Journal of New Technology and Materials (JNTM)
J. New Technol. Mater.
Vol. 13, N°01 (2023)75-80

OEB Univ. Publish. Co.

Polyaniline films as colorimetric food freshness indicator

Rey Ralph H. Virtucio* and Joel G. Fernando

Physics Department, Western Mindanao State University, Zamboanga City, Philippines

*Corresponding author, email: virtucio.rey@wmsu.edu.ph

Received date: March 30, 2023; accepted date: May 11, 2023

Abstract

The innate nature of polyaniline (PANI) films to visibly change its color from green to blue when exposed to basic volatile amines makes it an excellent candidate for spoilage indication. This is further studied at a low volatile concentrations where slight color changes are detected thru colorimetric image analysis. In this study, we have synthesized polyaniline films on a glass substrate and assessed the performance of the films as food freshness indicator through digital image colorimetry. The synthesized PANI films have an overall homogenous surface that is green in color. Well-dispersed globular nanostructures were revealed in the SEM micrographs. From the results of the Fourier-transform infrared (FTIR) spectroscopy and ultraviolet–visible (UV-Vis) spectroscopy, it was found that the films were in the doped emeraldine oxidation state. The RGB (red, green, and blue) color component values of the PANI film changed correspondingly with the time of exposure to spoiling milkfish samples. Moreover, based on the Pearson product-moment correlation, the green and red components best correlate with the pH values of the milkfish.

Keywords: polyaniline, colorimetry, total volatile basic nitrogen (TVBN), food freshness, sensor

1. Introduction

The quality of fish and meat products deteriorate as chemical changes become pronounced. These chemical changes are linked to the microorganisms that increase and spread after death [1]. For the fish, these specific spoilage microorganisms are the *Pseudomonas* spp [2]. During degradation, volatile amines such as trimethylamine (TMA), dimethylamine (DMA) and ammonia (NH3) are released. These are collectively known as total volatile basic nitrogen (TVBN) compounds and are potential spoilage indicators.

The degradation of the fish cannot be controlled but one has just to point out the tolerable stage of fish consumption when it is still safe to eat. Over the years, different methods arise to detect the presence of TVBN in fish products such as quantifying TVBN content and chromatography [3-4]. However, these standard methods are considered destructive, invasive, laborious, time consuming and expensive, hence it is not beneficial when it comes to mass production in the market. But, a new non-invasive, cost- and time-effective approach can replace these methods – the use of optical sensors or indicators integrated in smart food packaging system.

This concept can innovate the packaging system to address the global issues on food safety and food-borne diseases. Milkfish (*Chanos chanos*) is one of the top aquaculture commodities in the Philippines. It is considered as the backbone of the country's aquaculture and its supply chain from the producers to the consumers takes time [5-6]. This type of indicator will now facilitate convenience, preservation and protection during transportation, handling and storage and until the use of the fish products under the consumers' respective premises.

Sensors allow the species of interests to react with its chemically sensitive layer and the changes in the optical properties (absorbance, fluorescence, or intensity) are detected colorimetrically or spectrally. These sensors are already well established since the first pH indicator paper was developed. At present, most studies use pH sensitive dyes in sensor arrays such as methyl red for broiler chicken freshness, cresol red for TVBN monitoring in cod and orange roughy fishes, and Morsy group's 16 chemo-sensitive compounds for Atlantic salmon freshness monitoring [7-9]. But, these dyes are prone to leaching over time which can give an inaccurate result or false positive indication. Moreover, dyes' response to the pH change is affected by temperature – frozen condition to be in particular [10].

Polyaniline, a conducting polymer that has various applications, is one of the potential materials that can be developed into a sensor that is free from leaching, accurate, simple, low-cost, rapid, reliable, consumer-friendly, and non-invasive [10]. PANI is synthesized in different substrates such as glass and it changes conductivity and color due to pH change as a result of protonation in the polymer backbone. In a recent study, the distinct color change of the PANI film brought by the increasing volatile amines during milkfish spoilage period is studied spectrophotometrically as well as the determination of the onset of spoilage at room temperature [10]. In order to assess the freshness of packaged fish, the indicator is placed inside the packaging and the observed color change of the film is sensory evaluated. The change is just visible if there is a high TVBN content in the fish product. At low level TVBN concentration, distinguishing color differences could be hardly seen by a naked eye.

The slight color change of polyaniline films exposed to low concentration of TVBN compounds can be detected using digital image analysis. In this approach, high specification optical instruments such as digital and video cameras, mobile phones and scanners are used to take the digital images of the sensor element. The images are processed using an image-processing software such as Photoshop Pro and ImageJ by taking the RGB, Grayscale, CMYK or HSV values. The color components of the image are correlated to the pH values of fish and meat products, concentration of TVBN and microbial count.

In this study, we investigated the feasibility of employing digital image-based colorimetry to assess food freshness using of PANI films as sensing element. The thin films were synthesized on glass substrates via in-situ polymerization of aniline ($C_6H_5N_2$) using potassium dichromate ($K_2Cr_2O_7$) as oxidant. The physicochemical properties of the films were also determined as well as the effects of the spoiling fish samples on the color of PANI thin films.

2. Materials and Methods

2.1. Synthesis of PANI films

Polyaniline films on glass substrates were prepared via *in-situ* polymerization of aniline in an acidic medium using potassium dichromate as oxidant. To begin the polymerization, the substrates were immersed in a mixture of aniline (3.01 mL) and potassium dichromate (3.24 g) that were dissolved separately in hydrochloric acid (HCl) solutions (2.0 M). The samples were then removed after 30 minutes and rinsed successively with water and 1.0 M HCl.

2.2. Characterization

The synthesized PANI films were characterized using SEM-EDS (JEOL) for its morphology and UV-Visible (Perkin Elmer, UK) spectrometer from 400 nm to 900 nm for its UV Visible absorption spectra and Fourier transform infrared Spectrometer (Perkin Elmer, UK) for the FTIR spectra within the range of 650-4000 cm^{-1}.

2.3. Fish Spoilage Study

2.3.1 Preparation of the Fish Sample.

Freshly caught milkfish obtained from a local fish pond located at Zamboanga City, Philippines were placed inside a sealed container in order to avoid externally induced contamination when it is transported to the laboratory. These were skin cleaned without its innards and main bone. Aseptic techniques (e.g., the use of disposable gloves and flame sterilized scalpel) were applied to avoid the contamination of the samples.

2.3.2 PANI Film response to Fish Sample.

Three milkfish (200 g) samples were aseptically placed separately in three identical sterilized containers. PANI films, that were never in direct contact with the samples, were fixed in the inner side of the cap facing the fish samples, 5 cm above it. A reference container without any fish sample was also prepared as control and the color of the film was also observed. The pH values of the samples were periodically analyzed using a digital pH meter (Eutech Instruments Cyberscan pH 11). Photographs of the PANI films that were mounted in a light box illuminated by three eight-watt compact fluorescent lamps, were also taken at the same time. The RGB values of the digital images were taken using the ImageJ application, a public domain, Java-based image analysis software. The same part of the films that are homogenous were selected to avoid a wide range of values on the color components.

3. Results

3.1. Synthesis of PANI Films

The temperature profile of the reaction mixture was obtained and a typical course of polymerization was observed as shown in Figure 1. The temperature was virtually constant for 15 seconds and this denotes the induction period of the oxidation of aniline under acidic conditions [11]. This short induction period is due to the high dopant concentration. During this stage, aniline oligomers were formed and eventually, the nucleates were adsorbed on the surface of the glass substrates [13]. Polymerization period commenced in the 16th second where the temperature rapidly increased at a rate of 0.39 °C/s since it is an exothermic reaction. PANI chain formations began in this phase which is stimulated by the aniline oligomers [12]. The medium reached the maximum temperature in the 2nd minute and started to drop in the 5th minute which signals the end of the polymerization process (post-polymerization period).

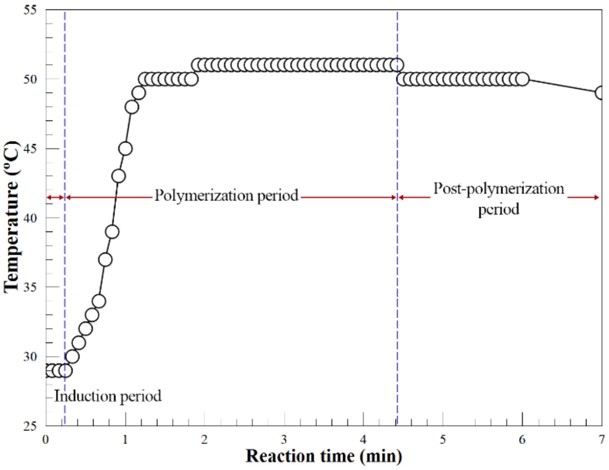

Figure 1. Temperature profile of the reaction mixture observed during the preparation of polyaniline films.

3.2. Scanning Electron Microscopy

The glass substrates were successfully coated with polyaniline films during the oxidation of aniline in an acidic media via surface or adsorption polymerization as shown in Figure 2(a). This was only achieved because the substrate was introduced to the reaction mixture before the induction period [13].

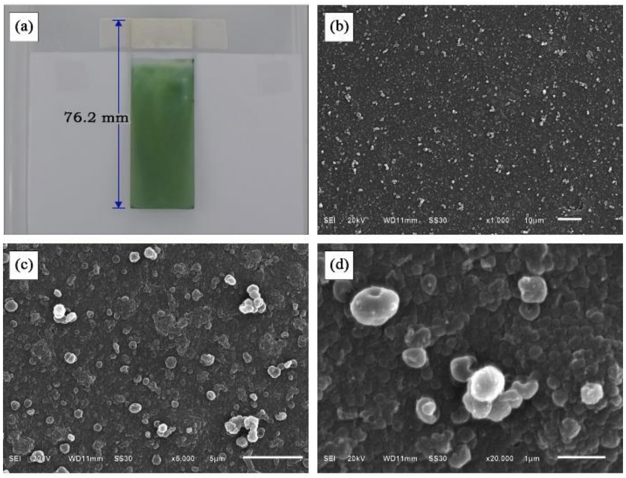

Figure 2. (a) Polyaniline thin films deposited on glass substrates and its surface morphology at three different magnifications: (b) 1 000, (c) 5 000 and (d) 20 000×.

The surface morphology of the polyaniline thin film was investigated and based on the SEM images, the film consists of well-dispersed globular nanostructures with an average diameter of 390 nm as shown in Figure 2(b) to 2(d). The globular nanostructured morphology is suitable for gas sensing application because gas molecules can interact on the maximized surface area of the material.

This PANI film with brush-like structure was globular in nature because of its dependence on the hydrophilicity of the flat macroscopic substrate which played an important role in the deposition of PANI [13]. Hydrophobic nucleates adsorbed and aggregated on the available surfaces and PANI chains subsequently started to grow forming globular structures (Figure 3). Secondary adsorption of nucleates occurred on top of the grown ones resulting to an increase of surface roughness and fused globular structures [11].

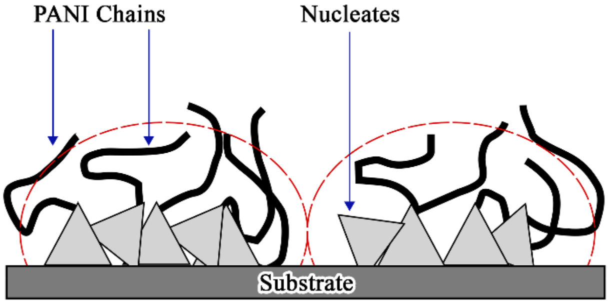

Figure 3. Globular formation model of PANI film [13].

3.3. UV-Vis Spectroscopy

The UV-Vis absorbance spectra of the PANI film at 400-900 nm region is shown in Figure 4. The absorption band at longer wavelengths (above 800 nm) indicates the presence of polarons. This suggests that the film is in doped state which makes the film green and conducting [14]. The peak at 423 nm is also one of the characteristic peaks of PANI salt which also proves the film to be protonated [15]. Another property that can be derived from the optical absorption measurements is the thickness of the PANI film. Film's thickness d_f is computed using the relation given by Sapurina et. al. [11] and the thickness of the film is found out to be 77.12 nm.

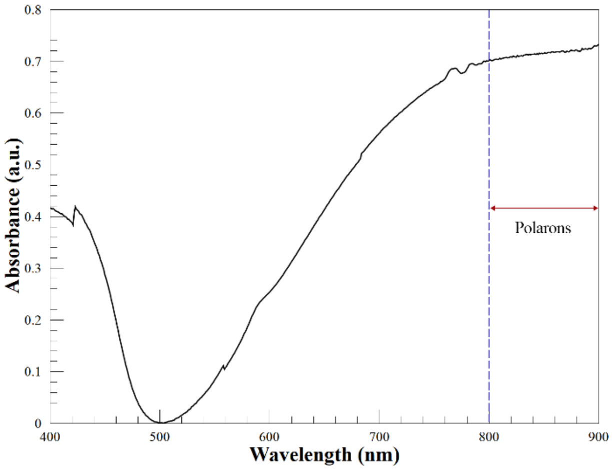

Figure 4. Absorption spectra of PANI film deposited on the glass substrate.

3.4. Fourier Transform Infrared Spectroscopy

Figure 5 shows the FTIR absorbance spectra of the polyaniline film. The main peaks at 1570 cm^{-1} and 1488 cm^{-1} correspond to the C=C stretching of the quinoid (Q) and benzenoid (B) rings, respectively [16]. The shoulder that appears near the quinoid ring at 1610 cm^{-1} is linked to the –C=C– stretching [15]. The absorption band at 1302 cm^{-1} is caused by the C–N stretching of the secondary aromatic amine that strengthened during the protonation of PANI [17]. Lastly, absorption band due to the C–N$^{+\bullet}$ stretching vibration in the polaron structure was found 1243 cm^{-1}. It is a band characteristic of PANI which implies that it is conducting and protonated [16].

The conducting nature of the film is further verified by the broad absorption band between 1700 cm^{-1} and 2700 cm^{-1}, which is due to the absorption of the free charge-carriers in the doped polymer [18]. The sharp peaks in the 2800-3200 cm^{-1} region are named H-peaks which are related to the interchain hydrogen bonding that exists only if the PANI chains are regularly aligned on the substrate. The hydrogen bonding is also supported by the energy peak at 2850 cm^{-1} [19]. The oxidation state of the polyaniline was also determined by taking the ratio of the absorption intensity of the quinoid peak (I_Q) and the benzenoid peak (I_B) and the computed Q/B_{ratio} is 0.8948.

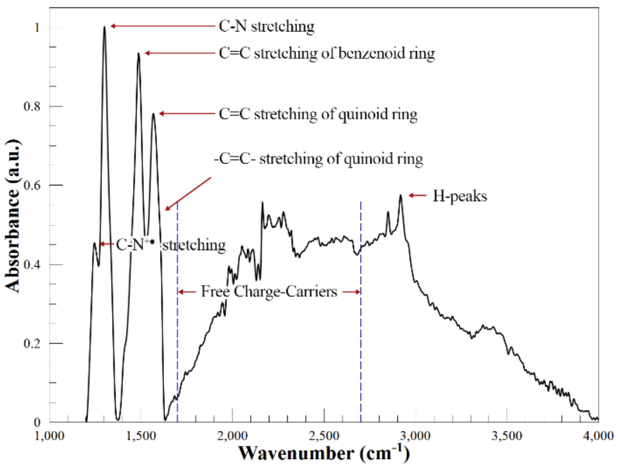

Figure 5. FTIR absorbance spectra of PANI films prepared on glass substrates.

This indicates that the synthesized PANI film on the glass substrate is in the emeraldine oxidation state since the ratio is close to unity and this agrees with the results on the UV-Vis absorption spectra.

3.5. Fish Spoilage Study

3.5.1. pH of the milkfish.

Figure 6 shows the pH values of the milkfish samples at room temperature. According to Gram and Huss, fish product has a high post mortem pH in the flesh that is greater than 6.0 [2]. The average pH value during the whole experiment varied from 5.96 to 6.30. It is more or less constant for the first 8 hours and began to rise at the 10th hour.

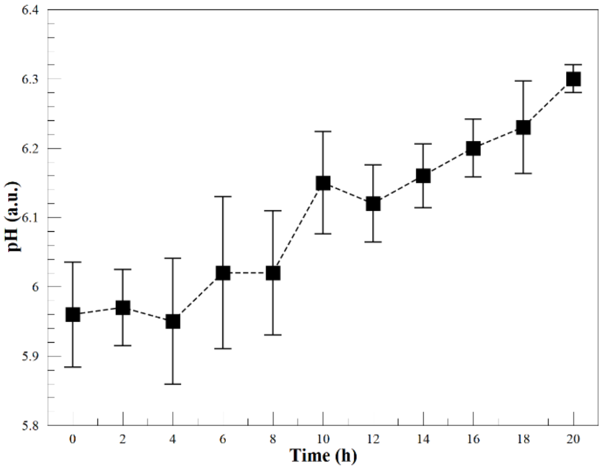

Figure 6. The pH values of the milkfish samples at room temperature.

3.5.2. PANI film response to fish sample.

The PANI film images were taken every two hours and a homogenous part of the film's image was cropped and analyzed using the ImageJ program to measure the RGB component values and its mean. Generally, the green component values are higher than blue and red because of the film's dominant color.

Figure 7 shows the RGB component values and the mean RGB value of the reference PANI film. Its component values and mean follow almost a horizontal trend line throughout the duration of the experiment. This implies that the air and humidity don't influence the color changes in the PANI film.

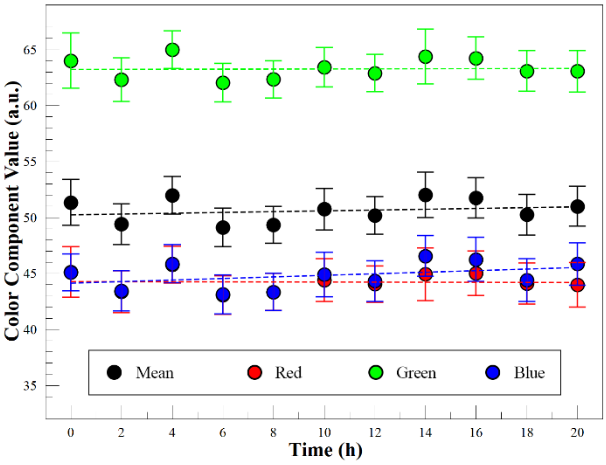

Figure 7. The color response of the reference PANI film exposed to ambient air.

Figure 8 shows the color response of the PANI film indicator. It was placed in close proximity with the 200 g fish sample and it changed its color from green to blue as the fish began to spoil. For the first 12 hours, no drastic color change was detected in the image analysis. It's also visually evident on the film. This indicates that the milkfish sample kept under room temperature was still fresh and only small amount of volatile amines was released at a relatively slow rate. In a similar study, the milkfish samples were also fresh within 10-12 hours and the spectrophotometer detected a color change at the 8th hour [10].

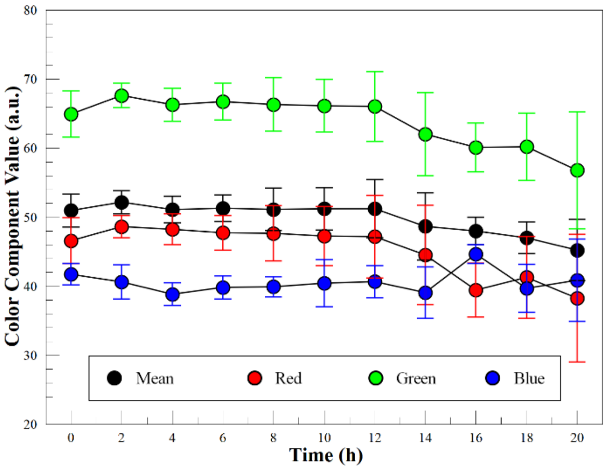

Figure 8. The color response of the PANI film exposed to spoiling milkfish.

Although not observable visually by a naked eye, a drop in the green component value was detected using the image analysis in the 14th hour. The drop indicates the onset of spoilage and denotes the transition of the film's color from green to blue. This trend was observed in all set ups for the green and red values while the blue values increased in the 16th hour. The drop in the red value is a contributing factor that influence the blueness of the deprotonated PANI film sensor.

The headspace becomes more basic because of the volatile amines produced during the milkfish spoilage. The exposed films undergone the dedoping process by a deprotonation mechanism in the amine groups of the emeraldine salt as shown in Figure 9 [20]. The protons on the –NH– groups of the polyaniline are transferred to NH3 molecules and other compounds released in the spoiling milkfish. The ammonia turned into ammonium ions NH_4^+ and PANI is converted into its base form. The counterion, X-, is induced close to the polymer chains to balance the positive charges.

3.5.3. pH and color correlation.

In order to evaluate the colorimetric sensitivity of the PANI film-based sensor, the pH values of the milkfish were correlated to each of the RGB component value. The Pearson product-moment correlation coefficient was computed to assess the relationship between the different color components of the films and the pH values of the fish samples. There was strong negative correlation between the pH values and green color component (r=-0.852, n=11 and p=0.001) at 0.01 level of significance. A scatter plot summarizes the result in Figure 10. Increase in the pH of the fish sample was correlated with the increase in green and red color (r=-0.856, n=11, p=0.001 and α=0.01) components. On the other hand, the blue component is not correlated with the pH of the fish sample (r=0.242, n=11, p=0.474 and α=0.05).

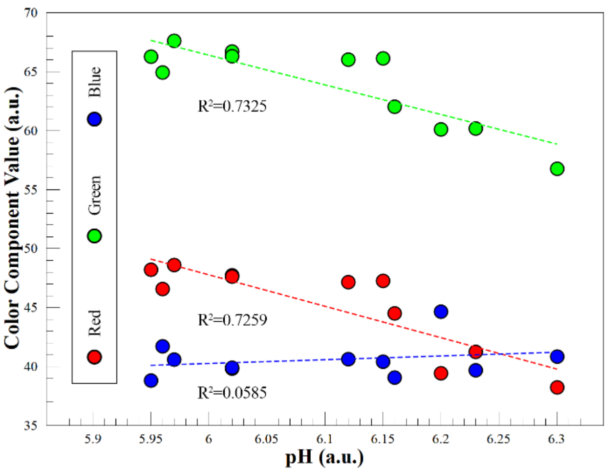

Figure 10. Linear relationship between the pH values and color component values.

4. Conclusion

The synthesized PANI films on glass substrates have an overall homogenous surface that is green in color. Well-dispersed globular nanostructures were revealed in the SEM micrographs. From the FTIR and UV-Vis analysis, it was found out that the films were in the doped emeraldine oxidation state. The color component values of the PANI film changed correspondingly with the time of exposure to spoiling milkfish samples. Moreover, based on the Pearson product-moment correlation, the green and red components of the RGB colors best correlate with pH values of the milkfish.

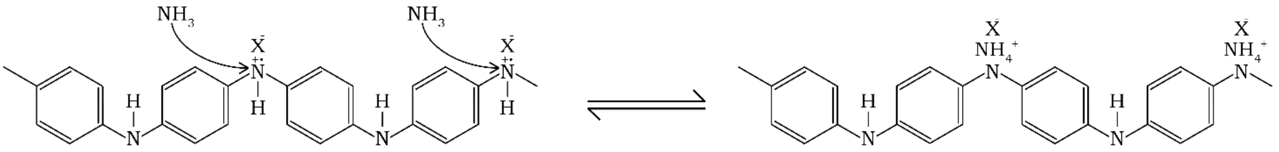

Figure 9. Schematic of the interaction between PANI and ammonia gas [19].

Acknowledgement

The researchers would like to thank Western Mindanao State University for making this research possible. Additionally, the researchers extend their appreciation to Engel Narvaez and Herbert Barba for supplying the fish samples.

References

[1] N. Hamada-Sato, K. Usui, T. Kobayashi, C. Imada, E. Watanabe, “Quality assurance of raw fish based on HACCP concept”, Food Control. 16 (2005) 301–307.

[2] L. Gram, H.H. Huss, “Microbiological spoilage of fish and fish products”, Int. J. Food Microbiol. 33 (1996) 121–137.

[3] R. Lv, X. Huang, J.H. Aheto, L. Mu, X. Tian, “Analysis of fish spoilage by gas chromatography–mass spectrometry and electronic olfaction Bionic System”, J Food Saf. 38 (2018).

[4] C. Ruiz-Capillas, W.F.A. Horner, “Determination of trimethylamine nitrogen and total volatile basic nitrogen in fresh fish by Flow Injection Analysis”, J. Sci. Food Agric. 79 (1999) 1982–1986.

[5] Philippine Statistics Authority 2021 “Fisheries Statistics of the Philippines 2019-2021” Available at: https://psa.gov.ph/tags/fisheries-statistics-philippines

[6] A. Gaitan, J. Toledo, M. Arnaiz, E. Ayson, J. Altamirano, R. Agbayani, N. Salayo, C. Marte . “Milkfish *Chanos chanos* cage culture operations” (2014) ISSN 0115-5369

[7] B. Kuswandi, Jayus, R. Oktaviana, A. Abdullah, L.Y. Heng, “A novel on-package sticker sensor based on

methyl red for real-time monitoring of broiler chicken cut freshness", Packag. Technol. Sci. 27 (2013) 69–81.

[8] L. Byrne, K.T. Lau, D. Diamond, "Monitoring of headspace total volatile basic nitrogen from selected fish species using reflectance spectroscopic measurements of ph sensitive films", Anal. 127 (2002) 1338–1341.

[9] M.K. Morsy, K. Zór, N. Kostesha, T.S. Alstrøm, A. Heiskanen, H. El-Tanahi, et al., "Development and validation of a colorimetric sensor array for fish spoilage monitoring, Food Control. 60 (2016) 346–352.

[10] B. Kuswandi, Jayus, A. Restyana, A. Abdullah, L.Y. Heng, M. Ahmad, "A novel Colorimetric Food Package label for fish spoilage based on polyaniline film", Food Control. 25 (2012) 184–189.

[11] I. Sapurina, A. Riede, J. Stejskal, "*In-situ* polymerized polyaniline films", Synth. Met. 123 (2001) 503–507.

[12] J. Stejskal, I. Sapurina, "Polyaniline: Thin films and colloidal dispersions (IUPAC technical report)", Pure Appl. Chem. 77 (2005) 815–826.

[13] J. Stejskal, I. Sapurina, M. Trchová, "Polyaniline nanostructures and the role of aniline oligomers in their formation", Prog. Polym. Sci. 35 (2010) 1420–1481.

[14] N.V. Blinova, I. Sapurina, J. Klimovič, J. Stejskal, "The chemical and colloidal stability of polyaniline dispersions", Polym. Degrad. Stab.. 88 (2005) 428–434.

[15] J. Fernando, R. Vequizo, M. Odarve, B. Sambo, A. Alguno, R. Malaluan, R. Candidato Jr, J. Gambe, M. Jabian, G. Paylaga, F. Bagsican F, H. Miyata, "Physico-chemical effects of supercritical carbon dioxide post polymerization treatment on HCl-doped polyaniline prepared via oxidative chemical polymerization," IOP Conf. Ser.: Mater. Sci. Eng. 79 (2015) 012010

[16] J. Stejskal, M. Trchová, I.A. Ananieva, J. Janča, J. Prokeš, S. Fedorova, et al., "Poly(aniline-co-pyrrole): Powders, films, and colloids. thermophoretic mobility of colloidal particles", Synth. Met. 146 (2004) 29–36.

[17] I. Sapurina, A.Y. Osadchev, B.Z. Volchek, M. Trchová, A. Riede, J. Stejskal, "*In-situ* polymerized polyaniline films", Synth. Met. 129 (2002) 29–37.

[18] M. Trchová, I. Šeděnková, J. Stejskal, "*In-situ* polymerized polyaniline films 6. FTIR spectroscopic study of Aniline Polymerisation", Synth. Met. 154 (2005) 1–4.

[19] C.-G. Wu, Y.-R. Yeh, J.-Y. Chen, Y.-H. Chiou, "Electroless surface polymerization of ordered conducting polyaniline films on aniline-primed substrates", Polymer. 42 (2001) 2877–2885.

[20] A.H. Navarchian, Z. Hasanzadeh, M. Joulazadeh, "Effect of polymerization conditions on reaction yield, conductivity, and ammonia sensing of polyaniline", Adv. Polym. 32 (2013).