CHAPTER V

pH INDICATOR FILM BASED ON PLASTIC/CHROMOPHORES MODIFIED PCH NANOCOMPOSITE FILMS

5.1 Abstract

A color indicator for detecting fish and climacteric fruit freshness was developed based on polypropylene/methyl red modified functionalized porous clay heterostructure (APPCH-MR) nanocomposite films and low density polyethylene/bromothymol blue modified PCH (PCH-BTB) nanocomposite films respectively. The nanaocomposite was prepared by twin screw extruder and fabricated into nanocomposite film by compression molding. The color change of pH indicator was measured and expressed as the Hunter color system (L, a, b) values and total color difference (TCD) values or ΔE. Fish spoilage was accessed through the total volatile basic nitrogen (TVB-N) test and the result showed that the TVB-N values of fresh fish were excess the values of not acceptable for human consumption (30-35mg/100g sample) after 8 hours. The results showed that the increase of total volatile basic nitrogen (TVB-N) values from 0-8 hours corresponded to the color change of nanocomposite indicator films from red to light orange during fish spoilage. In addition, color change of indicator films from green to yellow correlated with standard CO_2 levels which can be compared to CO_2 levels from respiration during fruit ripening. These pH indicator films could be used to determine fish freshness and can be applied for detecting the quality of climacteric fruit by color change.

Keyword: Chromophores/ Nanocomposite films

5.2 Introduction

Nowadays food packaging systems are increasingly important in food products because the problem of post harvest fruits and vegetables or meat products are the limitation of their fresh and shelf-life. At present, freshness indicators are interesting techniques which are used to monitor the status of food spoilage. Freshness indicators provide the direct product quality from the information of microbial growth or chemical changes within food product during food deterioration such as pH changes, formation of toxic compounds, off-odors and gas formation. Thus, the indication of freshness indicator can be based on the reaction between the indicator and the metabolites produced during food deterioration. The development of pH-sensitive color indicator by using as a smart packaging is the one concept that can monitor the status of food spoilage by color change.

Chemical parameters, such as volatile amine levels, have been used to assess fish freshness. The occurrence of volatile basic compounds, such as ammonia, trimethylamine (TMA-N) and dimethylamine (DMAN), is one of the characteristic features attributed to chemical changes occurring in marine fish muscle during spoilage. Methyl red is a pH-indicator which was used to determine the fish freshness by color change after reacts with volatile amine.

The ripening of climacteric fruit associates with increasing in respiration and ethylene production. The respiration rate will rise up to the climacteric peak and then decline. The main product during respiration is simple molecules, such as carbon dioxide and water, with the concurrent production of energy. Thus, carbon dioxide or carbonic acid releasing from the climacteric fruits during ripening is applied for determining climacteric fruit freshness by color change of indicator such as bromothymol blue.

In this work, pH indicator film for detecting fish and climacteric fruit freshness was prepared based on polypropylene/chromophores (methyl red) modified functionalized porous clay heterostructure (PCH) nanocomposite films and low density polyethylene/chromophores (bromothymol blue) modified PCH nanocomposite films respectively. The capability to use the film as a pH indicator for indicated the direct quality of freshness was evaluated in term of change in Hunter (L, a, b) and total color difference (ΔE) value.

5.3 Experiment

A. Preparation of PP/APPCH-MR 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 170, 175, 180, 185, 190, 195, 200, 205, 210 and 215°C from hopper to die respectively and the screw speed was 50 rpm.

First, polypropylene (PP) was blended with 6%wt surlyn® in order to obtain PP/surlyn® pellet. Then, PP/surlyn® pellet was mixed with 2%wt of chromophores modified functionalized 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 PP/chromophores modified functionalized PCH was prepared by compression molding machine at 5 tons of force for 5 minutes. The processing temperature was 210°C.

B. Indicator Films Response to Standard Ammonia

25 ml of standard ammonia solution in various concentrations (10, 25, 50, 100, 250 and 500 ppm) were pipette into the well which was sealed to prevent the leakage of amine. The indicator film (PP/APPCH-MR nanocomposite films) was faced down inside the well that contained standard ammonia [1]. The experiment was kept at room temperature until the color response of indicator film saturated to change color. The color response of films at various weight ratio of APPCH and methyl red (10:1, 20:1 and 30:1) 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. 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

C. Determination of Total Volatile Basic Nitrogen (TVB-N) and Indicator Films Response to Fresh Fish Tissue

The total volatile basic nitrogen released during the fish spoilage was measured following the procedure.

1. Sample Preparation

Grind the fish sample by homogenizer and 3 g of homogenized sample was placed into a centrifuge tube. Then, 12 ml of 4% trichloroacetic acid (TCA) solution was added to the centrifuge tube and the tube was sealed and shaken to make sure that it was properly mixed. After that, the sample was left at room temperature for 30 min with stirring from time to time. The sample mixture was filtrated using Whatman paper no.1. When freshly prepared samples were not used within a day for further analysis, the filtrated solution must be kept at -18°C in vials.

2. Measuring of TVB-N

Sealing agent (Vaseline) was first applied to the top edge of convey's unit. The inner ring solution (1% boric acid mixed with 1 ml 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). Then, 1 ml of filtered sample extract was pipette into the outer ring of conway's unit. 1 ml of saturated K_2CO_3 solution was pipette into the outer ring of conway's unit and placed on the opposite side of the sample solution (to made sample extract into alkalines condition similar to that of volatile compound). The convey's unit was immediately shaken in order to mixed sample solution and K_2CO_3 solution without contaminating the inner ring of conway. Stand the samples were kept at room temperature for 3 h. After the color of boric solution changed from pink to green (Figure 3.1), following the generation of volatile base, this sample was titrated with 0.01 N HCl containing in a micro-burette until the color changed back to pink

3. Calculation

TVB-N can be calculated by following equation:

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

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

The color response of indicator films after indirect contact with fresh fish tissue was measured. Fish tissue samples of approximately 20 g were placed in separated individual wells. Each well was sealed to prevent the leakage of amine. Indirect contact between indicator films (PP/APPCH-MR nanocomposite films) and fish sample was obtained. The response of indicator film with fish sample was observed at room temperature every 3 hours for 12 hours. The color response of nanocomposite films at various weight ratios of APPCH and methyl red (10:1, 20:1 and 30:1) 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.

D. Preparation of LDPE/PCH-BTB Nanocomposite Films

The nanocomposite was prepared by using the 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.

E. 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 (LDPE/PCH-BTB nanocomposite films) 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.

F. Indicator Films Response to Fresh Banana

Fresh banana (Musa (AAA group) "Kluai Hom Thong") with green color and unripe was selected for the experiment. Banana of approximate the size (150 g) was chosen. The indicator film (LDPE/PCH-BTB nanocomposite film) at weight ratio of PCH: dye of 10:1 was placed in polypropylene bag with contained fresh banana. The bag was sealed to prevent the leakage of carbon dioxide and moisture. Color changes of indicator film after fruit started to ripen was observed.

G. Leaching Studies

Indicator dye (methyl red or bromothymol) 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 films were cut into the rectangular shape with 4x4 cm and soaked in 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 pH dye.

5.4 Result and Discussion

A. Indicator Films Response to Standard Ammonia

Ammonia is one of the compounds produced during fish spoilage. Thus, the study of sensor response to standard ammonia gas provided a model of the response to total volatile basic nitrogen (TVB-N).

After all sensor indirect contact with various concentrations of standard ammonia at room temperature, the color change from red to light orange was observed and the change of hunter color values corresponded with increasing the concentration of ammonia as shown in Table 5.1, 5.2 and 5.3. The increasing of pH correlated to concentration of ammonia was shown in Figure 5.2. The results showed that hunter a of the indicator containing methyl red decreased while hunter b increased after indirect contact with ammonia indicated that the color of methyl red turned from red to light orange. Moreover, TCD values or ΔE of the indicator films also changed continuously responding with standard ammonia. The data of ΔE (Figure 5.1) showed that ΔE continuously increased at low concentration of ammonia and remaining constant at high concentration of ammonia due to the saturation of color change. The color change of the indicator film at various weight ratios of APPCH and methyl red (10:1, 20:1 and 30:1) were shown in Figure 5.3.

The data indicated that the sensor was sensitive to low concentration of standard ammonia and the saturation of color change was obtained at high concentration of standard ammonia. Therefore, PP/APPCH-MR nanocomposite films can be applied for detecting TVB-N during fish spoilage.

Table 5.1 Change in hunter color (L, a, b) and total color difference (TCD) values of PP/APPCH-MR (10:1) nanocomposite films after indirect contact with standard ammonia

Concentration (ppm)	L	a	b	ΔL	Δa	Δb	ΔE
10	26.40	5.47	5.05	-0.35	-2.08	1.80	2.77
25	25.70	6.04	5.16	0.64	-2.42	1.70	3.02
50	27.10	6.12	5.58	-0.56	-2.34	1.84	3.03
100	25.31	6.43	5.95	-1.48	-1.42	2.91	3.56
250	25.91	6.75	6.20	-1.68	-2.10	2.67	3.79
500	25.79	7.09	6.87	-1.28	-1.41	3.33	3.83

Table 5.2 Change in hunter color (L, a, b) and total color difference (TCD) values of PP/APPCH-MR (20:1) nanocomposite films after indirect contact with standard ammonia

Concentration (ppm)	L	a	b	ΔL	Δa	Δb	ΔE
10	29.52	5.24	4.72	-0.61	-2.24	2.24	3.23
25	29.48	7.64	7.23	0.55	-2.96	3.26	4.44
50	26.96	6.65	5.87	-0.09	-2.67	2.62	3.74
100	28.53	6.27	5.27	-0.22	-2.33	2.51	3.43
250	28.97	6.79	5.79	0.89	-2.57	2.68	3.81
500	28.48	6.49	5.23	0.04	-2.34	2.24	3.23

Table 5.3 Change in hunter color (L, a, b) and total color difference (TCD) values of PP/APPCH-MR (30:1) nanocomposite films after indirect contact with standard ammonia

Concentration (ppm)	L	a	b	ΔL	Δa	Δb	ΔE
10	26.04	6.08	4.60	-0.32	-2.33	2.05	3.12
25	26.23	6.53	5.07	-0.39	-2.10	2.26	3.11
50	27.71	6.85	5.40	1.23	-2.94	2.92	4.32
100	27.74	7.40	6.03	0.69	-2.45	2.97	3.91
250	26.69	7.00	5.36	0.08	-2.83	2.60	3.84
500	26.32	7.73	5.35	0.40	-2.33	2.22	3.24

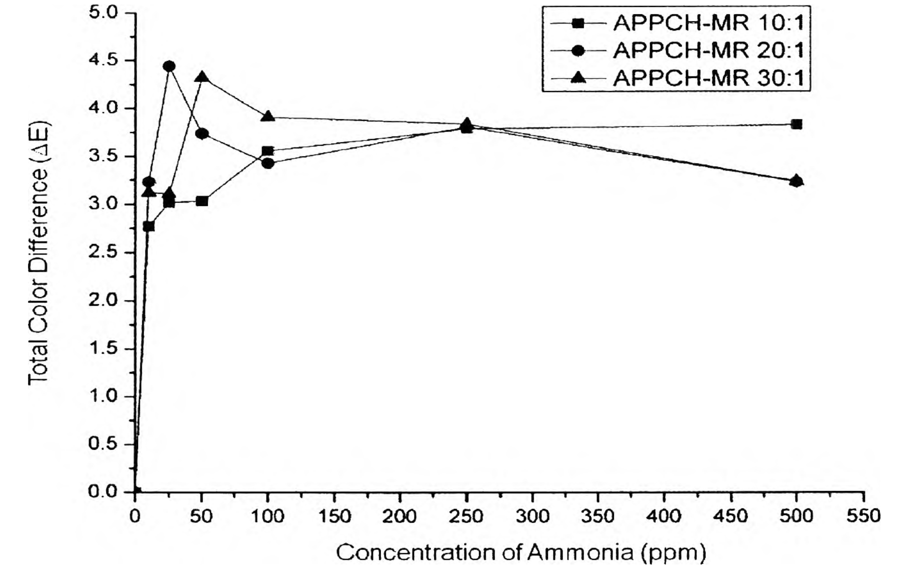


Figure 5.1 Changes in TCD values of the pH indicator films after indirect contact with standard ammonia during storage at room temperature.

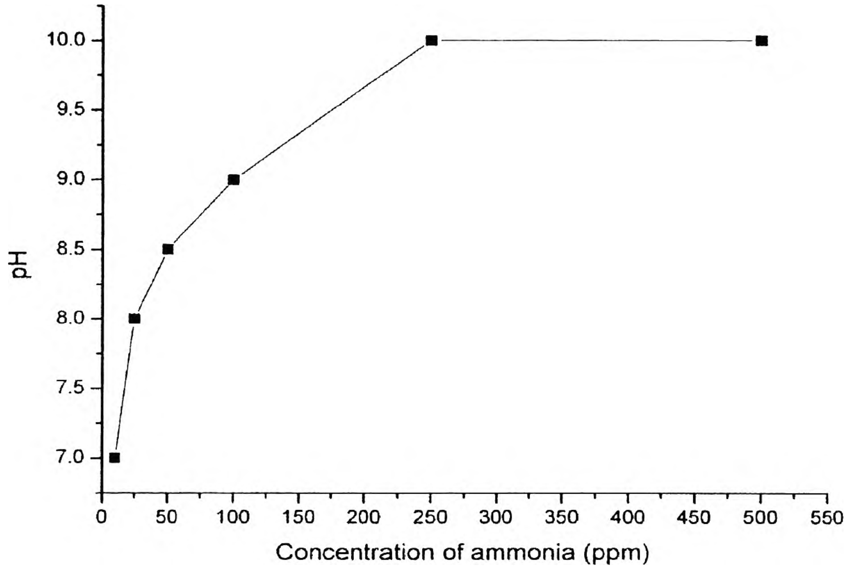
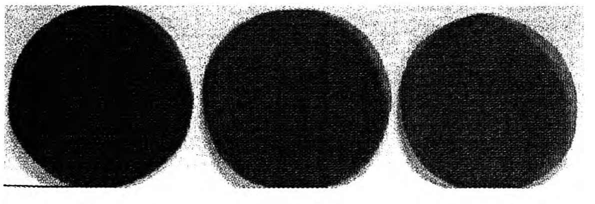
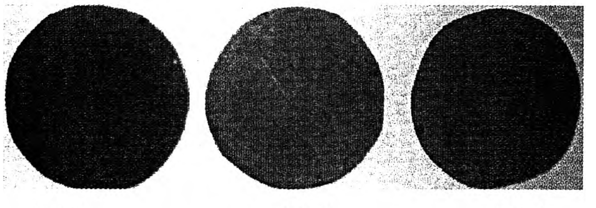


Figure 5.2 Changes in pH of standard ammonia.



10:1 20:1 30:1

(a)



10:1 20:1 30:1

(b)

Figure 5.3 Color changes of the pH indicator films (a) before and (b) after indirect contact with 25 ppm standard ammonia during storage at room temperature.

B. Fish spoilage and pH indicator film response to fish spoilage

Quality change and shelf life of Barramundi fish was determined by total volatile basic nitrogen (TVB-N) method because of the formation of volatile amine during the deterioration of fish. TVB-N releasing from fish such as ammonia, trimethylamine (TMA) and dimethylamine (DMA) is widely used as the useful index for determining the fish freshness. A level of 30-35 mg TVB-N/100g of fish muscle is the limit. Above the value of 30-35 mg TVB-N/100g, fish is not acceptable for human consumption [2].

In experiment, the changes in total volatile basic nitrogen (TVB-N) of fresh fish during storage at room temperature were measured as shown in Figure 5.4. The results showed that the TVB-N values continuously increased at the initial and medium step and remained constant at the final step of fish spoilage. The TVB-N values of fresh fish were excess the values of 30-35 mg/100g as the limit which fish is not acceptable for human consumption after 8 hours.

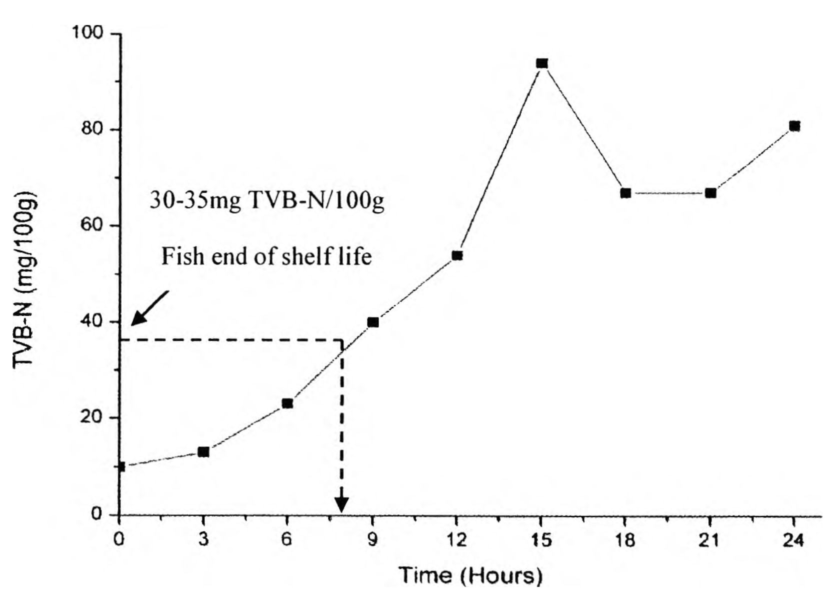
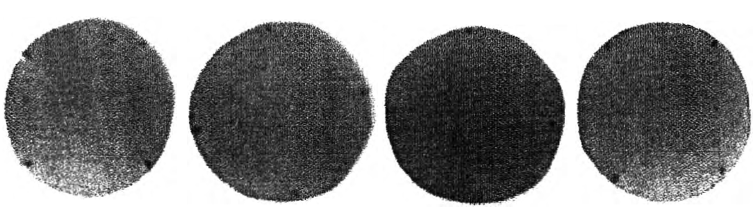


Figure 5.4 Changes in total volatile basic nitrogen (TVB-N) of fresh fish during storage at room temperature.

In addition, the result of indicator film response after in direct contact with fresh fish sample was observed. The result showed that the increasing of TVB-N values from 0-8 hours corresponded to the color change of nanocomposite indicator films from red to light orange during fish spoilage as shown in Figure 5.5. The increasing of pH during storage was shown in Figure 5.6. Hunter a of the indicator containing methyl red decreased while Hunter b increased after indirect contact with fresh fish sample correlated with the color of methyl red turned from red to light orange. The color change was described by the mechanism of pulling proton of ammonia molecule from methyl red in red form and then the structure changed into the yellow form as shown in Figure 2.9.

Moreover, the pH indicator film APPCH-MR 30:1 showed the highest color change compared to APPCH-MR 20:1 and APPCH-MR 10:1, respectively as shown in Table 5.4, Table 5.5, Table 5.6 and Figure 5.7. Thus, these results suggested that the PP/APPCH-MR nanocomposite films can be applied as the fish spoilage indicator.



0 3 6 9 (Hours)

Figure 5.5 Color changes of PP/APPCH-MR (30:1) nanocomposite films during fish storage at room temperature.

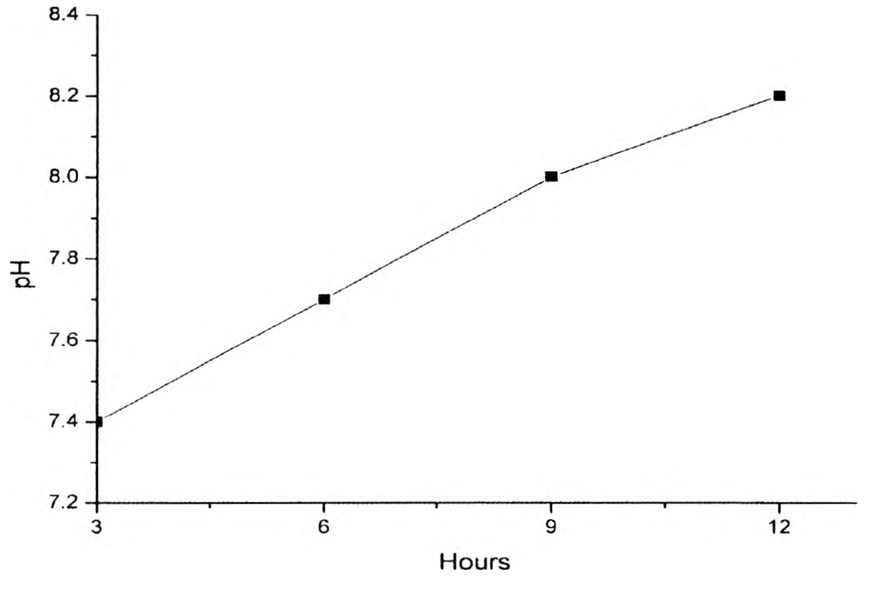


Figure 5.6 Changes in pH of total volatile basic nitrogen (TVB-N) of fresh fish during storage at room temperature.

Table 5.4 Change in hunter color (L, a, b) and total color difference (TCD) values of PP/APPCH-MR (10:1) nanocomposite films after in direct contact with fresh fish tissue

Time (Hours)	L	a	b	ΔL	Δa	Δb	ΔE
3	15.38	7.26	2.15	0	-1.24	1.61	2.03
6	14.35	6.45	2.59	0.15	-0.83	1.96	2.13
9	14.18	6.91	2.85	0.48	1.47	1.84	2.40
12	16.43	6.72	2.67	0.07	-1.71	1.95	2.59

Table 5.5 Change in hunter color (L, a, b) and total color difference (TCD) values of PP/APPCH-MR (20:1) nanocomposite films after in direct contact with fresh fish tissue

Time (Hours)	L	a	b	ΔL	Δa	Δb	ΔE
3	14.85	7.78	0.66	1.32	-1.81	2.59	3.42
6	14.76	8.25	1.01	1.09	-2.63	2.70	3.92
9	15.85	7.87	0.66	0.54	-2.53	3.37	4.25
12	17.63	8.16	0.72	1.71	-3.72	2.81	4.96

Table 5.6 Change in hunter color (L, a, b) and total color difference (TCD) values of PP/APPCH-MR (30:1) nanocomposite films after in direct contact with fresh fish tissue

Time (Hours)	L	a	b	ΔL	Δa	Δb	ΔE
3	16.02	6.72	-0.54	0.72	-1.71	2.74	3.31
6	17.53	7.78	-0.27	0.61	-2.62	3.25	4.21
9	17.57	7.91	-0.22	1.72	-2.61	3.88	4.98
12	19.69	8.12	-0.24	3.88	-3.67	3.40	6.06

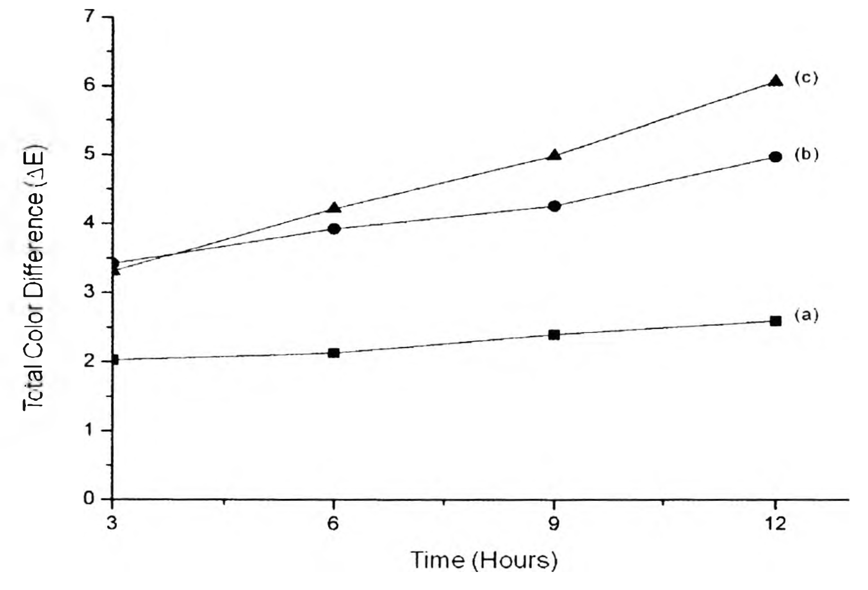


Figure 5.7 Changes in total color difference values (ΔE) of pH indicator film at various weight ratio of APPCH: MR (a) 10:1 (b) 20:1 and (c) 30:1 during fish storage at room temperature.

C. pH Indicator Film Response to Standard CO_2 and H_2O

Because the ripening of climacteric fruit associates with increasing in respiration and the main products from respiration of climacteric fruits such as carbon dioxide and water are generally to measure. Thus, carbon dioxide and water releasing before climacteric peak at the time that the fruit starts to ripen is the most important parameter to evaluate the freshness of the climacteric fruit by the color change of pH indicator films [3].

The color change of indicator films (LDPE/PCH-BTB nanocomposite films) after test with excess water and standard carbon dioxide (CO_2) at concentration from 0 to 150 ppm which can be compared with the production of CO_2 in the preclimacteric to climacteric phase during fruit ripening was shown in Table 5.7, Table 5.8, Table 5.9 and Figure 5.8. The result showed that the color of indicator film changed the color from green to yellow correlated with increasing in standard CO_2 levels as shown in Figure 5.9. The total color difference (ΔE) and hunter b values continuously increases at low concentration of standard CO_2 and remaining constant at high concentrations due to the saturation of color change. Moreover, the pH indicator film PCH-BTB 10:1 showed the highest sensitivity of color change compared to PCH-BTB 20:1 and PCH-BTB 30:1 at low concentrations of standard CO_2. The reaction of color change was described. Carbon dioxide combined with water and producing carbonic acid. Carbonic acid dissociated from parent molecule forming hydrogen ions (H^+) and bicarbonate ion (HCO^{3-}). Then, as a proton, a hydrogen ion combined with water molecule to form a hydronium ion (H_3O^+). Hydronium ions reacted with the bromothymol blue in basic form resulting in an acid form. The color change of indicator film was presented. The mechanism of color change was shown in Figure 2.10. Therefore, this pH indicator film can be applied for detecting the quality of climacteric fruit by color change.

Table 5.7 Change in hunter color (L, a, b) and total color difference (TCD) values of LDPE/PCH-BTB (10:1) nanocomposite films after indirect contact with standard carbon dioxide and excess water

Concentration (ppm)	L	a	b	ΔL	Δa	Δb	ΔE
30	29.83	-5.45	3.03	-1.06	0.27	0.65	1.19
60	29.28	-6.61	3.75	0.52	0.5	1.53	1.69
90	28.42	-5.83	2.92	0.35	0.36	2.25	2.31
120	28.56	-5.48	2.86	0.79	0.47	2.00	2.20
150	29.05	-5.28	2.40	0.03	0.63	2.08	2.17

Table 5.8 Change in hunter color (L, a, b) and total color difference (TCD) values of LDPE/PCH-BTB (20:1) nanocomposite films after indirect contact with standard carbon dioxide and excess water

Concentration (ppm)	L	a	b	ΔL	Δa	Δb	ΔE
30	32.75	-6.73	1.42	0.55	0.32	0.30	0.70
60	32.28	-5.65	1.06	0.15	0.33	1.18	1.23
90	32.41	-5.78	1.08	-0.21	0.16	1.54	1.56
120	31.69	-6.08	0.62	0.36	0.56	2.01	2.12
150	32.23	-5.62	0.70	-0.12	0.32	2.15	2.18

Table 5.9 Change in hunter color (L, a, b) and total color difference (TCD) values of LDPE/PCH-BTB (30:1) nanocomposite films after indirect contact with standard carbon dioxide and excess water

Concentration (ppm)	L	a	b	ΔL	Δa	Δb	ΔE
30	32.53	-5.87	1.64	-0.02	0.08	0.33	0.35
60	31.27	-5.39	0.70	-0.04	0.17	1.23	1.24
90	33.37	-5.97	1.18	-1.18	-0.17	2.05	2.37
120	33.15	-5.80	1.10	0.50	0.09	2.24	2.49
150	31.19	-5.70	0.78	0.16	0.16	2.23	2.24

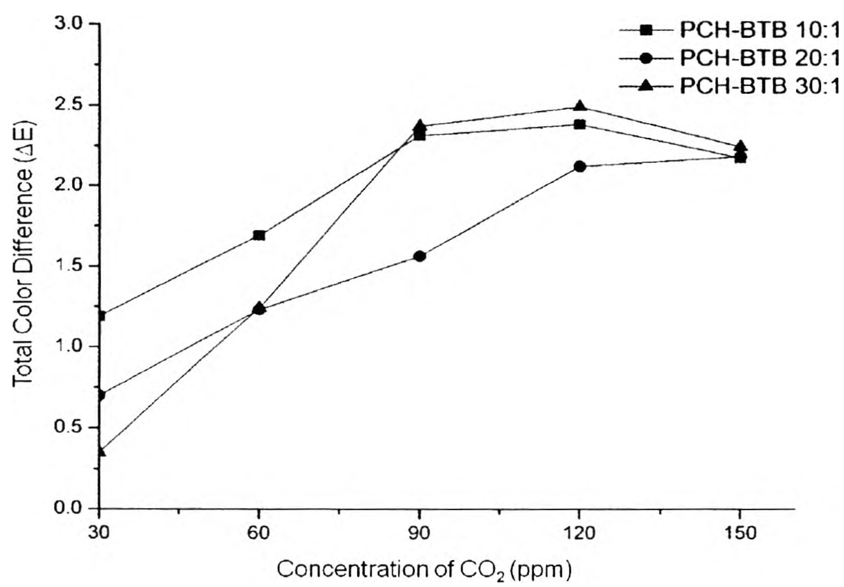


Figure 5.8 Changes in total color difference values (ΔE) of pH indicator film at various weight ratio of PCH: BTB (a) 10:1 (b) 20:1 and (c) 30:1.

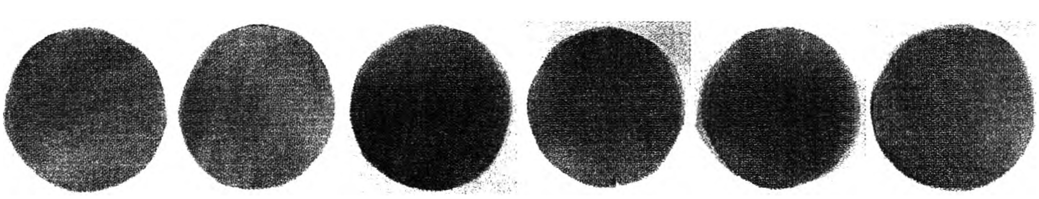


Figure 5.9 Color changes of LDPE/PCH-BTB (10:1) nanocomposite films after test with standard carbon dioxide at various concentrations (a) 0 ppm (b) 30 ppm (c) 60 ppm (d) 90 ppm (e) 120 ppm and (f) 150 ppm.

D. pH Indicator Film Response to Fresh Banana

The result of indicator film response after in direct contact with fresh banana was observed. The pH change during storage was shown in Figure 5.10. The result showed that the color of indicator film changed the color from green to yellow when banana started to ripen and remained constant due to the saturation of color as shown in Figure 5.11 and 5.12.

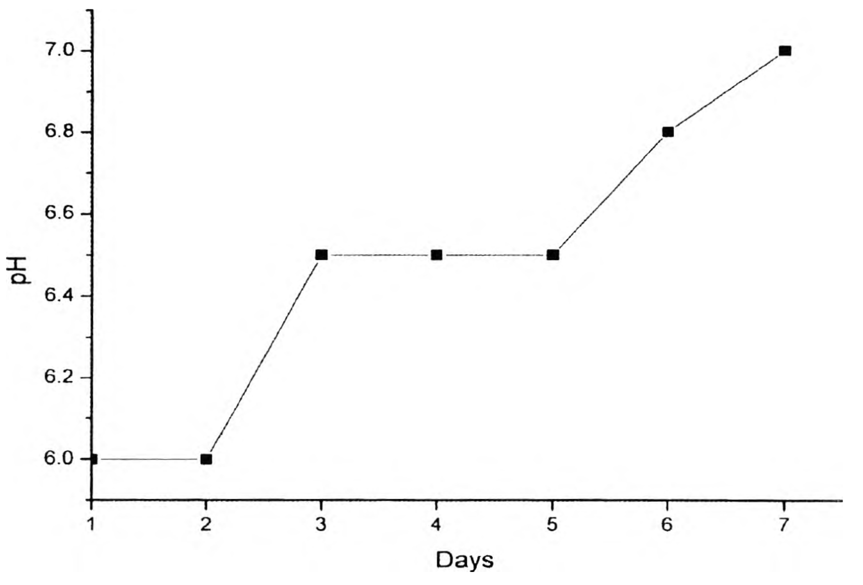


Figure 5.10 Changes in pH of fresh banana during storage at room temperature.

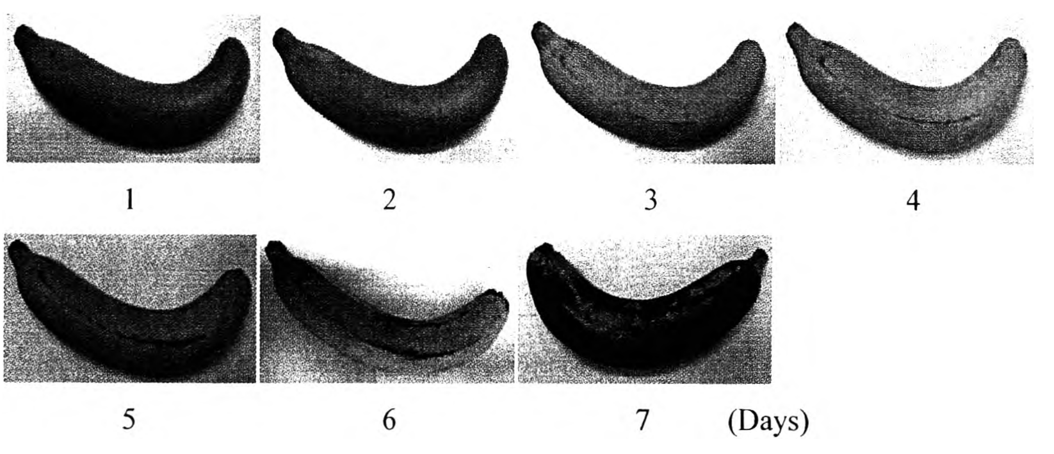


Figure 5.11 Reference bananas ripening at room temperature.

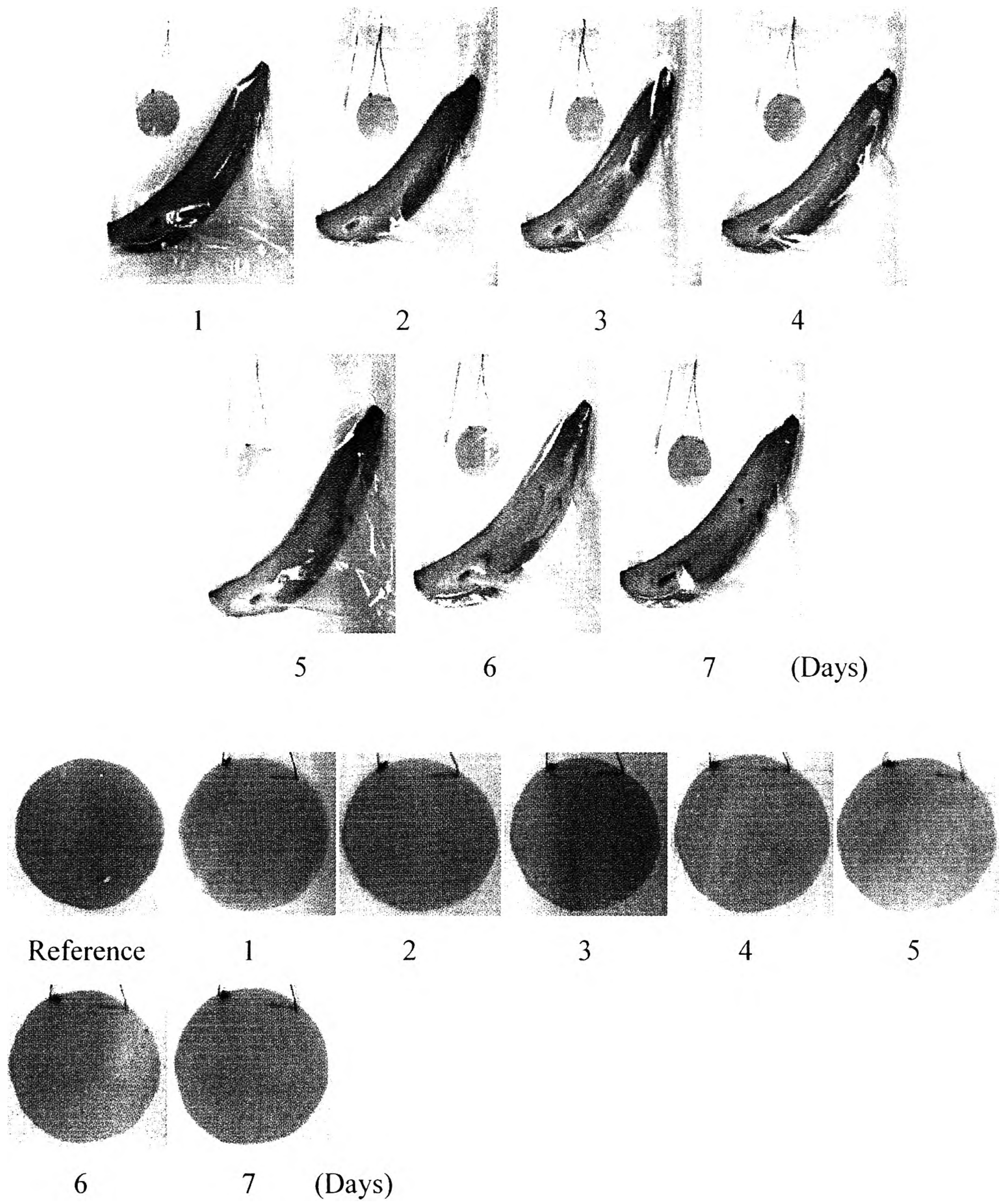


Figure 5.12 Color change of LDPE/PCH-BTB (10:1) nanocomposite film during banana ripening.

E. Leaching Studies

The leakage of the indicator dyes from the PP/APPCH-MR nanocomposite films and LDPE/PCH-BTB nanocomposite films with calibration curves was observed by UV-Vis spectrometer as shown in Figure 5.13 and Figure 5.14, respectively. After the both indicator films were soaked in water and carried out to detect the presence of pH dye. The results showed that the absorbance of water was about zero equaled to the absorbance of reference distill water. Thus, the results indicated that no have leakage of methyl red and bromothymol blue from both pH indicator films.

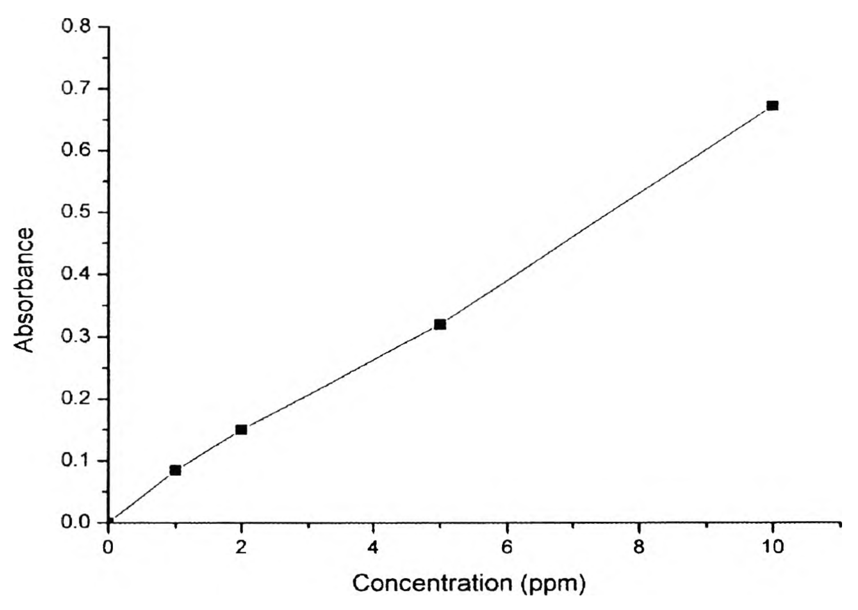


Figure 5.13 The leakage of methyl red from the PP/APPCH-MR nanocomposite films.

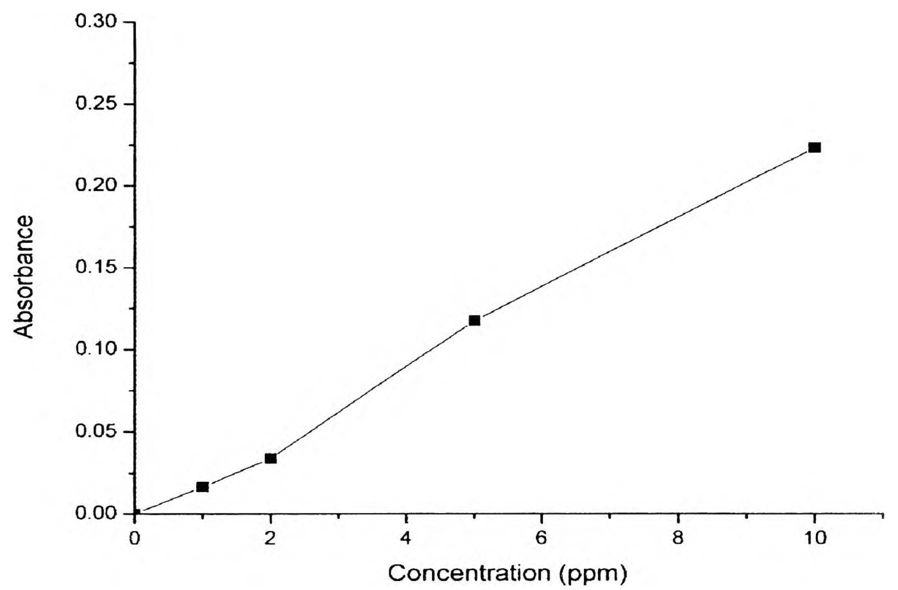


Figure 5.14 The leakage of bromothymol blue from LDPE/PCH-BTB nanocomposite films.

5.5 Conclusions

Indicator films for detecting fish freshness were prepared based on PP/APPCH-MR nanocomposite films. The fish freshness was indicated by the correlation between TVB-N values and color change from red to light orange during the fish lacked of freshness.

Indicator films for detecting climacteric fruit freshness were prepared based on LDPE/PCH-BTB nanocomposite films. These pH indicator films can be applied for detecting the quality of climacteric fruit by color change from green to yellow correlated with standard CO_2 levels, which can be compared to CO_2 levels from respiration in the climacteric phase during fruit ripening.

5.6 Acknowledgements

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5.7 References

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