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The potential application of red cabbage indicator film as smart packaging on tuna fillet

The potential application of red cabbage indicator film as smart packaging on tuna fillet

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Abstract. Edible chitosan film was used as a carrier for red cabbage pigment extract and then formed into a 2 x 2 cm smart indicator. The solvent extraction process in this study used 96 percent ethanol and a maceration-sonication method. Sensitivity indicators were evaluated on tuna fillet samples wrapped in plastic with the parameters of the analysis. This included determining the number of TVBN, TMA, and TPC as well as discoloration during testing at freezer (-6°C), cool temperature (10°C), and room temperature (28°C). The results indicated that visually smart discoloration indicator transitioned from early purple to blue to green. At freezer temperature (-6°C), the discoloration smart indicator takes 365 hours to transition from purple to green, compared to 149 hours at cool temperature (10°C) and 31 hours at room temperature (28°C). Discoloration of smart indicators occurs during storage at each temperature, which increases the value of TVBN, TMA, and TPC. The smart indicator pigmented red cabbage product has an excellent sensitivity, with a purple plot representing the condition of packaged fresh ingredients, blue (fit for consumption), and green (not fit for consumption).

1. Introduction

The information on food labels that manufacturers provide to consumers should be complete, particularly the expiration date, manufacture date, and health warnings, which are considered to be the top three most critical pieces of information on food labels [1]. Fish is one of the food products that is frequently displayed without a label, despite the fact that it is classified as a food that decays rapidly [2]. Thus, smart indicators are present to aid in the monitoring of fish quality by utilizing substances in plants that react with volatile ammonia found in fish [3].

Quality of tuna after post-capture had histamine levels ranged from 4.92 to 6.90 mg/kg in 5% of the samples [4]. The initial concentration of histamine was high (75-78 mg kg^{-1}) and stable for up to 8 days but then significantly decreased in all experiments (ice storage, vacuum packaging and MAP) reached 25-30 mg kg^{-1}, probably due to the presence of histamine-decomposing bacteria [5]. *Pseudomonas spp* is a bacteria that dominates tuna fish that causes a decrease in the quality of tuna fillets (Thunnus albacares) stored under vacuum at 3 °C, where the bacterial colony counts reached an average of 4.6 log10 cfu/g [6]. Storage of fresh yellowfin tuna below 4°C is good enough for a two-week product shelf life [7]. One of the efforts made to a preservative for freshly caught fish with nano-chitosan utilization. nano-chitosan

ilization does not appear to reduce bacterial counts, but TVB data shows just that the nano-chitosan treatment significantly suppressed bacterial activity and the effect was more pronounced at 28 ° C [8].

Smart indicators, such as time-temperature controllers and freshness indicators/sensors, are an innovation in packaging fresh materials. These smart indicator systems monitor the product's freshness, microbial growth, and chemical changes by measuring, for example, pH changes, gases (CO2, O2, H2S, ethylene, NH3, etc.) and volatile compounds, pathogens or their metabolites and toxins, time-temperature changes, and humidity [9–12].

Due to their low toxicity, eco-friendliness, ease of preparation, biodegradability, low cost, availability, renewable, and pollution-free properties, the use of smart indicator based on natural colors has emerged as an attractive alternative for food packaging [13]. There are many reports of the use of dyes contained in plant tissues as natural colors in food [14–17]. These dyes undergo color changes due to the presence of phenolic or conjugated compounds, such as anthocyanins [18]. One of plants that contains antochyanin is *Brassica oleraceae* (Red Cabbage). The color change in the BC-anthocyanin label was fixed dark red to dark blue, readily observable with the naked eye [19].

The purpose of this study was to determine the effect of storage temperature variation and discoloration time on the application of smart indicators of red cabbage extract and the form of indicator response to fish quality deterioration.

2. Materials and methods

2.1. *Materials*

Chitosan was purchased from CV. Bio Chitosan Indonesia (Jakarta, Indonesia). Red Cabbage was purchased from supermarket (Makassar, Indonesia). Yellowfin tuna was purchased from Paotere fish auction (Makassar, Indonesia). Alcohol 96% and glycerol was purchased from CV. Sentana (Makassar, Indonesia). Ammonium hydroxide, sodium hydroxide, hydrochloric acid, glacial acetic acid, formaldehyde, *plate count* agar and whatman filter paper 42 was taken from the chemical engineering laboratory of the state polytechnic of Ujung Pandang (Makassar, Indonesia).

2.2. *Preparation of the fish samples and storage conditions*

The fish was first cleaned and separated from its bones and skin (fillets). Fish fillets were stored in packaging containers using plastic wrap that has been attached to smart indicators to provide information through discoloration of the indicator. Samples were placed at three different temperatures namely room temperature (28ºC), cold temperature (10ºC) and freezer temperature (-6ºC). Samples were observed every hour and will be analyzed every time a discoloration occurs on the smart indicator.

2.3. *Smart indicator preparation*

The red cabbage was first mashed in a blender. Prior to maceration, the crushed red cabbage sample was placed in a 500 mL beaker and dissolved in 96 percent alcohol at a material:solvent ratio of 1:5 (w/v). Maseration was carried out according to Halik (2016) [20] with ultrasonic assistance at temperature 30ºC for 60 minutes resulting in a filtered exorcist using whatman paper 42.

This concentrated extract is then applied over edible film. The colored film are put in the freezer for 30 to 60 minutes until the color is attached to the film. The film sheet is cut to a size (2x2) cm which is then expressed as a sample smart indicator product. Edible film was made according to Warsiki-Putri (2012) [21] from 3 grams of chitosan dissolved in 70 ml of 1% glacial acetic acid with the addition of glycerol plasticizer as much as 1% of the volume of film made and heated in the oven at 50ºC for 24 hours.

2.4. Indicator Sensitivity Test

2.4.1. NH_3 steam indicator sensitivity test.

Smart indicator sensitivity is simulatively tested against NH_3 vapor resulting from NH_4OH 1N solution. in the testing process, smart indicators and red cabbage extract are placed on each beaker and inserted in the chumber that has contained a solution NH_4OH 1 N where this process lasts for 30 minutes.

2.4.2. Smart Indicator Sensitivity Test On Packaged Fresh Fish at room temperature (28°C), cold temperature (10°C), and freezer temperature (-6°C).

Smart sensitivity indicator tested using tuna fillets. Tuna fillets are placed in sterofoam wrapped in plastic wrap where the plastic wrap has previously been affixed to the indicator to prevent leakage in the packaging, around the container is tightly closed using sticky tape.

2.5 Chemical Analysis

2.5.1 Analysis of TVBN (Total Volatile Base Nitrogen).

Total volatile base nitrogen (TVBN) was carried out according to Halik (2016) [20]. Sample (5g) were crush by mortar, and then adding acetic acid solution 5% (30 ml) into the sample. The sample were silent during 5 minutes then separated extract by centrifuge. Once in centrifugation, two layers are formed. The top layer was pipetted as much as 5 mL and 5 mL of 2 M NaOH was added into volumetric flask and then distilled. the results of the distillate were accommodated/captured in a 250 mL erlenmeyer containing 15 mL of 0.01 M HCl. Three drops of phenol phytalain (PP) indicator were added and titrated with 0.01 M NaOH until a pink solution was reached.

$$TVBN\ \left(\frac{mgram\ N}{100gram}\right) = \frac{(V1 - V2)\ x\ N\ x\ BM}{M}$$

Where V1 and V2 represent the volumes of HCL required for titration without sample and with sample (ml); N is normality of NaOH (0.01M); BM is weight of nitrogen atom (14 g/ mol); M is sample weight (g)

2.5.2 Analysis of TMA (trimetil amin) .

Trimetil amin (TMA) was carried out according to Halik (2016) [20] from the results of TVBN analysis and added 1 mL of 16%. At the time of this addition, there is a discoloration from pink to colorless. Penetrated with NaOH 0.01 M until the solution changes color from colorless to pink.

2.5.3 TPC microbiology test (Total Plate Count) .

TPC microbiology test (Total Plate Count) was carried out according to [20] Halik (2016). Homogenized fish sample (1 g) in 10 mL physiological solution (0.9 % w/v NaCl) was prepared as the initial dilution (10^{-1}). The serial dilution was made by adding 1 mL of previous dilution into 9 mL physiological solution. The resulting dilutions (10^{-4} to 10^{-6}) were poured into PCA media.

Incubated petridish in reverse position in incubator for 24 hours. Observation and calculation of the number of colonies after the incubation period using the counter colony and counted using the formula:

$$N = \Sigma c\ x\ \frac{1}{\text{fp}}$$

Where N is number of colonies in the sample (colony/gram); Σcis number of colonies on all counted petridish; fp is dilution factor; M is sample weight (g)

The number of colonies is then counted according to the rules of the Standard Plate Count. The results of this test were adjusted to the Maximum Limit of Microbial Contamination (BMCM) of RSNI fresh fish meat 2729:2013.

3. Results and discussion

3.1 NH_3 steam indicator sensitivity test

Red cabbage is one of the agricultural products rich in anthocyanins [22]. Anthocyanin profile of red cabbage consists of twenty derivatives of cyaniding glucosides and the predominant in red cabbage was a nonacylated . to prove the sensitivity of this smart indicator of red cabbage, the NH_3 steam sensitivity test was conducted. The sensitivity of smart indicators was tested simulatively against NH_3 vapor resulting from NH_4OH solution 1 N.

Table 1. Results of time analysis and discoloration of smart indicator film and red cabbage pigment extract

Discoloration	Time (Minute)
Initial condition (purple)	0
Blue	Started at the 2nd minute
Green	Started at the 7th minute
Yellow	Started at the 30th minute (End of Testing)

Table 1 shows the color change in the smart indicator sample and extract. The color in the initial condition is purple. In the 2nd minute, it changed color to blue and in the 7th minute there was a change of color to green. This proves that the use of red cabbage pigment as an indicator dye on smart indicator products is excellent. As done by Sanches et al (2021) that red cabbage can be used as a dye on indicators because it contains anthocyanin, discoloration that occurs from purple to green [23].

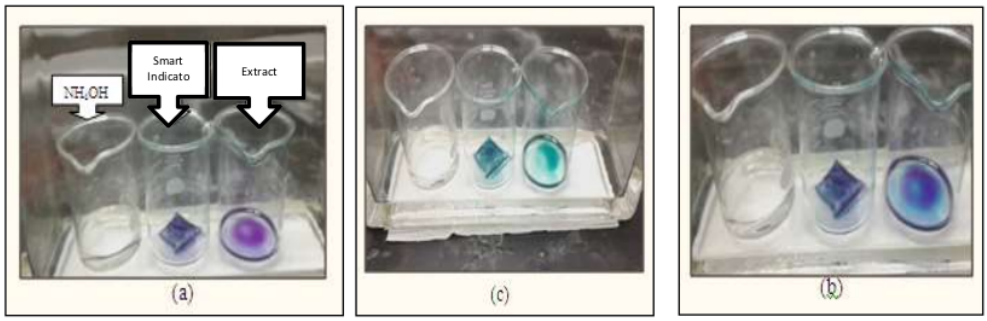

Figure 1. Discoloration of smart indicator, samples and pigment extracts in NH3 steam test (a) Initial condition of purple test (b) discoloration to blue (c) discoloration to green

3.2 Smart Indicator Sensitivity Test On Packaged Fresh Fish at room temperature (28°C), cold temperature (10°C), and freezer temperature (-6°C)

At this stage, smart indicator sensitivity tests are conducted on tuna fish to see if they work properly.

Table 2. Discoloring, TVBN, TMA, and TPC Value Test Results for tuna samples

Storage Temperature	Discoloring	Time (hours)	TVBN (mg N/100 g	TMA (mg N/100 g	TPC (coloni/g)
Room (28°C)	Purple	0	9.73	2.95	0.98×10^4
	Blue	6	17.51	7.12	21.98×10^4
	Green	31	37.71	13.26	267.63×10^4
Cold (10°C)	Purple	0	9.73	2.95	0.98×10^4
	Blue	8	16.87	5.92	19.17×10^4
	Green	149	36.05	11.82	241.92×10^4
Freezer (-6°C)	Purple	0	9.73	2.95	0.98×10^4
	Blue	10	15.68	5.33	18.2×10^4
	Green	365	32.99	11.09	237.82×10^4

From the table above it can be seen that the smart indicator has a good sensitivity to tuna fish, where there are discolorations at three different storage temperatures ranging from purple which indicates fresh to green which indicates the product is no longer suitable for consumption.

3.3 TVBN, TMA and TPC

During the testing process there are 4 parameters that become observational points (table 2) that are discoloration of smart indicators, TVBN, TMA and TPC. From table 2 it was seen that in the initial condition of tuna fillet samples obtained TVBN values of 9.73 mg N/100 g and TPC of 0.98×10^4. This result shows that the initial TVBN value is still below the maximum limit of 30 mg N/100 g indicating that the fish has a very fresh quality (Soekarto, 1990 in Halik, 2016), and the TPC value still meets the Maximum Limit of Microbial Contamination (BMCM) of fresh fish meat of 5×10^5 (SNI 2729:2013).

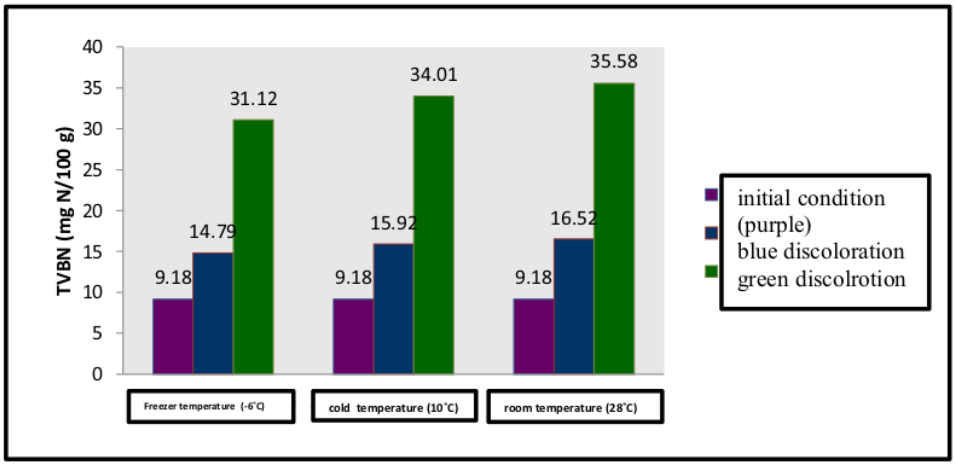

Figure 2. TVBN Value Graph on tuna fillet sample testing

The determination of TVBN, TMA, and TPC values is carried out every time there is a discoloration of the smart indicator. At room temperature (28°C), there is a discoloration of the smart indicator from the initial purple condition to blue after 6 hours of storage and obtained TVBN values of 17.51 mg N/100 mg and TPC of 21.98 x 10^4 colonies/gram. This also occurs in cold temperatures (10°C) and freezer temperatures (-6°C) that change color in the smart indicator to blue with a longer change time of 8 hours and 10 hours of storage with a TVBN value of 16.8 7 mg N/100 mg and 15.68 mg N/100 mg and TPC of 19.17 x 10^4 colonies/gram and 18.2 x 10^4 colonies/gram. These results show that TVBN and TPC values are still below the maximum limit indicating that the physical condition of tuna fillets at the change of color of the blue smart indicator is still in the fresh category and still worth consuming.

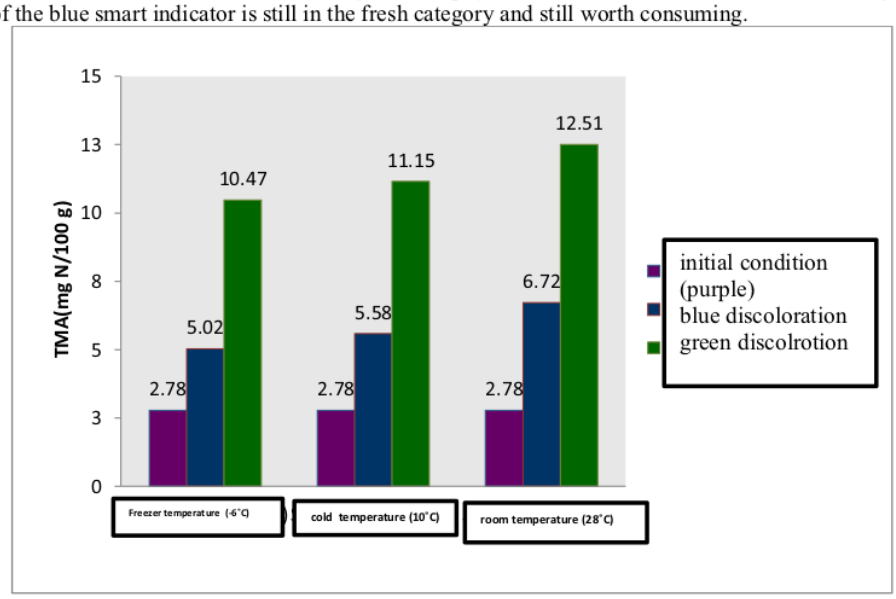

Figure 3. TMA value graph on tuna fillet sample testing

The increase in TVBN and TPC values reached 37.71 mg N/100 mg and 267.63 x 10^4 colonies/gram at room temperature (28°C), 36.05 mg N/100 mg and 241.92 x 10^4 colonies/gram at cold temperatures (10°C), 32.99 mg N/100 mg and 237.83 x 10^4 at freezer temperature (-6°C) with discoloration on green smart indicators after 6 days of storage at cold temperatures (10°C) and 15 days at freezer temperature (-6°C). The level of freshness of fishery products based on TVBN is grouped into four, namely very fresh fish with TVBN content of 10 mgN/100 g or smaller; fresh fish with TVBN content of 10-20 mgN/100 g; fish that are at the limit of freshness that can still be consumed with TVBN content of 20-30 mgN/100 g; rotten fish that cannot be consumed with TVBN content greater than 30 mgN/100 [20].

The increase in TVBN value is directly proportional to the increase in the number of bacterial colonies (TPC) as seen in table 2. The number of microbes will increase during storage. The longer the storage, the greater the growth rate of microbes

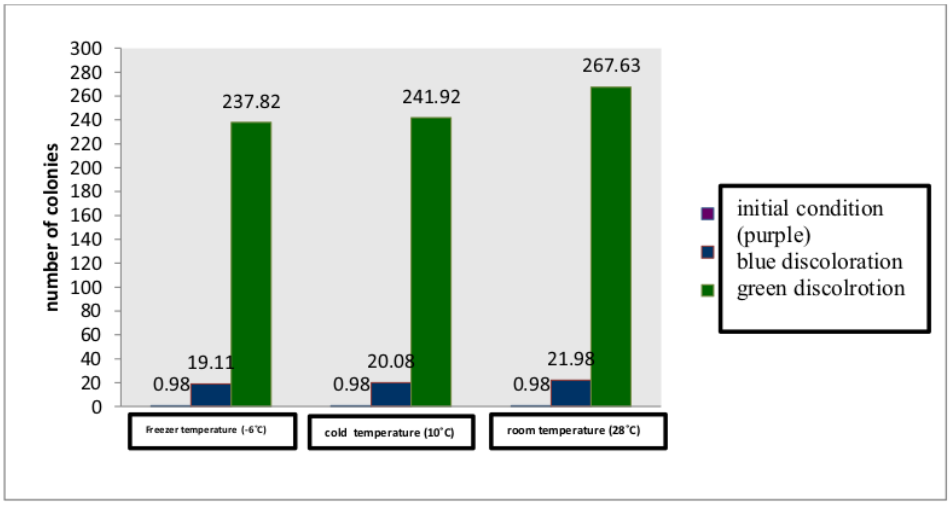

Figure 4. TPC Value Graph (colony/gram) on tuna fillet sample testing

According to [24] an increase in TVB value during storage due to protein degradation resulted in a number of volatile bases such as ammoniac, hydrogen sulfide and foul-smelling trimetilamin.

With the obtaining of the results of analysis on the determination of the number of TVBN, TMA, and TPC during the storage process, this is the basis of the indication of the decay process in the tuna fillet samples has taken place and produced a number of volatile base compounds that trigger the change in pH value in the smart indicator causing changes in the pigment of the smart indicator.

TVBN, TMA, and TPC values will increase during storage time. The longer the storage time, the greater the value of TVBN, TMA, and TPC. The effect of temperature during storage also has a significant impact on the length of decay time in fish meat and discoloration on smart indicators indicating the condition of tuna fillet samples. This can be seen from the smart indicator's discoloration process against tuna fillet samples which occurs faster at room temperature (28°C) than in cold temperatures (10°C) and freezer temperatures (-6°C). The results were conducted that at room temperature (28°C) for ± 31 hours the tuna fillet sample had decayed, while at cold temperatures (10°C) and freezer temperatures (-6°C) experienced a longer decay time of 149 hours (±6 days) and 365 hours (±15 days). This is in accordance with the literature which explains that anthocyanin stability is influenced by several factors including temperature, pH, light, and oxygen [25].

4. Conclusions

As a result, it can be concluded that storage temperature has an effect on the decay time of fish meat and the coloration of smart indicators. Discoloration from purple to blue to green occurs more rapidly at room temperature (28°C) than at low temperatures (10°C) and the freezer temperature (-6°C). The smart indicators' response to deterioration in fish quality is extremely sensitive, with purple representing extremely fresh fish, blue indicating the fish is still edible, and green indicating the fish has already decayed and is unfit for consumption.

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