CHAPTER III

EXPERIMENTAL

## 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

Dipalmitoylethy Hydroxyethylmonium Methosulfate under trade name Stepantex™ SP-90 shown in Figure 3.1 was purchased from Sunny World Co., Ltd.

$$C_{15}H_{31}\text{-}C\text{-}OCH_2CH_2 \diagdown \diagup CH_3$$
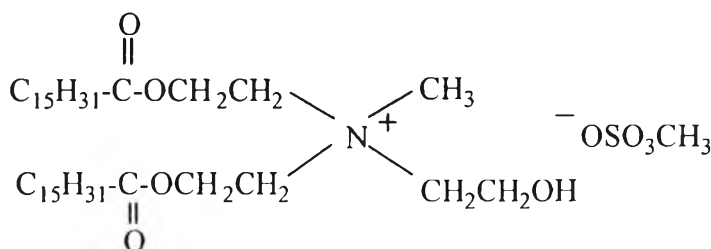
Figure 3.1 Chemical structure of STPANTEX™ SP-90.

3.1.3 Polymer

Polypropylene under trade name Polene NK 1126 (MFI 11 dg/min) was purchased from Thai Petrochemical Industry Public Co., Ltd (IRPC Co., Ltd).

3.1.4 Compatibilizer

Sodium-neutralized ethylene-co-methacrylic acid with MFI = 4 dg/min, under the trade name Surlyn® PC350, was purchased from DuPont Co Ltd.

3.1.5 Indicator

Bromocresol green ($C_{21}H_{14}O_5Br_4S$, M_w 698.02 g mol^{-1}), phenol red ($C_{19}H_{14}O_5S$, M_w 354.37 g mol^{-1}) was purchased from Labchem Chemical Co., Ltd.

3.1.6 Binder

Cellulose acetate (M_w 29,000 g mol^{-1}) was purchased from A.C.S.Xenon Limited Partnership.

3.1.7 Plasticizer

Dibutyl sebacate (DBP) was purchased from A.C.S.Xenon Limited Partnership.

3.1.8 Reagent

Boric acid (M_w 61.83 g mol^{-1}) was purchased from Merck.

Hydrochloric acid (HCl) 0.1M was purchased from Fisher Scitific.

Potassium carbonate (K_2CO_3) was purchased from Merck.

Trichloroacetic acid (TCA) was purchased from Carlo Erba Reagent.

3.1.9 Solvent

Ethanol, AR, 2.5 L was purchased from Carlo Erba Reagent.

Acetone, AR, 2.5 L was purchased from Carlo Erba Reagent.

3.1.10 Sample

Fresh Barramundi or Giant Perch was obtained from Sam yarn Market and filleted with aseptic techniques such as the use of disposable gloves, bactericide built-in cutting board, and flame sterilized scalpel were used to avoid sample contamination.

3.2 Equipment

3.2.1 X-ray Diffractometer (XRD)

The structure of organobentonite and its nanocomposites in PP was analyzed by wide angle X-ray diffraction (WAXD) using a Rigaku Model Dmax 2002 diffractometer with Ni-filtered Cu K_α radiation operated at 40 kV and 30 mA. The experiment was performed in the 2θ range of 2-20 degrees with scan speed 2 degree/min and scan step 0.01 degree. The crystal structures of PP/organoclay nanocompostes were also analyzed by Wide angle X-ray diffraction (WAXD) using a Ri-

gaku Model Dmax 2002 performed in the 2θ range of 2-40 degrees with the same scan speed and scan step as performed in organoclays.

3.2.2 Thermogravimetric Analysis (TGA)

- Thermogravimetric analysis (TGA) was used to study thermal stability of organomodified bentonite and PP/organoclay nanocomposites as compared to the pure PP. DTA curves-TG, collected on a Perkin-Elmer Pyris Diamond TG/DTA instrument. The degradation temperature, initial degradation temperature, weight loss, and final degradation temperature of the samples were determined. The nanocomposite pellets from twin screw extruder as well as PP pellets were loaded on platinum pan and heated from 30°C to 90°C at heating rate 10°C/min and flow under N_2 200 ml/min.

3.2.3 Differential Scanning Calorimeter (DSC)

The crystallization and melting behaviors of the PP/organoclay nanocomposites were measured with a Perkin-Elmer DSC 7 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.

3.2.4 Fourier Transform Infrared Spectrometer (FT-IR)

The FT-IR spectra of organoclay and nanocomposite film samples 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}.

3.2.5 Lloyd Universal Testing Machine

Tensile test of PP/organomodified bentonite nanocomposites films by blown film extrusion machine from Tang Packaging Co., of thickness of 45 µm. Samples operated according to ASTM D 882 were carried out by Lloyd Universal Testing Machine. The specimen was cut in machine direction into rectangular shape with 10x100 mm. Crosshead speed is 50 mm/mim.

3.2.6 Chroma Meter

The color changes of the indicator were measured through the HunterLab Model Colorflex with 45°/0° optical geometry and EasyMatch QC soft-

ware. The result expressed as Hunter system *(L, a, and b)* values and total color difference (TCD). The nanocomposite pH sensor was cut in circular shape with diameter of 5 cm.

3.2.7 Twin Screw Extruder

PP/organoclay nanocomposites were prepared by Collin D-8017 T20 twin screw extruder with L/D ratio of 30 and 25-mm-diameter. The operating temperatures were maintained at $80/160/180/190/200/210^\circ C$ from feed to die with screw speed of 50 rpm. Then the pellet samples were analyzed by TGA, DSC, and SEM.

3.2.8 Compression Molding Machine

The same thickness of 150 μm of PP/organoclay nanocomposites thin films were prepared by a Wabash V50H 50 ton compression molding machine. The mold containing the pellets was preheated at $200^\circ C$ for 5 minutes, and then compressed under 10 tons of force for 5 minutes. Then the molding was cooling to $50^\circ C$ under pressure. After that samples were cut into circular shape with 15 cm in diameter (150μm) for determining gas permeability and cut into rectangular shape with 2x2 cm for XRD analyzing. The thickness of the films was measured by using the peacock digital thickness gauge model PDN 12N by reading 15 points at random position over tested area and the results were averaged.

3.2.9 Blow Film Extrusion Machine

PP/organomodified bentonite nanocomposite films were prepared by blown film extrusion machine at Tang Packaging Co., Ltd. The screw speed was 50 rpm, die diameter of 150 mm, screw diameter was 45 mm, L/D was 26 and the processing temperature was $210^\circ C$. Then samples were analyzed by tensile testing and laminated with sensor disc to generate nanocomposite pH sensor film.

3.2.10 Centrifugal Ball Mill

Dried sediments were ground by FRITSCH Peluerisette 6 Centrifugal Ball Mill with rotational speed of 450 rpm in forward and reverse milling directions for 1 hr. The particle size of the power was less than 44 μm.

3.2.11 Scannig Electron Microscope

The dispersion of organomodified bentonite in polymer matrix was determined by using SEM (JEOL/JEM 5800 LV). The selected samples were dipped and fractured in liquid nitrogen. Then the samples were sputtered with gold before viewing under a scanning electron microscope (SEM) operating at 15 kV.

3.2.12 Gas Permeability Testing

Gas permeability tester, Oxygen Permeation Analyzer Model 8000, Illinois Instrument Inc., was used to determine oxygen permeability constants of pristine PP and nanocomposite films. Gas permeation experiment was investigated following the procedure described in ASTM D1434. The condition was proceed at 23 °C with oxygen flow rate is 50 cm^2/min. The films were prepared from a compression-molded sheet with the same thickness of 150 μm and were cut into circular shape with 15 cm in diameter. The thickness of the films was measured by using the peacock digital thickness gauge model PDN 12N by reading 15 points at random position over tested area and the results were averaged.

3.2.13 UV-Vis Spectrometer

The indicator dyes were dissolved in water to prepare the standard solution of 1, 2, 5, and 10 ppm. The absorbance at 615 nm of the indicator dye standard solutions was observed by UV-vis spectroscopy (SHIMADZU model UV-2550) with medium scan rate and sampling the data every 1 nm.

3.3 Methodology

3.3.1 Preparation of Organomodified Bentonite

350 g of Na-bentonite, (Mac-Gel® GRADE SAC) supplied by Thai Nippon Co., Ltd. Thailand, was swollen in water 1.05 liter for 24 hr. STPANTEX™ SP-90 as an alkyl ammonium ion (2.0CEC) was dissolved in 100 ml of ethanol. The whole swollen clay was then mixed with STPANTEX™ SP-90 solution with vigorous stirring for 2 hr at 80°C. After that the mixture was homogenized stirring for 20 min at 80°C. The sediment was filtrated and washed with hot water several times to remove to excess salts. It was dried in a vacuum oven at 100°C overnight and ground

into powder using centrifugal ball mill (FRITSCH Peluerisette 6) before being screened through a mesh [#]325.

3.3.2 Characterizations of Organomodified Bentonite

The variations of the interlayer spacing of Na-BTN and organo-bentonite were studied by wide-angle X-ray scattering. The thermal stability was determined by thermogravimetric analysis. The intercalation of the cationic surfactant was studied using infrared spectra collected using an FTIR spectrometer.

3.3.3 Preparation of PP/Organoclay Nanocomposites

The master batches containing 50 wt% organomodified clays with compatibilizer, Surlyn®, were initially prepared using a Model T-20 co-rotating twin-screw extruder (Collin) with L/D=30 and D=25 mm. The operating temperatures of extruder were performed at 80, 170, 180, 190, 200, and 210°C from hopper to die, respectively, with 50 rpm of screw speed.

The master batch was then added to PP and Surlyn® in appropriate amounts to obtain nominal contents of 1, 3, and 5 wt% clays in the nanocomposites. In this preparation, the compatibilizer was fixed at 6 wt%. Each composition was dried in a vacuum oven at 80°C for 12 h for moisture removal and then premixed by a tumble mixer for 10 min before introducing into the twin-screw extruder by Collin D-8017 T20 twin screw extruder with L/D ratio of 30 and 25-mm-diameter. The operating temperatures were maintained at 80/160/180/190/200/210°C from feed to die with screw speed of 50 rpm to yield strands of the nanocomposites. Then, the extruded thick strand was quenched immediately in water, and pelletized.

PP/organoclay nanocomposites films were prepared by blow film extrusion machine at Tang Packaging Co., Ltd. The screw speed was 50 rpm, screw diameter was 45mm, L/D was 26 and the processing temperature was 210°C. Finally, molded samples were immediately sealed in a plastic bag and placed in a vacuum desiccator for a minimum of 24 h prior to material property evaluation.

3.3.4 Characterizations of PP/Organoclay Nanocomposites

The change of interlayer distance and PP matrix crystal structure was measured by XRD.

The clay contents of the nanocomposites were measured by TGA. Crystallization and melting behavior of PP changed with the addition of the organoclay were investigated by DSC.

The mechanical properties tests were conducted according to ASTM D882 using Lloyd universal testing machine equipped with an extensometer. The tests were operated at a crosshead speed of 50 mm/min. The data was recorded at room temperature without preconditioning of the samples. The sample dimension is 10x100 mm.

Gas barrier property of the PP/clay nanocomposite films were investigated by using gas permeability tester. The gas permeability constants of PP/clay nanocomposite films were determined at 23 °C with oxygen flow rate is 50 cm^2/min.

The dispersion of OBTN in PP/clay nanocomposite films were investigated by SEM.

3.3.5 The pH Measurement of fish tissue and TVB-N

The pH measurement of fish tissue was adopted from method used by National Food Institute, Thailand.

20 g portions of tissue were homogenized in 10 ml of distilled water, followed by pH determination with a pH meter. Conversely, a pH of TVB-N was measured by using a pH paper. The pH paper was placed on a lid of 7x4 cm of polypropylene cup. Experimental design for pH measurement of TVB-N is shown in figure 3.2 below.
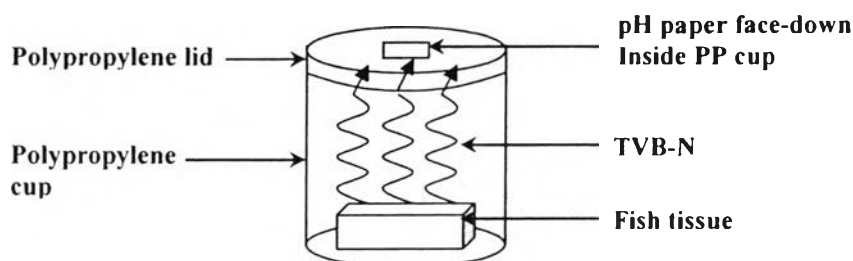
Figure 3.2 Experimental design for pH measurement of TVB-N.

3.3.6 Determination of Total Volatile Basic Nitrogen (TVB-N)

Determination of TVB-N will be adopted from method used by Fish Inspection and Quality Control Division (FIQD), the Department of Fisheries (DOF), Thailand.

3.3.7.1 Sample Extraction

In order to preserve the freshness of sample, grinding of the sample by homogenizer was performed. 3 g of homogenized sample was placed into a centrifuge tube. After that 12 ml of 4% Trichloroacetic acid (TCA) solution was added to the centrifuge tube, the tube was sealed and vigorously shaked to make sure that it was properly mixed. The sample was then left at room temperature for 30 min with stirring from time to time. The sample mixture was filltered using Whatman paper no.1. When freshly prepared samples were not used within a day for further analysis, The filtered solution must be kept at -18°C in vials, and to prevent the breaking of vial, sample must not be filled.

3.3.7.2 Measuring of TVB-N

Sealing agent (Vaseline) was first applied to the top edge of a conway's unit. The inner ring solution (1% boric acid mixed with 1ml of indicator) was pipette and placed into the inner ring of Conway's unit (volatile compounds from sample extract would diffuse into boric acid salt and these salts would be reduced to HCl-salts by strong HCl during titration). 1 ml of filtered sample extract was pipette into the outer ring of the conway's unit. 1 ml of saturated K_2CO_3 solution was pipette into the outer ring of the conway's unit and placed on the opposite side of the sample (to made sample extract into alkalines condition similar to that of volatile compound). The conway's unit was immediately covered and shaked gently to dissolve the samples and mix it with K_2CO_3 without contaminating the inner ring of conway (triplicate for each sample). Stand the samples were kept at room temperature for 3 hr. After the color of boric acid solution changed from pink to green, following the generating of volatile base, this sample was then titrated with 0.01 N HCl containing in a micro-burette until the color changed back to pink. Experimental design for determination of TVB-N is shown below (see figure 3.3).

Note: Blank test was carried out using 1ml of 4%TCA instead of sample extraction

3.3.7.3 Calculation

$$\text{TVB-N (mg/100g)} = \frac{(V_S-V_B)\times(N_{HCl}\times A_N)\times[\ W_S\times(M/100)+V_E]\times100}{W_S}$$

Where, V_S = Titration volume of 0.01 N HCl for sample extract (ml)

V_B = Titration volume of 0.01 N HCl for blank (ml)

N_{HCl} = Normality of HCl (= 0.01 N × factor of HCl)

A_N = Atomic weight of nitrogen (14.00)

W_S = Weight of tissue sample (g)

M = Percentage moisture of tissue sample (Assume 80%)

V_E = Volume of 4% TCA used in extraction
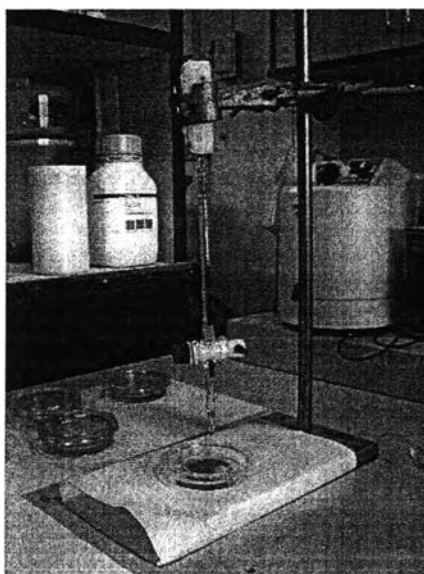
Figure 3.3 Experimental design for determination of TVB-N.

3.3.7 Microbial Analysis

Microbial analysis was adopted from method used by Pacquit *et al,.* (2007).

Samples of approximately 20 g was removed from the same Barramundi filets, under the same aseptic conditions, and placed in zip lock bags. Then, the samples were allowed to spoil at room temperature under the same conditions.

Aerobic Plate Count (APC) or Total Viable Count (TVC) was determined, using the pour plate method, on plate count agar and results were correlated with the sensor response.

3.3.8 The nanocomposite pH Sensor Fabrication

The pH sensor fabrication was adopted from method used by Pacquit *et al,.* (2007).

A typical pH sensor solution contained a binder, such as cellulose acetate (70%, w/w), a dye, such as bromocresol green (BCG) (1, 3, 5%, w/w), and a plasticiser, dibutyl sebacate (DBP) (30%, w/w). The mixture was sonicated for 45–60 min until dissolution was completed. Subsequently, the pH sensor was made by using an automatic pipette and simply deposited 3–4 µl of the pH sensor solution on a mirror substrate. Then, spin-coated at 1,000, 2,000 and 3,000 rpm for approximately 2 min. Films generated at different rotational speed would be varied in thickness. The coated discs were peeled from the mirror substrate then placed in an open container, subsequently in a dark cupboard for overnight at room temperature to complete the drying process. Thickness of the obtained pH sensors at different rotational speed were measured using a micrometer. The pH sensor discs of 5cm diameter were attached onto PP/clay nanocomposite films using a laminating machine (at 160°C).

3.3.9 Sensor Response to Standard Ammonia

Study of sensor response to standard ammonia was adopted based on the method used by Xiuchen (2000).

The main classes of volatile compounds which characterize the spoilage odor of fish such as amines were tested to estimate the sensitivity of the color response. Ammonia was selected from the amine group. Standard ammonia

(10-1000ppm) in aqueous solutions was prepared in various concentrations. 25 ml aliquots of ammonia standard were pipetted into a polypropylene cup (7x4cm), and closed with nanocomposite pH sensor films. Each cup was sealed with fast cure epoxy to create a permanent gas-tight seal and prevent leakage of amines. Experiment was kept at room temperature for 12 hr. Subsequently, investigation of film thickness, amount of dye and clay content were performed in the same method and measured two times each.

Color changes of the pH sensor were measured from the pH sensor with a Chroma Meter and expressed as Hunter system (L, a, and b) values and total color difference (TCD). The TCD value (ΔE) was calculated by the following equation (Francis, 1983):

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

Here, ΔL is the brightness difference between sample and target, Δa the redness difference between sample and target, and Δb is 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.

3.3.10 Study of Films Thickness, Amount of Dye, Clay Content, and quantity of fish tissue on Color Response of nanocomposite pH sensors to TVB-N

Investigation of film thickness was carried out based on the method used by Pacquit et al,. (2007).

For a set of sensors, fourteen replicate fish meat samples of approximately 20 g were placed in separate individual polypropylene caps. Each cap was sealed with fast cure epoxy to create a permanent gas-tight seal and prevent leakage of amines. Samples were at no time indirect contact with the sensors (i.e. only headspace was sampled). The response of fish tissue samples, allowed to spoil at room temperature, was monitored every 3 h with chroma meter for 42 h. Experimental design for fish spoilage monitoring is shown in figure 3.4 below.

Same measurements were carried out for the nanocomposite sensor film of different thickness prepared by changing spin coating speeds (1000, 2000,

and 3000 rpm), amount of dye (1, 3, and 5 %wt BCG), and clay content from 1-5%wt Clay. Moreover, the color change was also monitored for different fish meat content (20, 40, and 60g) and for the nanocomposite sensor film with various dye content.

Color changes of the pH sensor were measured from the pH sensor with a chroma meter as performed in sensor response to standard ammonia.

The degree of spoilage of fresh fish was assessed by Aerobic Plate Count (APC) and Total Volatile Basic Nitrogen (TVB-N), and was compared with Hunter color values of the nanocomposite pH sensors.
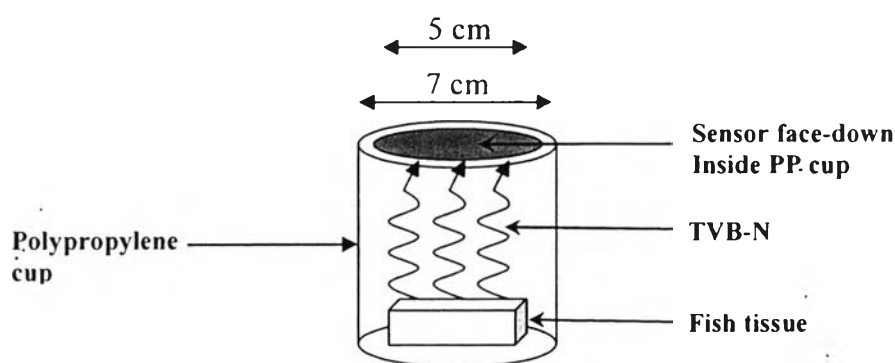
Figure 3.4 Experimental design for fish spoilage monitoring.

3.3.11 Leaching Studies

Indicator dyes (BCG) were dissolved in water to prepare standard solutions of 1, 2, 5, and 10 ppm. Then the absorbance of the standard solutions was investigated by using UV-vis spectroscopy (SHIMADZU model UV-2550) with medium scan rate and sampling the data every 1 nm. Spin-coated nanocomposite sensors were cut into rectangular shape with 4x4 cm and soaked with 10 ml of water in a small container for approximately 48 h, after which wavelength spectrum of the water (400–700 nm) was carried out to detect the presence of BCG, if any. The absorbance of the indicator dye standard solutions was observed by UV-vis spectroscopy. Calibration curve was plotted between absorbance versus concentration.