CHAPTER IV

ANALYSIS OF FISH SPOILAGE

4.1 Abstract

The development of a smart packaging in the form of nanocomposite pH sensor spots that monitor spoilage in fish is described. The sensor contains dye (pH sensitive) that indicates by a visible color change the presence of volatile spoilage compounds such as trimethylamine (TMA), ammonia (NH_3) and dimethylamine (DMA) collectively known as total volatile basic nitrogen (TVB-N). [1] The releases of TVB-N are in high enough concentrations in headspace to be monitored by a color change in a pH-sensitive sensor. [2] The pH sensor was fabricated by using a spin-coater and was attached to PP/clay nanocomposite films using a laminating machine (at 160°C) to respond to the headspace TVB-N released from selected fish species during spoilage. Trials on Barramundi or Giant Perch enabled real time monitoring of their spoilage and the sensor responses were found to correlate to changing micro-bial populations and TVB-N production. The color changes of nanocomposite pH sensors were measured and expressed as Hunter values as well as total color differ-ence (TCD). TCD values of bromocresol green (BCG) type indicator also changed continuously. The color changes of the nanocomposite indicator film correlated well with Aerobic Plate Count (APC), and TVB-N value of fresh fish. According to the changes in Hunter color values of the nanocomposte indicator film in the packages of fresh fish during storage at ambient temperature, the results show that the color of BCG type-film turned from initially yellow to finally blue. The color changes of the developed indicator properly represented the degree of deterioration of fresh fish. Moreover, the nanocomposite pH sensor films both could be employed as an effec-tive smart packaging technology for evaluating fresh fish and extent shelf life of fresh fish due to an improvement of oxygen barrier property of packaging by adding organoclay nanocomposite.

4.2 Introduction

Smart packaging is one of the innovative food packaging concepts that have been introduced as a response to the continuous changes in current consumer demands and market trends. In the fisheries industry, there is a large amount of emphasis in developing rapid methods to evaluate fish freshness by using general quality indicators. One concept to meet this requirement is by using a smart packaging in cooperation with a simple freshness color indicator that monitors the microbial breakdown products in the headspace of the packaged fish. When fish spoils, it releases a variety of basic volatile amines which are detectable with appropriate pH indicating sensors through visible color changes to the spoilage-volatile compounds that contribute to a quantity known as total volatile basic nitrogen (TVB-N) and the response of changing microbial populations (aerobic plate count (APC) or total viable count (TVC)).

In this research, the pH sensor for determination of amine spoilage product in packaged fish based on PP/clay nanocomposites was focused and fabricated. Bromocresol green, which indicates through a visible color change (yellow to blue) to the spoilage volatile compounds known as total volatile basic nitrogen (TVB-N) was attached on nanocomposite films by using a laminating machine (at 160°C). The color changes of the pH sensor were measured and expressed as Hunter values as well as total color difference (TCD). TCD values of bromocresol green (BCG) type indicator also changed continuously. The color changes of the nanocomposite indicator film correlated well with APC, and TVB-N value of fresh fish. According to the changes in Hunter color values of the nanocomposte indicator film within the packages of fresh fish during storage at ambient temperature, the results show that the color of BCG type-film turned from initially yellow to finally blue. The color changes of the developed indicator properly represented the degree of deterioration of fresh fish. In addition, leaching of the dye was assessed over time to evaluate the suitability of the sensor formulation for food packaging application.

4.3 Experimental

A. The pH Measurement of fish tissue and TVB-N

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

20 g portions of tissue were homogenized in 10 ml of distilled water, followed by pH determination with a pH meter. Conversely, a pH of TVB-N was measured by using a pH paper. The pH paper was placed on a lid of 7x4 cm of polypropylene cup. Experimental design for pH measurement of TVB-N is shown in Figure 4.1 below.
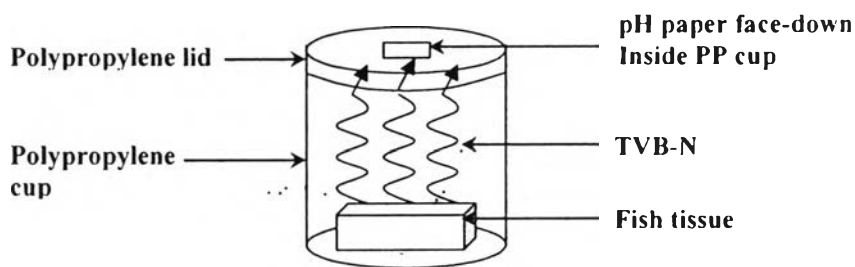
Figure 4.1 Experimental design for pH measurement of TVB-N.

B. Microbial Analysis

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

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

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

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

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

a) Sample extraction

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

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 1ml of indicator) was pipette and placed into the inner ring of Conway's unit (volatile compounds from sample extract would diffuse into boric acid salt and these salts would be reduced to HCl-salts by strong HCl during titration). 1 ml of filtered sample extract was pipette into the outer ring of the conway's unit. 1 ml of saturated K_2CO_3 solution was pipette into the outer ring of the conway's unit and placed on the opposite side of the sample (to made sample extract into alkalines condition similar to that of volatile compound). The conway's unit was immediately covered and shaked gently to dissolve the samples and mix it with K_2CO_3 without contaminating the inner ring of conway (triplicate for each sample). Stand the samples were kept at room temperature for 3 hr. After the color of boric acid solution changed from pink to green, following the generating of volatile base, this sample was then titrated with 0.01 N HCl containing in a micro-burette until the color changed back to pink. Experimental design for determination of TVB-N is shown below (see Figure 4.2).

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

c) Calculation

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

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 × 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
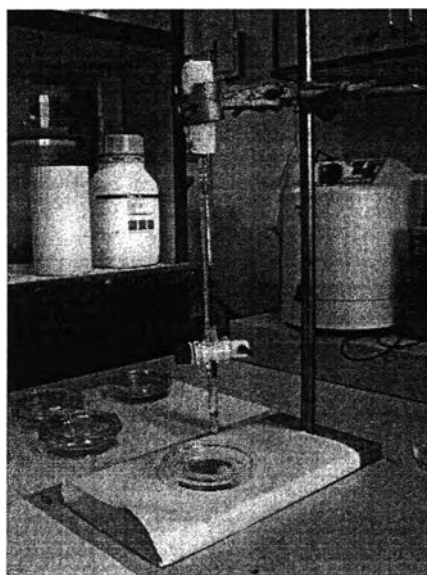

Figure 4.2 Experimental design for determination of TVB-N.

4.4 Results and Discussion

A. The pH measurement of Fish Tissue and TVB-N

The pH is usually chosen as the major quality attributes and deterioration indexes of fresh fish because they can represent the characteristic of the spoiled fish. Variations in values of pH during storage are depicted in Figure 4.3.

The pH value ranged from 6.85 to 7.58 for whole tissue and from 7.3 to 8.1, for TVB-N respectively, during the 42 hours storage period. The pH of live fish muscle is closed to the value 6.5–7. Post mortem pH can vary from 6.0 to 7.1 depending on season, species and other factors. [3] According to the literature, the pH is about 6.0–6.5 for fresh fish, and it increases during storage. The limit of acceptability is usually 6.8–7.0. [4] On storage, the pH values increased gradually. Increase in pH may be attributed to the production of volatile base compounds by bacterial activity. [5] Thus, the results in the pH indicate that formation of TVB-N. The high pH could be an additional factor explaining the elevated and late formation of TVB-N.
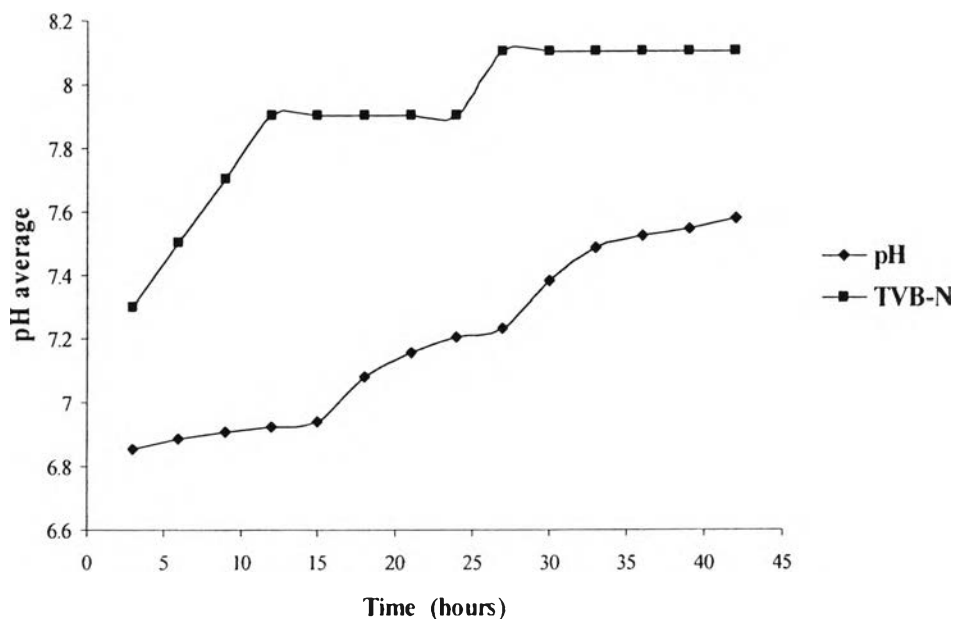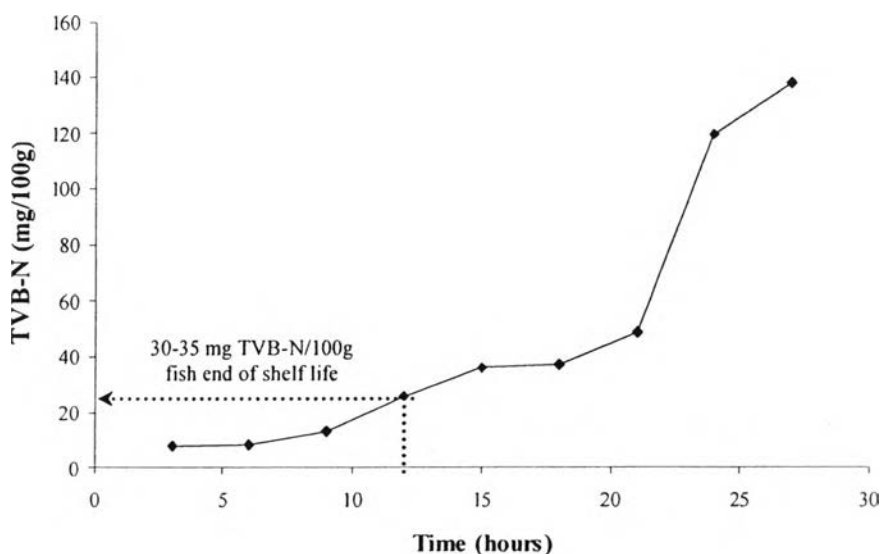

Figure 4.3 Changes in pH of barramundi filets during storage at 25°C.

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

Odor is also 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. [6] 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.

TVB-N in fish is mainly composed of ammonia, trimethylamine (TMA) and dimethylamine (DMA). A level of 30–35 mg TVB-N/100 g of fish muscle is usually regarded as spoiled. [7] Changes in TVB-N values are shown in Figure 4.4. Values were found to increase in all samples during storage at 25°C. However, TVB-N value of 30-35 TVB-N/100 g of fish muscle was reached after about 12 h. as the limit of TVB-N value which spoilage fish is not acceptable for human consumption.

Figure 4.4 Changes of TVB-N of barramundi filets during storage at ambient temperature.

However, TVB-N values showed significant fluctuation for all fish samples as a function of storage period indicating that TVB-N is a poor indicator of fish freshness, as also proposed by. [8, 9, 10] So in this work, APC characterization as a complementary method for finding fish spoilage period was selected.

C. Microbial Analysis

It is difficult to use reported bacterial counts in the literature to define exact spoilage thresholds as they can vary depending on the catch season, catch maturity, geographical location and above all fish species. [11] However, Characterization of the levels of spoilage specific microbial populations will be determined over the same timescale as in this investigation to enable the critical time of spoilage to be determined. This would need to be characterized for each species of interest, as the spoilage time is likely to be different for all species. [12] Fresh fish quite heterogeneous micro-flora. Often during storage, a specific bacteria group, known as specific spoilage organisms (SSO), will outgrow the others and cause the most chemical changes often. [13]

Koutsoumanis (2001) and Olafsdottir *et al.* (1997 and 2004) both reported APC values of 10^7cfu/g for fresh fish samples to reach end of shelf life. [14, 15] Figure 4.5 shows ranges of microbial population commonly associated with spoilage in barramundi fish and the specific level of 10^7cfu/g was reached after about 12 h. In Figure 4.5, APC were found to slowly increase from approximately 10^5 cfu/g during the initial 6 h but sharply rise from then on, reaching values of 10^7 cfu/g at approximately 12 h. At this point, the APC value is 21×10^7cfu/g for barramundi samples to reach end of shelf life.
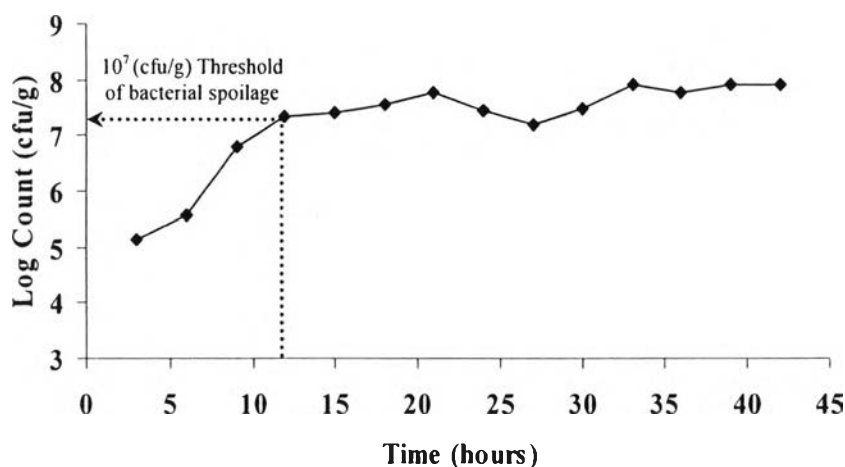
Figure 4.5 Changes in APC of barramundi during storage at ambient temperature.

4.5 Conclusions

Quality changes and shelf life of whole Barramundi fish stored at room temperature were monitored by pH measurement, TVB-N method, and microbiological analyses (APC). An estimation of the samples' shelf-life can be made based on the following criteria: (a) the pH of 6.8-7.0, (b) the TVB-N limit of 30-35 mg /100g of fish muscle, and (c) the level of 10^7cfu/g for APC. By combining these criteria the shelf-life of our samples was 12 hours for the Barramundi fish stored at room temperature. The presence of exceeding pH, TVB-N and microbial count standard value in fish samples may be related to bacterial activity during fish spoilage. Subsequently, this work investigated the color change of the sensor that relate to this spoilage period to inform the color change of nanocomposite pH sensor after fish spoilage in latter step.

4.6 Acknowledgements

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4.7 References

[1] Pacquit, A., Lau, K.T., and Diamond, D. (2004) Development of a colorimetric sensor for monitoring of fish spoilage amines in packaging headspace. IEEE Sensors, 365–367.

[2] Byrne, L., Tong Lau, K., and Diamond, D. (2002) Monitoring of headspace total volatile basic nitrogen from selected fish species using reflectance spectroscopic measurements of pH sensitive films. Analyst, 127, 1338–1341.

[3] Simeonidou, S., Govaris, A., and Vareltzis, K. (1998) Quality assessment of seven Mediterranean fish species during storage on ice. Food Research International, 30, 479–484.

[4] Ludorff, W. and Meyer, V. (1973) Fische und Fischerzeugnisse. Verlag Paul Parey, Hamburg, Berlin, 308 pp.

[5] Cann, D., Smith, G. L., and Houston, N. G. (1983) Further studies on marine fish stored under modified atmosphere packaging. Aberdeen: Ministry of Agriculture Fisheries and Food, Torry Research Station.

[6] Olafsdottir, G., Martinsdottir, E., Oehlenschlager, J., Dalgaard, P., Jensen, B., and Unde, I. (1997) Methods to evaluate fish freshness in research and industry. Food Science and Technology, 8, 258–265.

[7] Milanes, M. (2004) Characterization of sensory indexes of artic charr (salvelinus) and determination of its shelf life. Fishries training programme, The United Nations University, Islands.

[8] Papadopoulos, V., Chouliara, I., Badeka, A., Savvaidis, I.N., and Kontominas ,M.G. (2003) Effect of gutting on microbiological, chemical, and sensory properties, of aqua-cultured sea bass (Dicentrarchus labrax) stored in ice. Food Microbiol, 20, 414–420.

[9] Kyrana, V.R., Lougovois, V.P., and Valsamis, D.S. (1997) Assessment of shelf-life of maricultured gilthead sea bream (Sparus aurata) stored in ice. Food Science and Technology, 32, 339–347.

[10] Tejada, M. and Huidobro, A. (2002) Quality of farmed gilthead seabream (Sparus aurata) during ice storage related to the slaughter method and gutting. European Food Research Technology, 215, 1–7

[11] Pacquit, A., Frisby, J., Diamond, D., Lau, K.T., Farrell, A., Quilty, B., and-Diamond, D. (2007) Development of a smart packaging for the monitoring of fish spoilage. Food Chemistry, 102, 466–470.

[12] Byrne, L., Tong Lau, K. and Diamond, D. (2002) Monitoring of headspace total volatile basic nitrogen from selected fish species using reflectance spectroscopic measurements of pH sensitive films. Analyst, 127, 1338–1341.

[13] Gram, L., and Huss, H. (1996) Microbiological spoilage of fish and fish products. Food Microbiology, 33, 121-137.

[14] Koutsoumanis, K. (2001) Predictive modeling of the shelf life of fish under non-isothermal conditions. Applied and Environment Microbiology, 67, 1821–1829.

[15] Olafsdottir, G., Nesvadba, P., Natale, C. D., Careche, M., Oehlenschlager, J., and Tryggvadottir, S. V. (2004) Multisensor for fish quality determination. Food Science and Technology, 15, 86–93.