

CHAPTER III

EXPERIMENTAL SECTION

3.1 Materials

3.1.1 Polymer Matrix

Low-density polyethylene (LDPE) extrusion film grade LD1902F, obtained from Cementhai Co., Ltd. was used as a polymer matrix. The melt flow index was 2.0 g/10 min and the density was 0.919 g/cm³.

3.1.2 Compatibilizer

Ethylene vinyl acetate copolymer (EVA) grade N8038, supplied by Thai Petrochemical Industry Co., Ltd. was used as a compatibilizer. The melt flow index (2.16 kg/190°C) and the density were 2.3 g/10 min and 0.941 g/cm³, respectively. The vinyl acetate content was 18%.

3.1.3 Biodegradable Additive

Banana starch was used as a biodegradable additive. Preparation of banana starch will be described in the next section.

3.2 Instrument

Instruments	Model	Manufacturer
Twin screw extruder	D8017	Collin
Chill roll cast film	ECS – T10	Collin
Universal testing machine	LR 100k	LLOYD
Scanning electron microscope	JSM 5800 LV	Jeol
Thermalgravimetric analyzer	TGA 7	Perkin Elmer
Differential scanning calorimeter	DSC 7	Perkin Elmer
Fourier transform infrared spectroscopy	Impact 400D	Nicolet

3.3 Experimental Procedure

3.3.1 Banana Starch Preparation

Banana starch was extracted according to Lii's method. First, green banana was peeled and sliced into small pieces. It was then suspended in 0.05 N NaOH. The mixture was ground in a moulinex for 2 minutes until it was homogenized. The slurry was filtered through sieve (120 μm pore size) with distilled water. Prime starch was sedimented from the filtrate. The starch was washed several times with distilled water until the supernatant layer was substantially free of color. Finally, starch was dried in an air oven at 80 °C and kept in a dessicator until being used.

3.3.2 Plastic Film Preparation

Banana starch was dried in an air oven at 80 °C for 24 hr prior to use for the preparation of blends. A twin screw extruder was used to mix the blend composition. For LDPE/Starch blends, four different levels of starch were used, namely 5,10,15 and 20 wt%. In these blends, EVA was used as a compatibilizer at three different amounts that are 5,10 and 20 wt% based upon starch content. Starch – based

LDPE films were prepared using a chill roll cast film. The processing conditions for the twin screw extruder and chill roll cast film are shown in Table 3.1.

Table 3.1: Processing Conditions for Starch – Based LDPE Blends

Processing Parameter	Operating Value	
	Twin Screw Extruder	Chill Roll Cast Film
Extruder Temperature Profile (°C)		
Zone 1	60	135
Zone 2	130	145
Zone 3	135	150
Zone 4	140	150
Zone 5	145	150
Zone 6	150	160
Zone 7	-	160
Zone 8	-	160
Screw Speed (rpm.)	25	25

3.4 Characterization of Banana Starch

3.4.1 Scanning Electron Microscopy (SEM)

Granule morphology of banana starch was examined by a scanning electron microscope (SEM). Prior to the examination, starch was dried overnight in air oven at 80°C and mounted on stub with double sticky tape. Stub was then coated with a thin evaporated layer of gold in order to improve conductivity and prevent electron charging on the surface. The SEM was operated at 15 kV to image the samples.

3.4.2 Compositional Analysis

Chemical composition of banana starch was analyzed by the Association of Official Analytical Chemists, AOAC method at the Cassava and Starch Technology Research Unit of the Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, KAPI. Starch, protein, lipid, ash and amylose were determined quantitatively.

3.4.3 Chemical Structure of Banana Starch

Fourier Transform Infrared Spectroscopy (FTIR) technique was used to characterize chemical structure of banana starch. Sample was measured in the form of powder, which was prepared by KBr disc. The samples were scanned at a frequency range of $4000 - 400 \text{ cm}^{-1}$ with 32 consecutive scans and 4 cm^{-1} resolution.

3.4.4 Thermal Properties

3.4.4.1 Differential Scanning Calorimeter (DSC)

About 3.0 mg of starch was placed in a sealed aluminum cell. Before sealing, deionized water was added with the ratio of 30/70 by weight of starch/water. The sample was initially heated with a heating rate of $10^\circ\text{C}/\text{min}$ from 40°C to 100°C under nitrogen atmosphere. The gelatinized temperature (T_g) of starch was obtained from the peak maxima.

3.4.4.2 Thermogravimetric Analyzer (TGA)

Measurements were performed under nitrogen atmosphere using a heating rate of $20^\circ\text{C}/\text{min}$. Approximately 7.0 mg of banana starch was heated from 50°C to 600°C . Temperature degradation of starch can be estimated from the onset of the degradation.

3.4.5 Density Measurement

The density of starch was measured using the picnometric technique. The method involves the use of a picnometer of known volume (V_{pyc}). The starch to be evaluated was weighed (W_{st}) and put into the picnometer. Then, the picnometer containing the starch was filled with diethyl ether of known density (ρ_{d}) and reweighed ($W_{\text{pyc+st+d}}$). The weight and thus the volume of diethyl ether can be found from the weight difference. Finally, the density of the starch can be determined from the following equation:

$$\rho_{\text{st}} = \frac{W_{\text{st}}}{V_{\text{pyc}} - (W_{\text{d}} / \rho_{\text{d}})} \quad (\text{Eq. 3.1})$$

Where:

ρ_{st} is the density of the starch (g/cm^3).

W_{st} is the weight of the starch contained in the picnometer (g).

V_{pyc} is the volume of the picnometer (cm^3).

W_{d} is the weight of diethyl ether contained in the picnometer (g).

ρ_{d} is the density of diethyl ether (g/cm^3).

3.4.6 Moisture Absorption

Banana starch was dried in a hot-air oven for 24 hours at 80°C and cooled in a desiccator. The dried starch was placed in an enclosure containing distilled water. Starch was removed and then weighed to determine the weight change or moisture uptake periodically. Starch was immediately placed back after each measurement. At first, the weight change of starch were measured at every 1 hour for 6 hours and then at every 2 hours. The weight changes of each sample were monitored

until there is no further weight change, i.e., the moisture absorption reached equilibrium. Five specimens were measured for each sample and the results were averaged to obtain a mean value. The moisture absorption was calculated as the weight difference and reported as percent increase in the initial weight. It can be calculated from the following equation:

$$M (\%) = \frac{W_i - W_o}{W_o} \times 100 \quad (\text{Eq. 3.2})$$

Where:

- M = moisture content (%).
- W_i = current specimen mass (g).
- W_o = oven-dry specimen mass (g).

3.5 Characterization of LDPE/Starch Film

3.5.1 Scanning Electron Microscopy (SEM)

SEM was used to observe the surface and fractured surface of the LDPE/banana starch films. For fractured surface investigation, the blend films were fractured immediately after freezing in liquid nitrogen and were sputter – coated with gold to avoid charging under the electron beam.

For surface morphology analysis, the blend films were also coated with a thin layer of gold prior to the examination.

3.5.2 Chemical Structure of Films

Fourier Transform Infrared spectroscopy (FTIR) was used with 32 consecutive scans at a resolution of 4 cm^{-1} . The samples were scanned at a frequency range of $4000 - 400\text{ cm}^{-1}$. Pure LDPE and LDPE/starch blend films were mounted on standard FTIR sample plates using a removable magnetic cover.

3.5.3 Thermal Properties

3.5.3.1 Differential Scanning Calorimeter (DSC)

DSC was used to further evaluate compatibility between polymeric components. Approximately 8.50 mg. of film was heated from 40°C to 200°C , cooled to 40°C and heated again to 200°C at a heating and cooling rate of $10^\circ\text{C} / \text{min}$ for each trial. The melting temperature (T_m) and heat of fusion (ΔH_f^*) of the samples were obtained from the peak maxima and the area under the peaks, respectively. The percent crystallinity of the LDPE phase was calculated using the following equation:

$$\% \text{ Crystallinity} = \frac{\Delta H_f^*}{\Delta H_f^\circ} \times 100 \quad (\text{Eq. 3.3})$$

where :

ΔH_f° is the heat of fusion for 100% crystalline LDPE.

ΔH_f^* is the heat of fusion of the semi-crystalline LDPE blends.

3.5.3.2 Thermogravimetric Analyzer (TGA)

Approximately 7 mg of each film sample was heated at a heating rate of 20°C from 200°C to 600°C under nitrogen atmosphere. Prior to do the

experiment, the samples were dried in a vacuum oven at 60°C for 24 hours. The onset of degradation temperature for each sample was recorded.

3.5.4 Moisture Absorption of Films

Moisture absorption of the pure LDPE and the LDPE blend films were measured using a sample size of 1.5 cm wide by 20 cm long. Similar to other experiments, the films were dried in a hot-air oven at 60°C for 24 hours before starting the experiment. The dried film samples were then placed in an enclosure containing distilled water. The measurement was performed following the same procedure of the banana starch, as previously explained. The moisture absorption was calculated as the weight difference and reported as percent increase in the initial weight, as already shown in equation 3.2.

3.6 Biodegradation Procedure

3.6.1 Activated Sludge

The polymer blends in the form of thin films were cut into the size of 1.5cm x 20cm. The films were then exposed to activated sludge for 8 weeks in the wastewater treatment plant at the *Punjapol Fiber Container* company in Samutsakhon province. The temperature (25 ± 1 °C) and pH7 of the activated sludge were kept constant during the 8 weeks of the experiment. As will be described in the next section, the rate of aerobic biodegradation was followed by measuring the percentage weight loss and tensile properties of the film samples after being exposed to activated sludge at every 2, 4, 6 and 8 weeks, respectively. The change in physical appearance of the film surfaces was also observed by SEM analysis.

3.6.2 Enzymatic Degradation

Samples were cut into 1.5cm x 20cm squares and weighed before being placed in 40ml vial. Enzyme solution consisting of 0.1 M acetate buffer pH6, 8.3ml of Termamyl and 54 mM of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ was prepared. After adding the enzyme solution into the vial containing film samples, the vial was put into a water bath, with continuously shaking and heated at 70°C. Specimens were removed for testing at every 2 hours. After removal, specimens were washed with distilled water and dried under vacuum at 60°C for 24 hours to remove traces of moisture before testing. Samples were then weighed to determine the percentage of weight loss. The evidence of enzymatic degradation was also confirmed by the SEM analysis.

3.7 Evaluation of the Degradation

3.7.1 Tensile Properties

Measurements of the tensile properties such as tensile strength and elongation at break were performed according to the ASTM D882 method using universal testing machine before and after biodegradation testing in activated sludge. A 100 N load cell was used to measure the force. Since the films prepared by chill roll cast film process were biaxially orientated in the machine direction (MD) and the transverse direction (TD); their tensile properties were measured in both directions. Measurements were performed using a 20 mm/min crosshead speed and 20% strain rate. For each sample, five uniaxial specimens with dimensions of 1.5 cm wide, 20 cm long and 0.096 ± 0.017 mm thickness were tested and the results were averaged to obtain a mean value. The gauge length used in the experiment was 10 cm.

3.7.2 Weight Loss

The amount of starch removal from the films was determined by weighing the samples before and after degradation. The percentage weight loss was calculated using the following equation:

$$\text{Weight loss (\%)} = \frac{W_i - W_f}{W_i} \times 100 \quad (\text{Eq. 3.4})$$

Where:

W_i = initial weight of sample before degradation (g).

W_f = final weight of sample after degradation (g).

3.7.3 Scanning Electron Microscopy (SEM)

Electron micrographs were obtained from the film samples collected before and after biodegradation testing in activated sludge and enzymatic treatment. Each samples were cleaned using a soft brush and dried in a vacuum oven at 60 °C for 24 hours. The surfaces of the film specimens were coated with gold prior to an examination.