



Intelligent and Active Packaging System Application in Evaluating and Maintaining the Tuna (*Thunnus* Sp.) Fillets' Quality at Cold Temperature

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Abstract

This study developed intelligent and active packaging combined for tuna fillets. The purposes of this study were to determine the colour change of packaging indicator labels and the effectiveness of edible coatings (citronella oil concentrations of 0%, 0.5%, and 1%), as well as to establish a correlation between the colour analysis values of intelligent indicator and observed spoilage criteria of tuna stored at refrigerated temperatures ($4\pm 1^{\circ}\text{C}$). The parameters tested included the determination of Total Volatile Bases Nitrogen, Total Plate Count, pH, and sensory analysis. The test was carried out every three days for 18 days, with two replications. The findings indicated that the intelligent indicator label (Bromothymol blue + Methyl Red (1:1)) changed colour from deep red to yellow and then green. Edible coating with 0.5% citronella oil gave the best result in this study. The correlation between the colour analysis of the intelligent indicators and the parameters of the fish spoilage test is positive, suggesting a consistent trend pattern in determining the degree of decay in tuna fillets. Correlation coefficient values vary from 0.98 to 0.99, showing a significant correlation. The combination of intelligent and active packaging in a single packaging system allows for monitoring tuna's quality while increasing its shelf life.



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Introduction


Fish is an excellent source of protein, omega-3 long-chain unsaturated fatty acids, and micronutrients such as vitamins A, D, and B, as well as iron, zinc, selenium, and iodine.^{1,2} Fish with a variety

of nutritional qualities are an essential source of food for humans since they can meet the body's dietary needs. According to the Food Agriculture Organization (FAO), fish provides 17% of the animal protein consumed by the world's population.^{3,4}

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However, fish is a highly susceptible commodity to deterioration.⁵ Freshly caught fish undergo quality changes due to autolysis, bacterial activity, and lipid oxidation.^{6,7} This makes good handling necessary to maintain the quality of fishery products and prolong its shelf life. Thus, the nutritional value of fish is preserved until the product reaches the consumer. In general, changes in fish quality can be detected through changes in sensory attributes such as aroma, texture, and colour. However, this is limited by the ability of consumers to identify quality attributes related to the level of freshness of fish.

One technology that is able to evaluate the quality of fish during storage and make it easier for consumers to determine the level of freshness of fish is intelligent packaging. Intelligent packaging is a type of packaging technology designed to provide information about the quality level of food ingredients or products.⁵ Intelligent packaging with food quality indicators (FQI) is able to monitor the occurrence of quality degradation or product damage through colour changes that occur due to chemical or biological reactions in packaged food products. The indicator's colour change is caused by pH changes as a result of the production of volatile amine molecules.⁸ This intelligent packaging can assist reduce the risk of product damage-related loss and provide a more precise evaluation of the product's condition.^{9,10}

Applications of intelligent packaging can be integrated with active packaging that further called smart packaging. Active component in packaging might extend the food shelf life and improve food safety as well as maintain the packaged item's quality.^{6,11} Active packaging technology is created by including certain components that can control the release or absorption of certain substances and control the environmental conditions of the packaged food or food ingredients into the packaging system.¹² Manufacturing active packaging can be done in a variety of methods, including through the extrusion process, by dissolving the active component into the solvent, by adding the active component to edible coatings, or by mixing the active component with fillers in paper manufacturing.¹³⁻¹⁸ The active ingredients used could be chemicals capable of absorbing oxygen, ethylene, moisture, carbon dioxide, taste/odor, or substances capable of

releasing carbon dioxide, as well as antimicrobials, antioxidants, and flavors.^{5,19}

Active packaging and intelligent packaging play an important role as technology enables novel solutions for preserving and extending the shelf life of food products.¹² Research on intelligent packaging and active packaging separately has been applied extensively to fishery products. However, research on the application of intelligent and active packaging combined in one system is still limited, particularly for fisheries commodities with low temperatures for marketing purposes. Riyanto *et al.* (2014) utilized an intelligent indicator to determine the freshness of Double whip threadfin bream fillet (*Nemipterus nematophorus*).²⁰ Hakim *et al.* (2016) applied acetic acid and edible coating as active packaging on red tilapia fillets stored in cold temperatures ($4\pm 1^\circ\text{C}$).²¹ Yolanda *et al.* (2020) combined active and intelligent packaging on Tuna fillet stored at room temperature. Yolanda *et al.* (2020) found that with 15 % of garlic extract addition to the packaging system, the Tuna fillet can only last up to 10 hours.¹⁸ Thus, in this research, we tried to extend the shelf life of tuna fillet by applying different kinds of active compound and also with cool storage.

Based on the explanation above, this study was conducted to investigate the feasibility of combining intelligent and active packaging in a single packaging device for fisheries commodities, particularly tuna. In this study, an edible coating containing citronella oil was used as an active packaging material capable of inhibiting microbial growth and providing a selective barrier to gas and water vapor transfer. This study aimed to determine the colour change profile of intelligent indicators and the effectiveness of edible coatings as active packaging applied on tuna fish fillets, as well as to establish the correlation between the colour analysis measurements of the intelligent indicators and various parameters of the tuna fish spoilage test when stored under refrigeration at cold temperatures ($4\pm 1^\circ\text{C}$).

Materials and Methods

Materials

Fresh tuna was obtained from the Paotere fish auction in Makassar City. Other materials used were filter paper (Whatman No. 1), bromothymol blue (BTB, Arkitos), methyl red (MR, Arkitos), nutrient

agar (NA, Merck), ethanol (95%), glacial acetic acid, commercial sago starch (Alini), pure essential citronella oil (food-grade, M&H FARM), food-grade CMC (Foodchem, E466), glycerol (Merck, CAS No: 56-81-5), and aquadest.

Indicator Solutions Production

The indicator solution was made in a 1:1 ratio using methyl red (MR) and bromothymol blue (BTB) at a concentration of 0.1 % (w/v). After that, the indicator solution pH was adjusted to 2.55 using glacial acetic acid.²²

The Production of the Label of Intelligent Indicator

Uniformly, the Whatman paper no. 1 was cut to a size of 2 × 4 cm square and then immersed in 10 ml of the MR + BTB solution (1:1) pH 2.55 at room temperature (28 ± 2°C) for 12 hours. Following that, the label was then dried using an electric dryer and stored in a sealed container.²²

Edible Coating Production as Active Packaging

The edible coating is made by homogenizing sago starch with demineralized water at a 1:10 ratio. Following that, a filter cloth was used to filter the starch solution. After heating the starch solution to 55°C, 10% glycerol and citronella oil were added (with different ratios based on the treatment applied). Following that, it is swirled and heated to a temperature of 70°C. When the temperature was consistent at 70°C, the starch solution was then added with 1% CMC to create an edible coating.

Intelligent and Active Packaging Application on Tuna Fish Fillet

Fresh tuna fish was prepared in a sterile environment to obtain tuna fillets with a size of ±60g/package. Tuna fish fillets were submerged for 1 minute in the coating solution before being dried with a hairdryer. A coated tuna fish fillet was placed in a Styrofoam container. Following that, the label was placed in a plastic wrap then the plastic wrap was applied as the Styrofoam cover.

Colour Quantification of Indicator Label

The colour of the indicator label was quantified with a chromameter (Minolta CR-300); the indicator's colour is determined by attaching the chromameter to the indicator label that is placed in the package.

After that, the tool will display the values of L* (brightness), a* (chromatic colour red green), and b* (blue-yellow chromatic colour). Then, the colour value of the intelligent indicator label is obtained by calculating the hue value with the formula:

$$^{\circ}\text{Hue} = \tan^{-1} \frac{b^*}{a^*}$$

pH Measurement

Prior to pH measurements, the pH meter (HORIBA LAQUATwin) was calibrated. The pH of the sample was determined by dissolving 2 g of refined tuna fish fillet in 18 ml of demineralized water. Following homogenization, the sample's pH was determined by dropping it on the flat sensor. The pH meter will interpret the pH value of the sample, which is marked with the test complete indicator symbol on the monitor.

Total Volatile Bases Nitrogen (TVBN) Measurement

TVBN test was carried out based on SNI 2354.8:2009. 1 g of fine tuna fish was placed in a container along with 3 ml of a 7% TCA solution. The sample was homogenized for approximately 10 minutes and then filtered to obtain the filtrate. Following that, 2 ml of boric acid (H₃BO₃ 2%) was added to the inner side of the Conway cup, and 1 ml of the sample filtrate was added to the outer side. Add 1 ml of saturated K₂CO₃ solution to the outside half of the Conway dish prior to sealing. Circularly move the Conway cup until the two liquids in the outer Conway cup are combined. After 24 hours at room temperature, the colour indicator produced from a combination of methyl red and bromothymol blue (2:1) was applied in a concentration of up to 2 drops to the inner cup of Conway and then titrated with 0.02 N HCl until a pink hue developed. As with the blank, 1 ml of 7% TCA solution was substituted for the filtrate. The TVBN value was calculated using the equation:²³

$$TVB (mgN\%) = \frac{(V_{\text{sample}} - V_{\text{blank}}) \times N_{\text{HCl}} \times 14.007 \times 100}{\text{Sample mass}}$$

Explanation:

V_{sample} = HCl titration volume on the sample (ml)

V_{blank} = HCl titration volume on blank (ml)

N HCl = Normality of HCl

14.007 = atomic weight of nitrogen

Sample mass = mass of analyzed sample

The Total Bacterial Count of Tuna Fish (Tpc Method)

The TPC analysis on fish fillet samples was performed based on SNI 2331.3: 2015. 10 g mashed tuna was added to an Erlenmeyer holding 90 ml sterile physiological solution and homogenized using a vortex. The procedure was represented as the initial dilution (10⁻¹). Serial dilution was performed until 10⁻⁶. A total of 1 ml of suspension was obtained from dilutions 10⁻² to 10⁻⁶ using a sterile pipette and then placed on duplicate sterile petri dishes. Then it was added with 15 ml of warm Nutrient Agar (NA) medium and incubated for 2 x 24 hours at a temperature of 34-36°C. A petri dish containing between 30-300 colonies were counted. Additionally, the number of colonies per cup was

determined using the TPC technique in accordance with equation 11:

$$N = \frac{\sum c}{((1 \times n1) + (0.1 \times n2)) \times (d)}$$

Explanation:

- N = Number of colonies formed, colony per ml or colony per gram
- Σc = Number of colonies on all petri dishes counted
- n1 = Number of petri dishes on the first dilution counted
- n2 = Number of petri dishes on the second dilution counted
- d = The First dilution counted

Table 1: Description of the fish quality attribute values

Rated attributes	Value Description			
	0	1	2	3
Slimy	Not slimy	Slightly slimy	Slimy	Very slimy
Texture	Compact/elastic	Slightly soft	Soft	Very soft
Colour	Dark red	Red	Brown	Dark Brown/Grey
Aroma	Neutral	Slightly fishy	Fishy	Rotten fish smell
General acceptance	Like	Slightly like	Unlike	Very unlike

Sensory Analysis of Quality Index Method (QIM)

Sensory analysis of tuna fish fillets during storage with edible coating treatment and different concentrations of citronella oil was carried out by twenty untrained panelists. Attributes assessed include general appearance, texture, slime, and aroma. The rating scale for each parameter is 0-3, with the criteria of 0 being excellent quality and 3 being low quality (Table 1). The value of each attribute is the sum of the values of all its parameters. The sum of all attribute values is the total score of the Quality Index (QI).²⁵

Data Analysis

Each data obtained from this study was examined using analysis of variance (ANOVA) with two replications, and the differences between treatments were further evaluated using Duncan's test. The software used to calculate is Microsoft Excel 2010 and SPSS version 16. The correlation between the intelligent indicator label parameters is compared

with the data for each tuna fillet decay parameter through the Pearson Correlation test using SPSS version 16 software and displayed in a graph with the Sigma Plot software version 12.0.

Results and Discussion

Response to Colour Change Indicator Label

During storage of packaged tuna fish fillets at cold temperatures, there is a change in the colour of the indicator label in response to changes in the quality of packaged fish fillets. The indicator label is dark red when the tuna fillet is still fresh and continues to change colour until it turns yellow, which indicates the tuna fish fillet has started to deteriorate.

Intelligent indicator labels are expected to be able to evaluate the condition of the product through a significant change in colour (hue value) during storage. In this study, the colour change from red to green was obtained. The freshness of fish fillets can be seen from the colour on the intelligent

indicator label. The red colour indicates that the fish is in its fresh condition. Fish should be consumed immediately when the label is orange to yellow, while green indicates that the fish is rotten and no more fit for consumption.

The graph of the colour change profile of the intelligent indicator label for various edible coating treatments can be seen in Figures 1, 2, 3, and 4.

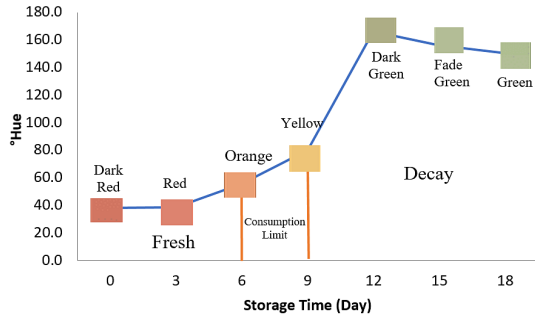


Fig. 1: Intelligent indicator label colour change profile on untreated samples

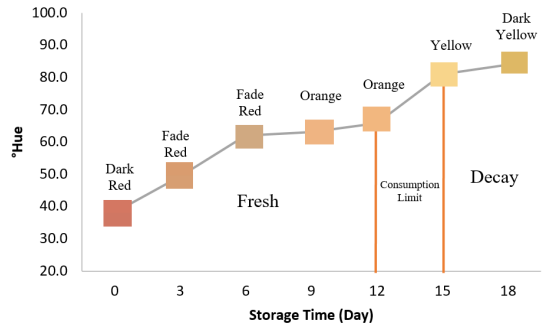


Fig. 3: Intelligent Indicator Label Colour Change Profile on Samples with Edible Coating with the Addition of Citronella Oil 0.5%

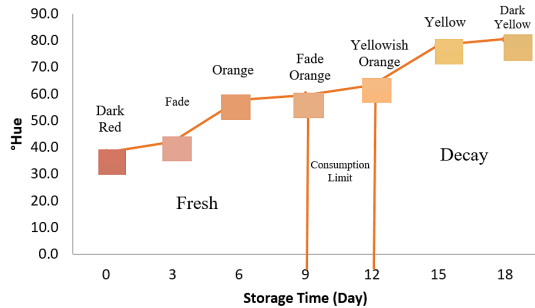


Fig. 2: Intelligent Indicator Label Colour Change Profile on Samples with Edible Coating without Citronella Oil

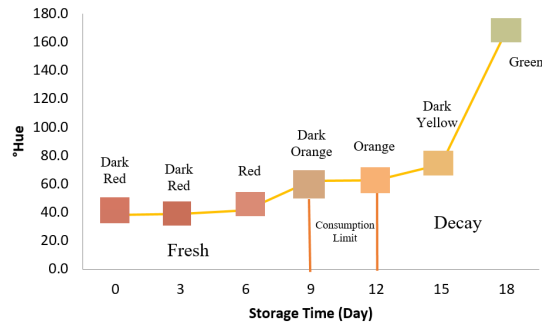


Fig. 4: Intelligent Indicator Label Colour Change Profile on Samples with Edible Coating with the Addition of Citronella Oil 1%

Table 2: Hue Value (°) of Intelligent Indicator Label

Storage time (day)	Treatment				Average
	Untreated	Edible coating without citronella oil	Edible coating + citronella oil 0.5%	Edible coating + citronella oil 1%	
0	38.20 ± 0.28	38.20 ± 0.28	38.20 ± 0.28	38.20 ± 0.28	38.20 ± 0.00 ^a
3	38.70 ± 0.04	42.20 ± 0.15	49.30 ± 0.04	39.00 ± 1.00	42.30 ± 4.95 ^b
6	55.60 ± 0.08	57.80 ± 0.03	62.00 ± 0.03	41.70 ± 0.10	54.27 ± 9.10 ^c
9	80.00 ± 0.03	59.70 ± 0.10	63.10 ± 0.16	62.20 ± 0.06	66.25 ± 9.14 ^d
12	166.30 ± 0.42	63.30 ± 0.04	65.80 ± 0.04	62.80 ± 0.08	89.55 ± 51.18 ^e
15	156.00 ± 0.64	78.50 ± 0.71	81.10 ± 1.41	73.80 ± 0.01	97.36 ± 39.24 ^f
18	150.60 ± 0.42	80.60 ± 0.28	84.00 ± 5.66	168.60 ± 0.08	120.95 ± 45.06 ^g
Average	97.91 ± 57.77 ^D	60.04 ± 16.20 ^A	63.35 ± 16.22 ^B	69.47 ± 45.73 ^C	

The mean value with different superscript letters indicated a significant difference based on Duncan's test at the 5% level ($p < 0.05$).

Colour measurement in this study uses a chromameter tool that is able to express colour in numerical form. The resulting data is expressed as the value of L*, a*, and b*. These three values are international standards for colour measurement.²⁶ According to the results obtained, the values of L, a, and b will be converted to hue values. Hue value is a hue that could be captured by the human senses so that it gives a difference from one colour to another. The hue value is a figure of the 360° axis, which is divided into four quadrants. Each quadrant represents a colour division consisting of red-yellow in quadrant I, yellow-green in quadrant II, blue-green in quadrant III, and blue-purple in quadrant IV.²⁷ The Hue value of the intelligent indicator label is presented in Table 2.

The intelligent indicator label Hue rises during storage, as seen in Table 2. On day 0 of the experiment, the Hue value of intelligent indicator on average is 38.20°, which is in the red colour spectrum. After 18 days of storage, there was a noticeable changing colour in the intelligent indicator with an average hue value of 120.95°. From table 2, it can be noticed that after 18 days of storage, the change in the intelligent indicator with the application of edible coating without the adding of citronella oil and sample with adding of 0.5% citronella oil is lower with Hue values of 80.6° and 84°, respectively which were in the yellow colour range, compared to the intelligent indicator without edible coating (150.60°)

and with 1 % citronella oil (168.60°), which were in the green colour range. In the average it can be seen that the administration of different treatments showed a significant difference in the Hue level of the fish.

Changes in hue value of intelligent indicator labels during the storage period of tuna fish fillets were caused by changes in environmental conditions in the packaging with increased volatile base compounds produced by microbes and enzymes that decompose fish protein. The volatile base compounds formed will accumulate in the packaging and cause the pH in the packaging to increase that further affecting the colour of intelligent indicator. This is in accordance with the statement of Alasalvar (2002),²⁸ which states that the results of protein decomposition by bacterial and enzyme activities produce up to 95% ammonia and CO₂. The compounds formed from the protein degradation contain nitrogen and are basic and volatile (volatile bases) which are stated as Total Volatile Bases Nitrogen (TVBN). The colour changes that occurs after 18 days of storage indicated that sample treated without the addition of citronella oil and with 0.5% of citronella oil deteriorated less than other two samples (untreated and treated with 1% citronella oil). Based on the explanation, it can be suggested that edible coating without citronella oil and with 0.5% of citronella oil gives the best effect in slowing the decay rate of the product.

Table 3: pH Value of Tuna Fillet

Storage time (day)	Treatment				Average
	Untreated	Edible coating without citronella oil	Edible coating + citronella oil 0.5%	Edible coating + citronella oil 1%	
0	5.84 ± 0.20	5.84 ± 0.20	5.84 ± 0.20	5.84 ± 0.20	5.84 ± 0.00 ^a
3	6.38 ± 0.11	6.36 ± 0.06	6.38 ± 0.01	6.35 ± 0.07	6.37 ± 0.01 ^b
6	6.73 ± 0.04	6.77 ± 0.05	6.29 ± 0.01	6.28 ± 0.11	6.52 ± 0.27 ^c
9	7.99 ± 0.39	7.07 ± 0.10	7.01 ± 0.05	6.71 ± 0.02	7.20 ± 0.55 ^d
12	8.04 ± 0.04	7.29 ± 0.01	6.98 ± 0.06	7.74 ± 0.07	7.51 ± 0.49 ^e
15	8.17 ± 0.01	7.70 ± 0.99	7.33 ± 0.04	7.96 ± 0.03	7.79 ± 0.36 ^f
18	8.41 ± 0.01	8.05 ± 0.05	7.74 ± 0.04	8.11 ± 0.16	8.08 ± 0.27 ^g
Average	7.37 ± 1.02 ^C	7.01 ± 0.76 ^B	6.80 ± 0.66 ^A	7.00 ± 0.92 ^B	

The mean value with different superscript letters indicated a significant difference based on Duncan's test at the 5% level ($p < 0.05$).

pH

The tuna fish fillets pH is one of the indicators applied to determine tuna quality. The changes in the pH value of tuna fish fillets during storage are presented in Table 3.

As can be seen in table 3, the pH value of tuna fish fillets rises along with the storage duration. In average, it started with 5.84 in day 0 and continue to rise to 8.08 in day 18 of storage.

Furthermore, it was observed that an increase occurred in all the applied treatments. After 18 days of storage, it can be depicted that the pH in the sample treated with the addition of 0.5% citronella oil (7.74) and edible coating without the addition of citronella oil (8.05) was lower than that of the treatment without the use of edible coating (8.41), and with the addition of 1% citronella oil (8.11).

The difference in elevating the pH of fish fillets in various treatments with varying quantities of citronella oil on edible coatings is due to citronella oil antimicrobials inhibiting microbial development at varying concentrations. Because the pH value is proportional to the total number of bacteria present in fish, the antimicrobial activity of citronella oil applied to edible coatings might indirectly slow the rate of pH increase in tuna fish during storage. It was expected the pH would increase in positive correlation with the increase of citronella oil applied

in packaging. However, the pH value obtained in this study indicated that adding 0.5 % citronella oil was more effective at slowing the rate of pH increase compared to adding 1% citronella oil. The inadequate suppression of 1 % citronella oil in edible coatings compared to 0.5 % and 0 % citronella oil is assumed to be due to the essential oil's ability to boost the growth of other undesirable bacteria. This is consistent with the statement of Davidson *et al.* (2005)²⁹ that although essential oils can eliminate certain bacterial populations, they can also create a favorable environment and encourage the growth of other undesirable microorganisms due to changes in microbial ecology. Besides that, according to Bagamboula *et al.* (2004)³⁰ the use of essential oils as antimicrobials in foodstuffs can potentially eliminate the antimicrobial effect due to their volatile and lipophilic properties. The data from table 3 also suggests that the treatment of edible coating without citronella oil and with 0.5% of citronella oil give the best effect in slowing the decay rate of the product. It can be concluded that the treatment with the application of 0.5% citronella oil keep the pH lower significantly compared to the other treatments.

Total Bacterial Count in Tuna Fish (TPC Method)

The TPC value of tuna fillet is one of the quality indicators used to evaluate the total bacteria contained in the seafood. Table 4 depicts the change in the TPC value of tuna fish fillets during storage.

Table 4: Total Bacterial Count (TPC, log CFU/mL) of Tuna Fish

Storage time (day)	Treatment				Average
	Untreated	Edible coating without citronella oil	Edible coating + citronella oil 0.5%	Edible coating + citronella oil 1%	
0	3.31 ± 0.04	3.31 ± 0.04	3.31 ± 0.04	3.31 ± 0.04	3.31 ± 0.00 ^a
3	3.89 ± 0.24	3.52 ± 0.71	3.41 ± 0.11	3.40 ± 0.18	3.56 ± 0.24 ^b
6	5.47 ± 0.64	4.82 ± 0.42	4.15 ± 0.14	4.61 ± 0.28	4.76 ± 0.50 ^c
9	5.87 ± 0.03	4.98 ± 0.03	4.25 ± 0.28	4.72 ± 0.14	4.96 ± 0.67 ^d
12	6.66 ± 0.08	5.71 ± 0.08	5.10 ± 0.71	6.48 ± 0.03	5.99 ± 0.72 ^e
15	7.96 ± 0.18	7.76 ± 0.09	6.42 ± 0.04	7.96 ± 0.02	7.53 ± 0.74 ^f
18	8.20 ± 0.28	8.07 ± 0.12	7.48 ± 0.07	8.12 ± 0.08	7.97 ± 0.34 ^g
Average	5.91 ± 1.87 ^D	5.45 ± 1.88 ^B	4.87 ± 1.57 ^A	5.51 ± 2.04 ^C	

The mean value with different superscript letters indicated a significant difference based on Duncan's test at the 5% level ($p < 0.05$).

The addition of citronella oil at various doses has an effect on increasing the TPC value during storage, as demonstrated by quantitative data on TPC changes during storage. As can be seen on day 0, tuna fish fillets had a TPC value of log 3.31 (2.1×10^3 CFU/ml) that made it categorized as fresh product (SNI 2729:2013 set that the limit for the number of TPC microbes in fish is 5×10^5 CFU/g or log 5.70³¹). Referred to the standard set by SNI, it can be seen that the fish product in average remained fresh until the 9 days of storage. However, individually, the results of this data indicates that tuna fish fillets no longer meet the feasibility of consumption on the 9th day for untreated sample, 12th day storage for sample with treatment of edible coating without the addition of citronella and 15 days with the application of 0.5% citronella oil and 12 days with the application of 1% citronella oil.

The variation in the increase in TPC number of fish fillets in various treatments with varying concentrations of citronella oil on the edible coating was due to the variable suppression of microbial growth by citronella oil antimicrobials at various concentrations. Various studies on the antibacterial activity of citronella oil against various microorganisms have been reported. Silveira *et al.* (2012) said that citronella oil was more active than other essential oils against 12 types of microorganisms.³² Some of them are microbes that cause spoilage in fish, such as *Escherichia coli*, *Bacillus cereus*, *Pseudomonas aeruginosa* and

Enterobacter aerogenes. Naik *et al.* (2010) also reported that citronella oil was effective against gram-negative and gram-positive bacteria.³³ The mechanism of antibacterial action in citronella oil is the breakdown of the phospholipid bilayer, which results in the loss of cellular components enzymes and the destruction of genetic material.³⁴ According to Moore-Niebel *et al.* (2012),³⁵ Citronella oil acts as an antibacterial agent by limiting the production of biofilms, eliminating existing biofilms, and specifically targeting bacterial cells.

The explanation in previous paragraph lead to expectation that the concentration of the citronella oil applied should have shown positive correlation with the length of the product remain fresh and is expected to be inversely proportional to the TPC value. However, the data shows that administration of 0.5% citronella oil kept the product fresh longer than the application of 1% of citronella oil. This is suspected as the effect of essential oil that stimulates the growth of other undesired microorganisms. This is consistent with the statement of Davidson *et al.* (2005) 29 that although essential oils can eliminate certain bacterial populations, they can also create a favorable environment and encourage the growth of other undesirable microorganisms due to changes in microbial ecology. This explanation indicated that varying amounts of citronella oil have an influence on the TPC value of tuna fish fillets during storage and that the 0.5% keep the fish fresh longer compared to other treatments.

Table 5: Total Volatile Bases Nitrogen (TVBN, mg N/100 gram) Value of Tuna Fillet

Storage time (day)	Treatment				Average
	Untreated	Edible coating without citronella oil	Edible coating + citronella oil 0.5%	Edible coating + citronella oil 1%	
0	2.71 ± 0.14	2.71 ± 0.14	2.71 ± 0.14	2.71 ± 0.14	2.71 ± 0.00 ^a
3	5.32 ± 0.03	4.76 ± 0.40	5.32 ± 0.01	3.64 ± 0.06	4.76 ± 0.79 ^a
6	6.16 ± 0.08	5.32 ± 0.40	6.44 ± 0.40	4.62 ± 0.59	5.64 ± 0.83 ^a
9	18.55 ± 5.44	16.45 ± 0.49	14.35 ± 1.48	12.95 ± 5.44	15.58 ± 2.45 ^b
12	77.35 ± 12.37	35.00 ± 0.99	19.60 ± 14.85	42.35 ± 2.47	43.58 ± 24.43 ^c
15	90.16 ± 10.37	40.04 ± 9.50	33.04 ± 3.17	47.18 ± 0.59	52.61 ± 25.69 ^d
18	124.88 ± 5.94	77.84 ± 4.75	62.72 ± 0.79	76.72 ± 2.38	85.54 ± 27.11 ^e
Average	46.45 ± 50.4 ^c	26.02 ± 27.32 ^b	20.60 ± 21.30 ^a	27.17 ± 28.72 ^b	

The mean value with different superscript letters indicated a significant difference based on Duncan's test at the 5% level ($p < 0.05$).

Total Volatile Bases Nitrogen (TVBN)

The Total Volatile Basic Nitrogen value of tuna fillet is used to determine the quality of tuna by evaluating the amount of volatile compounds generated by microorganisms. Table 5 illustrates the change in the TVBN value of tuna fish fillets during storage.

According to the table 6, the treatment without edible coating resulted in a rise in total QI, while the treatment with different doses of citronella oil had varying outcomes but tended to increase. According to quantitative data, tuna fillets treated

without the addition of edible coating were rejected by panelists on the 12th day of storage, but samples with the addition of citronella oil were rejected by the panelists on the 18th day of storage with the criteria of very slimy, very soft texture, foul-smelling, and generally very disliked by the panelists (score 15). The ANOVA test was used to assess if there was a significant difference between the addition of citronella oil at various concentrations and changes in the sensory analysis value (QI value) of tuna fish fillet during storage.

Table 6: Quality Index (QI) Value of Tuna Fillet

Storage time (day)	Treatment				Average
	Untreated	Edible coating without citronella oil	Edible coating + citronella oil 0.5%	Edible coating + citronella oil 1%	
0	5.10 ± 0.64	5.10 ± 0.64	5.10 ± 0.64	5.10 ± 0.64	5.10 ± 0.00 ^a
3	5.21 ± 0.44	6.21 ± 0.10	4.71 ± 0.67	4.45 ± 1.11	5.15 ± 0.78 ^a
6	7.23 ± 0.32	4.98 ± 1.66	6.75 ± 0.07	5.65 ± 0.42	6.15 ± 1.02 ^b
9	7.23 ± 0.32	7.58 ± 0.60	7.18 ± 0.67	5.23 ± 0.53	6.80 ± 1.06 ^b
12	15.00 ± 0.00	7.91 ± 0.39	6.41 ± 0.42	7.07 ± 1.63	9.10 ± 3.99 ^c
15	15.00 ± 0.00	5.03 ± 0.11	9.20 ± 0.21	7.55 ± 0.92	9.19 ± 4.23 ^c
18	15.00 ± 0.00	15.00 ± 0.00	15.00 ± 0.00	15.00 ± 0.00	15.00 ± 0.00 ^d
Average	9.97 ± 4.78 ^c	7.40 ± 3.57 ^{AB}	7.76 ± 3.52 ^B	7.15 ± 3.63 ^A	

The mean value with different superscript letters indicated a significant difference based on Duncan's test at the 5% level ($p < 0.05$).

The variation in the rise in QI value was due to the addition of citronella oil in various quantities is because citronella oil and an edible coating added to tuna fillets have antibacterial properties.³⁹ The sensory quality attributes tested are related to the TPC value of tuna fish fillets. The greater the TPC value of the tuna fillet, the worse the fish's quality, which has an influence on the sensory characteristics evaluated. The findings of this study indicate that the addition of 1% citronella oil treatment received better sensory ratings compared to other treatments based on the panelist's assessment. This suggests that citronella oil has a positive effect on the sensory value of tuna fillets as stated by Preedy (2016), that essential oils added to edible films can improve the sensory properties of food.³⁸ However, it was observed that the TPC values were higher in the addition of 1% citronella compared to the 0.5% citronella oil treatment; thus this study suggested

addition of 0.5% citronella oil is recommended for meat preservation.

Correlation between the value of the intelligent indicator's colour analysis and several parameters of tuna fish spoilage

The correlation between changes colour measurement of the indicator and the decay parameter of tuna fish fillet quality is presented in Figures 5, 6, 7, and 8.

Based on the figure 8 the pattern of the relationship between changes in the colour of the intelligent indicator label and all parameters of tuna fillet spoilage on average experienced the same pattern of improvement in detecting changes in tuna fillet spoilage. The correlation value between colour change and fish spoilage parameters (TPC, TVBN,

pH, and sensory analysis) was analyzed through multiple correlation tests to determine the level of strength and direction of the linear relationship of two or more independent variables to the dependent variable. Significance of the correlation it is based on the value of the correlation coefficient including

0.00-0.20 (negligible), 0.21-0.40 (low), 0.41-0.70 (moderate), 0.71-0.90 (high), 0.91-0.99 (very high), and 1.00 (perfect) 40. The results of the multiple correlations between indicator colour changes and tuna spoilage parameters can be seen in table 7.

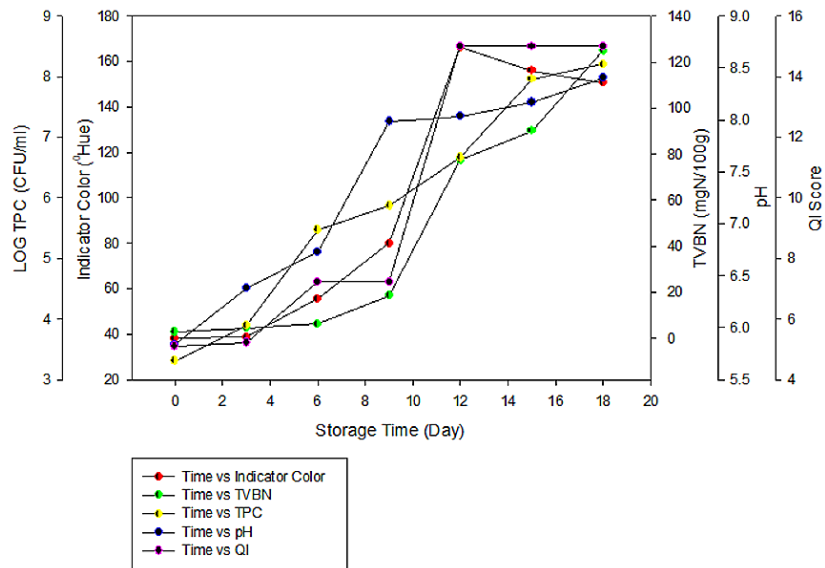


Fig. 5: Correlation of Colour Changes on Indicator Labels with All Spoilage Criteria in untreated samples

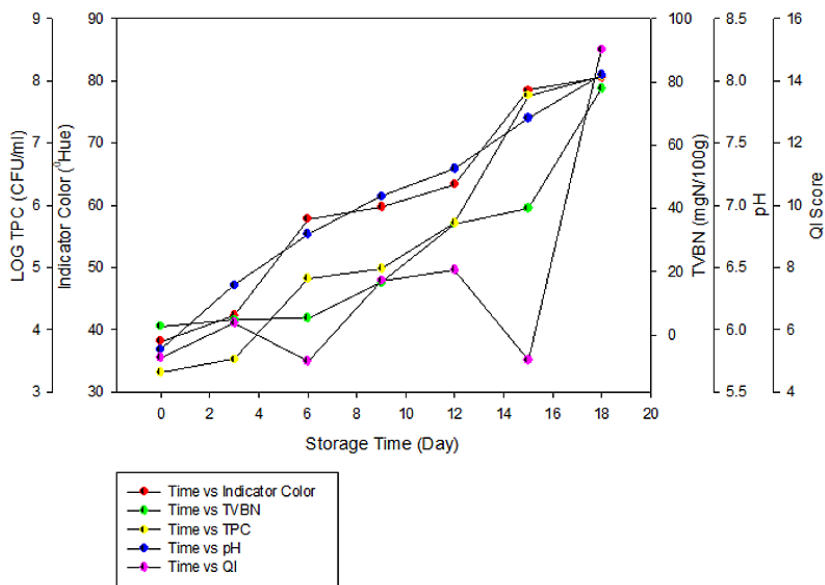


Fig. 6: Correlation of Colour Changes on Intelligent Indicator Labels with All Spoilage Parameters with the Use of Edible Coating without Citronella Oil

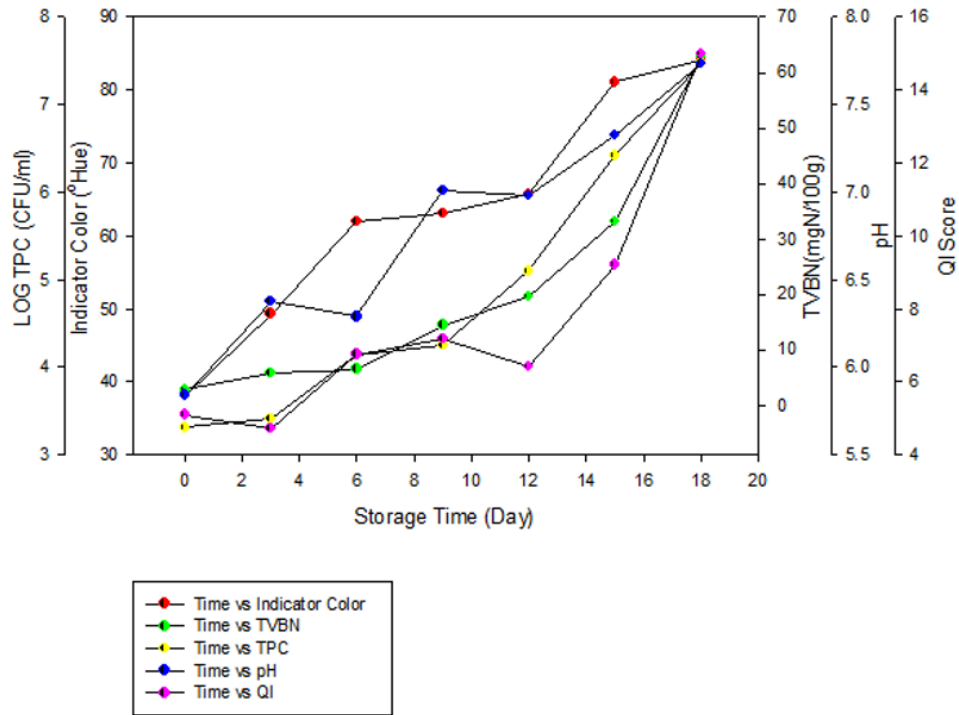


Fig. 7: Correlation of Colour Changes on Intelligent Indicator Labels with All Spoilage Parameters with the Use of Edible Coating contained citronella Oil 0.5 %

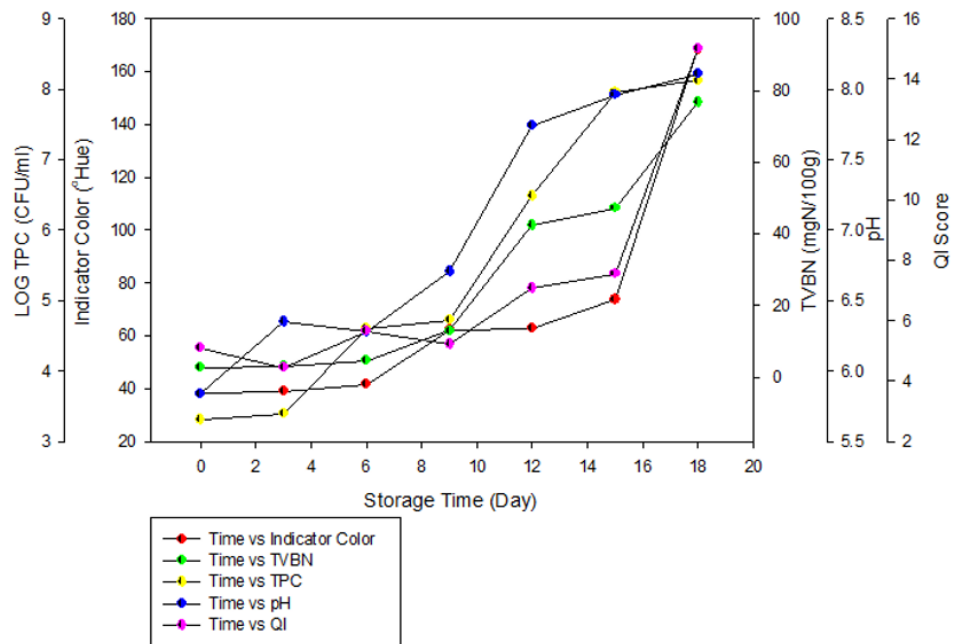


Fig. 8: Correlation of Colour Changes on Intelligent Indicator Labels with All Spoilage Parameters with the Use of Edible Coating contained citronella Oil 1%

Table 7: The correlation coefficient between the colour change and fish spoilage parameters

Treatments	Correlation coefficient	Significance value
Untreated	0.999	0.003
Edible coating without Citronella oil	0.998	0.007
Edible coating contained 0.5% Citronella oil	0.992	0.032
Edible coating contained 1% Citronella oil	0.986	0.056

The significance value < 0.05 indicates a significant relationship, the significance value > 0.05 indicates that there is no significant relationship, so there is a strong (very high) relationship or correlation between indicator colour changes and tuna fillet spoilage parameters

Conclusions

Through this research, it can be concluded that the smart packaging system employing intelligent packaging indicator label of Metyl Red + Bromothymol blue (1:1) can be applied to evaluate the tuna fish fillets quality marked by a change in colour from dark red to yellow, then green. And a 0.5% concentration of citronella oil addition to edible coating maintains the quality of tuna fish fillets best among other concentrations applied with a maximum storage period of 15 days as it is more effective in inhibiting microbial growth, which is the main parameter causing spoilage of fishery products. The level of correlation between the colour analysis value of the intelligent indicator and the effectiveness of edible coating on various

fish spoilage test parameters shows a positive correlation which shows the linear trend pattern in detecting the level of the spoilage of tuna fish fillets. The correlation coefficient value ranges from 0.98-0.99, which indicates a strong relationship.

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Conflicts of Interest

The authors declare no conflict of interest.

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