



## CHAPTER V

### PP/ORGANOCLAY NANOCOMPOSITE INTELLIGENT PACKAGING BASED ON PEDOT:PSS AND COPPER SENSORS

#### 5.1 Abstract

To date, a rapid development in techniques for evaluating the meat freshness has gained a considerably attention from the food industry. The development of an “intelligent packaging” that monitors the microbial breakdown products in the headspace of packaged meat is described. Poly(3,4-ethylenedioxythiophene):Poly(sodium-4-styrenesulfonate) (PEDOT:PSS) and copper (II) ion ( $\text{Cu}^{2+}$ ) are selected due to their sensitivity to the meat spoilage by changing the color of the films. PEDOT:PSS will change color from clear blue to deep blue after being stimulated by volatile base from aged chicken meat. Additionally,  $\text{Cu}^{2+}$  sensors turn color from light blue to green then to brown. For this work, PEDOT:PSS and  $\text{Cu}^{2+}$  has been coated on to polypropylene(PP)/organoclay nanocomposite films via a spin-coating technique. The change in color were measured through a Chroma Meter and reported in Hunter ( $L$ ,  $a$ , and  $b$ ) values and total color difference (TCD). The results indicated that there is a correlation among the color change of the composite films, TVB-N, and microbial populations. TCD increases with increasing concentration and thickness of sensors, amount of meat and decreasing clay content of the nanocomposite film substrates.  $\text{Cu}^{2+}$  sensor is more sensitive to volatile base than PEDOT:PSS sensor. Finally, it can be concluded that this intelligent packaging could be chosen as an effective tools for detecting the meat spoilage.

#### 5.2 Introduction

The quality, freshness and safety of food are required by consumers due to changes in the way of food production, distribution, storage and retail [1]. Therefore, food safety assurance has been critically important to consumer confidence. Various approaches have been used for the determination of food quality. Currently, most packaged food only offers an expiry date which depends on the suitable temperature

of storage at every step of the distribution process. For highly perishable foods such as fish, meat, milk, there is a definite risk that the product may be spoiled when sold to the customer despite being well within its expiry date. Public health concerns and the reputation of supplier are consequently impacted [2].

To meet the growing demands of consumer freshness and safety in food products, intelligent packaging system has become an alternative method to determine food quality and to warn distributors, suppliers and consumers about the state of the product. It has been defined as packaging system which monitors the condition of packaged foods to give information about the quality of the packaged food during transportation and storage [1].

Meat quality can be determined by intelligent packaging. By this technique, many chemical methods have been used as indices of deterioration of meat quality. They usually measure the amounts of breakdown products derived from enzymatic, bacterial or oxidative activities. Total volatile basic nitrogen (TVB-N) is one of those products formed by the bacterial degradation; therefore, it has been implicated as indicator of meat products [3].

Polypropylene (PP)/organoclay nanocomposites coated with poly(3,4-ethylenedioxythiophene):Poly(sodium-4-styrenesulfonate) (PEDOT:PSS) or copper ion ( $\text{Cu}^{2+}$ ) can be used as intelligent packaging for meat spoilage based on the detection of TVB-N produced during aging of meat. Since PEDOT:PSS exhibits a high degree of visible light transparency in the oxidized state, while being an opaque blue in the reduced state, it gives color of opaque blue when it receives some electrons from total volatile base [4]. Additionally,  $\text{Cu}^{2+}$  turn color from light blue to green then to brown after receiving some electrons, resulting in the change of oxidation number from +2, to +1, and then 0, respectively [5].

In this research work, packaging film based on PP organomodified clay nanocomposite film fabricated with PEDOT:PSS and  $\text{Cu}(\text{NO}_3)_2$  by varying concentration of sensor, sensor thickness, %clay content in nanocomposite film, the amount of meat samples, and type of sensor will be prepared. The capability to use this film as intelligent packaging is demonstrated in term of change in total color difference (TCD).

## 5.3 Experimental

### A. Materials

Tenderloin of chicken was purchased from the same filets, under the same aseptic conditions at Samyan market. Sodium activated bentonite (Na-BTN), under the trade name Mac-Gel<sup>®</sup> (GRADE SAC), with cationic exchange capacity (CEC) of 44.5 meq/100 g clay, was supplied by Thai Nippon Co., Ltd. Thailand. Dipalmitoylethy hydroxyethylmonium methosulfate was purchased from Sunny World Co., Ltd. Sodium- neutralized ethylene-co-methacrylic acid with MFI = 4 dg/min used as a compatibilizer was purchased from DuPont Co Ltd. Polypropylene (PP) with MFI = 11 dg/min, under the trade name, Polene NK 1126, was purchased from Thai Petrochemical Industry Public Co., Ltd (IRPC Co., Ltd). Poly(vinyl acetate) emulsion was purchased from TOA Chemical Industries Limited. 3,4-ethylenediethoxythiophene (EDOT) was purchased from Sigma-Aldrich. Copper (II) nitrate ( $\text{Cu}(\text{NO}_3)_2$ ) was purchased from Univar. Poly(sodium-4-styrenesulfonate) (PSS),  $M_w$  500,000  $\text{g mol}^{-1}$ , Iron (III) sulfate hydrate ( $\text{Fe}_2(\text{SO}_4)_3$ ) and Sodium peroxodisulfate ( $\text{Na}_2\text{S}_2\text{O}_8$ ) were purchased from Sigma-Aldrich. Boric acid ( $\text{H}_3\text{BO}_3$ ,  $M_w$  61.83  $\text{g mol}^{-1}$ ) was purchased from Merck.

### B. Preparation of Organomodified Bentonite (OBTN)

350 g of Na-bentonite, Na-BTN, was swollen in 1.05 liters of distilled water for 24 hr. A cationic surfactant with 2.0 equivalents of CEC was dissolved in 100 ml of ethanol at 80°C and was added to the bentonite dispersion and stirred vigorously for 3 h. The resulting organomodified bentonite, denoted as OBTN was filtered and washed with hot water several times to remove the excess surfactant. Then, it was dried in vacuum oven at 100°C overnight, ground, and screened through a 325 mesh sieve [6].

The characterizations of organomodified bentonite were described in chapter 4.

### C. Preparation of PP/Organoclay Nanocomposite Films

PP/organoclay nanocomposites were prepared by conventional melt intercalation a Collin D-8017 Model T-20 co-rotating twin-screw extruder with L/D=30 and D=25 mm. The operating temperatures of extruder were maintained at 80, 170, 180, 190, 200, and 210 °C from hopper to die respectively. The screw speed was maintained at 50 rpm. The master batches containing 50 wt% organomodified clays with the compatibilizer was added to PP and Surlyn<sup>®</sup>, fixed at 6 wt%, in appropriate amounts to obtain nominal contents of 1, 3, and 5 wt% clays in the nanocomposites. Each composition was premixed by a tumble mixer for 10 min before introducing into the twin-screw extruder and extruded through a single strand die. The yielded single strands of the PP/clay nanocomposites were obtained and solidified by cool water, palletized and then dried in a vacuum oven at 80°C before characterizations by SEM, TGA, and DSC [6].

The nanocomposites films were prepared by blown film extrusion machine. The nanocomposites pellets were dried in vacuum oven at 80°C for 12 h prior to blowing. Following extrusion conditions were employed; the rotational speed of screw of 50 rpm and blowing ratio of 1.52. The barrel and mold temperature were 210 °C. The thickness of the films was controlled to about 45 μm [7, 8]. The nanocomposite films from blow molding were used for preparing intelligent film and studying mechanical properties.

The characterizations of PP/organoclay nanocomposites were described in chapter 4.

### D. Synthesis of PEDOT:PSS Sensor

Synthesis of PEDOT:PSS sensor was carried out by following the experiment by Louwet *et al.* [9].

PEDOT:PSS was prepared by mixing of 10.65 g (75 mmol) of 3,4-ethylenedioxythiophene (EDOT), 439 g of 5.99 wt% PSS solution and 21.4 g (104 mmol) Na<sub>2</sub>S<sub>2</sub>O<sub>8</sub> in 2062 ml of distilled water. After initial stirring at room temperature for 10 min, 187 mg of Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> was added and the mixture was stirred vigorously for 24 h resulting in an aqueous PEDOT:PSS.

PEDOT which was synthesized from this procedure was in the doped state which has clear blue color. PSS acts as counter ion for charge compensation of PEDOT.

FT-IR spectrometer was used to investigate the PEDOT:PSS, polymerized in this work.

### *E. Preparation of Intelligent Films*

The sensor fabrication was carried out by following the experiment established by Pacquit *et al.* [10].

A typical sensor solution contained a binder, poly(vinyl acetate) and a sensor, PEDOT:PSS (1, 3, 5% w/w) and  $\text{Cu}(\text{NO}_3)_2$  (1, 3, 5, 10% w/w). The mixture was vigorously stirred for 30 min and degas in an ultra sonic bath for 30 min to remove the bubbles. A sensor solution was then spin-coated at 1000, 2000, 3000 rpm onto nanocomposite film substrate discs by using an automatic pipette and depositing 0.5 ml of the sensor solution. The obtained intelligent films with circular shape of 7 cm diameter were heated at 60 °C for 10 min or left at room temperature overnight to dry. After that, each intelligent film was laminated at 120 °C by lamination machine to improve adhesion between nanocomposite film and sensor. The thickness of sensor was measured by using the peacock digital thickness gauge model PDN 12N.

In the present work, the effect of the following parameters on the color change of PEDOT:PSS and  $\text{Cu}^{2+}$  sensors were investigated.

- 1) Effect of concentration of sensor
  - 1, 3, and 5 %w/w for PEDOT:PSS sensor
  - 1, 3, 5, and 10 %w/w for  $\text{Cu}^{2+}$  sensor
- 2) Effect of sensor thickness by controlling spin-coating speed
  - 1000, 2000, and 3000 rpm
- 3) Effect of clay content in nanocomposite film
  - 1, 3, 5 %wt clay
- 4) Effect of the amount of meat tissues
  - 25, 50, 100, and 150 g

### 5) Effect of type of sensor

- PEDOT:PSS sensor
- $\text{Cu}^{2+}$  sensor

### F. Meat Spoilage Trial

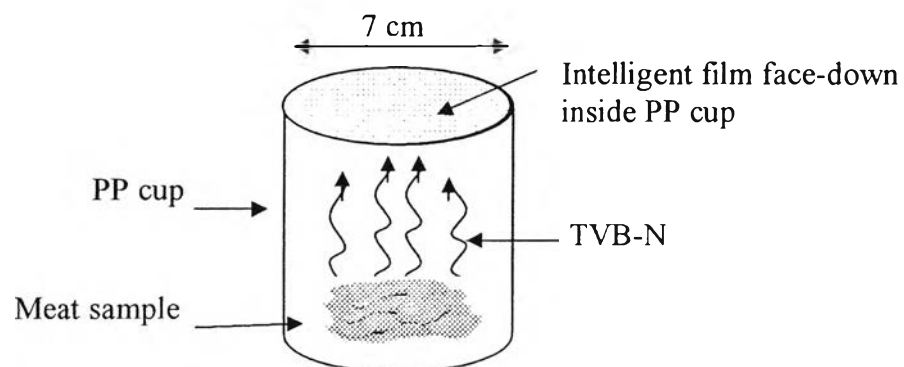
Tenderloin of chicken was obtained from the same filets, under the same aseptic conditions at Samyan market. Meat spoilage trial was carried out by following the experiment established by Byrne *et al.* [11].

Aseptic techniques such as the use of disposable gloves, bactericide built-in cutting board and flame sterilized scalpel were used to avoid sample contamination.

For a set of sensors, fifteen replicate meat samples were placed in separate individual polypropylene cups. Each intelligent film was sealed on the top of PP cup that contains meat tissue inside with fast cure epoxy to create a permanent gas-tight seal and to prevent leakage of amines. Samples were at no time indirect contact with the sensors (i.e. only headspace was sampled). The sensors responses were monitored every 3 h with a Chroma Meter.

In this study, PEDOT:PSS sensors displayed a color change from clear blue to deep blue and  $\text{Cu}^{2+}$  sensors change color from blue to brown after receiving some electrons.

Experimental design for meat spoilage monitoring is shown in Figure 3.2 below.



**Figure 5.1** Experimental design for meat spoilage monitoring.

### G. Evaluation of Intelligent Film

The responses of meat samples, allowed to spoil at room temperature, to different sensors of various thicknesses (1000, 2000, and 3000 rpm), sensor concentrations (1, 3, 5, and 10 %w/w for Cu<sup>2+</sup> and 1, 3, and 5%w/w for PEDOT:PSS), clay contents (1, 3, 5 %wt) in the nanocomposite substrates, the amount of meat tissues (25, 50, 100, and 150 g), and type of sensors (PEDOT:PSS and Cu<sup>2+</sup> ion) were investigated.

Color changes of the intelligent films were measured according to ASTM D65 by using a Chroma Meter, HunterLab<sup>®</sup> with 45°/0° optical geometry, software of Easy Match QC, diffuse/45°, and reported in Hunter system ( $L^*$ ,  $a^*$ , and  $b^*$ ) values and total color difference (TCD). The TCD value ( $\Delta E$ ) was calculated by the following equation [12]:

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

Here,  $\Delta L$  is the brightness difference between sample and target,  $\Delta a$  the greenness-redness difference between sample and target, and  $\Delta b$  is the blueness-yellowness difference between sample and target. The target color is (93.13, -0.96, 1.69) corresponding to ( $L^*$ ,  $a^*$ , and  $b^*$ ) for white standard color in Hunter system. The intelligent films, which were tested with meat at any time, were cut into circular shape with 5 cm in diameter and were measured by reading 3 points at random position over tested area.

### H. Microbial Analysis of Meat Samples

Microbial analysis was used to determine the degree of meat spoilage. The number of microorganism, which is more than  $10^7$  cfu/g is considered as spoilage [13].

Microbial analysis was carried out by following the experiment established by Pacquit *et al.* [14].

Samples of approximately 50 g was removed from the same meat filets, under the same aseptic conditions, and placed in zip lock bags. The samples were then allowed to spoil at room temperature under the same conditions and every 3 h, along

with every sensor response measurement, a sample was transferred into a freezer. Aerobic Plate Count (APC) or Total Viable Count (TVC) was determined, using the pour plate method, on plate count agar. Plates were counted after 48 h incubation at 30 °C and results were correlated with the sensor response.

Aseptic techniques such as the use of disposable gloves, bactericide built-in cutting board and flame sterilized scalpel were used to avoid sample contamination.

### *I. Determination of Total Volatile Basic Nitrogen (TVB-N)*

Volatile amine, such as trimethylamine (TMA), dimethylamine (DMA), and ammonia are products of microbial degradation and are collectively known as total volatile basic nitrogen (TVB-N). TVB-N can be also used to determine the degree of meat spoilage. A level of 30-40 mg TVB-N/100g of meat sample is usually regarded as spoiled [3].

Determination of TVB-N was carried out by following the experiment by Fish Inspection and Quality Control Division (FIQD), the Department of Fisheries (DOF), Thailand.

#### *a. Sample Extraction*

The meat sample was ground by homogenizer. After that 3 g of homogenized sample was placed into a centrifuge tube followed by adding 12 ml of 4% Trichloroacetic acid (TCA) solution. The tube was sealed and vigorously shaken to obtain good mixing. The sample was then left at room temperature for 30 min with stirring from time to time. The sample mixture was filtered using Whatman paper No. 1. When prepared samples were not used within a day, the filtered solution must be kept at -18°C in vials.

#### *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). After that 1 ml of filtered sample extract was pipetted into the outer ring of the Conway's unit. 1 ml of saturated  $K_2CO_3$  solution was then pipetted into the outer ring of the Conway's unit and placed on the opposite side of the sample (to make sample extract into alkaline condition similar to that of volatile compound). The Conway's unit was immediately covered and shaken gently to dissolve and mix the extract samples with  $K_2CO_3$  solution without contaminating the inner ring of Conway's unit (triplicate for each sample). Then, 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.

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

### c. Calculation of TVB-N

TVB-N value was calculated by the following equation

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

- 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)
  - $N_{\text{HCl}}$  = Normality of HCl (= 0.01 N  $\times$  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

### J. Volume Electrical Conductivity of Sensor to Meat Spoilage

The volume electrical conductivity ( $\sigma_{dc}$ ) was measured via two-point probe technique using Keithley 8009 Resistivity Test Fixture with dc voltage from 1 to 100

V. The sensors, which were tested with meat any time, were peeled off from nano-composite films before testing. The sensor films were placed in the Keithley 8009 test fixture. The voltage was applied across the sample, and the current was read by the electrometer. Then, the volume electrical conductivity was calculated via the following equation:

$$R = \frac{V}{I}$$

$$\rho_v = \frac{22.9V}{tI}$$

$$\sigma_{dc} = \frac{1}{\rho_v},$$

Where,  $R$  is the resistance (ohm),  $V$  is the voltage (volts),  $I$  is the current (amperes),  $\rho_v$  is the volume resistivity (ohm centimeters),  $t$  is the sample thickness (centimeters) and  $\sigma_{dc}$  is the volume conductivity (siemens per centimeter).

### *K. Leaching Studies*

The  $\text{Cu}^{2+}$  powders were dissolved in water to prepare the standard solution of 1, 5, 10, 20, and 40 ppm. Intelligent films were cut into rectangular shape with 4x4 cm and were soaked with 10 ml of water for approximately 48 h, after which a wavelength spectrum of the water (200-900 nm) was carried out to detect the presence of sensor by using UV-Vis spectroscopy (Shimadzu model UV-2550) with medium scan rate and sampling the data every 1 nm.

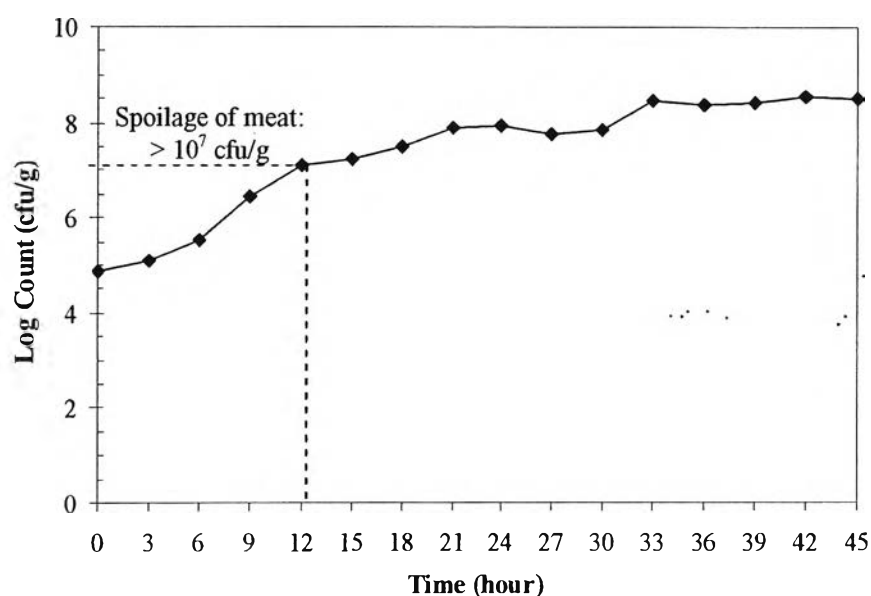
## **5.4 Results and Discussion**

### *A. Microbial Analysis*

Microbial analysis was used to determine the degree of meat spoilage. The number of microorganism, which is more than  $10^7$  cfu/g is considered as spoilage [13].

Spoilage of poultry is generally restricted to the outer surfaces of the skin and cuts and has been characterized by off-odors, which appears at a bacterial load

between  $10^7$  and  $10^8$  cfu/g, sliminess generally occurs shortly after the appearance of off-odors, with log counts of about 8 cfu/g, and various types of discolorations [1, 2]. Fig. 5.2 shows the range of microbial population commonly associated with spoilage of meat samples. The bacterial level of  $10^7$  cfu/g, considered as spoilage of meat was reached about 12 h. The aerobic plate counts gradually increased during the initial time, reaching values of  $10^7$  cfu/g at approximately 12 h before stabilizing at 33 h about  $10^8$  cfu/g.

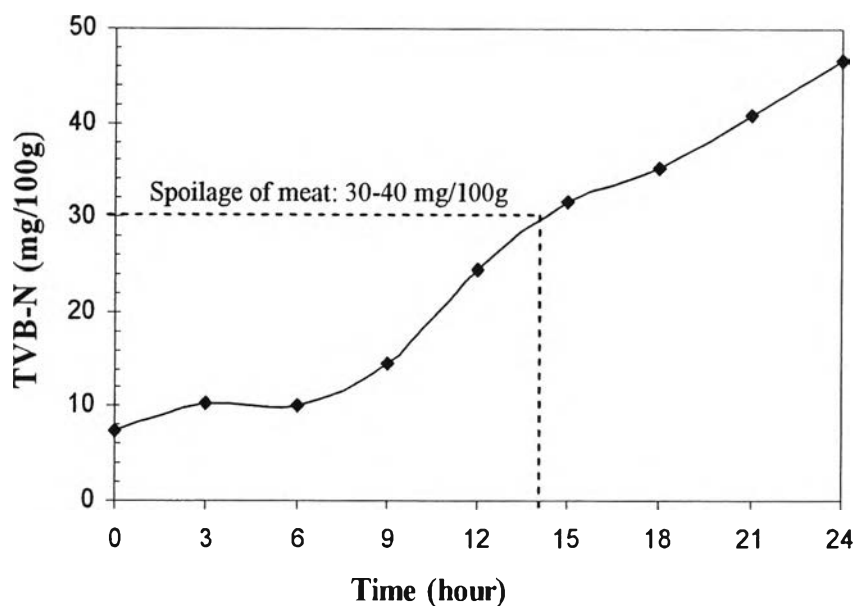


**Figure 5.2** Aerobic plate count of meat samples during storage time at room temperature.

#### B. Determination of Total Volatile Basic Nitrogen (TVB-N)

TVB-N was also used as deterioration index of fresh meat. A level of 30-40 mg TVB-N/100g of meat sample is usually regarded as spoiled [3]. TVB-N value of meat samples during storage time at room temperature is shown in Fig. 5.3. The TVB-N curve was found slowly increasing and the value of 30 TVB-N/100g, defined as spoilage was reached after 12 h.

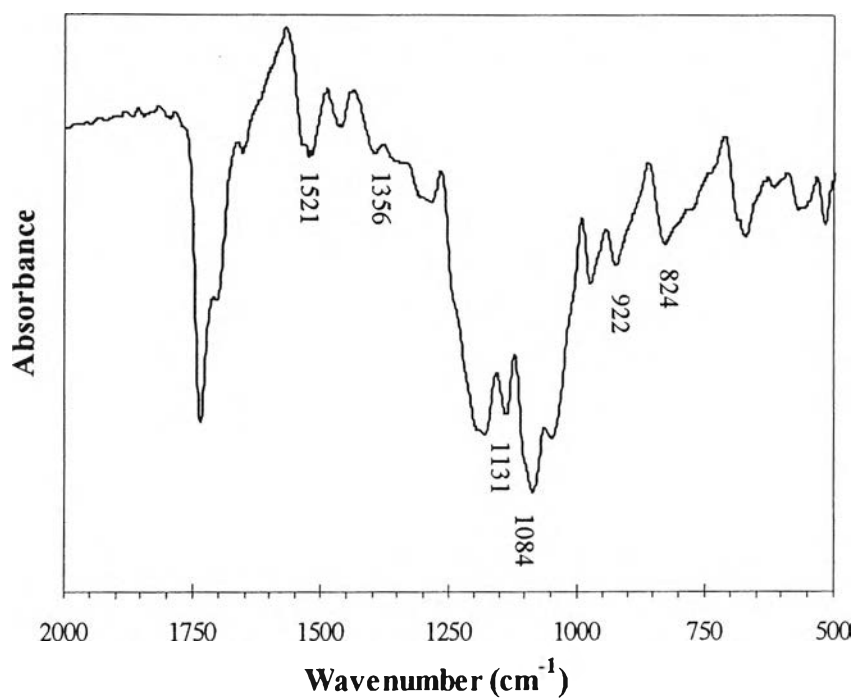
From the results of aerobic plate counts and TVB-N, it was observed that meat, left at room temperature, about 12 h is considered as spoilage and does not acceptable for consumption.



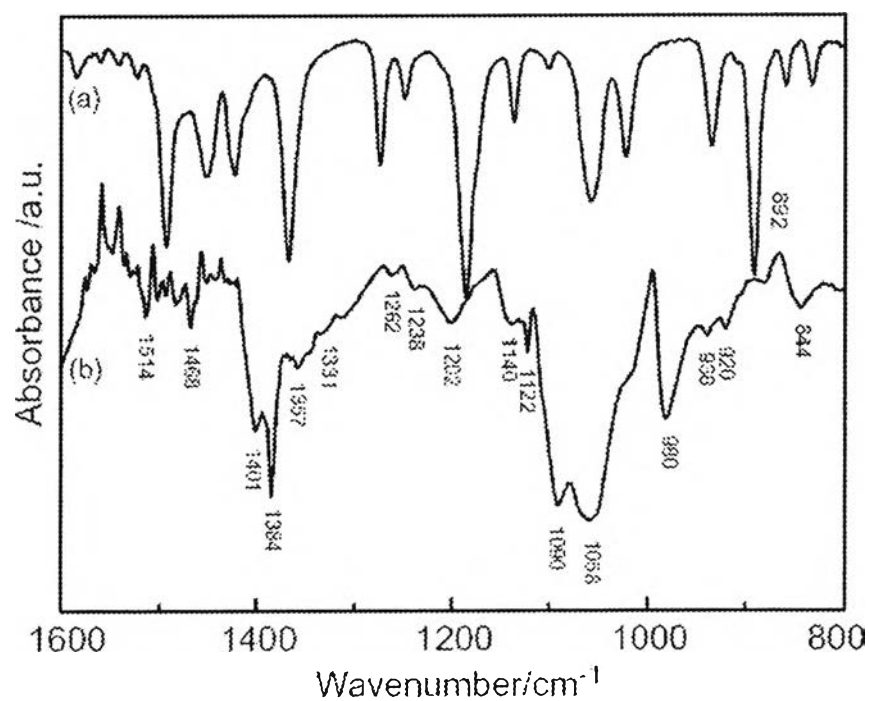
**Figure 5.3** TVB-N values of meat samples during storage time at room temperature.

### C. Characterization of PEDOT:PSS Sensor

FT-IR measurements were carried out to confirm molecular structures of the resulting PEDOT/PSS. FT-IR spectra of PEDOT/PSS polymer which was synthesized and used as reference are presented in Fig. 5.4 and Fig. 5.5, respectively [15]. The IR band at  $892\text{ cm}^{-1}$ , as shown in Fig. 5.5 (a), was ascribed to the bending mode of C-H bond in EDOT monomer. However, it disappeared after the polymerization (as shown in Fig. 5.4), indicating the formation of PEDOT molecular chains with  $\alpha$ - $\alpha$ -coupling. And the vibrations at  $1521$  and  $1356\text{ cm}^{-1}$  in Fig 5.4 were corresponding to the C=C and C-C stretching in the thiophene ring, bands at  $824\text{ cm}^{-1}$  could be ascribed to vibration modes of C-S bond in the thiophene ring, peaks at  $1131$  and  $1084\text{ cm}^{-1}$  were assigned to the stretching modes of the ethylenedioxy group in the molecule, peak at  $922\text{ cm}^{-1}$  was corresponding to the ethylenedioxy ring deformation mode. Those results indicated clearly that PEDOT is polymerized in this preparation [15].



**Figure 5.4** FT-IR spectra of PESOT:PSS as prepared.



**Figure 5.5** FT-IR spectra of PESOT:PSS as reference of: (a) EDOT monomer and PEDOT:PSS polymer [15].

#### D. The Concentrations of Sensors: Difference in Sensor Response

All intelligent films indirect contact with the meat samples responded to the increasing volatile basic amines generated by spoilage with color change from clear blue to deep blue for PEDOT:PSS sensor and from light blue to green then to brown for  $\text{Cu}^{2+}$  sensor. Since PEDOT:PSS exhibits a high degree of visible light transparency in the oxidized state, while being an opaque blue in the reduced state, it gives color of opaque blue when it receives some electrons from total volatile base. Additionally,  $\text{Cu}^{2+}$  turn color from light blue to green then to brown after receiving some electrons, resulting in the change of oxidation number from +2, to +1, and then 0, respectively. In this work, meat was allowed to spoil at room temperature and sensors were monitored every 3 h until 45 h, which is no further color change.

Both sensors with different concentrations were used to investigate the color change while the thickness of 44  $\mu\text{m}$ , 5%wt clay in nanocomposite film, and amount of meat samples of 100 g were controlled. Fig. 5.6 shows the effect of concentration of PEDOT:PSS sensors on changes in Hunter color values. Hunter  $L^*$ ,  $a^*$  and  $b^*$  were almost no color change during initial stage; however, they gradually changed at the middle stage and remain constant at the final stage.

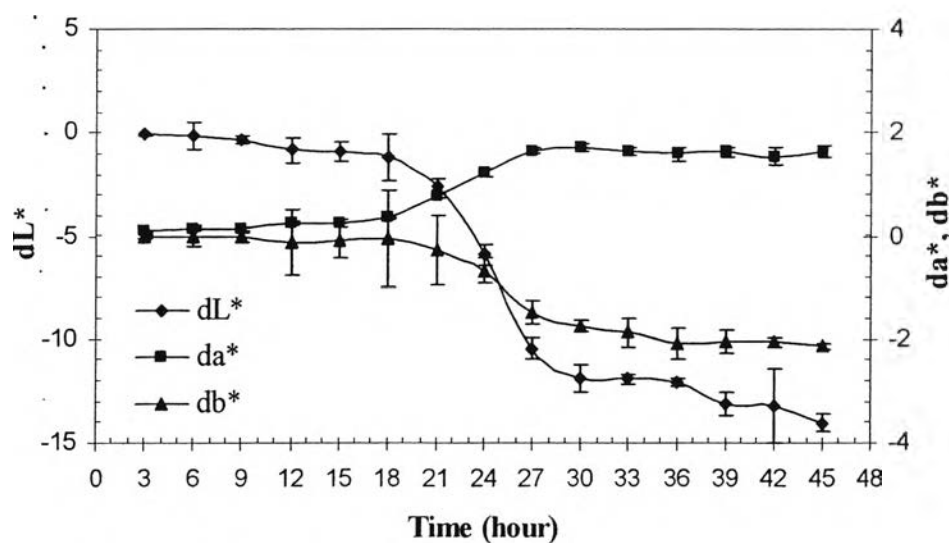
It is generally known that TCD values more than 5.0 can be easily detected by human eyes and more than 12.0 may imply absolutely different color spaces [16]. The TCD values of PEDOT:PSS sensors with various concentrations were calculated and shown in Fig. 5.7. The TCD values of three sensors increased with increasing storage time when fresh meat became deteriorated. Although responses in all concentrations were similar in time and range; however, sensors with the highest concentration, which was 5% w/w PEDOT:PSS, showed the greatest TCD valued among these sensors. This is due to the higher amount of materials on the film, compared to others. From these results, 5% w/w PEDOT:PSS was selected for further studies.

Fig. 5.8 shows the effect of concentration of  $\text{Cu}^{2+}$  sensors on changes in Hunter color value of  $\text{Cu}^{2+}$ . Hunter  $L^*$ ,  $a^*$ , and  $b^*$  of slowly changed at the initial stage, sharply changed at the middle stage, and remain constant at the final stage.

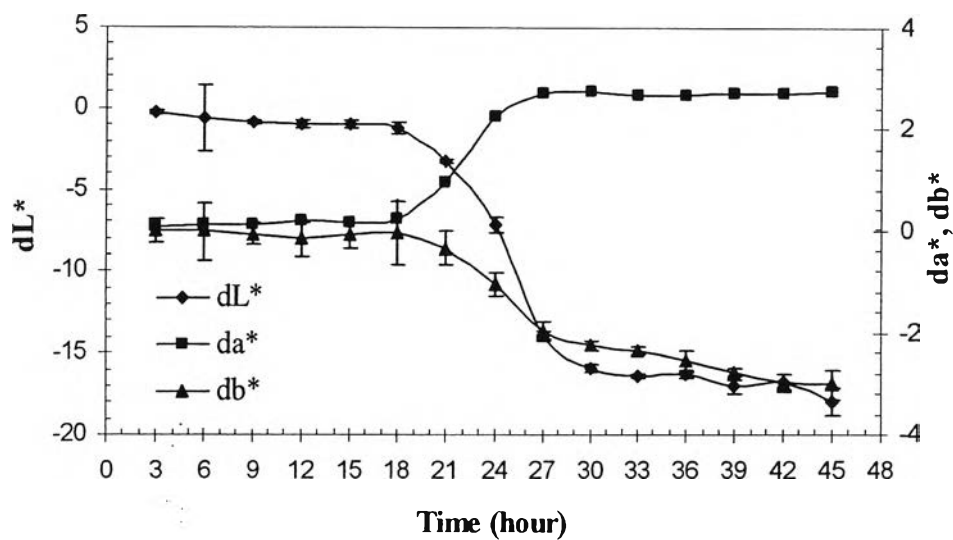
The effect of concentration of  $\text{Cu}^{2+}$  sensors on changes in TCD values is presented in Fig. 5.9. The TCD values of three sensors also changed continuously

with the responses of sensors and gradually rose with storage time as meat became spoiled. The sensor responses in all four concentrations were difference in time and range. 10% w/w  $\text{Cu}^{2+}$  responded to spoilage of meat samples faster than 5%, 3%, and 1% w/w  $\text{Cu}^{2+}$ , respectively. The reason of this result is similar to that of PEDOT:PSS sensor which is the greater amount of materials on the film, compared to the others. However, the response of 10% w/w  $\text{Cu}^{2+}$  sensor was not much different from 5% w/w  $\text{Cu}^{2+}$  sensor. Therefore, 5% w/w  $\text{Cu}^{2+}$  was selected for further studies.

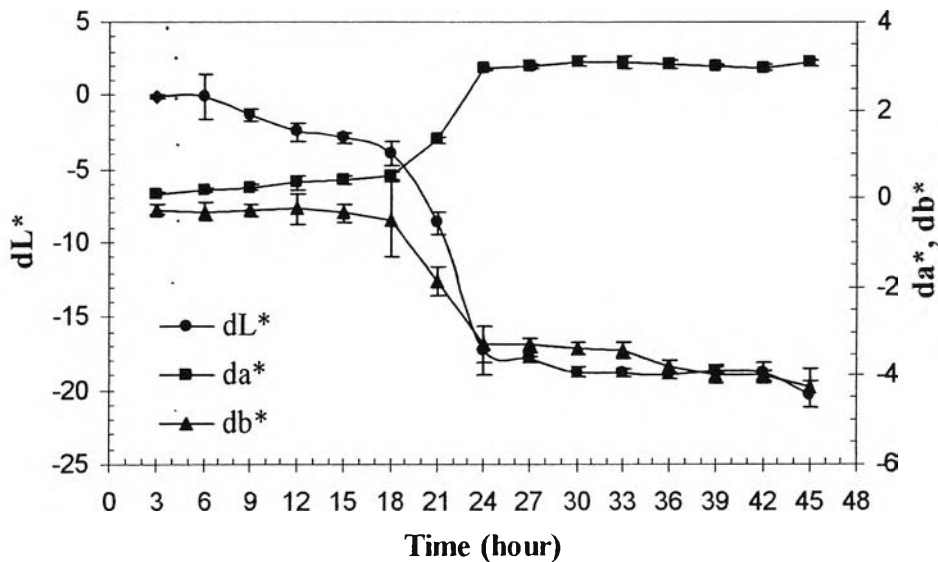
From the microbial and TVB-N analysis, the spoilage of meat started about 12 h. TCD value of 5% w/w PEDOT:PSS at 12 h was around 2 and that of 5% w/w  $\text{Cu}^{2+}$  was about 17. It was concluded that the PEDOT:PSS sensor was difficult to notice by human eyes whereas the  $\text{Cu}^{2+}$  sensor was easy to detect by naked eyes at the onset of meat spoilage due to high TCD values.



(A)



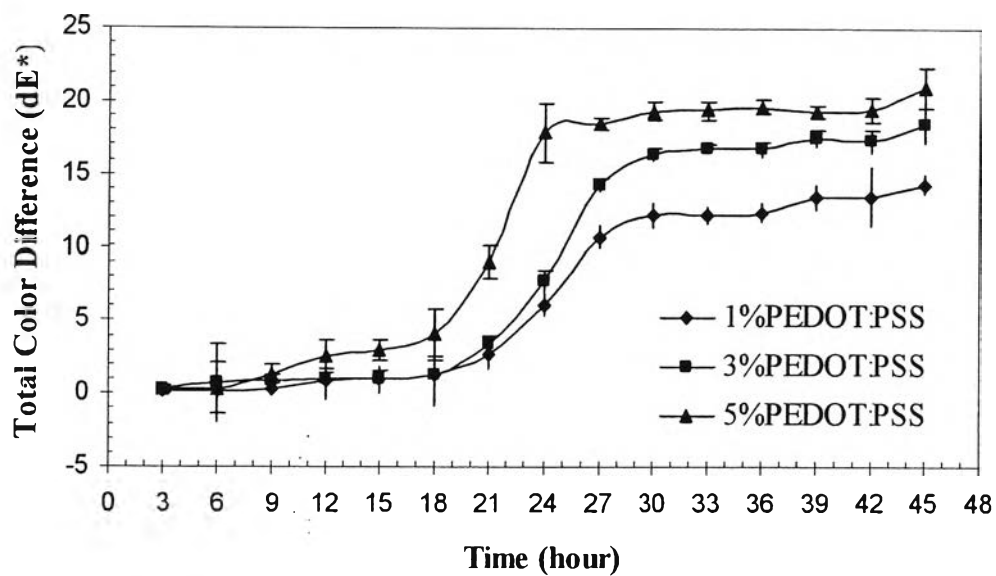
(B)



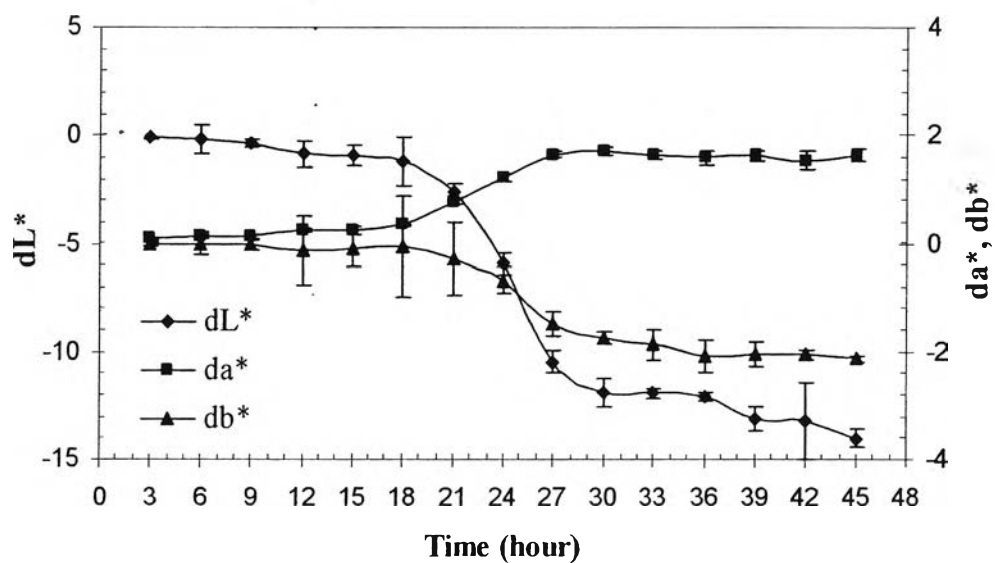
(C)

**Figure 5.6** The effect of concentration of PEDOT:PSS sensors on changes in Hunter color values of (A) 1% w/w PEDOT:PSS and (B) 3% w/w PEDOT:PSS (C) 5% w/w PEDOT:PSS.

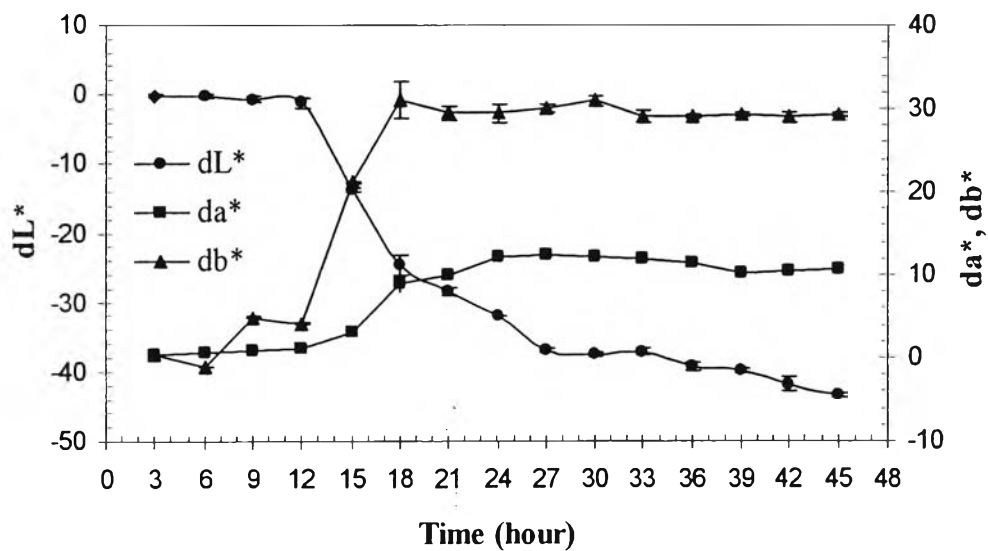




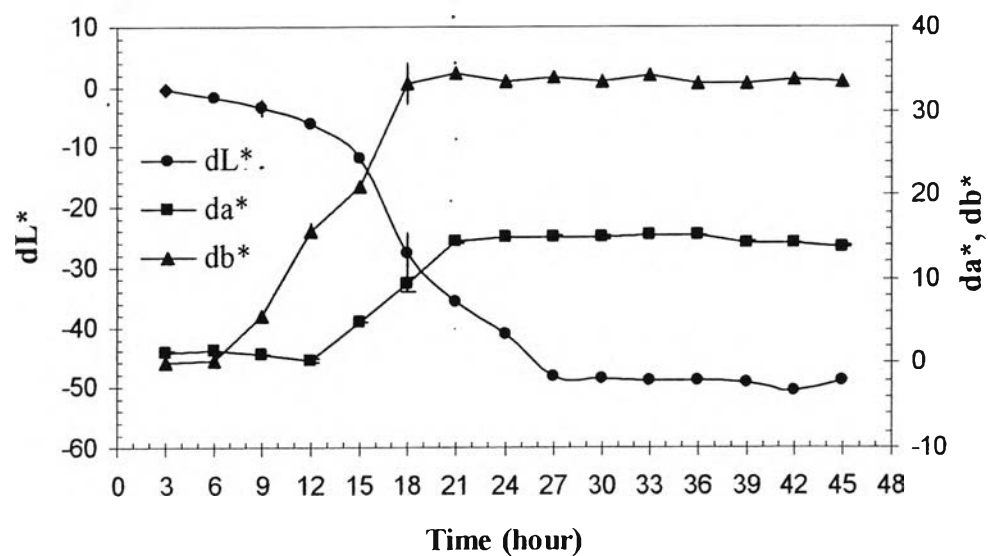
**Figure 5.7** The effect of concentration of PEDOT:PSS sensors on changes in TCD values.



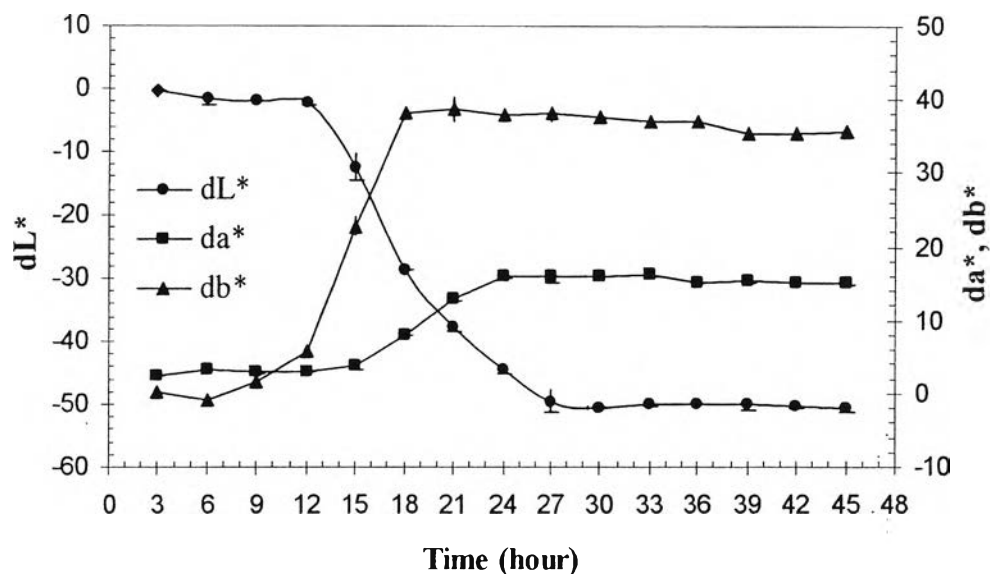
(A)



(B)

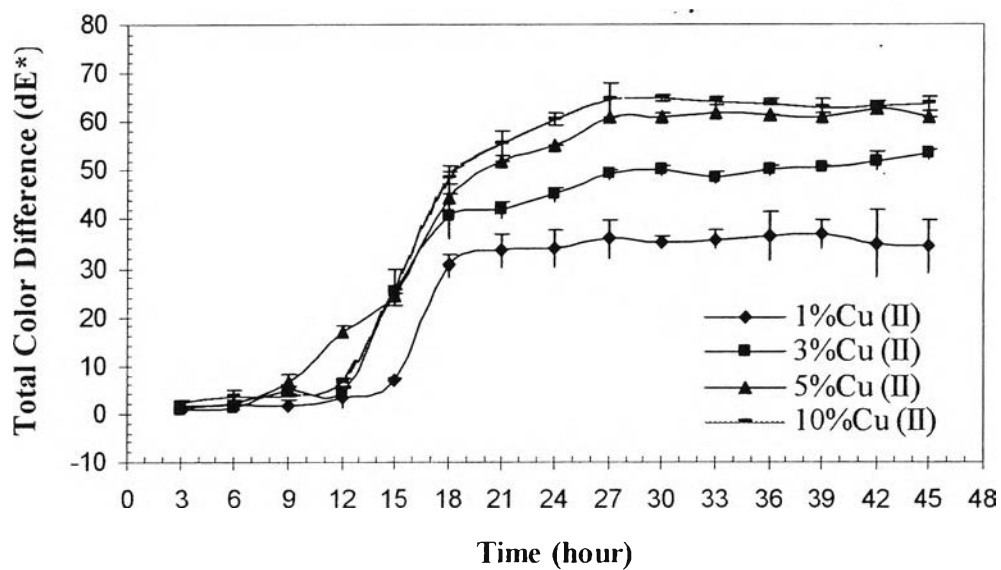


(C)



(D)

**Figure 5.8** The effect of concentration of  $\text{Cu}(\text{NO}_3)_2$  sensors on changes in Hunter color values of: (A) 1% w/w  $\text{Cu}(\text{NO}_3)_2$ , (B) 3% w/w  $\text{Cu}(\text{NO}_3)_2$ , (C) 5% w/w  $\text{Cu}(\text{NO}_3)_2$ , and (D) 10% w/w  $\text{Cu}(\text{NO}_3)_2$ .



**Figure 5.9** The effect of concentration of  $\text{Cu}(\text{NO}_3)_2$  sensors on changes in TCD values.

### E. Sensor Thickness: Difference in Sensor Response

PP/organoclay nanocomposite films were spin-coated with the sensor solution at 1000, 2000, and 3000 rpm in order to generate different sensor thickness, which was determined by using the peacock digital thickness gauge model PDN 12N. The effect of spin-coating speed on sensor thickness is reported in table 4.1. The results indicated that the higher the spin-coating speed, the thinner sensor film with the average thickness results of 44.40, 23.47, 16.67 at a spin-coating rate of 1000, 2000, and 3000 rpm, respectively.

**Table 5.1** Effect of spin-coating speed on thickness of both sensors without substrate

Spin-coating speed (rpm)	Thickness ( $\mu\text{m}$ )
1000	$44.40 \pm 7.10$
2000	$23.47 \pm 4.03$
3000	$16.67 \pm 3.60$

The effect of sensor thickness on color change of both sensors was investigated. These sensors were the same concentration of sensor of 5% w/w, 5%wt clay in nanocomposite film, and amount of meat samples of 100 g. As depicted in Fig. 5.10, Hunter L\* and b\* of all three thicknesses of PEDOT:PSS sensors were slowly decreasing and almost no color change, respectively during initial 18 h but decreased sharply before stabilizing at 24 h, while Hunter a\* was almost no color change during initial 18 h but sharply increasing before stabilizing at 24 h.

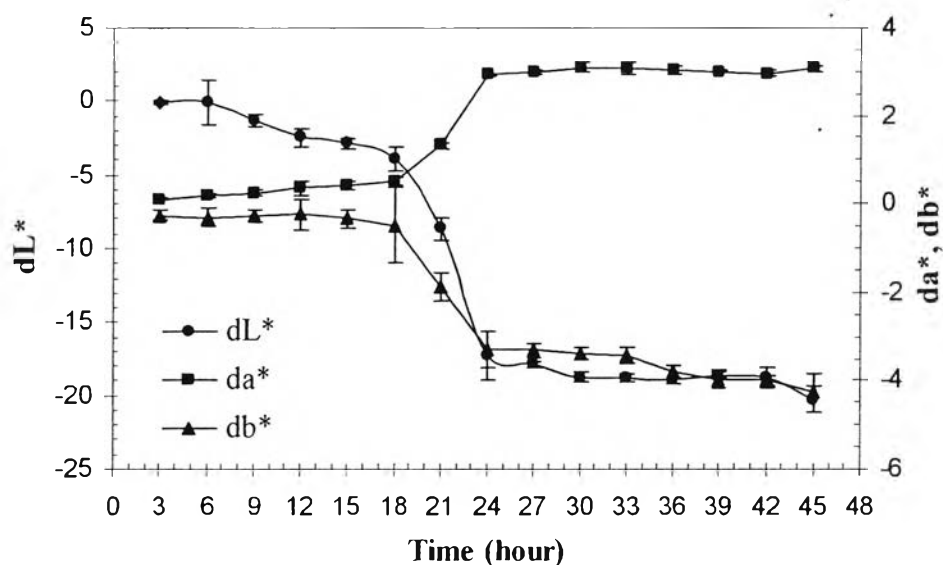
The TCD values of all thicknesses of PEDOT:PSS sensors with various thicknesses were calculated and shown in Fig. 5.11. It was observed that the TCD values of these sensors increased with storage time and similar in time and range. However, sensors with the greatest thickness (1000 rpm) showed higher TCD values than those coated at 2000 or 3000 rpm. This is due to the greater amount of materials on the film, compared to the thinner film [14]. Therefore, this thickness was selected for further studies.

In case of  $\text{Cu}^{2+}$  sensor, as shown in Fig. 5.12, Hunter L\* of  $\text{Cu}^{2+}$  sensor decreased slowly during initial 12 h but decreased sharply before stabilizing at 27 h,

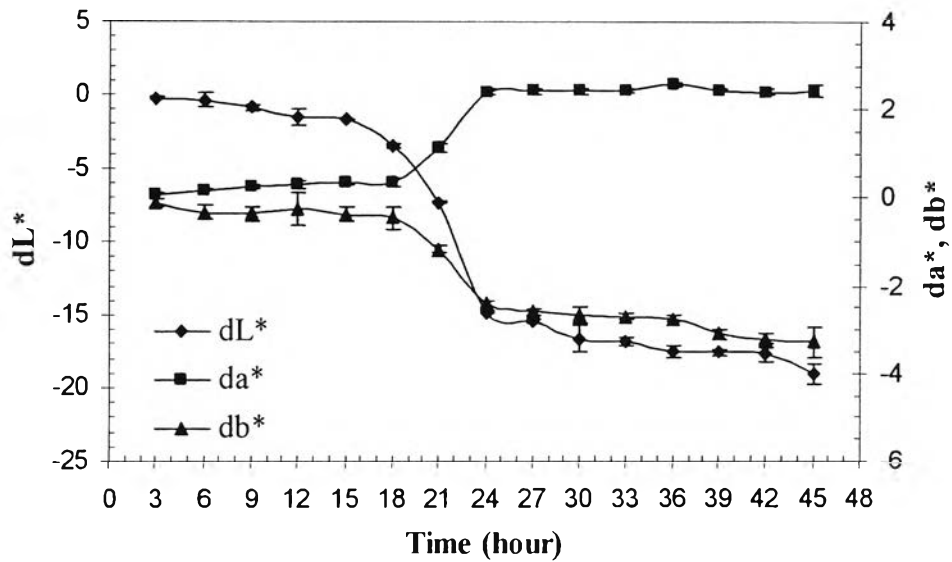
while Hunter  $a^*$  and  $b^*$  was almost no color change during initial stage but significantly increased before stabilizing at 21 and 18 h, respectively.

The TCD values of  $\text{Cu}^{2+}$  sensors with various thicknesses were calculated and shown in Fig. 5.13. The TCD values of three sensors also changed continuously with the responses of sensors and significantly increased with storage time. The responses in all three thicknesses were not difference in time and range. However, the thickest sensors, coated at 1000 rpm, provided more intense color change to eye than those coated at 2000 or 3000 rpm. The reason of this result is similar to that of PEDOT:PSS sensor. So, a spin-coating speed of 1000 rpm was selected for further studies.

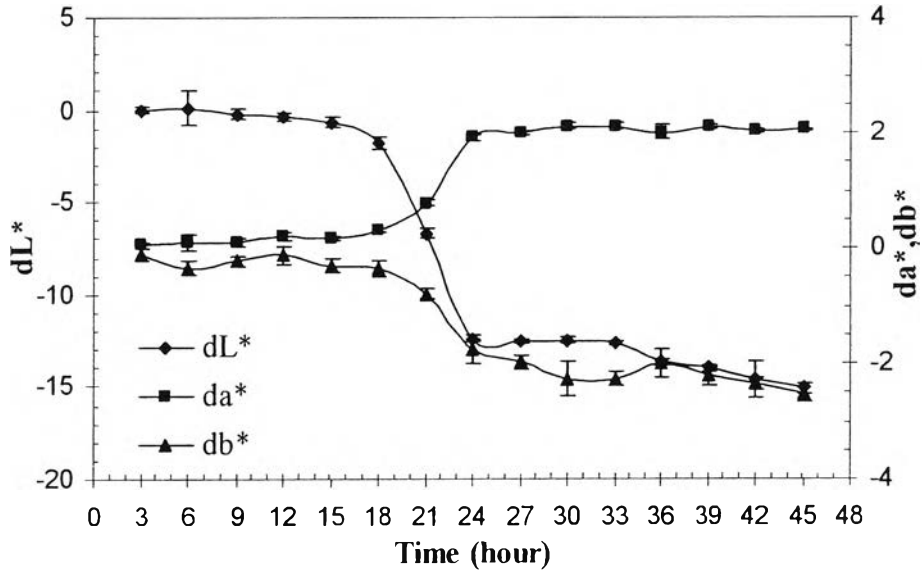
The color change of PEDOT:PSS sensor, coated at 1000 rpm at the point of meat spoilage was still difficult to detect by the naked eye, whereas that of  $\text{Cu}^{2+}$  sensor was absolutely visible to the naked eyes ( $\text{TCD} > 12$ ).



(A)

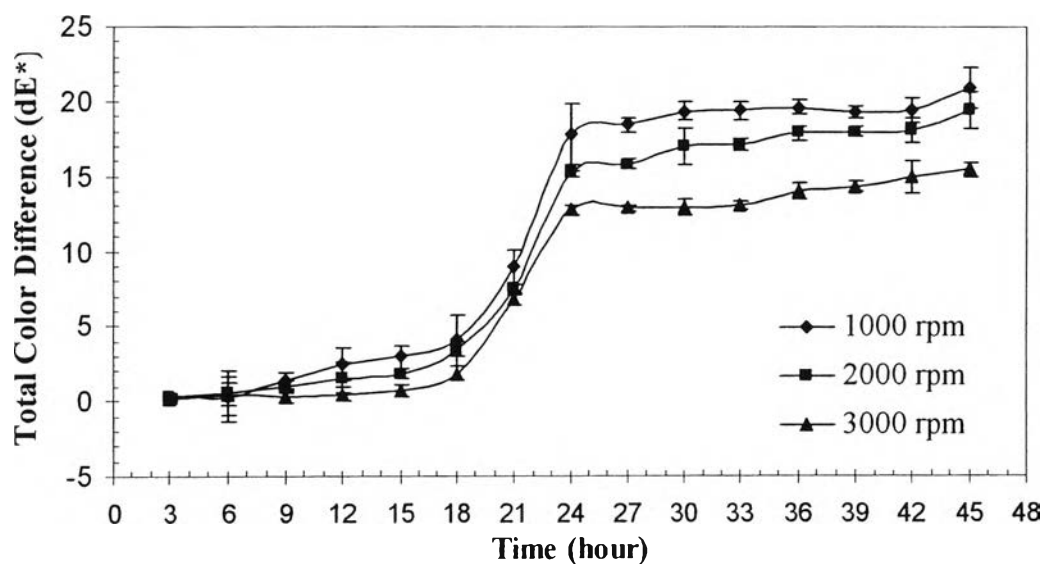


(B)

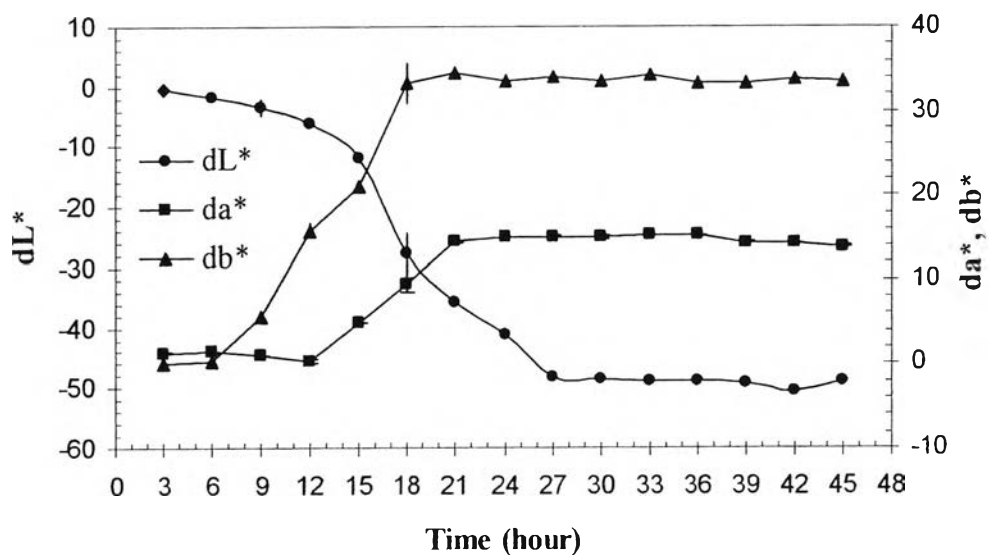


(C)

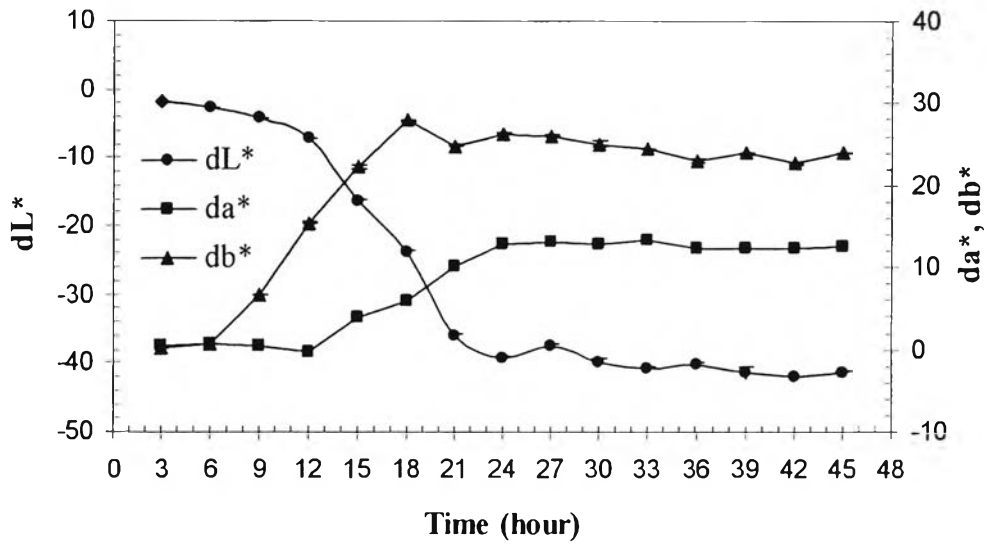
**Figure 5.10** The effect of sensor thickness on changes in Hunter color values of PEDOT:PSS sensors: (A) 1000 rpm, (B) 2000 rpm, and (C) 3000 rpm.



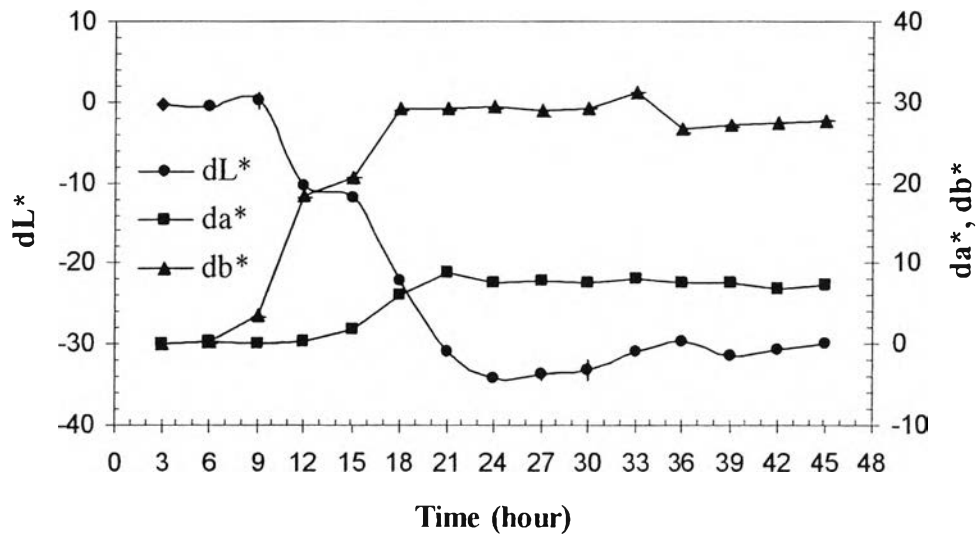
**Figure 5.11** The effect of sensor thickness on changes in TCD values of PE-DOT:PSS sensors.



(A)



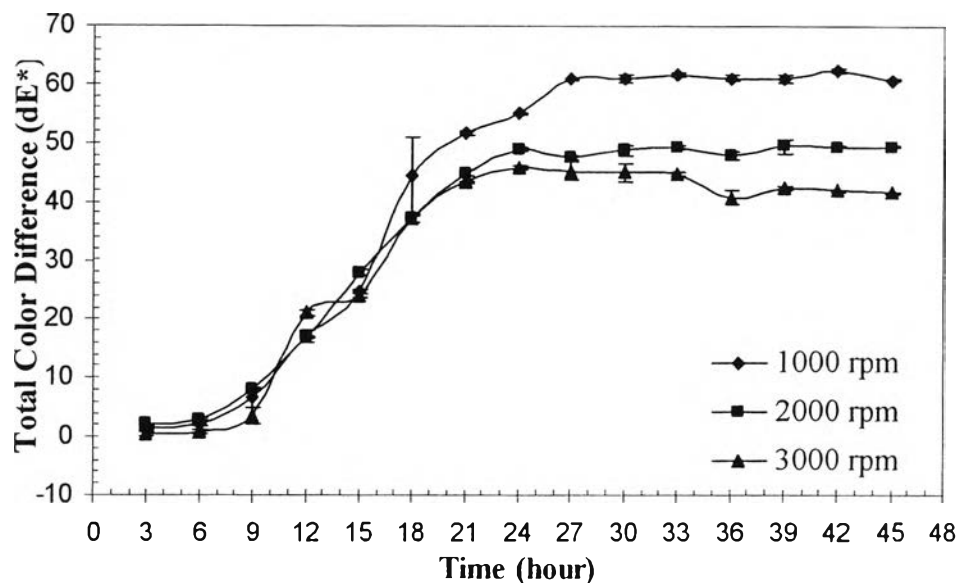
(B)



(C)

**Figure 5.12** The effect of sensor thickness on changes in Hunter color values of  $\text{Cu}(\text{NO}_3)_2$  sensors: (A) 1000 rpm, (B) 2000 rpm, and (C) 3000 rpm.



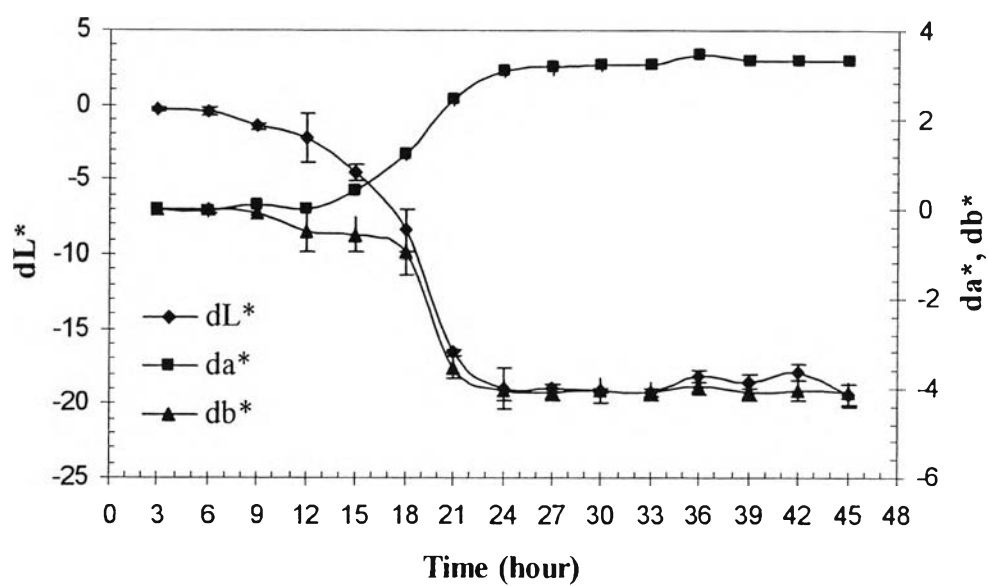


**Figure 5.13** The effect of sensor thickness on changes in TCD values of  $\text{Cu}(\text{NO}_3)_2$  sensors.

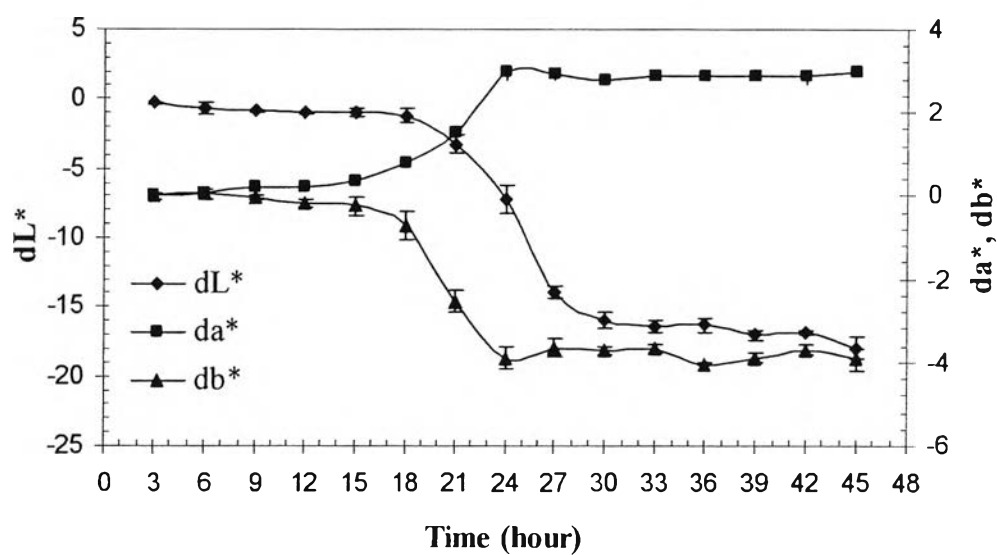
#### F. The Amount of Clay Content in Nanocomposites: Difference in Sensor Response

The effect of clay contents in nanocomposite films on the responses of sensors with the same the concentration of sensor of 5% w/w, spin-coating speed of 1000 rpm, and amount of meat tissue of 100 g was investigated. Fig. 5.14 and Fig. 5.16 show the change in Hunter color value of PEDOT:PSS and  $\text{Cu}^{2+}$ , respectively with different amount of clay contents. Hunter  $L^*$ ,  $a^*$  and  $b^*$  of all clay contents of both sensors were found slowly changed at the initial stage, sharply changed at the middle stage before stabilizing at the final stage.

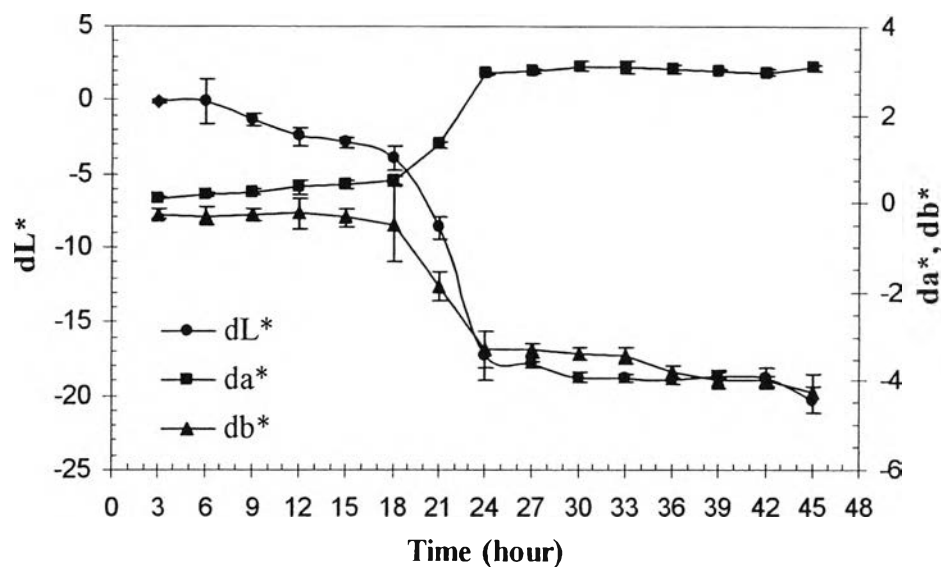
The TCD values of both sensors were presented in Fig. 5.15 and Fig. 5.17, respectively. It was founded that the TCD values of both sensors at 1%wt clay gives the faster response than 3%wt clay and 5%wt clay, respectively. This is explained by the improvement of gas barrier properties with high clay contents in nanocomposite films, which can inhibit the growth of aerobic bacteria and retard spoilage of meat [17]. Therefore, 5%wt clay in nanocomposites was selected to extend the shelf life of meat as well as to determine meat freshness.



(A)

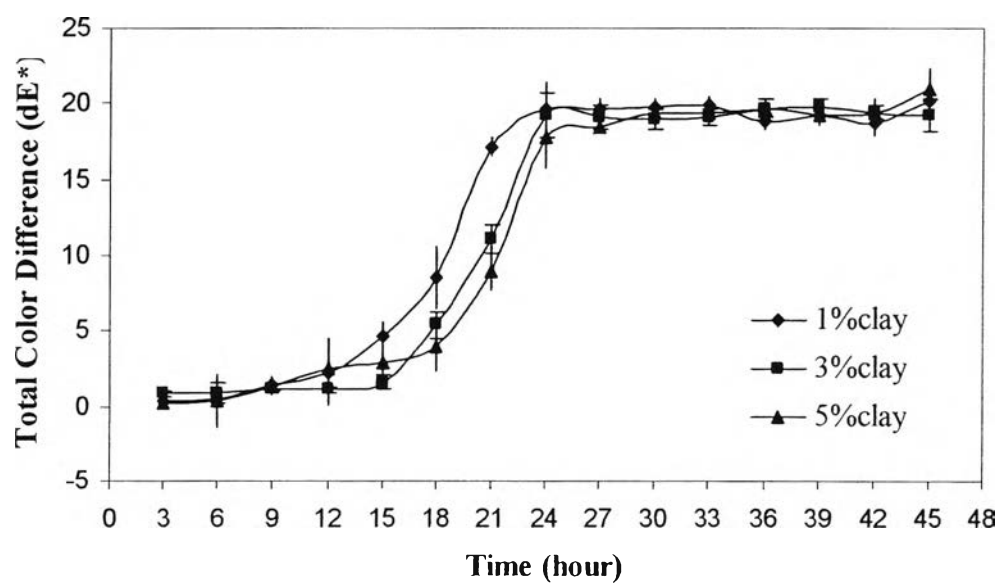


(B)

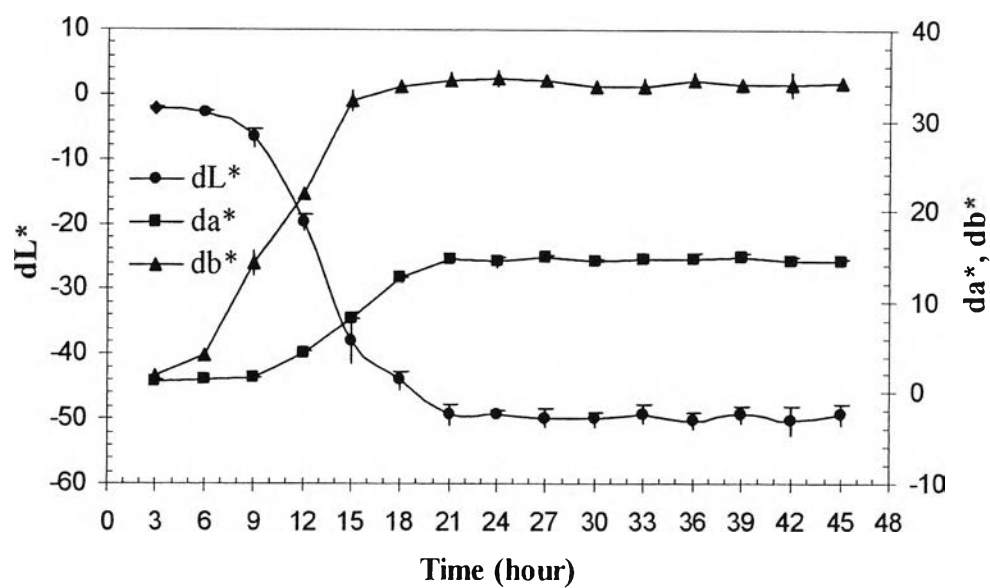


(C)

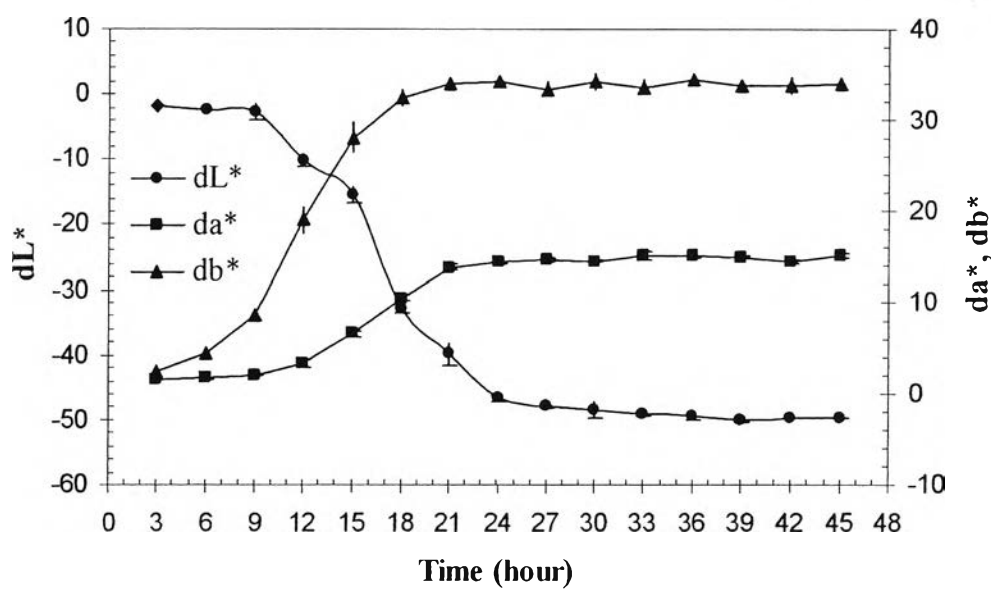
**Figure 5.14** The effect of clay contents in nanocomposite film on changes in Hunter color values of PEDOT:PSS: (A) 1%clay and (B) 3%clay , and (B) 5%clay.



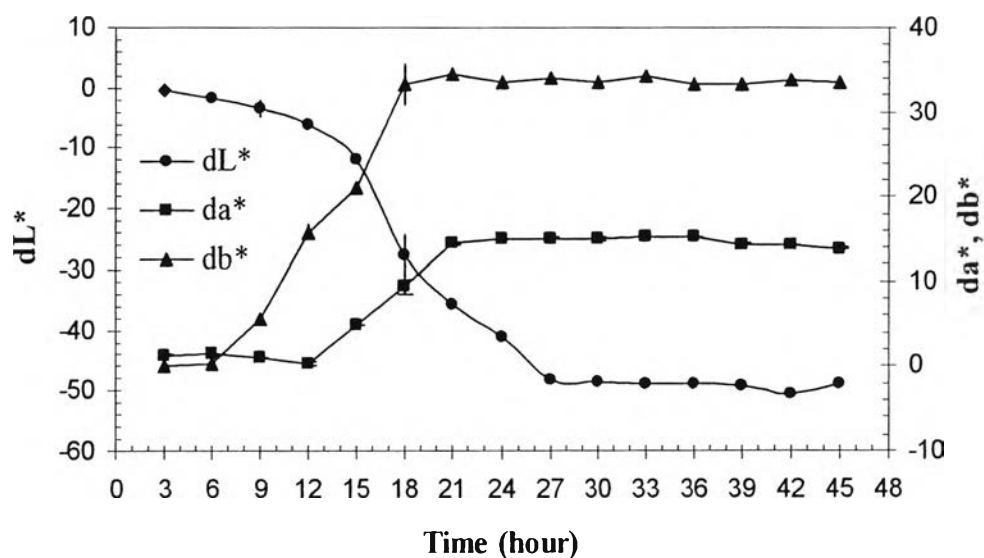
**Figure 5.15** The effect of clay contents in nanocomposite film on changes in TCD values of PEDOT:PSS sensor.



(A)

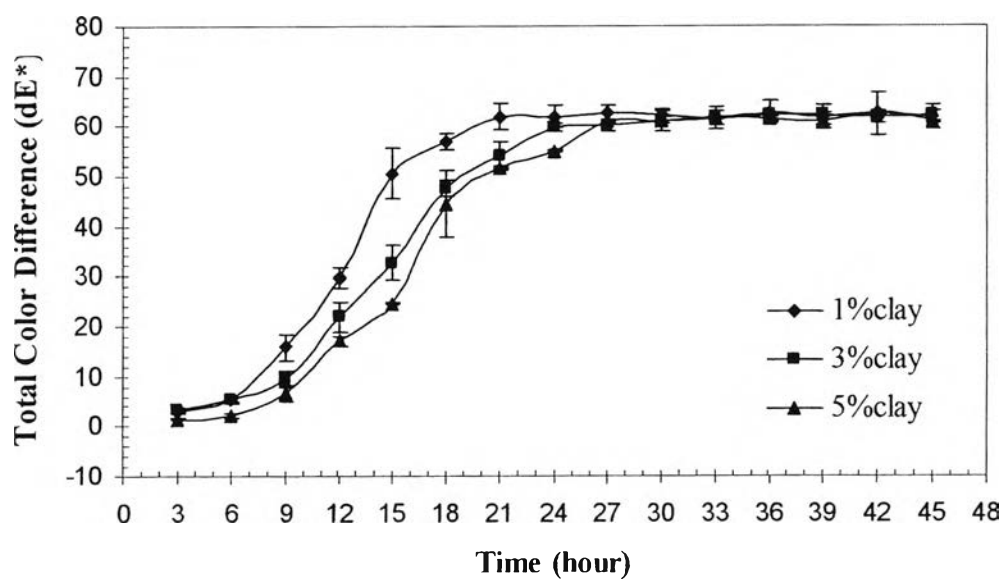


(B)



(C)

**Figure 5.16** The effect of clay contents in nanocomposite film on changes in Hunter color values  $\text{Cu}(\text{NO}_3)_2$  sensors: (A) 1%clay, (B) 3%clay, and (C) 5%clay.

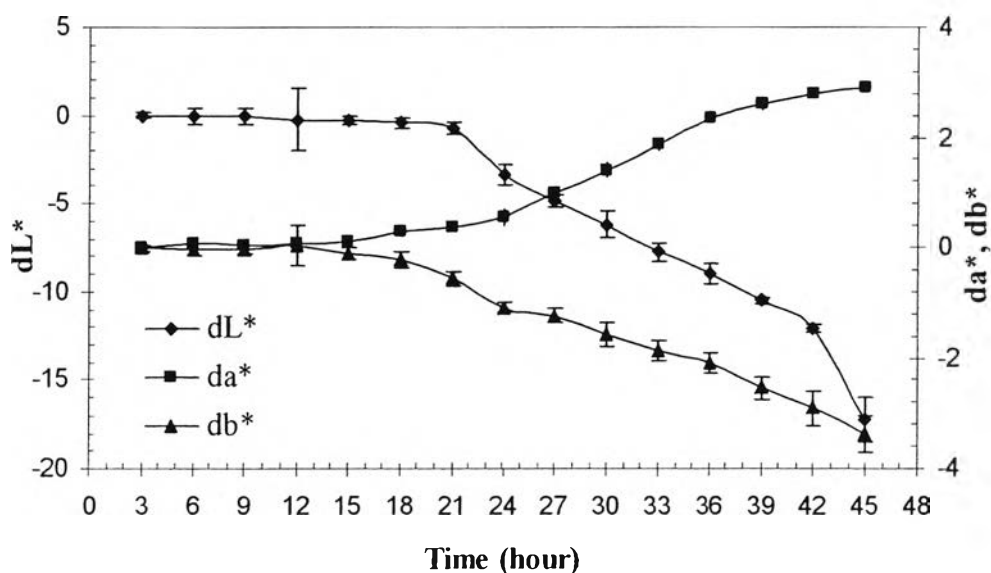


**Figure 5.17** The effect of clay contents in nanocomposite film on changes in TCD values of  $\text{Cu}(\text{NO}_3)_2$  sensors.

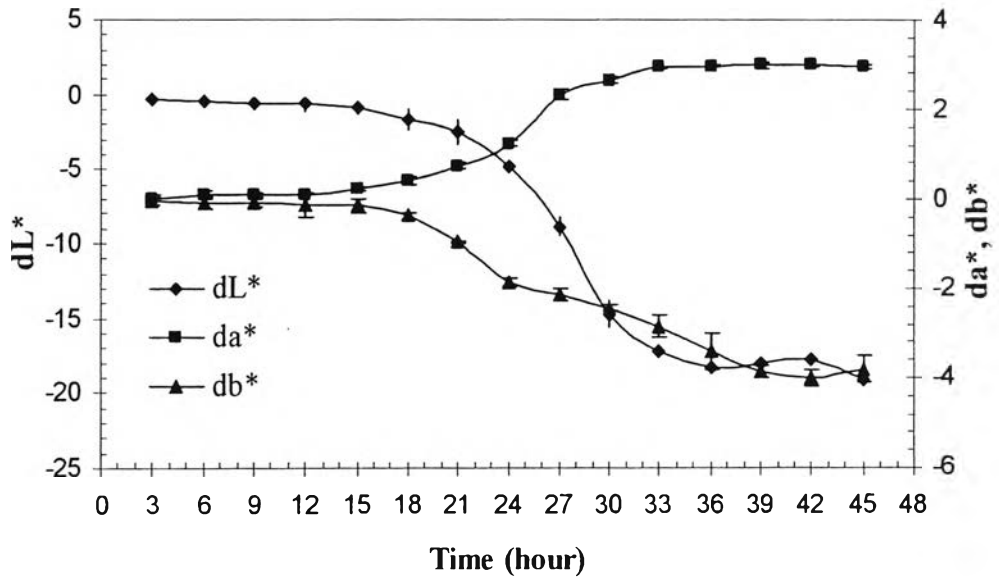
### G. The Amount of Meat Samples: Difference in Sensor Response

The sensors with the same concentration of sensor of 5% w/w, spin-coating speed of 1000 rpm, and 5% clay in nanocomposite film were used to investigate the effect of amount of meat samples on the color change of sensors. Fig. 5.18 and Fig. 5.20 shows the changes in Hunter color value of both sensors with different clay contents in nanocomposite film. Hunter  $L^*$ ,  $a^*$  and  $b^*$  of these sensors were slowly changed at the initial stage, significantly changed at the middle stage before remain constant at the final stage except PEDOT:PSS at meat samples of 25 g which was still gradually changed.

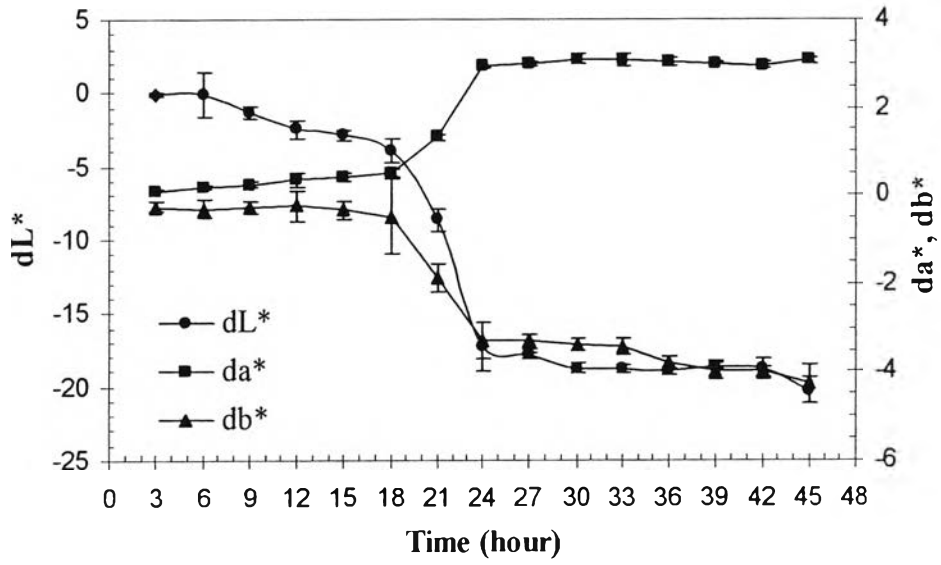
In case of TCD values of both sensors, as depicted in Fig. 5.19 and Fig. 5.21, meat samples of 150 g showed the higher value than 100 g, 50 g, and 25 g, respectively. It can be concluded that the sensor responses depend on the amount of meat samples. The higher the amount of meat samples, the higher TVB-N, the higher electron resulting in the faster sensor response.



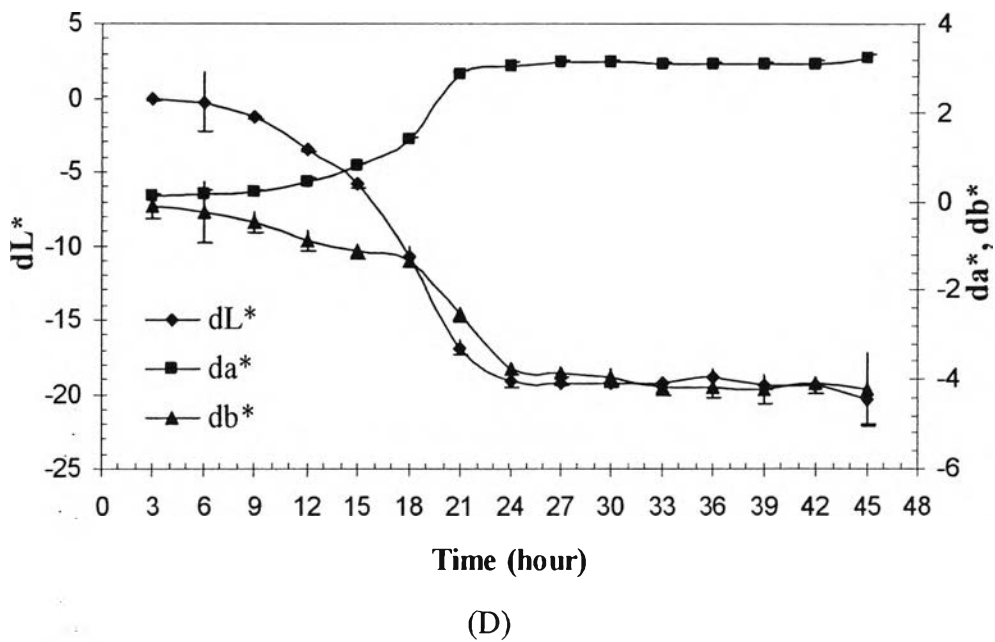
(A)



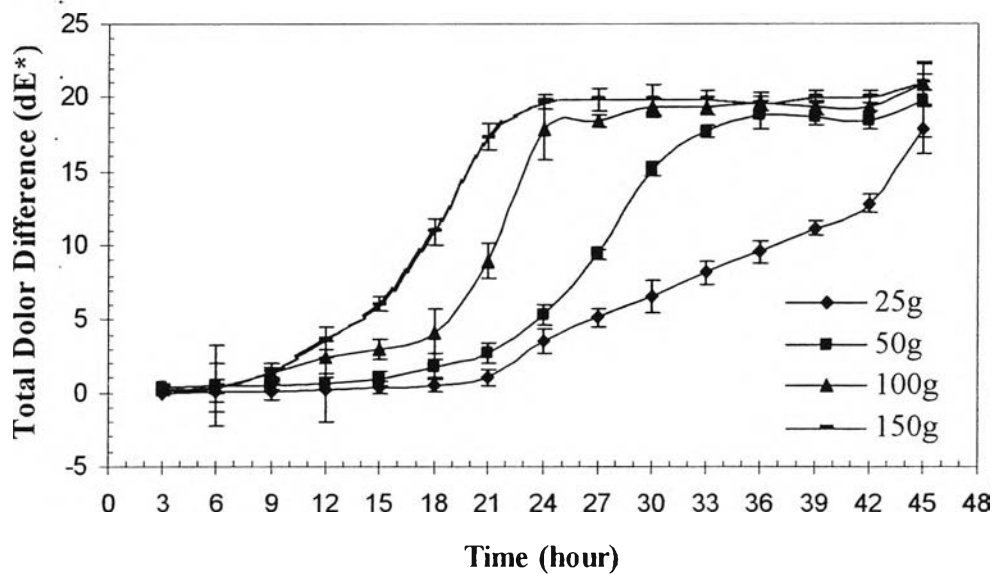
(B)



(C)

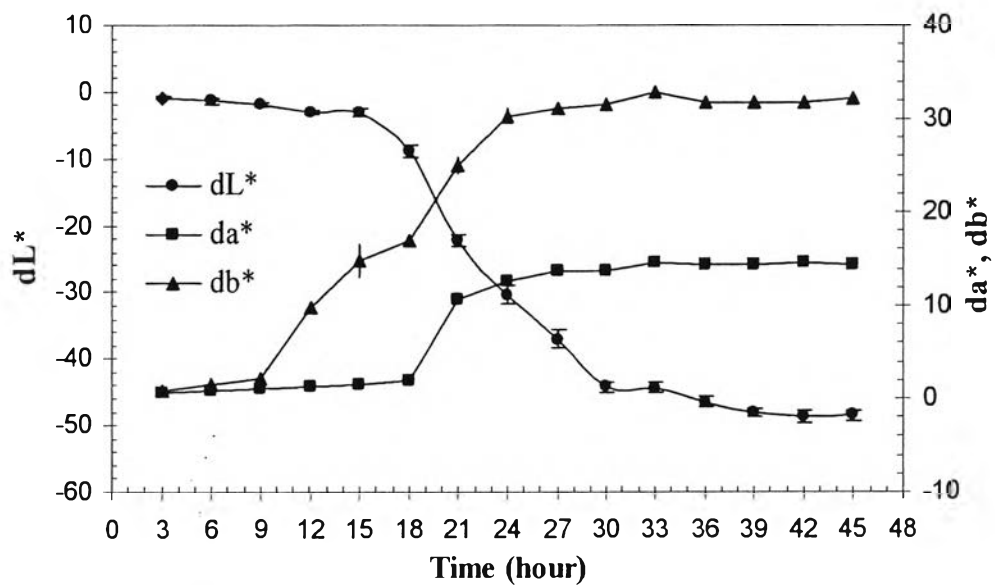


**Figure 5.18** The effect of amount of meat samples on changes in Hunter color values of PEDOT:PSS sensors: (A) 25 g, (B) 50 g, (C) 100 g, and (D) 150 g.

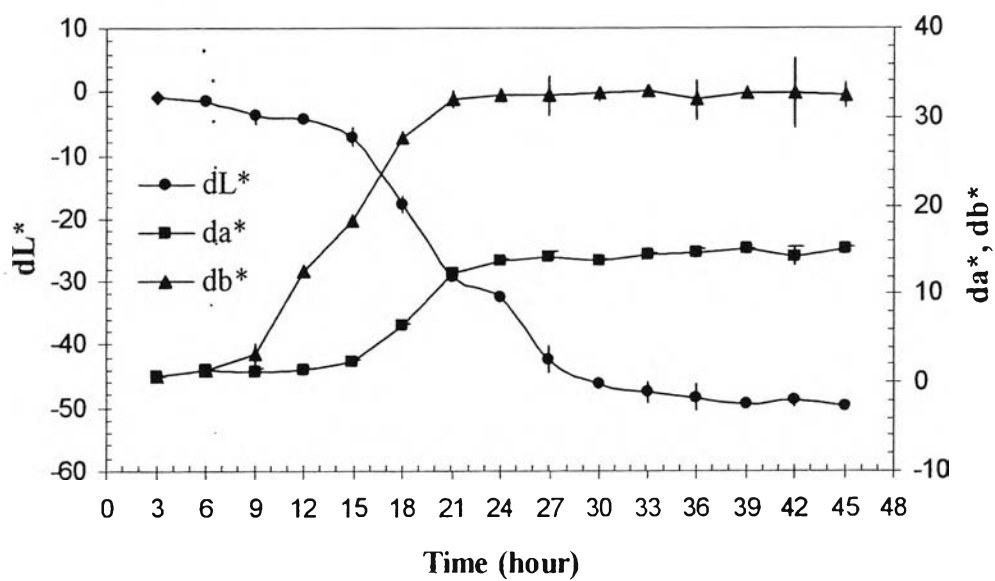


**Figure 5.19** The effect of amount of meat samples on changes in TCD values of PEDOT:PSS sensors.

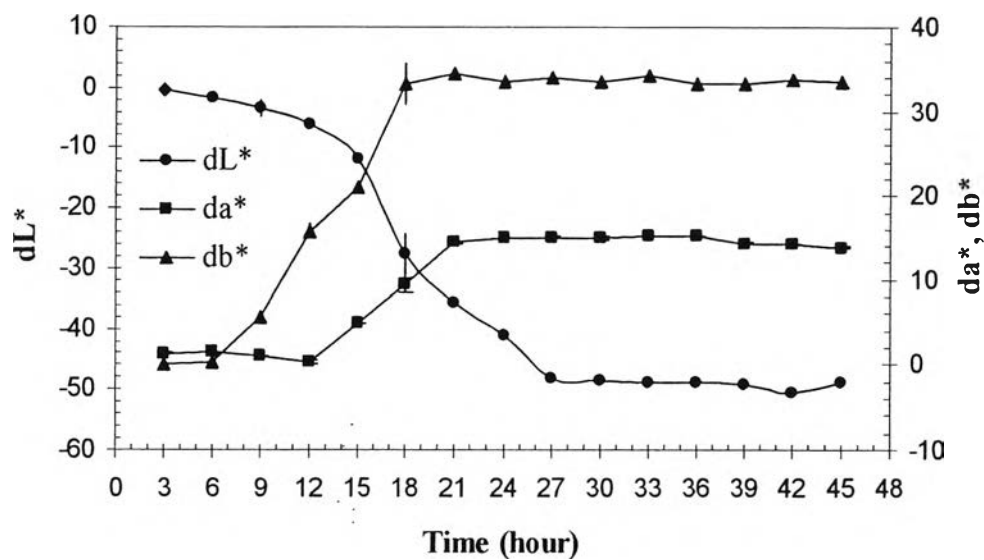




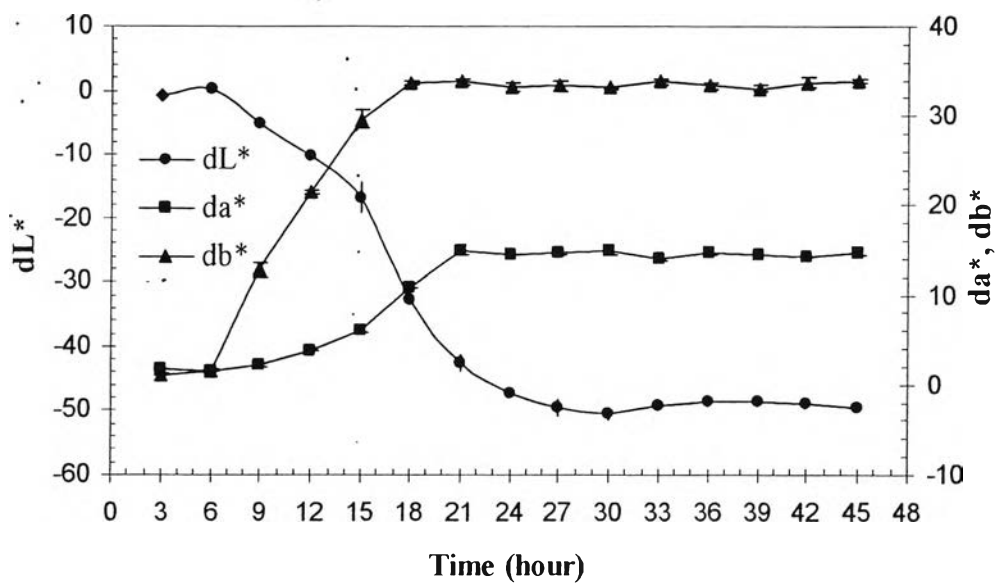
(A)



(B)

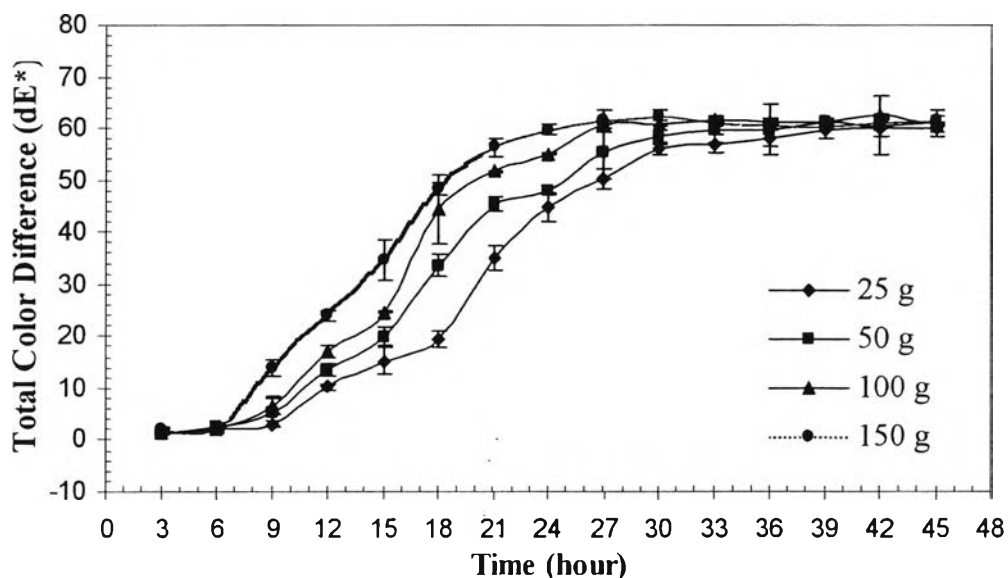


(C)



(D)

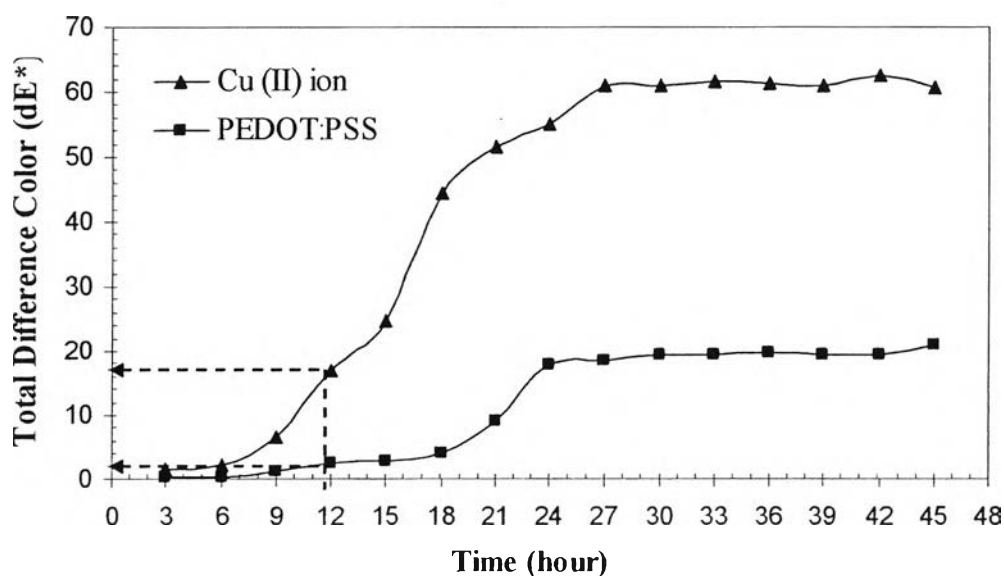
**Figure 5.20** The effect of amount of meat samples on changes in Hunter color values of  $\text{Cu}(\text{NO}_3)_2$  sensors: (A) 25 g, (B) 50 g, (C) 100 g, and (D) 150 g.



**Figure 5.21** The effect of amount of meat samples on changes in TCD values of  $\text{Cu}(\text{NO}_3)_2$  sensors.

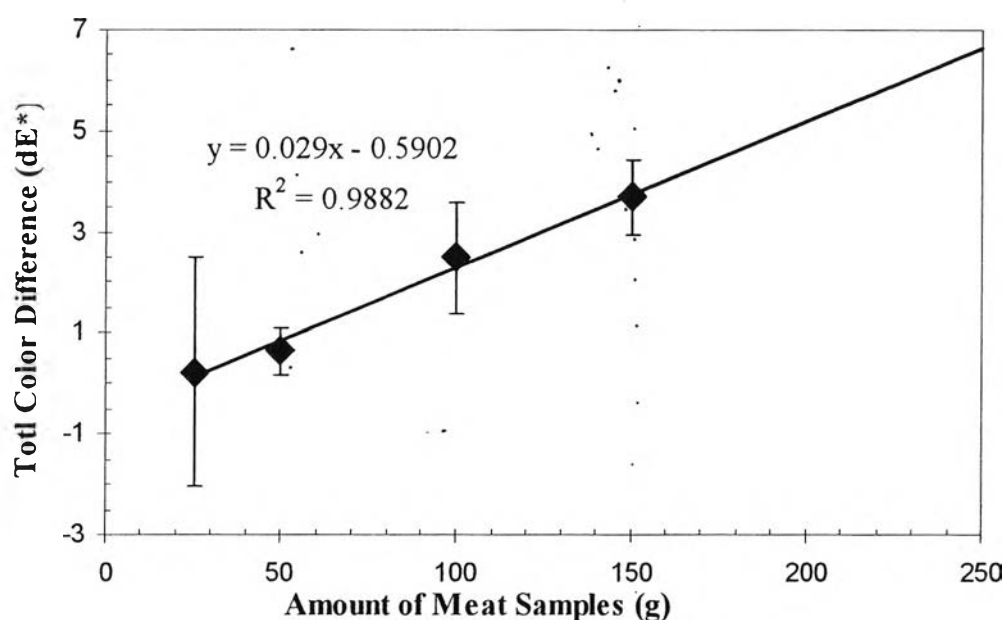
#### H. The Type of Sensor: Difference in Sensor Response

Both types of sensors are compared in Fig. 5.22. It was clear that the  $\text{Cu}^{2+}$  sensor showed higher response than PEDOT:PSS and was easy to observe the color change at the point of spoilage (12 h) with the naked eyes. Hence,  $\text{Cu}^{2+}$  is suitable sensor to determine meat freshness.



**Figure 5.22** Changes in TCD values of both sensors, PEDOT:PSS and  $\text{Cu}^{2+}$  sensors.

Although PEDOT:PSS sensor is low sensitivity and difficult to see the color difference at the point of spoilage (12 h); however, the sensor response depends on the amount of meat samples. If the amount of meat samples is enough, the color change of this sensor will be detected by the human eyes. Fig. 5.23 shows the effect of amount of meat samples on change in TCD values of PEDOT:PSS sensors at 12 h with different meat contents whereas the concentration of PEDOT:PSS of 5% w/w, spin-coating speed of 1000 rpm, and 5% clay in nanocomposite film were the same. As shown in Fig. 5.23, the TCD values reached values of 5 which is easy to detect by the naked eyes, about 200 g. It could be assumed that this sensor can be effective when the amount of meat samples is more than 200g.

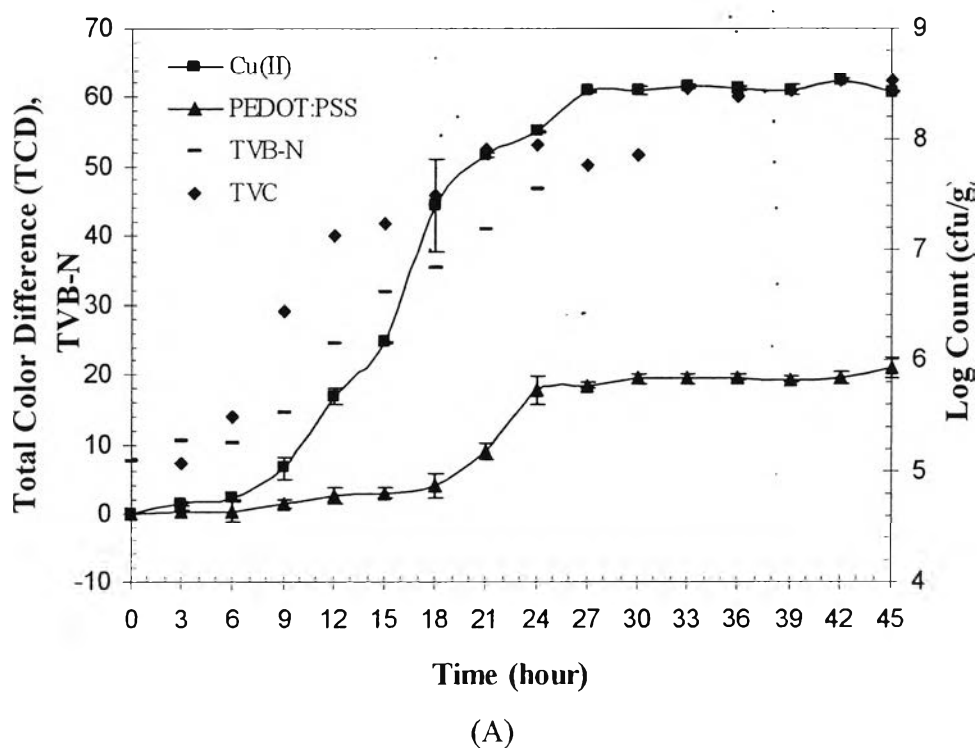


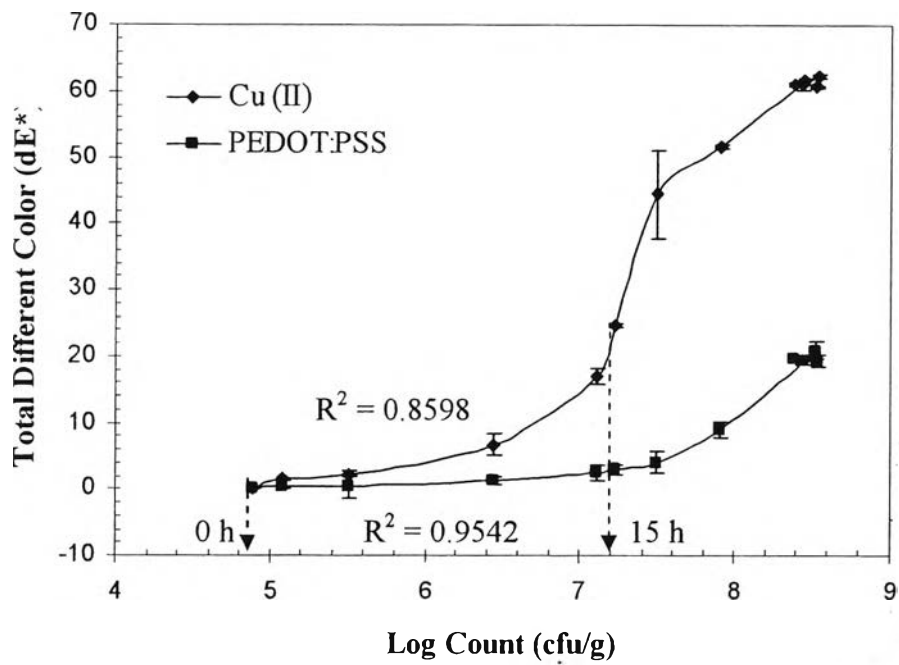
**Figure 5.23** The effect of amount of meat samples on change in TCD values of PEDOT:PSS sensors at the point of spoilage (12 h).

### *I. Correlation of Sensor Response to Meat Spoilage*

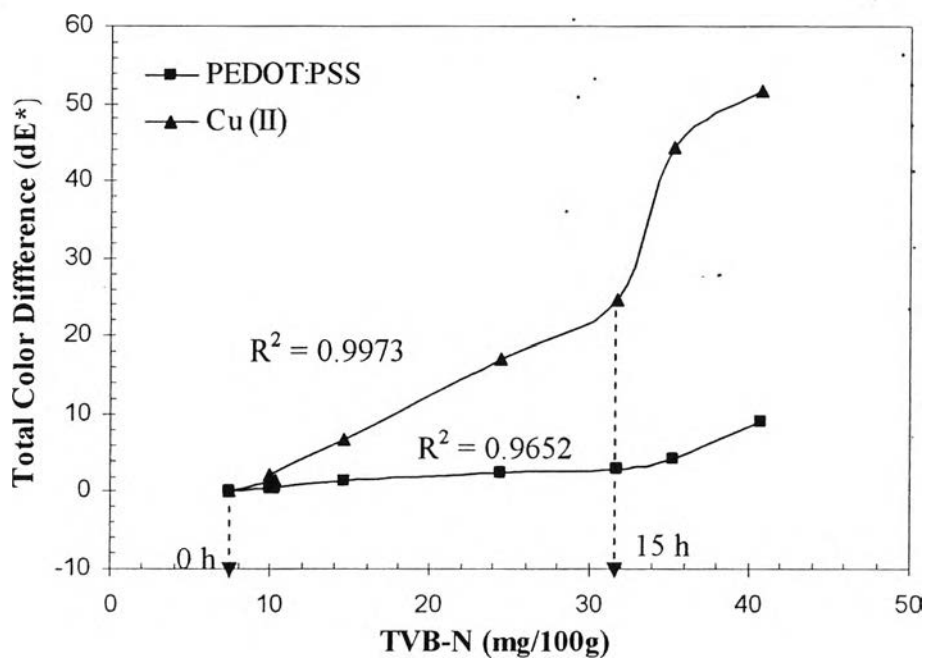
Fig. 5.24 shows the correlation of change in microbial population, TVB-N values, and sensor responses of both sensors with the same concentration of sensor of 5% w/w, spin-coating speed of 1000 rpm, 5% clay contents in nanocomposite film, and the amount of meat samples of 100 g. During initial state, the microbial

population increased sharply whereas TVB-N values and responses of both sensors increased slowly. So, there was a delay between the increase in microbial population and the sensor responses. This delay is inherent as volatile base generation follows the rise in microbial population. In the other word, microorganisms assimilate meat as their nutrients, resulting in the increase in population and then, generate volatile base. A delay between the increase in microbial population and appearance of the chemical spoilage markers, including TVB-N, has been previously observed by Pacquit *et al.* (2006, 2007). As shown in Fig 5.24 (B), the determination of coefficients ( $R^2$ ) for responses of both sensors with respect to microbial population of fresh meat were 0.8594 for  $\text{Cu}^{2+}$  and 0.9542 for PEDOT:PSS sensors. In case of TVB-N values, the determination coefficients ( $R^2$ ) for both sensor responses were 0.9973 for  $\text{Cu}^{2+}$  and 0.9652 for PEDOT:PSS sensors.





(B)

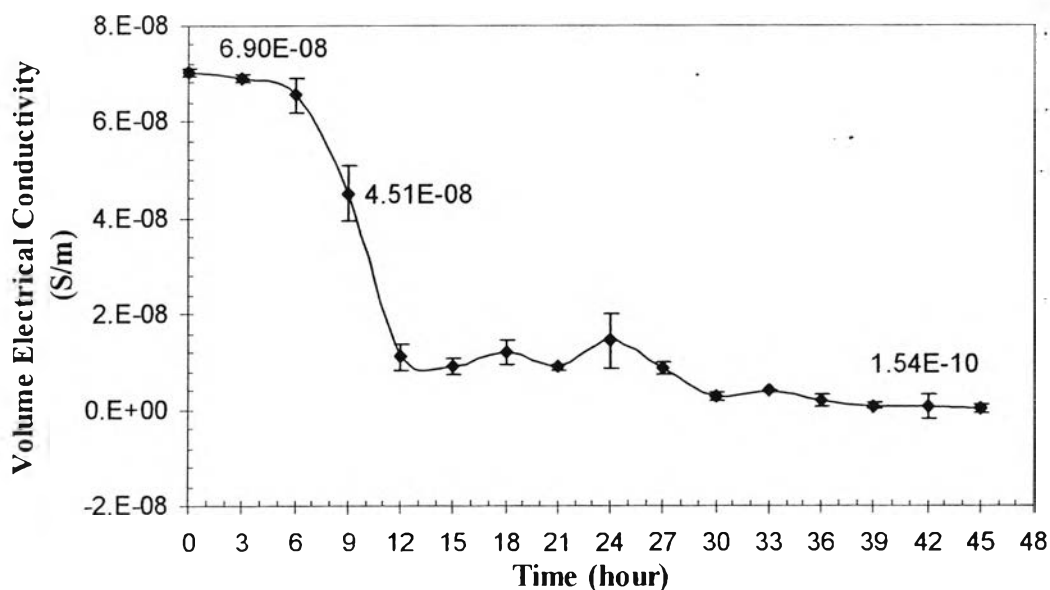


(C)

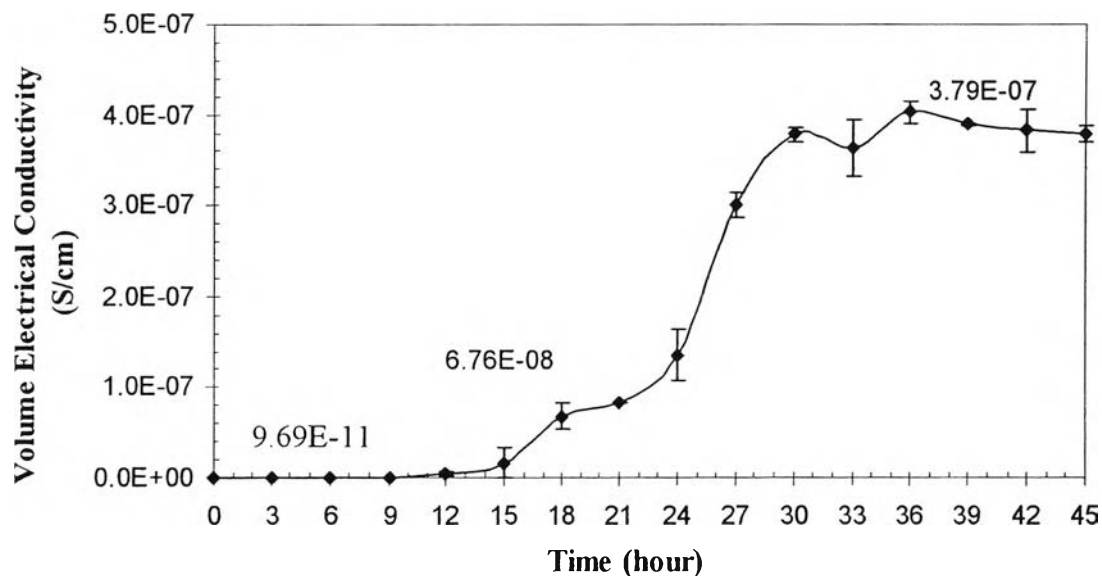
**Figure 5.24** Correlation of change in microbial populations of fresh meat and sensor response of both sensors: (A) curve of log count and TCD values with time, (B) curve between log count of microbial population and TCD values, and (C) curve between TVB-N and TCD values.

### J. Volume Electrical Conductivity of Sensor to Meat Spoilage

The volume electrical conductivity of PEDOT:PSS and  $\text{Cu}^{2+}$  was investigated to confirm receiving electron from total volatile basic nitrogen (TVB-N) and the results are shown in Fig. 5.25 and 5.26, respectively. The volume electrical conductivity of PEDOT:PSS sensor decreased with increasing storage time. This phenomenon is due to capturing electrons from volatile base led to the formation of a neutral polymer backbone and a decrease in charge carriers, resulting in a decrease of conductivity [18]. In case of  $\text{Cu}^{2+}$  sensor, volume electrical conductivity was increased when storage time increased. After receiving some electrons,  $\text{Cu}^{2+}$  sensors change to  $\text{Cu}^{1+}$ , then Cu metal, resulting in an increase of volume electrical conductivity. This showed that both sensors can be either detected by color change or conductivity due to redox reaction between sensing chemicals and total volatile basic nitrogen (TVB-N). Moreover, PEDOT:PSS that is not quite effective as color sensor becomes effective as electrical sensor to detect meat spoilage at 12 h.



**Figure 5.25** Electrical conductivity of 5% w/w PEDOT:PSS, spin-coated at 1000 rpm onto 5%clay nanocomposite films, of meat samples of 100 g.

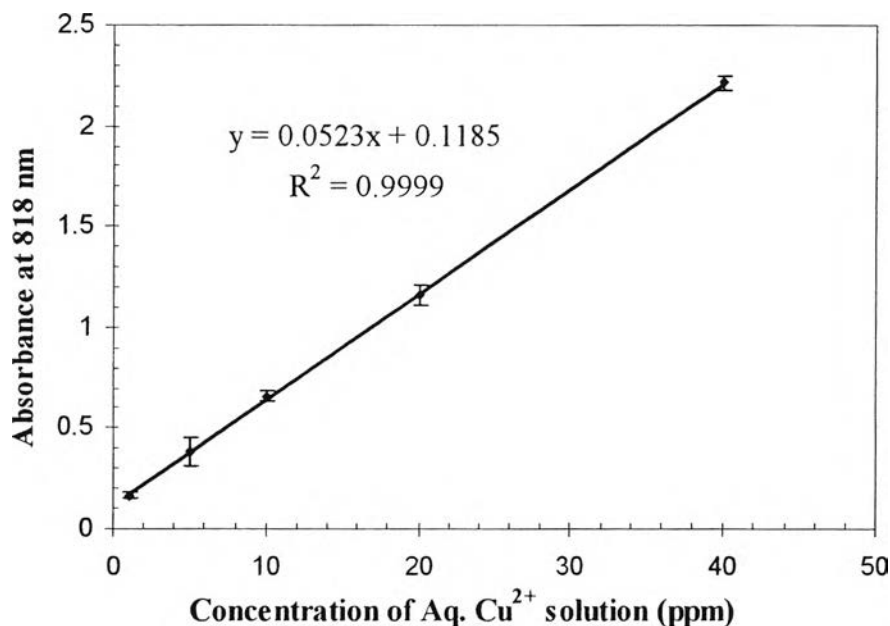


**Figure 5.26** Electrical conductivity of 5% w/w  $\text{Cu}(\text{NO}_3)_2$ , spin-coated at 1000 rpm onto 5% clay nanocomposite films, of meat samples of 100 g.

### K. Leaching Studies

Due to higher sensitivity than PEDOT:PSS sensor,  $\text{Cu}^{2+}$  is suitable sensor to determine meat freshness. Before using  $\text{Cu}^{2+}$  sensor in food packaging, the leakage of  $\text{Cu}^{2+}$  sensor should be studied. In this study, 5% w/w  $\text{Cu}^{2+}$  spin-coated at 1000 rpm onto 5% clay in the nanocomposite film of meat samples of 100 g was chosen to investigate the leakage of the  $\text{Cu}^{2+}$  sensor using UV-Vis spectrometer. The weight of this sensor film which was cut into rectangular shape with 4x4 cm was 325 mg. The calibration curve at 818 nm is shown in Fig. 5.27. The amount of  $\text{Cu}^{2+}$  sensors that leaked out to water was just only 0.77 ppm/325 mg of sensor. It is not exceed limitation of residual Cu of the U.S. Food and Drug Administration (FDA) which is 1.3 ppm. Therefore, this sensor was safe and suitable for food packaging application.





**Figure 5.27** The Calibration curve of the standard aqueous Cu<sup>2+</sup> solution.

#### 5.4 Conclusions

The evaluation of PP/organoclay nanocomposite intelligent packaging based on PEDOT:PSS and Cu<sup>2+</sup> sensors was demonstrated. The response to chicken meat at room temperature for sensors with various thicknesses, sensor concentrations, clay contents in the nanocomposite film, the amount of meat samples, and type of sensor (PEDOT, Cu<sup>2+</sup>) were studied. The color changes of the intelligent films were measured by using a Chroma Meter and reported in Hunter system ( $L^*$ ,  $a^*$ , and  $b^*$ ) values and total color difference (TCD). It was found that sensors with the greatest thickness (1000 rpm) showed higher response than those coated at 2000 or 3000 rpm. This is due to the greater amount of material on the film sensors, compared to the thinner film. The sensors with the highest concentration showed the greatest response among those sensors. The reason of this result is similar to that of sensor with various thicknesses. The sensor response at 1%wt clay in nanocomposite films gives the faster response than 3%wt clay and 5%wt clay, respectively. This is explained by the improvement of gas barrier properties with high clay contents in nanocomposite films. The sensor responses of 150 g of meat samples showed the higher value than 100 g, 50 g, and 25 g, respectively due to higher volatile base concentration in the

package-headspace. From the comparison of both sensors, it was clear that the  $\text{Cu}^{2+}$  sensor showed higher sensitivity than PEDOT:PSS sensor and easy to observe the color change at the point of spoilage (12 h) with the naked eyes. However, if the amount of meat samples is more than 200 g, the PEDOT:PSS sensor will be effective. A delay between the increase in microbial population and the sensor response was observed. This delay is inherent as volatile base generation follows the rise in microbial population. The receiving electron of PEDOT:PSS and  $\text{Cu}^{2+}$  sensors from total volatile basic nitrogen (TVB-N) was confirmed by the result of electrical conductivity.

The results presented in this study indicated that PP/organoclay nanocomposite film coated with  $\text{Cu}^{2+}$  sensor could be employed as an effective chromic intelligent packaging for evaluating meat freshness due to absolutely visible to the naked eyes at the onset of meat spoilage, 12 h, thus enabling the “real-time” monitoring of spoilage. Moreover, the leakage of  $\text{Cu}^{2+}$  is below the limitation of residual Cu of the U.S. Food and Drug Administration (FDA). However, nanocomposite film coated with PEDOT:PSS sensor cannot be used as an effective chromic intelligent packaging since it cannot be easily observed by human eyes. In addition, both sensors could be used as electrical-intelligent packaging.

## 5.5 Acknowledgements

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