

CHAPTER III

EXPERIMENTAL PROCEDURES

3.1 Materials

3.1.1 Clay Mineral

The commercial sodium activated bentonite Mac-Gel[®] (GRADE SAC), Na-BTN, with cationic exchange capacity (CEC) of 49.74 meq/100 g clay and surface area of 31.0 m²/g, supplied by Thai Nippon Chemical Industry Co., Ltd. Thailand, was purified before use.

3.1.2 Polypropylene

The commercial polypropylene powder (PP) under trade name 2300K (MFI 4 dg/10 min) supported from T.H.L. Industry Co., Ltd. was used as received. The properties of resin provided are reported in the Table 3.1.

Table 3.1 Resin properties of polypropylene (PP) powder 2300K

Physical properties	Value	Unit	Method
Melt Flow Index	4	g/10min	DIN 53735
Tensile Strength at Yield	29	N/mm ²	DIN 53455
Modulus of Elasticity	1300	N/mm ²	DIN 53457
Impact Strength (at 0 °C, -20 °C)	70, 30	kJ/m ²	DIN 53453
Notched Impact Strength (at 23 °C, 20 °C)	6.0, 3.0	kJ/m ²	DIN 53453
Heat Distortion Temperature (Load 0.46 N/MM ²)	80	°C	DIN 53461

The homopolymer polypropylene (PP) pellet under trade name POLIMAXX NK1126 IRPC LOT 0111204058 supplied by May Enterprises Co., Ltd. was used as received. The properties of resin provided are reported in the Table 3.2.

Table 3.2 Resin properties of polypropylene (PP) NK 1126

Physical properties	Value	Unit	Method
Melt Flow Index (2.16 kg/230°C)	11	g/10min	ASTM D1238
Tensile Strength at Yield	35	N/mm ²	ASTM D638
Elongation at Yield	29	%	ASTM D638
Charpy Notched Impact Strength (at 23 °C)	3	mJ/mm ²	DIN 53453
Flexural Modulus (1% SECANT)	1450	MPa	ASTM D790
Rockwell Hardness	105	R-Scale	ASTM D785
Heat Distortion Temperature (Load 0.45 N/MM ²)	100	°C	ASTM D 648

The Processing temperature is 100–200°C. However, the actual processing conditions depend on mold design, power of machine, equipment and other environments.

3.1.3 Ethylene Vinyl Acetate

Ethylene Vinyl Acetate, EVA pellet under trade name Polene MV1055 (18% vinyl acetate) LOT 12080161 was supplied by TPI POLENE Public Company (TPIPL).

3.1.4 Dry Sappan Heartwood

Dry sappan wood (*Caesalpinia sappan* Linn.) powder was purchased from a traditional drug store in Singburi, Thailand.

3.2 Chemicals

3.2.1 Silver (I) nitrate

Silver (I) nitrate, AgNO₃ was purchased from Carlo Erba and used as received. CAS NO. 7761-88-8

3.2.2 tri-Sodium citrate dihydrate

Tri-sodium citrate dihydrate was purchased from Carlo Erba and used as received. CAS NO. 6132-04-3

3.2.3 Dicumyl peroxide (DCP)

Dicumyl peroxide, DCP was purchased from Fluka and used as received. CAS NO. 80-43-3

3.2.4 γ -Methacryloxypropyltrimethoxysilane (MPS)

γ -Methacryloxypropyltrimethoxysilane, MPS was purchased from Aldrich and used as received. CAS NO. 2530-85-0

3.2.5 Ethanol

Absolute ethanol, AR grade was purchased from RCI Labscan and used as received. CAS NO. 64-17-5

3.2.6 Glacial acetic acid

Glacial acetic acid, AR grade was purchased from RCI Labscan and used as received. CAS NO. 64-17-5

3.3 **Instruments**

3.3.1 Ball mill (FRITSCH, pulverisette 6)

3.3.2 Mechanical Stirrer (ALC, quiet-SL)

3.3.3 pH Meter (EUTECH, pH510)

3.3.4 Centrifugator (KUBOTA, 5922/220V)

3.3.5 Soxhlet (VELP Scientifica, SER 148)

3.3.6 Vacuum Oven (Medcenter Einrichtungen GmbH, MMM)

3.3.7 Plasma Generator (PT-1, Nantong Sanxin Plastics Equipment)

3.3.8 Co-rotating twin screw extruder (Labtech. types LTE-20-32 & LTE-20-40)

3.3.9 Compression molding machine (Labtech, type LP 50)

3.3.10 Blown film extrusion machine (Labtech, type LTE-20-30)

3.3.11 Field Emission-Scanning Electron Microscope, FE-SEM (HITACHI, S-4800)

3.3.12 UV-VIS spectrophotometer (SHIMADZU, UV-1800)

- 3.3.13 FTIR spectrometer (Thermo Nicolet, Nexus 670)
- 3.3.14 X-ray Diffraction Microscope, XRD (Rigaku, Dmax 2002)
- 3.3.15 Differential Scanning Calorimeter, DSC (Mettler Toledo, DSC 822e)
- 3.3.16 Thermogravimetric Analyzer, TGA (Pyris Diamond, TG/DTA)
- 3.3.17 Thickness gauge (Peacock-PDP1, PDN-20)
- 3.3.18 Universal Testing Machine (Lloyd, LRX)
- 3.3.19 Oxygen Permeation Analyzer (Illinois Instrument, Model 8500)
- 3.3.20 Vapor Permeation Tester (LYSSY, Model L80-4000)
- 3.3.21 Color Reader (HunterLab, ColorFlex EZ)

3.4 Methodology

3.4.1 Preparation of Purified Clay

The sodium bentonite clay was first vigorously stirred into a deionized water at 700 rpm overnight by a mechanical stirrer. After that, the supernatant (swollen clay) was separated by centrifugation at 10,000 rpm for 15 minutes and lastly dried, sieved by mesh#400 and kept in a desiccator before use.

3.4.2 Modification of Bentonite (BEN)

1.5 wt.% of MPS solution was prepared in 70 vol.% of ethanol aqueous solution pH 4 adjusted by glacial acetic acid and then stirred for 45 min of hydrolysis to silanol. 50 g of bentonite were added respectively into MPS solution. The mixture was stirred at 110 °C for 24 hr. The plenty of absolute ethanol was used for the removal of excessive MPS during suction filtrating and the modified clay (MBEN) was finally dried at 80 °C in vacuum oven for 24 hr. The clay was pulverized by ball mill and then sieved by mesh#400 and kept in a desiccator prior to use.

3.4.3 Preparation of PP-Clay Nanocomposite Masterbatch via Plasma-based Process and Chemical Reaction

The PP and modified clay were mixed in the co-rotating twin-screw extruder (Labtech types LTE-20-32 & LTE-20-40 screw diameter 20 mm) with L/D ratio 40:1, named "PPC-MPS". The operation temperature was performed at 100, 180, 185, 190, 190, 190, 190, 195, 200, and 200 °C from hopper to die, respectively

and the screw speed at 10 rpm. Secondly, the PP and modified clay were mixed in the same co-rotating twin-screw as well as at the same screw speed and processing temperatures as the former does. After exiting die, the extrudate of nanocomposites was immediately treated by the plasma generator (PT-1 PLASMA TREATER) operated at 6 kV and 10 kHz for 5 sec at the distance of 4 cm from its nozzle to the extrudate (Figure 3.1), so-called “PPC-PLASMA”. Meanwhile, the others called “PPC-0.1DCP, PPC-0.2DCP, and PPC-0.3DCP” represented the nanocomposites which were prepared by mixing PP, modified clay including 0.1, 0.2, 0.3 phr of DCP in the same extruder at the same processing temperatures and screw speed as the plasma-based process was previously carried out. In conclusion, the formulations of nanocomposites designed in this procedure are reported in the Table 3.3.

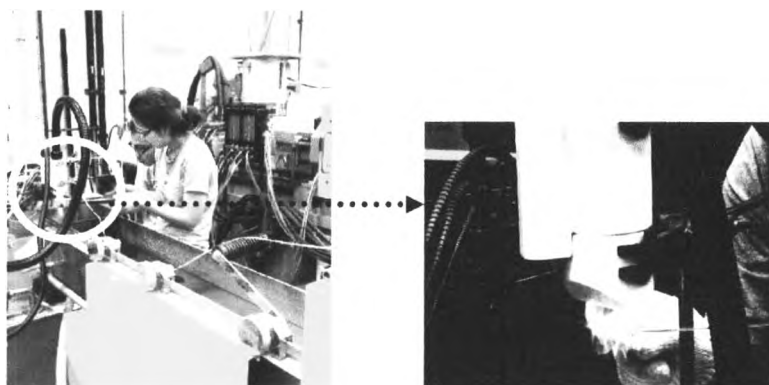


Figure 3.1 Plasma treater unit.

Table 3.3 Designing formulations of nanocomposite compounds

SAMPLE CODE	PP (wt%)	Modified Bentonite (wt%)
PP	100	-
PPC-MPS	95	5
¹ PPC-PLASMA	95	5
² PPC-0.1DCP	95	5
³ PPC-0.2DCP	95	5
⁴ PPC-0.3DCP	95	5

¹ prepared by the plasma process

^{2, 3, and 4} prepared by the chemical process

3.4.4 Preparation of Silver Nanoparticle-Loaded Clay (SBEN)

50 g of purified bentonite were firstly stirred in AgNO₃ solution at room temperature overnight. The silver-ion clay was then reacted with C₆H₅Na₃O₇·2H₂O at 1 : 3 molar ratio of AgNO₃ to C₆H₅Na₃O₇·2H₂O at 90 °C for 20 min to obtain a silver nanoparticles clay (SBEN). The as-synthesized silver nanoparticle-loaded clay was measured their shape and size by a transmission electron microscope (TEM) as well as their crystal structure by an X-ray diffractometer (XRD).

3.4.5 Modification of Silver Nanoparticle-Loaded Clay (MSBEN)

The as-prepared silver nanoparticle-loaded bentonite (SBEN) was modified via silanation process. 1.5 wt.% of MPS solution was prepared in 70 vol.% of ethanol aqueous solution pH 4 adjusted by glacial acetic acid and then stirred for 45 min of hydrolysis to silanol. 50 g of the clay were added respectively into the MPS solution. The mixture was stirred at 110 °C for 24 hr. The plenty of absolute ethanol was used for the removal of excessive MPS during suction filtrating and the modified clay was finally dried at 80 °C in vacuum oven for 24 hr. The clay was pulverized by ball mill and then sieved by mesh#400 and kept in a desiccator prior to use.

3.4.6 Preparation of Polypropylene-Modified Bentonite Masterbatch and Polypropylene-Modified Silver Nanoparticles-Loaded Bentonite Masterbatch via a Plasma-based Processing

Both the PP and MBEN (3.4.2) and PP and MSBEN (3.4.3) were mixed in the co-rotating twin-screw extruder (Labtech types LTE-20-32 & LTE-20-40 screw diameter 20 mm) with L/D ratio 40:1. The operation temperature was carried out at 100, 180, 185, 190, 190, 190, 190, 195, 200, and 200 °C from hopper to die, respectively and the screw speed at 10 rpm. During exiting die, the melt extrudate of each of the compounds was immediately treated by the plasma generator (PT-1 PLASMA TREATER) operated at 6 kV, 10 kHz for 5 sec at the distance of 4 cm from its nozzle to the extrudate (Figure 3.1).

3.4.7 Preparation of Active Films

The masterbatch of PP-modified bentonite (PPC-PLASMA) and PP-modified silver nanoparticles-loaded bentonite was further mixed with PP pellet in the ratio of PP : modified clay = 99 : 1 wt% (each compounds containing 1 wt.% of modified clay) in the co-rotating twin-screw extruder (Labtech types LTE-20-32 & LTE-20-40 screw diameter 20 mm) with L/D ratio 40:1. The operation temperature was performed at 100, 180, 185, 190, 195, 195, 195, 195, 200, and 200 °C from hopper to die, respectively and the screw speed at 30 rpm. Each of the nanocomposites was transformed into active films by using a Labtech (model LTE20-30) single-screw extruder with L/D = 40 (D = 20 mm and L = 800) and annular die with outer diameter of 70 mm and inner diameter of 68.5 mm. The operating temperature of blown film extrusion machine was operated in the range of 180 – 200 °C and the rotational screw speed was fixed at 60 rpm with the blow up ratio of 2 : 1.

3.4.8 Preparation of natural dye-carboxy methyl cellulose (SAP-CMC)

The dried sappan heartwood powder was extracted in a deionized water at the ratio of 1:4 (w/v). After that, the solid residue was filtered out. 200 g of carboxymethyl cellulose (CMC) was mixed with the dye extract at the ratio of 0.5:100 (w/v) and finally spray dried at 150 °C to sappan dyed-carboxymethyl cellulose (SAP-CMC) powder.

3.4.9 Fabrication of pH Indicator films based on EVA-SAP-CMC

The Dyed-CMC powder and ethylene vinyl acetate pellet were dried in a vacuum oven at 60 °C for 24 hours prior to use. The ethylene vinyl acetate (EVA) were melt-mixed with 1, 3, and 5 wt% of the Dyed-CMCs using 5 phr of the ethylene glycidyl methacrylate (EGMA) as a compatibilizer via the co-rotating twin screw extruder (Labtech types LTE-20-32 & LTE-20-40) with an L/D ratio of 40 and screw diameter of 20 mm. The temperature profiles of the barrel were 95 – 105 °C from the hopper to the die, and the screw speed was 30 rpm. Afterward, the extruded pellets were dried in a vacuum oven at 60 °C for 24 hours before being hot-compressed into thin film of ~150 µm in thickness. The composite was hot-compressed using compression molding machine (Labtech type LP 50) at 140 °C by pre-heating time of 15 min, compressing time of 5 min at pressure of 1500 psi., and cooling time of 5 min to thin film of sensor.

3.4.10 The Response of the Sensor Films to Standard Ammonia

According to the Figure 3.2, 25 ml of standard ammonia solutions in various concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 1, 5, 10, 15, 20, 25, 30, and 35 mg/mL) were pipetted into the polypropylene cup which was sealed to prevent the leakage of ammonia. Sensor films were faced-down inside the cup. The experiment was kept at room temperature for 6 hours. The color response of films at was measured by a color reader (HunterLab, ColorFlex EZ). Three different areas for each film were recorded. The result was recorded on Hunter color system, in which the outcome was expressed as the Hunter color L, a, b values and total color difference (TCD) or ΔE . The TCD value was calculated by following equation:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (\text{Eq. 3.1})$$

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 the Hunter system.

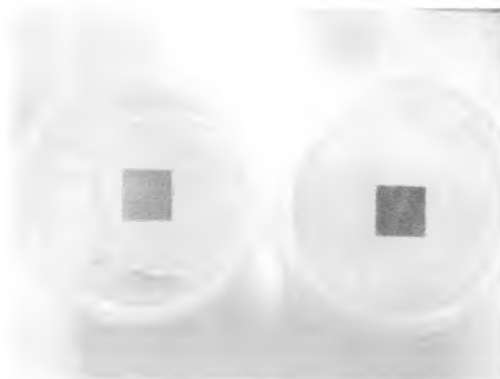


Figure 3.2 Experimental design for the response of the sensor films to different concentrations of standard ammonia (left) the original color of a sensor film and (right) the color change of sensor film at the ammonia concentration of 10 mg/mL after 6 hr of test time.

3.4.11 The Response of the Sensor Films to Fresh Fish Storage

The experimental design was set up according to the Figure 3.3. A fresh white perch (*Oreochromis niloticus* L.) approximately 800 g was selected for the experiment. The indicator film was laminated within polypropylene film (6 cm x 9 cm) with containing 100 g of fresh fish. The sensor was laminated on the PP film by distance of 1/3 of PP film. The packaging was sealed to prevent the leakage of TVBN. The color change of indicator film after the sample started to deteriorate was observed at 3, 6, 9, and 12 hours during the storage.

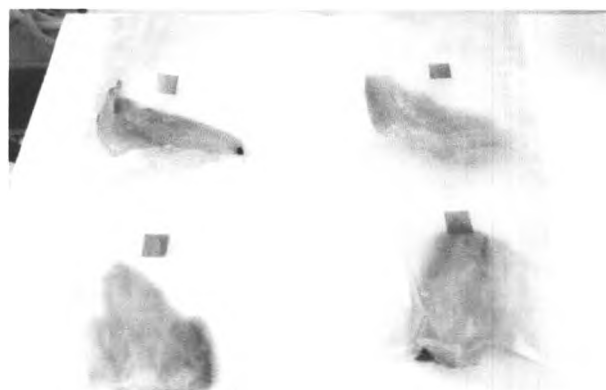


Figure 3.3 Experimental design for fish spoilage test.

3.4.12 Leaching studies

Indicator dye (SAP-CMC) was dissolved in water to prepare standard solutions of 1, 2, 5, 10, 20, and 100 ppm. The absorbance of standard solution was then investigated by the UV-Vis Spectrophotometer (Shimadzu Model UV-1800). The sensor films were cut into the rectangular shape with 2 x 2 cm and soaked in 30 mL of deionized water in a small container for approximately 24 hr as designed for “ Direct leaching studies ” (Figure 3.4). Another experiment was carried out by attaching a faced-down sensor film on the lid of PP cup containing 30 mL of deionized water for 24 hr, designated as “ Indirect leaching studies ” (Figure 3.5). Afterward, the wavelength spectrum in range of 400 to 700 nm was carried out to detect the presence of pH dye. Each of the leaching studies was performed in 3 replication experiments.

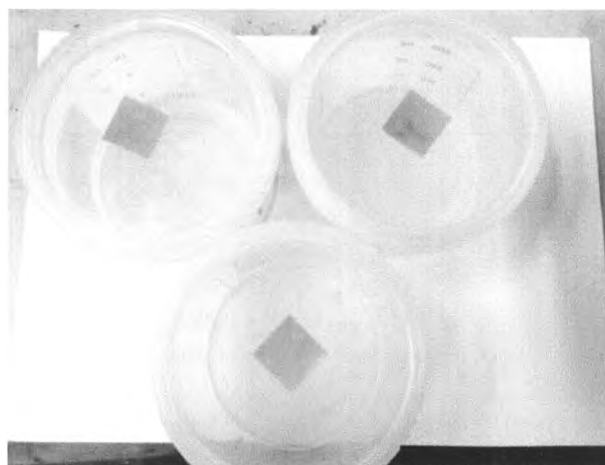


Figure 3.4 Experimental design set up for fish spoilage test designated as “Direct leaching study”.



Figure 3.5 Experimental design set up for fish spoilage test designated as “Indirect leaching study”.

3.5 Characterization

3.5.1 Field Emission-Scanning Electron Microscope (FE-SEM)

For the Chapter IV, the dispersion of bentonite in polymer matrix was determined by using an FE-SEM (HITACHI, S-4800) coupled with energy dispersive X-ray spectrometer (EDS). The selected samples were dipped and fractured in liquid nitrogen. Then the samples were sputtered with platinum before viewing under an FE-SEM/EDS operated at 20 kV.

In the Chapter VI, for the nanocomposite extrudates, the dispersion of bentonite in polymer matrix was determined by using an FE-SEM (HITACHI, S-4800) coupled with energy dispersive X-ray spectrometer (EDS). The selected samples were dipped and fractured in liquid nitrogen. Then the samples were sputtered with platinum before viewing under the FE-SEM/EDS operated at 20 kV with the magnifications of 1K and 4.5K.

In part of nanocomposite films, the cryogenic-fractured surface of cross-sectional area of the films was investigated by using an SEM (HITACHI, S-2500). The selected films were dipped and fractured in liquid nitrogen. The samples were then sputtered with platinum before viewing under an SEM operated at 15 kV with the magnification of 10K.

3.5.2 X-ray Diffractometer (XRD)

The crystal structure of bentonite and silver nanoparticles in bentonite was analyzed by wide angle X-ray diffraction (WAXD) using a Bruker AXS model Diffractometer D8 with Ni-filtered Cu K_α radiation operated at 40 kV and 30 mA. The experiment was performed in the 2θ range of 5 – 80 degrees with scan speed 2 degree/min and scan step 0.01 degree.

The crystal structure of PP-modified clay nanocomposites including the nanocomposites containing various contents of silver nanoparticles was also analyzed by small angle X-ray diffraction (SAXD) using a Rigaku Model Dmax 2200 Ultima+/Cu lamp performed in the 2θ range of 2 – 80 degrees with the same scan speed and scan step as performed in the nanoclay.

3.5.3 Fourier Transform Infrared Spectrometer (FT-IR)

The FT-IR spectra of bentonite and modified bentonite were collected by using a Nicolet Nexus 670 FT-IR spectrometer over a wave number range of 4,000 – 400 cm⁻¹ with 32 scans at a resolution of 2 cm⁻¹.

3.5.4 Differential Scanning Calorimeter (DSC)

The crystallization and melting behaviors of the active films were measured with a Mettler Toledo, DSC 822e analyzer. All operations were performed under a nitrogen atmosphere. The samples were first heated from 30°C to 200°C at a heating rate of 10°C/min in order to eliminate the influence of thermal history and then cooled down at a rate of 10°C/min from 200°C to 30°C to observe the melt crystallization behavior and lastly heated up from 30°C to 200°C at a heating rate of 10°C/min so as to observe the melt behavior. The crystallinity can be calculated with the following formula:

3.5.5 Thermogravimetric Analysis (TGA)

The samples were analyzed by TGA using a Perkin-Elmer Pyris Diamond TG/DTA instrument under N₂ flow of 200 ml/min. The heating process was conducted from 30 to 900°C at a rate of 10°C/min.

3.5.6 Lloyd Universal Testing Machine

Tensile test of the active film (45 μm in thickness) by blown film extrusion machine operated according to ASTM D882 was carried out by Lloyd

Universal Testing Machine. The specimen was cut in machine direction into rectangular shape with 100 x 100 mm. Crosshead speed is 50 mm/min.

3.5.7 Gas Permeability Testing

Vapor Permeation Tester Model L80-4000, LYSSY was used to determine water vapor permeability of neat PP and PP nanocomposite films. Water vapor permeation experiments were investigated following procedure described in ASTM E398. The test was performed at 38 °C with water vapor pressure of 49.7 mmHg. The blown films were cut into circular shape with 15 cm in diameter. The thickness of films was measured using peacock digital thickness guage PDN 12N by reading 15 points at random position over test area.

Oxygen Permeation Analyzer Model 8500, Illinois Instrument Inc., was used to determine oxygen permeability of neat PP and PP nanocomposite blown films. Gas permeation experiments were investigated following procedure described in ASTM D3985. The test was carried out at 25 °C with oxygen flow rate of 40 cm²/min. The blown films were cut into circular shape with 11.3 cm in diameter. The thickness of films was measured using peacock digital thickness guage PDN 12N by reading 15 points at random position over test area.

3.5.8 Antifungal test of active film

Active films were tested by the agar diffusion technique. The method was carried out in Luria-Bertani (LB) medium solid agar Petri dish. PP-clay nanocomposite films were cut into a disc shape of 2.0 x 2.0 cm² and then sterilized by autoclaving at 120 °C for 15 minutes and finally placed on *Escherichia coli* and *Staphylococcus aureus*-cultured agar plates, which would be then incubated at 37 °C for 24 hours. The zone of inhibition or clear zone would be measured and recorded.

3.5.9 Color Reader

The pH-indicator film was cut in rectangular shape with 2x2 cm before measurement. The color changes of the indicator were measured through the HunterLab Model Colorflex with 45°/0° optical geometry and EasyMatch[®] QC software. The result expressed as Hunter system (*L**, *a**, and *b**) values and total color difference (TCD, ΔE) (Tassanawat *et al.*, 2007).

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (\text{Eq. 3.1})$$

Where, ΔL is the brightness difference between sample and target ($+\Delta L^*$ = lighter and $-\Delta L^*$ = darker), Δa^* is the redness difference between sample and target ($+\Delta a^*$ = redder and $-\Delta a^*$ = greener), and Δb^* is the yellowness difference between sample and target ($+\Delta b^*$ = yellower and $-\Delta b^*$ = bluer) (Hunter Associates Laboratory, 2008).