Indonesian Journal of Applied Research IJAR

SAPPAN (*Caesalpinia sappan* L.) EXTRACT-BASED LABEL FOR DETECTION OF CHICKEN MEAT SPOILAGE

Volume 2 Issue 3
(December 2021)
e-ISSN 2722-6395
doi: 10.30997/ijar.v2i3.146

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ARTICLE INFO

Article history:
Received: 15-09-2021
Revised version received: 22-09-2021
Accepted: 24-11-2021
Available online: 08-12-2021

Keywords:
Indicator label; sappan extract; spoiled chicken meat; smart packaging

How to Cite:
Kurniawan, M. F., Fitrilia, T., Chosidan, H. N., & Nacing, N. (2021). SAPPAN (*Caesalpinia sappan* L.) EXTRACT-BASED LABEL FOR DETECTION OF CHICKEN MEAT SPOILAGE. *Indonesian Journal of Applied Research (IJAR)*, 2(3), 153-159. https://doi.org/10.30997/ijar.v2i3.146

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ABSTRACT

Brazilin in sappan is sensitive to pH, which make it possible as indicator of food quality. This research aimed to investigate performance of sappan extract as colorant for indicator label applied in chicken meat packaging. The samples were stored at room temperature for 5 days and refrigerator temperature for 7 days. They were then tested for color change of label RGB (Red Green Blue), pH and TVB-N (Total Volatile Base-Nitrogen) of the meat. Data were analyzed using ANOVA at significant level of 5%. Indicator label showed the reduced RGB during storage, while pH and TVB-N increased significantly ($p<0,5$). In case of room temperature, the color change into pink occurred in day 3 with pH 6.72 and TVB-N 1.84 mg N/100g. Meanwhile, for samples at refrigerator temperature, the label color turned into pink in day 6, with pH 6.56 and TVB-N 2.09 mg N/100g. In this regard, indicator label could detect the depletion of chicken meat quality as indicated by color shift from yellow to pink when pH and TVB-N were at critical value. This result provides significant output for development of sappan extract for indicator of meat spoilage. The recommendation in this study is the need to validate the quality of chicken meat by microbe and sensory testing.

1. INTRODUCTION

Fresh chicken meat is highly susceptible to microbial deterioration since it is rich in protein and fat. As a consequence, substantial changes in chemical composition occur during storage, distribution and retailing, as indicated by reduction of chicken meat quality which make it unsafe to consume (Hajrawati et al., 2016). Diminished meat attributes result from formation of amine compounds characterized as volatile components such as trimethylamine (TMA), ammonia, and dimethylamine (DMA), as well as change of pH (Riyanto et al., 2014).

The main mechanism of how the chicken meat spoils is chemical reaction forming gasses and other chemical groups, thus the spoiled product is visually difficult to estimate. To eradicate this challenge, smart packaging can be one of the promising methods. The packaging is equipped with specially designed indicator which enables to monitor the rate of meat spoiling as well as estimate its shelf life (Singh et al., 2018).

Indicator label is developed from colorant and carrier materials aiming to retain color indicator. Simply, colorant added in the label is able to inform product freshness and condition, besides expired date. Pacquit et al. (2006) detected fish meat deterioration using a sensor highly sensitive to presence of volatile amine compounds, developed using pH sensor of bromocresol green. In addition, the use of natural colorants was also applied, in which they were known as flavonoid present in various plants such as rosella flower (Ismed et al., 2018), *Aerva sanguinolenta* leaf (Nofrida et al., 2013), suji (*Pleomele agustifolia*) leaf and strawberry fruit (Rahardjo & Widjanarko, 2014), and purple sweet potato (Imawan et al., 2018). Sappan wood (*Caesalpinia sappan L.*) is also a potential source of natural colorant for predicting chicken meat spoilage.

As reported by Rondão et al. (2013), brazilin is a colorless phenolic compound and regarded as main active component of sappan. However, it can change into brazilein, a dark red colored compound, after easily oxidized in presence of light. Brazilein is then widely applied as colorant in textile industry and also used as chemosensor to detect cyanide (CN^-) and fluoride (F^-) ions. Adawiyah and Indriati (2003) reported the color alteration of brazilin upon pH changes: yellow in acid condition and purplish red in base condition. The results suggest applicability of brazilin as color indicator. This present work aimed to evaluate performance of sappan extract as indicator of chicken meat spoilage.

2. METHODS

2.1. Sappan Extraction

The protocol of extraction followed method of FR & Azmi (2017). Sappan powder (sieved through 40-mesh screen) was mixed with ethanol 96% and macerated in Ultrasonic Cleaning Bath (Branson 5800) at 20 kHz for 60 min under atmospheric pressure. The extract solution was filtered through Whatman No 1 paper. Following pH testing, the extract was used as colorant in the indicator label.

2.2. Preparation of Indicator Label

The label was made using method prescribed by Imawan et al. (2018). Whatman No. 1 paper was cut 2×2 cm and submerged in sappan extraction solution for 5 min. Subsequently, the paper label was dried using blower at room temperature.

2.3. Application of the labels in meat packaging

The 2×2 cm indicator label was placed inside styrofoam box as a packaging of 50 g chicken meat. The box with chicken meat and indicator was wrapped with cling wrap film and stored at refrigerator (2-5°C) for 7 days and room temperature (25-30°C) for 5 days. Each sample was daily evaluated for color changes in the labels and meat quality, following method of Iskandar (2014) with modification.

2.4. Color Measurement

Color intensity of the labels was evaluated. The label was scanned using a scanner machine, cropped and tested for its profile of RGB using software ImageJ (Nurfawaidi et al., 2018).

2.5. pH measurement

The pH of chicken meat was determined. The crushed meat (1 g) was mixed with 10 mL of distilled water and tested for pH using pH meter. The calibrated cathode was merged in sample solution and left until the pH was detected. Once used, the cathode was rinsed with distilled water and drained prior to further use (Nurfawaidi et al., 2018).

2.6. Determination of Total Volatile Base

Total volatile base (TVB) of chicken meat was determined as prescribed by SNI (Indonesia, 2011). Sample (10 g) was crushed and mixed with 100 mL of perchlorate acid 6% and stirred for 5 min. The mixture was filtered to collect filtrate. Filtrate (50 mL) was transferred into distillation tube and dropped with phenolphthalein and anti-foaming silicon. Sample-containing tube was set at desiccator and added with 10 mL of NaOH 20% (indicated by red color appearance). In the apparatus, Erlenmeyer containing 100 mL of H_3BO_4 3% and 3-5 drops of Tashiro (methyl red and methylene blue) indicator solution was also set. The sample was distillated for about 10 min to collect 100 mL of distillate, with total volume of 200 mL in the Erlenmeyer (solution displayed as green color). Blank solution was also set to replace sample with perchlorate acid 6% using similar procedures. Distillate solution (sample and blank) was titrated with HCl 0.02 N, until purple color occurred. Titration was performed 2 replications. TVB was calculated as follows:

$$\text{TVB} = (\text{mgN}/100\text{ g}) = \frac{(Vc - Vb) \times N\ HCl \times Ar\ N \times\ Fp}{sample\ weight} \times 100$$

where Vc is volume of HCl in sample titration, Vb is volume of HCl in blank titration, Ar N is atom weight of Nitrogen (14.007), and Fp is dilution factor.

2.7. Statistical Analysis

All experiments were performed at triplicates. Data collected were statistically evaluated using One-Way ANOVA and Duncan test at significance of 5% in software SPSS 25 to observe the changes of RGB, pH, and TVB-N during storage.

3. RESULTS AND DISCUSSION

Sappan extract appears yellow in color at pH 5. This is in accordance with a report by FR & Azmi, (2017) that ethanolic extract of sappan at pH 2-6 was orange-yellow in color. The yellow color of the extract is still consistent when it is applied in the indicator label. The indicator was applied in chicken meat packaging with different storage conditions, i.e. room temperature (5 days) and refrigerator (7 days). As the results, there is a considerable color change between two treatments (Table 1).

Table 1 Color changes of sappan-indicator labels in samples at different storages

Storage conditions	Days							
	0	1	2	3	4	5	6	7
Room temperature (25 - 30°C)								
Refrigerator temperature (2 - 5°C)								

Sappan color indicator demonstrates a color shifting as progress of storage time (Table 1). At room temperature, early color change occurred from yellow to pink (at day 1), while at refrigerator temperature, the color changed into pink at day 6. FR & Azmi (2017) reported that ethanolic sappan extract color was reliant on pH. The color can be altered as pH levels: reddish yellow at acid condition (pH 2-6), red at neutral condition (pH 7), and purplish red at alkaline condition (pH 8-12). In this experiment, the color change in the indicator label is quantified according to total RGB. Besides, TVB-N of samples was also determined.

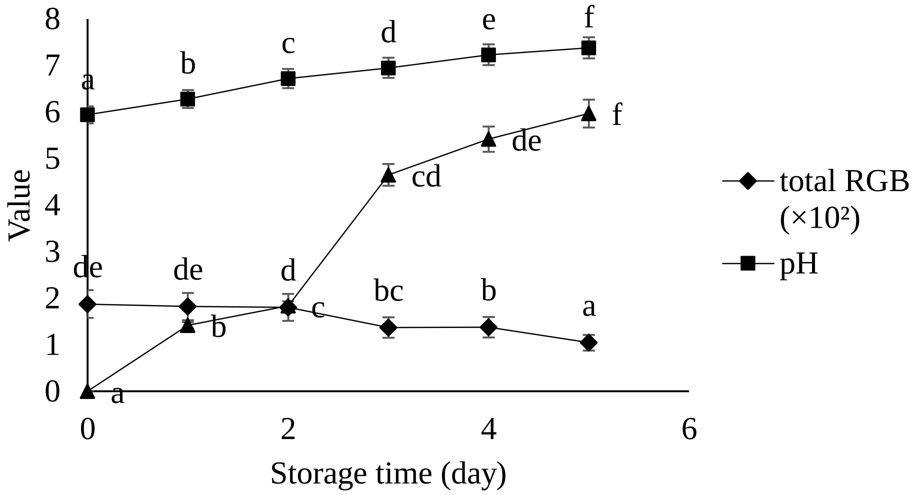

Figure 1 Changes in total RGB, pH, and TVB-N in samples stored at room temperature

The results demonstrated that storage conditions significantly altered total RGB, pH and TVB-N of samples ($p<0{,}05$), as depicted in Figure 1. Total RGB tended to decrease as the increase in storage period. On the contrary, pH and TVB-N increased along with storage time. This confirmed previous finding by Nurfawaidi et al. (2018) reporting that total RGB declined in the indicator label with colorant methyl red and bromocresol purple, while levels of pH and TVB of the samples increased along with storage time. Talukder et al. (2017) also found similar characteristic, in which levels of pH, ammonia, and TVB-N increased during storage at room temperature. The rise of pH results from proteolytic activity by bacteria accounting for formation of NH_3, leading to reduction of protein content. During storage, alkaline condition was more intense which provokes the microbial deterioration (Suradi, 2012). Furthermore, microbial activity during product deterioration was responsible for production of TVB-N.

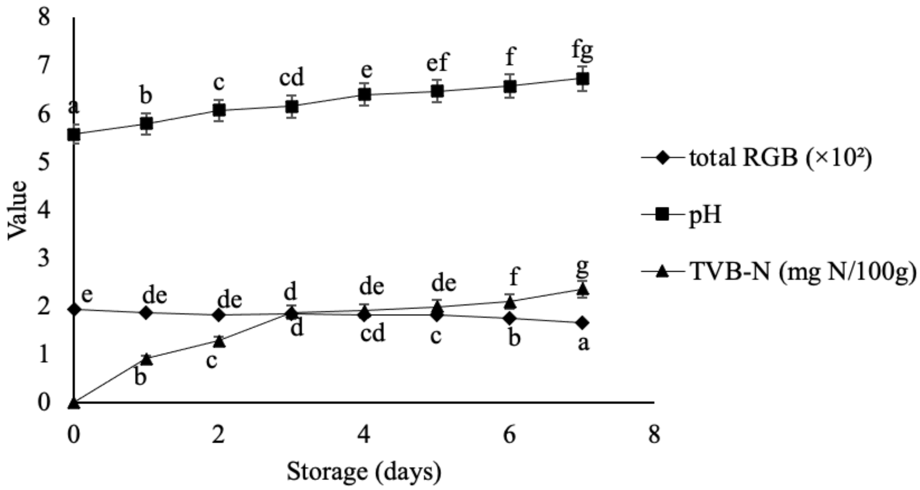

Figure 2 Changes in total RGB, pH, and TVB-N in samples stored at refrigerator temperature

In case of refrigerator temperature, the alteration of RGB, pH, and TVB-N of samples occurred with the same trend as those in room temperature. Total RGB was found to decline, while pH and TVB-N increased remarkably (Figure 2). Samples stored in refrigerator were kept for 7 days, since the change did not appear in the early period of storage. This is understandable that refrigerated meat is less susceptible against microbial attack. Soeparno (2005) reported that metabolic rate of microbial activity relied heavily on temperature. Lower temperature can retard microbial decomposition, extending shelf-life of the product. Compared with samples stored at room temperature, TVB-N of samples at refrigerator temperature did not change significantly. TVB-N of meat stored at room temperature increased drastically (4,65 mg N/100 g) at day 3, while in refrigerator, the highest value of TVB-N was 2,09 mg N/100g in day 6.

As reported by Soeparno (2005), pH level of fresh chicken meat ranges from 5.5 to 6.0. The initial sign of meat destruction occurred when pH was >6.5 (Nitiyacassari, 2019). Therefore, deterioration of refrigerated meat started at pH 6.56 in day 6, while the decay occurred in day 2 (pH 6.72) for samples stored at room temperature. TVB-N represents amount of NH_3 released in the packaging. This induces accumulation of base compounds, resulting in alkaline condition. These compounds actively react with indicator colorant causing color changes from yellow to red. As displayed in Figure 3, pink color represents meat decay, specifically at pH >6.0 and TVB-N >1 mg N/100g.

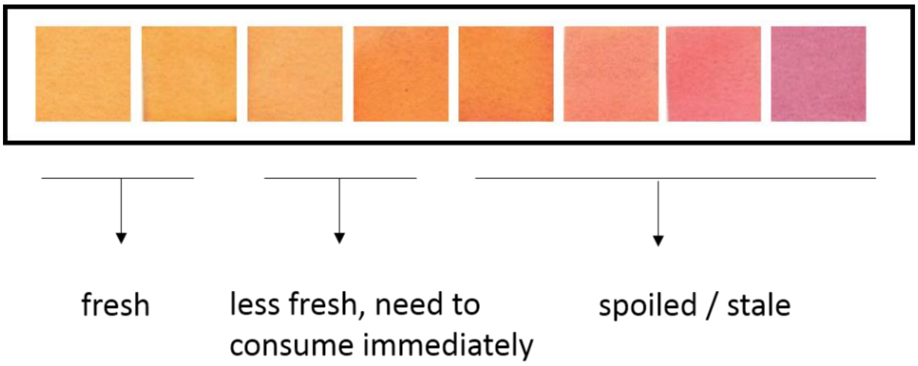

Figure 3 Colors indicating status of meat freshness. Left: fresh; middle: less fresh, need to consume immediately; right: spoiled / stale

Figure 3 exhibits color variation representing freshness of the meats. Color in these labels is meaningful indicator particularly for temperature-sensitive products. Yellow color means that the product is at good quality. When it turns into light-dark orange, the meat needs to cook immediately. Pink and bluish red color indicates spoiled product, suggesting that the meat should not be consumed. Color display in the label informs product freshness to consumers, enabling them to be more selective. Color change may also indicate improper storage conditions.

4. CONCLUSION

Sappan extracts containing brazilin, used for development of quality indicator for chicken meat. Sappan indicator label shows color changes as response to chemical condition during storage. The label also enables to show color change from yellow to pink when pH and TVBN reached critical limit of meat freshness. The developed indicator represents a simple and visual method to detect quality degradation of flesh foods. The recommendation in this study is the need to validate the quality of chicken meat by microbe and sensory testing

ACKNOWLEDGMENT

The authors gratefully acknowledge the Institute for Research and Community Service, Universitas Djuanda Bogor, for funding this research through internal research grant No. 150/LPPM/K-X/VIII/2021

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