CHAPTER III
EXPERIMENTAL SECTION

3.1 Materials

High-density polyethylene (HDPE) extrusion film grade 3355A, obtained from Thai Petrochemical Industry Co. Ltd., was used as the polymeric matrix. The melt flow index (2.16 kg/190°C) was 0.06 g/10 min and the density was 0.95 g/cm^3. The HDPE incorporated a conventional thermal stabilizer of undisclosed composition.

Tapioca starch, supplied by Siam Modified Starch Co., Ltd., was used as a biodegradable additive. Tapioca starch consists of amylose and amylopectin.

Natural rubber (TTR5L), suppied by Victor & Prosper Limited Partnership was used as an "autooxidant" to promote oxidative degradation of the HDPE.

Zinc stearate [$Zn(C_{17}H_{35}COO)_2$], suppied by Imperial Industrial Chemicals Thailand Co., Ltd., was used as a transition metal salt to catalyze the oxidative degradation of the HDPE. The melting point was 120 - 124°C and the metal content was 10.0 - 11.5%. The bulk density was 0.2 - 0.3 kg/lt.

The mixture of the autooxidant and the transition metal salt will be referred to as a "prooxidant" to accelerate the degradation process.

The enzyme ∝-amylase, commercial name "Termamyl", was kindly supplied by Novo Industry. Termamyl is a liquid enzyme preparation containing an outstanding heat-stable ∝-amylase produced by a selected strain

of *Bacillus licheniformis*. Termamyl 120L (120 KNU/g) is an endoamylase which will hydrolyze 1,4-alpha glucosidic linkages in amylose and amylopectin. Starch is therefore rapidly broken down to soluble dextrins and oligosaccharides. One kilogram of Novo alpha-amylase unit (1 KNU) is the amount of enzyme which breaks down 5.26 g starch per hour using Novo Nordisk's standard method.

3.2 Experimental Procedure

3.2.1 Plastic Samples Preparation

Most of the samples prepared contained additives which in different ways promoted degradation whilst pure HDPE was used as a control sample. The starch-based HDPE blends containing various additives are shown in Table 3.1.

Table 3.1 Starch-HDPE Blends Containing Various Additives

Component (s)	Starch (%)	NR:Zinc stearate (%)
HDPE	0	0
HDPE:St	15, 30	0
HDPE:St:NR:ZnSt	5, 10, 15, 20, 30	5:1

* The abbreviations St, NR and ZnSt refer to starch, natural rubber and zinc stearate, respectively.

Well-dried tapioca starch and prooxidant additive were mixed together with HDPE on a Lab Tech LRM 110 two-roll mill. The starch was predried in a hot air-oven at 110°C for 2 hours to reduce the moisture content

prior to use. The starch-HDPE blends were blended on the two-roll mill for 15 min. The blends were then taken off in the form of a thick sheet and comminuted in a shredder. The shredded starch-HDPE blends were compression molded using a Wabash V50H press to produce flat sheets of thickness 1.98 mm using a 16.5 × 13 cm window-frame mold. The mold used in this work was made from chromium plated stainless steel. The blends were pressed at 180°C with 15 tons force for 12 min before cooling to room temperature. Finally, specimens were press-cut from the sheet for further degradation analysis.

3.3 Degradation Procedure

3.3.1 Part I : Thermal Oxidative Degradation

The effect of oxidative degradation on samples' properties were studied using an oven aging test which involved heating the plastic samples in the presence of oxygen (air).

The plastic samples were placed in the forced-air oven (Laboratory air circulation oven model UT 6), in which the natural circulation of air was possible at 80°C and 100°C. Aged specimens were removed from the oven at regular intervals in order to study any changes in mechanical properties and melt flow index (MFI). Fourier Transform Infrared Spectroscopy (FTIR) analyses were also performed on the aged specimens.

3.3.2 Part II : Biodegradation of Starch

Specimens were cut into 15×15 mm squares and weighed (each specimen weighed approximately 0.02 - 0.03 g) before being placed in a 125

ml Erlenmeyer flask. A reaction mixture consisting of 25 ml of 0.05 M acetate buffer (pH 6.0), 1 ml of Termamyl 120L ∝-amylase (120 KNU/g) and 54 mM $CaCl_2 2H_2O$ was added to the flask which was then heated in a water bath at 80°C, with shaking, for 12 hours. A similar set of specimens were treated in the same manner except without ∝-amylase. Specimens were removed for testing every 3 hours. After removal, specimens were washed with distilled water and dried under vacuum at 35°C for 5 hours to remove traces of moisture before testing. Specimens were then weighed to determine the percentage of weight loss. Specimens were also observed under the Scanning Electron Microscope (SEM).

3.3.3 Part III : Combined Thermal Oxidative Degradation and Biodegradation

The 15 mm × 15 mm square samples weighing approximately 0.02 - 0.03 g were preheated in the forced-air oven at 80°C for 20 days before enzymatic treatment. The samples were transferred to a 125 ml Erlenmeyer flask and a reaction mixture of 25 ml. of 0.05 M acetate buffer (pH 6.0), 1 ml. of Termamyl 120L ∝-amylase (120 KNU/g) and 54 mM $CaCl_2 2H_2O$ was added. The flask was heated in a water bath at 80°C, with shaking, for 12 hours. The same set of specimens were treated in the same manner except without ∝-amylase. Specimens were removed at regular interval of 3 hours to determine the percentage of weight loss. After removal, specimens were washed with distilled water and dried under vacuum at 35°C for 5 hours to remove traces of moisture before testing. Specimens were also observed under the Scanning Electron Microscope (SEM).

3.4 Evaluation of the Degradation

3.4.1 Mechanical Properties

Dumbbell-shaped test pieces were cut from molded sheet approximately 2 mm thick. Tensile properties were measured at room temperature (26°C) using an Instron Universal Testing Machine, Model 4206, in the extension mode in accordance with test procedure ASTM D638-91. ASTM D638-91 Type I specimens were used in which the width of the narrow section was 13 mm, the length of the narrow section was 57 mm with gauge length of 50 mm and a crosshead speed of 50 mm/min. From the stress-strain curves obtained, the following properties (average of four specimens) were calculated: tensile strength at yield and elongation at maximum load.

Tensile strength at yield is the tensile stress at the yield point sustained by the specimen during a tension test. It can be defined as follows:

$$\text{Tensile strength at yield } (\sigma_y) = \frac{\text{Load at yield point}}{\text{Original cross-sectional area}}$$

The percentage of elongation at yield is the ratio, expressed in percentage, of the extension (change in gauge length) to the original gauge length at yield point. It can be calculated from the following equation:

$$\text{Percent elongation at yield } (\varepsilon_y) = \frac{\text{Extension}}{\text{Original gauge length}} \times 100$$

3.4.2 Melt Flow Index (MFI)

The changes in the molecular weight of the samples were determined indirectly, by means of MFI, using a ZWICK model 4150 Extrusion Plastometer. The MFI is inversely proportional to the molecular weight. MFI is the weight (in g/10min) of extruded material passing through an orifice (D = 2.1 mm, H = 8 mm) at 190°C under a load of 2.16 kg (ASTM D1238). The melt-flow index is calculated by weighing a specimen piece which was automatically cut off at the exit of the orifice after a given time by the extrudate cutter. An average was taken of five measurements.

3.4.3 Fourier Transform Infrared (FTIR) Spectroscopy

The extent of polyethylene oxidation was followed by measuring the level of ketone carbonyl (1715 cm^{-1}) absorbances using a BIO-RAD FTS 45A Fourier Transform Infrared (FTIR) spectrometer with 64 scans at a resolution of 4 cm^{-1}. A frequency range of 4000 - 400 cm^{-1} was observed using a deuterated triglucinesulfate detector (DTGS) with a specific detectivity, D*, of 1×10^{9} $cmHz^{1/2}W^{-1}$. The thickness of each piece of film was measured to 0.08 ± 0.007 mm. with a micrometer gauge. Polyethylene film, free from wrinkles, were mounted on standard FTIR sample plates using a removable magnetic cover or removable tape.

Carbonyl index (CI), defined as the ratio of carbonyl and methylene (1465 cm^{-1}) absorbances, was used to express the measured levels of carbonyl compounds (Albertsson et al., 1987)

$$CI = \frac{\text{Carbonyl absorbance } (1715\ cm^{-1})}{\text{Methylene absorbance } (1465\ cm^{-1})}$$

3.4.4 Scanning Electron Microscopy (SEM)

Changes in the surface morphology of the starch-HDPE films was examined by secondary electron images, using a JEOL JSM 5200 scanning electron microscope, SEM. The operating voltages were in the range of 10 - 25 kV. Prior to examination, the surface of the specimen was coated with a thin evaporated layer of gold under vacuum for 15 minutes in order to improve conductivity and prevent electron charging on the surface. Micrographs of the samples were taken at magnifications ranging from 500 to 3300 to identify cracks, holes and other changes on the surface of samples due to degradation.

3.4.5 Weight loss

The amount of starch removal from the films was determined by weighing the samples before and after every 3 hours of treatment.

- Weight loss with enzyme present was the amount of starch hydrolysed in the presence of ∝-amylase.
- Weight loss in the absence of enzyme was the amount of starch leached in buffer solution.
- The percentage weight loss due to starch hydrolysis was calculated using the following equation:

$$\% Wt_{st.\ hyd} = \% Wt_{enz} - \% Wt_{w/o\ enz}$$

Where:

$\% Wt_{st.\ hyd}$ = % weight loss due to starch hydrolysis

$\% Wt_{enz}$ = % weight loss with enzyme

$\% Wt_{w/o\ enz}$ = % weight loss without enzyme

- The percentage starch hydrolysis was calculated using the following equation:

$$\% \text{ Starch hydrolysis} = \frac{\% \text{ Wt}_{\text{st. hyd}}}{\text{Initial \% starch}} \times 100$$

Where:

$\% \text{ Wt}_{\text{st. Hyd}}$ = % weight loss of the specimen due to starch hydrolysis