

3. PROCEDURE

3.1 Materials

3.1.1 Clay Mineral

Commercial sodium activated bentonite Mac-Gel[®] (GRADE SAC), Na-BTN, with cationic exchange capacity (CEC) of 44.5 meq/100 g clay, was supplied by Thai Nippon Co., Ltd. Thailand.

3.1.2 Surfactant

Cetyl trimethyl ammonium bromide (CTAB) was purchased from Italmar Co., Ltd.

Dodecylamine (DDA) was purchased from Aldrich.

3.1.3 Chemical Substances

Tetraethoxysilane (TEOS) was purchased from Aldrich.

3.1.4 Polymer

Low density polyethylene under trade name (PETLIN LD C150Y) (MFI 5 dg/10min) was purchased from PETLIN (MALAYSIA) Sdn Bhd.

3.1.5 Compatibilizer

Sodium-neutralized ethylene-co-methacrylic acid (MFI = 4 dg/10min) under trade name Surlyn[®] PC350 was purchased from DuPont Co., Ltd.

3.1.6 Indicator

Bromothymol Blue ($C_{27}H_{28}Br_2O_5S$, MW 624.4 g/mol) was purchased from Ajax Finechem, Australia.

3.1.7 Reagent

Hydrochloric acid 37% was purchased from Calro Erba.

Boric acid was purchased from (H_3BO_3) was purchased from Calro Erba.

Potassium carbonate (K_2CO_3) was purchased from Calro Erba.

Trichloroacetic acid (TCA) was purchased from Calro Erba.

3.1.8 Solvent

Methanol was purchased from RCI Labscan Limited.

3.2 Equipment

3.2.1 X-ray Diffractometer (XRD)

The X-ray diffractometer using Bruker AXS model Diffractometer D8 was used to identify the interlayer spacing of Na-bentonite and modified clay. The experiment was performed in the 2θ range of 2-10 degrees with scan speed 2 degree/min and scan step 0.01 degree.

3.2.2 Fourier Transform Infrared Spectrometer (FT-IR)

The functional group of Na-bentonite and modified clay were identified by Nicolet Nexus 670 FTIR spectrometer. FTIR was carried out in the transmission mode with 64 scans between 4000-400 cm^{-1} at a resolution of 4 cm^{-1} .

3.2.3 Scanning Electron Microscope (SEM)

The surface morphology of Na-bentonite and modified clay were observed by using S-4800 field emission scanning electron microscope. Then, the dispersion of the PCH in polymer matrix was determined. The specimens were coated with platinum before observation.

3.2.4 Surface Area Analyser (SAA)

The pore size, surface area and pore volume of porous samples were measured by nitrogen adsorption-desorption isotherms on surface area analyzer using Quantachrome Autosorb-1. The samples were degassed at 250°C for 17 h in a vacuum furnace before analysis.

3.2.5 UV-Vis Spectrometer

The indicator dye (bromothymol blue) was dissolved in water to prepare standard solution. Optical absorption spectrum of standard solution and the solution of leaching of dye from nanocomposite film was observed by UV-Vis using Shimadzu Model UV-1800.

3.2.6 Twin Screw Extruder

The nanocomposites were prepared by using co-rotating twin-screw extruder (Labtech) with $L = 80$ and $D = 20$ mm. The operation temperature was performed at 170, 175, 180, 185, 190, 195, 200, 205, 210 and 215°C from hopper to die respectively and the screw speed was 25 rpm for prepared LDPE/Clay nanocomposites.

3.2.7 Compression Molding Machine

The nanocomposite films were prepared by compression molding machine using Wabash V50H. The mold containing the pellet was preheated at 130°C for 5 minutes and then compression at 5 tons of force for 5 minutes for prepared LDPE/Clay nanocomposite films. Then, the molds were cooled to 50°C.

3.2.8 Gas Permeability Testing

Oxygen Permeation Tester, Illinois model 8000, was used to determine the oxygen gas transmission rate through LDPE films and LDPE/clay nanocomposite films. Gas permeation experiment was investigated following the procedure described in ASTM D 3985-05 at 23°C. The films were prepared by compression molding and were cut into circular shape with 15 cm in diameter.

3.2.9 Calorimetric Spectrophotometer

The nanocomposite films were measured the color change by the HunterLab Model Colorflex with 45°/0° optical geometry and EasyMatch® QC software. The result was expressed as Hunter color system (L, a, b) values and total color difference (TCD) or ΔE . The nanocomposite films were cut into the circular shape with diameter of 4 cm. The TCD value was calculated by following equation:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

Where, ΔL = The brightness difference between sample and target
 Δa = The redness difference between sample and target
 Δb = The yellowness difference between sample and target

The target color is (93.13, -0.96, 1.69) corresponding to (L, a, b) for white standard color in Hunter system.

3.2.10 Thermogravimetric Analyzer (TGA)

Thermogravimetric analysis (TGA) was used to study the thermal stability of LDPE/clay nanocomposite films compared to LDPE films. The degradation temperature of samples was determined by Perkin Elmer Pyris Diamond TG/DTA instrument. The pellets were loaded on platinum pan and heated from 30 to 900°C at heating rate 10°C/min and flow under N₂ 200 ml/min.

3.2.11 Differential Scanning Calorimeter (DSC)

The crystallization and melting behavior of LDPE/clay nanocomposite films compared to LDPE films were measured by Differential Scanning Calorimeter (DSC) using Met.tler DSC822 STARe System. First, the pellet samples was heated from 30°C to 200°C at heating rate of 10°C/min in order to eliminate the influence of thermal history and then cooled down from 200°C to 30°C to observe melt crystallization behavior. After that, reheated to 200°C to observe melting behavior.

3.2.12 Lloyd Universal Testing Machine

Tensile test of nanocomposite films was measured under ASTM D 683. The specimen was cut into rectangular shape with 10x100 mm and cross head speed of 50 mm/min.

3.3 Methodology

3.3.1 Preparation of Climacteric Fruit Freshness Indicator

3.3.1.1 *Preparation of Porous Clay Heterostructure (PCH)*

The obtained organoclay prepared with the surfactant (Cetyl trimethyl ammonium chloride, CTAC) was added to a neutral amine such as dodecylamine and tetraethoxysilane (TEOS) in the follow molar ratio: organoclay/dodecylamine/TEOS = 1/20/150. In first step, organoclay was mixed with dodecylamine and stirred at 50°C for 30 min. After that tetraethoxysilane (TEOS) was added to the mixture allowed reacting for 4 h at room temperature under continuous stirring. After reaction, the modified clay was filtrated from the solution and washed with methanol. The modified clay was dried at room temperature for 24 h. For extraction process, 1 g of as-synthesized PCH was added to 45 ml of methanol and 5 ml of concentrated HCl and refluxed for 2 h. Then, the modified clay was filtered off and washed with methanol and water. After that, it was air-dried at room temperature overnight before screened through a mesh #325.

The interlayer spacing of modified clay was studied by small angle X-ray diffractometer. The intercalation of cationic surfactant between the layers of bentonite was studied using FTIR spectra.

The surface morphology of porous clay compared to the pristine clay was observed by SEM images. The pore diameter, pore volume and surface area of porous clay compared to the pristine clay were characterized by surface area analyzer.

3.3.1.2 Preparation of Chromophores Modified PCH

The PCH was modified with pH dye (bromothymol blue) at weight ratio of PCH: dye of 10:1, 20:1 and 30:1. First, bromothymol blue was dissolved in solution of 0.05 M NaOH. Then, bromothymol blue solution was added into the PCH under continuous stirring to obtain chromophores modified PCH. After that the modified clay was filtered off and dried in vacuum oven before screened through a mesh #325. The bromothymol blue modified PCH was named PCH-BTB.

The morphology of PCH-BTB was observed by SEM images. The results of pore diameter, pore volume and surface area after incorporated bromothymol blue onto PCH were measured by surface area analyzer.

3.3.1.3 Preparation of Nanocomposite Film

The nanocomposite was prepared by using twin-screw extruder (Labtech) with $L = 80$ and $D = 20$ mm. The operation temperature was performed at 130°C from hopper to die respectively and the screw speed was 25 rpm.

First, low density polyethylene (LDPE) was blended with 6%wt surlyn[®] in order to obtain LDPE/surlyn[®] pellet. Then, LDPE/surlyn[®] pellet was mixed with 2 %wt of chromophores modified PCH to obtain the nanocomposite. Each composition was dried in vacuum oven for moisture removal and premixed in tumble mixer before extruded through the twin screw extruder. Then, the extruded nanocomposite was quenched immediately in water and pelletized. The obtained pellet was dried in vacuum oven.

Nanocomposite films of LDPE/chromophores modified PCH was prepared by compression molding machine at 5 tons of force for 5 minutes. The processing temperature was 130°C .

3.3.1.4 Indicator Films Response to Standard Carbon dioxide

The main products from respiration of climacteric fruits such as carbon dioxide and water were used to test the sensitivity of color response of indicator films. The indicator film was placed in glass chamber with contained

standard carbon dioxide and water. Standard carbon dioxide in various concentrations (0, 30, 60, 90, 120 and 150 ppm) and the excess water was prepared into 600 ml glass chamber. Then, the glass chamber was closed by the rubber stopper with the rubber septum in the middle of the stopper. Color changes of indicator film at weight ratio of PCH: dye of 10:1, 20:1 and 30:1 after reacted with carbon dioxide and water was measured by the calorimetric spectrophotometer. The result was expressed as Hunter color system (L, a, b) values and total color difference (TCD) or ΔE .

3.3.1.5 Leaching Studies

Indicator dye (bromothymol blue) was dissolved in water to prepare standard solutions of 1, 2, 5 and 10 ppm. Then the absorbance of standard solution was investigated by UV-Vis Spectroscopy (Shimadzu Model UV-1800). The indicator nanocomposite films were cut into the rectangular shape with 4x4 cm and soaked with 10 ml of water in a small container for approximately 48 h. Then, the wavelength spectrum in range of 400 to 700 nm was carried out to detect the presence of bromothymol blue.