BIODEGRADABLE BIOCOMPOSITE STARCH BASED FILMS BLENDED WITH CHITOSAN AND WHEY PROTEIN

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FAUZIAH SHAHEEN BINTI SHEH RAHMAN

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

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Universiti Malaysia Pahang

MAY 2010

"I hereby declare that this thesis entitled "Biodegradable Biocomposite Starch Based Films Blended with Chitosan and Whey Protein" is the result of my own research except as cited references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree".

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DATE	:	7 MAY 2010

I dedicate this entire work to my family especially to my beloved parents (Papa and Mama), whose patient, support and companionship have facilitated my study, and made my life enjoyable, to my lovely sisters (Sis Azmah, Sis Nazia, Azra and Shaesta) and my grateful brothers (Big B, Nikz, Didi and Guddu) for their enduring faith and unconditional love in good times and bad.

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ABSTRACT

The use of synthetic plastic materials as a food packaging causes serious hazards to the environment. The introduction of biodegradable materials, which can be disposed directly into the soil, can be one possible solution to this problem. The objectives of this work were to produce biodegradable biocomposite films and study the characterization of starch-based films blended with chitosan and whey protein. The films were synthesized by using the mixing process and the casting method. The characteristics of the blend films with different tapioca starch composition (1, 2, 3, 4 and 5 g/100 mL) were evaluated using Universal Testing Machine, Fourier transform infrared spectroscopy (FTIR), differential scanning calorimeter (DSC), Thermogravimetric Analysis (TGA), scanning electron microscope (SEM) observation and biodegradability using microbiological degradation test and soil burial degradation test. Among them, the biodegradable blend films compatibilized with 1% of tapioca starch content showed good mechanical properties and had the highest thermal stability. The FTIR confirmed that tapioca starch, chitosan and WPI were compatible and inter-molecular hydrogen bonds existed between them. Moreover, the SEM analysis with 1% of tapioca starch content showed a compatible, smooth and homogenous structure of the composite film. The microbiological degradation test indicated that the growth of *A. Niger* colony increases as the tapioca starch content was increased. In soil burial test, a rapid degradation occurred for all the films in the initial 10 days followed by 100% composting within 18 days. As a conclusion, the film fabricated had potential application in future to be used as food packaging because it can enhanced foods quality and at the same time protected the environment.

ABSTRAK

Penggunaan bahan sintetik plastik sebagai pembungkus makanan boleh menyebabkan masalah yang serius terhadap persekitaran. Pengenalan bahan bio-urai dimana ia boleh dibuang secara terus ke tanah, boleh menjadi salah satu penyelesaian untuk masalah ini. Tujuan kajian ini dijalankan adalah untuk menghasilkan filem biokomposit biodegrdadasi dan mengkaji ciri-ciri filem berasaskan kanji dicampur dengan kitosan dan tepung protein dadih (WPI). Filem ini dihasilkan dengan menggunakan proses pencampuran dan kaedah tuangan. Filem yang terhasil dengan komposisi campuran kanji ubi kayu yang berbeza (1, 2, 3, 4 dan 5 g/100 mL) ini telah dicirikan dengan menggunakan mesin pengujian, spektroskopi inframerah transformasi Fourier (FTIR), kalorimeter pengimbasan perbezaan (DSC), analisi termogravimetri (TGA), mikroskopi pengimbasan elektron (SEM) dan biodegradasi menggunakan kaedah mikrobiologi dan kaedah timbus tanah. Di antara filem yang telah dihasilkan, kanji ubi kayu dengan komposis 1% menunjukkan sifat mekanik yang kukuh dan mempunyai kestabilan terma yang tertinggi. FTIR menegaskan bahawa kanji ubi kayu, kitosan dan WPI bersesuaian dan wujud ikatan hidrogen diantaranya. Tambahan pula, SEM analisis bagi komposisi 1% kanji ubi kayu menunjukan struktur yang serasi, halus dan homogen. Uji kaji degradasi mikrobiologi menunjukkan bahawa pertumbuhan koloni *A. Niger* meningkat dengan pertambahan komposisi kanji ubi kayu. Dalam uji kaji timbus tanah, degradasi pantas berlaku untuk semua filem pada 10 hari terawal diikuti dengan 100% kompos dalam masa 18 hari. Kesimpulannya, filem yang terhasil ini mempunyai aplikasi berpotensi di masa hadapan untuk digunakan sebagai pembungkus makanan kerana ia dapat meningkatkan kualiti makanan dan dalam masa yang sama dapat melindungi alam sekitar.

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LIST OF NOMENCLATURES

$^{\circ}C$	-	Celsius
CD	-	Compact disk
DSC	-	Differential Scanning Calorimetry
E	-	Elongation or modulus of elasticity
FTIR	-	Fourier Transformed Infrared Spectroscopy
ΔH	-	Melting or gelatinization enthalpy
SEM	-	Scanning Electron Microscopy
Tc	-	Crystallization point
T_d	-	Decomposition temperature
Tg	-	Glass transition temperature
TGA	-	Thermogravimetric Analysis
Tm	-	Melting temperature
TS	-	Tensile Strength
TV	-	Television
WPI	-	Whey protein isolates

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Nowadays, million tons of plastic are produced annually all over the world. The production and consumption continue to increase each year. It plays a significant role in our daily life and has become a widely used material due to its wide array of applications particularly as packaging materials.

Petrochemical based plastics such as polyolefin, polyesters and polymides have been increasingly used as packaging materials because of their availability in large quantities at low cost and favorable functionality characteristics such as good tensile and tear strength, good barrier properties to oxygen and aroma compounds and heat seal ability. However, these plastics are made of petroleum-based materials that are not readily biodegradable and therefore lead to environmental pollution, which pose serious ecological problem (Ali *et al*., 2008). The most obvious form of pollution associated with plastic packaging is waste plastic dump to the landfills. Plastics are very stable and therefore settle in the environment in prolong time after been discarded, especially if they are shielded from direct sunlight by being buried in landfills. It can leach harmful chemicals that eventually spread into groundwater sources which may cause numerous illnesses to human health.

In order to overcome these problems, several studies are concentrated on the development of new biodegradable packing materials that can be manufactured with the utilization of environmentally friendly raw materials. Among the natural

polymers, starch has been considered as one of the most promising candidates for future materials because of its attractive combination of price, abundance and renewable in addition to biodegradability. Starch naturally occurs in a variety of botanical sources such as wheat, corn, yam, potatoes and tapioca (Salleh and Muhamad, 2008). The preponderance of amylose in starch increases the strength of the films. However, biodegradable products based on starch, possess many disadvantages, mainly attributed to the water solubility, brittle nature of starch films and poor mechanical properties. Therefore, it needs to be treated first by either plasticization or blending with other materials.

Plasticizers are added to polymers to reduce brittleness, since they work as spacers polymer chains, decreasing inter-molecular forces and thus increasing flexibility and extensibility of polymers. They must be compatible with the film forming polymers (Wu *et al*., 2009). Common plasticizers for starch are glycerol and other low-molecular-weight-polyhydroxy-compounds, polyethers and urea. Glycerol was the most suitable plasticizer with respect to mechanical properties and transparency.

Moreover, one of the effective strategies to overcome the poor mechanical properties of starch based films, while preserving the biodegradability of the material, is to associate starch with chitosan. Chitosan, a 1,4 linked-2-deoxy-2-aminoglucose, prepared by *N*-deacetylation of chitin (Xu *et al*., 2005). It is relatively low cost, widespread availability from a stable renewable source, that is, shellfish waste of the sea food industry (Bourtooma and Chinnan, 2007) and appears as a natural antimicrobial candidate for the incorporation because it can inhibit the growth of a wide variety of fungi, yeasts and bacteria (Salleh and Muhamad, 2008).

Whey protein is the collection of globular proteins isolated from whey, a by-product of cheese manufactured from cow's milk. It can be used for production of biodegradable films. Whey protein films have low tensile strength and high water vapor permeability due to the high proportion of hydrophilic amino acid in their structures (Ghanbarzadeh and Oromiehi, 2008). According to B. Sen Gupta and Magee (2007), these films were found to retard moisture loss and oxygen diffusion.

It also showed good tensile strength and moderate elongation. The films had apparently no flavor or taste that interfered with those of the food. The films also have excellent mechanical and barrier properties.

Hence, this study will focus on the preparation and characterization of biodegradable blend films from starch-chitosan-whey protein as well as their microstructure, thermal properties and biodegradibility. The method applied to produce starch-chitosan-whey protein blend films is by using casting technique.

1.2 Problem Statement

Plastic is the "highlander" material, living extremely long lives, used in countless products that could potentially have service over decades. The production and disposal of plastics contribute to an array of environmental problems, which pose serious ecological problem. The most obvious form of pollution associated with plastic packaging is waste plastic sent to landfills. Plastics are very stable and therefore settle in the environment in prolong time after been discarded, especially if they are shielded from direct sunlight by being buried in landfills. It can leach harmful chemicals that eventually spread into groundwater sources which may cause numerous illnesses to human health. Other than that, we are also facing problem with the depletion of fossil fuels sources, which are connected to plastics as their raw materials. Crude oil is a product of fossils and therefore it is not renewable. Hence, a possible solution to overcome the problems is by using biodegradable composite. Thus by blending starch, chitosan and whey protein it would produce bio-composite film which offer biodegradability and subsequently reduce the amount of pollution caused by polymers to the environment.

1.3 Research Objective

The objectives of this research are to produce biodegradable biocomposite films and to study the characteristics of starch-based films blended with chitosan and whey protein.

1.4 Scopes Of The Study

The scopes of this study are:

1. To produce biodegradable biocomposite films from tapioca starch blended with chitosan and whey protein.
2. To investigate the influence of starch composition on biodegradable biocomposite films (1, 2, 3, 4 and 5 g/ 100 mL).
3. To determine the mechanical properties of the biodegradable blend films using Universal Testing Machine.
4. To identify the interactions and/or chemical bonds between tapioca starch, chitosan and whey protein by using Fourier Transform Infrared Spectroscopy (FTIR).
5. To study the thermal properties and stability of blend films by using Thermogravimetric Analysis (TGA) and Differential Calorimetry Scanning (DSC).
6. To observe the microstructure of films by using scanning electron microscope (SEM).
7. To determine the biodegradability of blend films by microbial degradation using *Aspergillus Niger* and soil burial degradation test.

CHAPTER 2

LITERATURE REVIEW

2.1 Polymers: Synthetic Polymers and Natural Polymers

Polymers are long chain giant organic molecules that are assembled from many smaller molecules called monomers. Polymers consist of many repeating monomer units in long chains. A polymer is analogous to a necklace made from many small beads (monomers). Another common name for many synthetic polymers is plastic which comes from the Greek word "plastikos", suitable for molding or shaping. Many objects in daily use from packing, wrapping and building materials include half of all polymers synthesized. Other uses include textiles, TV's, CD's, automobiles and many other all are made from polymers. There are many types of polymers including synthetic and natural polymers (Ophardt and Losey, 2003).

Synthetic polymers can be classified as addition polymers, formed from monomer units directly joined together or condensation polymers, formed from monomer units combining such that a small molecule, usually water, is produced during each reaction (Joesten, 2008). Some examples of synthetic polymers are polyethylene, polyester, polyurethane and many more. These types of polymers basically cannot be degraded and thus need to be recycle in order to avoid landfills problems.

Natural polymers are large molecules, produced by plants and animals that carry out many life-sustaining processes in a living cell. The cell membranes of plants and the woody structure of trees are composed in large part of cellulose, a

polymeric carbohydrate. Carbohydrates, which comprise one of the three classes of foodstuffs, contain carbon, hydrogen and oxygen atoms. They can be classified as monosaccaharides, disaccharides and polysaccharides (Moore *et al*., 2005).

2.2 Film Forming Materials

Film forming materials, such as starches, chitosan, proteins and glycerol are used for different purposes in a biomaterial and/or edible film preparation process. Biopolymers like starches and proteins create the basic network structure of the film. However, films prepared from biopolymers are often too fragile to stand handling, *e.g.*, bending or stretching. Thus, they have to be plasticized using low molecular weight substances, such as polyols (glycerol, xylitol, sorbitol, sugar and maltitol), which decrease interactions between the biopolymer chains (Wu *et al*., 2009).

Biopolymers used in film preparation are often carbohydrates or proteins extracted or separated from plants, animal tissues or animal products. The storage carbohydrate in plants is starch. Depending on the plant the starch is formed in different parts of the plant, *e.g.*, grain, tuber or root. Other carbohydrates found in the plants *e.g.* the cell wall include cellulose and pectin (Keeling, 1998). Some carbohydrates, such as alginate and carrageenan, are found in seaweeds (Larotonda *et al*., 2005). Biopolymers extracted from animal products or parts are also used in edible film manufacturing. Chitosan is widespread available in shellfish waste of the sea food industry and have an ability to form a good films (Bourtooma and Chinnan, 2007). Casein and whey proteins are separated from milk and they are often used to prepare films (Ghanbarzadeh and Oromiehi, 2008) and (B. Sen Gupta and Magee, 2007).

Out of all of the film forming materials mentioned in this section, this literature review focuses mainly on the starch, chitosan, whey protein and polyols.

2.2.1 Starch

2.2.1.1 Sources for Starch

Starch is the major carbohydrate reserve in plant tubers and seed endosperm where it is found as granules (Buleon *et al*., 1998), each typically containing several million amylopectin molecules accompanied by a much larger number of smaller amylose molecules. By far the largest source of starch is corn (maize) with other commonly used sources being wheat, potato, tapioca and rice. Amylopectin (without amylose) can be isolated from 'waxy' maize starch whereas amylose (without amylopectin) is best isolated after specifically hydrolyzing the amylopectin with pullulanase (Vorwerg *et al*., 2002). Genetic modification of starch crops has recently led to the development of starches with improved and targeted functionality (Jobling, 2004).

2.2.1.2 Starch Structure and Composition

Starch molecules arrange themselves in the plant in semi-crystalline granules. Each plant species has a unique starch granular size: rice starch is relatively small, about 2μm, and potato starch has larger granules about up to 100μm (Wikipedia). Native starches from different botanical sources vary widely in structure and composition, but all granules consist of two major molecular components, amylose (20-30%) and amylopectin (70-80%), both of which are polymers of α-D-glucose units in the C_1conformation. In amylose (Refer Figure 2.1), these are linked -(1 → 4)-, with the ring oxygen atoms all on the same side, whereas in amylopectin about one residue in every twenty is also linked -(1 → 6)- forming branch-points as shown in Figure 2.2 (Nowjee, 2004).

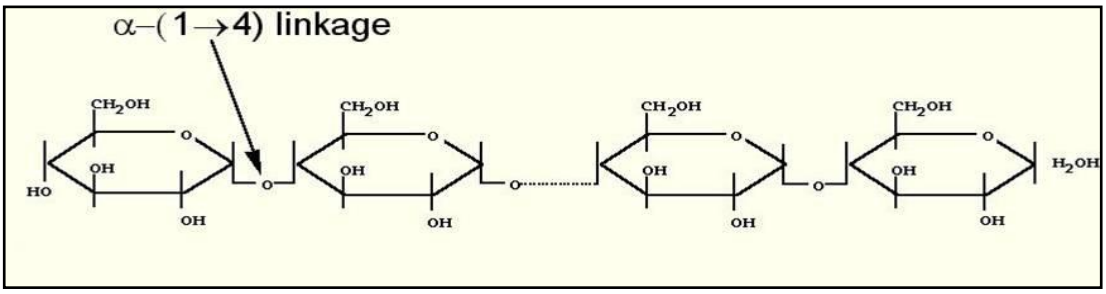

Figure 2.1: Amylose molecules (Nowjee, 2004)

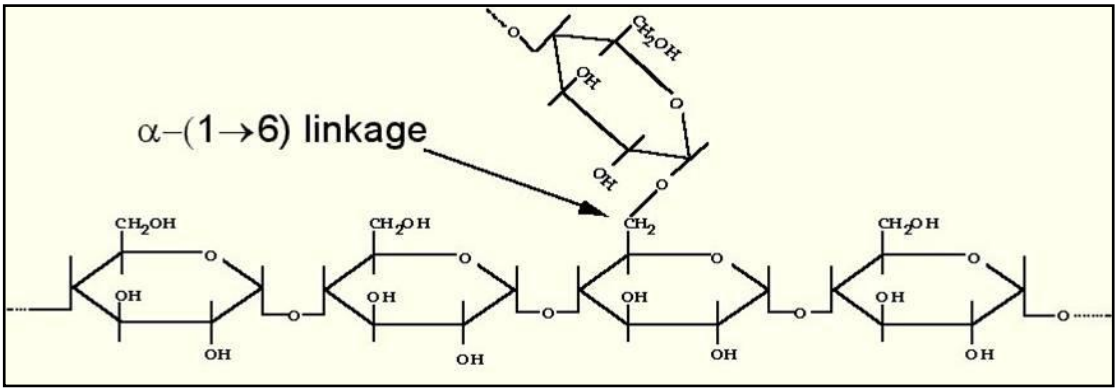

Figure 2.2: Amylopectin molecules (Nowjee, 2004)

A typical feature of starch is that it becomes soluble in water when heated. The granules swell and burst, the semi-crystalline structure is lost and the smaller amylose molecules start leaching out of the granule. This process is called starch gelatinization. During cooking the starch becomes a paste and gets its viscosity. During cooling or prolonged storage of the paste, the semi-crystalline structure partially recovers and the starch paste thickens. This is mainly caused by the retrogradation of the amylose. This process is also responsible for the staling, hardening of bread and the water layer on top of a starch gel (syneresis). Some cultivated plant varieties have pure amylopectin starch without amylose, known as waxy starches. The most used is waxy maize. Waxy starches have less retrogradation, the viscosity of the paste will be more stable. Also high amylose starch, amylomaize, is cultivated for the use of its gel strength (Wikipedia).

2.2.2 Chitin and Chitosan

2.2.2.1 Structural and Characterization of Chitin and Chitosan

Chitin and chitosan are known biodegradable natural polymers based on polysaccarides, which are extracted from various animals and plants. Chitin exists widely in cell walls of some microorganisms such as fungi, molds and yeasts and in the cuticular and exoskeletons of invertebrates such as crustaceans, mollusks, crab, shrimps, lobster, squid and insects (Knaul *et al.*, 1999). Chitosan exists naturally only in a few species of fungi. Chitin and chitosan consist of 2-acetamido-2-deoxy-β-D-glucose and 2-amino-2-deoxy-β-Dglucose as repeating units respectively. Chitin is chemically identical to cellulose except that secondary hydroxyl group on the alpha carbon atom of the cellulose molecule is substituted with acetoamide groups (Refer Figure 2.3).

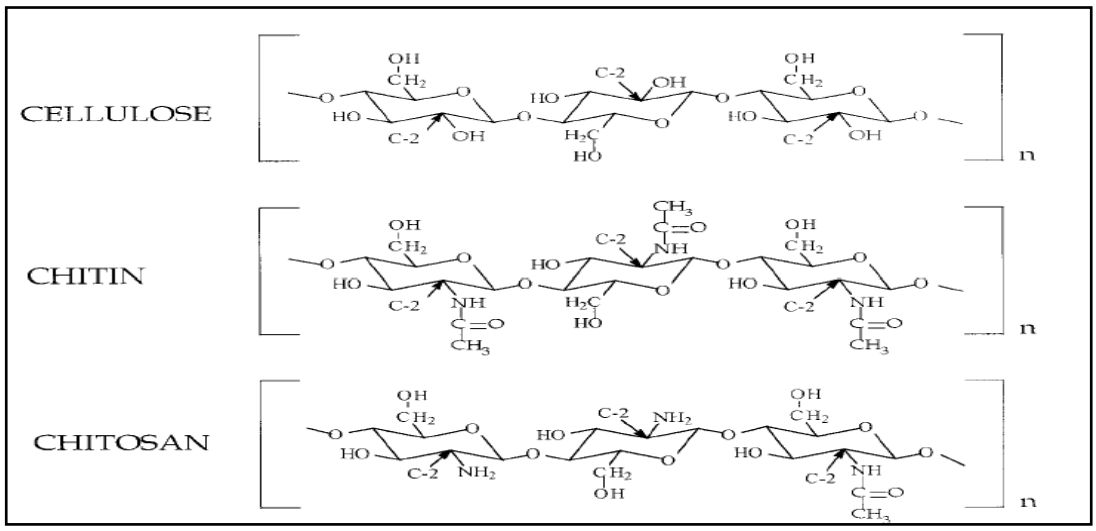

Figure 2.3: Comparison of Molecular Structure of Cellulose, Chitin, Chitosan (Knaul *et al*., 1998).

Chitosan is the N-acetylated form of chitin. Figure 2.4 shows the deacetylation reaction of chitin.

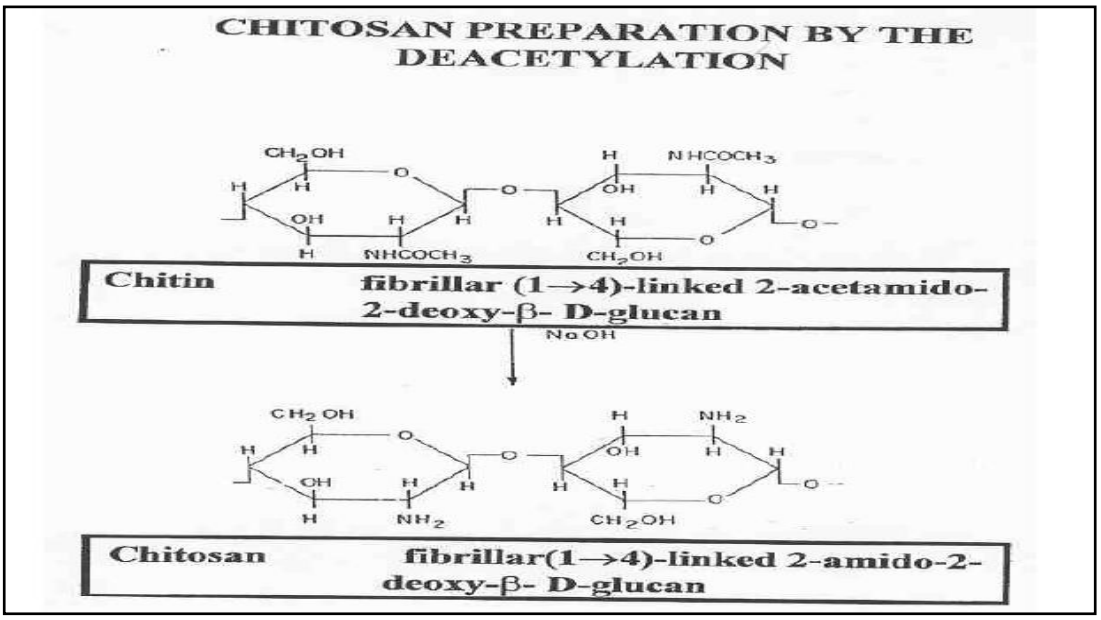

Figure 2.4: The chitosan production with deacetylation of chitin (Melichar, 2007).

Chitosan is insoluble in water and is soluble in acidic solvent below pH 6. Organic acid such as acetic formic and lactic acids are used for dissolving chitosan and most commonly used is 1% acetic acid solution (pH is about 4.0). Solubility in inorganic acids is quite limited. Chitosan is soluble in 1 % hydrocloric acid but insoluble in sulfuric and phosphoric acids. Chitosan solution's stability is poor above about pH 7. At higher pH, precipitation or gelation will occur. Chitosan solution forms poly-ion complex with anionic hydrocolloid and provides gel. Figure 2.5 shows the chitosan preparation from chitin and chitosan solution in acetic solvent (Onar and Sariisik, 2002).

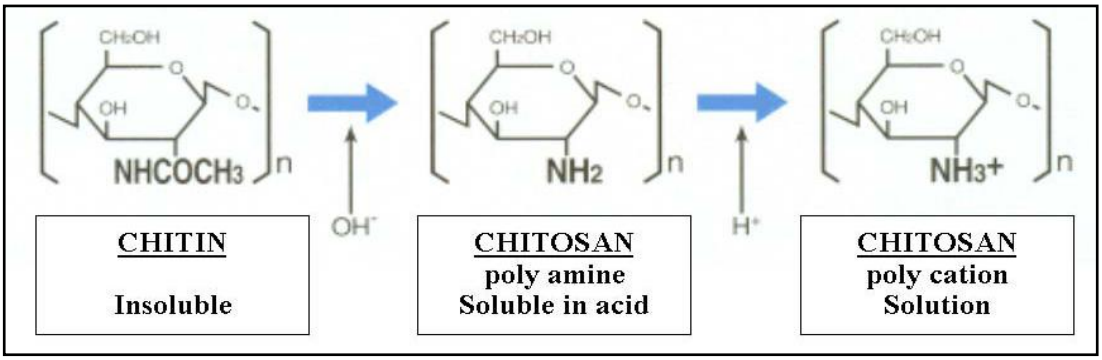

Figure 2.5: Chitosan preparation from chitin and chitosan solution in acidic solvent (Onar and Sariisik, 2002).

2.2.2.2 Chitin and Chitosan Manufacturing Process

In most studies, chitin and chitosan fibers are produced by a wet-spinning process, but rarely by a dry-spinning process. When using wet-spinning process to produce the fibers, the two polymers firstly are dissolved in a solvent and then the polymer solution is extruded via fine holes (especially through a viscose-type spinneret) into a non-solvent (coagulant) at 45-50 0C. The polymer precipitates out in the form of a filament, which can be washed, drawn and dried to form the fibers (Onar and Sariisik, 2002). Figure 2.6 shows the chitin and chitosan manufacturing process.

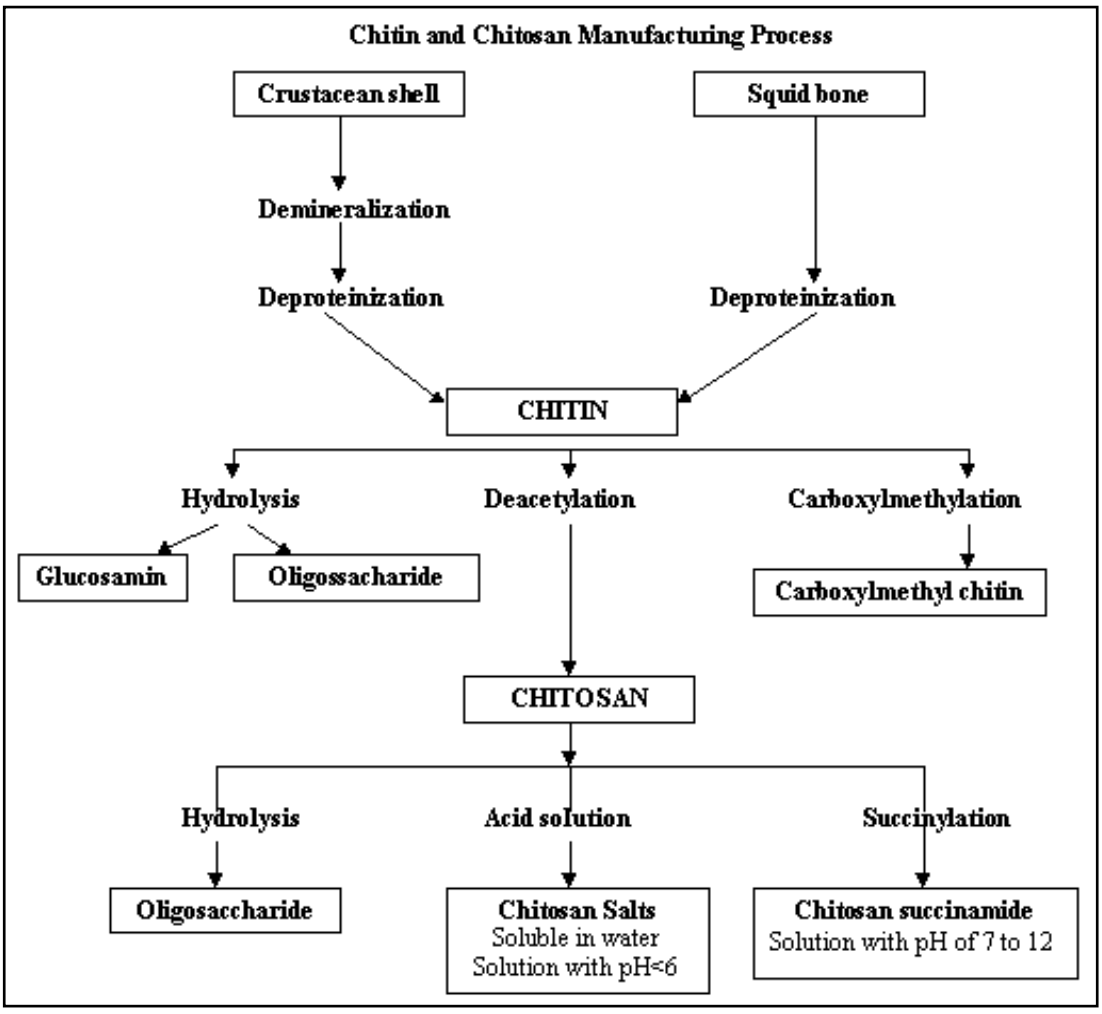

Figure 2.6: Chitin and chitosan manufacturing process (Onar and Sariisik, 2002).

2.2.3 Whey Protein

Whey is a by-product of the cheese making process. Production of a kilogram of cheese yields about 9 kilograms of liquid whey. The exact composition of whey is dependent on the source of milk and the manufacturing process. It commonly consists of a rich blend of lactose, proteins and minerals and can be used as a low cost source of proteins, carbohydrates vitamins and minerals. Normal milk contains about 78% of caseins, the remainder being the whey proteins as shown in Table 2.1. Raw whey is not suitable for direct consumption owing to high levels of water (93.5%) and lactose (4.5-5.0%). Usually whey is dried to a powder form for food related applications, in particular as a protein supplement in processed food. Whey proteins can be concentrated by ultrafiltration. The product is used extensively in processed food and beverages as a protein supplement. In addition to their nutritional values, whey proteins improve rheological properties of the food material during processing (B. Sen Gupta and Magee, 2007).

Table 2.1: Typical composition of Whey Protein
(B. Sen Gupta and Magee, 2007)

Protein	Concentration, kg/m^3	pH
β- Lactoglobin	2.7	5.2
α- Lactalbumin	1.2	4.5
Immunoglobins	0.65	5.5
Bovine serum	0.4	4.7
Lactoferrin	0.1	9.0

Whey proteins produce transparent, bland, flexible water-based edible films with excellent oxygen, aroma and lipid barrier properties at low relative humidity (Perez-Gago and Krochta, 2000). They also show good tensile strength and moderate elongation (B. Sen Gupta and Magee, 2007). Whey protein- based films have an extra edge over many other biopolymer films because of excellent nutritional value, bland flavor and capability to carry flavorings and functional ingredients (Li and Chen, 2000).

2.2.4 Polyols

Polyols (polyalcohols) are low molecular weight carbohydrates which are used in food, non-food, health care and pharmaceutical applications. They are increasingly used to provide the sweetness of various products or replace sucrose in confectionery. Polyols are used in chewing gum, because they do not contribute to development of dental caries and they neutralize pH in the mouth. Moreover, polyols are also used as plasticizers in the edible films.

Commonly, polyols are produced by the hydrogenation process in which hydrogen is added to the carbonyl group of saccharides. Depending on the starting materials of the hydrogenation process polyols are divided into three categories which are hydrogenated monosaccharides, hydrogenated disaccharides and mixtures of hydrogenated polysaccharides. The hydrogenation process of monosaccharides, such as D-glycerose, D-xylose, D-glucose and D-mannose, yields glycerol, xylitol, D-glucitol (sorbitol) and mannitol, respectively and correspondingly the hydrogenation process of disaccharides, such as maltose or lactose, yields maltitol and lactitol, respectively. Most of the polyols, such as erythritol, xylitol and sorbitol, appear as crystalline powders which have their characteristic melting temperatures, whereas glycerol is a melt (Talja, 2007).

Monosaccharide-based polyols, such as glycerol and sorbitol, are widely used as plasticizers in edible film applications because of their plasticization ability due to their low molecular weights. Plasticizer is added to the film to give better handling properties like flexibility and elasticity. Plasticizer decreases interactions between biopolymer chains, such as amylose and amylopectin, thus preventing their close packing which results in lower degree of crystallinity in the film. Pores and cracks in the film could be also prevented by using plasticizers. Polyols are good plasticizers because of their low molecular weight and *Tg*. Generally, the lower the *Tg* of the plasticizer the less it will be needed to obtain plasticized film. This is fairly important because at the high plasticizer content phase separation of the plasticizer may occur (Talja, 2007). Table 2.2 shows the characteristic properties of various monosaccharide- and disaccharide-based polyols.

Table 2.2: Characteristic properties of various monosaccharide- and disaccharide-based polyols: molecular weight (*Mw*, g mol–1), onset of the glass transition (*Tg*, °C) and melting (*Tm*, °C) temperatures, *Tm/Tg* ratio and melting enthalpy (Δ*H*, J g–1) (Talja, 2007)

Polyol	M_w[a]	T_g	T_m	T_m/T_g[b]	ΔH	Reference
Monosaccharide-based						
Glycerol	92	–86	-	-	-	Murthy (1996)
Erythritol	122	–45[c]	118	1.71	323	Barone *et al.* (1990)
Xylitol	152	–29	95	1.51	226	Roos (1993)
Sorbitol	182	–9	99	1.41	154	Roos (1993)
Mannitol	182	11[d]	167–170	1.53	-	Yu *et al.* (1998)
Disaccharide-based						
Maltitol	344	39	149	1.35	147	Roos (1993)
Lactitol	344	50	-	-	-	Jouppila *et al.* (2007)

[a] Calculated from molecular structures presented in Whistler and BeMiller (1997).
[b] Calculated in Kelvins.
[c] The T_g from the present study (I).
[d] Extrapolated value by Yu *et al.* (1998).

2.3 Biopolymer Films

2.3.1 Film Formation Process

Most biopolymers are hydrophilic and thus, water is the solvent used most often to dissolve biopolymers to obtain film forming solutions. Instead of water some other solvents with or without water can be used to dissolve biopolymers. Usually, heating with solvent is needed to disrupt the native structure of the biopolymer to obtain a film forming solution. Plasticizer is added to the film forming solution at a convenient stage of the process to obtain flexible and elastic films which are often desired.

There are various biomaterial film forming processes such as casting, spraying, extrusion and thermo-molding. The most common process to produce films on a laboratory scale is casting, which is used to produce free films for testing. In this process, a film forming solution is cast on a non-adhesive surface. Water or solvent is evaporated from the solution in order to form the film. As a result of solvent evaporation, biopolymer increases with the result that hydrogen bonds are formed and basic film structure is created (Talja, 2007).

2.3.2 Mechanical Properties

Usually, in the mechanical testing of the film a stress-strain experiment is carried out where a film sample is stretched at a constant rate until it breaks. Tensile properties indicate how the material will react to forces being applied in tension. Tensile tests are used to determine the modulus of elasticity, elastic limit, elongation, proportional limit, reduction in area, tensile strength, yield point, yield strength and other tensile properties. Figure 2.7 shows the stress strain curve. The stress-strain curve relates the applied stress to the resulting strain and each material has its own unique stress-strain curve.

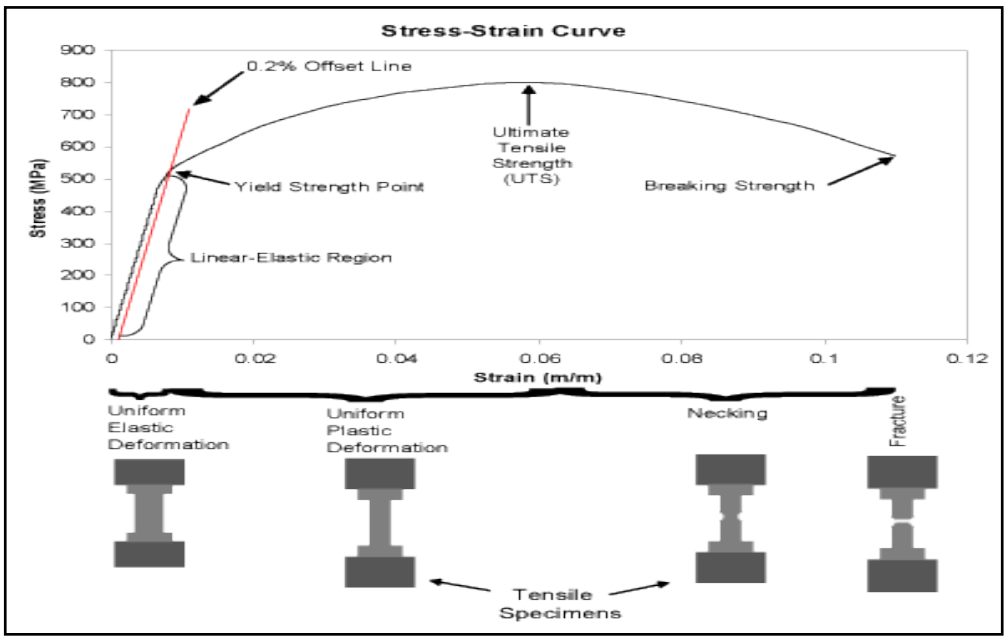

Figure 2.7: Stress-Strain Curve (NDT Resource Centre)

As can be seen in the Figure 2.7, the stress and strain initially increase with a linear relationship. This is the linear-elastic portion of the curve and it indicates that no plastic deformation has occurred. In this region of the curve, when the stress is reduced, the material will return to its original shape. In this linear region, the line obeys the relationship defined as Hooke's Law where the ratio of stress to strain is a constant.

The slope of the line in this region where stress is proportional to strain is called the modulus of elasticity or Young's modulus. The modulus of elasticity (E) defines the properties of a material as it undergoes stress, deforms and then returns to its original shape after the stress is removed. It is a measure of the stiffness of a given material. To compute the modulus of elastic, simply divide the stress by the strain in the material. Since strain is unitless, the modulus will have the same units as the stress, such as kpi or MPa. The modulus of elasticity applies specifically to the situation of a component being stretched with a tensile force (NDT Resource Centre).

In ductile materials, at some point, the stress-strain curve deviates from the straight-line relationship and Law no longer applies as the strain increases faster than the stress. From this point on in the tensile test, some permanent deformation occurs in the specimen and the material is said to react plastically to any further increase in load or stress. The material will not return to its original, unstressed condition when the load is removed. In brittle materials, little or no plastic deformation occurs and the material fractures near the end of the linear-elastic portion of the curve.

With most materials there is a gradual transition from elastic to plastic behavior and the exact point at which plastic deformation begins to occur is hard to determine. Therefore, various criteria for the initiation of yielding are used depending on the sensitivity of the strain measurements and the intended use of the data. For most engineering design and specification applications, the yield strength is used. The yield strength is defined as the stress required to produce a small, amount of plastic deformation. The offset yield strength is the stress corresponding to the intersection of the stress-strain curve and a line parallel to the elastic part of the

curve offset by a specified strain (in the US the offset is typically 0.2% for metals and 2% for plastics) (NDT Resource Centre).

The ultimate tensile strength (UTS) or, more simply, the tensile strength, is the maximum engineering stress level reached in a tension test. The strength of a material is its ability to withstand external forces without breaking. On the stress-strain curve above, the UTS is the highest point where the line is momentarily flat. However, since the UTS is easy to determine and quite reproducible, it is useful for the purposes of specifying a material and for quality control purposes.

The conventional measures of ductility are the engineering strain at fracture (usually called the elongation) and the reduction of area at fracture. Both of these properties are obtained by fitting the specimen back together after fracture and measuring the change in length and cross-sectional area. Elongation is the change in axial length divided by the original length of the specimen or portion of the specimen. It is expressed as a percentage. Since, an appreciable fraction of the plastic deformation will be concentrated in the necked region of the tensile specimen, the value of elongation will depend on the gage length over which the measurement is taken. The smaller the gage length the greater the large localized strain in the necked region will factor into the calculation (NDT Resource Centre).

Elongation at break for brittle plastic samples is usually 1–2% of their original length and stress increases linearly with strain until break (Sperling, 1992). For brittle/glassy biopolymer films reported values of elongation at break vary from 3 to 9%. These films have a high Young's modulus and tensile strength (Biliaderis *et al*., 1999). Anyhow, slightly increased elongation at break has been observed for biopolymer films which were in the glassy state without plasticizer (Lazaridou and Biliaderis, 2002), (Lazaridou *et al*., 2003) and (Chang *et al*., 2000). In these films water content varied approximately from 5 to 15% and they still remained in the glassy state in which brittle to ductile transition was observed (Lazaridou and Biliaderis, 2002), (Lazaridou *et al*., 2003) and (Chang *et al*., 2000). Similar brittle to ductile transition induced by water have been reported for the gelatinized starch in

the glassy state (Nicholls *et al.*, 1995). Lazaridou and Biliaderis (2002) have stated that plasticizer addition also induces the brittle to ductile transition in the glassy state.

In the mechanical testing of the films, which are in the rubbery state, above *Tg*, different mechanical properties of the films are obtained as compared to those of the glassy films. Clearly lower values of Young's modulus and tensile strength and higher values of elongation at break of the biopolymer films have been reported for the rubbery films than for the glassy ones (Biliaderis *et al*., 1999), (Lazaridou *et al*., 2003) and (Mali *et al*., 2006). Lazaridou *et al.* (2003) have shown for films made of pullulan and sorbitol that significantly increased values of elongation at break when the films turned gradually from brittle to rubbery state due to increasing water content. The values of Young's modulus and tensile strength of the pullulan sorbitol film decreased simultaneously when elongation at break increased due to water or/and polyol plasticization (Singh *et al.*, 2003). Similar trends in mechanical properties have been reported for the polyol plasticized films prepared from starch (Mehyar and Han, 2004) and (Alves e*t al.*, 2007), other polysaccharides (Debeaufort and Voilley, 1997) and proteins (Anker *et al*., 1999).

The effect of amylose content on the starch-based films has been studied previously. In these studies, films have often been prepared from physical blend of amylose and amylopectin which is plasticized with various polyols. The amylose content affects the crystallinity of the starch film, which is often linked to the mechanical properties. The increasing crystallinity of amylose and amylopectin in the film increases Young's modulus and tensile strength simultaneously decreasing elongation at break (Talja, 2007).

2.3.3 Fourier Transformed Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is a powerful tool for identifying types of chemical bonds in a molecule by producing an infrared absorption spectrum that is like a molecular "fingerprint". FTIR is most useful for identifying chemicals that are either organic or inorganic. It can be utilized to quantitate some components of an unknown mixture. It can be applied to the analysis of solids, liquids and gasses (West Coast Analytical Service). Figure 2.8 shows the characteristic wavelength regions for different vibrations (Modern Techniques in Chemistry: Infrared Spectroscopy, 2005).

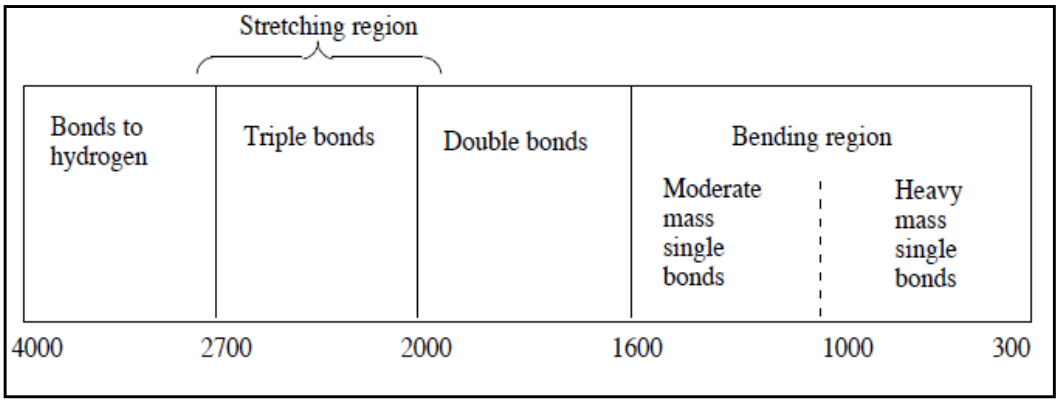

Figure 2.8: Characteristic wavelength regions (in wavelength, cm-1) for different vibrations (Modern Techniques in Chemistry: Infrared Spectroscopy, 2005).

According to previous reports (Mathew and Abraham, 2008), (Xu *et al*., 2004) and (Norashikin and Ibrahim, 2009), starch film showed a broad band at 3413 to 3350 cm^{-1}, which corresponded to the hydrogen bonded hydroxyl groups, while the bands at 1648 cm^{-1} and 1467 to 1458 cm^{-1} were assigned to the δ(O-H) bendings of water. The bands from 763 to 1136 cm^{-1} corresponded to the C-O bond stretching.

Xu *et al*. (2004) have shown for films made of starch and chitosan, the chitosan spectrum showed broad band at 3351 cm−1 was the O-H stretching, which overlapped the N-H stretching in the same region. The band at 1578 cm−1 was the N-H bending (amide II). A small peak near 1655 cm−1 was due to the C=O stretching (amide I), and a peak at 1741 cm−1 suggested the presence of a carbonyl

group in the film. Similar chitosan spectrums have been reported for the biodegradable films made of chitosan and corn husk (Norashikin and Ibrahim, 2009).

In whey protein films, the IR spectrum for peptide bonds of WPI was found to exhibit characteristic absorption at 1645 cm^{-1}, which were attributed to the δN-H bending vibration of amides. The other characteristic peaks of the peptide group of WPI appeared at 1240 cm^{-1} and 1076 cm^{-1}, which were attributed to the δC-N bending (Zaleska *et al*., 2001).

2.3.4 Thermal Analysis Methods of Polymers

Thermal analysis is defined as a group of methods based on the determination of changes in chemical or physical properties of material as a function of temperature in a controlled atmosphere. Thermal analysis is a good analytical tool to measure thermal decomposition of solids and liquids, solid-solid and solid-gas chemical reactions, material specification, purity and identification, inorganic solid material adsorption and phase transitions. Based on this information, one can characterize polymers, organic or inorganic chemicals, metals, semiconductors and other common classes of materials. The principal techniques of thermal analysis are Differential Scanning Calorimetry (DSC) and Thermogravimetry Analysis (TGA).

2.3.4.1 Thermogravimetric Analysis (TGA)

TGA is based on the measurement of the weight loss of the material as a function of temperature, time and/or atmosphere (Lobo and Bonilla, 2003). Mass is measured continuously to determine the decrease of mass. Temperature may be increased following a temperature program. The temperature program may hold temperature constant to measure loss of mass as a function of time.

TGA is used to determine polymer degradation temperatures, residual solvent levels, absorbed moisture content and the amount of inorganic (non-combustible) filler in polymer or composite material compositions. It can also assist in de-formulation of complex polymer products (Independent Polymer Technology Ltd, 2006). Knowledge of the kinetic parameters associated with thermal degradation constitutes an important tool in estimating the thermal behavior of composites under dynamic conditions (Kim *et al*., 2004). Figure 2.9 below shows the thermogravimetric analyzer.

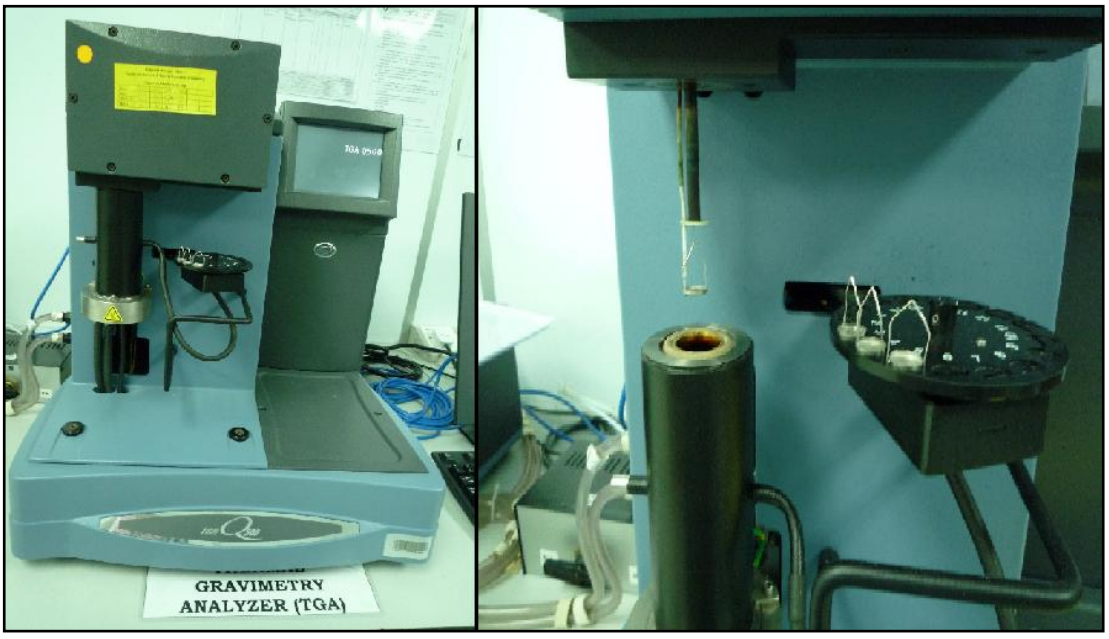

Figure 2.9: Thermogravimetric Analyzer

Thermogravimetric data can be used without further analysis for estimating the composition of blends when they contain components for which the temperature range of thermal degradation is not overlapped. Previous study by Vega *et al*. (1996) on the starch-polyethylene blends are a good example of components with well separated temperature ranges of degradation. In this case it is quite simple to obtain the blend composition from the partial weight losses corresponding to starch and polyethylene. When substantial overlapping of the weight losses of the different components occurs, a more detailed analysis of the thermograms is required. In these cases the derivative of the weight loss curve can be used to determine the contribution of each component to the total weight loss. However, derivative

analysis requires also an integration in order to obtain the amount of each element present in the blends. Such integration usually introduces considerable errors due to uncertainties in the determination of the initial and final temperature of degradation for each element (Vega *et al*., 1996).

2.3.4.2 Differential Scanning Calorimetry (DSC)

DSC is an analytical technique that measures the heat flow to or from a sample specimen as it is subjected to a controlled temperature program in a controlled atmosphere. In other word, a DSC curve. Although DSC is used in many different industries, its applications and use in the plastics industry is widely accepted. It is used to characterize materials for melting points, softening points and other material and material-reaction characteristics such as specific heat, percent crystallinity and reaction kinetics. The heat of fusion is the amount of energy added to the material divided by its mass. It is determined from a thermal curve by calculation the area under the melting peak. This is important information for process engineering as a plastic processing system is designed (Lobo and Bonilla, 2003).

Figure 2.10 shows the glass transition temperature (*Tg*), temperature at which an amorphous polymer or an amorphous part of a crystalline polymer goes from a hard brittle state to a soft rubbery state, the crystallization point (*Tc*), temperature at which a polymer crystallizes upon heating or cooling and the melting point (*Tm*), temperature at which a crystalline polymer melts (Intertek Plastics Technology Laboratories).

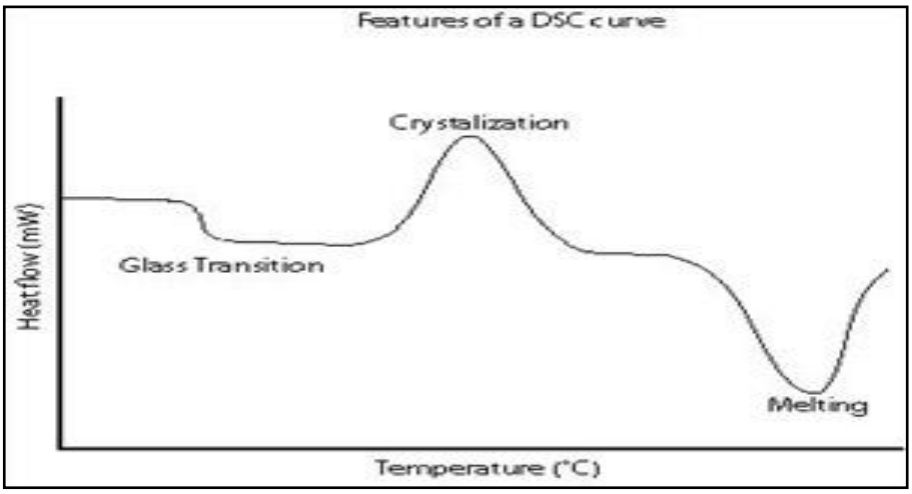

Figure 2.10: A schematic DSC curve demonstrating the appearance of several common features (Fleming Polymer Testing & Consultancy)

According to previous report (Talja, 2007), at the gelatinization temperature range of starch, one or more peaks may be obtained depending on the water content presented. A single symmetrical endotherm at the gelatinization temperature range had been reported for rice starch-water mixtures containing more than 60% water and more than one peak at lower water contents (Talja, 2007).

Traditionally, the glass transition temperature (*Tg*) had been used to study interaction and miscibility of polymer blends. If the blend would have one medially shifted *Tg*, the blend would have a good miscibility among components. If multiple *Tg* would be found in a blend, it would suggest poor miscibility (Sun *et al*, 2007).

Chitosan has no clear glass transition temperature (*Tg*) though many researchers had tried to determine it and a wide range of *Tg* from 161°C to 203°C had been reported (Nam and Lee, 1997), (Ahn *et al*, 2001) and (Sakurai *et al*., 2000). Although chitosan had some crystalline regions, its crystalline melting temperature (*Tm*) could not be found because strong inter and/ or intra-molecular hydrogen bonds lead to rigid-rod polymer backbone, which, like cellulose, would degrade before reaching its melting point (Sun *et al*., 2007).

2.3.5 Scanning Electron Microscope (SEM)

The Scanning Electron Microscope (SEM) is a microscope that uses electrons rather than light to form an image. There are many advantages to using the SEM instead of a light microscope.

The SEM has a large depth of field, which allows a large amount of the sample to be in focus at one time. The SEM also produces images of high resolution, which means that closely spaced features can be examined at a high magnification. Preparation of the samples is relatively easy since most SEMs only require the sample to be conductive. The combination of higher magnification, larger depth of focus, greater resolution and ease of sample observation makes the SEM one of the most heavily used instruments in research areas today (Swapp, 2009).

According to Salleh *et al*. (2009), the surface of pure chitosan films was relatively smooth, homogenous and showed continuous matrix without cracks with good structural integrity. It was flat and compacted with very sparsely distributed small particles without any phase separation. The blend films of starch-chitosan various ratio also exhibit such patterns, the intensity of which reduced with the decreasing concentration of starch. Chitosan microdomains were dispersed within the starch matrix in the blend films with relatively good interfacial adhesion between the two components and were similar to the surface cellulose/ carboxymethylated-chitosan blends (Li *et al*., 2002). Similarly, native potato starch showed ovate shapes and its surface was smooth (Wu *et al*., 2008).

2.3.6 Biodegradability

Biodegradation is the decay or breakdown of materials that occurs when microorganisms use an organic substance as a source of carbon and energy. Biodegradation is a microbial process that occurs when all of the nutrients and physical conditions involved are suitable for growth. Temperature is an important

variable, keeping a substance frozen can prevent biodegradation. Most biodegradation occurs at temperatures between 10 and 35°C. Water is essential for biodegradation (Erickson and Davis). Lack of water-solubility and the size of the polymer molecules, microorganisms are unable to transport the polymeric material directly into the cells where most biochemical processes take place, rather, they must first excrete extracellular enzymes which depolymerize the polymers outside the cells (Refer Figure 2.11).

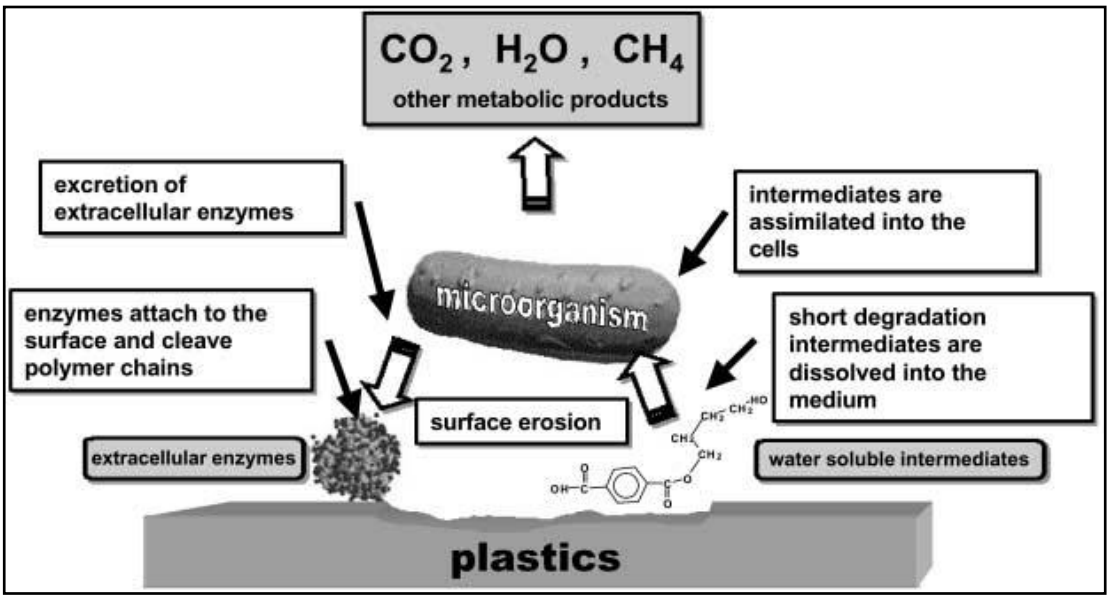

Figure 2.11: General mechanism of plastics biodegradation (Muller, 2003)

As a consequence, if the molar mass of the polymers can be sufficiently reduced to generate water-soluble intermediates, these can be transported into the microorganisms and fed into the appropriate metabolic pathway(s). As a result, the end-products of these metabolic processes include water, carbon dioxide and methane (in the case of anaerobic degradation), together with a new biomass. (Muller, 2003).

Environmental factors not only influence the polymer to be degraded, they also have a crucial influence on the microbial population and on the activity of the different microorganisms themselves. Parameters such as humidity, temperature, pH, salinity, the presence or absence of oxygen and the supply of different nutrients have

important effects on the microbial degradation of polymers and so these conditions must be considered when the biodegradability of films is tested (Muller, 2003).

Another complicating factor in films biodegradation is the complexity of the film materials with regard to their possible structures and compositions. In many cases, films do not consist simply of only one chemical homogeneous component, but contain different polymers (blends) or low molecular weight additives (e.g., plasticizers). All of the above described factors must be considered when measuring the biodegradation of films and interpreting the results and this makes the testing of films biodegradability a highly interdisciplinary process.

2.3.6.1 Microbiological Degradation

A degradation of polymers may proceed by one or more mechanisms, including microbial degradation in which microorganisms such as fungi and bacteria consume the material. The increased surface area produced by this action and subsequent fragmentation of the product enhances the auto-oxidation of the polymer (Orhan *et al*., 2004).

Aspergilli produce a wide variety of diseases. Like the zygomycetes, they are ubiquitous in nature and play a significant role in the degradation of plant material as in composting. Similar to Candida and the Zygomycetes, they rarely infect a normal host. The organism is distributed world-wide and is commonly found in soil, food, paint and air vents. They can even grow in disinfectant. There are more than one hundred species of aspergilla. The most common etiologic agents of aspergillosis are *Aspergillus fumigates*, *Aspergillus niger*, and *Aspergillus flavus.* Aspergilli require 1-3 weeks for growth. The colony begins as a dense white mycelium which later assumes a variety of colors, according to species, based on the color of the conidia. The hyphae are branching and septate. The species differentiation is based on the formation of spores as well as their color, shape and texture (DiSalvo, 2008).

Aspergillus Niger (*A. Niger*) is a fungus that generates oxalic and citric acid when grown on sucrose substrate. Figure 2.12 shows a typical optical microscopic characterization of *A. niger,* which is filamentous. These individual filaments are termed hyphae. The hyphae grow only at the tip and extend their branching regularly behind the tips. They reproduce by sexual and asexual modes, however, the final product of their reproduction system is conidospores (Raghavan *et al*., 1990).

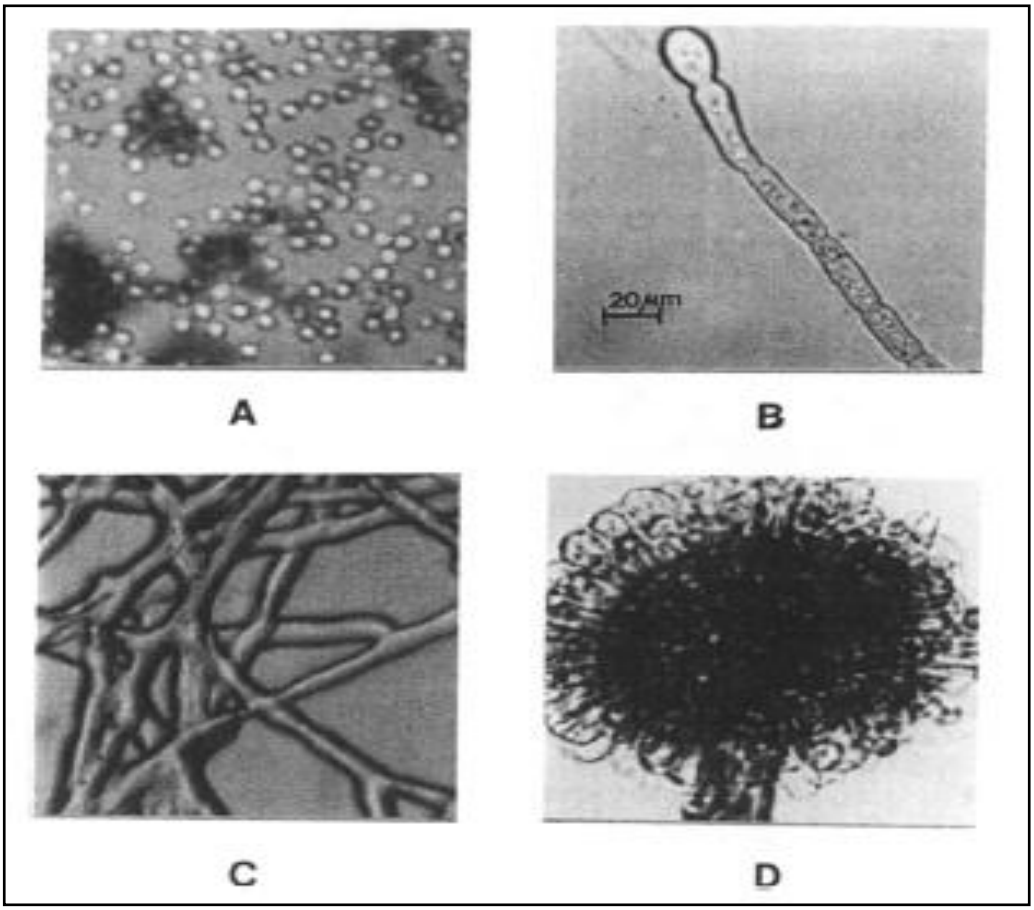

Figure 2.12: Morphological characteristics of *Aspergillus niger* during its growth from spores (A) through budding (B),production of hyphae network (C) and finally to fruiting body (conidia) and condiospores (D) (Raghavan *et al*., 1990)

The present study investigated the fungal communities that developed on the surface of films. Ali *et al*. (2008) stated that the growth of *A. Niger* colony increases as the starch content was increased. The granular starch presented on the surface of the polymer film was attacked by fungi and therefore, this weakens the polymer

matrix and increased the surface volume ration, hydrophilic and permeability of the film.

According to previous reports (Salleh and Muhamad, 2008) and (Rhim *et al*., 2006), chitosan appeared as a natural antimicrobial candidate because it can inhibited the growth of a wide variety of fungi, yeasts and bacteria. Similar results have been reported for the chitosan antibacterial properties prepared without and with deproteinization process (No *et al*, 2003).

2.3.6.2 Soil Burial Degradation

Progressive biodegradation of film fragments is predicted once the film is plugged in to the top soil. The microorganisms present in the soil use the plastic material as carbon source for their growth and thereby degrade the films. Soil burial degradation test have been widely conducted for films biodegradation because of the similarity to actual conditions of use and disposal. The biodegradation of films proceeds actively under different soil conditions according to their properties, because the microorganisms responsible for the degradation differ from each other and they have their own optimal conditions in the soil (Vijaya and Reddy, 2008) and (Orhan *et al.*, 2004).

According to previous study (Li and Chen, 2002), the transparent WPI films began to degrade in two days and become darker in color over the time. Moreover, the films were eroded significantly and lost their original shape completely after 7 days. Norashikin and Ibrahim (2009) stated that the compactness of the films was destroyed as the degradable time was increased. The corn husk film was shown to degrade within a period of 7-9 months and achieved 100% degradation at 270 days.

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter presents the detail procedure for the film preparation of the biodegradable blend films. The blend films and their performance were characterized by using Universal Testing Machine, Fourier Transform Infrared Spectroscopy (FTIR), Thermogravimetric Analysis (TGA), Differential Scanning Calorimeter (DSC), Scanning Electron Microscopy (SEM), and biodegradability test using microbiological degradation and soil burial degradation.

3.2 Materials and Methods

3.2.1 Materials

Tapioca starch (Bunga Merah Brand) used was obtained from local supermarket. Chitosan (84% deacetylated) was purchased from R&M Chemicals, Essex, U.K and whey protein isolates were obtained from Ultimate Nutrition, Inc, Farmington, USA. Glycerol and acetic acid were purchased from Systerm. Fungi *Aspergillus Niger* (*A.niger*) used for the microbiological degradation was purchased from The Department of Microbiology, MARDI, Serdang, Malaysia. The soils for the soil burial test were taken from the field nearby the university campus.

3.2.2 Methods

3.2.2.1 Films Preparation

Tapioca starch was dissolved in distilled water at concentration of 1, 2, 3, 4, and 5 g/ 100 mL by heating the mixtures on hot plate and stirred until it gelatinized at temperature of 85 ± 2°C for about 5-15 minutes. The chitosan solution was prepared by dispersing 20 g of chitosan in 1000 mL of acetic acid (1% v/v) and stirred overnight until it completely dissolved. Whey protein isolates (WPI) solution was prepared by dissolving 10 g of WPI in 100 mL distilled water. The solution was heated to 90 ± 2°C for 15-30 minutes while stirring continuously to denature the whey protein. A series of starch-chitosan-WPI blend were prepared by mixing 100 mL of the starch solution (1, 2, 3, 4, and 5 g/ 100 mL) with 100 mL of the chitosan solution (2 g/100 mL) and 100 mL of the whey protein solution (10 g/100 mL). The solutions were mixed by gentle stirring with a magnetic stir bar until the solution becomes homogenized. Glycerol was added as 40% (w/w) of the total solid weight in the blend solutions. The resulting solutions were degassed for several hours. Then, the solutions were poured and casted onto flat, leveled, non-stick glass trays and leaved it in the oven at 55°C for 10 h undisturbed. After the film was completely dried, the film then been peeled off from the glass plate. Film thickness was controlled by consistently casting the same amount (250 mL) of film-forming solution.

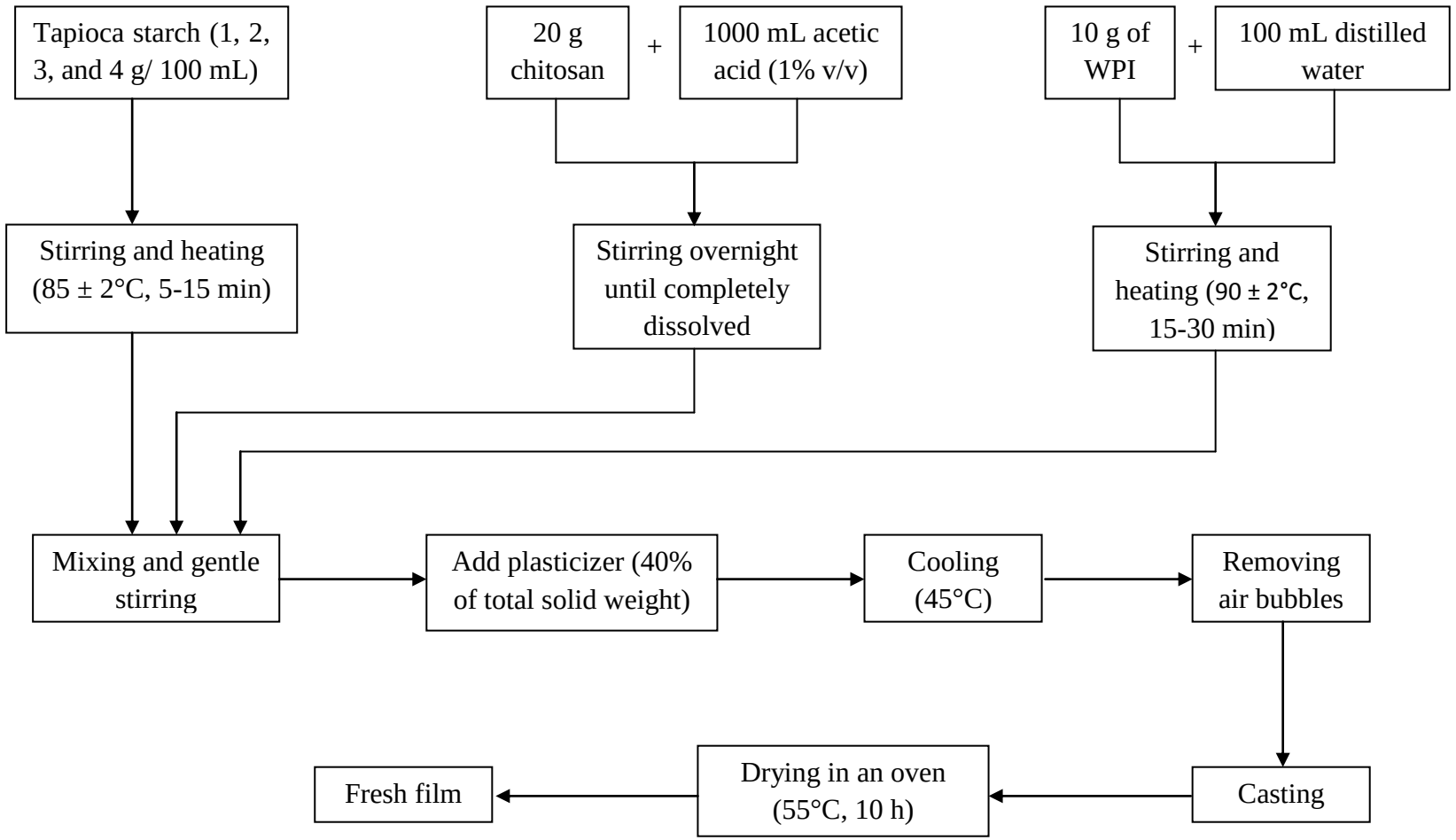

Figure 3.1 The films formation process

3.2.2.2 Mechanical Properties

The mechanical tests were performed using a Universal Testing Machine brand Shimadzu, fitted with 5kN static load cell. The films were cut into dumbbell shape with 12.5mm wide and 16.5 mm long. The tensile properties were measured at a crosshead speed of 5 mm/min and an initial grip separation of 115 mm.

3.2.2.3 FT-IR Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) identifies chemical bonds in a molecule by producing an infrared absorption spectrum. The FTIR spectroscopy was performed using FTIR Nicolet Avatar 370 DTGS. The FTIR generates an infrared spectral scan of samples that absorb infrared light. FTIR spectra were recorded between 400 and 4000 cm-1 with a piece of film 2 cm in diameter. Spectral output was recorded in absorbance as a function of wave number.

3.2.2.4 Thermogravimetric Analysis (TGA)

Thermogravimetric Analysis (TGA) was performed to study the degradation characteristic of the biodegradable blend films. Thermal stability of the biodegradable blend films was determined using TGA Q500 series Thermo gravimetric Analyzer (TA Instruments) with a heating rate of 10 °C/min in a nitrogen environment. It has a weighing capacity 2 mg to 8 mg and final temperature 600°C.

3.2.2.5 Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) identifies the melting temperature of the film. The thermal properties of the film with a weight about less than 5 mg was performed by a DSC Q1000 series (TA Instrument) under nitrogen atmosphere, with a flow capacity of 25 ml/min from 30 to 300°C at a heating rate of 20 °C/min.

3.2.2.6 Scanning Electron Microscopy (SEM)

SEM was used to characterize the microstructure of the samples. SEM was performance with SEM EDX Spectrometer EVO 50, operating at an acceleration voltage of 15kV. The surface of the biodegradable blend films were coated with gold under vacuum for SEM observation.

3.2.2.7 Microbiological Degradation Test

Fungi *Aspergillus Niger* (*A.niger*) was used for the microbiological degradation. The agar plate of *A.niger* was prepared using Sabaraud Dextrose Agar (SDA). The dilution and spread method was used in order to get the same concentration of *A.niger* in all agar plates. A 0.1 mL of 10^{-1} dilution of *A.niger* culture was transferred into each agar plates and were uniformly spread the transferred liquid over the entire plates. The dried samples were cut into 2.5 cm x 2.5 cm square specimens and faced on the surface of the agar in the petri dish containing *A.niger*. Thereafter, the films were observed for evidence of fungi *A.niger* growth.

3.2.2.8 Soil Burial Degradation Test

Soil burial degradation was performed as described by Yun *et al*., (2008) with a slight modification. The garden pots with a medium size were filled with soil taken from a field around Universiti Malaysia Pahang (UMP). The biodegradable blend films were cut into 2.5 cm x 2.5 cm pieces and buried in the soil at the depth 5 cm. The pots were placed in an uncovered placed. The soil was kept moist by sprinkling water at regular time interval to maintain the humidity. The excess water was drained through the hole at the bottom of the pot. The degradation of the specimen was determined at a regular time interval (3 days) by taking the specimen carefully from the soil and washing it gently with distilled water to remove the soil. The specimen was dried in an oven until constant weight was obtained. Weight losses of the specimens with time were used to indicate the degradation rate in the soil burial test.

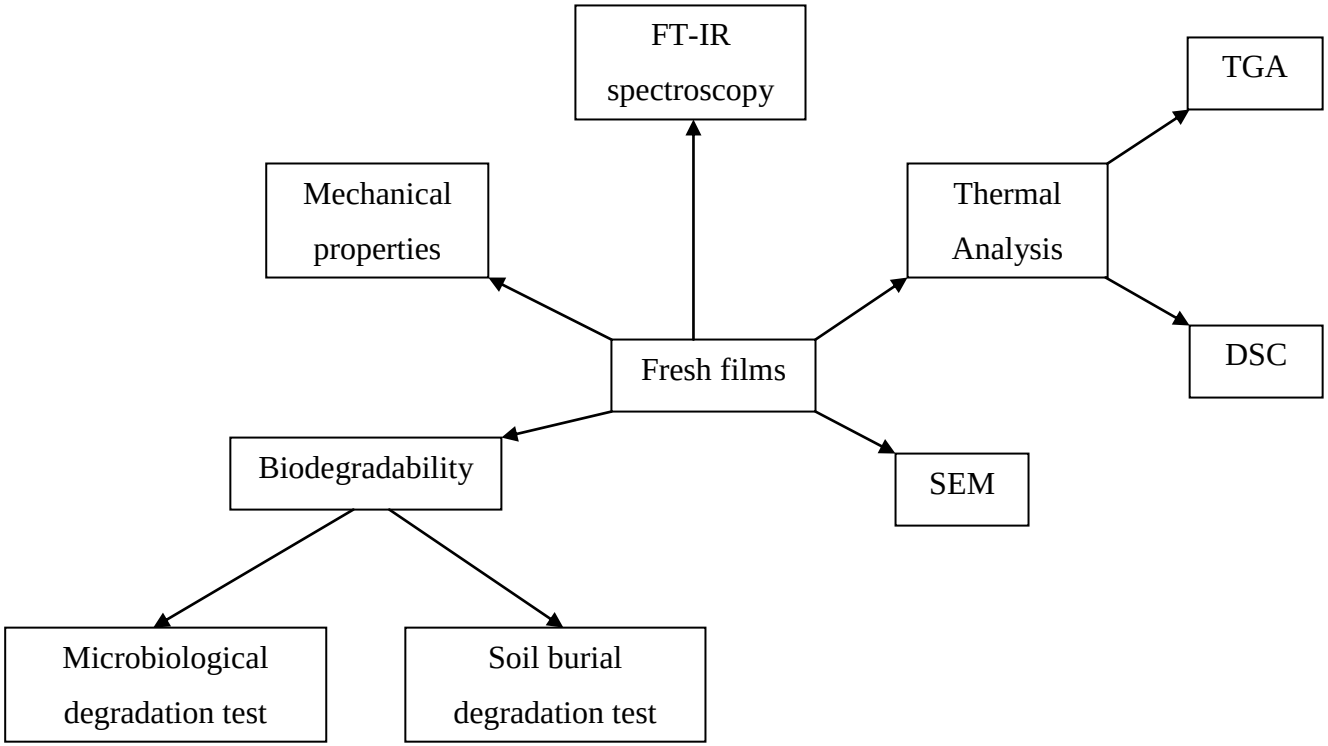

Figure 3.2 The experimental plan of the present study

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This chapter provides a detail discussion about the results analysis of biodegradable biocomposite starch-based films blended with chitosan and whey protein. The results obtained were compared with the findings from previous researchers.

4.2 Mechanical Properties

The tensile strength and elongation at break of the starch film and starch-chitosan-WPI films (biodegradable blend films) with different starch contents were shown in Figure 4.1 and Figure 4.2. The tensile strength provides a measure of film strength, whereas the elongation to break is an indicator of the flexibility of the materials (Pierro *et al*., 2006).

Compared to the control films, the tensile strength values of the biodegradable blend films are much higher and the maximum tensile strength of biodegradable blend films occurred at 1% of starch content. However, as the tapioca starch content were increased, the tensile strength of the biodegradable blend films decreased. The decrease in tensile strength with increasing tapioca starch content may occur because intra-molecular hydrogen bonds were formed rather than inter-

molecular hydrogen bonds, resulting in a phase separation between the tapioca starch, chitosan and whey protein (Xu *et al*., 2005).

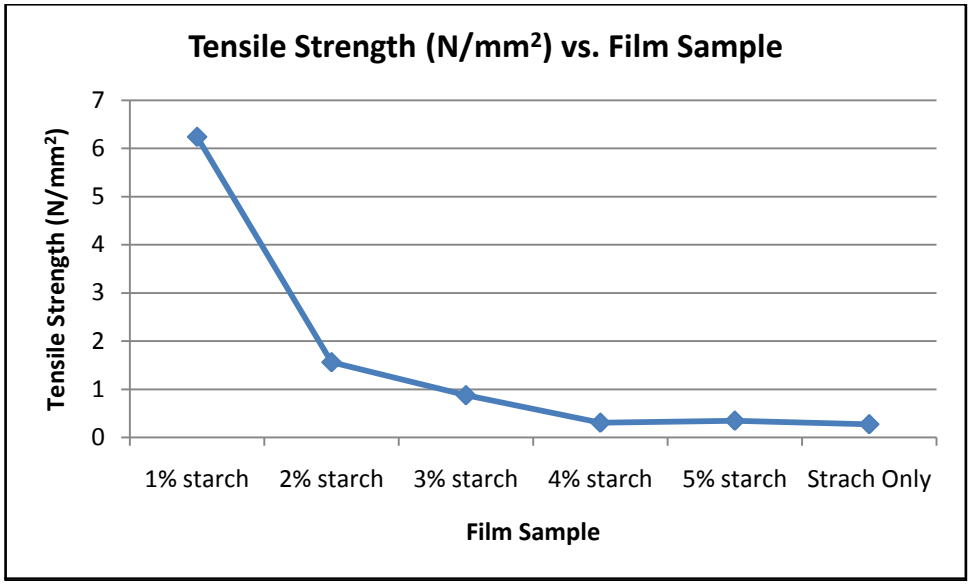

Figure 4.1: Tensile strength (N/mm^2) for starch film and biodegradable blend films with different starch contents.

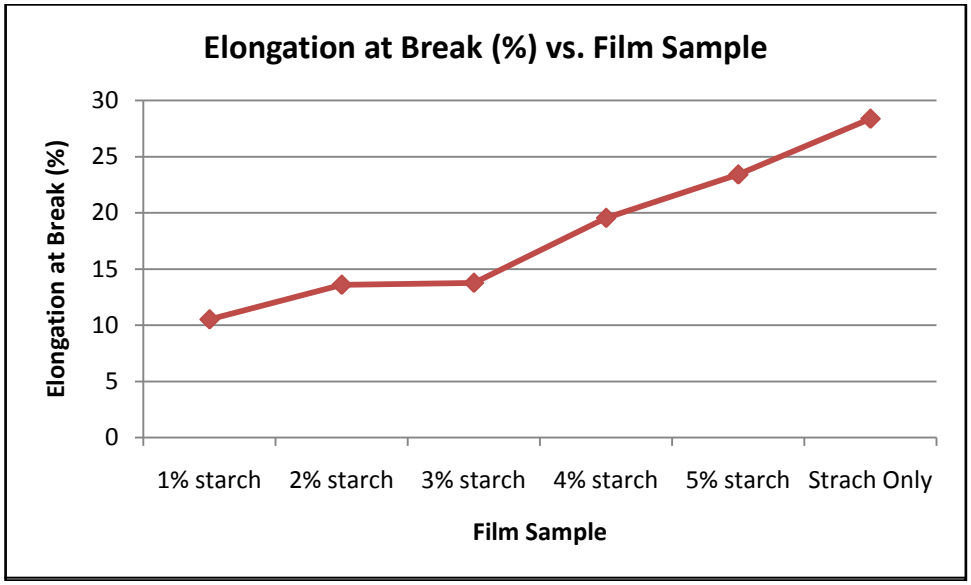

Figure 4.2: Elongation at break (%) for starch film and biodegradble blend films with different starch contents.

The highest tensile strength value of biodegradable blend film at 1% of starch content was attributable to a high formation of inter-molecular hydrogen bonding between NH_3^+ of the chitosan backbone and OH^- of the starch. The amino group (NH_2) of chitosan were protonated to NH_3^+ in the acetic acid solution, whereas the ordered cystalline structured of starch molecules were destroyed with the gelantanization process, resulting in the OH^- groups being exposed to readily form hydrogen bonds with NH_3^+ of the chitosan (Xu *et al*., 2005) and (Bourtoom and Chinnan, 2007). The number of NH_3^+ groups decreased with increased starched content in film-forming solution. As the tapioca starch content increased, the amount of glycerol also increased since the total solid content increased. According to Chae and Heo (1997), it was considered that the addition of glycerol resulted in the excessive disruption of hydrogen bonds and hydrophobic interactions between whey protein molecules.

The result showed that the elongation at break values of the biodegradable blend films behaved inversely to the tensile strength values, increasing from 10% to a maximum of 25% when the starch content was 5%. Elongation at break was increased, probably due to the decreased crystallinity of tapioca starch in the blend films. Moreover, the introduction of plasticizer into the polymeric matrix also resulted in higher elongation values as these decreases the intermolecular attractive forces, improving the films flexibility and extensibility. These results were strongly supported from the previous research finding by Mathew and Abraham (2008).

4.3 FT-IR Spectroscopy

FTIR spectroscopy was used to determine the interactions between the chemical used to produce the films. The infrared spectra of starch film and biodegradable blend films with different starch contents were shown in Figure 4.3.

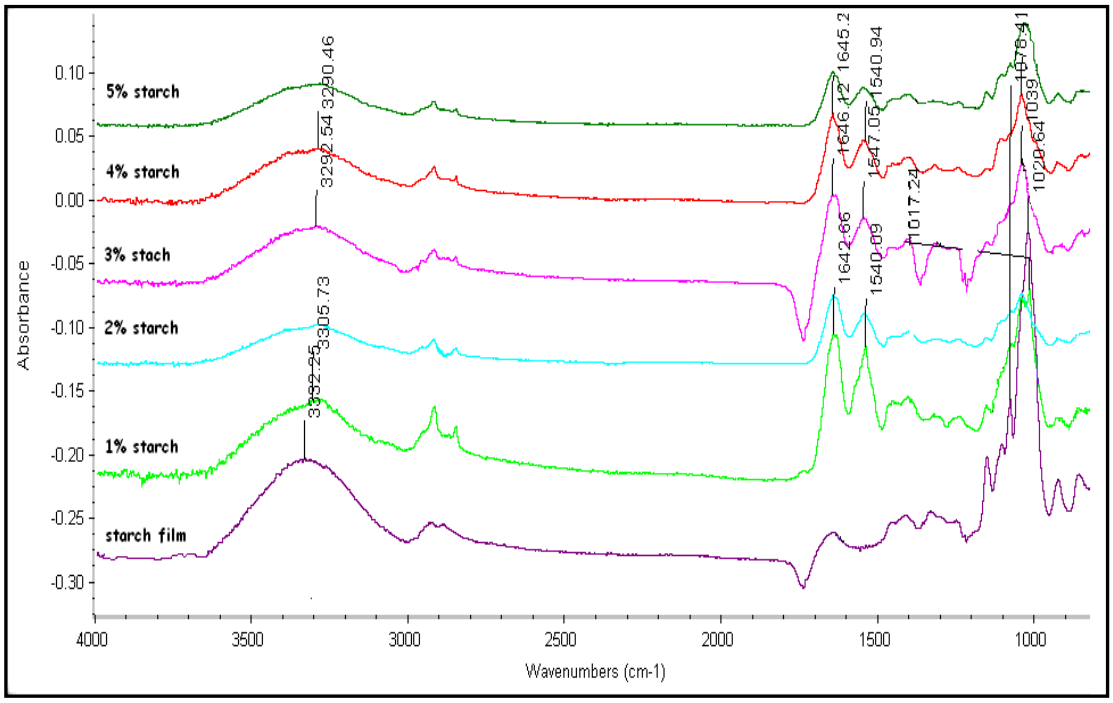

Figure 4.3: The infrared spectra of starch film and biodegradable blend films with different starch contents.

The broad band of starch film and biodegradable blend films at 3600 cm^{-1} to 3000 cm^{-1} was the O-H stretching, while the small peak at 2900.00 cm^{-1} was corresponded to the C-H stretching (Mathew and Abraham, 2008) and (Bourtoom and Chinnan, 2007).

In the spectrum for starch film, the peak at 1648 cm^{-1} and 1332.75 cm^{-1} were assigned to the δ(O-H) bendings of water (Xu *et al.*, 2005) and (Bourtoom and Chinnan, 2007). The bands at 924.29 cm^{-1} to 1151.82 cm^{-1} was due to δC-O bond stretching.

In the spectrum of biodegradable blend films, the results were similar to previous reports (Xu *et al*., 2005), (Bourtoom and Chinnan, 2007) and (Mathew and Abraham, 2008). The amino group peak of chitosan was found to exhibit characteristic absorptions at 1640 cm^{-1} to 1560 cm^{-1}, which were attributed to the N-H bending vibration of primary amines and to the presence of acetylated groups, respectively (amide II). A small peak at 1670 cm^{-1} to 1600 cm^{-1} was due to the C=O

stretching (amide I). The IR spectrum for peptide bonds of WPI was found to exhibit characteristic absorption at 1645 cm^{-1}, which were attributed to the δN-H bending vibration of amides. The other characteristic peaks of the peptide group of WPI appeared at 1240 cm^{-1} and 1076 cm^{-1}, which were attributed to the δC-N bending (Zaleska *et al*., 2001).

According to previous reports (Norashikin and Ibrahim, 2009) and (Xu *et al*., 2005), when several components are mixed, the physical blends versus chemical interactions are affected by changes in the characteristic spectra peaks. In the spectrum of biodegradable blend films, the amino group peak of chitosan and the amide group of WPI shifted from 1020.00 cm^{-1} to 1540 cm^{-1} and 1646 cm^{-1} cm with addition of tapioca starch. This phenomenon pointed out that interactions were presented between the hydroxyl group of tapioca starch and the amino group of chitosan and amide group of WPI. This is consistent with other results. The peak of the hydroxyl groups could not be used to evaluate the interactions because of the effects of content of glycerol and water.

4.4 Thermogravimetric Analysis (TGA)

The thermogravimetric analysis was used to determine the weight loss of the material as it is heated (Norashikin and Ibrahim, 2009). The threshold decomposition temperature indicated the highest processing that could be used (Xu *et al*., 2004). The films were run for thermal gravimetric analysis and the result for starch film and biodegradable blend films with different starch content were shown in Figure 4.4.

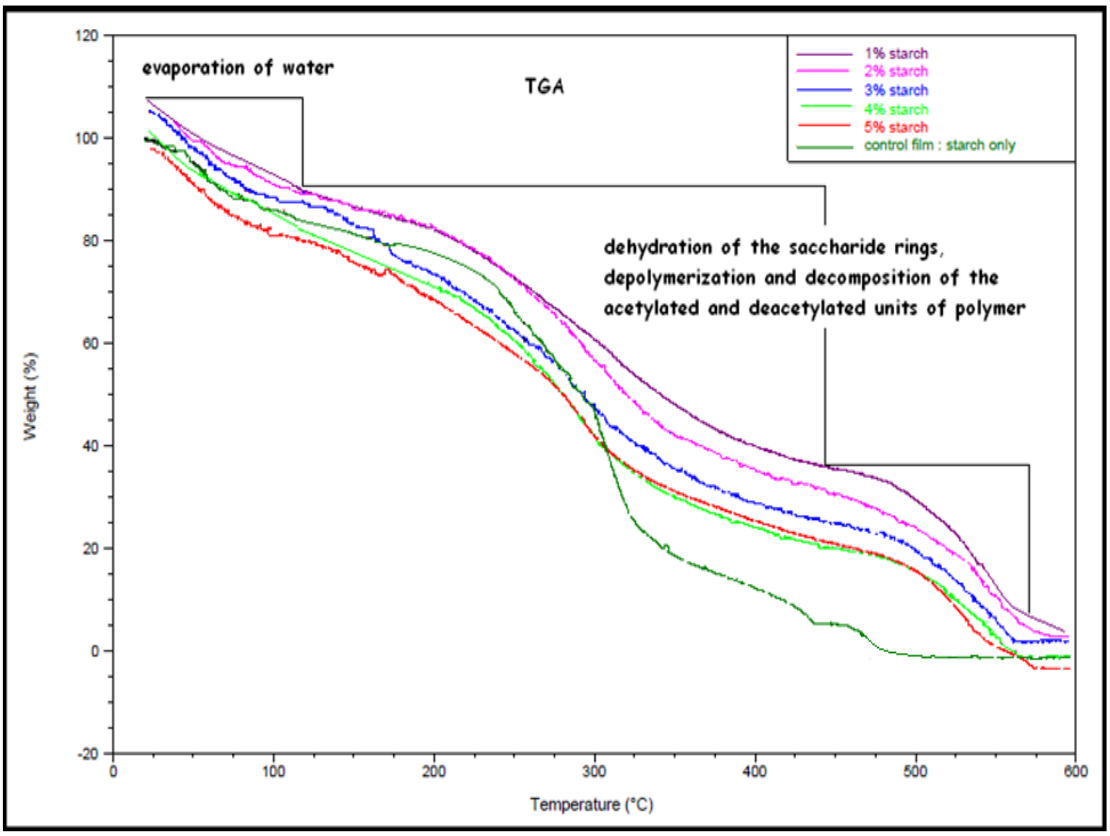

Figure 4.4: The TG curve of starch film and biodegradable blend films with different starch contents.

Thermogravimetric data can be used without further analysis for estimating the composition of blends when they contain components for which the temperature range of thermal degradation is not overlapped (Vega *et al*., 1996). Starch-chitosan-WPI blends are a good example of components with well separated temperature ranges of degradation. In this case it is quite simple to obtain the blend composition from the partial weight losses corresponding to starch, chitosan and WPI. When substantial overlapping of the weight losses of the different components occurs, a more detailed analysis of the thermograms is required (Vega *et al*., 1996). In these cases the derivative of the weight loss curve in Figure 4.3 can be used to determine the contribution of each component to the total weight loss.

The results above demonstrated that, the starch film and biodegradable blend films with different starch content exhibited decomposition in three stages. The initial weight loss of all samples at approximately 100 °C due to the evaporation of

water, while the weight loss in the second range (200°C-350°C) and the third stage (450°C) corresponded to a complex process including the dehydration of the saccharide rings, depolymerization and decomposition of the acetylated and deacetylated units of polymer (Mathew and Abraham, 2008) and (Duangdao and Charoenkongthum, 2002). The weight loss was used to estimate the percentage of each component in the blends (Vega *et al*., 1996).

From the TG curve result shows that the starch film is stable up to 250°C with a maximum rate of decomposition occurring at 265.32°C and ended at 329.16°C with a weight loss of 76.43%. Further heating to 600°C resulted in carbonization and ash formation (Norashikin and Ibrahim, 2009).

For the biodegradable blend films, the weight loss clearly occurred at approximately 300°C. At this temperature, the biodegradable blend films began to degrade and therefore, this was defined as the degradation temperature of biodegradable blend films (T_d). Clearly, the T_d shifted towards low temperature as the amount of tapioca starch increased (Duangdao and Charoenkongthum, 2002).

The biodegradable blend film with 1% starch started to degrade at 313.2°C, whereas the film containing 5% starch began to degrade at 298.5°C. Less than 6% residue was left at 600°C. Certainly, the tapioca starch content affected the amount of weight loss of the blend films at this stage. The results were similar to previous report (Duangdao and Charoenkongthum, 2002). Therefore, these results confirmed the effect of starch content on the thermal stability of the biodegradable blend films. As shown, it is clearly that the thermal stability of the films decreased with respect to the amount of tapioca starch. Among them, the biodegradable blend films compatibilized with 1% starch had the highest thermal stability.

4.5 Differential Scanning Calorimetry (DSC)

To further understand the structure and interaction between the components, DSC study of the film was performed and the results for the DSC curve were presented in Figure 4.5 for starch film and Figure 4.6 to Figure 4.10 for biodegradable blend films with different starch content. Whereas, Table 4.1 shows the melting temperature, *Tm* (°C) of biodegradable blend films with different starch contents.

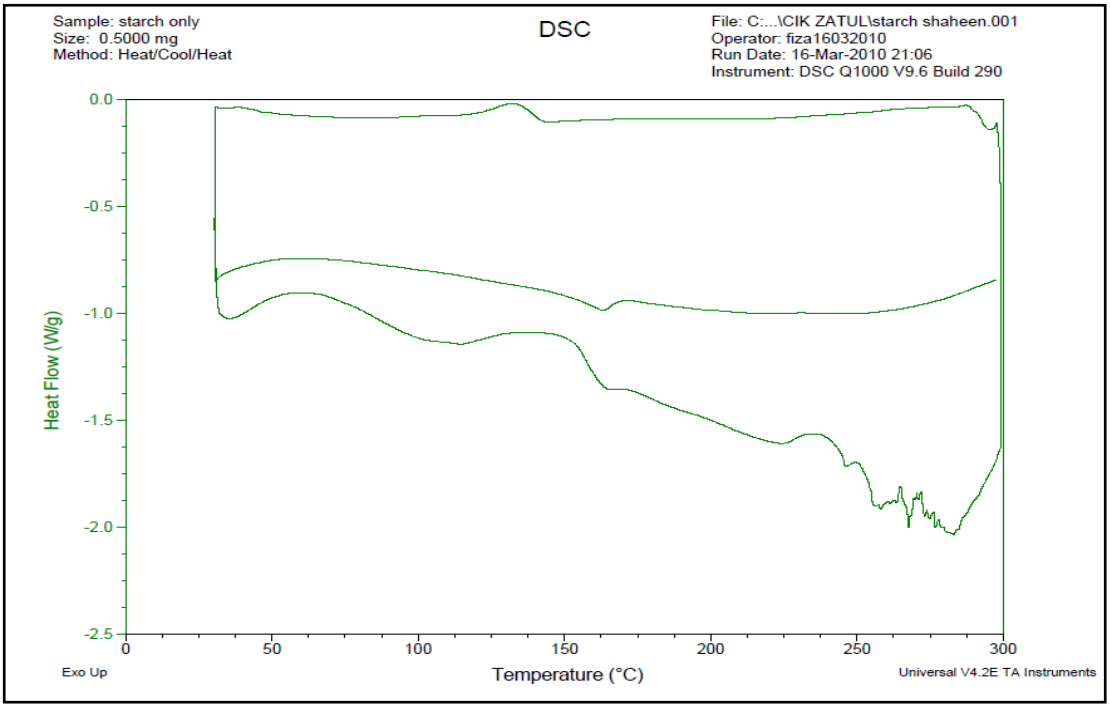

Figure 4.5: DSC curve of starch film.

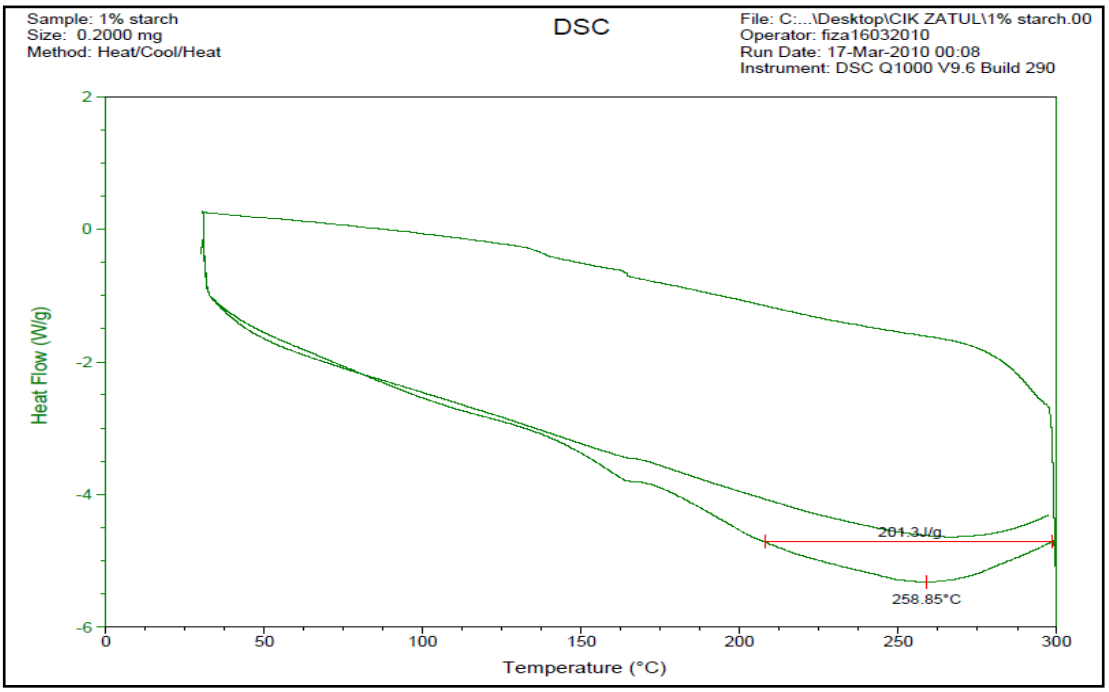

Figure 4.6: DSC curve of biodegradable blend film with 1% of starch content.

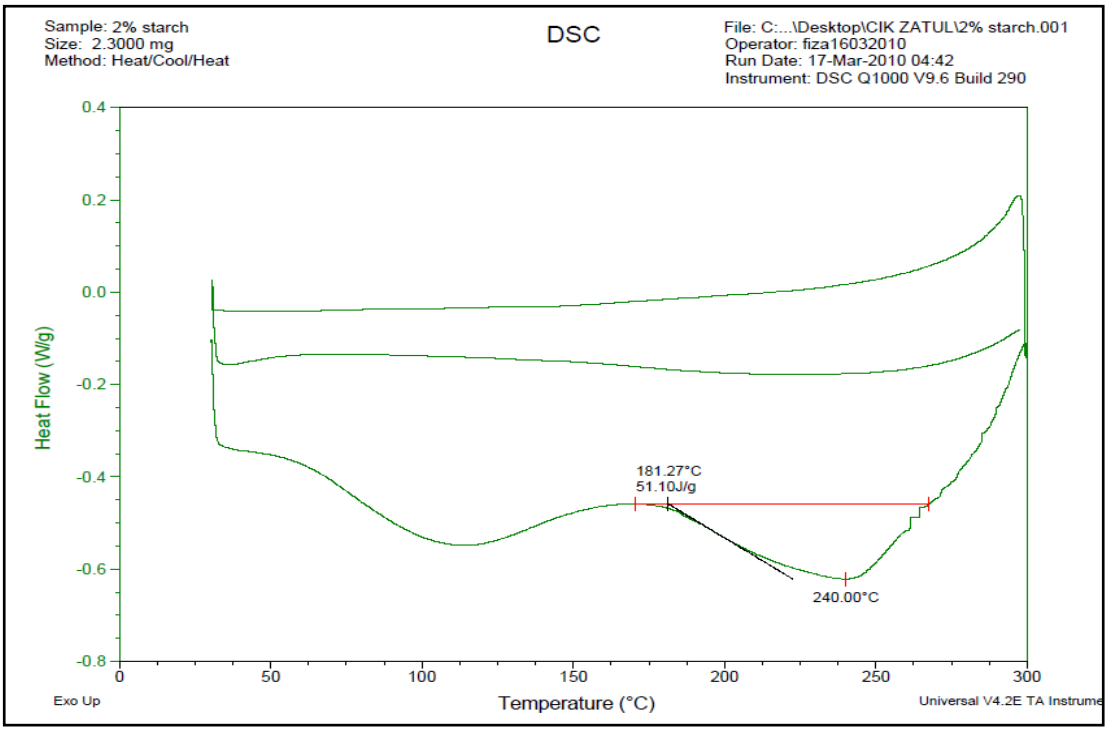

Figure 4.7: DSC curve of biodegradable blend film with 2% of starch content.

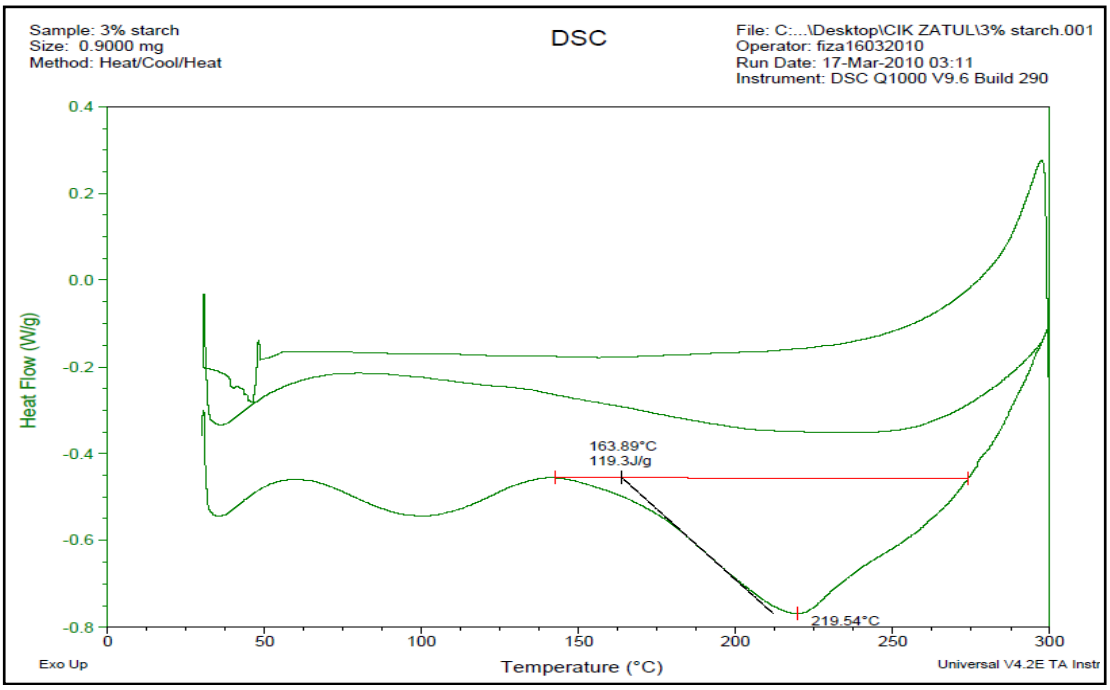

Figure 4.8: DSC curve of biodegradable blend film with 3% of starch content.

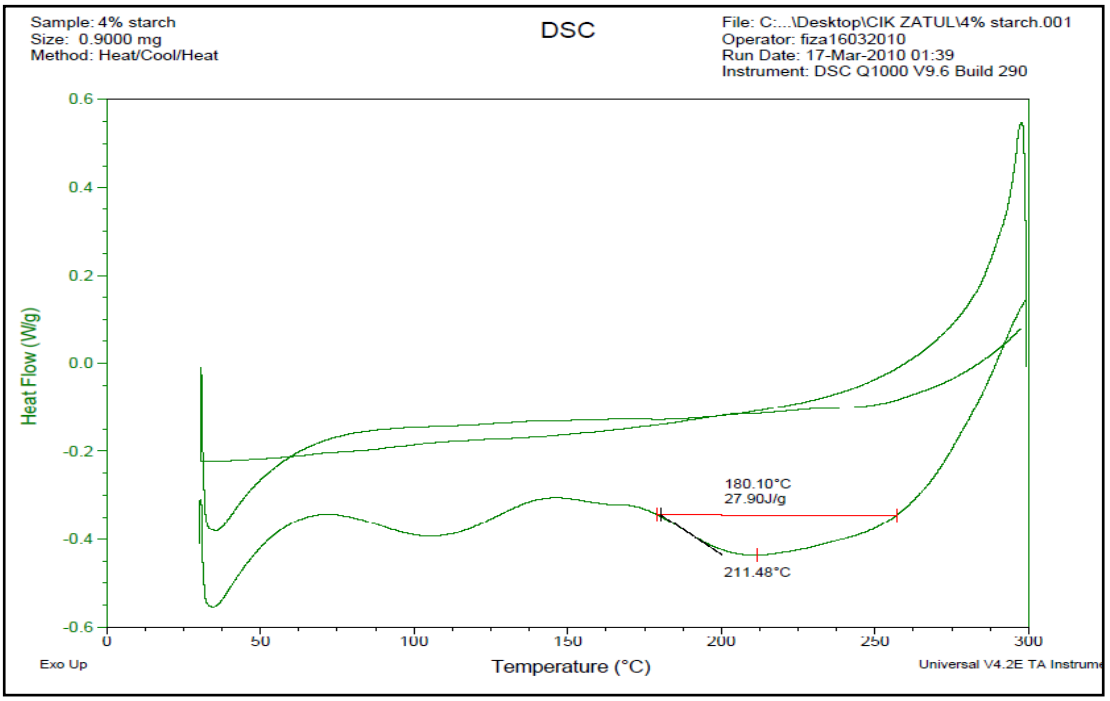

Figure 4.9: DSC curve of biodegradable blend film with 4% of starch content.

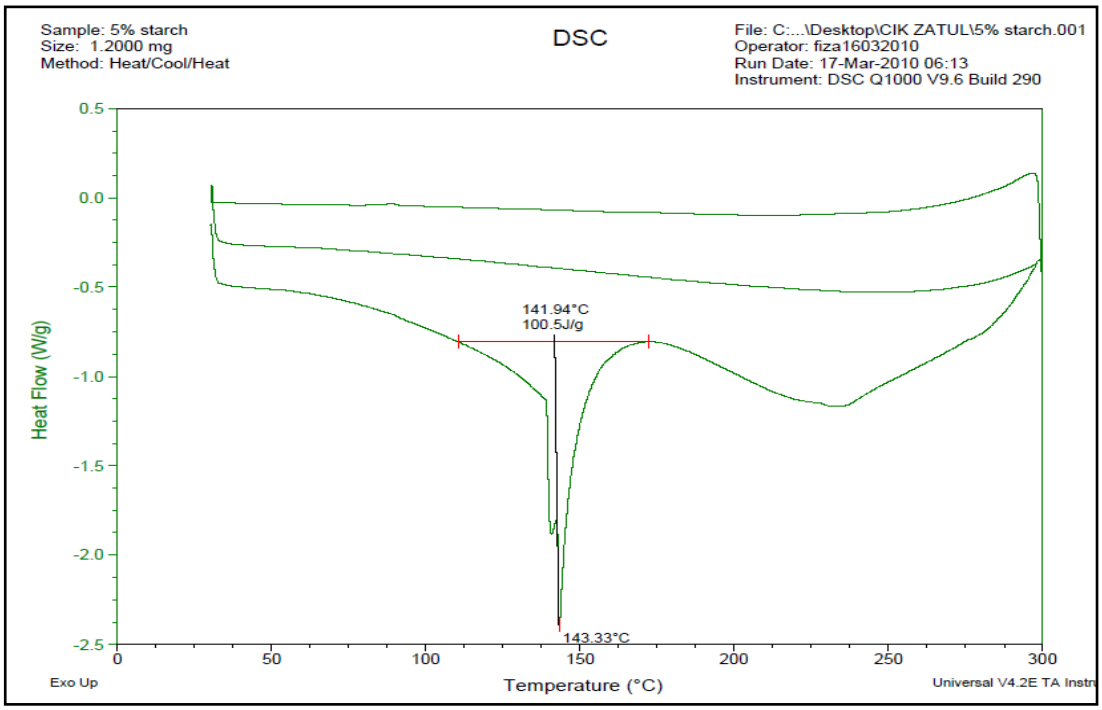

Figure 4.10: DSC curve of biodegradable blend film with 5% of starch content.

Table 4.1: Melting Temperature, *Tm* (°C) of biodegradable blend films with different starch content.

FILM SAMPLE	MELTING TEMPERATURE, *Tm* (°C)
Blend Film with 1% Starch	258.85°C
Blend Film with 2% Starch	240.00°C
Blend Film with 3% Starch	219.54°C
Blend Film with 4% Starch	211.48°C
Blend Film with 5% Starch	143.33°C

Like any other starch, tapioca starch has no melting temperature, but gelatinization and degradation temperature (Duangdao and Charoenkongthum, 2002). The gelatinization temperature range obtained from the DSC thermogram of tapioca starch is presented in Figure 4.5. According to previous report (Talja, 2007), at the gelatinization temperature range of starch, one or more peaks may be obtained depending on the water content present. A single symmetrical endotherm at the

gelatinization temperature range has been reported for rice starch-water mixtures containing more than 60% water and more than one peak at lower water contents (Talja, 2007). In the present study, one peak in the gelatinization temperature range was observed. As can be seen, the gelatinization temperature ranges from 72 to 78°C (Duangdao and Charoenkongthum, 2002).

The endothermic peaks that occurred over a large temperature range (~35°C-100°C) were attributed to water loss, represented the energy required to vaporize water present in the film samples (Dhanikula and Panchagnula, 2004). The results above demonstrated that as the amount of tapioca starch increased, the melting temperature of biodegradable blend films were decreased. Comparing the difference between starch film and biodegradable blend films, there was an apparent decreased in the heat of fusion as the amount of tapioca starch increased (Duangdao and Charoenkongthum, 2002). This is probably due to the decreased crystallinity of starch in the blend films and also formation of starch intra-molecular hydrogen bonds rather than inter-molecular hydrogen bonds which resulted in a phase separation (Xu *et al*., 2005). The melting processes for all films occurred at range of 140°C.

The films had distinctive exothermal peaks at 167–168°C. These exothermal peaks were attributed to crystallization, during which heat was released after the macromolecules became closely packed (Sun *et al*., 2007). This result demonstrated that the blend films of tapioca starch-chitosan-WPI had the capability to crystallize. The ability for blended components to crystallize in a physically blend system is important in terms of miscibility and stability. During crystallization, system entropy was decreased since molecules become more closely and orderly packed due to the polar interactions, i.e., mainly hydrogen bond interactions between amine groups of chitosan and the amide groups of WPI in this blending (Sun *et al*., 2007). The lower energy state of a blending system would make such a blend system thermodynamically more stable. The induced crystals could also serve as physical cross-linkers to improve the stability of the system.

4.6 Scanning Electron Microscopy (SEM)

The microstructures of the starch film and biodegradable blend films with 1%, 3% and 5% starch content were investigated through SEM micrographs and the results were as provided in Figure 4.11 to Figure 4.14.

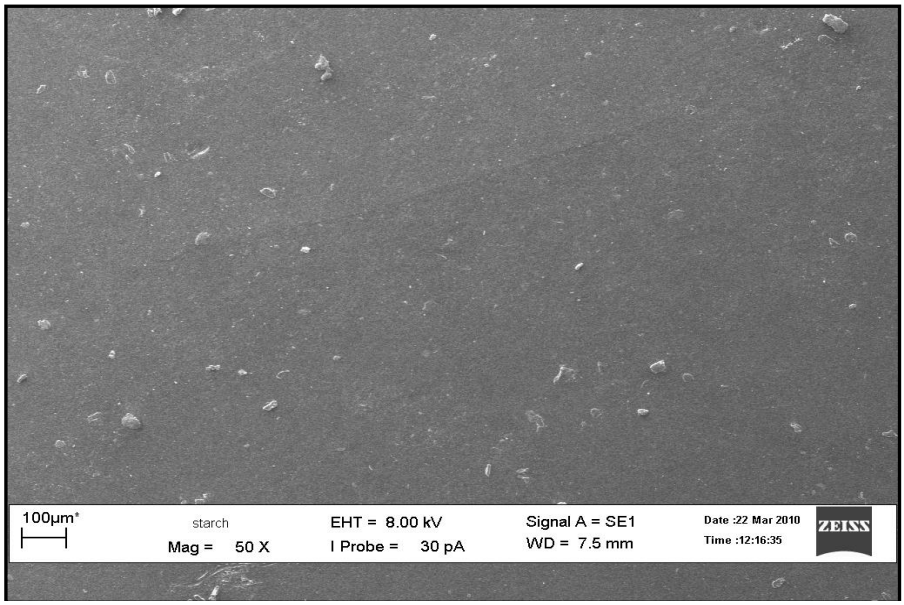

Figure 4.11: SEM micrograph of the surface of starch film.

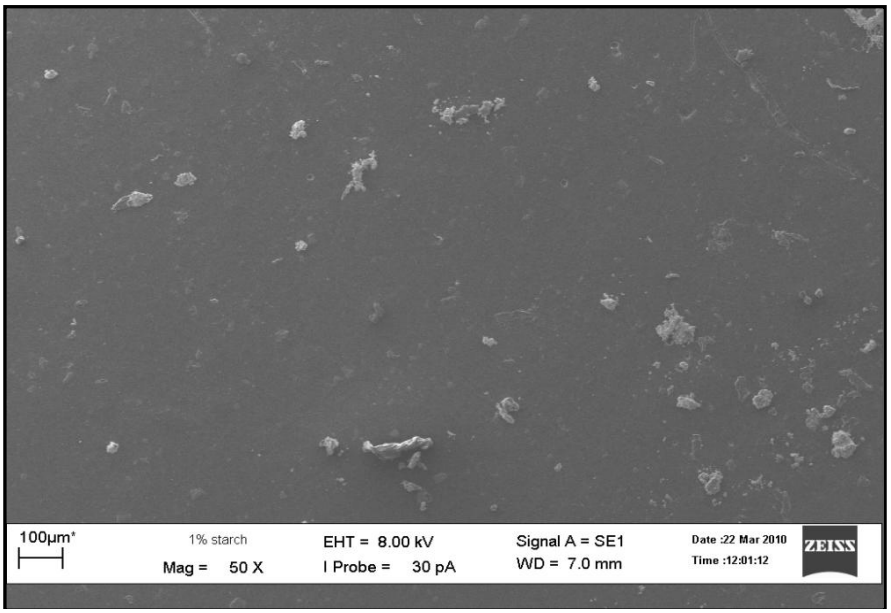

Figure 4.12: SEM micrograph of the surface of biodegradable blend film with 1% starch.

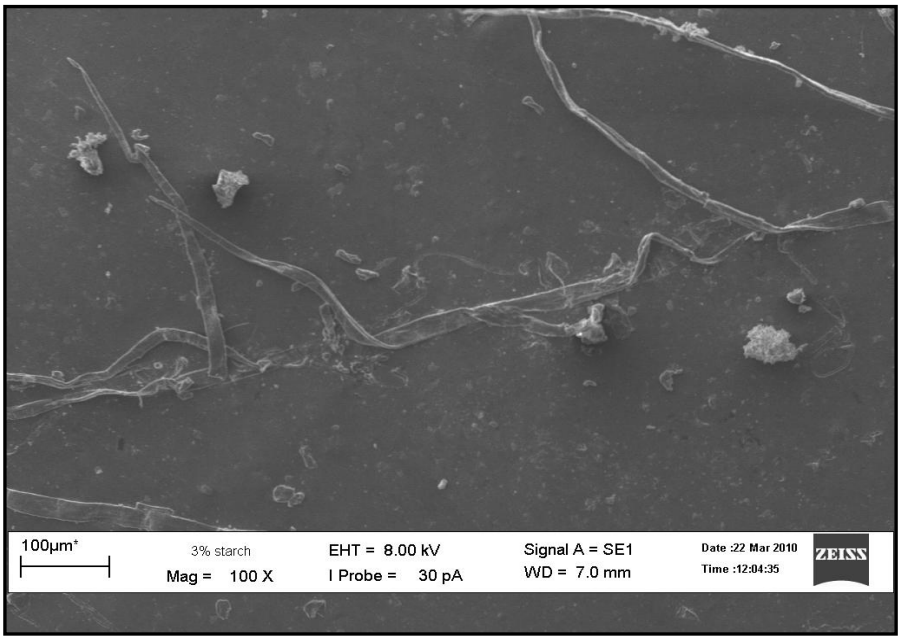

Figure 4.13: SEM micrograph of the surface of biodegradable blend film with 3% starch.

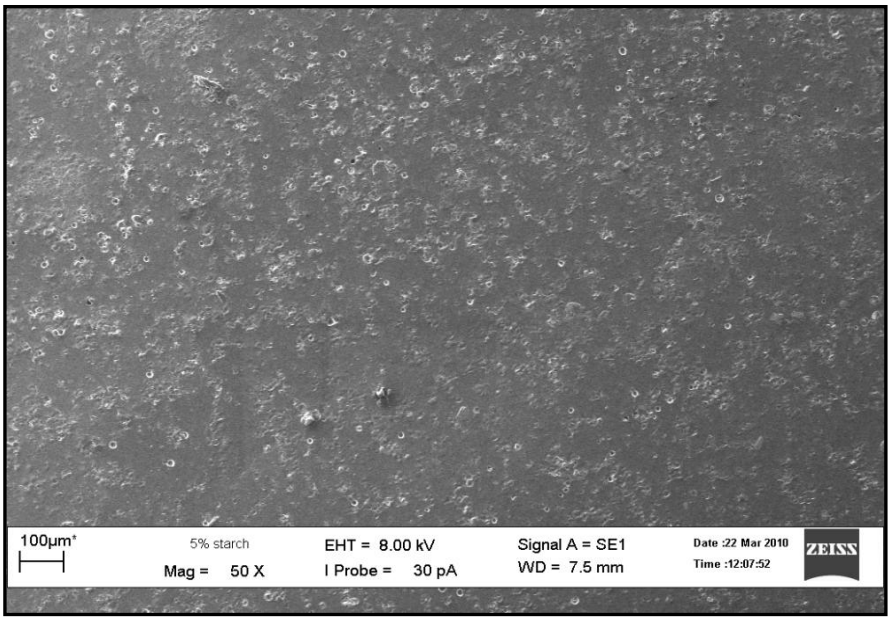

Figure 4.14: SEM micrograph of the surface of biodegradable blend film with 5% starch.

In general starch-based films blended with chitosan and WPI obtained were compacted, translucent and presented good flexibility and elastic. In addition they were easy to handle when dried off. The oven-dried blend films had a pale yellow colour, which could be attributed to the preferential drying of the surface layers as well as the addition of chitosan and WPI in the blend films. The starch films, however, were white in colour. Addition of starch content provided more transparent films (Salleh *et al*., 2009).

Clearly, it was evident that there were significant structural differences among the four samples. Starch film in Figure 4.11 shows a relative smooth morphology. The result was in accordance with those of the previous studies (Wittaya, 2009) and (Salleh *et al*., 2009). Biodegradable blend film with 1% starch content in Figure 4.12 shows a smooth surface with no visible pores and has a good compact structure and may be used as an edible film wrap (B. Sen Gupta and Magee, 2007). This indicated that the film had a good compatibility with the components and plasticizer (Mathew and Abraham, 2008). Whereas, biodegradable blend film with 3% starch content in Figure 4.13 shows a bilayer and folded structure but it is free from pinholes. Biodegradable blend film with 5% starch content in Figure 4.14 shows multiple pores and it is unsuitable for use as a food wrap. While poor formation would reduced structural strength of a film and pores would adversely affected the barrier properties (B. Sen Gupta and Magee, 2007).

4.7 Degradation of Films

Degradability of polymers was a critical functionality in their application. Currently, no official standard method had been established in determining the biodegradability of polymers. The enzyme method, the microbiological method, and the soil burial method had been used by different researchers (Yun *et al*., 2008). Moreover, the biodegradability was also recorded through diverse indexes even in the same method. In order to get an overall biodegradability, the microbiological method and soil burial method were performed simultaneously in the present study.

4.7.1 Microbiological Degradation Test

Table 4.2 shows the rating of fungal growth for neat starch and various compositions of biodegradable blend films. From the results, it shows that for starch films and blend films with 5% of starch content most of the specimen surfaces were covered by fungi growth. Meanwhile, there were less than 75% and 25% of fungi covered the specimen surface for blend films with 3% of starch content and blend films with 2% and 1% of starch content.

Table 4.2: Assessment of fungi growth for each formulations of biodegradable blend films.

Film Samples	Rating* of Fungal Growth
Starch	3
5% Starch	3
4% Starch	3
3% Starch	2
2% Starch	1
1% Starch	1

* Rating: 1- growth covering less than 25% of the specimen surface, 2- growth covering less than 75% of the specimen surface, 3- growth covering more than 75% of the specimen surface

This indicated that the growth of *A. Niger* colony increased as the tapioca starch content was increased. The granular starch presented on the surface of the films was attacked by fungi. This weakens the polymer matrix and increased the surface volume ratio, hydrophilic and permeability of the films (Ali *et al*., 2008).

Figure 4.15 shows the visual of fungi growth covering surface of starch film and biodegradable blend films with different starch content.

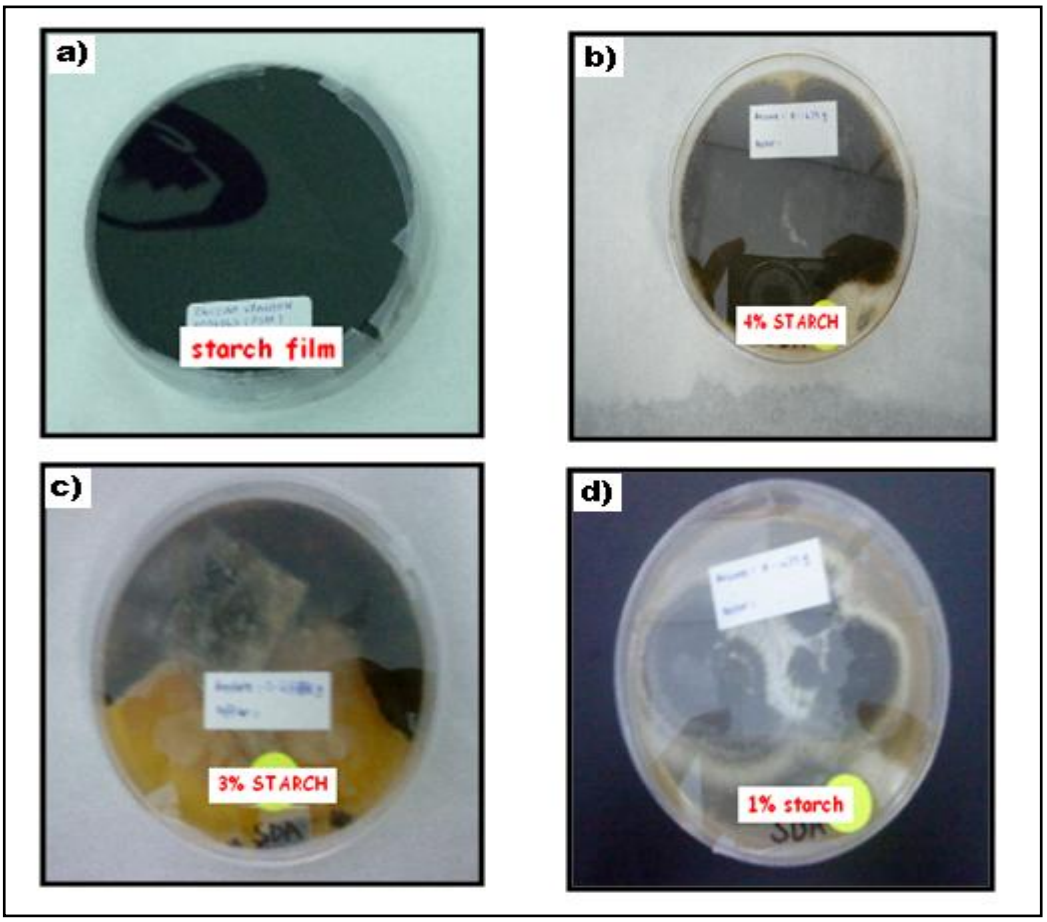

Figure 4.15: Visual of fungi growth covering surface of a) starch films, b) blend films with 4% starch content, c) blend film with 3% starch content, and d) blend films with 1% starch content.

As can be seen in Figure 4.15, there were less *A. Niger* covered the surface of blend film at 1% of starch content. This might be because of the appearance of chitosan as a natural antimicrobial candidate, which inhibited the growth of the fungi due to its cationic property (Salleh and Muhamad, 2008) and (Rhim *et al.*, 2006). The results were in accordance with those of the previous studies (Ali *et al*., 2008) and (Rhim *et al*., 2006), where the growth of *A. Niger* colony increased as the tapioca starch content was increased.

4.7.2 Soil Burial Degradation Test

The soil burial test provided a realistic environment where soil humidity, temperature, types and the amount of microorganisms were less in control and changed with seasons (Yun *et al*., 2008). All the tested specimens had the same shape and size in order to avoid the effects of the film's shape on its biodegradability. The loss of weight of the biodegradable films monitored by means of sample collected from the soil at regular time interval. The films were buried in the soil and the sample was removed for evaluation at 3 day interval.

Figure 4.16 shows the scanned pictures of blend films before and after composting test. The transparent films began to degrade in 3 days and become darker in color over time. The results were similar to previous report (Li and Chen, 2000). The films were eroded significantly and lost their original shape completely after 10 days.

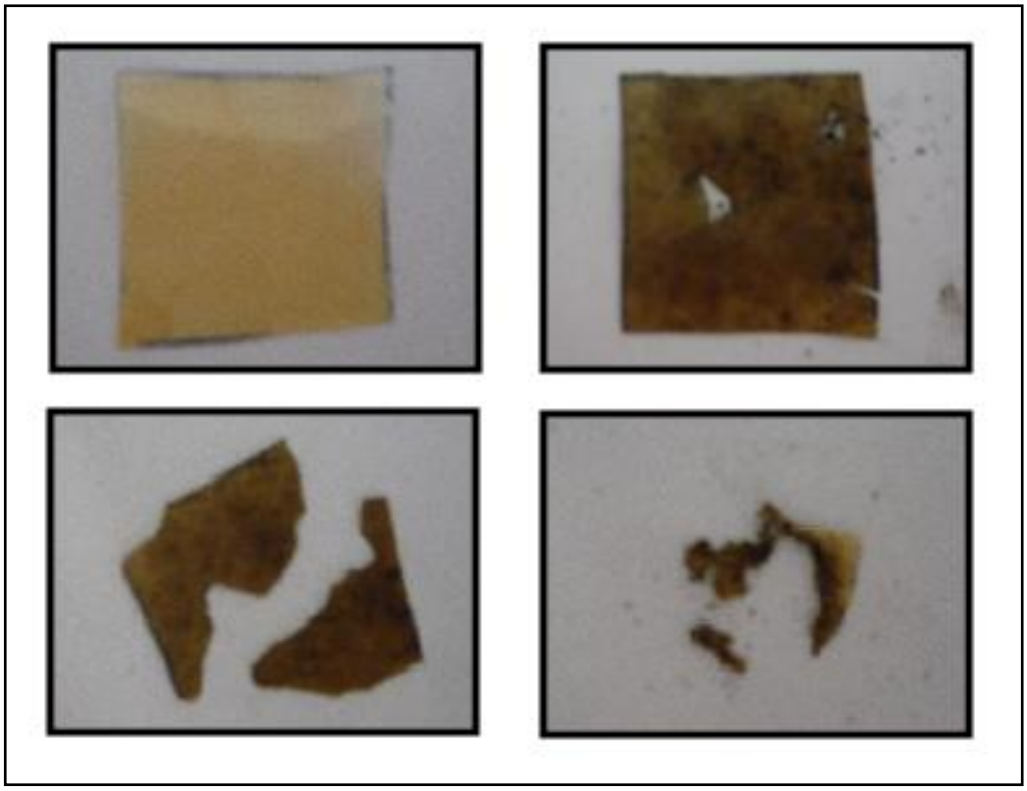

Figure 4.16: Scanned pictures of blend films before and after composting burial test.

The evolution of the degradation in soil of the biodegradable film during the experimental period was shown in Figure 4.17.

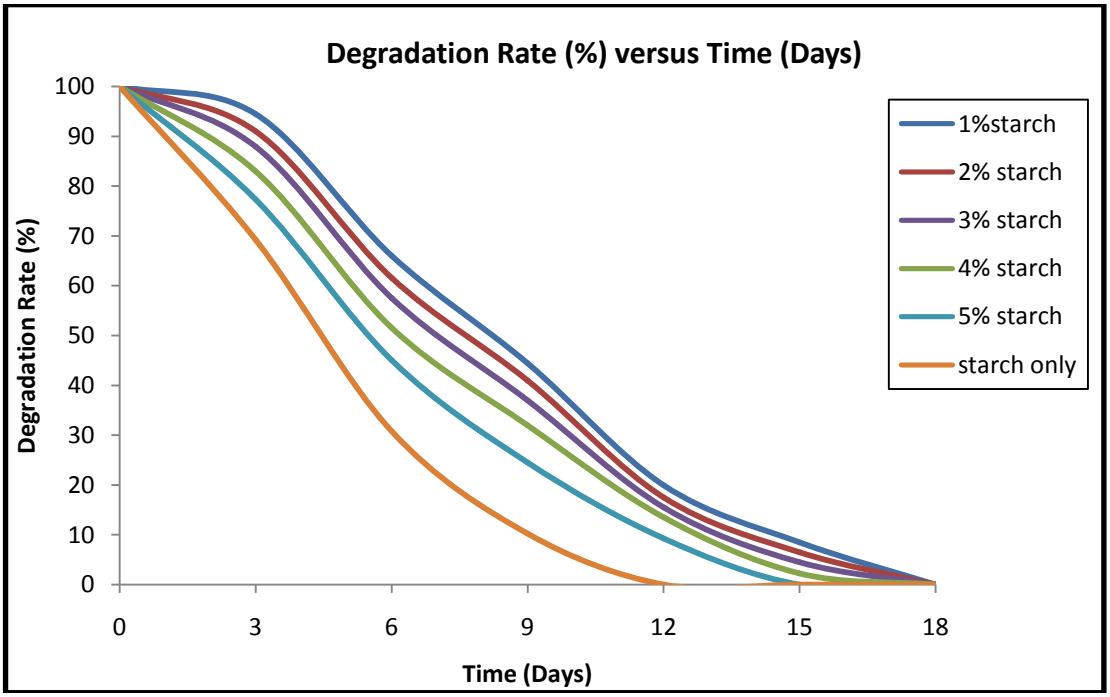

Figure 4.17: Degradability of starch film and blend films with different starch content in the soil burial test.

From the Figure 4.16 as shown above, it can be seen that with the increased of degradable time, the compactness of the films was destroyed (Norashikin and Ibrahim, 2009). The films had shown a rapid degradation within a period of 3-10 days (Yun *et al*., 2008). More than 80% weight loss of total solids was observed for the films in 10 days. The films were shown to start degrading after 3 days and achieved 100% degradation within 18 days.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

Biodegradable blend films with different starch content were synthesized by using the mixing process and the casting method. The characteristics of the blend films with different tapioca starch composition (1, 2, 3, 4 and 5 g/100 mL) were evaluated using Universal Testing Machine, Fourier transform infrared spectroscopy (FTIR), differential scanning calorimeter (DSC), Thermogravimetric Analysis (TGA), scanning electron microscope (SEM) observation and biodegradability using microbiological degradation test and soil burial degradation test. Among the films fabricated, the biodegradable blend films compatibilized with 1% of tapioca starch content showed good mechanical properties and had the highest thermal stability. The FTIR confirmed that tapioca starch, chitosan and WPI were compatible and inter-molecular hydrogen bonds existed between them. Moreover, the microstructure analysis showed that blend film with 1% of tapioca starch content had a smooth surface with no visible pores and had a good compact structure and may be used as an edible film wrap.

For the microbiological degradation test, the results demonstrated that more than 75% of the specimen surface were covered by fungi growth for the blend film with 5% of starch content while less than 25% of fungi were covered the specimen surface for blend films with 2% and 1% of starch content. This indicated that the growth of *A. Niger* colony increased as the tapioca starch content was increased. In

soil burial test, a rapid degradation occurred for all the films in the initial 10 days, followed by 100% composting within 18 days.

The films produced from biodegradable biocomposite starch based films blended with chitosan and whey protein had potential application to be used as food packaging because it can enhanced foods quality and at the same time protected the environment.

5.2 Recommendation

In order to know the influence of starch and protein types on the enhancement of the blend films, different starches and proteins could be introduced in the future study. Besides, an antimicrobial agent such as lauric acid also could be used in order to extend the food shelf life. Moreover, in order to get better results for the microbiological degradation test, the samples should be weighted prior to inoculation with *A. Niger* and reweighed to determine the weight loss of the films.

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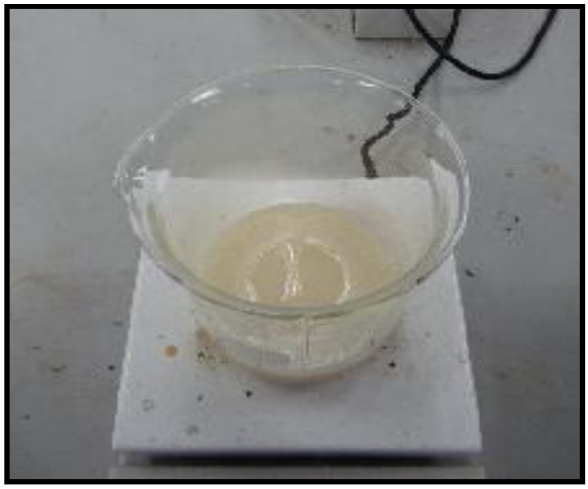
APPENDICES

Appendix A: Films Preparation

Materials preparation

Starch solution
(85 ± 2°C, 5-15 min)

Chitosan solution



Whey protein solution
(90 ± 2°C, 15-30 min)

Mixed solution

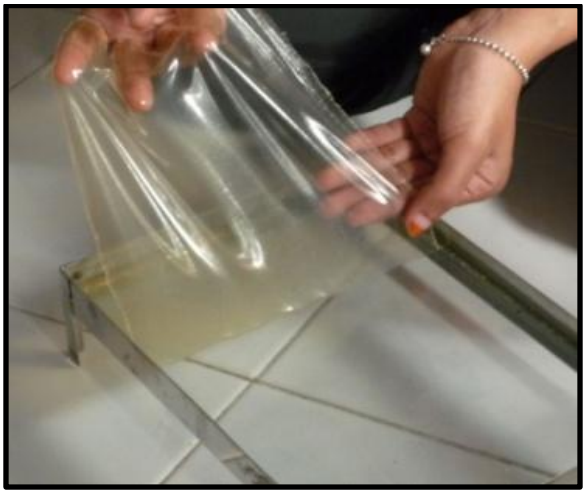
+

Add plasticizer (40% of total solid weight)

Casting

Drying in an oven
(55°C, 10 h)

Dried film



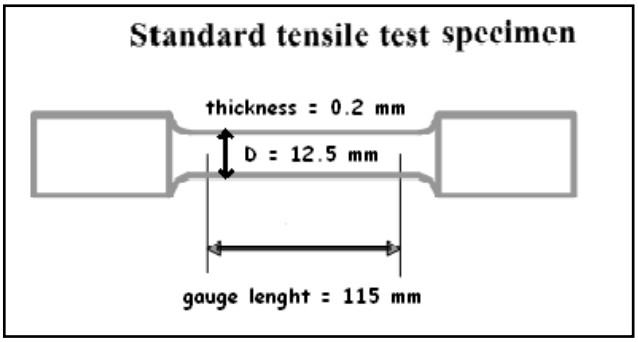
Peeling off film

Fresh film

Films Testing

Appendix B: Films Testing

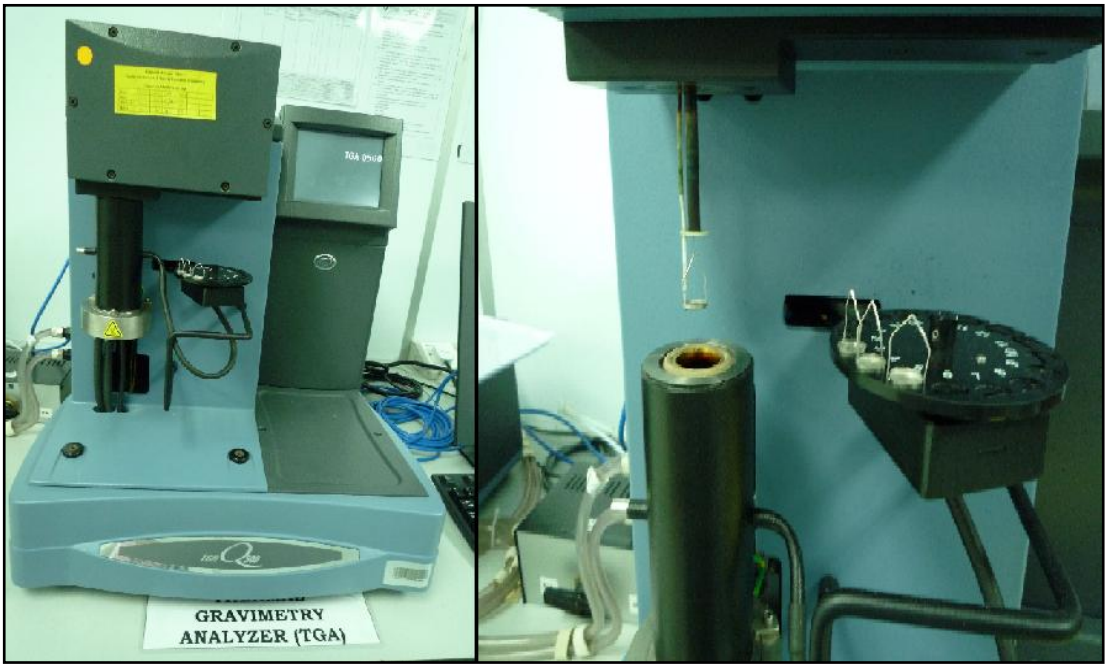
i) Mechanical Properties Test



ii) FTIR Spectroscopy

IR Spectrometer

iii) Thermogravimetric (TGA) Analyses



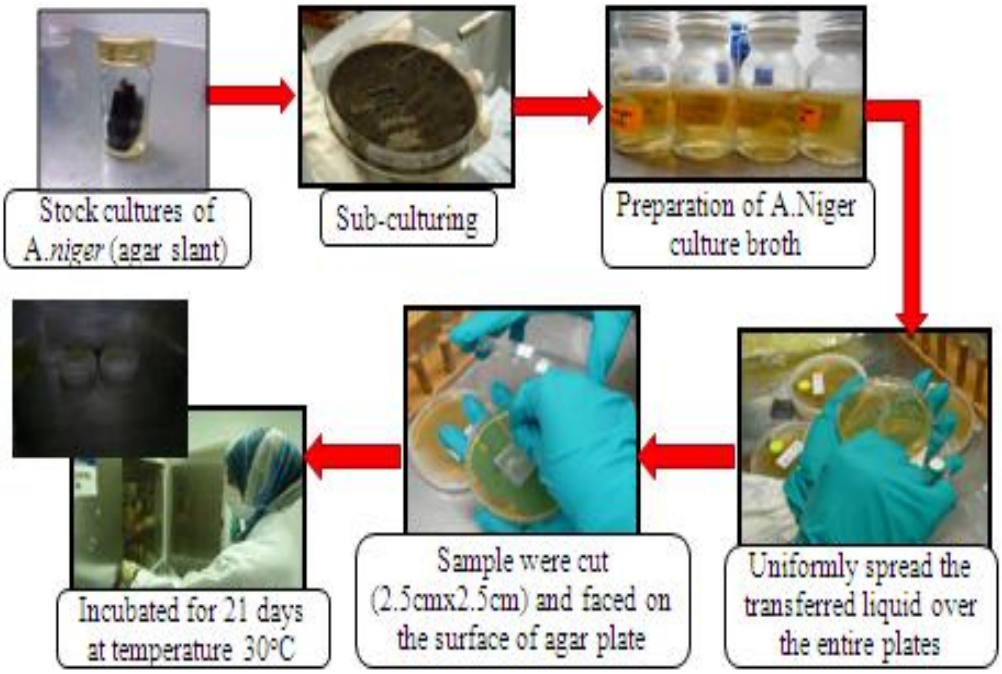
Thermogravimetric Analyzer

iv) Differential Scanning Calorimetry (DSC)

v) Microstructural Studies

SEM EDX Spectrometer EVO 50

vi) Microbiological Degradation Test



Appendix C: Stress Strain Curve For Tensile Test

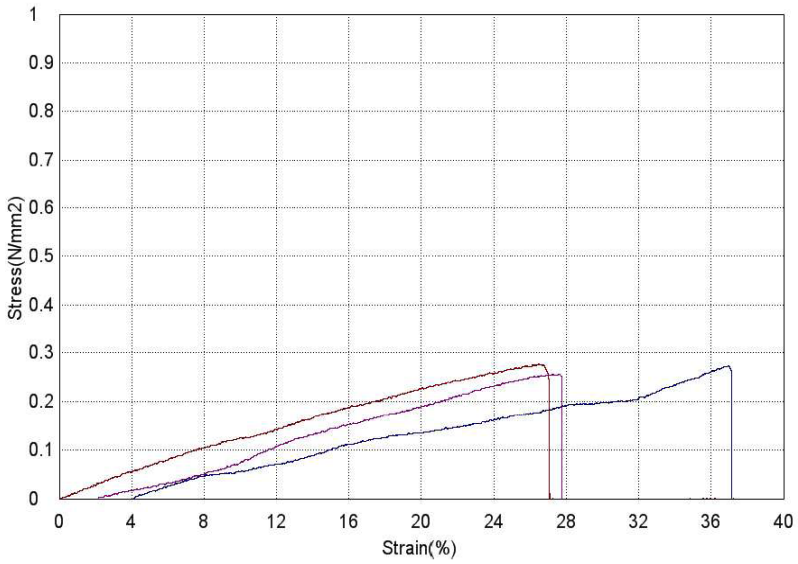
Title

Biodegradable Biocomposite Starch-based Films Blended with Chitosan and WPI

Key Word		Product Name	
Test File Name	starch only.xtak	Method File Name	tensile- zatul student.xm
Report Date	2006/12/01	Test Date	2006/11/30
Test Mode	Single	Test Type	Tensile
Speed	5mm/min	Shape	Plate
No of Batches:	1	Qty/Batch:	5

Name Parameters Unit	Elastic Force 10 - 20 N N/mm2	Max_Force Calc. at Entire A N	Max_Stress Calc. at Entire A N/mm2	Max_Strain Calc. at Entire A %
1 _ 2	-.-	0.92506	0.27753	26.5091
1 _ 3	-.-	0.97672	0.25636	25.5997
1 _ 5	-.-	1.06017	0.27395	32.9853
Average	-.-	0.98732	0.26928	28.3647
Standard Devi	-.-	0.06818	0.01133	4.02731
Maximum	-.-	1.06017	0.27753	32.9853
Minimum	-.-	0.92506	0.25636	25.5997

Name Parameters Unit	Break_Stress Sensitivity: 10 N/mm2	EASL1_Stroke Force 1 N mm	EASL1_Strain Force 1 N %
1 _ 2	-.-	-.-	-.-
1 _ 3	-.-	-.-	-.-
1 _ 5	-.-	36.5151	31.7522
Average	-.-	36.5151	31.7522
Standard Devi	-.-	-.-	-.-
Maximum	-.-	36.5151	31.7522
Minimum	-.-	36.5151	31.7522



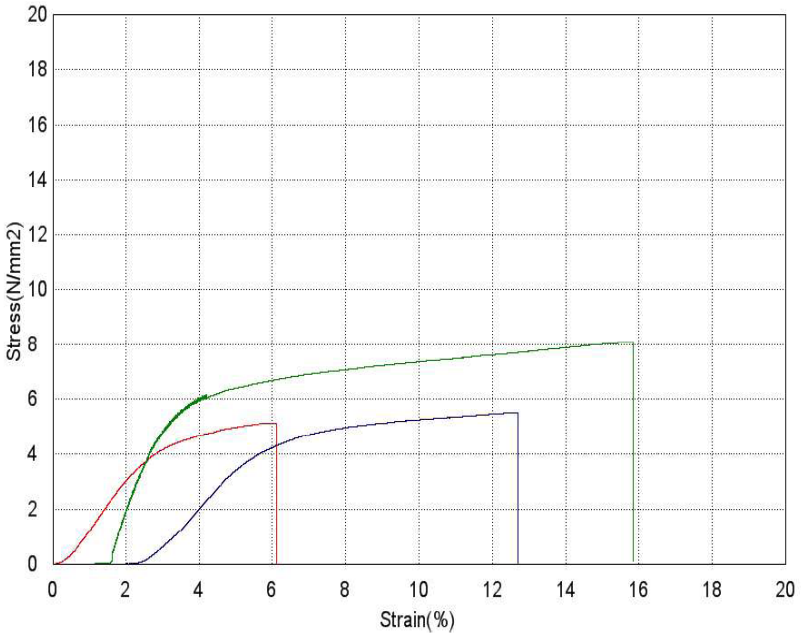
Title

Biodegradable Biocomposite Starch-Based Films Blended with Chitosan and WPI

Key Word		Product Name	
Test File Name	1% starch-1.xtak	Method File Name	tensile- zatul student.xm
Report Date	2006/12/01	Test Date	2006/11/30
Test Mode	Single	Test Type	Tensile
Speed	5mm/min	Shape	Plate
No of Batches:	1	Qty/Batch:	5

Name Parameters Unit	Elastic Force 10 - 20 N N/mm2	Max_Force Calc. at Entire A N	Max_Stress Calc. at Entire A N/mm2	Max_Strain Calc. at Entire A %
1 _ 1	-.-	5.20786	5.14204	6.10344
1 _ 4	-.-	7.95126	8.06743	14.7636
1 _ 5	-.-	5.38588	5.51832	10.6708
Average	-.-	6.18167	6.24260	10.5126
Standard Devi	-.-	1.53510	1.59151	4.33225
Maximum	-.-	7.95126	8.06743	14.7636
Minimum	-.-	5.20786	5.14204	6.10344

Name Parameters Unit	Break_Stress Sensitivity: 10 N/mm2	EASL1_Stroke Force 1 N mm	EASL1_Strain Force 1 N %
1 _ 1	-.-	1.03364	0.89881
1 _ 4	-.-	0.89365	0.77708
1 _ 5	-.-	1.54979	1.34764
Average	-.-	1.15903	1.00784
Standard Devi	-.-	0.34557	0.30050
Maximum	-.-	1.54979	1.34764
Minimum	-.-	0.89365	0.77708



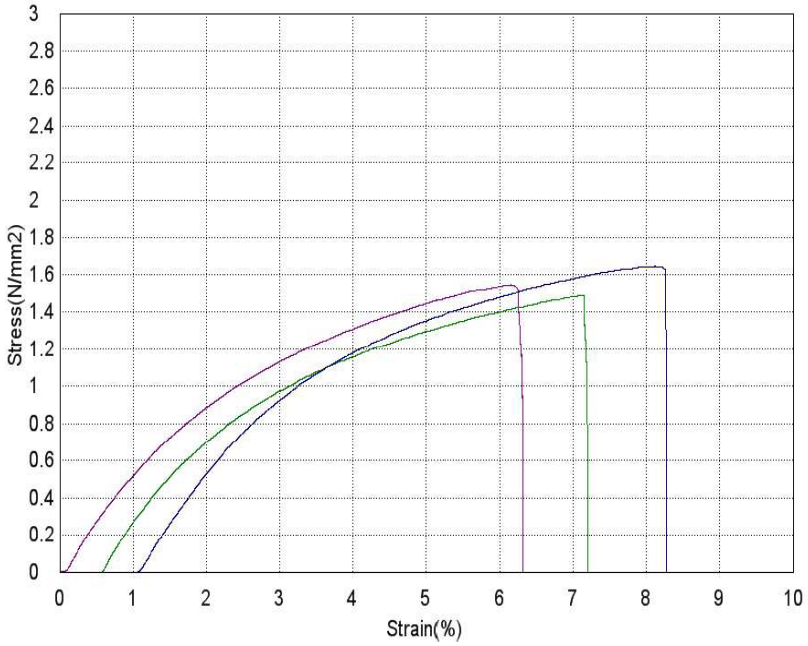
Title

Biodegradable Biocomposite Starch-based Films Blended with Chitosan and WPI

Key Word		Product Name	
Test File Name	2% starch.xtak	Method File Name	tensile- zatul student.xm
Report Date	2006/12/01	Test Date	2006/11/30
Test Mode	Single	Test Type	Tensile
Speed	5mm/min	Shape	Plate
No of Batches:	1	Qty/Batch:	5

Name Parameters Unit	Elastic Force 10 - 20 N N/mm2	Max_Force Calc. at Entire A N	Max_Stress Calc. at Entire Al N/mm2	Max_Strain Calc. at Entire Al %
1 _ 3	-.-	3.92199	1.54409	17.0714
1 _ 4	-.-	3.77814	1.48746	13.4077
1 _ 5	-.-	4.17312	1.64296	10.2946
Average	-.-	3.95775	1.55817	13.5912
Standard Devi	-.-	0.19990	0.07870	3.39213
Maximum	-.-	4.17312	1.64296	17.0714
Minimum	-.-	3.77814	1.48746	10.2946

Name Parameters Unit	Break_Stress Sensitivity: 10 N/mm2	EASL1_Stroke Force 1 N mm	EASL1_Strain Force 1 N %
1 _ 3	-.-	0.85006	0.73918
1 _ 4	-.-	0.86166	0.74927
1 _ 5	-.-	0.86270	0.75017
Average	-.-	0.85814	0.74621
Standard Devi	-.-	0.00702	0.00610
Maximum	-.-	0.86270	0.75017
Minimum	-.-	0.85006	0.73918



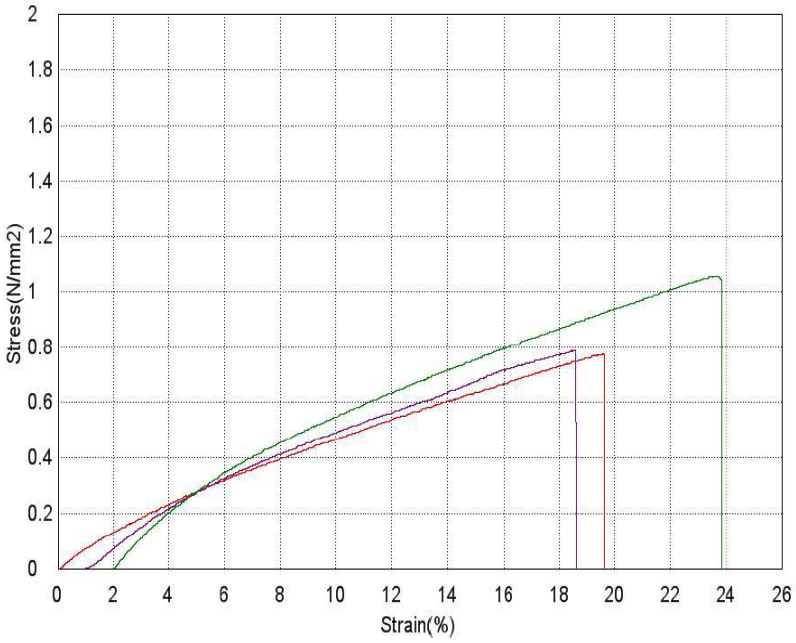
Title

Biodegradable Biocomposite Starch-based Films Blended with Chitosan and WPI

Key Word		Product Name	
Test File Name	3% starch.xtak	Method File Name	tensile- zatul student.xm
Report Date	2006/12/01	Test Date	2006/11/30
Test Mode	Single	Test Type	Tensile
Speed	5mm/min	Shape	Plate
No of Batches:	1	Qty/Batch:	5

Name Parameters Unit	Elastic Force 10 - 20 N N/mm2	Max_Force Calc. at Entire A N	Max_Stress Calc. at Entire A N/mm2	Max_Strain Calc. at Entire A %
1 _ 1	-.-	1.95980	0.77401	15.5157
1 _ 3	-.-	1.99000	0.78594	12.2707
1 _ 4	-.-	2.67108	1.05493	13.5165
Average	-.-	2.20696	0.87163	13.7676
Standard Devi	-.-	0.40222	0.15886	1.63701
Maximum	-.-	2.67108	1.05493	15.5157
Minimum	-.-	1.95980	0.77401	12.2707

Name Parameters Unit	Break_Stress Sensitivity: 10 N/mm2	EASL1_Stroke Force 1 N mm	EASL1_Strain Force 1 N %
1 _ 1	-.-	9.15100	7.95739
1 _ 3	-.-	7.56504	6.57829
1 _ 4	-.-	5.57330	4.84634
Average	-.-	7.42978	6.46067
Standard Devi	-.-	1.79268	1.55886
Maximum	-.-	9.15100	7.95739
Minimum	-.-	5.57330	4.84634



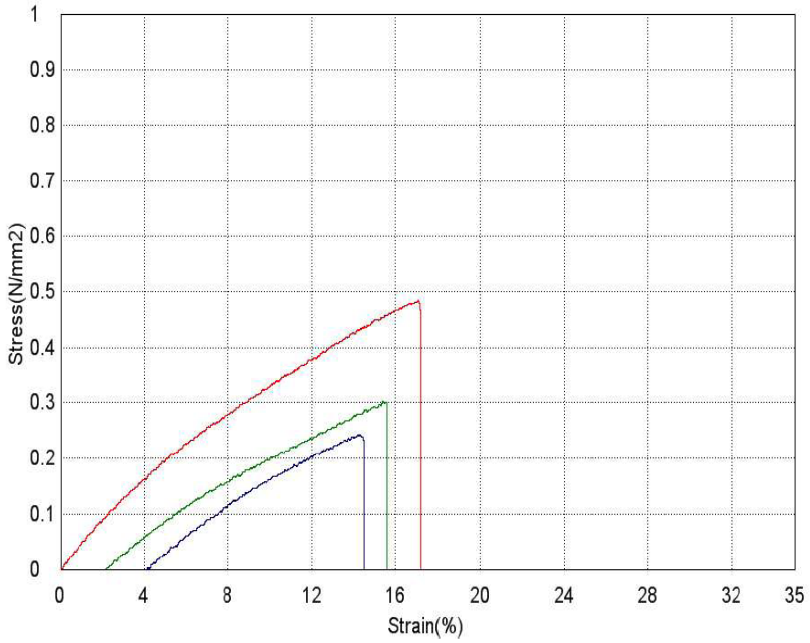
Title

Biodegradable Biocomposite Starch-Based Films Blended with Chitosan and WPI

Key Word		Product Name	
Test File Name	4% starch.xtak	Method File Name	tensile- zatul student.xm
Report Date	2006/12/03	Test Date	2006/12/01
Test Mode	Single	Test Type	Tensile
Speed	5mm/min	Shape	Plate
No of Batches:	1	Qty/Batch:	5

Name Parameters Unit	Elastic Force 10 - 20 N N/mm2	Max_Force Calc. at Entire A N	Max_Stress Calc. at Entire A N/mm2	Max_Strain Calc. at Entire A %
1 _ 1	-.-	1.20322	0.48361	19.6099
1 _ 4	-.-	0.75181	0.30218	17.5534
1 _ 5	-.-	0.60558	0.24340	21.4570
Average	-.-	0.85354	0.34306	19.5401
Standard Devi	-.-	0.31154	0.12522	1.95274
Maximum	-.-	1.20322	0.48361	21.4570
Minimum	-.-	0.60558	0.24340	17.5534

Name Parameters Unit	Break_Stress Sensitivity: 10 N/mm2	EASL1_Stroke Force 1 N mm	EASL1_Strain Force 1 N %
1 _ 1	-.-	14.8839	12.9426
1 _ 4	-.-	-.-	-.-
1 _ 5	-.-	-.-	-.-
Average	-.-	14.8839	12.9426
Standard Devi	-.-	-.-	-.-
Maximum	-.-	14.8839	12.9426
Minimum	-.-	14.8839	12.9426



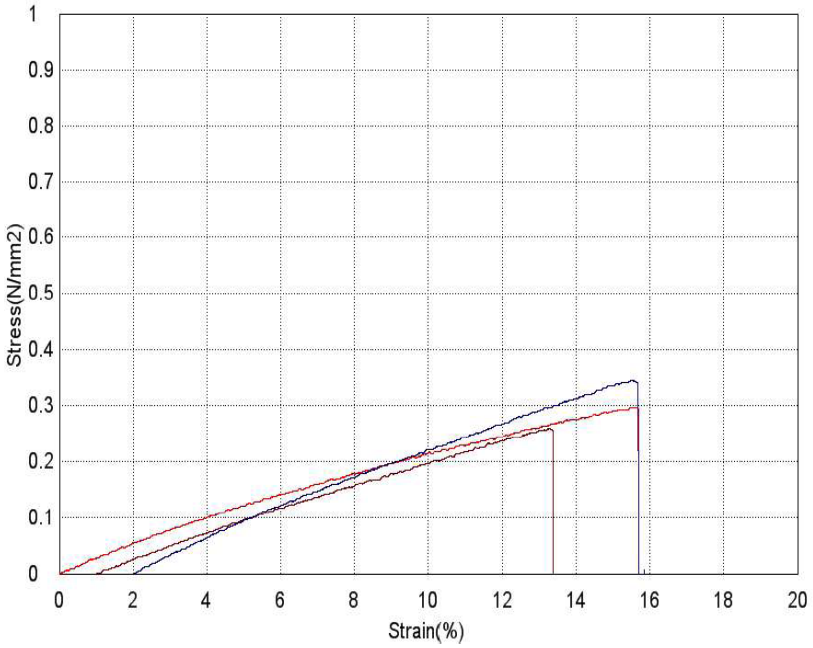
Title

Biodegradable Biocomposite Starch-based Films Blended with Chitosan and WPI

Key Word		Product Name	
Test File Name	5% starch.xtak	Method File Name	tensile- zatul student.xm
Report Date	2006/12/01	Test Date	2006/12/01
Test Mode	Single	Test Type	Tensile
Speed	5mm/min	Shape	Plate
No of Batches:	1	Qty/Batch:	5

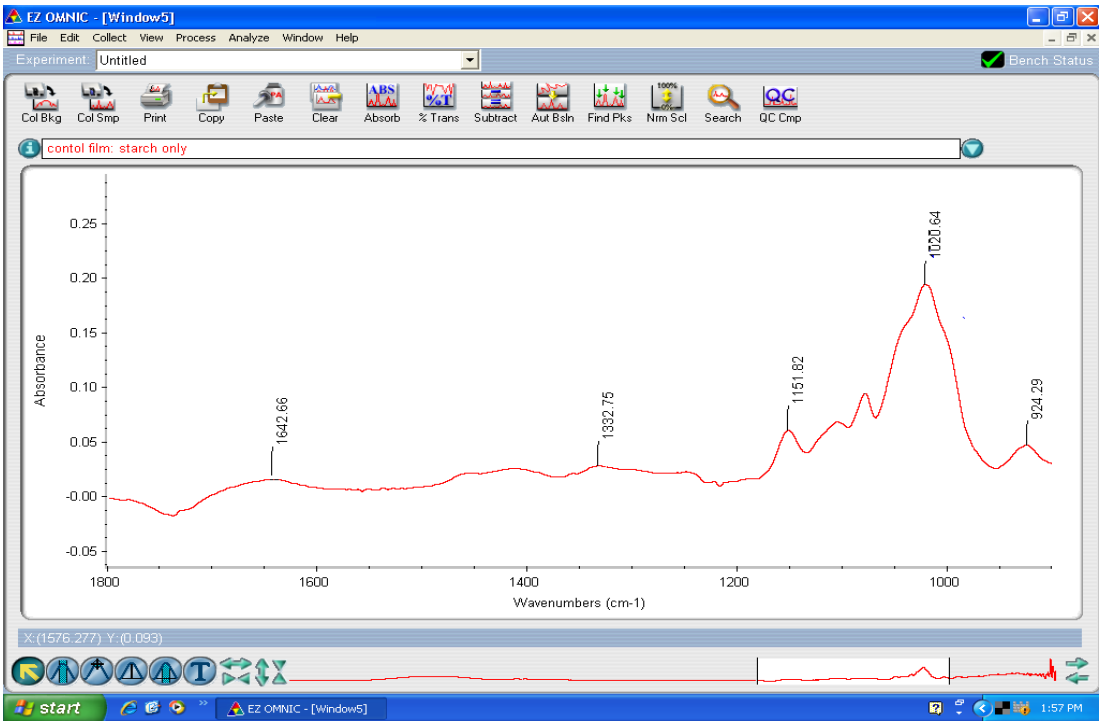
Name Parameters Unit	Elastic Force 10 - 20 N N/mm2	Max_Force Calc. at Entire A N	Max_Stress Calc. at Entire A N/mm2	Max_Strain Calc. at Entire A %
1 _ 1	-.-	0.89328	0.29776	17.0714
1 _ 2	-.-	0.77963	0.25988	23.4475
1 _ 5	-.-	1.03633	0.34544	29.6816
Average	-.-	0.90308	0.30103	23.4002
Standard Devi	-.-	0.12863	0.04287	6.30523
Maximum	-.-	1.03633	0.34544	29.6816
Minimum	-.-	0.77963	0.25988	17.0714

Name Parameters Unit	Break_Stress Sensitivity: 10 N/mm2	EASL1_Stroke Force 1 N mm	EASL1_Strain Force 1 N %
1 _ 1	-.-	-.-	-.-
1 _ 2	-.-	-.-	-.-
1 _ 5	-.-	14.7695	12.8431
Average	-.-	14.7695	12.8431
Standard Devi	-.-	-.-	-.-
Maximum	-.-	14.7695	12.8431
Minimum	-.-	14.7695	12.8431

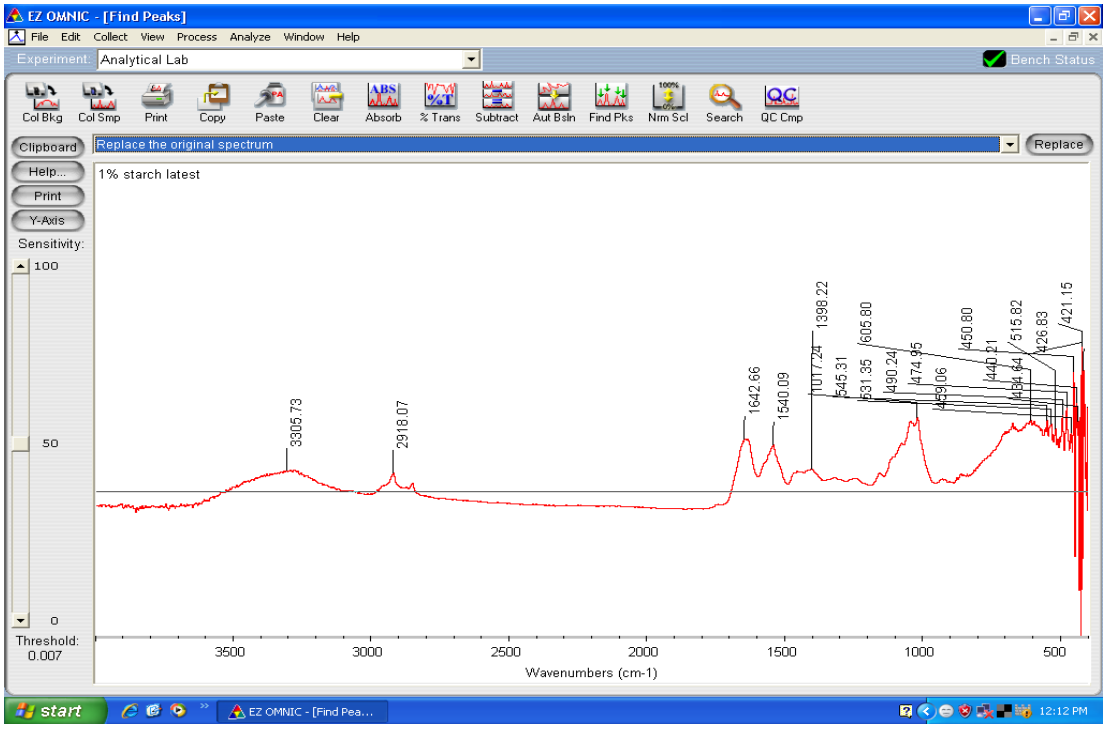


Appendix D: FT-IR Spectra

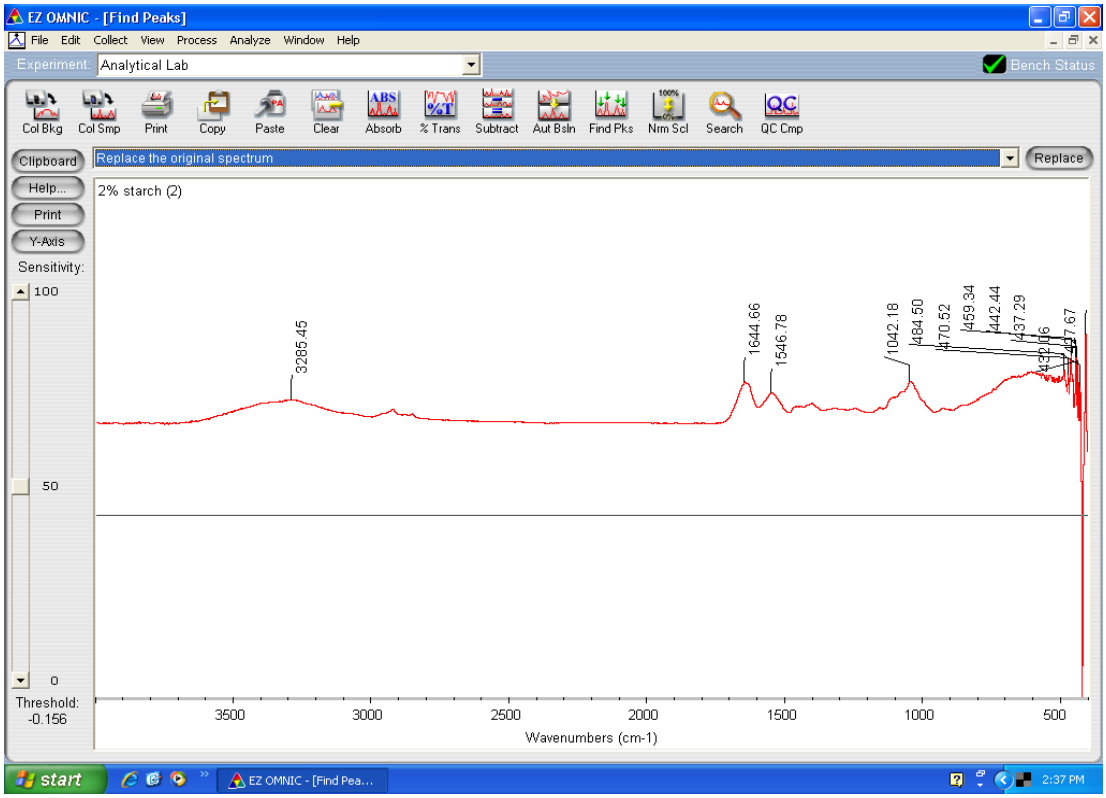
i) Control film: starch only



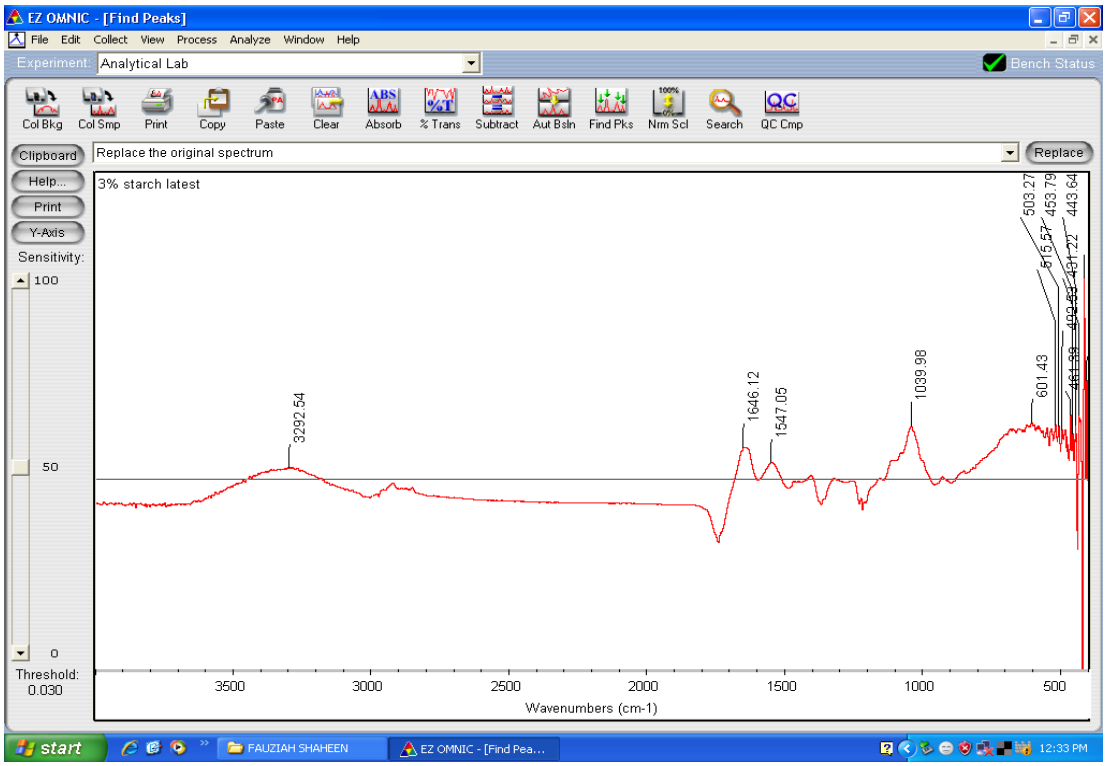
ii) Blend film with 1% starch content



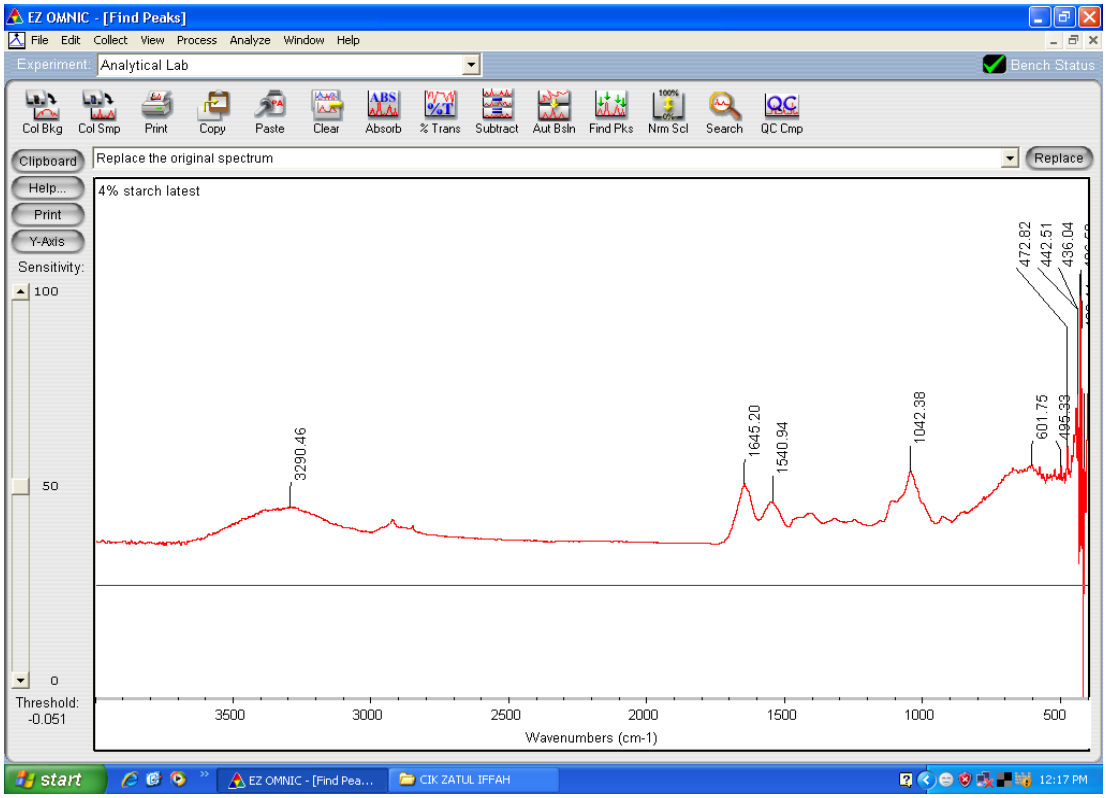
iii) Blend film with 2% starch content



iv) Blend film with 3% starch content



v) Blend film with 4% starch content



vi) Blend film with 5% starch content

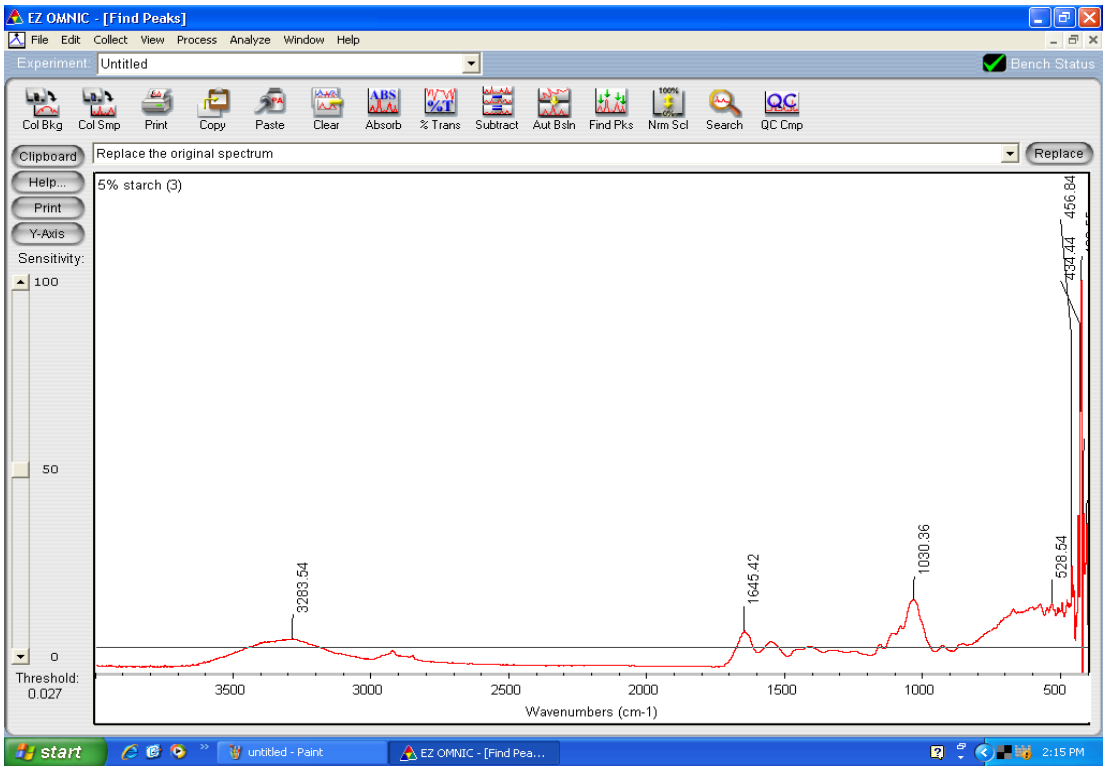
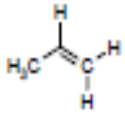
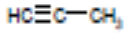
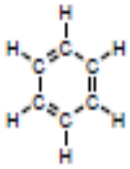
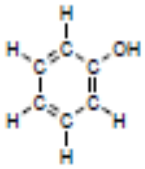
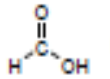
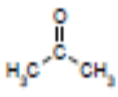
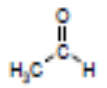
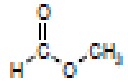
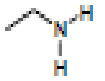
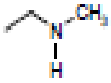
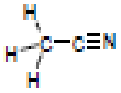
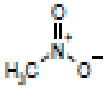
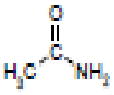


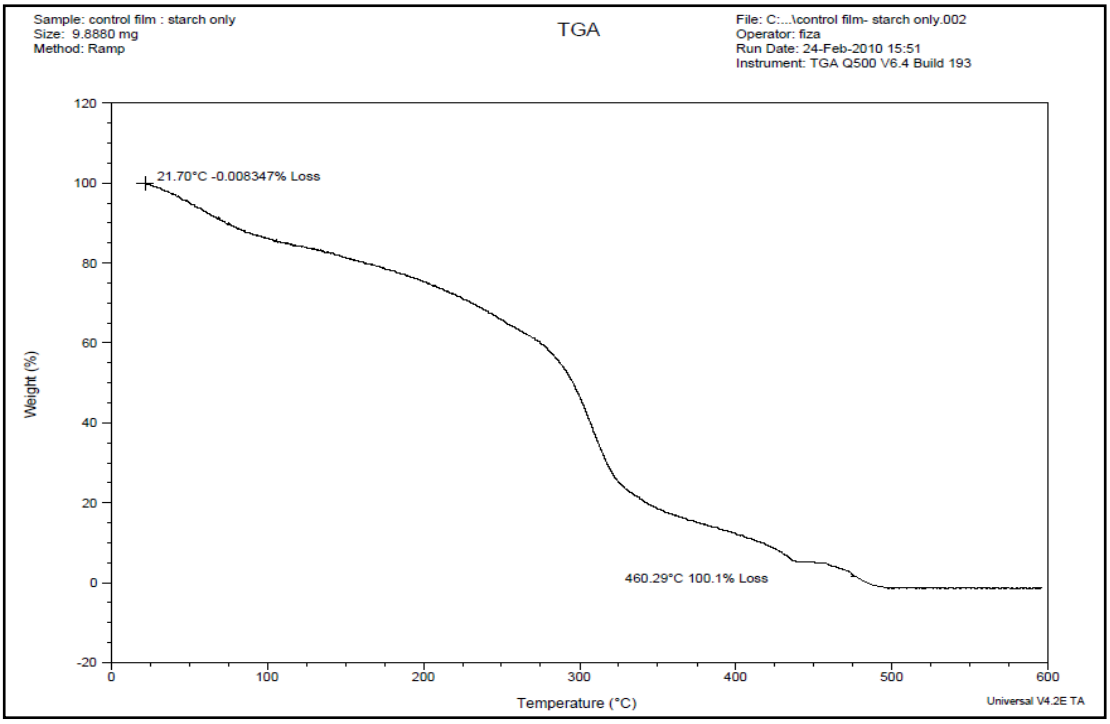
Table C.1: Principal IR Absorption for Certain Functional Groups

Functional Group Names & Example compounds	**Absorption Ranges(cm^{-1})** [Look for a single absorption in these regions, unless stated otherwise.]	**Type of Vibration causing IR absorption**
Alkanes:  Methane	**3000-2800** (Note: The absorptions can be seen as several distinct peaks in this region.)	H-C-H Asymmetric & Symmetric Stretch
	1500-1440	H-C-H Bend
Alkenes:  1-Propene	**3100-3000**	C=C-H Asymmetric Stretch
	1675-1600	C-C=C Symmetric Stretch
Alkynes:  Propyne	**3300-3200**	≡C—H Stretch
	2200-2100	C≡C Stretch
Aromatic Rings:  Benzene	**3100-3000**	C=C-H Asymmetric Stretch
	1600-1580	C-C=C Symmetric Stretch
	1500-1450	C-C=C Asymmetric Stretch
Phenols & Alcohols:  Phenol  Methanol (Alcohol)	**3600-3100** (Note: Phenols MUST have Aromatic Ring Absorptions too.)	Hydrogen-bonded O-H Stretch (This peak usually appears much broader than the other IR absorptions.
Carboxylic Acids:  Formic Acid	**3400-2400** *(This peak always covers the entire region with a **VERY BROAD** peak.)*	Hydrogen-bonded O-H Stretch *(Note: This peak can obscure other peaks in this region.)*
	1730-1650	C=O Stretch
Ketones:  Acetone	**1750-1625**	C=O Stretch
Aldehydes:  Ethanal	**1750-1625**	C=O Stretch
	2850-2800	C-H Stretch off C=O
	2750-2700	C-H Stretch off C=O

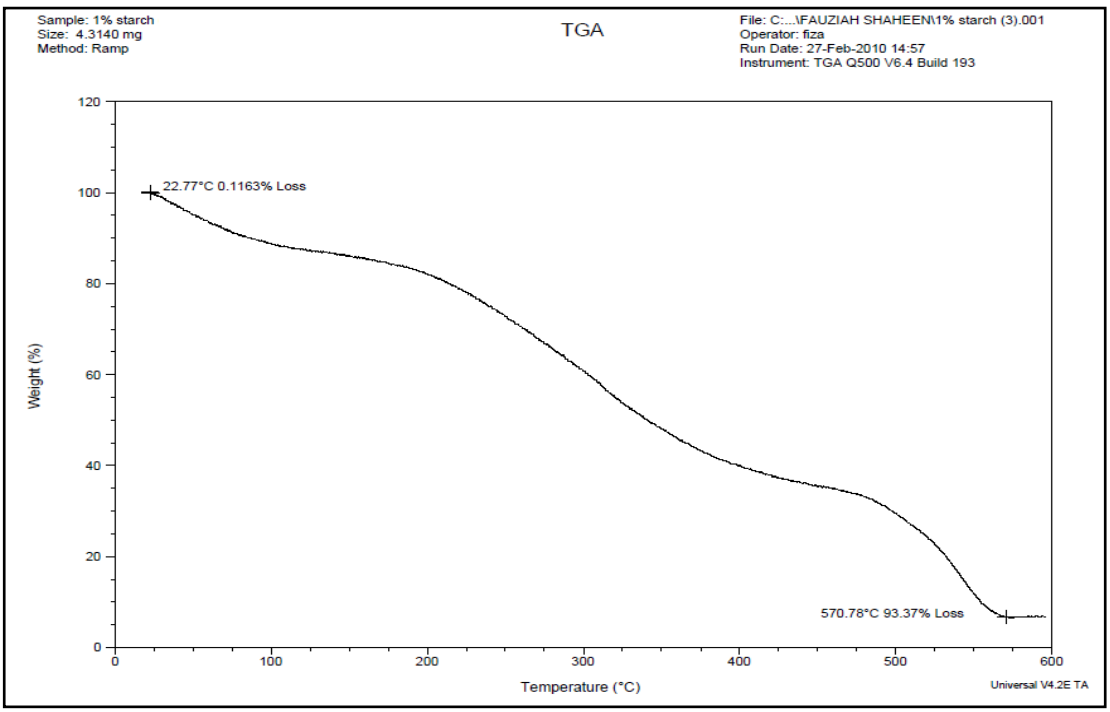
Functional Group Names & Example compounds:	**Absorption Ranges(cm^{-1})** [Look for a single absorption in these regions, unless stated otherwise.]	**Type of Vibration** causing IR absorption
Esters:  Methyl Formate	**1755-1650**	C=O Stretch
	(1300-1000)	(C-O Stretch)
Ethers:  Diethyl Ether (aka-Ethyl Ether)	**(1300-1000)**	(C-O Stretch)
Amines—Primary:  Ethylamine	**3500-3100 (TWO PEAKS!)**	N-H Stretch
	1640-1560	N-H Bend
Amines—Secondary:  N-Methylethylamine	**3500-3100 (ONE PEAK!)**	N-H Stretch
	1550-1450	N-H Bend
Nitriles:  Methanenitrile	**2300-2200**	C≡N Stretch
Nitro Groups:  Nitromethane (Note: Both peaks are <200 cm-1 apart.)	**1600-1500**	N=O Stretch
	1400-1300	N=O Bend
Amides:  Methanamide	**3500-3100**	N-H Stretch (similar to amines)
	1670-1600	C=O Stretch
	1640-1550	N-H Bend

Appendix E: TG Curve

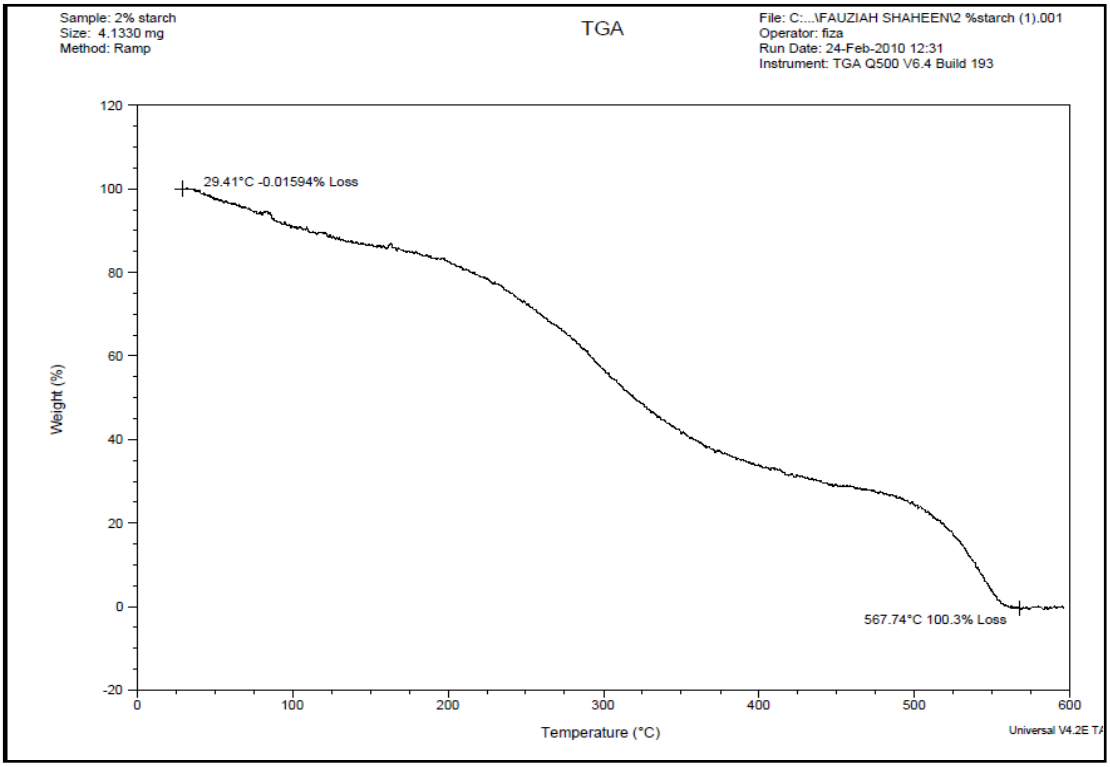
i) Control film: Starch only



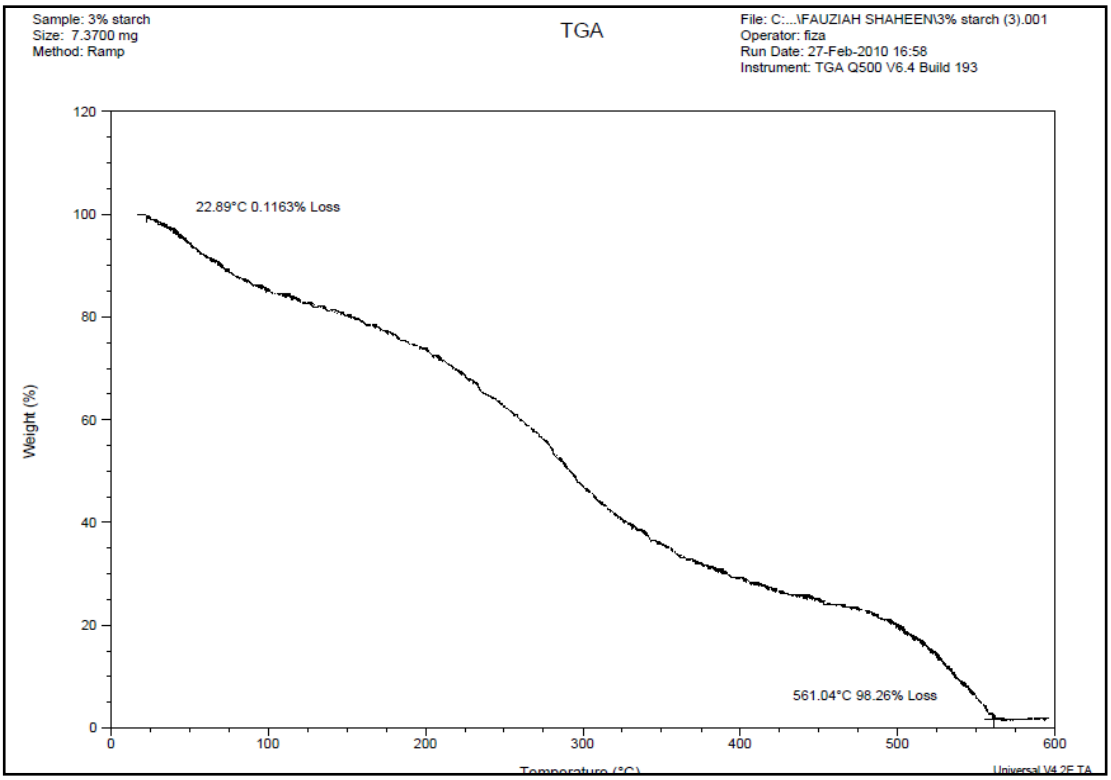
ii) Blend film with 1% starch content



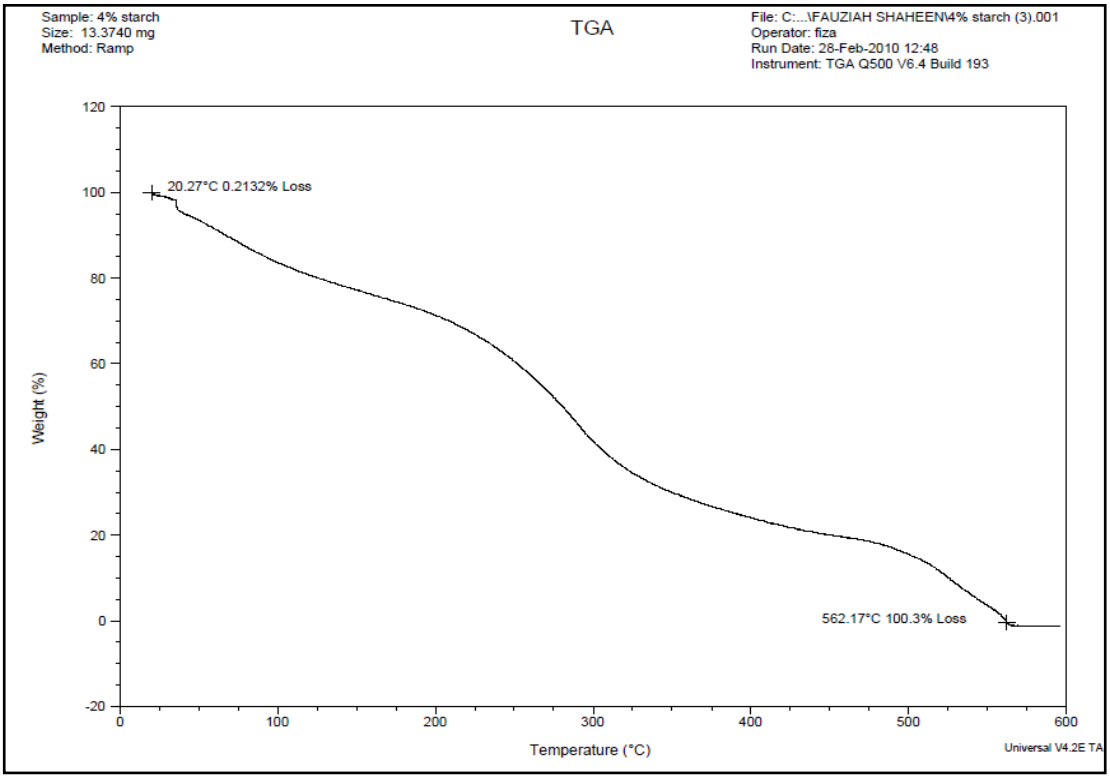
iii) Blend film with 2% starch content



iv) Blend film with 3% starch content



v) Blend film with 4% starch content



vi) Blend film with 5% starch content