CHAPTER VII
DYE INDICATOR AND FISH SPOLIAGE DETECTION OF MANGOSTEEN-DYED ETHYLENE VINYL ACETATE FILM

7.1 Abstract

Natural dyed film was prepared and studied the feasibility for using as indicator for food spoilage. Red dye was extracted from mangosteen pericarp using citric acid and mixed with ethylene vinyl acetate (EVA) in a twin-screw extruder at 120 ^{o}C to produce dye compound. The dye film was prepared by using mangosteen extracted solution 2, 4, and 6 wt%. It is found that the TVBN value of 40.32 mg/100g of fish meat was reached after about 9 hr of storage indicating that the spoilage fish was not acceptable for human consumption. Then, the color of the 4 wt% magosteen/EVA film in the fish spoilage test was changed gradually with the lower C* than those in the ammonia test. The relationship between the change in color and time is equal to $C^* = 0.01767t + 0.0104$ where t is time in hrs. The R^2 of the linear fitting of the data is 0.98. The sensitivity of the mangosteen/EVA films to detect the fish spoilage is better than those in the ammonia solution test due to lesser basic environment.

7.2 Introduction

Volatiles amines, such as trimethylamine (TMA), ammonia (NH_3) and dimethylamine (DMA) contribute to a quantity known as total volatile basic nitrogen (TVB-N), are the characteristic substances responsible for the fishy odour and flavour encountered in fish after having past the initial phase of freshness. TVB-N levels increase as a result of bacterial metabolism. In an enclosed food package, as the fish product spoils, a pH increase occurs over time within the headspace which can be detected with an appropriate pH indicating sensor. The fundamental characteristic of pH indicator dyes that change color when placed in an acidic or basic environment is the key element of this freshness indicator. Pacquit *et. al.* (2007) used the Bromocresol green or BCG to be pH indicator dye for fish spoilage

with a presence of quaternary ammonium salt to form an ion pair. They reported that their invented sensor response was found to correlate with bacterial growth patterns in cod and whiting fish samples thus enabling the ''real-time'' monitoring of spoilage. These colorimetric sensors offers the potential of developing dynamic ''best-before'' dates that may lead to important and exciting improvements in the quality assurance sector.

Mangosteen (*Garcinia mangostana* Linn., GML) is a fruit cultivated in tropical areas especially in Thailand, Malaysia, the Philippines and Indonesia. Experimental studies have demonstrated that extracts of GML have antioxidant, antitumoral, antiallergic, anti-inflammatory, antibacterial, and antiviral activities. The pericarp of GML is a source of xanthones and other bioactive substances. Prenylated xanthones isolated from GML have been extensively studied; some members of these compounds possess antioxidant, antitumoral, antiallergic, anti-inflammatory, antibacterial, antifungal and antiviral properties. Xanthones have been isolated from pericarp, whole fruit, heartwood, and leaves.

The most studied xanthones are α-, β-, and γ-mangostins ($C_{20}H_{22}O_5$, Fig.7.1), garcinone E, 8-deoxygartanin, and gartanin(Pedraza-Chaverri, 2008). Moreover, a substantial amount of red pigment, mainly are cyanidin-3-sophoroside and cyanidin-3-glucoside as shown in Fig.7.2 can be isolated from the fruit pericarp of mangosteen (Palapol, 2008). Their color can change with pH; solutions of the compound are red at pH < 3, violet at pH 7-8, and blue at pH > 11. The red pigment from the fruit pericarps of mangosteen can be used as a natural dye source for dyeing, with associated benefits in use with respect to reduced health hazards, lower toxicity and allergic reactions. Chairat *et al.* (2007) extracted the fruit pericarp of mangosteen in aqueous acidic solution (15% w/v citric acid, dried weight 1 g in 5 ml solution) and obtained dark red dye solution for dyeing of cotton and silk. (Chairat *et al.*, 2007). However, there has been no report for attempt to use mangosteen dye extraction in food packaging.

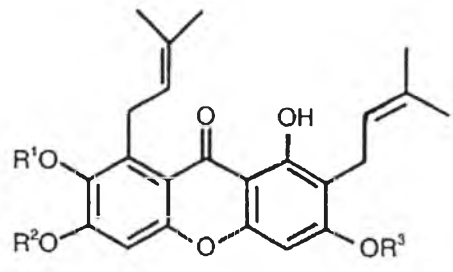

Figure 7.1 Chemical structure of xanthones in mangosteen pericarp.
α-Mangostin (αM)R^1 = Me, R^2 = R^3 = H
β-Mangostin (βM)R^1 = R^3 = Me, R^2 = H
γ-Mangostin (γM)R^1 = R^2 = R^3 = H

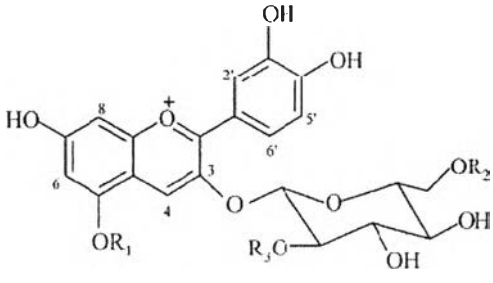

Figure 7.2 Chemical structure of cyanidin-3-glucoside (R_1=R_2=R_3=H) and cyanidin-3-sophoroside (R_1=R_2=H and R_3=glucosyl)

Figure 7.3 FTIR spectrum of mangosteen shell before (a) and after (b) dyes extracted.

This chapter was studied the feasibility for using mangosteen dye extracted from mangosteen pericarp in aqueous acidic solution as the indicator for ammonia sensing during food spoilage in ethylene vinyl acetate film.

7.3 Experimental

7.3.1 Materials

Ethylene vinyl acetate (EVA) was purchased from Dupont. Fresh mangosteen fruits (Garcinia mangostana Linn) were purchased from local fresh food markets in Bangkok or Samut Sakorn. Ammonia 30 wt% (as NH_3) was purchased from Panreac. The density of the ammonia concentration is 0.892-0.898.

7.3.2 Extraction of Mangosteen Pericarp Dye in Acidic Solution

Fresh mangosteen fruits were washed with tap water several times to remove impurities such as dirt, insect pesticide. Mangosteen pericarps were removed from the whole fruits and air dried for 12 hours. Air dried pericarps were then crushed into small pieces with a kitchen blended, and dried in a vacuum oven at 60 ^{o}C for 12 hours to remove moisture. Then, oven dried pericarps were crushed again in a kitchen blended to make them into fine powder. Fine powder mangosteen pericarps were extracted in aqueous acidic solution (15% w/v citric acid, dried weight 1 g in 5 ml solution) for 4 hours (Chairat *et al.*, 2007). They were kept cold at 5 ^{o}C in a refrigerator for further use.

7.3.3 Preparation of Mangosteen-dyed EVA Film

Fresh mangosteen dye was compounded with ethylene vinyl acetate (EVA) in a Haake Rheomex PTW-16 co-rotating twin-screw extruder with D = 16 mm and L/D = 25. The operating temperature of extruder was set at 120 ^{o}C with a screw speed of 60 rpm. The weight ratio of mangosteen dye to EVA pellets was varied 2, 4, and 6 wt% based on dye solution extracted from the previous step. MGT-dyed EVA compound was fabricated into film samples by hot pressing at 120 ^{o}C using a hot pressing machine (PR1D-W300L350, Chareon Tut, Thailand).

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

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

a) Sample Extraction

In order to preserve the freshness of sample, grinding of the sample by homogenizer was preformed. 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 minute with stirring from time to time. The sample mixture was filtered using Whatman paper no.1. When freshly prepared samples were not used within a day for further analysis, the filtered solution were kept at -18 ^{o}C in vials, and to prevent the breaking of vial, sample must not be filled.

b) 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 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). 1 ml of filtered sample extract 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 alkaline condition similar to that of volatile compound). The Conway's unit was immediately covered and shaken gently to dissolve the samples and mix it with K_2CO_3 without contaminating the inner ring convey (triplicate for each sample). Stand the samples were kept at room temperature for 3 hours. 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 Fig.7.5) *Note* : Blank test was carried out using 1 ml of 4% TCA instead of sample extraction

c) Calculation

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

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 x 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

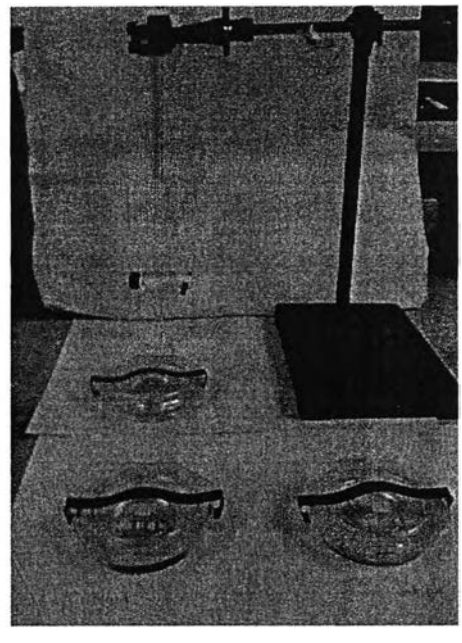

Figure 7.4 Experimental design for determination of TVB-N.

7.3.5 Determination of Ammonia Sensing by Mangosteen-dyed EVA Film (MGT/EVA)

MGT/EVA films were cut into rectangular strips of 1.5 cm wide and 3.0 cm long. Prior the test, these strips were labeled in number individually, and measured their lightness and color in Lab system using a color reader in a control light box. Three different areas for each strip were recorded.

Ammonia solution with concentration of 5.0, 10.0, 15.0, 20.0, 25.0, and 30.0 mg/ml, were prepared from 30 w/w% ammonia solution. The pH of ammonia solution was measured with a pH meter (CyberScan pH1000). One strip of MGT/EVA film (1.5 x 3.0 cm) was immersed in a glass bottle having 20 ml of each ammonia solution. Their caps were sealed with plastic film to ensure no leak of ammonia vapor during the test. The time duration for ammonia sensing was set at 12 hours, and then these strips were removed, washed with distilled water, and dried with tissue paper. Then, the tested strips were measured their lightness and color by a color meter in a control light box. The difference of lightness and color were compared and reported.

7.3.6 Ammonia Sensing in Fish Spoilage Test by Mangosteen-dyed EVA Films

MGT/EVA films were cut into rectangular strips of 1.5 cm wide and 3.0 cm long. Prior the test, these strips were labeled in number individually, and measured their lightness and color in Lab system using a color reader in a control light box. Three different areas for each strip were recorded.

100 gram of fresh white perch meat was placed in a glass bottle as shown in Figure 7.6, and pH of fresh fish was determined using pH indicator paper. Strips of MGT/EVA films with measured color were placed in a sealed bottle for 20 hours to sense the ammonia gas released from spoilage fish meat.

Figure 7.2 Setup for ammonia sensing (fish spoilage) by mangosteen-dyed EVA films.

7.4 Results and Discussion

7.4.1 Color of Mangosteen Dyed EVA Film

Mangosteen dye solution extracted following procedure explained in Chairat *et al.* (2007) is dark red dye in aqueous acidic solution (pH = 2). After adding dye solution into EVA by melt processing, the color tends to change from red into brown. Fig.7.6, Fig.7.7, and Fig.7.8 show color of mangosteen-dyed EVA films compared to the pure EVA films under the same hot-pressing condition. The mangosteen-dye EVA films are reddish brown which the color is darker with respect to the weight ratio of mangosteen dye adding into EVA. These films were measured their color by a color reader in Lab system and reported in Table 7.1. It is seen that the higher concentration of mangosteen dye added into EVA the darker film are as evidenced in lower of lightness (L). The yellowness (+b) was higher with respect to the concentration of dye.

Table 7.1 Lightness and color in Lab system of pure EVA and mangosteen-dyed EVA films (MGT/EVA)

Film sample	Lab measurement			
	L	a*	b*	C*
Pure EVA film	39.7	-1.1	-0.5	1.30
2 wt% MGT/EVA film	39.4	-0.4	0.1	0.40
4 wt% MGT/EVA film	39.3	-0.4	0.8	0.90
6 wt% MGT/EVA film	40.2	-0.2	0.8	0.80

Note: $C^* = \sqrt{a^2 + b^2}$

Figure 7.1 Comparison between pure EVA and 2 wt% mangosteen-dyed EVA films.

Figure 7.2 Comparison between pure EVA and 4 wt% mangosteen-dyed EVA films.

Figure 7.3 Comparison between pure EVA and 6 wt% mangosteen-dyed EVA films.

7.4.2 Total Volatile Basic Nitrogen (TVB-N) of Spoiled White Perch

Odor is another one of the most important parameters to evaluate fish Freshness. During storage of fish the odor undergoes changes from fresh odor, to sweet then stale odors and until the final phase of spoiled or putrid odors. Volatile compounds contributing to odor changes can be measured to evaluate the freshness and spoilage of fish. During the deterioration of fish amines are formed. So, the measurements of total volatile basic nitrogen (TVB-N) is the another method that was used in this work as an indicator of quality for fish and fish products due to the easy and inexpensive method. Table 7.2 reports the total volatile nitrogen occurred during the storage of white perch (Lates calcarifer) fresh meat.

Table 7.2 Total volatile basic nitrogen (TVBN)

Hours	0.01 HCl (ml)	TVB-N (mg/100g)
0	0.15	10.08
3	0.20	13.44
6	0.20	13.44
9	0.60	40.32
12	0.80	53.76
15	1.00	67.20
18	1.00	67.20
21	1.00	67.20
24	1.00	67.20

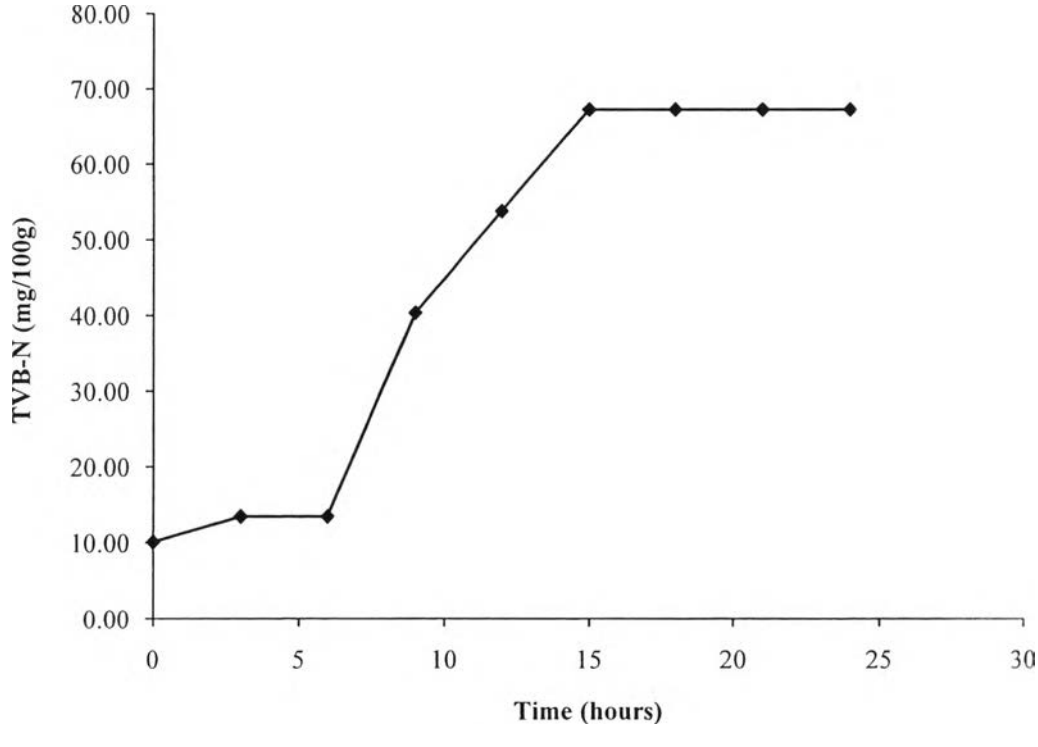

Figure 7.9 The concentration of TVB-N as a function of time.

TVB-N in fish is mainly composed of ammonia, trimethylamine (TMA) and dimethylamine (DMA). A level of 30-35 mg TVB-N/100g of fish meat is usually regarded as spoiled. Changes in TVB-N values are presented graphically in Fig.7.9. Values were found to increase during storage of white perch meat at 25 °C. TVBN value of 40.32 mg/100g of fish meat was reached after about 9 hr of storage indicating that the spoilage fish was not acceptable for human consumption. The TVBN value of 67.20 mg/100g of fish meat was reached after about 15 hr as the limit of TVB-N value. Therefore, the storage time for fish meat in the ammonia sensing test would be set up with the time period over 9 hr.

7.4.3 Ammonia Sensing by Mangosteen-dyed EVA Film

Fig.7.10 shows the change in color of the mangosteen-dyed EVA film after contacted with ammonia solution in different concentration. It is seen that the mangosteen dyed film changed its color after contacted with ammonia solution. This result from the acidic species (from citric acid) in mangosteen dye reacted with basic ammonia solution, causing the mangosteen dye changed its color from dark brown-red to be dark brown. With the 2 and 4 wt% dye adding into EVA film, the linear relationship between color change and ammonia concentration was obtained and presented in Fig.7.11 and 7.12. The correlation between the data and linear fitting is presented in the figure using Origin program. The steeper slope in the 4 wt% dye indicates better sensitivity to detect change in ammonia concentration. With the 6 wt% dye, the correlation between color change and ammonia concentration is not obtained. This is due to dark brownish EVA films produced from using high amount of dye in the film and high acidic content in the film that alkaline species in ammonia solution would not be enough to react with.

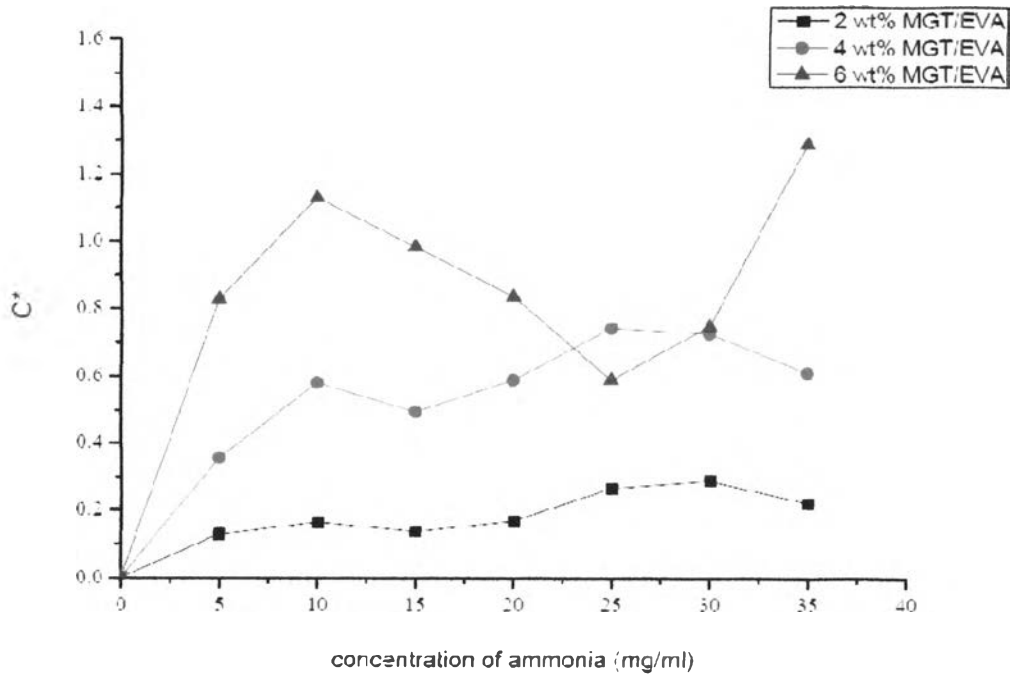

Figure 7.1 Change in color (C*) of mangosteen-dyed EVA film after contacted to ammonia in different concentration.

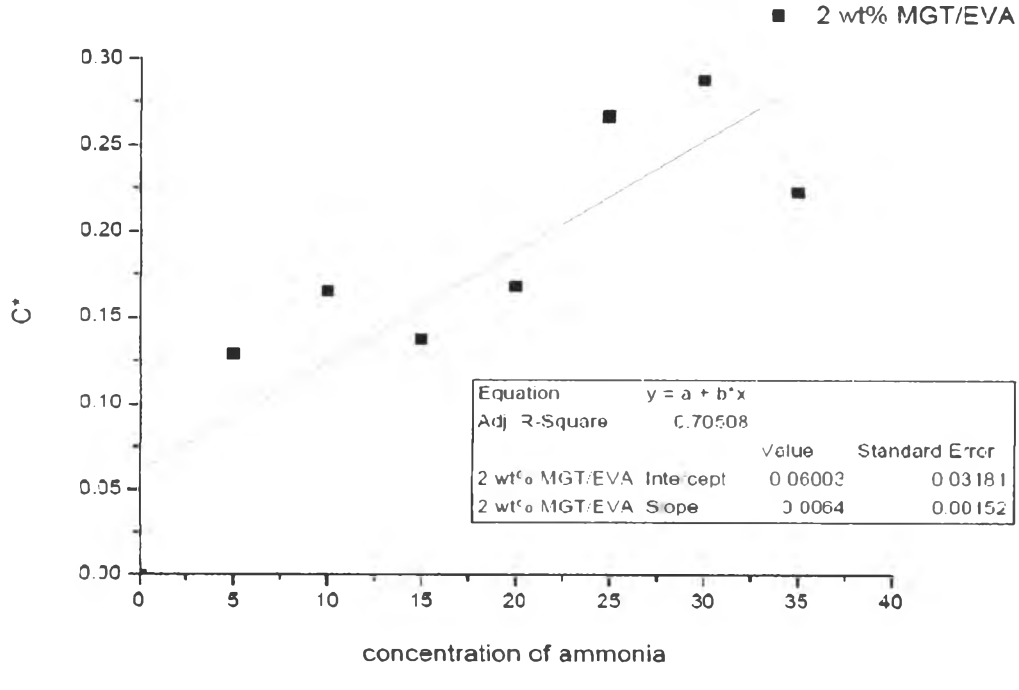

Figure 7.2 Change in color (C*) of 2 wt% mangosteen-dyed EVA film after contacted to ammonia in different concentration.

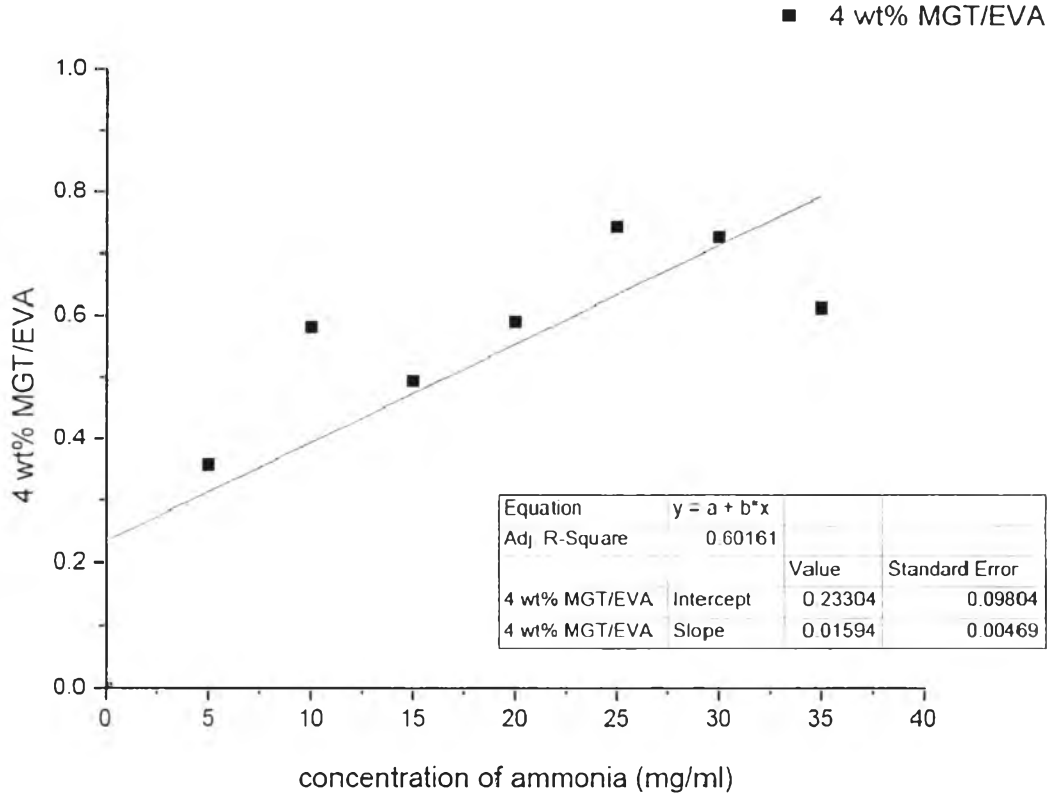

Figure 7.12 Change in color (C*) of 4 wt% mangosteen-dyed EVA film after contacted to ammonia in different concentration.

7.4.4 Fish Spoilage Test

The fundamental characteristic of pH indicator dyes that change color when placed in an acidic or basic environment is the key element of this freshness indicator. Fig.7.13 shows the change in 4 wt% MGT-EVA film in the fish spoilage test. It is seen that the color of the 4 wt% MGT-EVA film was changed gradually with the lower C* than those in the ammonia test. The relationship between the change in color and time is equal to $C^* = 0.01767t + 0.0104$ where t is time in hr. The R^2 of the linear fitting of the data is 0.98.

The sensitivity of the MGT/EVA films to detect the fish spoilage is better than those with the ammonia solution test results from lesser basic environment. The pH during the fish spoilage is reported to vary from 6.85 - 7.58, while pH of the ammonia solution is about 11. The gradual change in color is

detected with the color reader; however, they were not easy to differentiate with naked eyes. Fig.7.14 and Fig.7.15 present photo of film samples after the ammonia and the fish spoilage tests. It is seen that the color of film sample was changed in ammonia test from red into yellow more visually than those in the fish spoilage test.

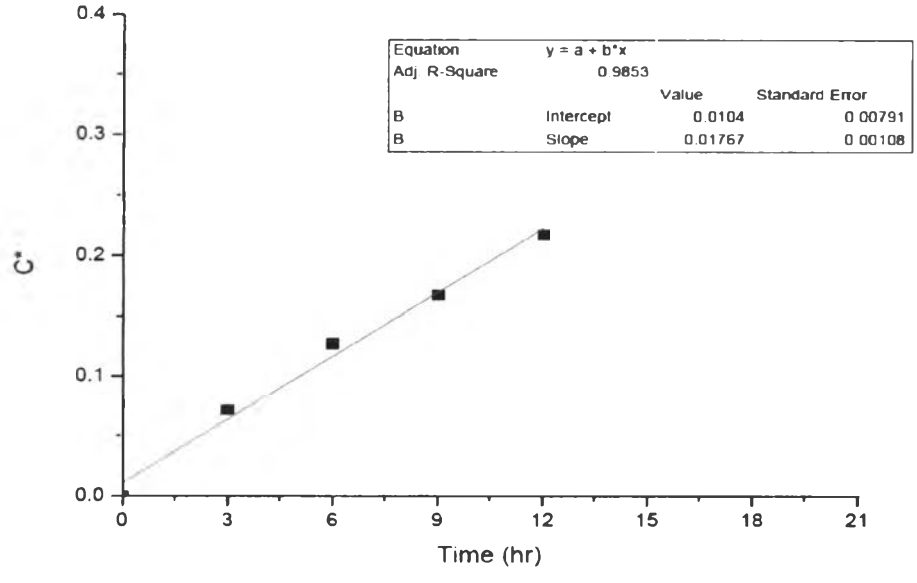

Figure 7.13 Change in color (C*) of 4 wt% mangosteen-dyed EVA films after contacted to fresh fish spoilage in various time. Relationship is expressed in linear with R^2 of 0.98.

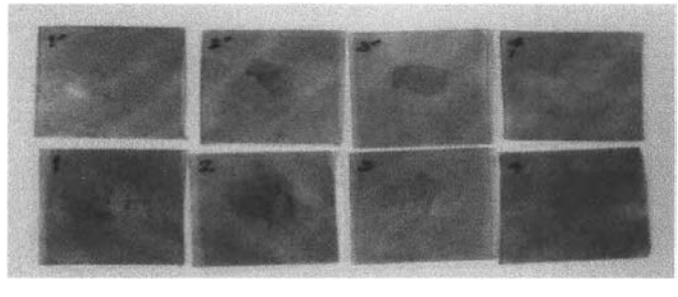

Figure 7.14 Change in color of 4 wt% mangosteen-dyed EVA films after tested in fish spoilage test in various times (the bottom row). The number 1, 2, 3, and 4 refer to test time of 3, 6, 9, and 12 hours, respectively. The top row is the samples exposure in a glass bottle filling with 10 ml of ammonia 40 mg/ml.

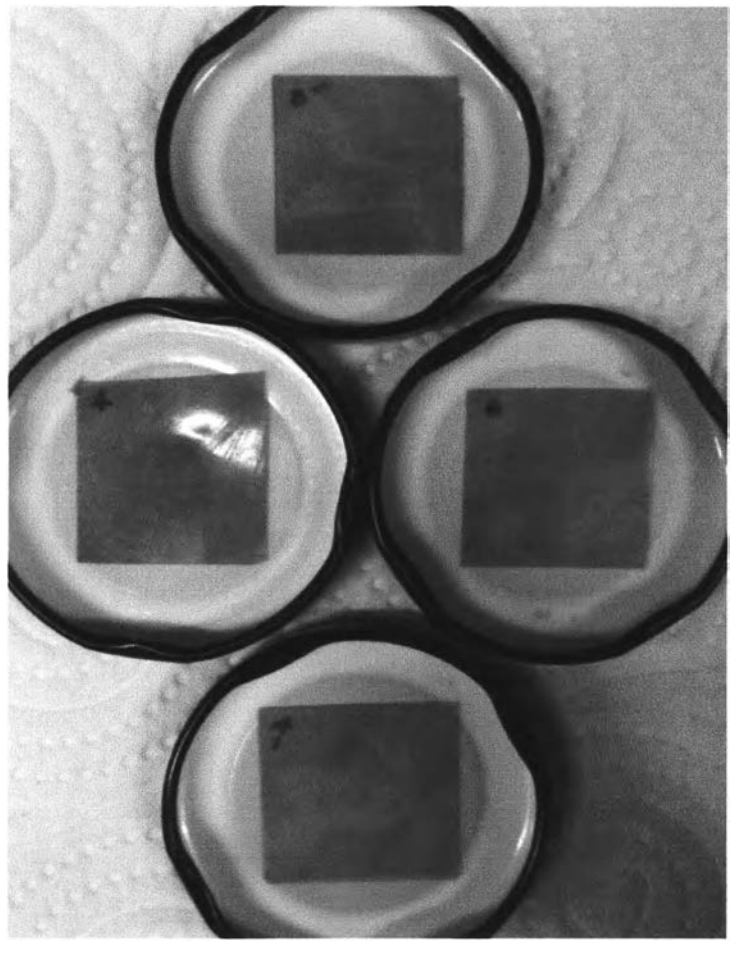

Figure 7.15 Change in color of 4 wt% mangosteen-dyed EVA film after tested in fish spoilage test for 12 hours. The top row shows the original dyed film sample, the middle row shows the film samples after tested in fish spoilage test, and the bottom row is the film sample after tested in ammonia.

Fig. 7.16 shows laminated films between PP/OBEN-CuNP5 film and 4 wt% MGT-EVA films. The laminated film was produced by hot-pressing with temperature of one side of 160 °C (for heating PP/OBEN films), and the other side was room temperature (MGT/EVF films). The setup for fish spoilage was performed similarly to the previous tests, except the glass bottles contained fresh fish were covered with the laminated films instead of tight sealed metal covers. As presented in Fig.7.17, the 4 wt% MGT/EVA film was placed inside the glass bottle which the

color of the MGT/EVA film could be seen thoroughly the laminated films. The laminated films were glued onto the glass bottles using cyanoacrylate-based adhesive. Weight of fresh fish meat used was 100 grams in all bottles. The fish meat did not contact with the film, where the head space between dye films and the fresh fish meat in the glass bottles was in the range of about 2 cm.

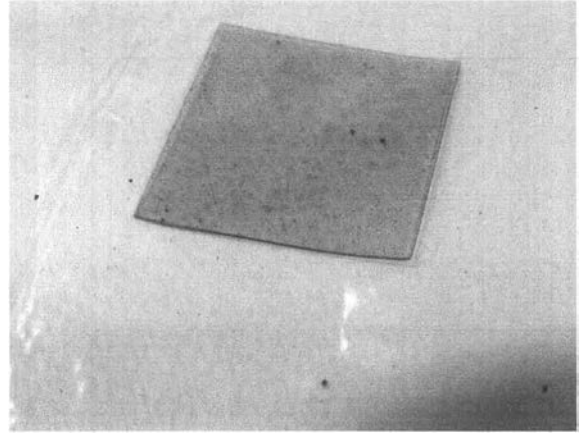

Figure 7.16 Laminated films between PP/OBEN-CuNP5 film and 4 wt% MGT-EVA films for fish spoilage test. The size of 4 wt%MGT/EVA film was 1.0 x 1.0 inch.

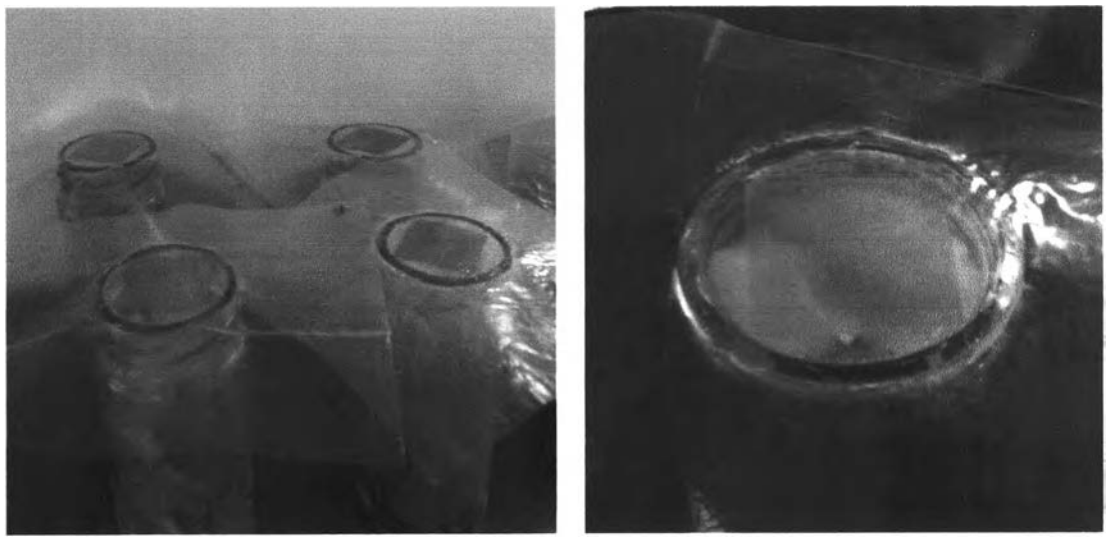

Figure 7.17 The fish spoilage setup showing the glass bottles contained 100 g of fresh fish covered with laminated films between PP/OBEN-CuNP5 film and 4 wt% MGT-EVA films.

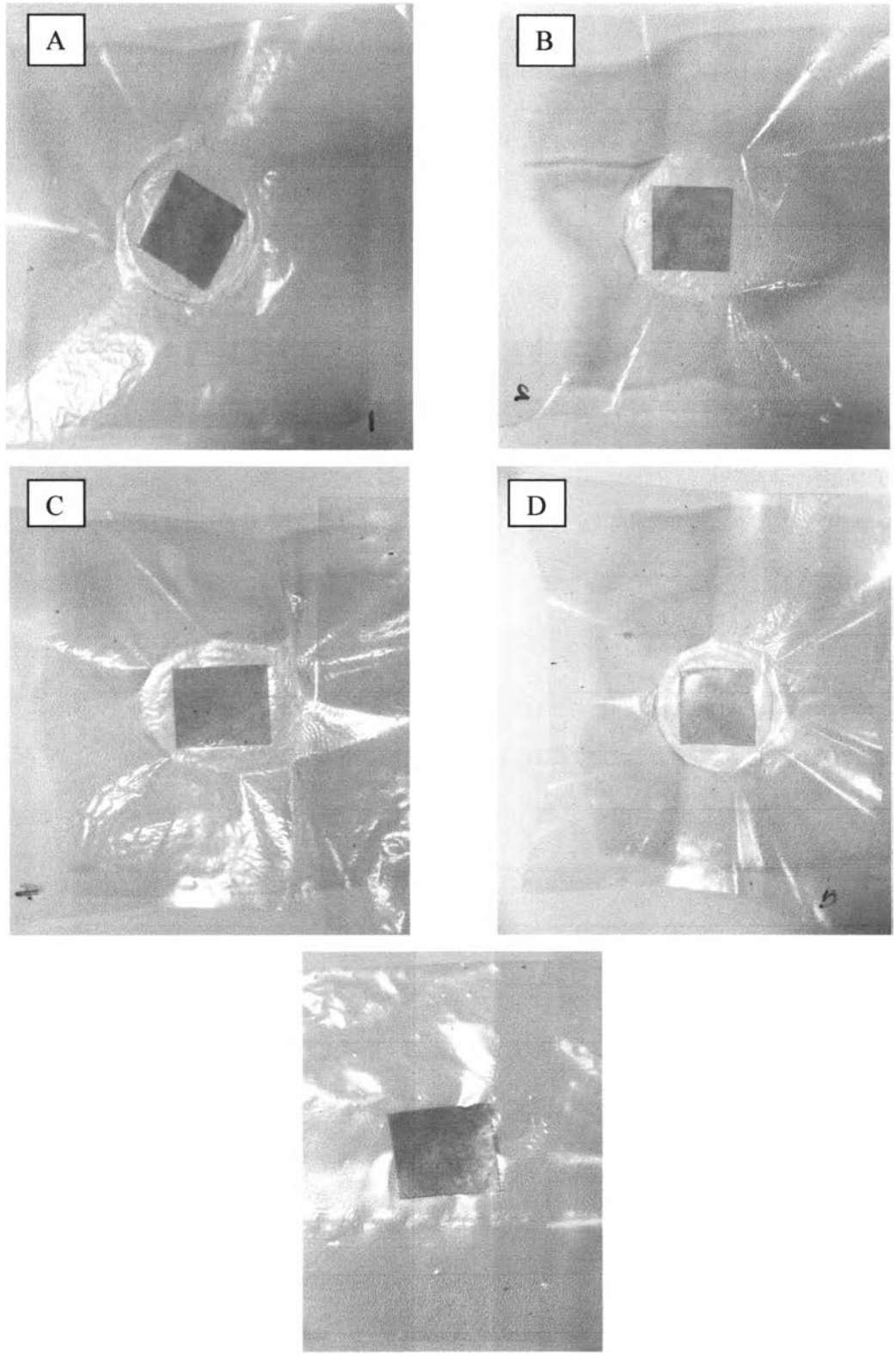

Figure 7.18 The color of the 4 wt% MGT-EVA films after the fish spoilage test with the time period of (a) 3 hours, (b) 6 hours, (c) 9 hours, (d) 12 hours, compared with the original film (e).

Fig.7.18 shows the changes in MGT/EVA color after the fish spoilage test with the time period of 3, 6, 9, and 12 hours, compared with the original dye film. It is found that the change in color of the MGT/EVA films was able to detect by naked eyes when the test time was over 9 hours. It indicates that the alkali volatiles from the fish spoilage reacted with the H^+ in the dye structures causing its color to change from reddish into brown. Fig.7.12 presents the change in color (C*) and lightness (L) after the fish spoilage test of the laminated film between 4 wt% MGT/EVA films and PP/OBE-Cu5 nanocomposite films. It is seen that the lightness of the dye film was lighten significantly after 9 hours, while the color was changed gradually.

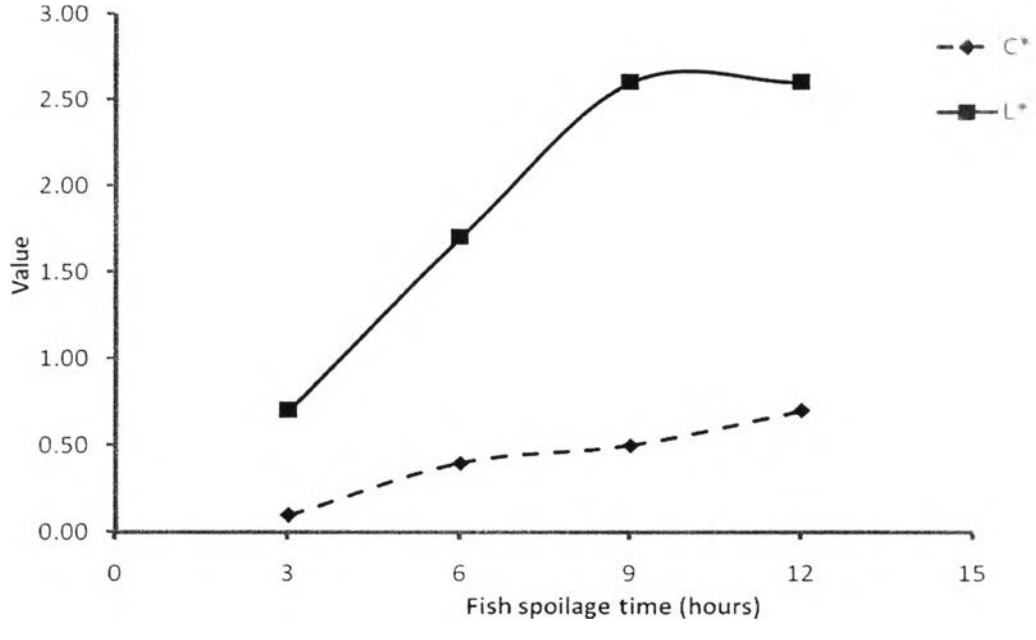

Figure 7.16 Change in color (C*) and lightness (L*) after the fish spoilage test of the laminated film between 4 wt% MGT/EVA films and PP/OBE-Cu5 nano-composite films.

7.5 Conclusions

Natural dyed film was prepared and studied the feasibility for using as a indicator for food spoilage. Red dye was extracted from Mangosteen pericarp using

citric acid and mixed with EVA in a twin-screw extruder at 120 °C to produce dye compound. The dye film was prepared by using mangosteen extracted solution 2, 4, and 6 wt%. In the ammonia sensing test, the steeper slope in the 4 wt% dye EVA film indicates better sensitivity to detect change in ammonia concentration. The TVBN value of 40.32 mg/100g of fish meat was reached after about 9 hr of storage indicating that the spoilage fish was not acceptable for human consumption. In the fish spoilage test, it is seen that the color of the 4 wt% MGT-EVA film was changed gradually with the lower C* than those in the ammonia test. The relationship between the change in color and time is equal to $C^* = 0.01767t + 0.0104$ where t is time in hrs. The R^2 of the linear fitting of the data is 0.98. The sensitivity of the MGT/EVA films to detect the fish spoilage is better than those in the ammonia solution test due to lesser basic environment. The gradual change in color is detected with the color reader; however, they were not easy to differentiate with naked eyes. The laminated films between 4wt% MGT/EVA and PP/OBEN-Cu5 was successfully prepared and they were able to detect the spoilage of the fresh fish.

7.6 References

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