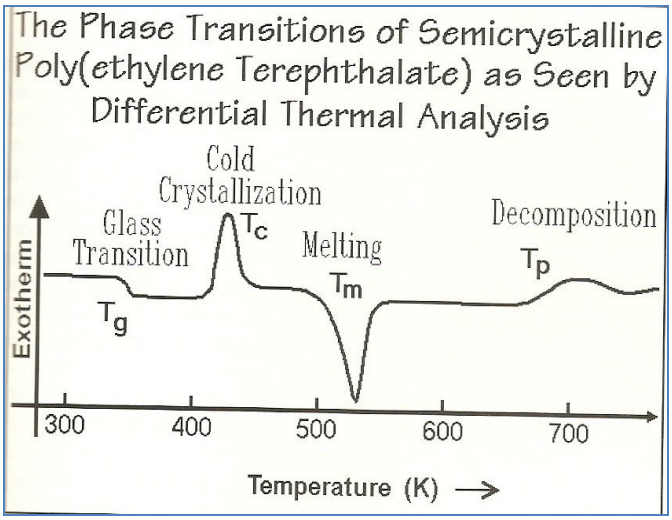


Figure 2.18: The typical trend of a DSC curve (Wunderlisch, 2005).

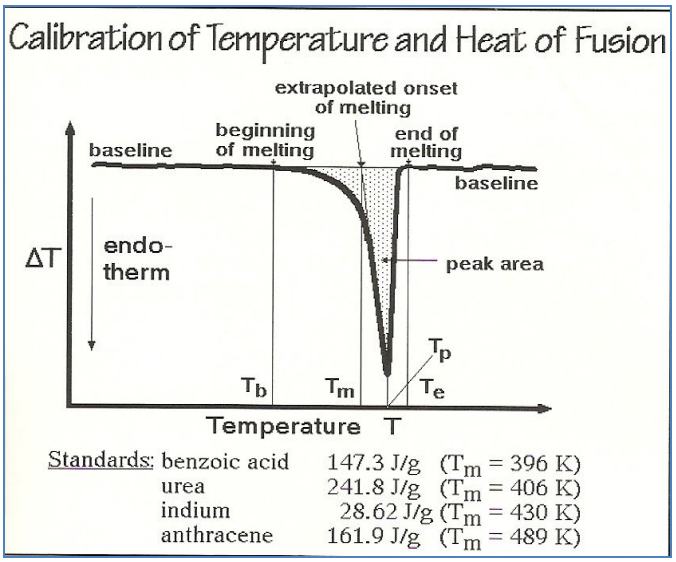


Figure 2.19**:** The typical melting point curve obtained through DSC analysis (Wunderlisch, 2005).

In addition to the ability of DSC in analyzing the mechanical properties of polymer blends, it is also well known that their Tg is an important criteria for the miscibility of components. In a completely miscible blend of two polymers, only one Tg, found between the Tg values of each pure polymer, will appear in DSC thermograms. If two components are only partially miscible, the Tg value of each component phase should be affected by the other one, and it is usually composition dependent (Suyatma *et al.*, 2004).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipment

The equipments that were used throughout the experiment are listed in Table 3.1 below.

Table 3.1: List of equipments with their brand name and model

Equipment	*Brand Name/Model*
Oven	HERAEUS/UT-6
Hot Plate and Magnetic Stirrer	BIBBY STERILIN/HB502
Ultrasonics Cleaner	DAIHAN/DH300H
Thermal Gravimetric Analyzer, TA	TA INSTRUMENTS/Q500TGA
Differential Scanning Calorimeter, DSC	TA INSTRUMENTS/Q1000
Fourier Transform Infrared Spectrometer, FTIR	THERMO NICOLET /370 DTGS
Atomic Force Microscope, AFM	SIRIM-UNIMAP

3.1.2 Chemicals and Raw Materials

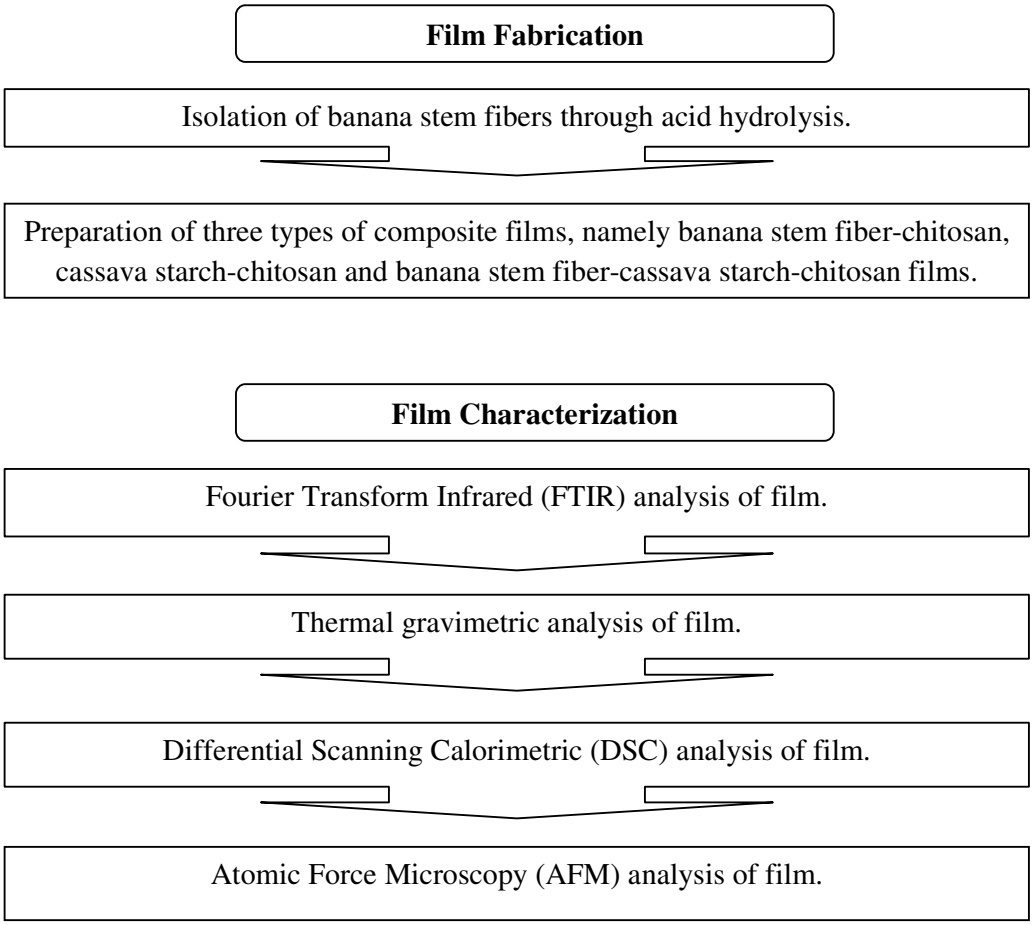
The chemicals and raw materials that were used throughout the experiment are listed in Table 3.2.

Table 3.2: List of chemicals and raw materials with their major supplier

Chemicals and Raw Materials	***Supplier***
Sodium Hydroxide, NaOH	Fisher Scientific
Hydrochloric Acid, HCl	SYSTERM
Polyethylene Glycol 400, PEG 400	R&M Chemicals
Chitosan (Low Viscous)	Fluka Analytical
Cassava Starch	Namye Food Industries Sdn. Bhd.
Banana Stem	Banana Plantation in Kuantan
Acetic Acid Glacial, CH_3COOH	R&M Chemicals

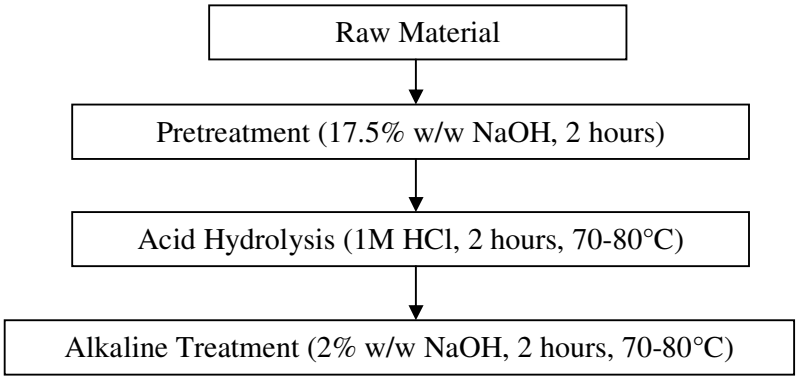
3.2 Methodology

3.2.1 Overview of methods



3.2.2 Film fabrication

3.2.2.1 Isolation of banana stem fibers through acid hydrolysis



A banana pseudostem (fake stem) was cut out of the whole of a banana tree that had born fruits before. The latex oozing out of the stem was washed away with distilled water beforehand. The stem was then dissected to obtain the soft central stalk. The soft central stalk obtained was subsequently cut into small pieces and soaked in a sodium hydroxide solution of concentration 17.5% w/w for 2 hours at room temperature. After the two-hour pretreatment, the stalk pieces were subjected to extensive rinsing with distilled water and later treated with 1M hydrochloric acid solution of 1%v/v at 80°C for 2 hours. Following the acid hydrolysis treatment, the stalk pieces were then treated with a sodium hydroxide solution of concentration 2% w/w for 2 hours at 80°C. The stalk pieces were then let to dry in the oven at 60°C for one night. Ultimately, when the stalk pieces were dry enough, they were blended into extremely short strands to obtain an almost powdery form of fibers.

Figure 3.1: The banana pseudostem is being cut off.

Figure 3.2: The soft central stalk is being shown.

Figure 3.3: The pseudostem is dissected and the central stalk is taken out.

Figure 3.4: Pieces of banana central stalk immersed in a solution of 17.5 % w/w sodium hydroxide at ambient temperature.

Figure 3.5: Pieces of banana central stalk taken out of the oven after being dried at 60°C. Acid hydrolysis has been carried out beforehand.

Figure 3.6: Blended banana central stalk. The fibers have been subjected to acid hydrolysis.

3.2.2.2 Preparation of banana stem fiber-chitosan composite film

4g of low viscous chitosan powder was weighed and placed inside a beaker. 100mL of 1% v/v 1M acetic acid was then added into the beaker containing the chitosan powder and heated on a hot plate to dissolve the chitosan powder resulting in a solution of chitosonium acetate. Next, 1g of banana stem fibers was weighed and dissolved in 100mL of distilled water. Following that, 5mL of PEG400 was consequently added as a plasticizer to enhance the film's properties. The solutions were then mixed and stirred at 300RPM for 8 hours for them to miscibilize. Degassing was carried out in an ultrasonic bath since there were bubbles present in the solution. The end solution was finally cast onto a glass plate. The cast film was allowed to dry and subsequently peeled off the glass plate.

3.2.2.3 Preparation of cassava starch-chitosan composite film

4g of low viscous chitosan powder was weighed and placed inside a beaker. 100mL of 1% v/v 1M acetic acid was then added into the beaker containing the chitosan powder and heated on a hot plate to dissolve the chitosan powder resulting in a solution of chitosonium acetate. For the next step, 2g of cassava starch was weighed and added into a beaker containing 100mL of distilled water. The starch solution was heated to 82 - 89°C to bring the solution to gelatinization. The solutions of chitosonium acetate and starch were afterwards mixed with 5mL of PEG400 and stirred at 300RPM for 8 hours for them to misciblize. Degassing was carried out in an ultrasonic bath right after that since there were air bubbles present in the solution. The end solution was then cast on a glass plate and left to dry overnight. Subsequently, the film was peeled off the glass plate when it was dry.

3.2.2.4 Preparation of banana stem fiber-cassava starch-chitosan composite film

4g of low viscous chitosan powder was weighed and placed inside a beaker. 100mL of 1% v/v 1M acetic acid was then added into the beaker containing the chitosan powder and heated on a hot plate to dissolve the chitosan powder to form a solution of chitosonium acetate. In the next step, 2g of cassava starch was weighed and placed inside a beaker containing 100mL of distilled water. The starch solution was heated on a hot plate to 82 - 89°C to bring the solution to gelatinization. 1g of banana stem fibers was finally weighed and placed inside a beaker containing 100mL of distilled water. All three solutions were afterwards mixed together with 5mL of PEG400 being added as plasticizer. Degassing was carried out in an ultrasonic bath to wring out the gas bubbles present in the solution. The end solution was stirred at 300RPM for 8 hours and cast onto a glass plate. The cast film was left to dry overnight and peeled off the next day when it was dry enough.

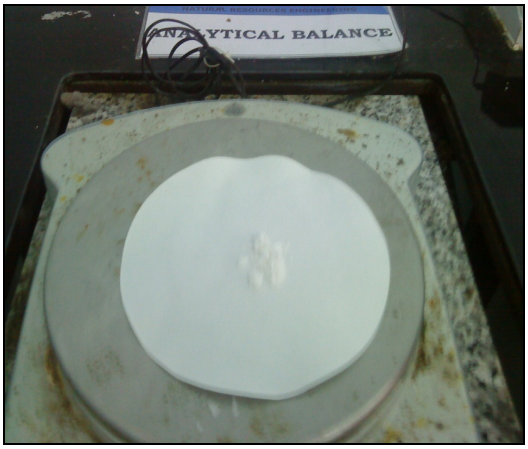


Figure 3.7: 2g of cassava starch is weighed on an electronic balance.

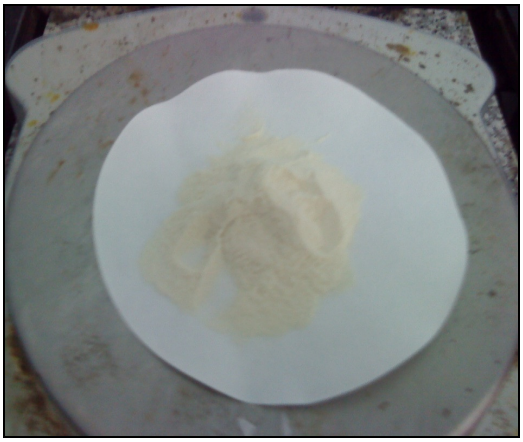


Figure 3.8: 4g of low viscous chitosan is weighed on an electronic balance.

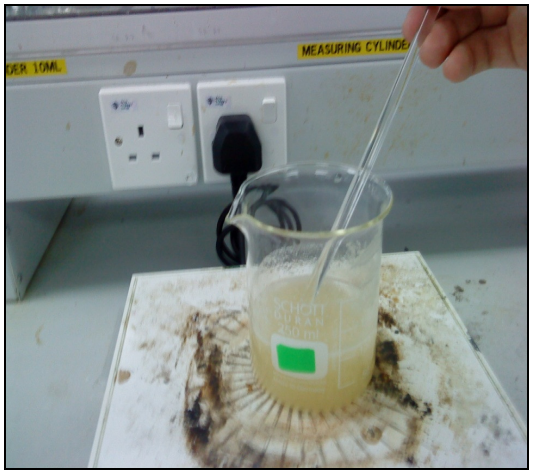


Figure 3.9: Chitosan is dissolved in a solution of acetic acid 1% v/v.

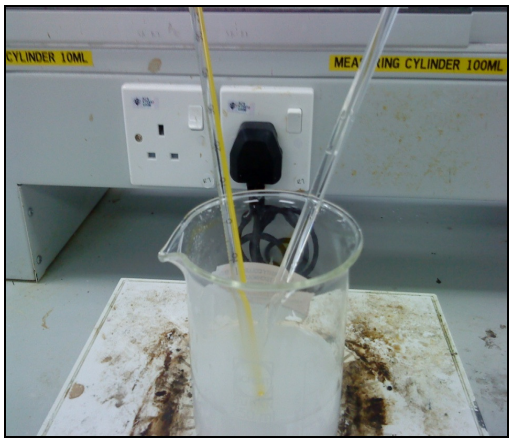


Figure 3.10: Cassava starch is dissolved in 100mL of distilled water and heated to 82 – 89°C.

Figure 3.11: Cassava starch forms a viscous and almost transparent solution after being brought to gelatinization.

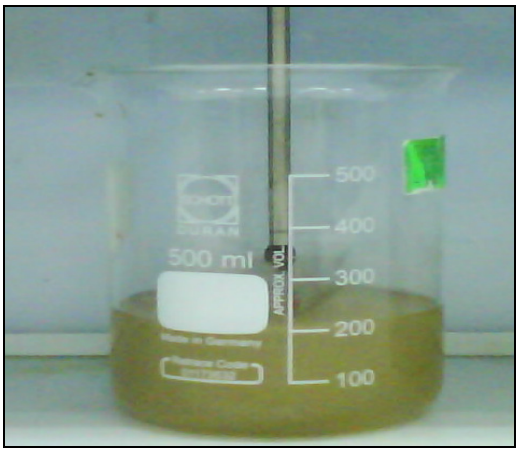


Figure 3.12: Solution of banana stem fiber-chitosan is stirred at 300RPM for 8 hours.

Figure 3.13: Cast solution is peeled off when dry.

3.2.3 Film characterization

3.2.3.1 Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared (FTIR) spectra were obtained for banana stem fiber-chitosan, cassava starch-chitosan, and banana stem fiber-cassava starch-chitosan films using the Thermo Nicolet 370DTGS model (Nicolet Analytical Instruments, Madison, WI). The spectra of the thin films were recorded through the Attenuated Total Reflectance (ATR) technique. The film sample was placed on top of the blue germanium crystal. The germanium crystal was washed with acetone solution before placing the film sample on top of it. Pressure was then applied on the film and the analysis was initiated. In each analysis, 16 duplicates of spectra in the region of 400 to 4000 cm^{-1} were recorded with a resolution of 4 cm^{-1}.

Figure 3.14: Drops of acetone solution are spread on the germanium crystal to clean it from any impurities.

Figure 3.15: Film sample is placed on top of the germanium crystal.

3.2.3.2 Thermal gravimetric analysis (TGA)

A TA instruments Q500TGA model consisting of a TG50 furnace, an M3 microbalance and a TA72 Graph Ware was used for thermal degradation measurements of the film samples. Each specimen was weighed and ensured to be in the range of 10 - 20 mg and placed on a platinum pan using a stainless tweezer so that fingerprints imprinting on the pans can be avoided. Fingerprints could affect the results generated later on. The pans were first tared by the instrument and later heated in the temperature range of 25 - 600°C at a heating rate of 10 °C/min under a nitrogen flow rate of 200 mL/min. All samples were analyzed for 16 duplicates.

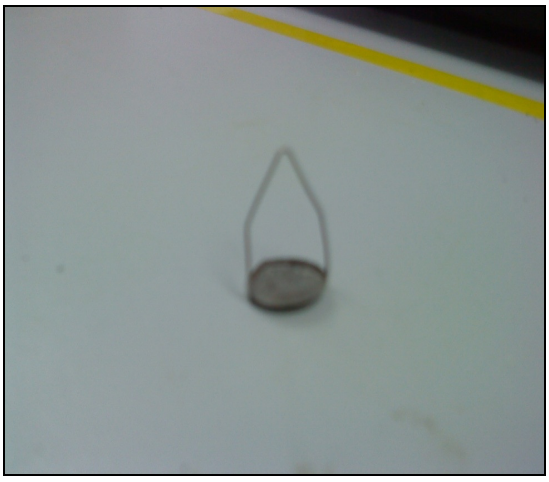


Figure 3.16: A platinum pan that holds a sample.

Figure 3.17: The pan with the sample is placed on the TGA analyzer.

3.2.3.3 Differential scanning calorimetry (DSC)

A TA Instruments Q1000 Series consisting of a DSC-30 cell and a TA72 Graph Ware was used for thermodynamic transitions evaluation of the film samples. Film sample of about 10 - 20 mg was weighed and placed in a standard pan using a stainless tweezer. A standard lid was then placed on top of the pan also using a stainless tweezer to avoid fingerprints imprinting on the pan and lid. Next, the pan and the lid were crimp pressed using the sample crimping press. DSC test was finally run on the pressed sample where it was heated from 25 to 300 °C at a heating rate of 10 °C/min under a nitrogen flow of 80 mL/min. All samples were analyzed for 16 duplicates.

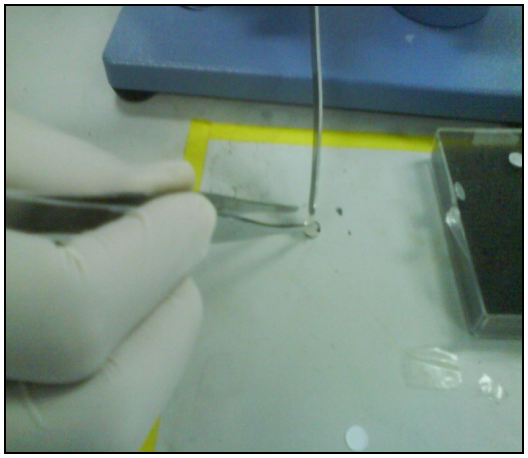


Figure 3.18: Film sample is placed in a standard pan using a stainless tweezer.

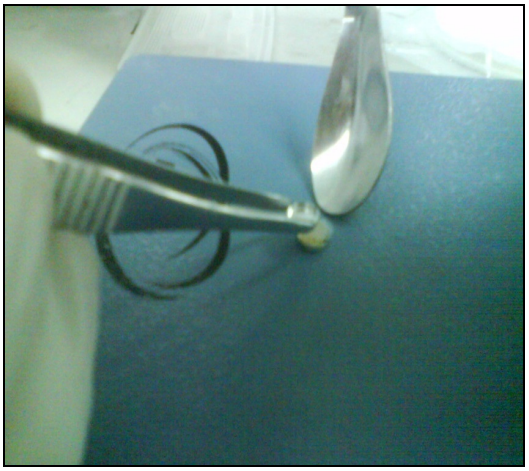


Figure 3.19: A standard lid is placed on top of the film sample.

Figure 3.20: The standard pan and lid are crimp pressed using a sample crimp press.

Figure 3.21: The crimped pan is placed into the DSC analyzer.

3.2.3.4 Atomic force microscopy (AFM)

The film samples were sent to SIRIM-UNIMAP for atomic force microscopy test. The sample was mounted on a sample stage driven by a piezo tube scanner, which was calibrated with standard gratings before use. A silicon microcantilever, which was a 2 N/m of spring constant and 70 kHz of resonance frequency made by Olympus Co., Japan, was used for the scanning. The scan rate was adjusted in the range 1.0–2.0 Hz depending on the image quality. Each scan line contains 256 pixels, and a whole image is composed of 256 scan lines. For acquisition of the surface

morphology and material distribution, both topographical and phase images were recorded. Since both difference between the material mechanical properties and topographic fluctuation can contribute to the phase contrast, the phase images were obtained under various conditions, such as at very fast or very slow scan rate, light, moderate, and hard tapping, and scan size from 2 μm to 500 nm, to ensure that the phase contrast pattern is mainly caused by mechanical and topographic reasons.

All analyses of the images were conducted at the software environment provided by the AFM manufacturer. The AFM provided qualitative data (topographic images) as well as quantitative date such as roughness. Roughness was calculated using root-mean square (rms) deviation of the heights of the various features imaged by AFM (Oses *et al*., 2009) as:

$$Rms = [(Z_1^2 + Z_2^2 + Z_3^2 + \ldots + Z_N^2)/N]^{0.5}$$

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Film samples

4.1.1 Banana stem fiber-chitosan film sample

Chitosan presents excellent film-forming capacity (Fernandez-Saiz *et al*., 2008). This was proven when the cast solution of banana stem fiber-chitosan formed a thin layer of film on the glass plate when left overnight to dry. In this study, the film sample fabricated from banana stem fiber and chitosan was peeled off the glass plate with ease when it was dry enough at ambient temperature. Sebastien *et al.* (2006) reported in their study that by having a higher PEG concentration, chitosan-based films can be removed from their support more easily and they also become more flexible.

The surface of the film was observed to be smooth and clean with slight roughness caused by the banana stem fibers. The banana stem fibers that thrust outward should have been the reason for the significant protrusion on the top surface of the film. The film, which was added with PEG400 as plasticizer, also showed considerable tensile strength when being subjected to pulling from all ends.

The flexibility of the film could also be observed when the film was drenched in water. Chitosan is hydrophilic and moisture sensitive (Sebastien *et al*., 2006). It became significantly rubbery and elastic when brought in contact with water. The original form of the film sample was however re-obtained when it was dry.

The film did not easily break or shatter into pieces either and that fact distinctly shows that its brittleness was very low. It was due to the usage of plasticizer that helped to reduce internal hydrogen bonding between polymer chains while increasing molecular volume (Mali *et al*., 2006).

Plasticizers are generally small molecules such as polyols like sorbitol, glycerol and polyethylene glycol (PEG) that intersperse and intercalate among and between polymer chains, disrupting hydrogen bonding and spreading the chains apart, which not only increases flexibility, but also water vapor and gas permeabilities (Bourtoom, 2008). Bourtoom (2008) put forth that glycerol and polyethylene glycol plasticized films exhibited flexible structure.

Some air bubbles were present in the film's structure however. The void space of air in the film could be the result of the low viscous chitosan used being permeable to gases. Also, the casting solution of chitosan might have had trapped air bubbles in it when heated. Air bubbles could rupture the film and render the films weak. In this case, the banana stem fibers can act as fillers or a bridge since they can fill up most of the bubble void space.

Astonishingly, the film sample resembled many of the conventional plastics in use today in many physical aspects. The high level of transparency of the film sample also makes it suitable as a packaging film.

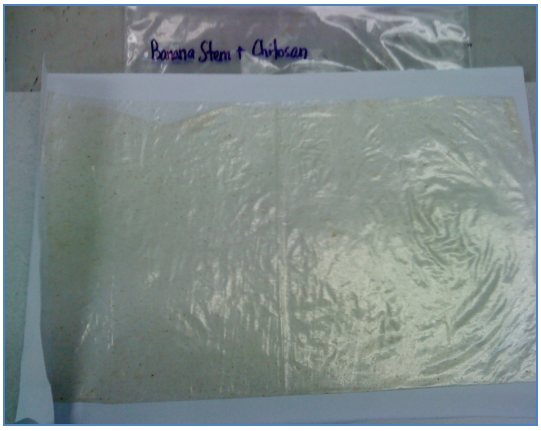


Figure 4.1: Banana stem fiber-chitosan film sample.

Figure 4.2: Banana stem fiber-chitosan film when brought in contact with water.

4.1.2 Cassava starch-chitosan film sample

Starch is a natural polymer which can be readily cast into films (Peressini *et al*., 2004). The film forming capabilities of starch were clearly observed in this study using cassava starch. The starch became very viscous and pasty when brought to gelatinization.

Starch in general does not form tough, pliable and unsupported films (Arvanitoyannis and Biliaderis, 1997). It has poor physical properties, but these are improved by blending starch with other materials. Thus, in this study, the cassava starch was blended with chitosan and the film sample fabricated was peeled off the glass plate when dry at ambient temperature with ease. The film sample had a coarse surface but otherwise smooth. The film which had PEG400 added as its plasticizer also showed significant tensile strength when subjected to pulling from all ends.

Water also has plasticizing effect on biodegradable films. Water is compatible with starch and is an effective plasticizer. With increasing content of water, starch shows both an increasing strain at break and stress at break (Jansson and Thuvander, 2004). This explains the rubbery condition of the film that was clearly noted when the film was washed with water. It became elastic and flexible

when brought in contact with water. The original film sample was re-obtained when it was dry.

The film did not easily crack or shatter into pieces either and it without doubt shows that its level of brittleness was very low. Cutter (2006) reported that plasticizers can be used to modify film mechanical properties, thereby imparting desirable flexibility, permeability, or solubility to the resulting film For example, adding glycerol, polyethylene glycol, or sorbitol to a film composition can reduce brittleness (Tharanathan, 2003). Otles and Otles (2004) suggested some common plasticizers for hydrophilic polymers such as starch like glycerol and other low molecular weight polyhydroxy compounds, polyethers and urea

There were some really small needle-like feel lumps present throughout the film's structure however which could have caused the surface to feel quite coarse. These jutting lumps might have been caused by the slight immiscibility between the chitosan and the cassava starch used. However, all in all, the film was somewhat intact. The combination of hydrogen bonding, opposite charge attraction between chitosan cations and negatively charged starch film surface, hydrophilicity, and compatible water activities provided a good adherence between the starch film and the chitosan compounds (Bangyekan *et al*., 2006).

Interestingly, the film sample resembled most of the conventional plastics in use today in many physical aspects even though the level of transparency of this film was quite low. The milky color was the result of the starch added as starch is vastly known for its pasty and milky form when it gelatinizes. The paste is white in color and therefore, this explains the sported milky color of the film sample. Starch, according to Viturawong *et al.* (2008), is hypoallergenic for many, bland in taste, white in color and, as a gel, is smooth in texture while Techawipharat *et al*. (2008) pointed out their interesting unique attributes in the food industry.

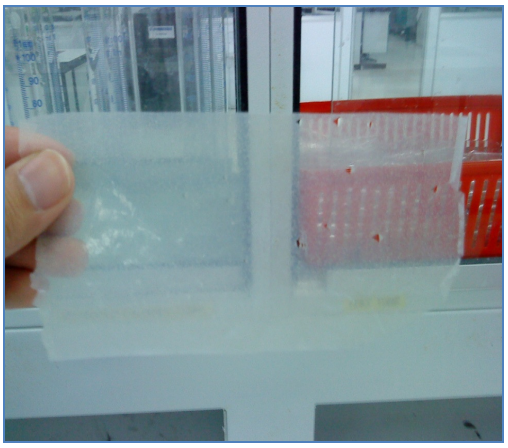


Figure 4.3: Cassava starch-chitosan film sample.

Figure 4.4: Cassava starch-chitosan film sample when brought in contact with water.

4.1.3 Banana stem fiber-cassava starch-chitosan film sample

Film characteristics are dependent on the cohesion of the polymeric matrix, which in turn is dependent on the structure of the polymer chains, the film obtainment process and the presence of plasticizer agents (Carmen *et al*., 2008).

The film forming capabilities of starch were readily observed in this study using cassava starch when the starch solution was brought to gelatinization. Despite that, it is also known that starch in general does not form tough, pliable and unsupported films (Arvanitoyannis and Biliaderis, 1997). Cassava starch film is

brittle and weak leading to inadequate mechanical properties (Bangyekan *et al*., 2006). The banana stem fiber-cassava starch-chitosan film sample was therefore designed and prepared with the cassava starch and chitosan compounds acting as a composite matrix to the fibers.

The film was peeled off the glass plate when dry at ambient temperature with ease. Sebastien *et al*. (2006) reported that preliminary assays showed difficulties in film recovery when formulated without plasticizers. The film sample had a very smooth and clean surface. The slight coarseness was caused by the banana stem fibers. The film which had PEG400 added as its plasticizer also showed significant tensile strength when being subjected to pulling from all ends.

Water is highly compatible with starch and therefore is also an effective plasticizer. As a matter of fact, both starch and chitosan are hydrophilic and retain considerable amount of water (Rueda *et al*., 1999). This obviously explains the stark rubbery condition of the film that was carefully noted when the film was soaked with water. It became elastic and flexible when brought in contact with water. The original film sample was however re-obtained when it was dry.

The film did not easily crack or shatter into pieces either and this came to show that its level of brittleness was very low. Plasticizers, which are low molecular components, increase the free volume of the material or the macromolecular mobility of the polymer, and consequently the polymeric network becomes less dense due to the decrease in intermolecular forces, thus improving the extensibility and flexibility of the films (Mendieta-Taboada *et al*., 2008).

In addition, plasticizer agents strongly affect the glass transition temperature (Tg) of biopolymer-based films (Mendieta-Taboada *et al*., 2008). Also, Bangyekan *et al*. (2006) proposed in their study that the combination of hydrogen bonding, opposite charge attraction between chitosan cations and negatively charged starch film surface, hydrophilicity, and compatible water activities were the reasons to a good adherence between a starch film and a chitosan film

The film sample resembled most of the conventional plastics in use today in many physical aspects even though the level of transparency of this film was quite low. This could be the result of the addition of cassava starch as starch is known for its pasty and gel-like condition when brought to gelatinization. There has also been lot of research on use of natural fibers in reinforcements (Mukhopadhyay *et al*., 2008). With respect to the blending of films to overcome the intrinsic deficiencies of starch and other polysaccharide films, several research reports have indicated that cellulosic fibers may be added to modified starch such as hydroxypropylated starch to enhance film mechanical properties. Cellulose microfibrils have been found to improve starch performance (Ban *et al*., 2006). For that reason, the banana stem fiber-cassava starch-chitosan film showing significant strength was partly contributed by the reinforcing fibrous materials.

Figure 4.5: Banana stem fiber-cassava starch-chitosan film sample.

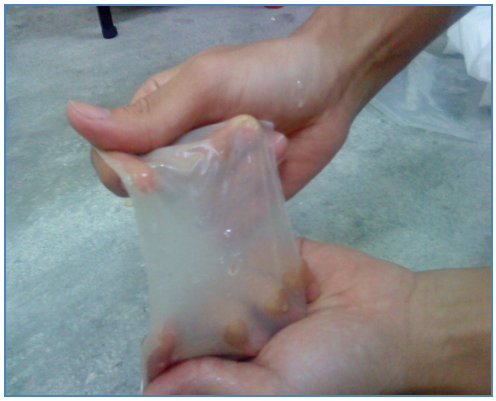


Figure 4.6: Banana stem fiber-cassava starch-chitosan film sample when brought in contact with water.

4.2 Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy determines the interactions and the biocompatibility between cassava starch, banana stem fibers, and chitosan. Specific interaction between blend components is often important to achieve blend miscibility (Reis *et al*., 2008). According to Reis *et al*. (2002), this spectroscopy provides information about structures, miscibility and analyses the chemical or physical interactions in the blend. Shifts in absorption position and changes in band contours, accompanying changes in molecular environment, may also suggest important structural details (Silverstein *et al*., 2005). Hydrogen bonding alters the force constant of both groups; thus, the frequencies of both stretching and bending vibrations are altered. The X–H stretching bands move to lower frequencies (longer wavelengths) usually with increased intensity and band widening. The stretching of the acceptor group, for example, C=O, is also reduced but to a lesser degree than the proton donor group. The H–X bending vibration usually shifts to a shorter wavelength when bonding occurs; this shift is less pronounced than that of the stretching frequency (Silverstein *et al*., 2005).

Table 4.1: Some characteristic infrared absorption peaks (Skoog *et al*., 2004).

	Functional group	**Wavenumber, cm^{-1}**	**Wavelength, µm**
O-H	Aliphatic and aromatic	3600 – 3000	2.8 – 3.3
NH_2	Also secondary and tertiary	3600 – 3100	2.8 – 3.2
C-H	Aromatic	3150 – 3000	3.2 – 3.3
C-H	Aliphatic	3000 – 2850	3.3 – 3.5
C≡N	Nitrile	2400 – 2200	4.2 – 4.6
C≡C-	Alkyne	2260 – 2100	4.4 – 4.8
COOR	Ester	1750 – 1700	5.7 – 5.9
COOH	Carboxylic acid	1740 – 1670	5.7 – 6.0
C=O	Aldehydes and ketones	1740 – 1660	5.7 – 6.0
$CONH_2$	Amides	1720 – 1640	5.8 – 6.1
C=C-	Alkene	1670 – 1610	6.0 – 6.2
Φ-O-R	Aromatic	1300 – 1180	7.7 – 8.5
R-O-R	Aliphatic	1160 – 1060	8.6 – 9.4

4.2.1 Infrared spectrum of banana stem fiber-chitosan film

In Figure 4.9, the strong and wide absorption peak at approximately 3410cm^{-1} can be observed in the region of 3000 – 3600cm^{-1}. According to Skoog *et al*. (2004), this region can be precisely attributed to the stretching of O-H and NH_2 bands of stretching, with the O-H band ranging from 3000 – 3600cm^{-1} and the NH_2 band stretching from 3100 – 3600cm^{-1}. Since these two functional groups fall in the same region, an overlap of peaks is therefore readily observed. The O-H and NH_2 functional groups show quite a high and broadened out absorbance rate in this region through the analysis of the IR spectrum in Figure 4.9. The structure of chitosan in Figure 4.7 below proves that the O-H band can be ascribed to chitosan since it contains the O-H stretching which can form hydrogen bonding with adjacent molecules, in its structure. The region of 3100 – 3600cm^{-1} can also be ascribed to the NH_2 functional group of chitosan. The main components of natural fibres are cellulose (α-cellulose), hemicellulose, lignin, pectins, and waxes (John and Thomas, 2008). Consequently, Figure 4.8 below proves the stretching region of 3000 – 3600cm^{-1} is also attributable to the banana stem fibers, also known as lignocellulosic fibers, which were present throughout the banana stem fiber-chitosan film sample that was put through the FTIR analysis.

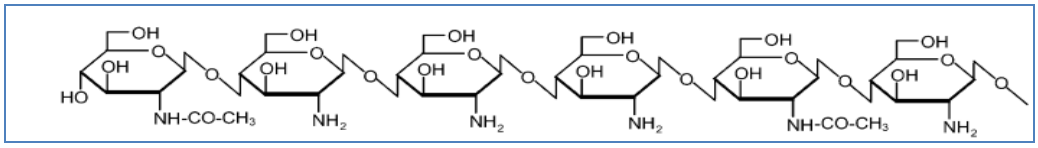


Figure 4.7: Chitosan (Tharanathan, 2003)

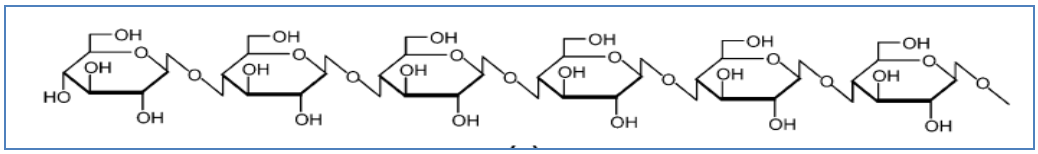


Figure 4.8: Cellulose (Tharanathan, 2003).

Perez-Mateos *et al*. (2009) suggested that the increased absorbance in the 3000 – 3600cm^{-1} range could be due to the adsorbed water molecules in gelatin film and was possibly brought about by the presence in the film of relatively high

amounts of glycerol and sorbitol as plasticizers. Therefore, the 3000 – 3600cm^{-1} region of the O-H stretching can also be ascribed to PEG400. Mathew and Abraham (2008) reported in their study that the O-H bending was inherent with the peak in another region in the range of 1200 – 1500cm^{-1}. To a stated extent, this should also further contribute to the fact that water or moisture content was present in the film sample.

Silverstein *et al*. (2005) wrote about the possibility of the fingerprint region being associated with the intermediate portion of the spectrum, 1300 – 900cm^{-1}. Another region in the spectrum which is attributable to chitosan is the region in the range of 1500 – 1700cm^{-1} wavenumber. The band at 1581 cm^{-1} was caused by the NH bending, which is a part of the amide functional group, $CONH_2$ (amide II). There is a small peak near 1655 cm^{-1} and it was due to the C=O stretching, another part of the amide functional group, $CONH_2$ (amide I). The slight peak in the region between 3000 – 2850cm^{-1} can be attributed to the C-H (Skoog *et al*., 2004) band of aliphatic chain of both the cellulosic fiber and chitosan. C-H is the backbone structure of both components. Besides that, the spectra at the highest peak in the spectra suggest the presence of the C-O-C stretching attributable to PEG400 (Wang *et al*., 2003). Spectra of natural fibers exhibit a strong band at 1034.36cm^{-1} due to the C-O-C symmetric stretching of dialkyl ether linkages and the C-O stretching vibration in cellulose, hemicellulose and some minor lignin contribution. This is consistent with what was reported by Bilba *et al*. (2007).

Xu *et al*. (2005) put forth the peak of chitosan film they observed to be at 1578cm^{-1}, in which they ascribed to the amide I functional group. Xu *et al*. (2005) also noted that the peak at 1741 cm−1 suggested the presence of a carbonyl group in the chitosan film, while Skoog *et al*. (2004) pointed out that the carbonyl functional group, C=O lies in the wavenumber range of 1640 – 1720cm^{-1}. These are also consistent with what Bourtoom and Chinnan (2008) reported for the amide I functional group. In this study, the banana stem fiber-chitosan film did portray slight interactions between them for the peak of the amide I group for the banana stem fiber-chitosan film was observed and read at 1581cm^{-1}. The shift was really a small one and thus suggests the slight or little interactions between the two compounds.

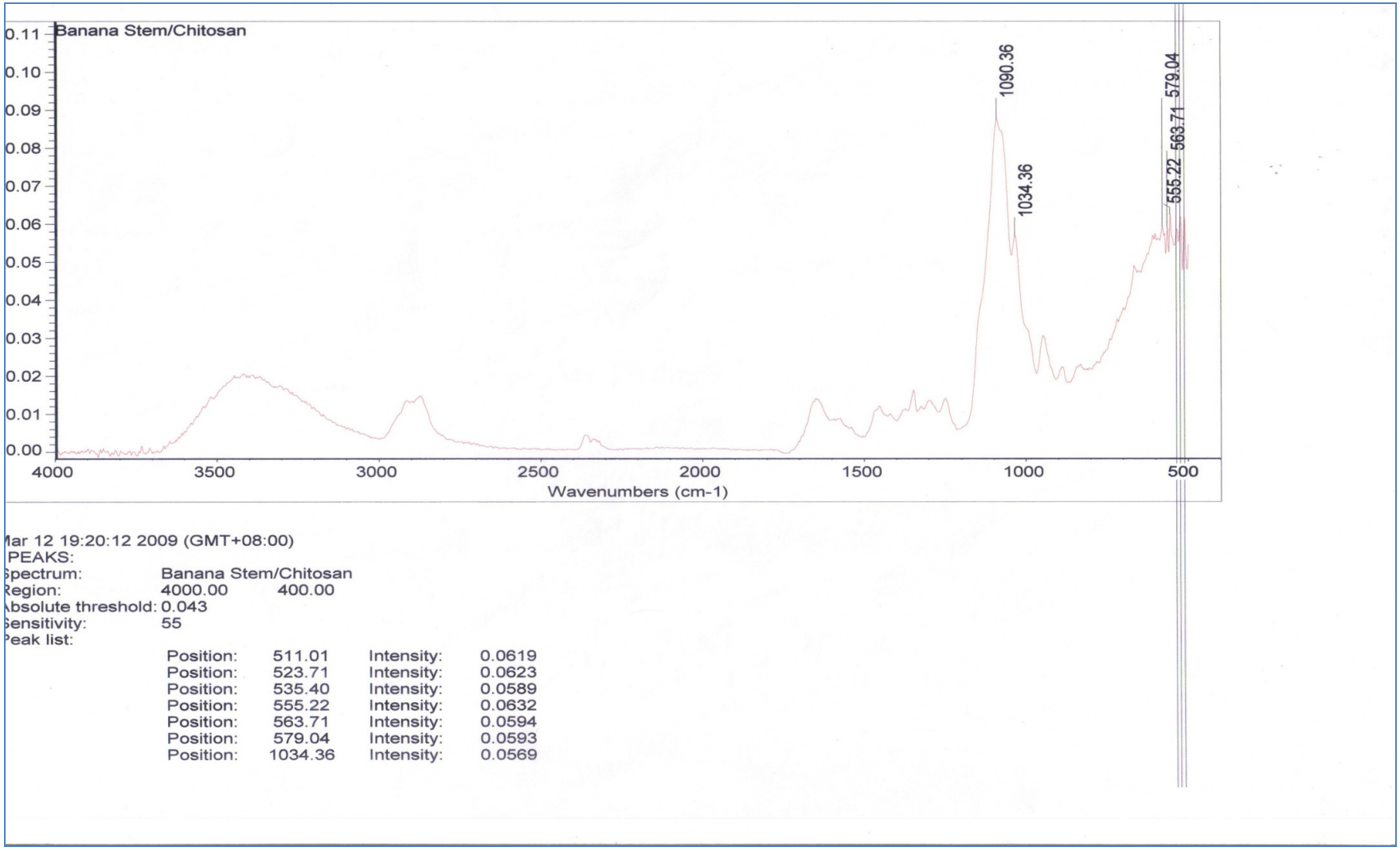


Figure 4.9: IR spectrum of banana stem fiber-chitosan film sample.

4.2.2 Infrared spectrum of cassava starch-chitosan film

In Figure 4.11, the significantly strong and wide absorption peak at approximately 3415cm^{-1} can be observed in the region of 3000 – 3600cm^{-1} wavenumber. According to Skoog *et al.* (2004), this region can be precisely attributed to the stretching of O-H and NH_2 bands of stretching, with the O-H band ranging from 3000 – 3600cm^{-1} and the NH_2 band stretching from 3100 – 3600cm^{-1}. Since these two functional groups fall in the same region, again an overlap of peaks is therefore readily observed. The O-H and NH_2 functional groups show quite a high absorbance rate in this region through the analysis of the IR spectrum in Figure 4.11. The structure of chitosan in Figure 4.7 further attests that the O-H band in this case can definitely be ascribed to chitosan. Also, the region of 3100 – 3600cm^{-1} can be ascribed to the NH_2 functional group of chitosan. Chitosan should be able to portray the characteristic peaks of the two band stretchings. Cassava starch consists of two types of molecules: amylose, a substantially linear polymer with a molecular weight of about 10^{-5}, and amylopectin, a highly branched polymer with very high molecular weight of about 10^{-7}.

Figure 4.10 below shows the structure of amylose. Amylopectin also has the same structure except for the fact that amylose is linear while amylopection is highly branched (Fessenden and Fessenden, 1998). Hence, the stretching region of the 3000 – 3600cm^{-1} wavenumber is also attributable to cassava starch. The O-H stretching in the region could have on its part suggested the presence of amylose and amylopectin components in the film as well.

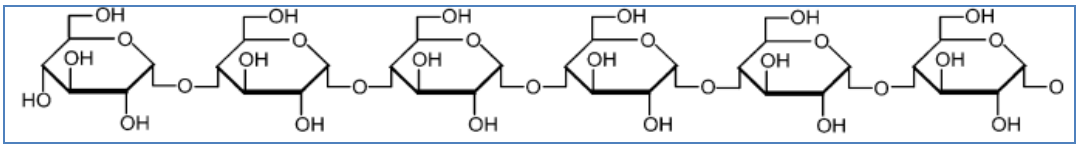


Figure 4.10: Amylose (Tharanathan, 2003)

Perez-Mateos *et al.* (2009) suggested that the increased absorbance in the 3000–3600cm^{-1} range could signify the presence of adsorbed water molecules in gelatin film and was possibly brought about by the presence of relatively high

amounts of glycerol and sorbitol as plasticizers in the film. The region of the O-H stretching could therefore be brought into significance by the PEG400 used in the film as plasticizer. Mathew and Abraham (2008) reported that the O-H bending was also inherent with the peak in the range of 1200 – 1500cm^{-1}. This further proves that slight amount of water or moisture content was present in the film.

Silverstein *et al*. (2005) also wrote about the possibility of the fingerprint region being associated with the intermediate portion of the spectrum, 1300 – 900cm^{-1}. Another region in the spectrum attributable to chitosan is the region in the range of 1500 – 1700cm^{-1} wavenumber. The band at 1590 cm^{-1} was contributed by the NH bending, which is a part of the amide functional group, $CONH_2$ (amide II). There's another significant narrow peak near 1660 cm^{-1} and it was due to the C=O stretching, another part of the amide functional group, $CONH_2$ (amide I). The slight peak in the region between 3000 – 2850cm^{-1} can also be attributed to the C-H (Skoog *et al*., 2004) band of aliphatic chain of both the cassava starch and chitosan components present in the film. C-H is the backbone structure of both components. Apart from that, the spectra at the highest peak in the spectra suggest the presence of the C-O-C stretching attributable to PEG400 (Wang *et al*., 2003). Xu *et al*. (2005) reported that when two or more substances are mixed, physical blends versus chemical interactions are reflected by the changes in characteristic spectra peaks. Bourtoom and Chinnan (2008) also suggested the same about changes in characteristic spectra being associated to the physical blends of two or more substances. Xu *et al*. (2005) put forth the peak of chitosan film they observed to be at 1578cm^{-1}, in which they ascribed to the amide I functional group. Xu *et al*. (2005) also pointed out that the peak at 1741 cm^{-1} suggested the presence of a carbonyl group in chitosan film.

The result was aesthetically consistent with what Bourtoom and Chinnan (2008) reported in their study for the amide I functional group. Hence in this case, the cassava starch-chitosan film did portray slight interactions between them for the peak of the amide I group can be readily observed and read at 1590cm^{-1}. The shift was really a small one and thus suggests the slight or little interactions between the two components.

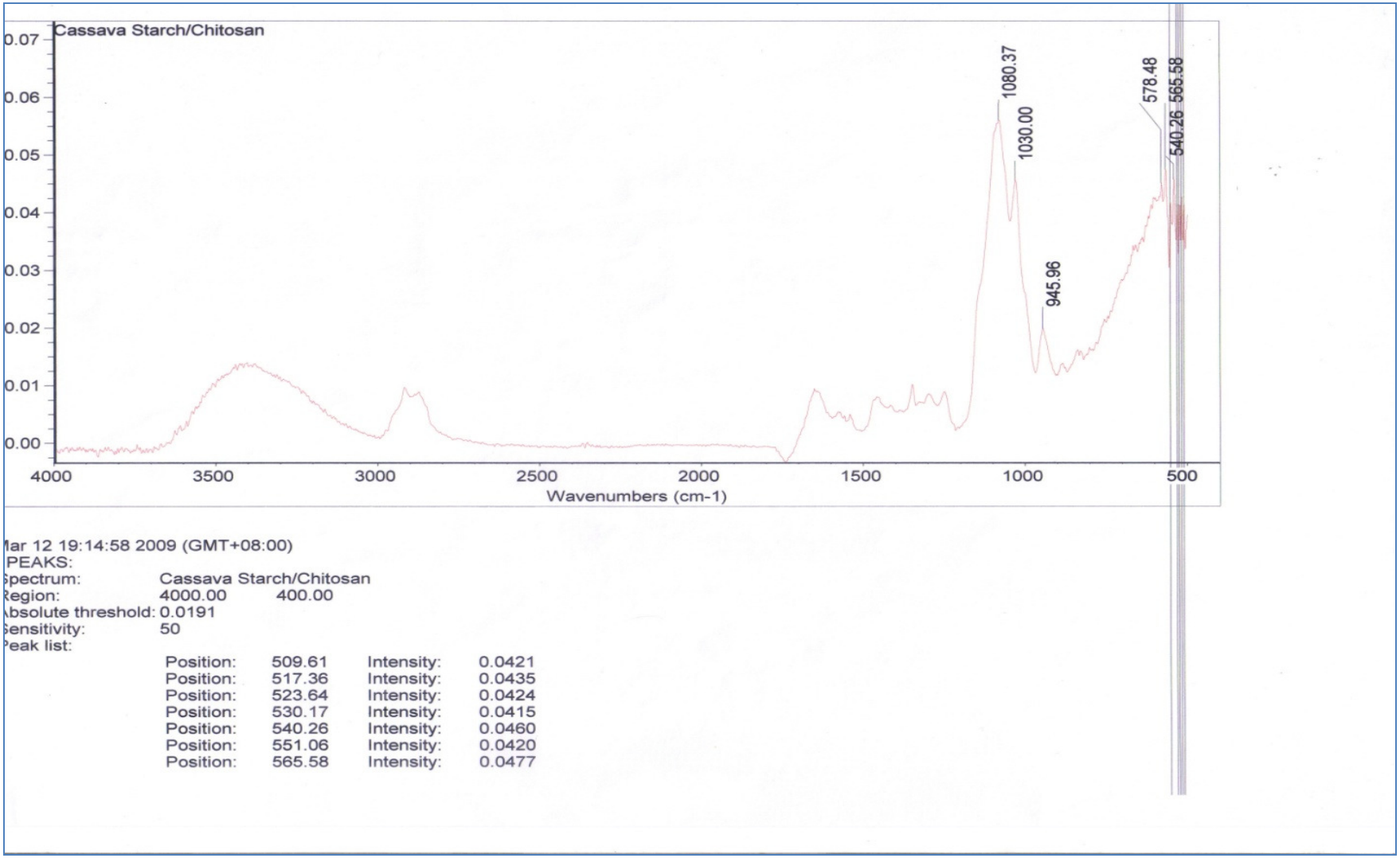


Figure 4.11: IR spectrum of cassava starch-chitosan film sample.

4.2.3 Infrared spectrum of banana stem fiber-cassava starch-chitosan film

In Figure 4.12, the strong and wide absorption peak at approximately 3410cm^{-1} is observed. According to Skoog *et al.* (2004), this region is attributable to O-H and NH_2 stretchings, with the O-H band ranging from 3000 – 3600cm^{-1} and the NH_2 band stretching from 3100 – 3600cm^{-1}. This region in Figure 4.12 appears a little broadened and stretched out compared to the same region in the IR spectra of the cassava starch-chitosan and banana stem fiber-chitosan films in Figure 4.11 and Figure 4.9 respectively. This could be caused by the higher interactions between the three compounds, namely the cellulosic fibers, cassava starch and chitosan, present in the banana stem fiber-cassava starch-chitosan film sample. The O-H and NH_2 bands fall in the same region, an overlap of peaks is thus readily observed in the IR spectrum of the film sample. The O-H and NH_2 functional groups show quite a high absorbance rate in this region through the analysis of the IR spectrum in Figure 4.12. The structure of chitosan in Figure 4.7 undoubtedly proves that the O-H band can in this case be ascribed to chitosan. The region of 3100 – 3600cm^{-1} wavenumber can also be ascribed to the NH_2 functional group of chitosan. The main components of natural fibres are cellulose (α-cellulose), hemicellulose, lignin, pectins, and waxes (John and Thomas, 2008). Hence, the stretching region of 3000 – 3600cm^{-1} is also attributable to the banana stem fibers.

Cassava starch consists of two types of molecules: amylose, a substantially linear polymer with a molecular weight of about 10^{-5}, and amylopectin, a highly branched polymer with very high molecular weight of about 10^{-7}. Figure 4.10 shows the structure of amylose. Amylopectin also has the same structure except for the fact that amylose is linear while amylopection is highly branched (Fessenden and Fessenden, 1998). Hence, the stretching region of the 3000 – 3600cm^{-1} is also attributable cassava starch. The O-H stretching in the region could have on its part suggested the presence of amylose and amylopectin components in the film as well.

Perez-Mateos *et al.* (2009) suggested the increased absorbance in the 3000–3600cm^{-1} range could signify the presence of adsorbed water molecules in the gelatin film and was possibly brought about by the presence in the film of relatively high

amounts of glycerol and sorbitol as plasticizers. Therefore, the region of O-H stretching could also be caused by PEG400 used in the film fabrication as plasticizer. Mathew and Abraham (2008) reported that the O-H bending was also inherent with the peak in the range of 1200 – 1500cm^{-1}. Silverstein *et al*. (2005) also wrote about the possibility of the fingerprint region being associated with the intermediate portion of the spectrum, 1300 – 900cm^{-1}. This portion of spectrum is extremely valuable in reference to the other regions.

Another region in the spectrum which is attributable to chitosan's structure is the region in the range of 1500 – 1700cm^{-1} wavenumber. The band at 1591 cm^{-1} was caused by the NH bending, which is a part of the amide functional group, $CONH_2$ (amide II). There's a small peak near 1665 cm^{-1} and it was due to the C=O stretching, another segment of the amide functional group, $CONH_2$ (amide I).

The slight peak in the region between 3000 – 2850cm^{-1} can also be attributed to the C-H (Skoog *et al*., 2004) band of aliphatic chain of both the cellulosic fiber and chitosan. C-H is the backbone structure of both components. In addition, the highest peaks in the spectra in Figure 4.12 suggest the presence of the C-O-C stretching which can be attributed to PEG400 (Wang *et al*., 2003). The spectra of natural fibers also exhibit a strong band at 1034.36cm^{-1} due to the C-O-C symmetric stretching of dialkyl ether linkages and the C-O stretching vibration in cellulose, hemicellulose and minor lignin contribution. This is consistent with what was reported by Bilba *et al*. (2007).

Xu *et al*. (2005) reported that when two or more substances are mixed, physical blends versus chemical interactions are reflected by the changes in characteristic spectra peaks. Bourtoom and Chinnan (2008) also suggested the same about changes in characteristic spectra being associated to the physical blends of two or more substances. Xu *et al*. (2005) put forth the peak of chitosan film they observed to be at 1578cm^{-1}, in which they ascribed to the amide I functional group. Xu *et al*. (2005) also suggested that the peak at 1741 cm−1 was ascribable to the presence of a carbonyl group in the film. Skoog *et al*. (2004) pointed out that the carbonyl functional group, C=O lies in the wavenumber range of 1640 – 1720cm^{-1}.

The IR spectral result obtained in Figure 4.12 is somehow consistent with what Bourtoom and Chinnan (2008) reported for the amide I functional group. For that very reason, in this case, the banana stem fiber-cassava starch-chitosan film did portray slight interaction between them for the peak of the amide I group was observed and read at 1591cm^{-1}. The shift was really a small one and thus had suggested the slight or little interactions between the three components. All three compounds, the lignocellulosic fibers, cassava starch and chitosan, have O-H ends with the exception of chitosan having both the O-H and $CONH_2$ functional groups, can form hydrogen bonding, or even slight interactions with each other and among themselves when brought in contact with each other.

4.2.4 Comparing the infrared absorption trend of the three film samples by their FTIR spectra

Table 4.2: Some important infrared regions that were analyzed from the three film samples.

IR regions (cm^{-1})	***Functional group***	***Banana stem fiber-chitosan***	***Cassava starch-chitosan***	***Banana stem fiber-cassava starch-chitosan***
3600 – 3000	Aliphatic and aromatic	Present	Present	Present
2000 – 2700	Amide II	Present	Present	Present
1740 – 1660	Aldehydes and ketones	Present	Present	Present
1720 – 1640	Amide I	Present	Present	Present

All three film samples portrays similar spectra which could suggest the high level of structural compatibility between them. Chitosan is known for its biocompatibility with other polysaccharides (Rivero *et al*., 2008; Phisalaphong and Jatupaiboon, 2008).

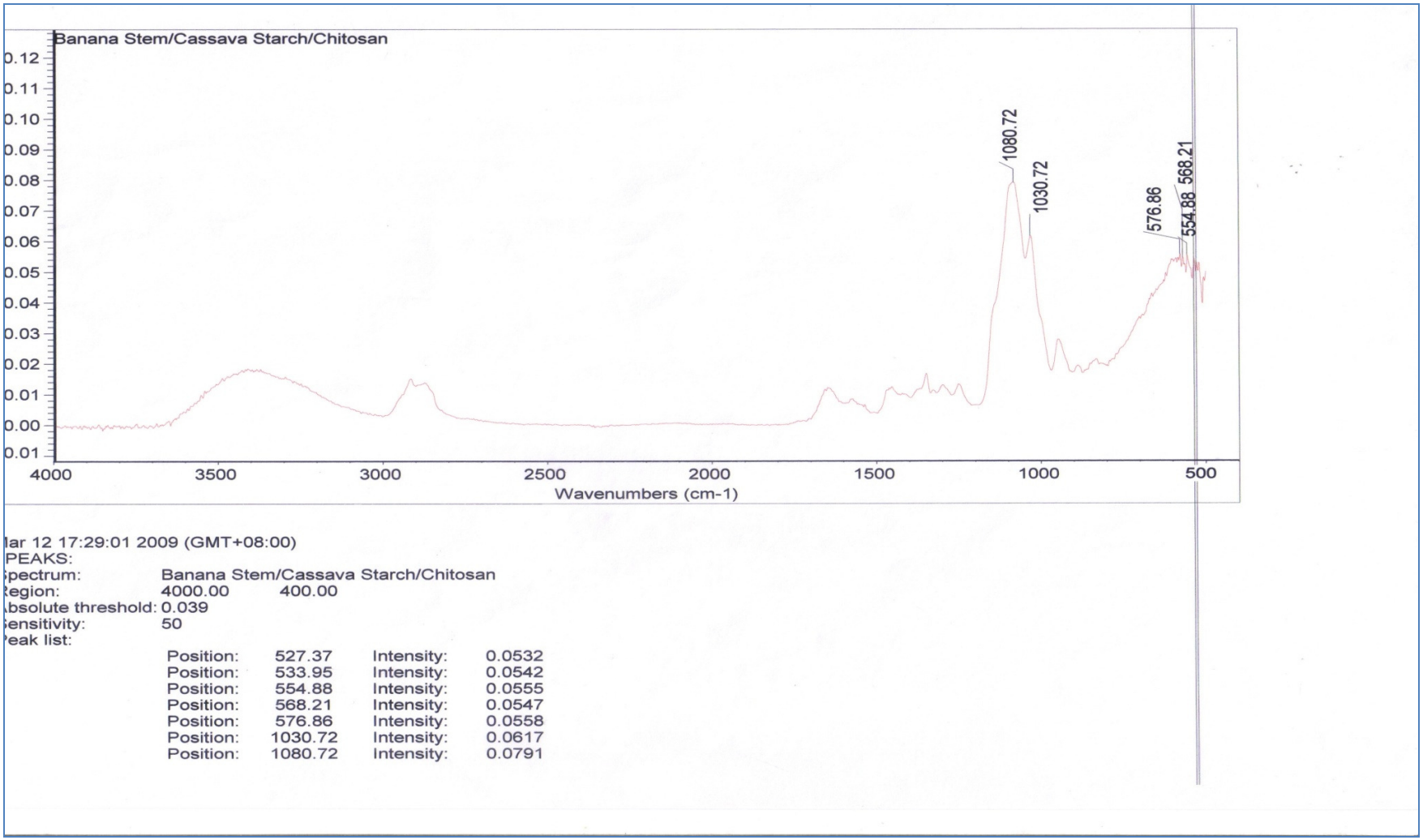


Figure 4.12: IR spectrum of banana stem fiber-cassava starch-chitosan film sample.

Bourtoom and Chinnan (2008) and Xu *et al*. (2005) pointed out in their studies that interactions were present between the hydroxyl group of starch and the amino group of chitosan. Xu *et al*. (2005) reported that when two or more substances are mixed, physical blends versus chemical interactions are reflected by the changes in characteristic spectra peaks. Bourtoom and Chinnan (2008) also suggested the same about changes in characteristic spectra being associated to the physical blends of two or more substances.

Cellulose, either obtained from plants or microbially produced, is the main candidate for polymeric matrix reinforcement constituent. By acetylation it could become soluble in water (Simkovic, 2008). It could be combined with chitosan, xylan, starch, or pectin due to hydroxyl, amine or carboxyl interaction without a covalent linking between individual polysaccharides (Simkovic, 2008). Composites could be formed by multilayer films consisting of layers of single type polysaccharides (Simkovic, 2008).

The O-H stretching region was not further being explained and brought into discussion in details when mentioning about the interactions between the compounds in all the three film samples. This was because there might have been some effects of water and PEG400, which were both present in the film samples, imparted on the IR spectra. These two compounds also have considerable impacts on the O-H stretching region of the 3000 – 3600cm^{-1} wavenumber.

4.3 Thermal gravimetric analysis (TGA)

4.3.1 Thermogravimetric traces for the decomposition of banana stem fiber-chitosan film sample

The banana stem fiber-chitosan film sample shows a thermal stability of up to approximately 270°C, also known as the onset temperature, T_{onset}. Lignocellulosic materials, specifically referring to the banana stem fibers, decompose on heating and when exposed to an ignition source by two different mechanisms. The dominant

mechanism at temperatures below 300˚C, degrades polymers by the breaking of internal chemical bonds; dehydration (elimination of water); formation of free radicals, carbonyl, carboxyl, and hydroperoxide groups; formation of carbon monoxide and carbon dioxide; and finally, the formation of reactive carbonaceous char (Rowell and LeVan-Green, 2005).

Three stages of decomposition trend are thus observed in Figure 4.13. The first stage is attributable to the loss of water or moisture content of the film sample. About 2.657% of water content of the film sample was lost when being heated up to about 175°C. The high crystalline structure of the cellulosic fibers might have rendered it hard for the film to trap water molecules in it, thus the low amount of water was recorded. PEG400 was reported in a study by Wang *et al*. (2003) to have a sharp drop in its TGA curve at 150°C. Hence, it can be concluded here that the decomposition trend in the range of 25-175°C might have also included the evaporation of the PEG400 compound as well. Fellinto *et al*. (2007) pointed out in their study that the loss of mass for PEG-chitosan films was due mainly to the loss of physically adsorbed water on the PEG solution.

At the second stage of the thermal decomposition trend, the intensity of the heating peaks at the 15^{th} minute at the oxidation temperature, T_o, of 273.38°C. The high energy put in to heat up the polysaccharide rings should be able to attest to that. The highest mass change occurs at this slope. According to Neto *et al*. (2005), pyrolysis of polysaccharides starts by a random split of the glycosidic bonds, followed by a further decomposition forming acetic and butyric acids and a series of lower fatty acids, where C_2, C_3 and C_6 predominates. The last prominent stage of decomposition starts at 473.92°C at the 35^{th} minute. This final onslaught could also be attributed to the dehydration and depolymerization of the polysaccharide rings of the banana stem fiber-chitosan film sample. The total percentage of weight change or weight loss stands at 93.23 while the total percentage of residue left behind is 6.917. The decomposition reaches a maximum of 553.39°C in Figure 4.13. The high residue composition might be caused by the amount of lignin present in the cellulosic fibers. Acid hydrolysis carried out would not have been enough to hydrolyze all of the lignin and the surface waxes that act as the encrusting substances.

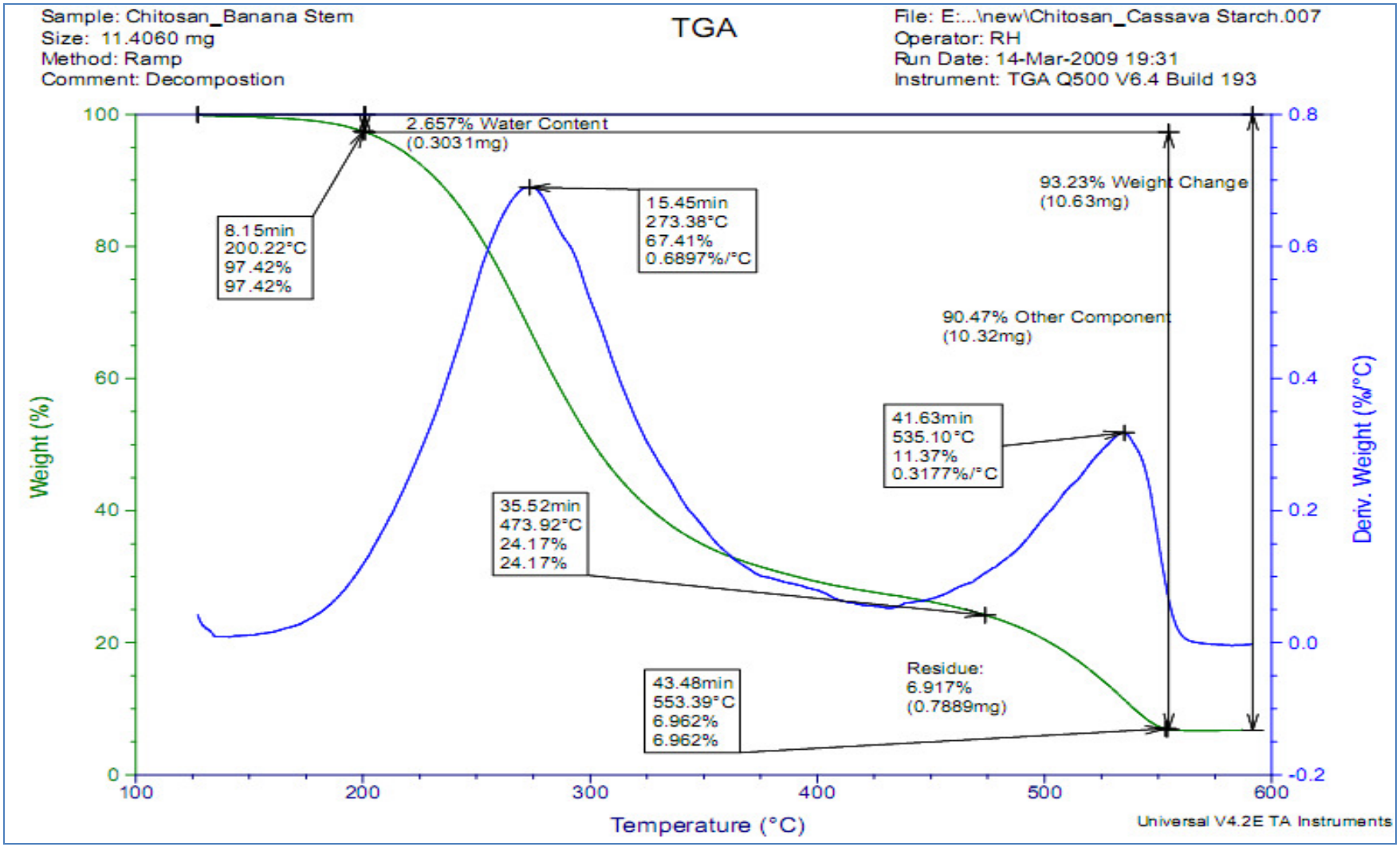


Figure 4.13: The decomposition trend of banana stem fiber-chitosan film sample.

4.3.2 Thermogravimetric traces for the decomposition of cassava starch-chitosan film sample

In Figure 4.14, the cassava starch-chitosan film sample in study shows a thermal stability of up to approximately 275°C, also known as the onset temperature, T_{onset}. From the bio-packaging perspective, that should be enough to hold off any early degradation.

Three stages of decomposition are being observed in Figure 4.14 in which the first stage is attributed to the loss of water or moisture content of the film sample. PEG400 was reported in a study by Wang *et al*. (2003) to have a sharp drop in its TGA curve at 150°C. Hence, it can be concluded here that the decomposition trend in the range of 25-175°C might have also included the evaporation of the PEG400 compound as well. Fellinto *et al*. (2007) pointed out in their study that the loss of mass for PEG-chitosan films was due mainly to the loss of physically adsorbed water on the PEG solution.

About 13.22% of water content of the film sample was lost when being heated up to 80.91°C. The water composition in the film sample of cassava starch-chitosan was significantly high.

The fact that both starch and chitosan are hydrophilic and permeable to moisture should explain the high water content in the film sample made from both compounds. The structure of these two polysaccharides, both having O-H functional groups with chitosan sporting extra amide group in which both of these functional groups could form interactions with other molecules, allow for the considerable level of hydrophilicity of the film sample.

At the second stage of the thermal decomposition trend, the intensity of the heating peaks at the 26^{th} minute at the oxidation temperature, T_o, of 288.27°C. The high energy put in to heat up the polysaccharide rings should be able to explain the breaking and opening of the bonds. The highest mass change occurs at this slope. According to Neto *et al*. (2005), pyrolysis of polysaccharides starts by a random split

of the glycosidic bonds, followed by a further decomposition forming acetic and butyric acids and a series of lower fatty acids, where C_2, C_3 and C_6 predominates. The last prominent stage of decomposition starts at 462.33°C at the 43rd minute. This final heat onslaught could also be attributed to the dehydration and depolymerization of the polysaccharide rings of the cassava starch-chitosan film sample. The total percentage of weight change or weight loss stands at 94.79 while the total percentage of residue left behind is 5.276. The decomposition reaches a maximum of 544.82°C in Figure 4.14.

The third stage, which takes over at temperatures above 300˚C, involves the cleavage of secondary bonds and formation of intermediate products such as anhydromonosaccharides, which are converted into low molecular weight products (oligosaccharides and polysaccharides), which lead to carbonized products (Kawamoto *et al*., 2003).

4.3.3 Thermogravimetric traces for the decomposition of banana stem fiber-cassava starch-chitosan film sample

In Figure 4.15, the banana stem fiber-cassava starch-chitosan film sample shows a thermal stability of up to approximately 280°C, also known as the onset temperature, T_{onset}. As a packaging material, the thermal stability value carried by the banana stem fiber-cassava starch-chitosan film would be consistent enough. Three stages of decomposition are readily observed in which the first stage is attributed to the loss of water or moisture content of the film sample. About 4.456% of water content of the film sample was lost when being heated up to 120°C.

PEG400 was reported in a study by Wang *et al*. (2003) to have sharp drop in its TGA curve at 150°C. Thus, it can be concluded here that the decomposition trend in the range of 25-160°C might have also included the evaporation of the PEG400 component as well. Fellinto *et al*. (2007) pointed out in their study that the loss of mass for PEG-chitosan films was due mainly to the loss of physically adsorbed water on the PEG solution.

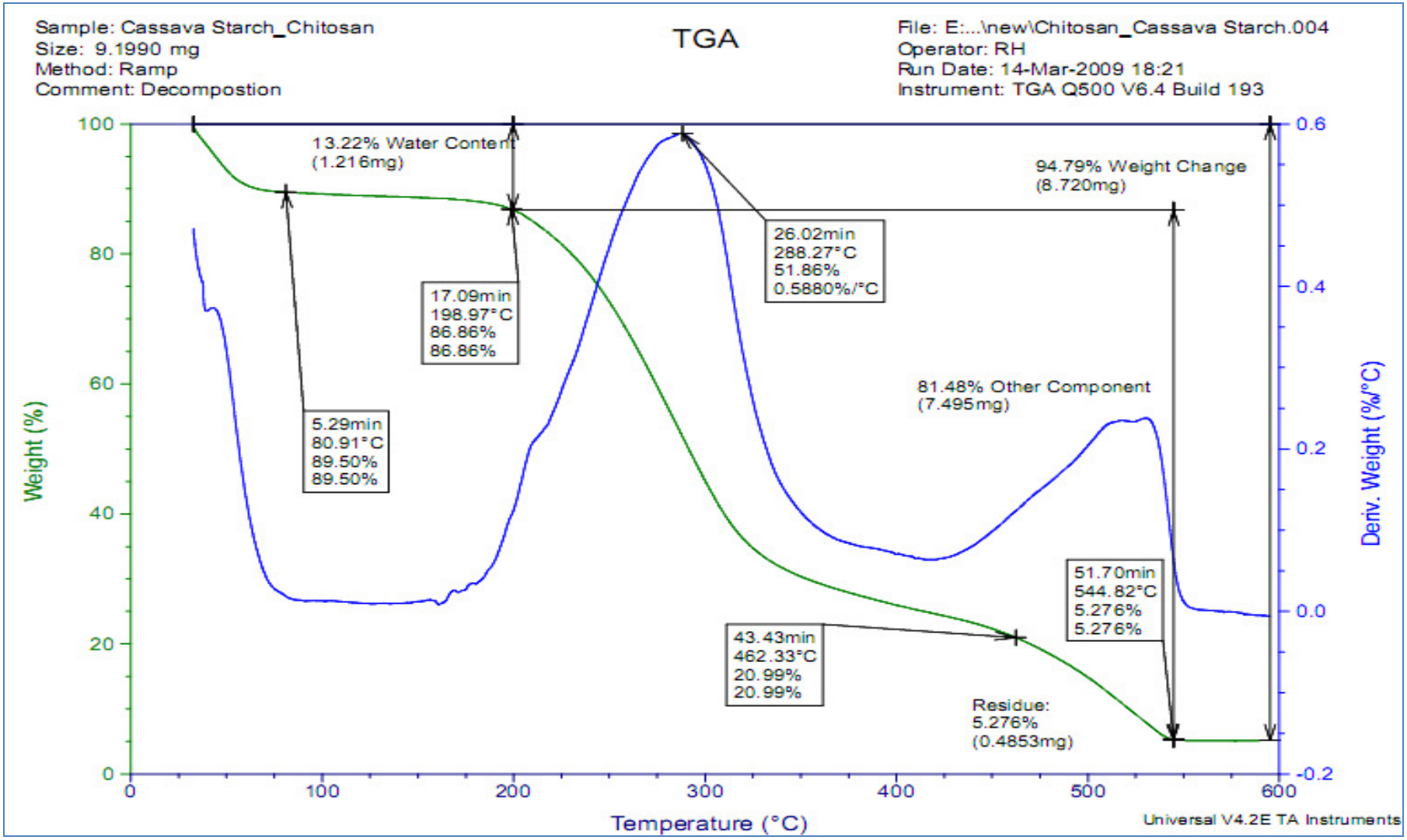


Figure 4.14: The decomposition trend of cassava starch-chitosan film sample.

Kawamoto *et al*. (2003) reported that lignocellulosic materials decompose on heating and when exposed to an ignition source by two different mechanisms. The first, dominant at temperatures below 300˚C, degrades polymers by the breaking of internal chemical bonds; dehydration (elimination of water); formation of free radicals, carbonyl, carboxyl, and hydroperoxide groups; formation of carbon monoxide and carbon dioxide; and finally, the formation of reactive carbonaceous char. The second mechanism, which takes over at temperatures above 300˚C, involves the cleavage of secondary bonds and formation of intermediate products such as anhydromonosaccharides, which are converted into low molecular weight products (oligosaccharides and polysaccharides), which lead to carbonized products

At the second stage of the thermal decomposition in Figure 4.15, the intensity of the heating peaks at the 24^{th} minute at the oxidation temperature of 294.82°C, T_o. The high energy put in to heat up the polysaccharide rings should be able to attest to that. The highest mass change takes place at this slope. According to Neto *et al*. (2005), pyrolysis of polysaccharides starts by a random split of the glycosidic bonds, followed by a further decomposition forming acetic and butyric acids and a series of lower fatty acids, where C_2, C_3 and C_6 predominates.

The last prominent stage of decomposition starts at 457.27°C at the 41^{st} minute. This final heat onslaught could also be attributed to the dehydration and depolymerization of the polysaccharide rings of banana stem fiber-chitosan film sample. The total percentage of weight change or weight loss stands at 98.261 while the total percentage of residue left behind was 1.739. The decomposition reaches a maximum of 558.81°C.

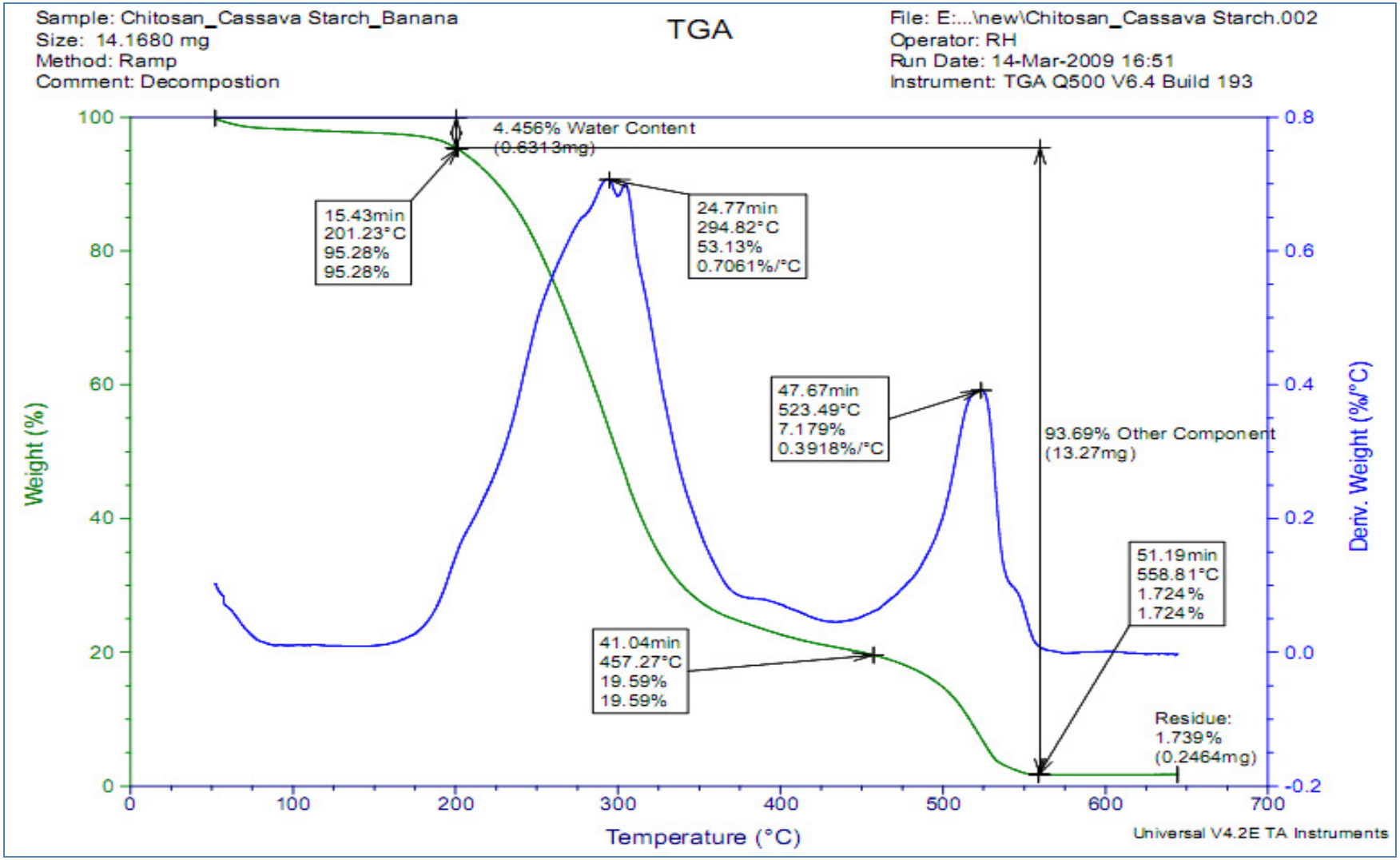


Figure 4.15: The decomposition trend of banana stem fiber-cassava starch-chitosan film sample.

4.3.4 Comparing the decomposition trend of the three film samples by their TGA curves

The decomposition trends of all three samples are almost the same with all three of them portraying three considerably significant stages of decomposition. Ash or residual contents of the film samples range from 1 – 10%.

When either organic compounds are decomposed or released at high temperature (500°C - 600°C), the remaining residue is the ash (Stephen, 1995). This residue consists of oxides and salts containing anions such as phosphates, chlorides, sulfates, and other halides and cations such as sodium, potassium, calcium, magnesium, iron, and manganese. The film samples fabricated are conclusively thermally stable up to 250°C, also known as onset temperature, T_{onset}, referring to the temperature when oxidation just begins. Hence, it should be appropriate for the three film samples of different compounds to be used as a packaging material for they are thermally stable up to 250°C. The thermal degradation of the material is therefore slow as pointed out in the TGA analysis and of significant importance as one of the assessments for the suitability of the film samples being made a packaging film.

The ash content of the film samples contains all the minerals present in the compounds that make up the constituents of the film samples. It can also contain any soil contaminants associated with the compounds as well. However, the ash content says nothing about the quality of the used compounds' mineral content and other much more sophisticated and expensive tests must be done to determine how much of different minerals (potassium, phosphorus, copper, zinc, manganese etc.) is provided by the said compounds.

Ash as reported is also present in lignocellulosics, especially straw which contains silica. Table 4.3 shows the ash composition of various biofibers in use today. The banana stem fiber-chitosan film sample recorded the highest ash or residual left over when being thermally degraded up to 600°C. This proves that the amount of minerals in the film is high. The banana stem fiber-cassava starch-chitosan film sample recorded the lowest ash content. Cassava starch-chitosan film sample

contained the highest moisture or water content and was most probably due to the fact that the film sample was highly permeable to water. Plus, both the compounds have the capabilities to bond with water molecules. The banana stem fiber-cassava starch-chitosan film sample had the lowest moisture content and it pointed to the fact that with the addition of more crystalline compounds, the degree of susceptibility of the film sample to water attack becomes lower. Cellulose crystallinity decreases its polar character. Then, cellulose addition into a starchy matrix decreases the global water content (Averous and Boquillon, 2004).

Table 4.3: The availability and ash composition of bio-fibers (Reddy and Yang, 2005).

Fiber source	***Availability (10^3 tons)***	***Ash (%)***
Corn stover	727	3.6 – 7.0
Pineapple leaf fiber	-	0.7 – 0.9
Coir	100	2.7 – 10.2
Bagasse	100	1.5 – 5
Banana	-	4.7
Wheat straw	568	6 – 8
Rice straw	579	14 – 20
Sorghum stalks	252	-
Barley straw	195	2 – 7

Table 4.4: The oxidation temperature, ash content, water content and onset temperature of the three film samples that were analyzed.

Film sample	***Oxidation Temperature, T_o (°C)***	***Ash(%)***	***Water(%)***	***Onset Temperature, T_{onset} (°C)***
Banana stem fiber-chitosan	273.38	2.657	6.917	260
Cassava starch-chitosan	288.27	5.276	13.22	275
Banana stem fiber-cassava starch-chitosan	294.82	1.739	4.456	280

4.4 Differential Scanning Calorimetry(DSC)

4.4.1 DSC curve of banana stem fiber-chitosan film sample

Cellulose forms the structural component of lignocellulosic fibres. Other than cellulose, hemicellulose and lignin forms the principal component of banana fibres (Pothan *et al*., 2006). Good adhesion between fibre and matrix leads to high values of dynamic modulus (Pothan *et al*., 2006). As has been mentioned in many journals, fiber treatment results in the removal of foreign materials, making the interfibrillar regions less dense and less rigid. This results in more matrix material being present. The fibrils, coming into existence by the dissolution of lignin and hemicellulose which are present in the natural fibers, are capable of arranging themselves ultimately resulting in more free space for bonding and interactions; refer Figure 4.16 (Pothan *et al*., 2006). A better fiber–matrix adhesion reduces the molecular mobility and thereby the damping values which leads to higher glass transition. In this case, the glass transition is observed at 22°C in Figure 4.17. The melting point is also observed to be higher than the plasticized chitosan films and will be discussed later on.

The hemicellulose present in plants is slightly cross-linked and is composed of multiple polysaccharide polymers with a degree of polymerization and orientation less than that of cellulose. Hemicellulose usually acts as filler between cellulose and lignin and consists of sugars including glucose, xylose, galactose, arabinose and mannose. Mechanically, hemicellulose contributes little to the stiffness and strength of fibers or individual cells (Reddy and Yang, 2005). Thus, it did not significantly affect the glass transition of the film sample under study.

Hemicellulose is more easily hydrolyzed into sugars than cellulose and therefore fibers containing a higher proportion of hemicellulose would be preferable for producing sugars, and eventually for fuels such as ethanol. Wheat and rice straw, corn stover and bagasse are, to a certain extent, favorable for producing carbohydrates because of their higher hemicellulose content and relatively larger availability. In comparison, coir and banana fibers have low hemicellulose content,

and using these fibers to produce carbohydrates might not be economical owing to the low conversion rates possible (Reddy and Yang, 2005).

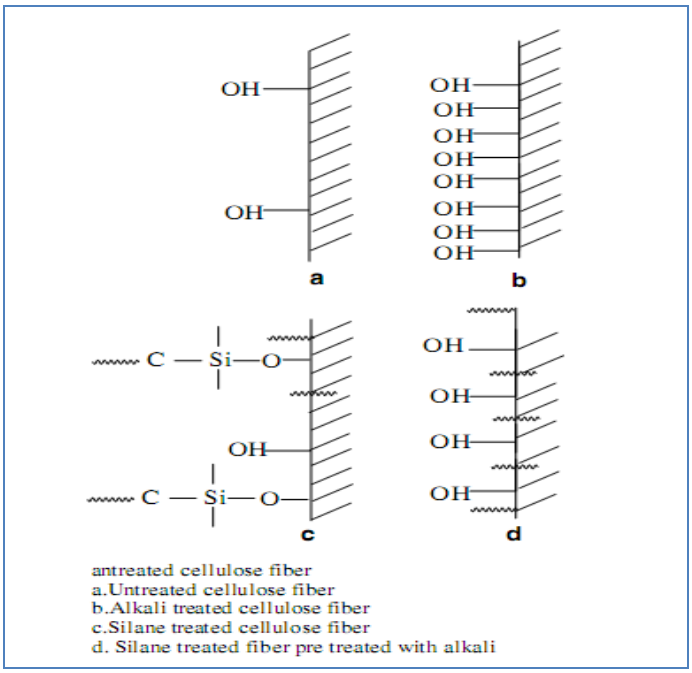


Figure 4.16: A schematic representation of interaction (Pothan *et al*., 2006).

Suyatma *et al*. (2004) reported in their study that the melting temperature of chitosan film was 97°C. Suyatma *et* al. (2004) also reported in their study that three blend compositions of chitosan/PLA, 90/10, 80/20, and 70/30 showed a slight decrease in melting temperature at about 93–94°C.

Those findings by Suyatma e*t al*. (2004) are somehow coherent with the current findings of the curve obtained for the banana stem fiber-chitosan film sample. The melting point of this film sample was recorded at 106.9°C in Figure 4.17. Thus, the degree of crystallinity of the film sample had been increased a little by the addition of the lignocellulosic fibers of the banana stem for the melting point had shifted right. Also, in the DSC curve obtained below, only one glass transition is readily observed. For that very reason, it suggests that the degree of miscibility between the fibers and the chitosan used was quite high.

There is a number of small curves 70 - 100°C. Chitosan film reveals a sharp and significant transition of endothermic peak centered at about 95-100°C, whereas broader and weak boiling peak of water was observed at the same temperature. It may be due to a certain amount of water evaporation contained in the chitosan film. Thus, Tae and Byung (2006) contended that the enthalpies for this endothermic peak represent the energy required to vaporize the water present in the films.

Therefore, the small curves that made up the noise in the range of 70 - 100°C can be attributed to the evaporation of water. The slight noise present could also signify the interactions between the water molecules with the film sample. The small endothermic curves thus bring forth the fact that the heat was absorbed to overcome the interactions between the OH ends of water with the OH ends of the other compounds present in the film, refer Figure 4.16. Also, the presence of only one prominent melting point endothermic peak signifies the miscibility between the compounds used to fabricate the film.

4.4.2 DSC curve of cassava starch-chitosan film sample

It has been reported that tapioca (cassava) starch's crystallinity, molecular order, and DSC enthalpy were completely lost at 69.5°C upon heating in water (Ratnayake and Jackson, 2007). Ratnayake and Jackson (2007) also noted in their findings that the irreversible granular swelling of cassava starch occurred at 70°C.

During the first portion of the phase transition of gelatinization, water absorbed by starch granules increases the mobility of starch polymers, especially amylose in amorphous domains. The results suggest that the increased polymer mobility facilitates rearrangement of these polymers. This polymer rearrangement includes polymer realignment and formation of new intermolecular bonds. Ratnayake and Jackson (2007) went on to prove that an increase in DSC enthalpy values with increasing lower temperature thermal pretreatments confirmed the phenomenon. Changes in relative crystallinities, at low temperatures, suggest that the

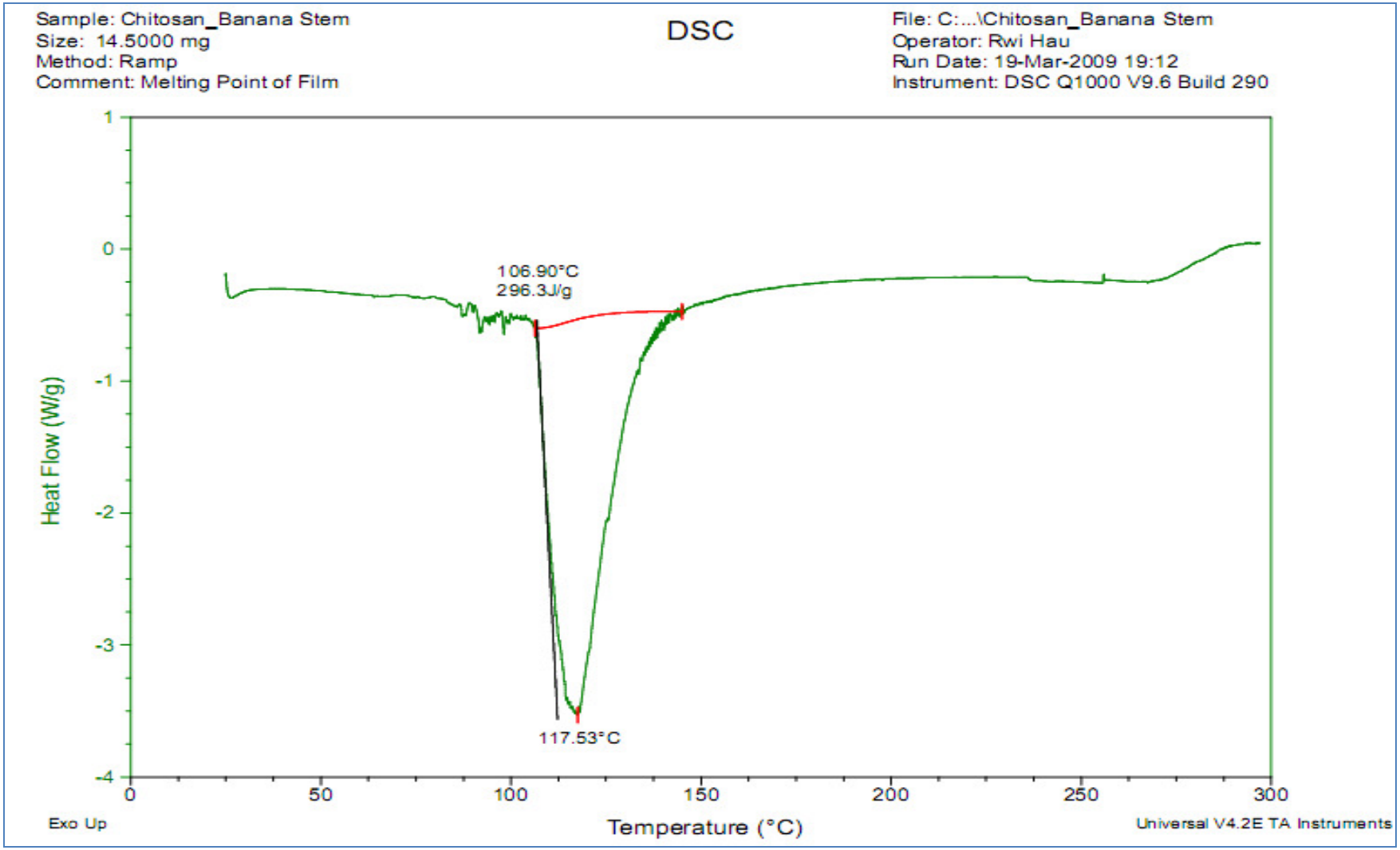


Figure 4.17: DSC curve of banana stem fiber-chitosan film sample.

structural changes also include alterations in the crystalline domains of granules. Changes in amorphous and crystalline domain occur simultaneously during any initial low temperature heat treatments (Ratnayake and Jackson, 2007).

Upon further heating, at increased temperatures, starch polymers become more mobile, reduce or lose their inter-polymer interactions, and starch granules break apart (Ratnayake and Jackson, 2007; Bilbao-Sainz *et al*., 2007). In summary, it can be concluded that starch phase transitions are three stage processes during which the following structural events take place:

a) water absorption by starch granules facilitates increased starch polymer mobility in the amorphous regions,
b) starch polymers in the amorphous regions rearrange often forming new intermolecular interactions,
c) with increasing hydrothermal effects, the polymers become more mobile and lose their intermolecular interactions and overall granular structure.

The energy absorbed by granules not only melts crystallite structures during gelatinization, but also facilitates 'rearrangement' or formation of new bonds among molecules at lower temperatures before gelatinization. An array of new molecular rearrangements and bonds having different stabilities is formed during this structural re-ordering process. This rearrangement process is different from annealing and the nature of this restructuring process, before granule break-down, depends on the starch type (Ratnayake and Jackson, 2007).

Koroteeva (2007) explained that from a thermodynamic point of view, the chains decreasing the T_m and H_m values could be attributed to defects destabilizing structure of crystalline lamellae, whereas the chains increasing the thermodynamic parameters could be ascribed to ones promoting suboptimal packing within crystalline lamellae.

Hence, it explains as to why the glass transition of the cassava starch-chitosan film began to shift leftward, as shown in Figure 4.18, decreasing for the fact that its level of crystallinity has shrunk. Suyatma *et al*. (2004) also reported in their study that chitosan powder has a single T_g at 194°C. Even though chitosan is more crystalline than starch, the fact that it did not help in greatly shifting the glass transition to the right was mainly due to the addition of PEG400. PEG400 acts as a plasticizer in many biodegradable films and plasticizers are well known for their roles in reducing the crystallinity of polysaccharides by boosting for chain mobility (Lazaridou and Biliaderis, 2002). The T_g of the cassava starch-chitosan film sample in the study was recorded at around 24°C in Figure 4.18.

With the glass transition shifting to the left, the melting temperature of the film sample was recorded at 109.09°C in Figure 4.18. There is a sharp narrow curve in the range of 70 - 100°C being observed. Chitosan film reveals a sharp and significant transition of endothermic peak centered at about 95-100°C, whereas broader and weak boiling peak of water was observed at same temperature. It may be due to a certain amount of water evaporation contained in the chitosan film. Thus, Tae and Byung (2006) contended that the enthalpies for this endothermic peak represent the energy required to vaporize the water present in the film.

Therefore, the small narrow curve, sometimes called as noise, in the range of 70 - 100°C can be attributed to the evaporation of water. Also highly obvious, there are slight interactions between the water molecules and the film samples which signify the absorbed heat at the endothermic curves required to overcome them. Also, the presence of only one prominent melting point endothermic peak signifies the miscibility between the compounds used to fabricate the film.

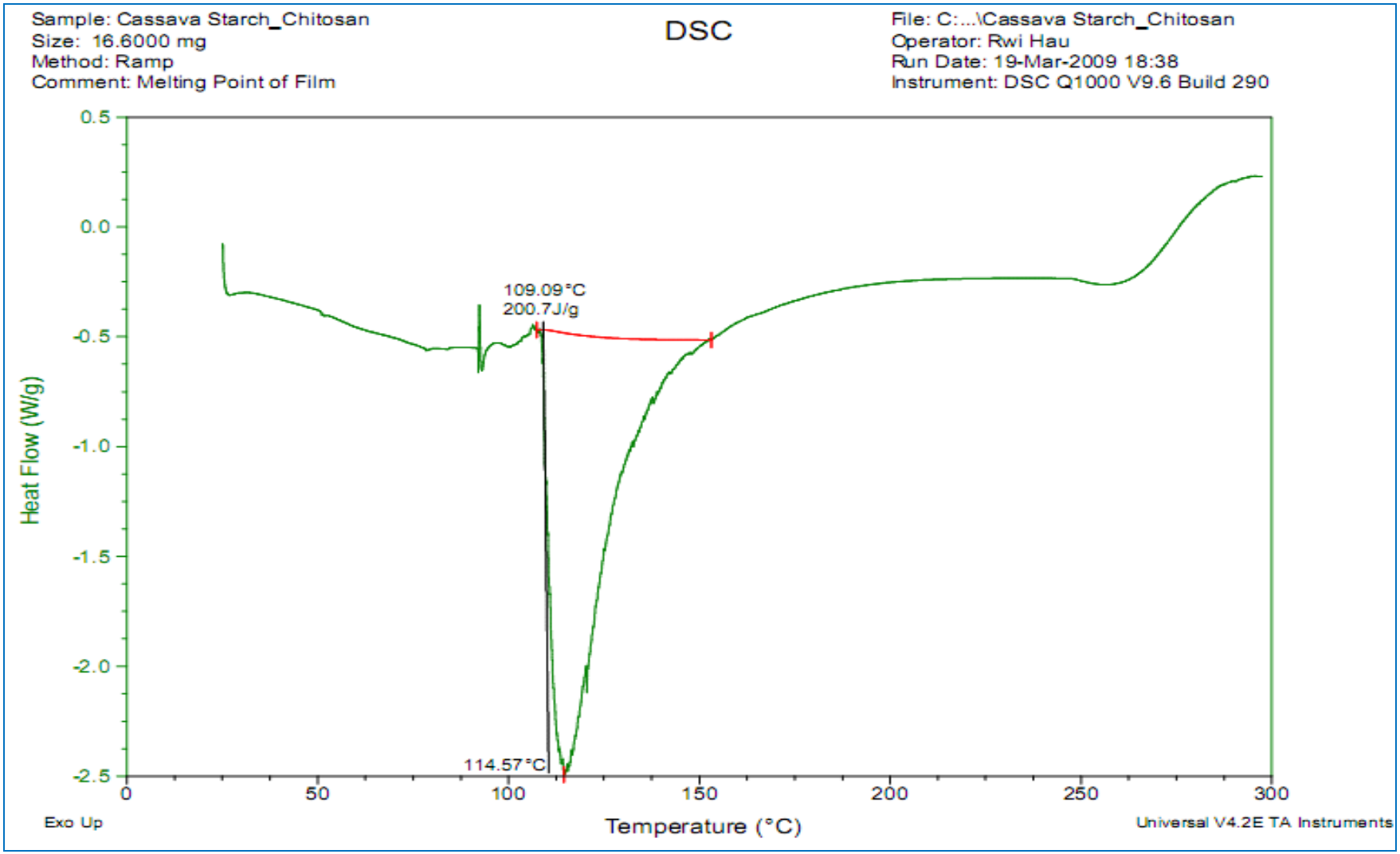


Figure 4.18: DSC curve of cassava starch-chitosan film sample.

4.4.3 DSC curve of banana stem fiber-cassava starch-chitosan film sample

Starch phase transitions during gelatinization, as pointed out by Bilbao-Sainz *et al*. (2007), are a three stage process during which the following structural events take place:

a) water absorption by starch granules facilitates increased starch polymer mobility in the amorphous regions,
b) starch polymers in the amorphous regions rearrange often forming new intermolecular interactions,
c) with increasing hydrothermal effects, the polymers become more mobile and lose their intermolecular interactions and overall granular structure.

The above stages rightfully explain the partial contribution to the fact that the glass transition, Tg, had shifted to the left. Even though chitosan is more crystalline than starch, the fact that it did not help to a certain degree in shifting the glass transition to the right was mainly due to the addition of PEG400. PEG400 acts as a plasticizer in many biodegradable films and plasticizers are well known for their roles in reducing the crystallinity of polysaccharides by boosting for chain mobility (Lazaridou and Biliaderis, 2002). The T_g of the banana stem fiber-cassava starch-chitosan film sample in the study was recorded at around 25°C in Figure 4.19. Mali *et al*. (2005) reported in their study that films formulated with low yam starch and high glycerol concentrations showed lower Tg values and Tg of the film formulated without plasticizer (control sample) was higher than those of films with glycerol (44.2°C); the plasticizer decreased Tg because it facilitates chain mobility.

As has been mentioned in many journals, fiber treatment would result in the removal of foreign materials, making the interfibrillar regions less dense and less rigid. This results in more matrix material being present. The fibrils, which have resulted by the dissolution of lignin and hemicellulose which are present in the natural fibers, are capable of arranging themselves ultimately resulting in more free space for bonding and interactions; refer Figure 4.16 (Pothan *et al*., 2006).

A better fiber–matrix adhesion reduces the molecular mobility and thereby the damping values which leads to higher glass transition. For that reason, the glass transition is observed at 25°C compared to the cassava starch-chitosan film sample at 24°C. With the glass transition shifting to the left by the gelatinization of starch and the addition of PEG400, the crystallinity of the whole structure of the film was contributed by the addition of banana stem fibers and thus the melting temperature of the film sample was recorded at 110.53°C, compared to the cassava starch-chitosan film sample whose T_g was recorded 109.09°C. Also, the presence of only one prominent melting point endothermic peak signifies the miscibility between the compounds used to fabricate the film.

4.4.4 Comparing the melting and glass transition trend of the three film samples by their DSC curves

For a series of polysaccharides, widely differing in their molecular structure branching, and conformation of glycosidic linkages, their glass transition temperature varies only by 1 - 20°C over a moisture range content range of 5 – 25% w/w. Moreover, these polymers exhibit parallel trends in their T_g. Moisture content plots are indicative of similar plasticization responses (Lazaridou and Biliaderis, 2002).

The glass transition of plasticized starch is sometimes difficult to be determined by DSC analysis, because the heat capacity change is quite low at the glass transition (Averous and Boquillon, 2004). The granules containing least stable crystallites start to change first upon heating. Water absorption by the granules lowers the melting points of crystallites, which results in quick melting of remaining crystallites. This process reduces the constraints of still remaining crystallites to lower their melting points. This cooperative process happens quickly when there is sufficient water and gives a narrow or single DSC endotherm (Ratnayake and Jackson, 2007).

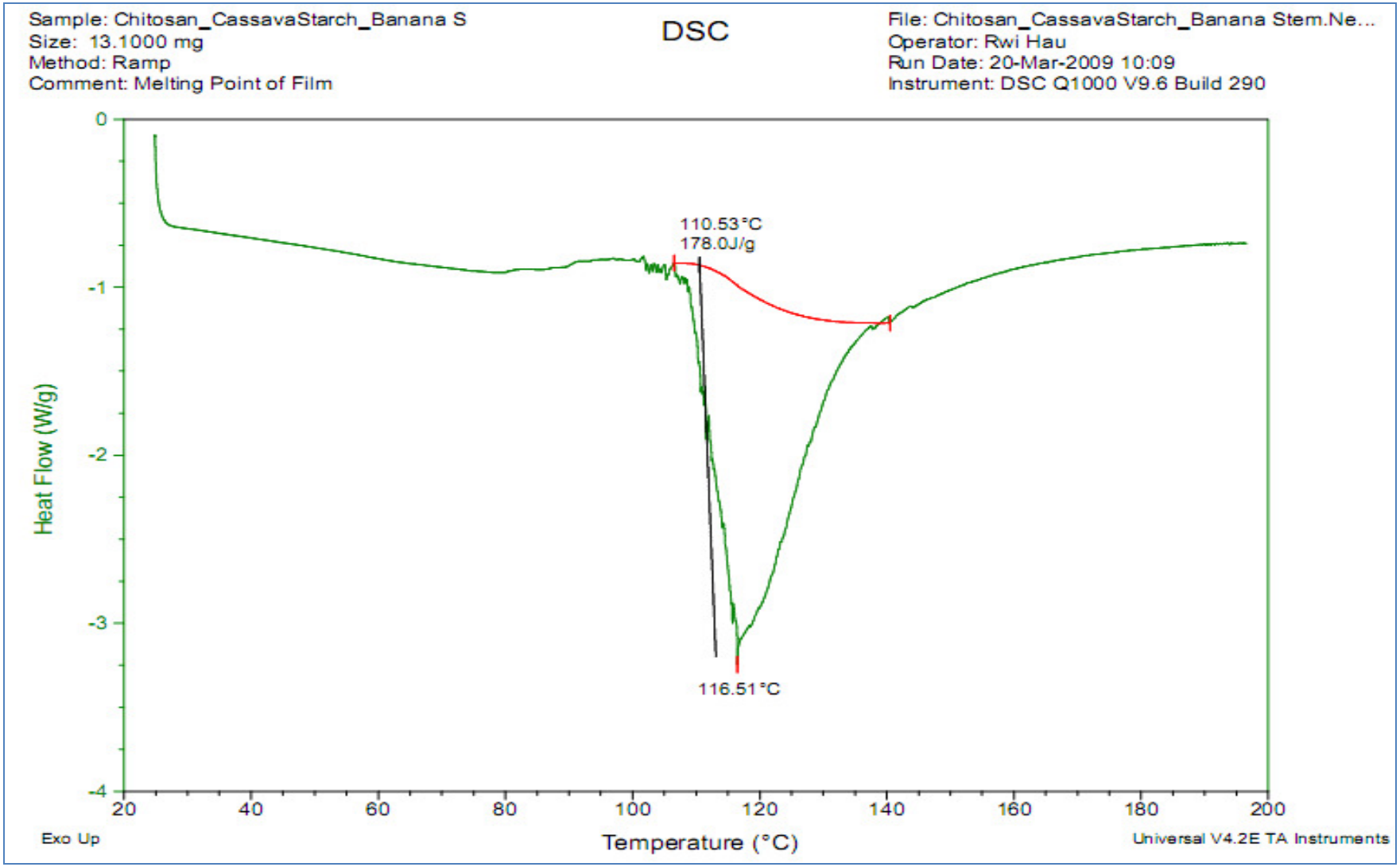


Figure 4.19: DSC curve of banana stem fiber-cassava starch-chitosan film sample.

Plasticizer also seems to limit crystal growth and recrystallization due to the interaction with the polymeric chains, interfering with polymer chain alignment due to steric hindrances. Thus, it also lends a hand in reducing the glass transition, Tg. In this study of biodegradable films, the addition of banana stem fiber has increased the melting point of chitosan film judging from what Suyatma *et al*. (2004) reported in their study that the melting temperature of chitosan film was at 97°C. Suyatma *et al*. (2004) also reported in their study that three blend compositions of chitosan/PLA 90/10, 80/20, and 70/30 showed a slight decrease in melting temperature at about 93 – 94°C.

Also in this study, the cassava starch-chitosan film sample recorded a melting temperature of 109.09°C while the addition of banana stem fiber significantly increased the film to 110.53°C. Comparing the cassava starch-chitosan film in this study to the chitosan film sample fabricated by Suyatma *et al*. (2004), it can be concluded that composite polymeric matrix imparts a higher interactions thus leading to higher melting point as more heat is needed to overcome the interactions of the polar compounds. Also, the present of only one prominent melting point endothermic peak signifies the miscibility between the compounds used to fabricate the film. The presence of more than one endothermic peak would suggest more than one structure characteristic to each of the compound used being present. If there is a considerable level of miscibility between the compounds used, it would mean each of the characteristic structure being replaced by a single new one.

Table 4.5: Glass transition and melting temperatures of the three film samples that were analyzed.

Film sample	T_g ***(°C)***	T_m ***(°C)***
Banana stem fiber-chitosan	22	106.9
Cassava starch-chitosan	24	109.09
Banana stem fiber-cassava starch-chitosan	25	110.53

4.5 Atomic force microscopy (AFM)

4.5.1 The topographic analysis of the three film samples

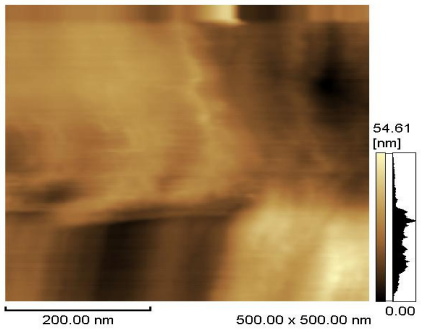


Figure 4.20: Banana stem fiber-chitosan film sample in 2D projection.

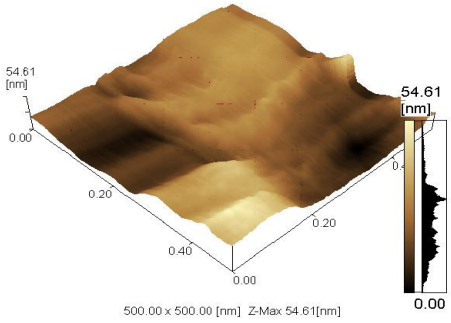


Figure 4.21: Banana stem fiber-chitosan film sample in 3D projection.

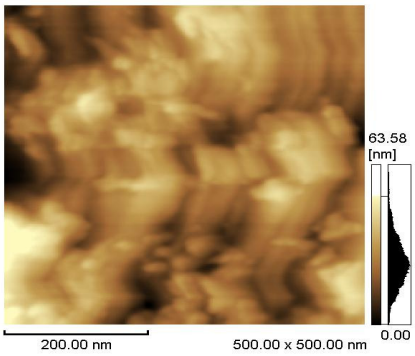


Figure 4.22: Cassava starch-chitosan film sample in 2D projection.

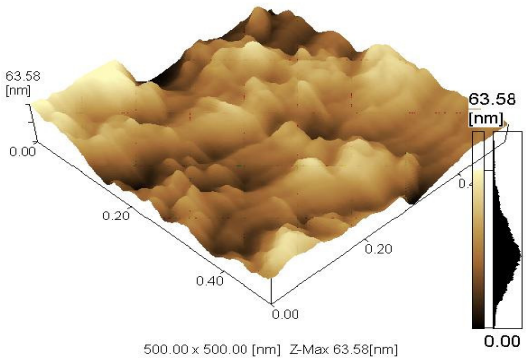


Figure 4.23: Cassava starch-chitosan film sample in 3D projection.

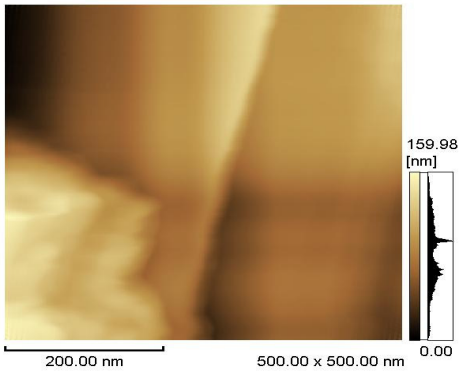


Figure 4.24: Banana stem fiber-cassava starch-chitosan film sample in 2D projection.

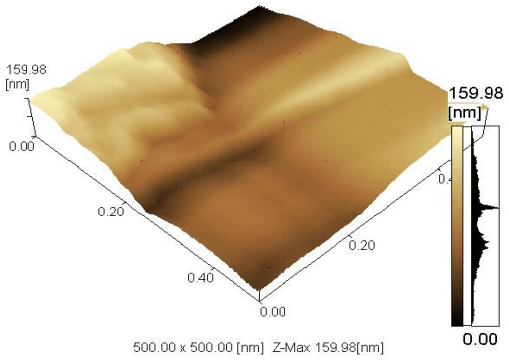


Figure 4.25: Banana stem fiber-cassava starch-chitosan film sample in 3D projection.

The AFM analysis was carried out to study the topographical surface of the film samples. The AFM's surface structure of the banana stem fiber-chitosan film sample appears to be quite smooth and clean with slight ruggedness. The banana stem fiber-cassava starch-chitosan film sample also appears rather smooth and clean with slight ruggedness. The cassava starch-chitosan film sample on the other hand appears a little too rough but otherwise, quite smooth. The high degree of roughness on the cassava starch-chitosan film sample which makes it look lumpy from the top could have been caused by the high level of immiscibility between the two compounds, the cassava starch and chitosan, used to fabricate it.

4.5.2 Surface roughness

Table 4.6: The surface roughness of the three film samples that were analyzed

Sample		***Surface Roughness (nm)***			
		Ra	***Ry***	***Rz***	***Rms***
Banana stem fiber-chitosan	0.5um X 0.5 um	8.195	53.923	28.410	9.820
Cassava starch-chitosan	0.5um X 0.5 um	9.298	76.363	53.681	11.775
Banana stem fiber-cassava starch-chitosan	0.5um X 0.5 um	24.997	159.073	137.643	30.133

The surface roughness calculated shows that the roughness of the film samples increases from the banana stem fiber-chitosan film sample with a value of 9.820, to cassava starch-chitosan film sample at 11.775 and finally, banana stem fiber-cassava starch-chitosan film sample at 30.133. The film sample with the most compounds added shows the highest surface roughness. The total mass of the compounds used could have also contributed to the surface roughness. The higher the amount of the compounds used, the higher the surface roughness due to the degree of immiscibility being increased.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The study carried out on the fabrication and characterization of the banana stem fiber-chitosan, cassava starch-chitosan and banana stem fiber-cassava starch-chitosan film samples was in fact successful as it most importantly met the objectives of the study besides giving a new in-depth insight into the usage of biofibers as reinforcing agents of biodegradable films with low mechanical strength.

The film samples were successfully fabricated and then being subjected characterization tests. In the FTIR analysis, the film samples showed considerable interactions between the amide group of the chitosan and the hydroxyl group of the starch and cellulosic fibers through the changes or peak shifting in the 2000-1600 cm^{-1} region. In the TGA analysis, all the film samples showed a thermal stability up to approximately 250°C with three prominent stages of decomposition.

In the DSC analysis, the melting points of the three film samples were analyzed. The banana stem fiber-chitosan's melting point stood at 106.9°C. The cassava starch-chitosan film sample on the other hand recorded a melting point of 109.09°C and the banana stem fiber-cassava starch-chitosan film sample recorded a melting point of 110.53°C. The glass transition of these three film samples ranged from 22°C for the banana stem fiber-chitosan film sample, to 24°C for the cassava starch-chitosan film sample and finally 25°C for the banana stem fiber-cassava starch-chitosan film sample.

For the AFM analysis, the surface roughness of the banana stem fiber-chitosan and banana stem fiber-cassava starch-chitosan film sample showed rather smooth and clean surfaces. The cassava starch-chitosan film sample showed rough and lumpy surface and this could have been caused by the immiscibility of the cassava starch and chitosan used.

5.2 Recommendation

The study carried out to fabricate the banana stem fiber-chitosan, cassava starch, banana stem fiber-cassava starch-chitosan film samples used PEG400 as plasticizer. Other poly-ol plasticizers such as glycerol, sorbitol, glycerin and PLA can be used to study the effects that they would be able to impart on the three types of film samples. Other non-harmful biodegradable additives can also be added to the films to further improve them in their mechanical strength, tensile strength, thermal properties and moisture and gas barrier.

For the TGA and DSC analyses, the heating rate used can most probably be lowered to a certain acceptable value to obtain more accurate and sound curves or peaks. Other types of heating methods, such as heat-cool-heat, can also be considered to study the thermal properties of the film samples.

Since the level of miscibility between the compounds used is also very important in order to ensure the compatibility of the structure of the films, it is therefore highly recommended that stirring at a higher speed and longer duration be carried out. Degassing should also be carried out to settle out trapped air bubbles in the casting solution. Casting knife can also be used to ensure smooth surface of films being cast onto the glass plate.

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