Characterization of tannin based colorimetric indicator and its application on fish packaging

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Abstract. This study aims to determine characteristics of the gel-type colorimetric indicator at various pH and under different storage conditions, then determine the indicator characteristic in the packaging of fish fillets during storage. The gel-type color indicator was synthesized with a concentration of 1%, 3%, and 5% gambir powder. The FT-IR spectra of the color indicator showed the presence of tannin functional groups, namely C=C aromatic rings, C-C phenolic, and C-H groups, respectively at wavenumbers 1517-1519, 1440-1475, and 752-761 1/cm. Moreover, the coefficient value of the Red-Green-Blue (RGB) of the indicator was changed over pH and did not appear to be consistent. Based on the Analysis of Variance (ANOVA) test, the concentration of gambir powder and duration of color indicator storage, respectively, had a significant effect (P <0.05) on the value of the RGB coefficient. The color indicator exposed to sunlight had a smaller RGB coefficient value than the RGB coefficient value of the indicator stored at room temperature and 5-7°C. The application of color indicators with a 1% Gambir powder concentration in fish fillet storage has been tested for 5 days at a temperature of 5-7°C. They showed that the RGB coefficient value of the indicator was proportional to changes in the pH value of fish fillets but not in line with changes in Total Volatile Based Nitrogen (TVBN) of fish fillets.

1. Introduction
Tannins are the main chemical components of gambir and generally used for dyes. Gambir is an extract of sap from the leaves and young twigs of the gambir plant (Uncaria gambir Roxb.). Gambir is a perennial plant; thus, its availability is endless. Consequently, it is very efficient to be used as a source of dyes in various industries such as the textile industry [1]. Tannin levels in gambir obtained from several locations in West Sumatra ranged from 34.79% to 42% [2]. The high tannin content in gambir offers gambir as a source of food coloring agent and can increase the commercial value of gambir.

Tannins consist of two types, namely condensed tannins and hydrolyzed tannins. The most dominant type of tannin in plants is condensed tannin [3], a polymer consisting of 2 to 50 (or even more) flavonoid units joined by carbon bonds; as a result, they are not easily hydrolyzed. Tannins in plants have a bitter taste, are generally soluble in water, and can be hydrolyzed by enzymes, bases, and acids. Tannins have a yellowish-white to light brown color and are easily oxidized. In addition, tannins
will also turn darker when exposed to direct light [4]. These color changes show the potential of gambir tannin to be applied as a color indicator in smart packaging.

Smart packaging can detect, record, and communicate food quality during transportation and storage [5]. Smart packaging is equipped with indicator labels containing compounds that act as sensors. The sensors are usually sensitive to environmental conditions, such as pH and temperature. The indicator will change its color if the condition of the packaged material deteriorates. One type of smart packaging color indicator is gel-type. The gel-type color indicator can be synthesized from starch, chitosan, and agar. Fabrication of smart packaging indicators made from a mixture of agar powder, Selenite Enrichment Broth (SEB), tapioca, and glycerol resulted in acceptable indicator physical properties [6]. A gel-type color indicator has also been synthesized from distilled water, tapioca, powdered agar, and 0.002% methyl red [7]. The color indicator becomes darker in color as the food quality decreases. However, the development of tannins as color sensors has few studies; thus, it is needed to study the synthesis of a gel-type color indicator at definite tannin concentrations.

Several studies related to the application of pigments as sensors in smart packaging have been carried out, including films based on purple sweet potato anthocyanin extract as a bio-indicator of chicken meat deterioration [8], cassava leaf chlorophyll to detect tilapia fillet decay [9], and label of smart packaging from bromothymol blue and phenol red as an indicator to monitor meat freshness [10]. Bromothymol blue and phenol red-based smart packaging indicator labels are sensitive to cold-stored fresh meat spoilage. The color change on the indicator label was apparent on the sixth day of storage and changed color from reddish-orange to purple on the sixteenth day.

In general, anthocyanin and chlorophyll have been widely applied as natural colors in smart packaging biosensors. Although tannin has also been used as a natural dye, studies have not provided any information about the concentration of tannin extract applied as a biosensor in fabricating color indicators. Different concentrations of pigment can produce different characteristics of color indicators. Therefore, this study synthesized a gel-type colorimetric indicator from tannin gambir and aimed to determine the characteristics of the indicator at various pH and under different storage conditions. The colorimetric indicator changes its color over food quality alteration. Thus the tannin indicator characteristic in the packaging of fish fillets during storage were determined.

2. Materials and Methods
2.1. Materials for indicator fabrication
The colorimetric indicators were synthesized from gambir powder from West Sumatera, containing 27.76% of total tannin. Other materials for gel compounds were agar powder, corn starch, and distilled water.

2.2. Fabrication of colorimetric indicator
The colorimetric indicators were synthesized by dissolving gambir powder, 2 g of agar powder, and 2 g of tapioca in 50 ml of distilled water. The concentration of gambir tannin powder was 1%, 3%, and 5% of the total solution. The solution was then heated on a hot plate and stirred until the temperature of the solution reached 85 °C. Then 20 ml of the solution was put into a petri dish and stored in a laminar flow for 8-12 hours until it formed a sheet. The gel-type color indicator had 80 gsm in thickness, and then it was cut into 2 x 2 cm sizes. A control indicator was synthesized as well as the colorimetric indicator without gambir powder addition.

2.3. Characterisation of colorimetric indicator
2.3.1. Functional group of tannin. FT-IR Spectrophotometer IRPrestige-21 (Shimadzu) was applied to determine the functional group on the indicator. The analysis was performed at 4 cm\(^{-1}\) and ten replications at a wavenumber of 400-4000 cm\(^{-1}\). First, the sample was prepared by cutting the indicator sheets into 4 cm\(^2\). Next, the sheet was placed on the sample holder and read using the FT-IR Spectrophotometer. Then the result showed a spectrum with a wave number.
2.3.2. **Red-Green-Blue (RGB) measurement of the indicator during storage.** The indicators were stored in three conditions, i.e., exposure to sunlight, room temperature, and low temperature at 5-7 °C. The color change of the indicator was observed for ten days of storage. Red-Green-Blue (RGB) testing was conducted using Adobe Photoshop on the color changes in the gel-type indicators.

2.3.3. **Red-Green-Blue (RGB) measurement of the Indicator at pH 3-10.** The color indicators were dipped in a solution having a pH range of 3-10. RGB testing using Adobe Photoshop was carried out on color changes in the indicators at different pH conditions.

2.4. **Application of the indicator for fish fillet storage.** The indicator with the addition of 1% gambir powder was applied to monitor fish fillets' deterioration. The fish fillet was cut into 2x3x3.5 cm and weighed 20 g, then it was placed in a plastic container (polypropylene) with a lid and stored in a cooler with a glass door at a temperature of 5-7 °C for 5 days. The color indicator was placed in the container and made indirect contact with the fish fillet. RGB testing was carried out on the indicators, then pH and Total Volatile Based Nitrogen (TVBN) were conducted on fish fillets before and during storage, as referred to Shukla et al. [11]

2.5. **Experiment design and data analysis**
A non-factorial completely randomized design was applied in this study consisting of tannin gambir concentrations at 1, 3, and 5% in solution. This study was carried out in 3 replications. Analysis of Variance (ANOVA) and least significant difference (LSD) tests were used for statistical data analysis of the indicator RGB measurement during storage under three conditions.

3. **Results and Discussion**

3.1. **Characteristics of the indicator by FT-IR spectroscopy**
Hydroxyl group (-OH) is one of the functional groups in tannin compounds where the peak at wave number 3600-3000 cm⁻¹ of FT-IR spectrum indicates the presence of a hydroxyl group. In addition, the peak of 1379 cm⁻¹ indicates the O-H bond of the phenolic or alcohol groups which is part of the tannin compound [12]. According to Figure 1, the peaks at wave numbers 1625 and 1523 cm⁻¹ indicate an aromatic bond C=C and the peak at wave number 1467 cm⁻¹ shows the presence of C-C phenolic groups. The peak at wave number 1288 cm⁻¹ refers to the C-O bond of the aromatic ring. Moreover, the presence of a peak at wave number 1082 cm⁻¹ indicates asymmetric C-O-C groups of ether and C-H deformation. Tannins are also characterized by the C-H functional group at 736 cm⁻¹ indicating a C-H bond in the aromatic ring of the tannin structure (Figure 1). It is also observed by Syarif et al. [13]. Since the gambir Asalan contains alcohol and phenol functional groups at wavenumbers 1636, 1520, and 1412 cm⁻¹. Moreover, carboxyl functional groups at wavenumbers 1296, 1250, 1203, 1149, 1080, and 1026 cm⁻¹ showed tannin compounds.

Figure 2 exhibited FT-IR spectra of colorimetric indicators and control indicators (without gambir powder). The peak at wave number 1517 cm⁻¹ indicates the C=C bond in the aromatic ring, which shows tannins in the gel-type colorimetric indicator. In the spectra, other functional groups, such as O-H, C-H, and C-C groups, also indicate chemical compounds of gel composition in the gel-type indicator. i.e., agar distilled water and starch. In addition, the gambir powder and the indicator spectra exhibit a peak of C=C bond vibration at a wavelength of 1523-1519 cm⁻¹, while the control indicator spectrum does not show any peak at that wavelength. Thus, the presence of tannins in the gel-type colorimetric indicator is indicated explicitly by the C=C bond of the aromatic ring functional group.
Figure 1. FT-IR functional group of tannin in gambir powder at 4000-500 cm\(^{-1}\) (a) and 1650-1350 cm\(^{-1}\) (b).

Figure 2. Spectra of the colorimetric indicator with 1% gambir powder addition and control indicators at 1650-1350 cm\(^{-1}\).
3.2. Characteristics of colorimetric indicator by RGB coefficient at pH 3-10

Colorimetric indicators of tannin concentration of 1% were decreased in the RGB coefficient value at pH 3 to 4. In contrast, the indicators with tannin concentrations of 3 and 5% increase the value of the RGB coefficient at pH 3 to 5 (Table 2). These indicate that the 1% tannin concentration indicators tend to be lighter in color under base conditions than under acidic conditions. Meanwhile, the gel-type indicators with 3 and 5% concentrations tend to be lighter in color in acidic solutions than in base conditions. Those are indicated by the higher value of the RGB coefficient, thus the brighter color of the gel-type colorimetric indicator. Organic compounds will alter their color with changing pH conditions [14]. The color change in the indicator with 1% tannin concentration is inversely proportional to the report of Firdausni et al., [15] that gambir powder in base pH solution was darker in color than in acidic pH. It is presumably due to the low percentage of gambir powder used to fabricate the gel-type colorimetric indicator. Moreover, the properties of the indicator are influenced by the indicator materials consisting of agar and starch.

Fluctuation in the RGB coefficient of the gel-type indicator treated with pH solution also occurred in the phenolphthalein-based colorimetric indicator. The phenolphthalein indicator changed its color at pH 7 and became colorless until pH 8. Then it turned to red at pH 10 and again colorless in a strong base solution. Color alteration of the indicator occurred due to the chromophore compound in phenolphthalein being changed by an acid-base reaction [14]. The study relates to the application of gambir extract as a colorant for food products with various types of color enhancers. It shows that gambir extract with the addition of 3% citric acid produced red color, 3% ascorbic acid produced red color, and 3% lime betel produced brownish-red color [15].

### Table 1. RGB coefficient of the indicator at pH 3-10

| pH | The concentration of gambir tannin powder |
|----|------------------------------------------|
|    | 1% | 3% | 5% |
| 3  | 162.67 | 93.33 | 80.67 |
| 4  | 151.00 | 94.00 | 88.00 |
| 5  | 164.00 | 94.67 | 89.00 |
| 6  | 145.00 | 91.33 | 86.00 |
| 7  | 166.33 | 89.33 | 83.33 |
| 8  | 164.33 | 85.33 | 82.67 |
| 9  | 170.33 | 88.33 | 87.33 |
| 10 | 149.00 | 96.00 | 85.00 |

3.3. Characteristics colorimetric indicator by RGB coefficient during storage

The colorimetric indicators have a fluctuation value of RGB coefficient when stored in 3 different conditions for 10 days (Table 2 and 3). According to ANOVA, gambir concentration and storage duration respectively have a significant effect (P ≤ 1) on RGB coefficient value on each condition of storage. The higher the RGB coefficient, the brighter color of the indicator, and vice versa. According to Table 2, the indicator with the highest concentration of gambir powder has the lowest RGB coefficient value indicating the darkest color of the indicator when the amount of gambir powder addition in gel composition increases, then the color of indicator becomes darker.

The RGB coefficient value of the indicator decreases consistently from day 3 to day 6 (Table 3). The BNT 5% test results show a significant decrease of the RGB coefficient after 3 days at each storage condition, supposedly because the tannin compound is starting to be sensitive to the environment as light exposure and temperature change. As a result, the tannins contained in the color indicator change their color to a darker color.

The gel-type colorimetric indicators stored under direct sunlight have a lower RGB coefficient value than those stored at room temperature and 5-7°C. Therefore, it indicates that the indicators
exposed to sunlight for 10 days tend to be darker in color than the indicators stored at other conditions. Furthermore, the tannin indicators are more sensitive to sunlight. The indicator turns darker because exposure of tannin in the indicator to direct light can lead to tannin oxidation which pro-oxidants can accelerate as sunlight [4].

**Table 2.** Effect of gambir concentration on RGB coefficient value of colorimetric indicator under different storage conditions.

| Concentration (%) | Under Sunlight Exposure | Room Temperature (°C) | Temperature 5-7°C |
|-------------------|------------------------|-----------------------|-------------------|
| 1                 | 156.04±0.277c          | 161.58±0.218c         | 166.23±0.309b     |
| 3                 | 112.40±0.308b          | 111.72±0.315b         | 103.35±0.318a     |
| 5                 | 96.30±0.333a           | 97.40±0.299a          | 99.30±0.277a      |

**Table 3.** Effect of duration storage to RGB coefficient of colorimetric indicator under different storage conditions

| Days | Under Sunlight Exposure | Room Temperature (°C) | Temperature 5-7°C |
|------|------------------------|-----------------------|-------------------|
| 0    | 127.49±3.80bc          | 127.49±3.81b          | 127.49±3.81bc     |
| 1    | 121.16±4.05b           | 125.24±3.31b          | 124.09±4.52b      |
| 2    | 134.64±3.36c           | 129.98±3.66bc         | 130.84±3.97bc     |
| 3    | 136.56±3.13c           | 138.31±3.61c          | 137.80±4.25c      |
| 4    | 125.60±3.65bc          | 130.60±3.55bc         | 128.69±3.87bc     |
| 5    | 116.80±3.23ab          | 121.98±3.97b          | 117.38±4.14ab     |
| 6    | 112.60±3.93ab          | 111.42±4.08a          | 112.87±4.14ab     |
| 7    | 121.84±3.17b           | 121.42±4.43b          | 133.00±4.95bc     |
| 8    | 117.82±3.49ab          | 119.00±4.01ab         | 114.36±4.22ab     |
| 9    | 110.76±3.27a           | 116.04±3.22ab         | 112.44±3.95a      |
| 10   | 112.09±3.19ab          | 117.73±4.00ab         | 113.60±4.27ab     |

3.4. Application of the indicator for fish fillet storage

Based on Figure 3, the RGB coefficient value of the indicator declined slightly until day 3 storage, then on day 4, it has slightly increased on day 5. It shows that the indicator changes to a darker color until the 3rd day of fish fillet storage. The determination of the lightness of the color indicator is known from the RGB coefficient value. The higher the RGB coefficient value, the lighter the color indicator.

The declining value of the RGB coefficient on the color indicator is directly proportional to decreasing pH value of the fish fillet. The pH value of fish fillets goes down until day 3 from 6 to 5.71, but then it slightly up to 5.8 on day 5. Nevertheless, this pH alteration is still in the pH range for fresh fish fillets. When the fish fillet experiences a decrease in pH value, the RGB coefficient of the indicator also decreases and vice versa. The pH of fish fillets in this study was still suitable for consumption. Tuna with pH 5.5 is still safe for consumption [16]. Fresh tuna fillets have pH values from 5.85 to 6.01 [17]. Decreasing the pH value of tuna fish fillets over the storage time can be caused by a biochemical process that aerobically and produces lactic acid. In cold storage conditions, fish fillets are in a pre-rigor state, which causes the pH value to decrease due to the anaerobic glycolysis process and produces lactic acid. Rising the pH value of fish fillets on the 4th day of storage can be caused by psychrophilic bacteria that increase volatile bases. Tilapia decreased its pH on the 4th day of storage, and then it increased until the 12th day and dropped back down on the 16th day. The pH value of red tilapia on the 12th day was above 7 and fell back below 7 on the last day of storage [18].
Skipjack tuna had a pH of 5.87 on the 7th-day storage at 5°C. Grey tuna had a pH of 5.49 on the 5th day of storage at 8°C [19, 20].

![Figure 3. Correlation of RGB coefficient of the colorimetric indicator with pH and TVBN of fish fillet during fish fillet storage.](image)

In addition, the parameter to determine fish quality is nitrogen content in fish determined by the Total Volatile Based Nitrogen (TVBN) method. TVBN values increased during 5 days of storage at 5-7°C (Figure 3). On the 3rd day, the fish fillets were still relatively fresh, while on the 5th day, the fish fillets were not classified as fresh but were still allowed to be consumed. Fish with a TVBN value of 10-20 mg N/100 g are categorized as fresh fish and TVBN of 20-30 mg N/100 g as edible fish [21]. Commission Regulation (EC) NO 2074/2005 also states that fish for consumption should have a TVBN value of < 35 mg N/100 g. Referring to Figure 3, although the fish fillets are still consumable after 5 days of storage, the quality of fish fillets has decreased. An increase in the value of TVBN indicates declining fish quality which can be caused by microbiological and biochemical changes in fish muscles. Then it leads to increased production of volatile compounds due to proteolytic microbial activity. Increasing TVBN values in this study is also in line with previous studies, which obtained TVBN values from 15.56 to 22.00 mg N/100 g and 17.36 to 26.88 mg N/100 g for fish stored for 7 days at a temperature of 4°C [22, 23]. The TVBN value is affected by the amount of nitrogen in the fish, determined by fishing season and type of fish [24, 25].

The RGB coefficient of the indicators is not directly proportional to the TVBN value of fish fillets for 5 days of storage. The RGB coefficient value decreases from day 0 to day 3, then increases on day 4, while the TVBN of fish fillet slowly rises during storage. Hence, it can be pointed out that the indicator is less sensitive to volatile nitrogen and more sensitive to changes in the pH of fish fillets.

4. Conclusions
This study showed that tannin functional groups on tannin-based colorimetric indicators indicated by C=C aromatic rings, C-C phenolic, and C-H groups. They appear at wavenumbers 1517-1519 cm⁻¹, 1440-1475 cm⁻¹, and 752-761 cm⁻¹. In addition, the indicator's color changes to a darker color with longer storage time and amount of gambir powder in gel composition. Oxidation of tannin in gel indicator composition by sunlight exposure can also cause the darker color of the indicator compared to other storage conditions. Alteration of the RGB coefficient of the indicator during storage of fish fillets is in line with changes of fish fillets pH, but it is not according to changes in the TVBN value of fish fillets.
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