ATLANTIS PRESS

Advances in Health Sciences Research, volume 26

2nd Bakti Tunas Husada-Health Science International Conference (BTH-HSIC 2019)

Utilization of Anthocyanin Extract from Rambutan Fruit Rind (*Nephelium lappaceum* l.) as an Indicator of the Quality on Freshness Meat

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***Abstract*— Chicken meat is the most consumed food. In Indonesia, meat is one of the contributors to poisoning because meat has a high protein and nitrogen content and environmental conditions suitable for the growth of microorganisms. Both dyes from nature and synthesis have been developed as the detection for the presence of substances or the occurrence of a process. One of the dyes from nature is anthocyanin. Making indicators containing anthocyanin dyes can be an alternative for identifying chicken meat safety. The purpose of this study was to know the ability of rambutan fruit rind extract as an indicator of chicken meat spoilage based on color changes. Rambutan fruit rind was extracted using the maceration method with methanol : HCl (9:1) solvent. The indicator solution was made from extract as much as 5%, to be applied to filter paper by soaking for 24 hours and centrifuging for 15 minutes, each room temperature dried and 60 ºC for overnight. Testing is based on observing the color changes in the indicator that have been applied to chicken meat at various storage times, and then compared to the litmus paper. The results showed that anthocyanin-based indicators can be used as an indicator of chicken meat spoilage, but are less sensitive compared to litmus paper**

***Keywords*: *anthocyanins, chicken meat, indicator, spoilage, rambutan fruit rind* (Nephelium lappaceum L.)**

I. INTRODUCTION

In 2018 according to data from the Indonesian Ministry of Agriculture, total meat production in Indonesia is 3.5 million tons per year starting from chicken, beef and buffalo meat (Ministry of Agriculture, 2017). The meat produced is cut the carcass in an edible offal area for a certain time. Generally, cicken meat has a shelf life of 5-7 days (at temperatures of 0-10 ℃). Chicken meat can experience a decrease in quality, caused by damage due to biochemical, physicochemical and microbiological processes. Besides that, the high protein and water content in chicken meat enables microorganisms (proteolytic) to grow, besides being supported by a suitable environment. Poisoning due to food consumption in Indonesia, one of which is widely reported in Surabaya which causes zoonosis disease (a disease that can be transmitted from animals to humans and vice versa) and several digestive health problems [11].

In recent years there have been many studies that have developed techniques for making detection systems of certain decomposition of materials using pH-based indicators. To reduce the consumption of decomposed chicken meat, it can be identified using indicators. This indicator is one of the developments to clarify the presence or absence of certain substances or interactions in a material, through visual changes such as color. Dyes have been widely used as materials or markers on indicators, such as anthocyanins [8].

In this study utilizing anthocyanin from rambutan fruit rind extract as a color change due to an increase in pH value, to see the ability of rambutan fruit rind extract as an indicator of chicken meat spoilage based on changes in color. Rambutan rind is used because it is one source of anthocyanin dyes that is still not widely used. Anthocyanin can be used as a natural pH indicator [13],[14].

II. MATERIAL AND METHOD

A. Tools and materials

The tools used in this study are scales, maceration bottles, *rotary evaporator* (Eyela®), erlenmeyers (Pyrex®), *Beaker glass* (Pyrex®), stirring rods (Pyrex®), spatulas, cotton, glass funnels (Pyrex®), test tube (Pyrex), dropper pipette, vaporizer cup (RRC®), silicate exchange rate, oven (Memmert®), furnace (Thermo Scientific), aluminum foil, *waterbath* (Memmert®), desiccator, litmus, filter paper (Whatman®), and UV-Vis spectrophotometer (Cary 60 Agilent®).

Test materials used in this study were the rambutan fruit rind, methanol, hydrochloric acid (HCl), NaOH (Merck®), buffer pH solutions 1-12 [1], and reagents for phytochemical screening (HCl 2 N; HCl 10% ; H_2SO_4 P; Wagner LP reagents, Mayer LP, Dragendorff LP; magnesium P powder; anhydrous acetic acid; chloroform; aquadest; 1% $FeCl_3$; ether; 10% NaOH; 10% NaCl and $CuSO_4$ (copper sulfate).

B. Sample Preparation

Previous, plants rambutan were determined at Jatinangor Herbarium Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Bandung. Rambutan rind samples (*Nepphelium lappaceum* L.) obtained were sorted wet and cut into small pieces. Rambutan rind samples are then washed thoroughly with running water then chopped and dried in an oven at temperatures below 60 ℃. Samples that have been

dried, sorted dry and weighed and then mashed using a blender to obtain rambutan fruit rind powder.

C. Procedure

Extraction

Extraction is done by the maceration method. A total of 300 grams of powdered rambutan fruit rind (*Nepphelium lappaceum* L.) was macerated using a mixture of methanol and 1% HCl (9:1). Substitution of solvents carried out for 1 x 24 hours [12]. The collected filtrate is then concentrated using a *rotary evaporator* until a thick extract is obtained. The resulting viscous extract was weighed and recorded by weight then stored in a refrigerator or freezer [6].

Phytochemical Screening

Rambutan fruit rind extract was analyzed qualitatively secondary metabolites in the form of flavonoids, saponins, polyphenols, tannins, quinones, alkaloids, steroids and triterpenes, monoterpenes and sesquiterpenes.

Anthocyanin Qualitative Test in Rambutan Fruit Rind

Rambutan rind extract was added with HCl 2 M then heated at 100 ℃ for 5 minutes, with the characteristic non-fading red color of anthocyanin and a red color change to blue-green, then slowly faded when added 2 M NaOH solution drop by drop [12].

Determination of Anthocyanin Levels in Rambutan Fruit Rind Extract

Anthocyanin extract from rambutan fruit rind was dissolved into methanol : 1% HCl solution and then the maximum wavelength was determination is performed in the range of 200-800 nm on a UV-Vis spectrophotometer. Then, the total anthocyanin content can be determined using the differential pH method [4],[9]. Anthocyanin extract was dissolved into buffer pH 1 and buffer pH 4.5. The solution was incubated for 15 minutes at room temperature then the absorbance was measured at the maximum wavelength of the sample at 700 nm. Then anthocyanin levels can be calculated by the equation:

$$\text{Anthocyanin levels} = = \frac{A \; X \; WM \; X \; DF \; X \; 1000}{\varepsilon X 1}$$

$$A = ((A\,\lambda_{max} - A_{700})_{pH\,1} - (A\,\lambda_{max} - A_{700})_{pH\,4,5})$$

Where A: absorbance at maximum wavelength; WM: molecular weight of compounds (g / mol) (Cyanidin-3-glucoside = 449.2 g / mol); DF: sample dilution factor; l: cuvette width (1 cm); and ε: molar absortivity constant (26,900 L / mol / cm).

Anthocyanin Characteristics of Rambutan Fruit Rind Extract as a Color Substance on Indicators

The characteristics of the dye are carried out by determining the color route at the addition of pH 1-12, using a buffer solution. Each solution from pH 1 to 12 as much as 2 ml was added with anthocyanin extract and color changes were observed so that a route was obtained according to the pH of the solution [18].

Anthocyanin Testing as an Indicator of Meat Spoilage

Indicator Preparation

The application of an indicator solution of 5% (g / 100 mL) extracts on the indicator media was carried out by two different methods namely indicator A made by soaking filter paper for 24 hours and drying at room temperature overnight [13][14], while indicator B is made by soaking filter paper measuring 1.5 cm x 5 cm in the indicator solution and centrifuged at 3000 rpm for 15 minutes then dried overnight at 60 °C[16].

Testing Indicator for Chicken Meat Spoilage

Chicken meat is a carcass that is taken directly after the cutting process. Chicken meat is stored at storage time with a variation of time 1, 3, 5, 7, and 14 days respectively at room temperature and the refrigerator or freezer. Chicken meat is given an airtight plastic and water package in a container that is applied to indicator paper and compared to litmus paper. Observations were made by looking at physical characteristics and color changes that occur in the indicator [13],[14].

III. RESULTS AND DISCUSSION

A. Preparation

The determined sample is rambutan fruit rind obtained from several regions in the Tasikmalaya area to ensure the identity of the plant based on its physiology and morphology. From the results of the determination conducted, rambutan fruit rind is a plant that comes from the species of *Nephelium lappaceum* L. with the *Sapindaceae* family.

B. Extraction

From 2 kg rambutan fruit rind, 900 grams of dried powder was obtained. A total of 300 grams was extracted using the maceration method with methanol and 1% HCl (9 : 1) solvent for 3 x 24 hours. The yield extraction obtained was 67.57%.

C. Phytochemical Screening

The results of phytochemical powder and extract of rambutan fruit rind can be seen in Table 1.

TABLE 1. RESULTS OF PHYTOCHEMICAL POWDER AND THE EXTRACT OF RAMBUTAN RIND (*Nephelium lappaceum* L.)

Phytochemical Group Compound	Powder	Extract
Flavonoids	(+)	(+)
Saponins	(+)	(+)
Polyphenols	(+)	(+)
Tanin	(+)	(+)
Quinone	(-)	(-)
Alkaloids	(-)	(-)
Steroids and Triterpenoids	(-)	(-)
Cepenoid and Monoterpenoid	(+)	(+)
Note : (+) detected; and (-) not detected.		

D. Anthocyanin Qualitative Test in Rambutan Fruit Rind

The anthocyanin qualitative test of rambutan fruit rind extract can be seen in Figure 1.

Figure 1. Qualitative anthocyanin test of rambutan fruit rind extract (HCl; left, and NaOH; right)

At the time of acid addition, anthocyanin is stable because it is in the form of the cavity flavilium which is indicated by the fading red color. In neutral conditions or flavilium cation bases become unstable as a result when added to the NaOH solution, the sample solution changes color ie it turns green and then fades slowly.

E. Determination of Anthocyanin Levels in Rambutan Fruit Rind Extract

The results of the determination of the wavelength showed that the rambutan fruit extract was at λmaks 530 nm. Anthocyanin is at a wavelength of 475 - 560 nm. This shows that the rambutan fruit extract contains anthocyanin type cyanidine [9]. Based on research conducted by Giusti and Wrolstad [4],[9], the total anthocyanin content can be determined using the differential pH method. Anthocyanin extract was dissolved into buffer pH 1 and buffer pH 4.5 and then absorbed at the maximum wavelength of 530 nm and 700 nm. Total anthocyanin level was calculated as equivalent to cyanidine 3-glucoside anthocyanin. The results of the determination of total anthocyanin levels calculated as cyanidine 3-glucoside using the differential pH method are 55.5269 mg / L.

F. Anthocyanin Characteristics of Rambutan Fruit Rind Extract as a Color Substance on Indicators

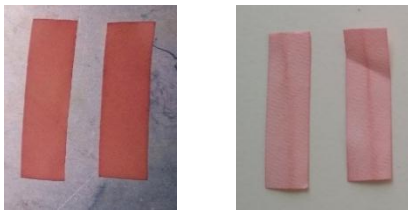
Characteristics of dyes in the addition of a buffer solution at pH 1-12, can be seen in Table 2. The red change in anthocyanin rambutan fruit rind extract on the addition of a buffer solution at a different pH can show the characteristics of anthocyanins that are not stable in a neutral or basic atmosphere. In the anthocyanin rambutan rind extract changes in pH 1-12 in succession from red, pink, brown to green.

TABLE 2. CHARACTERISTICS OF DYES OF RAMBUTAN FRUIT RIND EXTRACT (*Nephelium lappaceum* L.)

pH	Colour
pH 1	Red
pH 2	Red
pH 3	Red
pH 4	Pink
pH 5	Pink
pH 6	Light-brown
pH 7	Brown
pH 8	Brown
pH 9	Brown
pH 10	Brown
pH 11	Brown-green
pH 12	Green

G. Anthocyanin Testing as an Indicator of Meat Spoilage Indicator

The results of indicator preparation can be seen in Figure 2.



a b

Figure 2. Anthocyanin indicator of rambutan fruit rind (*Nephelium lappaceum* L.); (a) Indicator A, and (b) Indicator B.

In the method of making indicator A, the resulting media color of indicator paper is darker than the method of making indicator B which produces a faded red color. In indicator B, the effect of centrifugation can reduce the thickness of the extract, so that the absorption of the extract solution in the filter paper media lighter particles will be absorbed. During the centrifugation process, the substance will undergo particle

separation based on the weight of the particle to the density of the kite, so that the particle density is higher than the solvent down (sediment) and lighter particles float upwards [5].

TABLE 3 RESULTS OF PHYSICAL OBSERVATIONS (TEXTURE) OF CHICKEN MEAT STORAGE AT ROOM TEMPERATURE AND FREEZER WITH VARIATIONS IN STORAGE TIME

Days	Texture	
	Room (27℃)	***Freezer* (-15℃)**
1	Pretty elastic (springy)	Pretty elastic
3	Already elastic	Pretty elastic
5	Rather easily destroyed	Pretty elastic
7	Easily destroyed	Rather easily destroyed
10	Easily destroyed	Rather easily destroyed
14	Easily destroyed	Easily destroyed

TABLE 4 RESULTS OF PHYSICAL OBSERVATIONS (SMELL) OF CHICKEN MEAT STORAGE AT ROOM TEMPERATURE AND FREEZER WITH VARIATIONS IN STORAGE TIME

Days	Smell	
	Room (27℃)	***Freezer* (-15℃)**
1	Fishy	Fishy
3	Rather fishy smell	Rather fishy smell
5	The pungent odor	The pungent odor
7	The stench is very strong	The stench is very strong
10	The stench is very strong	The stench is very strong
14	The stench is very strong	The stench is very strong

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TABLE 5 TEST RESULTS OF CHICKEN MEAT SPOILAGE AT ROOM TEMPERATURE USING ANTHOCYANIN INDICATORS

Days	Room temperature			
	Indicator A	**Indicator B**	**Litmus blue**	**Litmus red**
0	red	red	red	red
1	red	red	Red-purple	Red-purple
3	Red-brown	Red-brown	Blue	Blue
5	Light-green	Light-green	Blue	Blue
7	Green	Green	Blue	Blue
10	Green	Green	Blue	Blue
14	Green	Green	Blue	Blue

TABLE 6 TEST RESULTS OF CHICKEN MEAT SPOILAGE AT FREEZER TEMPERATURE USING ANTHOCYANIN INDICATORS

Days	*Freezer* temperature			
	Indicator A	**Indicator B**	**Litmus blue**	**Litmus red**
0	red	red	Red-purple	red
1	red	red	Red-purple	red
3	red	red	Red-purple	red
5	red	red	Blue	Red purple
7	red	red	Blue	Blue
10	Red-brown	Red-brown	Blue	Blue
14	Green	Green	Blue	Blue

The results of observations of the physical characteristics of chicken meat can be seen in Table 3. Meat stored in the freezer has a longer shelf life than meat stored at room temperature Meat can stay in the freezer for 7 days but at room temperature the meat and only lasts 3-5 day. In testing the storage of meat at room temperature, it can be seen in Table 3.3 that both the indicator and litmus undergo color changes starting on day 3. While at freezer temperature (Table 3.4) an increase in basicity is seen starting on day 10, and on litmus paper red discoloration begins on days 5.

Compared to storage at room temperature, chicken meat stored in the freezer has a longer shelf life. The use of plastic in packaging chicken meat storage can also maintain the quality of chicken meat such as changes in pH. The requirement or pH value of chicken meat is below 6.

The interaction that occurs in the indicator is an interaction due to an increase in the ammonia base due to the influence of microorganisms that occur in chicken meat. This change in acidity to base is seen when the indicator paper changes color to light green to green. In testing anthocyanin indicators from rambutan fruit rind, anthocyanin can be used as an indicator of monitoring the quality of chicken meat through the process of decomposition. Both indicator A and indicator B show less sensitive results when compared to litmus paper, but have a better entity value in the testing of chicken meat spoilage. The value of this entity can be seen from the anthocyanin extract indicator of rambutan rind is more low-cost because the dyes used come from nature and can detect decay by changes in physical characteristics of chicken meat. This is also supported by research conducted by Ghollasi-mood et al [3] that at 0 ℃, chicken meat can last up to 300 hours.

IV. CONCLUSION

Based on research that has been done, anthocyanin from rambutan fruit rind extract (*Nephelium lappaceum* L.) can be used as an indicator of spoilage of chicken meat based on color changes. From testing the indicator of chicken meat decay anthocyanin-based indicator paper from rambutan fruit rind has a better entity value, but it is less sensitive when compared to litmus paper. It is recommended that this research be continued to increase the sensitivity of anthocyanin indicators and the use of other methods in anthocyanin testing as indicators on monitoring the quality of chicken.

ACKNOWLEDGMENT

This article is part of the assignment for the first author to complete bachelor studies (S1) in the STIKes Bakti Tunas Husada Tasikmalaya of Education. Thank you very much to Tresna Lestari, M.Si., Apt and Ade Yeni Aprillia, M.Si. who have contributed to the completion of this article.

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