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To cite this article: A H Julyaningsih et al 2020 IOP Conf. Ser.: Earth Environ. Sci. 486 012053

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The making of smart and active packaging on tuna fillet

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Abstract. Indicator label were made by immobilizing indicator solution on Whatman paper with treatments A1: Methyl Red/MR (pH 4.40), A2: Methyl Red/MR (pH 2.20), A3: Bromothymol Blue/BTB (pH 5.80), A4 : Bromothymol Blue/BTB (pH 2.90), A5 : Methyl Red + Bromothymol Blue / MR+BTB (1:1) (pH5.10), A6 : Methyl Red + Bromothymol Blue / MR+BTB (1:1) (pH 2.55). Whatman papers were immersed in the indicator solutions for 24 hours, dried then glued on the plastic cover of tuna fillet packaging. The color of each paper was observed every day. The effectiveness of the label indicator was evaluated by observing the color change of the indicator label of tuna fillet packaging. Edible coatings were made from sago starch with the addition of 0%, 0.5%, and 1% lemongrass oil. Tuna fillet was immersed in the coating solution for one minute then dried. The edible coating was analyzed every 3 days for 18 days using biological analysis (TPC), physical analysis (color, weight, and organoleptic) and chemical analysis (the value of TVBN, pH, and TBA) to assess the effectiveness of edible coating on maintaining tuna fish fillet quality. The results indicate that the best indicator solution for indicator label was a solution of Methyl Red + Bromothymol Blue (1:1) (pH 2.55). This indicator solution was the most sensible solution in showing the color change as the result of the tuna fillet quality degradation. The best edible coating treatment, when applied on fillet tuna, was edible coating treatment with the addition of 0.5% lemongrass oil.

1. Introduction

Storage in cold or freezing temperatures is one of the most common methods used in handling postharvest tuna. This method is considered quite effective in suppressing the rotten rate of fish by inhibiting the growth of spoilage microorganisms. However, storage in low temperatures is still not optimal in extending the shelf life of fish due to the activity of microorganisms and enzymes that naturally occur in fish tissue will continue to degrade the muscle protein so the fish is spoil. Moreover, tuna fish which is sold in the modern market cannot be checked directly for its freshness level by the 4M method (seeing, touching, pressing and smelling) [1] due to the presence of packaging. The shelf life label on a package is considered less able to interpret the packaged fish freshness.

Smart packaging has been studied as one of the technologies in interpreting the condition of packaging products. This packaging is able to provide information about the product conditions both in the transportation process and storage. One smart packaging type is a food quality indicator that identifies the changing color of indicators which is occurred due to the decline of food products quality packaged due to the spoilage process.

Food quality indicators on food product packaging that experience a pH change during the quality decreasing process can be made by immobilizing indicator solutions in Whatman paper. The pH value

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of fish will increase with the increasing ammonia levels of fish flesh due to protein degradation by spoilage bacteria [2]. This change in pH can be detected by the indicator label by showing the color change according to the fish quality condition packaged

The edible coating is a thin layer that is spread evenly on the surface of the food and is edible or safe for consumption. The edible coating serves as a barrier that is able to withstand mass transfer such as moisture, oxygen, lipids or as a food additives carrier that can preserve the food (antimicrobial) [3]. The edible coatings can be made from hydrocolloid materials that have a hydrophilic polymer consisting of many hydroxyl groups which are able to form gels when treated with water. One of the materials that can be developed as the edible coating is starch and one of the starch sources which has great potential in Indonesia is sago starch.

The edible coating can also be used as a carrier for preservative food additives, one of them is antimicrobial substances. Natural antimicrobial substances can be found in some commodity spices in Indonesia such as lemongrass, cloves, cinnamon, ginger, turmeric, pepper, garlic, and others. One of the commodity spices that have the potential as a naturally antimicrobial substance is lemongrass. Lemongrass contains active citronellal, geraniol and citronellol components which can be used as antibacterial and antifungal [4–6]

Edible coating with the addition of lemongrass oil to inhibit the fish spoilage rate is one of the applications of active packaging which is used as an alternative method for extending the shelf life of food products and for touching the character of smart packaging, it can be done by applying color sensor indicators that can interpret the condition of fish freshness which is packed both during the storage process and distribution process.

This research was occurred to make an indicator label that was able to interpret the quality of tuna fillets in packs as a smart packaging function and determined the best edible coating treatment applied to tuna fillets as an active packaging function.

2. Materials and methods

2.1. Materials

The Tuna fish were purchased from a local market (Potere market) in Makassar, Sulawesi Selatan and transported to the library in a dead state and immediately cleaned and stored in a refrigerator at 4°C. The methyl red (MR) and bromothymol blue (BRB) indicator solutions were used to determine the treatment of the best indicator solution in label paper making. Commercial sago starch, glycerol, and CMC are used to make coating formulations. Commercial lemongrass oil is added as an anti-microbial.

2.2. Preparation of the color indicator labels

Indicator labels were made with the following various treatments [7].

- A1: Methyl Red/MR (pH 4.40)
- A2: Methyl Red/MR (pH 2.20)
- A3: *Bromothymol Blue*/BTB (pH 5.80)
- A4: *Bromothymol Blue*/BTB (pH 2.90)
- A5: Methyl Red + Bromothymol Blue / MR+BTB (1:1) (pH5.10)

A6: Methyl Red + Bromothymol Blue / MR+BTB (1:1) (pH 2.55)

 $2x 4 \text{ cm}^2$ (Whatman) filter paper was immersed in an indicator solution at room temperature for 12 hours and dried using a hairdryer, the same method used by Hidayat et al., 2019 [8]. The successful immobilization process was shown by a change in color of the filter paper similar to the color of the original indicator solution.

2.3. Preparation of the coating- forming solutions and treatments Tuna fillet was immersed in the coating in the following formulation [9].

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B0: untreated

- B1: Sagoo starch + water (1:10)/ glycerol 10%
- B2: Sagoo starch + water (1:10)/ glycerol 10% and lemongrass oil 0.5%
- B3: Sagoo starch + water (1:10)/ glycerol 10% and lemongrass oil 1%

The coating solution prepared by mixing 50 grams of sago starch in 500 ml of distilled water added glycerol and citronella oil according to the treatment then heated while stirring at 55°C for 20 minutes. 1% CMC was added to the starch solution when the temperature was maintained at 70°C to produce a coating solution. After the coating solution was cooled at room temperature, tuna fish fillets were immersed in the coating solution for 1 minute then aerated until dry in the drying box. After that, the tuna fillet was packed in styrofoam, covered with clingwrap plastic and stored at $4 \pm 1^{\circ}$ C for 18 days

2.4. Microbiological analysis

The microbiological analysis method which was used was the Total Plate Count. One gram of tuna fillet sample was crushed and put into 9 ml of diluent solution. Then it was shaken until homogeneous with a vortex. Dilution and homogenization did until the 10^{-5} dilution rate. From each dilution, sample piped aseptically 1 mL to be put into sterile Petri dishes (fertilizing) in duplicate and added sterile sodium agar media (NA) as much as 15-20 ml. Immediately after pouring, the petri dish was moved on the table carefully to spread the microbial cells evenly, i.e. with a circular or number eight motion. After the media was cooled, the petri dish was incubated upside down in an incubator at 37 ° C for 2x24 hours. Microbiological data were transformed into logarithms of the number of colony-forming units (CFU/g). All treatments were performed three times.

2.5. Physical Analysis

2.5.1. Tuna Fillet Flesh Color. The surface color of tuna fillets before and after storage was measured by Minolta CR-300 chromameter. The scale used was scale L* (brightness), a* (chromatic color redgreen), and b* (blue-yellow chromatic color). Testing was done by putting sensors to tuna fillets and firing rays on two different parts [10]. Measuring was done three times for each section. Then the obtained data were averaged. All treatments were performed three times.

2.5.2. *Water loss Analysis.* Measurements of weight loss were defined through gravimetrically, ie comparing the difference in weight before storage with after storage.

2.5.3. Sensory Evaluation. Organoleptic tests were evaluated using the Quality Index Method (QIM) [11] by 20 semi-trained panelists. This test was done one day after the coating process. The tested parameters were included mucus, texture, color, smell, and general acceptance. The QIM test used a score scale of 0 - 3, where a score of 0 represented the best quality and a higher score represented the poor quality. Then the score for each parameter was added to get the overall score. This method gave a score of 0 (close to 0) for very fresh fish while a higher score indicates a bad fish.

Quality Parameters	Description points			
	0	1	2	3
Mucus	Absent	Slight	Moderate	Moderate or sticky
Texture	Firm or elastic	Slightly soft	Soft	Very soft
Smell	Neutral	Fishy	Stale	Spoiled
General acceptance	Like	Slightly Like	Dislike	Very dislike

Table 1. Description points of quality parameters

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2.6. Chemical analysis

2.6.1. Determination of total volatile basic nitrogen (TVB-N). The mashed tuna fillet sample was weighed as much as 2 g. Then the sample was put into a blender and added 75 ml of 7% TCA solution and mashed for 1 minute. The samples were filtered and tested for TVB-N levels. 1 ml of boric acid was poured into the inner chamber of the Conway dish, then the sample filtrate was poured into the outside of the Conway dish. The Conway dish was closed, then 1 ml of K₂CO₃ solution was added to the outside chamber. For blank, the filtrate was replaced with 5% TCA solution. Incubate the sample at 35° C for 2 hours. After incubating, the solution in Conway inner chamber, both blank and sample, titrated with 0.02 N HCl until it turned pink like blank's solution.

2.6.2. Determination of pH. Measurements were identified by using a 2-star Ori-pH pH meter, an MWW (101) model, USA. Before being used the ph meter was calibrated with a pH 7 buffer and pH buffer 4. 5 grams of sample was mashed and added with 50 ml of distilled water and stirred until evenly distributed. The pH value was measured by placing the sample on the ph meter sensor, and the pH value was seen on the pH-Meter screen.

2.6.3. Determination of Thio Barbituric Acid (TBA) value. The tuna fish fillet was weighed about 3g, put in a waring blender, added 50 ml of distilled water and mashed for 2 minutes. The sample was transferred quantitatively into a 1000 ml distillation flask while washing with 48.5 ml of distilled water and then added with 1.5 ml approximately 4 N HCL. The distillation set was run with the highest possible heating so that 50 ml of distillate was obtained for 10 minutes of heating. The obtained distillate was filtered and added 5 ml of TBA reagent: a solution of 0.02M thiobarbituric-acid in 90% glacial acetic acid. The dissolution process was accelerated by heating the distillate with the water bath. Then, the distillate was cooled with cold water, the Optical Density was read with a spectrophotometer at a wavelength of 528 nm with a blank solution as a zero point.

2.7. Data analysis

All analyses were run in triplicate (except microbiological analysis were performed in duplicate) All data were processed by analysis of variance (ANOVA) and tested further by Duncan test where significant differences in treatment were characterized by P < 0.05).

3. Results and discussion

3.1. The color indicator labels

Determining the best label indicators treatment was done qualitatively through its visual assessment of the indicator label color change which had been immobilized on Whatman paper along with the declining quality of the untreated tuna in the package.



Figure 1. The color change on indicator label in every treatment

Based on figure 1, treatments A1 and A6 were the most sensitive treatments that undergo the color change along with the declining quality of tuna fillets in the packaging. The A6 treatment was chosen as the best treatment even though the A1 treatment was also able to change its color along with the declining quality of the tuna fillet, but the A6 treatment was able to detect a greater pH range than A1 did due to the mixing of 2 different indicator solutions types. The A6 treatment produced an indicator label that was able to detect the pH range until it passed pH 7.6 so the label indicator changed its color from red to yellow and then blue.

Ammonia is a base protein metabolic result substance (above pH 7) so that the pH of fish increases. Methyl Red is a weak organic acid indicator solution that has a pH scale of 4.2-6.3 which will change color from red to yellow when in a base environment. Bromothymol Blue is a weak organic acid indicator solution with pH scale of 6 - 7.6. Bromothymol Blue will change color from yellow to blue in a base environment [12].

3.2. Microbiological analysis

Changes in the total microbial amount of various tuna fillet treatments can be seen in Figureure 2. The total microbial count of tuna fillets on day 0 was 5.7 log CFU / g which according to the literature reports that the bacteria counts of different freshwater fish species are between 2 and 6 log CFU / g [13]. The TPC threshold for fish products is 10-5 while the entire treatment sample had a value of TPC 10° on day 0. This was because of the fish used in this study were fish obtained from fishermen who had been stored a few moments after harvesting. So that tuna fillet was not recommended to be consumed in a raw form such as sushi. TPC is also a method in determining the total number of all types of bacteria that live in fillets not specific to gram-negative protei decomposing bacteria which are the main factors of spoilage to a fish [14].



Figure 2. Total plate count of tuna fillet during storage 4-C (B0: untreated; B1: edible coating+ lemongrass oil 0%; B2: edible coating + lemongrass oil 0.5%; B3: edible coating + lemongrass oil 1%)

Figure 2 showed an increase in the total number of microorganisms during the storage of tuna fish fillets on the control treatment, the use of edible coatings treatment and edible coating with the addition of lemongrass oil 0.5% and 1% treatments. The graph also showed that the total number of bacteria in the control treatment had the largest total number of bacteria compared to the total number of bacteria with the addition of edible coating treatments until the 9th day (P <0.05) and did not differ significantly when reaching the 12th day (P>0.05).

The three treatments of edible coating on tuna fish fillets could inhibit and even killed the decomposing bacteria that live there. The polysaccharide of sago starch which was the main ingredient in the edible coating could reduce the rate of oxidation and hydrolysis, especially in fishery products. The presence of oxygen is needed in some types of decomposing bacteria that are obligate aerobics to stay alive, for example, *Pseudomonas sp* bacteria. So that the presence of edible coating will reduce oxygen exposure from the environment which will reduce the growth rate of obligate aerobe bacteria. The edible coating is also able to reduce the rate of hydrolysis because water that can be obtained from the air in the environment will be hampered due to the polysaccharide layer of sago starch so the hydrolysis process will be inhibited [15]. Then the addition of lemongrass oil can reduce the growth rate of decomposing bacteria. Lemongrass oil is one type of natural anti-microbial that can kill microbes, mainly bacteria and fungi [16]. The essential oil in citronella oil is able to remove ions in cells, change cell permeability, block the process of proton pumps and reduce ATP products in bacterial cells [17].

The antimicrobial effectiveness of lemongrass oil on tuna edible fillet coating only worked effectively in the lag phase and exponential phase of bacterial growth, when reaching the stationary phase, the treatment of edible coating and the addition of citronella oil were not too different from the control treatment. The presence of lemongrass oil containing citral and prosthetic groups of metal ions such as Hg^{2+} , $CO_2 + and Ba^{2+}$ can be a competitive inhibitor for extracellular protease enzymes in degrading proteins so the bacterial protein metabolic activity is also reduced and causes bacterial growth decrease [18]. In the stationary and death phase, the amount of protein has decreased dramatically so that the food source of the bacteria has also been reduced. So, the effectiveness of lemongrass oil antibacterial activity also decreases.

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3.3. Physical analysis

3.3.1. Tuna filet flesh color. L * a * b * coordinates were used to determine the location of colors in a color diagram. L * or commonly called Lightness related to product brightness. a * is the coordinates for red/green and b * is the yellow or blue color coordinates [19]. The ANOVA test results showed no difference in the value of L * and b * between the edible coating treatments (p> 0.05) so that it can be concluded that both edible coating treatments and storage time did not affect the L* and b* value of fish flesh color. While the value of a * shows the difference between the control treatment and the edible coating treatment (p <0.05).



Figure 3. The change of a*value during storage 4^cC (B0 : untreated; B1: edible coating + lemongrass oil 0%; B2: edible coating + lemongrass oil 0.5%; B3: edible coating + lemongrass oil 1%)

Based on the figure 3 can also be concluded that the treatment of edible coating without the addition of lemongrass oil (B1) has the lowest a * value compared to the control treatment (B0) and the t edible coating with the addition of lemongrass oil 0.5% (B2) or 1% (B3) treatments. The value of a* in treatments B0, B2 and B3 did not look too much different during storage (p> 0.05). This was due to tuna fillet samples in treatment B1 derived from different fish body parts with tuna fish fillets treated B0, B2, and B3. Differences in the location of fillet sampling on a fish's body can affect the color of the fish flesh. The values of * L, * a and * b fillet on fishtail are greater than fillets from fish flesh in the body and the middle part of the fish body [20]. Areas of fish bodies that tend to have high physical activity have darker colored muscles and higher myoglobin [21].

3.3.2. Water loss analysis. Figure 4 showed the various differences in the treatment of edible coating on tuna fillets which did not give any difference in the weight loss value (p>0.05) due to the concentration of edible coating used in treatments B1, B2 and B3 were the same. The water loss value of control treatment was lower than the treatment of edible coating. This was due to the control treatment spoiling more quickly due to the decomposition process by decomposing bacteria from complex energy sources such as starch, protein, and fat into simple components. The number of decomposing bacteria in the control treatment was more so their energy sources were more rapidly decrease. The reduction of this energy source caused the weight loss of control treatment to be lower than the edible coating treatments [22]. The longer the storage, the higher the weight loss occurs due to tissue synthesis done by bacteria became a simpler substance [23].



Figure 4. The weight loss of tuna fillet during storage 4 °C (B0 : untreated; B1: edible coating + lemongrass oil 0%; B2: edible coating + lemongrass oil 0.5%; B3: edible coating + lemongrass oil 1%)

3.3.3. Sensory evaluation. Based on figure 5, it could be concluded that the longer the storage duration, the higher the organoleptic quality index score of tuna fillets in all treatments. It was caused the longer the storage duration, the more bacterial decomposition activity will affect all sensory parameters such as mucus, smell and texture of tuna fillets [24]. The worst total score of tuna fillet quality was 12 where the control treatment (B0) has reached that score on day 12, the treatment of edible coating without the adding of lemongrass oil (B1) reached a score of 12 on the 15th day and both treatments of edible coating with the addition of lemongrass oil 0.5% and 1% respectively reached a score of 12 on the 18th day. This was according to the results of TPC value which showed the highest peak bacterial population occurred on the 12th day for the control treatment. The edible coating of starch can reduce oxidation and hydrolysis rate which had an important role in the protein degradation process and fat oxidation [15].

The edible coating treatment had an effect on the results of sensory analysis (p < 0.05). Based on figure 4, it could also be concluded that the higher the concentration of lemongrass oil used in edible coating, the less organoleptic quality index score of tuna fillet, it caused better sensory quality based on the panelist's assessment. In addition to suppressing the growth of decomposing bacteria, the fragrant odor of citrus which is had by lemongrass oil was favored by panelists and it was able to disguise the rancid odor due to natural fat oxidation and spoilage odor due to the production of ammonia from protein degradation in tuna fillets [25]. Lemongrass oil consists of high citral content (> 45%) and its quality is determined by the amount of citral concentration [4].



Figure 5. Organoleptic quality index of tuna fillet during storage 4-C (B0 : untreated; B1: edible coating+ lemongrass oil 0%; B2: edible coating + lemongrass oil 0.5%; B3: edible coating + lemongrass oil 1%)

3.4. Chemical Analysis

3.4.1. Total Volatile Basic Nitrogen. Figure 5 showed the different treatment of tuna fillets at 4°C temperature storage caused by the increase of TVBN values during storage. This increase was caused by the activity of decomposing bacteria that degrade proteins as their energy source to become a simpler component, one of them was the volatile component [26].



Figure 6. TVBN value of tuna fillet during storage 4° C (B0 : untreated; B1: edible coating+ lemongrass oil 0%; B2: edible coating + lemongrass oil 0.5%; B3: edible coating + lemongrass oil 1%

Edible coating treatment showed a significant difference in TVBN value of tuna fillet (p < 0.05). Edible coating treatment and the higher concentration of lemongrass oil showed that the lower TVBN value produced. This was caused edible coating to have a function as a barrier that can reduce the exposure of oxygen needed by some decomposing bacteria to life [27] while lemongrass oil is a type

(IC-FSSAT)	IOP Publishing
IOP Conf. Series: Earth and Environmental Science 486 (2020) 012053	doi:10.1088/1755-1315/486/1/012053

of bacteriocidal essential oil that can kill decomposing bacteria [16]. Decomposing bacteria play an important role in degrading protein into volatile compounds which is base. The threshold for TVBN values for fish products is 30 mg N / 100 g. Lemongrass oil was effective in suppressing microbial growth rates both gram-positive and gram-negative bacteria so that lemongrass oil can be applied to food products to extend the shelf life of food products [28].

The control treatment had exceeded the threshold in the 9th day (34.30 mg N / 100 g) while the edible coating treatment without lemongrass oil (36.40 mg N / 100 g) and edible coating with the addition of 1% lemongrass oil (44.10 mg N / 100 g) reached the threshold on day 12. The edible coating treatment with the addition of 0.5% citronella oil just reached the TVBN threshold value on the 15th day with a TVBN value was 33.32 mg N / 100g.

B3 treatment with 1% lemongrass oil had a higher TVBN value compared to other edible coating treatments while B2 treatment with 0.5% of lemongrass oil had the lowest TVBN value then followed by treatment B1 without adding lemongrass oil. The B3 treatment had the highest concentration of lemongrass oil so the increase of fat content due to microbes activity further reduced the effectiveness of the antibacterial component of essential oils such as lemongrass oil [29].

3.4.2. *pH Value*. The threshold for pH values in tuna is 7 - 7.5 [30]. The pH value in fish was closely related to the TVBN value so that the higher the pH value indicated the more base the fish was. Bases in fish were obtained from the decomposing process of fish proteins by decomposing bacteria [2]. Figure.6 showed the longer storage duration, the higher the pH of tuna fillets. This was because of the TVBN value which also increased during storage due to the decomposing bacteria activity that degrades proteins into volatile substances form which is base. Figure 6 also showed that the edible coating treatment and the addition of lemongrass oil had an effect on the pH value of tuna fillets (p <0.05).



Figure 7. pH value of tuna fillet during storage 4°C (B0 : untreated; B1: edible coating+ lemongrass oil 0%; B2: edible coating + lemongrass oil 0,5%; B3: edible coating + lemongrass oil 1%)

The threshold for the pH value of fresh fish is 7-7.5. The control treatment (B0) had the largest pH value and the fastest one which reached the pH threshold compared to other edible coating treatments. The B0 treatment had reached the threshold on the 9th day with a pH reaching 8.02 and the pH value continued to increase until the end of the observation, B1 and B3 treatment reached the threshold on the 12th day while B2 treatment reached the threshold on the 15th day. This change of pH value had a

positive correlation with the change in the previous TVBN value. This was due to the presence of edible coatings that could inhibit the oxidation process and hydrolysis process in tuna fish so it reduced the growth rate of decomposing bacteria [27]. In addition, the addition of lemongrass oil could kill and reduce the growth of decomposing bacteria. Its application to food besides being able to maintain organoleptic properties also could inhibit the decomposing bacteria activity which causes a decrease in product quality [25]. The active content of citral in lemongrass oil (65-85%), mineral and geraniol can deactivate pathogenic bacteria in food [4].

3.4.3. Thiobarbituric acid Value. The maximum limit of the TBA value in fish products is 5 mg MDA / kg [31]. Figure.7 showed an increase in the TBA value during storage in all treatments until the 12th day due to the duration of exposure to oxygen causing unsaturated fatty acid oxidation occurs continuously [32]. TBA value decreased significantly when reaching the 15th day. due to the peroxide number of unsaturated fatty acids oxidation had reached its maximum point on day 12 so aldehyde production also decreased [33].



Figure 8. TBA value of tuna fillet during storage 4°C (B0 : untreatment; B1: edible coating+ lemongrass oil 0%; B2: edible coating + lemongrass oil 0.5%; B3: edible coating + lemongrass oil 1%)

Figure 7 above also showed that the edible coating treatment with the addition of 1% lemongrass oil (B3) had the highest TBA value compared to other treatments. Then followed by B2 treatment with edible coating and the addition of lemongrass oil 0.5%. The control treatment had the smallest TBA value compared to other treatments. The higher the concentration of lemongrass oil applied to the edible coating, the higher the TBA value of the sample. This was caused by lemongrass oil contains linoleic fatty acids (30.72%), oleic acid (28.17%), myristic acid (4.33%) and lauric acid (1.87%) [34]. Linoleic and oleic acid is one type of unsaturated fatty acids [35] then through the oxidation process and produce reactive unsaturated hydroperoxides. This hydroperoxide will be degraded and produce alcohol compounds, aldehydes and other unsaturated compounds with smaller molecules. Aldehydes are unstable and easily through the condensation polymerization reactions that produce rancid odors [33].

4. Conclusions

This study showed that the Methyl Red + Bromothymol Blue (1: 1) (pH 2.55) treatment was the best treatment of indicator solution in making indicator label paper while the treatment of edible coating with 0.5% lemongrass oil was the best treatment in making edible coating tuna fillet. The change color pattern of the label indicators applied for fillet tuna packaging changed color from red to yellow along with the declining quality of tuna fish fillets. The Active packaging treatment was able to extend the shelf life of tuna fish fillets from 9 days to 15 days at 4C cold temperature storage.

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