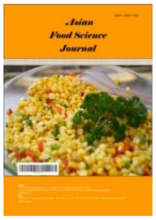

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Methyl Red Indicator on Smart Packaging as a Freshness Sensor for Tilapia Fillets

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Authors' contributions

This work was carried out in collaboration among all authors. Author KAS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KH and IR managed the analyses of the study. Author IR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This research aims to analyze the response of methyl red indicator film sensor label color changes on smart packaging in detecting the freshness of tilapia fillets over a shelf life of 10 hours at room temperature (± 25ºC). This research uses a comparative descriptive method. The parameters observed were pH values, total plate count (TPC) and total volatile base nitrogen (TVBN). The results of this research indicate that the response of the film label consists of several concentrations of methyl red namely control (0%); 0.05%; 0.5% and 1%. The best results from film sensor label response is 0.05% Methyl red concentration in detecting the freshness of tilapia fillets and showed the most rapid response to changing the color of the label at the observation of the 7th hours during storage of 10 hours with 8 times the observation time. The freshness of tilapia fillets has decreased quality with changes in pH that tend to fluctuate ie the pH has decreased and then rose again as the color changes are indicated by the film label from yellow to reddish-orange. Total volatile base nitrogen (TVBN) and total plate count (TPC) during the observation showed a trend towards the same graph results, namely, the longer shelf life will increase the decrease in freshness quality of tilapia fillets with marked decay. Therefore, the freshness level of tilapia fillets can be determined by the smart packaging sensor methyl red indicator label at room temperature storage (± 25ºC).

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Keywords: Smart packaging; mehtyl red; room temperature; shelf life; tilapia fillets.

1. INTRODUCTION

Food needs are increasing, especially fish in the fresh form. Fish with good quality and fresh without physical defects will have a competitive value with a high selling value on the market, but in reality in the process of handling fresh fish often encountered problems in an effort to maintain its quality, including the quality of fish that is not good as a result of poor handling not careful, not immediately carried out, unhygienic and the use of inappropriate temperatures during handling [1]. One product that is handling good quality so that it does not quickly decay by being made into fillets.

Tilapia fish (*Oreochromis niloticus*) is a type of freshwater fish that is commonly cultivated by the community because it can adapt to the environment as well as growth and resistance to very good diseases. Based on data from the Ministry of Maritime Affairs and Fisheries [2], tilapia fish is one of the second largest aquaculture products after milkfish from total aquaculture production in Indonesia. Tilapia fish production in 2011 reached 481,441 tons or 75.31% of the production target. However, when compared with 2010 production increased by 5.44%. Research on tilapia fish in Indonesia does not yet provide a complete description of the decay patterns and quality of tilapia specifically tilapia fillets products [3]. Fresh tilapia fillets are rapidly losing their quality because tilapia fish has high protein content and water content resulting in bacterial and enzyme activity in the fish fillets.

In developments of packaging technology, smart packaging has been created, a system capable of providing intelligent functions such as detection, recording, communication, and taste [4]. That is because this technology can provide information to consumers by producing chemical sensors and biosensors. Through these two sensors, it will be easy to monitor product quality and safety during marketing. Smart packaging sensor application development that is being done is smart packaging in the form of film. Usually, to facilitate use, the film is given an additional color as an indicator of quality. The use of synthetic dyes can be applied to detect deterioration in the quality of tilapia fillet products. One indicator that is usually used is Methyl red (MR). Methyl Red, also known as CI acid red 2, is a pH indicator that turns red when in an acidic atmosphere (pH below 4.4) and turns yellow in an alkaline atmosphere (pH above 6.2).

This packaging technique uses a method known as FQI (Food Quality Control). The working principle of FQI on fishery products as an acid-base indicator is that color changes occur due to changes in pH [5]. This package reacts to chemical or biological changes found in the packaging that indicates damage to the product [6]. The decrease in meat quality by enzymes and microbial activity produces alkaline compounds that will evaporate in the packaging and cause changes in pH in the packaging system so that it will be detected by indicators through color changes [7]. One of the potential ingredients as color carriers in FQI is chitosan. Chitosan-based films have strong, elastic, flexible and difficult to tear properties comparable to medium strength commercial polymers [8]. The combination film of chitosan and Polyvinyl Alcohol (PVA) can produce a better film because it increases thickness and tensile strength [9]. Polyvinyl Alcohol (PVA) is a vinyl alcohol polymer composed of monomer units such as ethylene and propylene.

Based on the problem of the deterioration of the quality of freshness of tilapia fillets in food products must be detected and informed to consumers by using smart packaging to detect a decrease in the quality of tilapia fish (*Oreochromis niloticus*) fillets with a methyl red pH indicator dye sensor which is seen from significant color changes. This study regarding smart packaging by referring to the FQI principle detects the decline in the quality of the tilapia fish (O*reochromis niloticus*) fillets. Sensor applications in the form of film labels that utilize the change in response color by using a pH indicator dye methyl red, chitosan and polyvinyl alcohol (PVA). This smart packaging can provide information to consumers about the condition of the product that occurs during storage for 10 hours.

2. MATERIALS AND METHODS

2.1 Material and Equipment

The materials used to make smart packaging film labels are chitosan, polyvinyl alcohol (PVA) and methyl red indicator dyes. Another material used is the material for testing, tilapia fish (*Oreochromis niloticus*) in the form of fillets obtained from the Ciroyom market, Bandung, West Java. Tilapia fish is taken from the Ciroyom market place while still alive with a weight of 1 kg. Tilapia fish is put into a plastic container that has been given oxygen so that the fish does not die, then transported to the place of screening and research in the laboratory of fisheries, the Faculty of Fisheries and Marine Sciences, Padjadjaran University. The tools needed for this research are fish filtering tools, analysis of pH values, analysis of total plate count (TPC) and total volatile base nitrogen (TVBN).

2.2 Methods

This research was conducted in several stages with the comparative descriptive experimental methods. The first stage is aimed at making a color indicator film from a mixture of chitosan and polyvinyl alcohol (PVA) with methyl red color indicator material. The second step was carried out to determine the change in the color of the smart packaging film labeling the tilapia fillets during storage at room temperature (± 25ºC).

2.2.1 Making smart packaging

Smart packaging is made by using chitosan-acetate and polyvinyl alcohol (PVA). The use of these materials is used based on research conducted by Byne modified [10]. The composition of the solution used is 1% chitosan-acetate solution and 1% polyvinyl alcohol (PVA) solution and the pH methyl red indicator with a comparison ratio of control (0%), 0.05%: 0.5%: 1%. Mixing the solution is done using a magnetic stirrer laboratory instrument for 40 minutes until the solution is completely mixed as a whole and also equipped with a hot plate (heating plate) to heat the solution so that the temperature is maintained. After the solution has been homogenized, printing is done (coated) using a small-sized tray container which is then dried in an oven blower at 50ºC for 24 hours. Smart packaging sensors that have become finished in the same size of 5 × 3 cm^2 ish are square-shaped. The flow chart of the process of making smart packaging sensors with chitosan-acetate, PVA and methyl red indicators as seen in the diagram in Fig. 1.

2.2.2 Smart packaging application against fillets

Tilapia fillets sample with a weight of 25 grams is placed in a plastic container then the smart packaging sensor is then placed upside down on the tilapia fillets sample. Film labels are affixed around the tilapia fillets so that the sensor color changes will be seen clearly. The testing/storage stage of the label/film indicator response is carried out at room temperature with a temperature of ± 25ºC stored in a plastic plate container, for 4 treatments namely control (0%), 0.05%: 0.5% and 1% on the methyl indicator red used. Analysis of freshness level of tilapia fillets was carried out by TVBN, TPC and pH values with a vulnerable shelf life of 10 hours in observations of the 0th, 3rd, 5th, 6th, 7th, 8th, 9th and 10th hours with room temperature storage conditions at a temperature (± 25ºC).

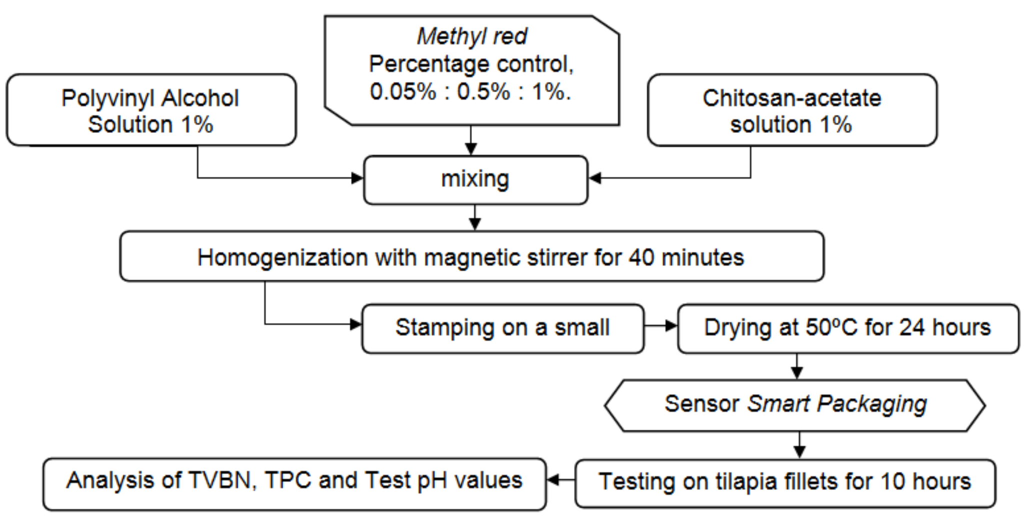


Fig. 1. Flow diagram of making smart packaging sensor and analysis of TVBN, TPC and pH values in observing the freshness level of tilapia for 10 hours

2.2.3 Measurement of fillets pH value

Samples of tilapia fillets that have been destroyed, then taken as much as 5 grams homogenized with 45 mL of distilled water, then determine the pH value of tilapia fillets using a pH meter.

2.2.4 Determination of Total Plate Count (TPC) fillets

Determination of Total Plate Count (TPC) is a how to calculate the number of microbes contained in a growing product on agar media at a predetermined temperature and incubation time. Tilapia fillets weighed 5 grams plus 90 ml NaCl 0.85%. Then it is dilution 10^{-1}, 10^{-2}, 10^{-3} to 1:1.000.000 by aseptic and Duplo. Furthermore, sterile plate count agar media (PCA) was poured at 45ºC as much as 15 ml and incubated at 30ºC in an upside-down position for 48 hours. Perform bacterial counts using the Quebec calculator adjusted for Standard Plate Count (SPC). The number of bacterial colonies counted was petri dishes which has a bacterial colony between 30-300 colonies.

2.2.5 Determination of Total Volatile Base Nitrogen (TVBN) fillets

Blended tilapia fillets of 10 grams plus 30 mL of distilled water. Then filtered to take nature. Boric acid was prepared along with a saturated K_2CO_3 solution that had been added, then distillation was carried out until a pink color change occurred and the distillation results were titrated with 0.01 N HCl. TVBN was determined by the formula:

$$\%N\ (\text{mg N/100 g}) = (j - i) \times \text{N HCl} \times \frac{100}{M} \times \frac{fp}{1} \times 14\ \text{mg N/100 g}$$

2.2.6 Data analysis

Data analysis from the Total Volatile Base Nitrogen Test (TVBN), the Total Plate Count (TPC) test and the pH value test were carried out in a comparative descriptive manner.

3. RESULTS AND DISCUSSION

3.1 Effect of Methyl Red Concentration on Smart Packaging

Changes in the pH value of tilapia fillets will affect changes in pH value on the film label because the compounds resulting from degradation of tilapia fillets meat will cause the label pH to increase and the color of the label to change color. The relationship between the quality of tilapia fillets with the smart packaging sensor label to tilapia fillets stored at room temperature (± 25ºC) in the vulnerable shelf life of 10 hours is shown in Table 1.

The results showed that the best concentration of methyl red was 0.05%. The film label with 0.05% methyl red gives a reddish-yellow color change in the process of decreasing the quality of tilapia fillets with the process of tilapia fillets decay during the observation time of 10 hours, which shows that the fastest compared to other treatments is at 7th hours. Changes in the color of film labels tend to continue to increase in the 8th hours until the 10th hours. whereas with a concentration of 0.5%, the color changes to reddish-yellow at 8th hours and 1% concentration experiences a reddish color change at the 8th hours. This shows that the lower the concentration of methyl red, the faster the color change of the film label and the more obvious the change in color compared to the higher concentration of methyl red, the more intense the color change will be, which will be difficult to see the color changes in the freshness of the tilapia fillets. This change in color is thought to be from the presence of volatile base components resulting from the degradation process of chemical compounds in tilapia fillets meat which tends to increase during the fish rot process.

3.2 Test the pH Value

Based on the change in pH, the storage time causes the pH of the meat to decrease even more so that the freshness level of tilapia fillets is seen that it has decreased in quality, but after that, an increase in pH value will occur. The results of observing the pH value during the process of decreasing the quality of tilapia fillets at an observation time of 10 hours showed that the pH value has fluctuated, this can be seen in the Graph of pH values on the tilapia fillets during storage at room temperature (± 25ºC) in Fig. 2.

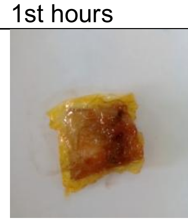
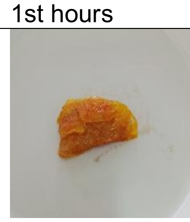
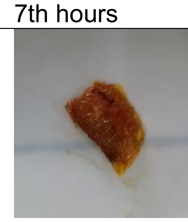
Seen in the graph that the beginning of storage with a range of 7.0 to the end of storage has 6.6 pH values. At a concentration of 0.05%, it was seen that the pH value during storage at room temperature decreased from storage at 0 to 7th hours and experienced an increase in pH again at the 8th hours to the 10th hours in tilapia fillets representing each concentration. the other. This happens because in fish meat there is no supply of oxygen so respiration will occur anaerobically which will produce lactic acid called the rigor mortis phase. The buildup of lactic acid in meat causes the pH of the meat to become more acidic during its shelf life at room temperature. According to Soeparno [11] after an animal dies there is no aerobic metabolism anymore because the blood circulation that carries oxygen to the muscle tissue is stopped so that the metabolism turns into an anaerobic system that causes the formation of lactic acid. The acidic atmosphere in fish meat according to Aksnes [12] causes the enzyme cathepsin to be active and breaks down proteins into simpler compounds in the form of peptides, amino acids, and ammonia which are alkaline, so that the pH rises back to neutral called the Post Rigor phase.

3.3 Test TPC (Total Plate Count)

The number of microbes has been the most important factor in determining the quality of food [13]. Both when processing fillets and removing stomach contents, cross-contamination between fish and the extent of surface exposed to microbial contamination in the environment can reduce the shelf life of fish [14]. Testing of Total Plate Count (TPC) aims to determine the microbes contained in fish meat so that the quality of meat can be known. Following is a table of values (CFU/ml) of TPC at room temperature storage (± 25ºC) presented in Table 2.

The test results showed that at the beginning of the storage amount of bacteria is 5×10^4 CFU/ml and continued to increase until the 10th with a value of 2.7×10^9 CFU/ml. It can be concluded that the longer the storage time of fish meat, the more microbes will grow. The maximum limit of bacteria according to (Indonesian National Standard) SNI 01-2729.1-2006 is 5×10^5 CFU/ml or with a log value of 5.70, indicating that tilapia fillets with the longest shelf life at the 6th hours with the number of bacteria is 2.3×10^5 CFU/ml. It can be concluded that the longer the meat storage time, the more microbes will grow. The measurement of the extent of damage to fish fillets can be seen from the number of bacteria that grow and develop in fish fillets meat.

Table 1. Correlation between quality of tilapia fillets with smart packaging sensor labels

Concentration of methyl red	Observation time	
0% (control)	 1st hours	 10th hours
0.05%	 1st hours	 7th hours
0.5%	 1st hours	 8th hours
1%	 1st hours	 8th hours

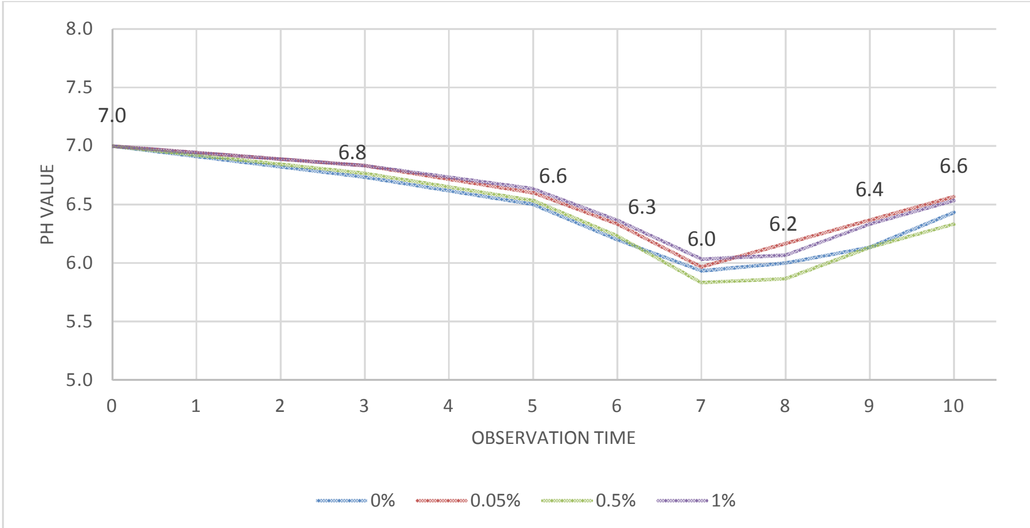


Fig. 2. Graph of pH value on tilapia fillets during room temperature storage

Table 2. TPC test results on the storage time of tilapia fillets at room temperature (±25ºC)

Dilution of sixth hours	The amount microbes cfu/ml
0	$1,5 \times 10^4$
3	$9,5 \times 10^4$
5	$2,1 \times 10^5$
6	$2,3 \times 10^5$
7	$9,5 \times 10^6$
8	$2,1 \times 10^7$
9	$1,8 \times 10^8$
10	$2,7 \times 10^9$

In a study conducted by Hasnedi [15], the TPC value of tilapia fillets also exceeded the fading limit at the 5th hours' observation, which was log 5.81 (8.1×10^5 CFU/ml) at a storage temperature range (30ºC). The same thing happened to the curet fish fillets which already contained more than 10^5 CFU/g at 3rd hours [16]. Based on this, the number of fish fillets microbes that have been carried out by Hasnedi [15] and Riyanto et al. [16] has also exceeded the upper limit of the number of microbes found in tilapia fillets with temperatures ranging from 25ºC to 27ºC at storage times of 3rd and 5th hours.

3.4 TVBN Value Test (Total Volatile Base Nitrogen)

The compounds formed as a result of protein decomposition are compounds containing nitrogen that are basic and volatile (volatile bases) which are all expressed as Total Volatile Base Nitrogen (TVBN) [17]. The results of the TVBN values of each tilapia fillets with room temperature storage are presented in Fig. 3.

Table 3. Quality standards for freshness of fish

Fish quality	TVB-N value (mg N/100 g)
Very fresh	<10
Fresh	10-20
Limit consumed	20-30
Rotten	>30

Based on the results that can be seen from Fig. 3, the value of TVBN during storage at room temperature ranges from 5.41 to 30.27 mg N/100 g. The results of TVBN value analysis during the tilapia fillets rot during the 10th hours' observation period showed that there was an increase in the value of TVBN in line with the longer observation time. In the 0th hours, the TVB value was 5.41 to 8.69 mg N/100 g for each treatment which was classified as very fresh according to Table 3. After that, at the fifth hour of storage, the fish included in the fresh with a TVB value of 14.46 to 18.55 mg N / 100 g. At the 10th hours fish included in the limit of consumption are TVB values ranging from 22.56 to 30.27 mg N/100 g.

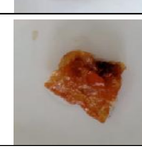
If you see a tendency to increase the value shows that the pattern of existing data is the same, which is increasing in line with the longer time of observation of the decay process of tilapia fillets. In a study conducted by Hasnedi [15], the value of TVBN tilapia fillets also increased during storage, namely at the 0th, 5th, 10th and 15th hours respectively at 8.40 mg N/100 g, 14.56 mg N/100 g, 28.42 mg N/100 g, and 52.36 mg N/100 g.

3.5 The Relationship between Smart Packaging Sensor Labels and Methyl Red Indicators of Various Fish Quality Deterioration Parameters

The relationship between the detection results of the freshness level of tilapia from the color change of the smart packaging sensor based on Chitosan-PVA and the Methyl Red indicator with the value generated from the fish rot parameters such as TVBN, TPC and pH values is very important to know the level of sensitivity and effectiveness of the sensor in detecting the freshness of tilapia fillets.

Color changes that occur are seen at the time of observation at 0 to 7th hours from yellow to reddish yellow and increasingly visible color changes on the label at the 8th hours to the end of the 10th hours' observation to red. The longer the storage time of fish has increased the value of TPC, TVBN, and pH which means the quality of the fish decreases which in line affects the

Table 4. Correlation between quality of tilapia fillets with smart packaging sensor labels

Observation time	pH analysis	TPC analysis (cfu/ml)	TVBN analysis (mg N/100 g)	Sensor smart packaging
0	7,0	$1,5 \times 10^4$	7,14	
3	6,8	$9,5 \times 10^4$	-	
5	6,6	$2,1 \times 10^5$	16,46	
6	6,3	$2,3 \times 10^6$	-	
7	6,0	$9,5 \times 10^7$	-	
8	6,2	$2,1 \times 10^7$	-	
9	6,4	$1,8 \times 10^9$	-	
10	6,6	$2,7 \times 10^9$	26,20	

Remarks: - no observations were made

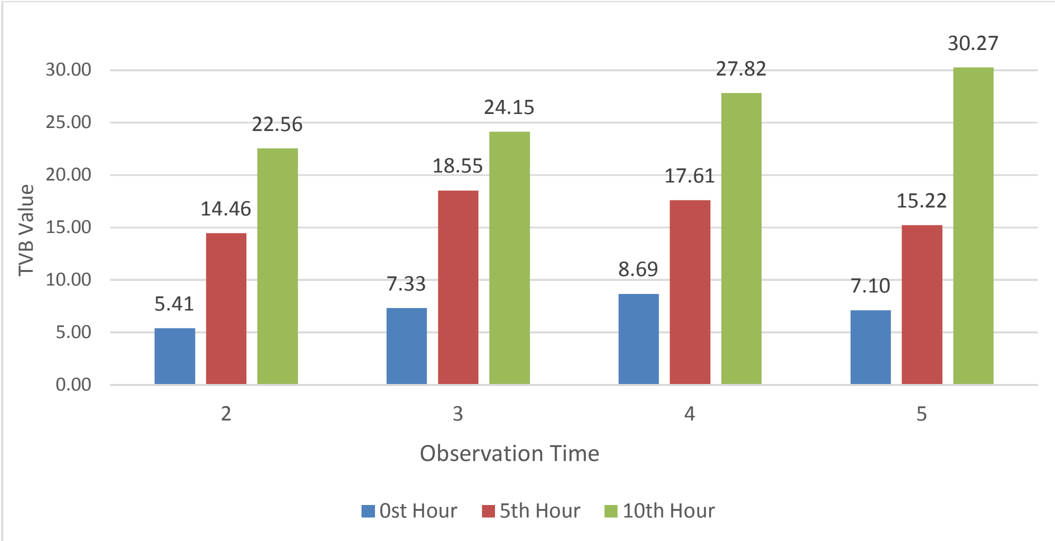


Fig. 3. TVBN value bar chart at room temperature storage

color changes on the smart packaging sensor label. The sensor label shows the color change at the 7th hours to the 10th hours to reddish yellow with a TVBN value of 26.20 mg N/100 g which means tilapia fillets must be consumed immediately based on Table 4. This color change is by the visual state of tilapia fillets that have changed color to pale. Based on the TPC value, the tilapia fillets did not meet the maximum limit at the 6th hours with a range of 2,3 × 10^6 CFU/ml bacteria, while the TVBN value of the tilapia fillets was still fresh to the limit for consumption. The higher the level of TVBN, the more the number of psychrophilic microbes will grow. This is by the statement of [18] stated that TVBN values from tilapia fillet samples would increase according to the growth of microorganisms, indicating volatile basic compounds especially those produced from the metabolic activity of decomposing microorganisms. However, differences in TPC and TVBN results obtained due to the method of calculating the number of microbes used are not selective against spoilage bacteria in fish, but counting all microbes contained in these fish, whereas not all microbes contained in fish are spoiled.

At the pH value fluctuations where this affects the other parameters, it can be seen that with the increasing number of bacteria the higher the pH value (more alkaline). This is due to the longer storage period, the number of bacteria will increase and produce alkaline metabolites such as TVBN and ammonia which is the result of an overhaul of chemical compounds found in meat so that the pH value will rise [18].

The longer the storage, the TVBN levels will increase and will cause the pH of the meat also increases (more alkaline). The increase in TVBN levels is caused by the activity of spoilage microorganisms that produce alkaline metabolites, causing an increase in pH in fishery products [6].

4. CONCLUSIONS AND RECOMMENDATIONS

Based on the research that has been done, it can be concluded that the response of film sensor labels in detecting freshness of tilapia fillets is characterized by a concentration of 0.05% Methyl red which shows the best response by experiencing the fastest change in label color at 7th hours observation time with a span of 10th hours during storage at room temperature (± 25ºC). There was a change in the color of the film sensor label which was originally yellow in the 0th hours to reddish-yellow at 7th hours which shows a decrease in freshness of fish. Suggestions for continuing this research that is the need to implement the application of film sensor labels to detect the freshness of fish under various conditions and storage techniques at different temperatures.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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