



Chemosensor Strip from Kepok Banana Bracts Extract (*Musa paradisiaca* L.) for Detection of Tuna Freshness

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Abstract

Anthocyanins as chemosensory compounds for amines have been tested in this study. Because anthocyanins are sensitive to pH changes, while amines have an alkaline nature, they can cause structural changes in anthocyanins, resulting in changes in the color of anthocyanins. The source of anthocyanins was the Kepok banana bracts (*Musa paradisiaca* L.), which were extracted using a mixture of ethanol:HCl 0.15% (3:2). The types of anthocyanin compounds were characterized using a UV-Vis spectrophotometer. The anthocyanin content obtained varied from 1.26 mg/100 g to 5.08 mg/100 g. The type of anthocyanin in the Kepok banana bracts was found as a cyanidin-3-rutinoside with maximum absorption at 513 nm at pH 1. The color of anthocyanin extract varied with changes in pH; it turned red in acid and faded in neutral solutions. The green color in the alkaline solution changes to brownish-yellow was associated with anthocyanin degradation. The color change at different pH indicates that banana bracts are regarded as a potential chemosensory compound to detect tuna freshness. The chemosensor was applied to a cellulose-based strip and exhibited a color change that corresponded to the increase in pH and was comparable to the results of the pH meter measurement. The structural changes of anthocyanin before and after the tuna freshness test were identified by the FTIR-ATR, indicating a change in the anthocyanin structure. Tuna freshness began to diminish after being stored for 12 and 24 hours at room temperature, marked by a color change of the paper strip to colorless and blackish gray.

1. Introduction

The high nutritional content of tuna makes it one of the most popular foods globally [1]. However, tuna is perishable during processing and storage; some rogue sellers also sell chemically-treated metamorphic meats to make them look fresh, which will cause food poisoning [2]. As a result of this phenomenon, the freshness of tuna is quite challenging to detect without using indicators. The freshness of tuna itself should be an essential concern because of the high content of biogenic amines in spoiled tuna, which can be harmful to health. One type of biogenic amine is histamine. The higher histamine content is directly proportional to the level of fish spoilage, where high histamine content can cause histamine poisoning [3]. Thus, detecting fish freshness is necessary

to prevent various diseases caused by the consumption of spoiled fish.

Measurement of histamine or trimethylamine (TMA) content can be an accurate indicator in measuring fish freshness. In a previous study, fish freshness detectors have been developed, such as the abient-pressure HePI-MS for directly measuring trimethylamine levels in samples [4], the HPLC method [3], and other detectors. Unfortunately, these detectors require expensive equipment that takes up space and time. Chemosensor is a simple, relatively inexpensive, and efficient analytical method. The use of chemosensors to detect tuna freshness can be the right solution for measuring fish freshness by the wider community and various food industries.

Anthocyanins are flavonoid compounds widely found in plants as producers of plant pigments that appear in various colors, from orange and red to blue and purple [5]. The basic chemical structure of anthocyanins is 3,5,7-trihydroxy-2-phenylbenzopyran [6]. Anthocyanins can interact with amine compounds through hydrophobic, electrostatic, and hydrogen bonding interactions [7]. Amines compounds that can provide an alkaline pH atmosphere can cause changes in the structure of anthocyanins, causing color changes [8]. This makes anthocyanins a compound that can be used as a chemosensory compound.

Anthocyanins, as natural plant pigments [9], are widely found in grapes and raspberries [10], red cabbage [4], sweet potatoes [3], and the crown of some flowers [11]. The source of anthocyanins used in this study was the Kepok banana bracts (*Musa paradisiaca* L.). The content of anthocyanin dyes in banana bracts, which can detect biogenic amines in tuna, can be used as a chemosensor [12]. The banana bracts are a source of anthocyanin that has not been widely used and is one of the commodities that is readily available, especially in Indonesia. Because the banana is not a seasonal fruit, the bracts can be readily available at an affordable price throughout the year. The anthocyanin content in red cabbage can be equivalent to anthocyanin in banana bracts, which has been widely studied [12]; thus, banana bracts still have potential as a source of anthocyanins. Apart from its high anthocyanin content, the utilization of banana bracts is also an effort to recycle waste because the part used is the outer bracts that are generally rarely used or have no economic value.

As previously described, anthocyanins extracted from various fruits can be used as active compounds for chemosensors. The extraction step of natural dyes plays a vital role in the yield and purity of the obtained extract, so selecting the suitable solvent should be of particular concern. Anthocyanins from natural products can be extracted using various solvents, such as HCl, methanol, ethanol, and acetone [13]. Extraction using acetone followed by methanol or acidified ethanol exhibited the best values for anthocyanin content [13]. However, since acetone and methanol have high toxicity, ethanol can be safely used in the food industry instead [14].

The application of anthocyanins as chemosensors is effortless by employing cellulose-based strips. The hydroxy group in anthocyanin compounds can form glycoside bonds with glucose compounds [15, 16, 17]. These glycoside bonds cause anthocyanins easily bound to cellulose polymers. Chemosensor compounds applied to cellulose-based strips have been proven to facilitate the application of chemosensors and can provide apparent color changes [18]. The changing color of the anthocyanin strips can detect changes in a narrow range according to the pH of spoiled fish. Another advantage is that these anthocyanin strips can be a much more affordable solution than existing fish freshness labels. Employing anthocyanin strips for chemosensor applications is expected to provide time and cost efficiency and enhance the stability and sensitivity of anthocyanin as chemosensors.

2. Methods

2.1. Tools and Materials

The tools were laboratory glassware, containers, blender, mortar, pistil, dark bottles, analytical balance (Libror Scale EV-330 Shimadzu), Büchner funnel, evaporator, and vials. The instruments were UV-Vis spectrophotometer (Shimadzu UV-1800) and FTIR-ATR Nicolet iS5 from ThermoScientific.

Kepok banana bracts were purchased from Sayon Jogja (e-commerce), tuna was purchased from the Kranggan traditional market in Yogyakarta, Indonesia. Ethanol (analytical grade), HCl solution, and NaOH pellets were supplied from E-Merck. pH buffer (pH 1, 4, 6.9, and 10), distilled water, and Whatman Grade 42 filter paper were obtained from the general chemical store.

2.2. Extraction of Anthocyanin from Banana Bracts

The banana bracts were washed and then crushed using a blender and soaked in saltwater. Banana bracts (150 g) were dried in an oven at 70°C for 24 hours. Each dried and wet banana bracts was weighed as much as 83.45 g and then dissolved in 250 mL of 0.15% ethanol:HCl mixture (3:2). Sonication was performed for 20 minutes at around 51°C. The results were filtered and then evaporated at 55°C and pressure of ± 100 mBar. The solution was placed in a dark bottle lined with aluminum foil and stored in a refrigerator at about 14°C [19].

2.3. Determination of Anthocyanin Content

The anthocyanin filtrate solution from banana bracts (3 mL) was adjusted to pH 1 or 4.5 and added the appropriate buffer solution until the volume of the solution became 10 mL. The absorbance was measured at 700–200 nm using a UV-Vis spectrophotometer [12]. The determination of anthocyanin content was repeated three times.

2.4. Sensitivity of Anthocyanins at Various pH

The appropriate buffer (8 mL) was added to 0.675 mL anthocyanin filtrate solution. After that, the solution was adjusted to pH ranging from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 13.7. The solution was put into a 10 mL volumetric flask followed by a buffer up to the mark. The absorbance of each solution was measured at 700–200 nm with a UV-Vis spectrophotometer.

2.5. Preparation of Anthocyanin Strip

Whatman Grade 42 filter paper was cut into 5 x 1 cm, then heated in an oven for 10 minutes at 70°C. The dried filter paper was put into anthocyanin solution (9 mL), centrifuged for 4 minutes, and then dried in the oven for 10 minutes at 70°C. The strips were tested by adding a dilute NaOH solution.

2.6. Tuna Freshness Detection

Qualitative identification of spoilage levels of tuna meat was determined by observing the change in the color of the filter paper. The naked human eye can easily detect this color change. The tuna sample was first mashed using a blender, and then 10 g of tuna meat was put into

the vial. Each vial was stored with time variations of 0, 3, 6, 12, and 24 hours at room temperature and in the refrigerator (0°C). Each sample was attached to an anthocyanin strip for ±10 seconds.

A total of 10 mL of distilled water was added to the vial and stirred briefly to obtain the extract. The anthocyanin strip was immersed in the extract and measured using a pH meter. The effectiveness of anthocyanin strip and anthocyanin solution as a chemosensor was compared by adding 5 drops of anthocyanin extract to tuna meat extract. The strips used for the tuna freshness test were then observed for structural changes of anthocyanin using the FTIR-ATR. All color changes were observed and recorded. The freshness analysis of tuna using anthocyanin strips was compared with the total volatile basic nitrogen (TVB-N) test data at the same meat storage time range (0, 3, 6, 12, and 24 hours).

2.7. Analysis Method

The anthocyanin content was calculated as cyanidin-3-glucoside using the following equation:

$$A = (A_{510} - A_{700})pH_1 - (A_{510} - A_{700})pH_{4.5}$$

$$\text{Anthocyanin content (mg/kg)} = \frac{A \times V \times MW}{\epsilon \times W \times 1000} \times 10^6 \quad [24]$$

UV-Vis absorption (absorbance) and FTIR-ATR transmittance data were processed using OriginPro 2018 software.

3. Results and Discussion

3.1. Anthocyanin Content in Banana Bracts

The method of isolation of anthocyanins from banana bracts was sonication. Sonication utilizes ultrasonic rays associated with the cavitation phenomenon; therefore, this method can work more efficiently, quickly, cost-effectively, and gives high reaction yields [19, 20]. The extraction results showed a difference in color where the wet banana bracts formed a bright red solution while the dry ones had a brown solution. Drying samples at 70°C would inhibit the isolation of anthocyanin compounds from banana bracts due to degradation. The high temperature and the duration of heating cause the degradation of anthocyanins to become brown chalcone products [21]. Further tests were conducted on the wet banana bracts extract.



Wet banana blossom extract Dried banana blossom extract

Figure 1. Anthocyanin extracts from wet and dried banana bracts

The solvent concentration and the solvent to solute ratio significantly affected the extraction of anthocyanins. A high ethanol concentration as a solvent allows a high extraction yield. However, pure ethanol is not recommended because the low water content can reduce the effectiveness of binding hydrophilic anthocyanin compounds. In addition, the effect of the solvent to solute ratio is also directly proportional to the number of anthocyanins obtained [19].

Anthocyanin stability is influenced by pH, temperature, and light exposure [21]. The anthocyanin structure changes reversibly at different pH conditions. High temperatures accelerate the rate of anthocyanin degradation. The presence of oxygen and interactions with other compounds are also possible at high temperatures. Anthocyanins can undergo photo-oxidation with the minor product p-hydroxybenzoic acid at constant light exposure. Hydroxy-substituted anthocyanins at C-5 undergo fluorescence; therefore, phytochemical decomposition occurs more effortlessly than unsubstituted compounds [22]. In this study, the banana bracts that had been crushed were immersed in salt water to prevent spoilage. Extracted anthocyanin solutions were also stored in dark room at low temperatures. Anthocyanin degradation occurred when the extract was stored at 27°C for 10 days in light room, marked by a color change to red-orange. Figure 2 shows the color of the solution at various storage variations.

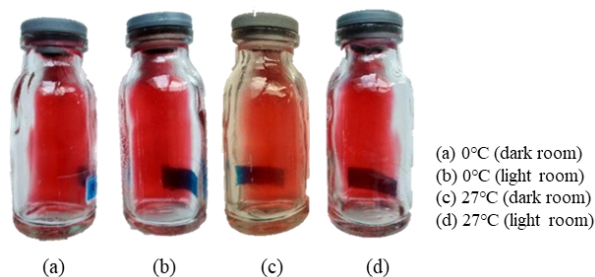


Figure 2. Stability of anthocyanins stored for 10 days

The red anthocyanin solution was determined quantitatively by using the pH differential method. At pH 1, anthocyanins are generally in the form of colored flavylium or oxonium cations. While at pH 4.5, anthocyanins are present in the form of carbinol or colorless hemicals. This principle causes the pH differential to provide a reasonably accurate and rapid measurement of total anthocyanins [23].

Figure 3 shows the absorption in UV-Vis spectra from 700–200 nm for anthocyanin solutions at pH 1 and 4.5. The anthocyanin content in the Kepok banana bracts can be determined based on the absorption at 510 and 700 nm. The calculation of anthocyanin levels using the differential method repeated 3 times resulted from different Kepok banana bracts are 1.26 mg/100 g, 4.72 mg/100 g, and 5.08 mg/100 g.

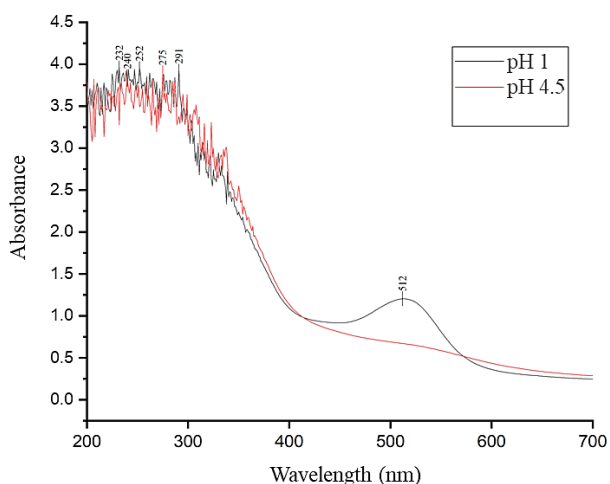


Figure 3. UV-Vis spectra for anthocyanin solutions at pH 1 (blue) and pH 4.5 (red)

3.2. Determination of Anthocyanin Compounds in Banana Bracts

Anthocyanin compounds have several types that can be classified according to Table 1.

Table 1. Anthocyanin structure [7]

Anthocyanin structure	R1	R2	Anthocyanins
	-OH	-H	Cyanidin
	-OH	-OH	Delphinidin
	-H	-H	Pelargonidin
	-OCH ₃	-H	Peonidin
	-OCH ₃	-OH	Petunidin
	-OCH ₃	-OCH ₃	Malvidin

The type of anthocyanin in the Kepok banana bracts extract was qualitatively concluded based on UV-Vis spectra (Figure 2). UV-Vis spectra of banana bracts extract at pH 1 showed maximum absorption at 236, 281, and 513 nm wavelengths. The peak at 514 nm was identified as cyanidin-3-rutinoside, while at 512 nm was peonidin-3-glucoside [24]. The absorption for peonidin and cyanidin compounds was similar [25]. However, another study that also conducted anthocyanin extraction concluded that the predominant compound was cyanidin-3-rutinoside which had UV-Vis absorption at 281 nm [26]. In addition, 80% of the compounds contained in the banana bracts are cyanidin-3-rutinoside [5]. Therefore, the main anthocyanin compound in the banana bracts was predicted to be cyanidin-3-rutinoside (Figure 4).

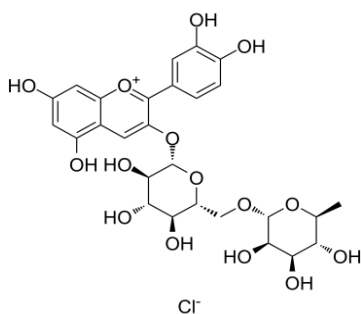


Figure 4. Cyanidin-3-rutinoside structure

3.3. Effect of pH and Stability of Anthocyanins

The maximum absorption of anthocyanin solution changes for a solution of pH 1 to 13.7, as shown in Figure 5. There was a hypsochromic shift (blueshift) from acidic to alkaline medium. Changes in the maximum absorption of anthocyanins occurred at pH 1 at a wavelength of 513 nm to 403 nm at pH 13. A wavelength of 400 nm indicates an increase in absorbance from acidic to alkaline medium. Color changes and shifts in the peak of anthocyanin absorption are caused by structural changes of anthocyanin at each pH [27]. This shift in wavelength in the visible region indicates that anthocyanins play a significant role as chemosensory compounds involving differences in pH.

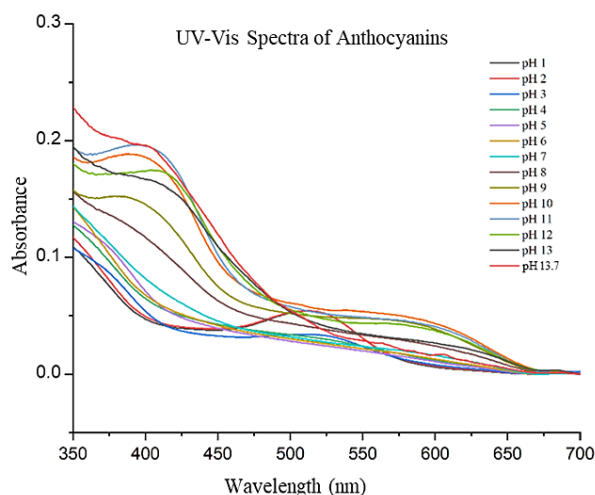


Figure 5. UV-Vis spectra of anthocyanin solutions at all pH

Through the research that has been done, the color of the anthocyanin solution is different at each pH. When anthocyanins were exposed to acidic conditions (pH < 7), the color of the solution became redder. Anthocyanins are much more stable at acidic pH due to the presence of flavylium cations. Meanwhile, under alkaline conditions (pH > 7), the color of the solution faded and turned green to yellow (Figure 6).



Figure 6. The color of anthocyanin solutions at pH variations

As the pH increases, the anthocyanin structure will be degraded [28] and cause instability in anthocyanin. The anthocyanin became colorless starting from pH 4 because their structure began to form a pseudo base that underwent tautomerization between the keto-enol form and produced alpha diketones. Furthermore, it formed a quinoidal base at pH 9. There was a hypochromic shift with increasing pH due to the loss of conjugation in the anthocyanin structure. At pH 11, anthocyanin began to degrade completely to form chalcone compounds [29, 30]. This structural change is depicted in Figure 7.

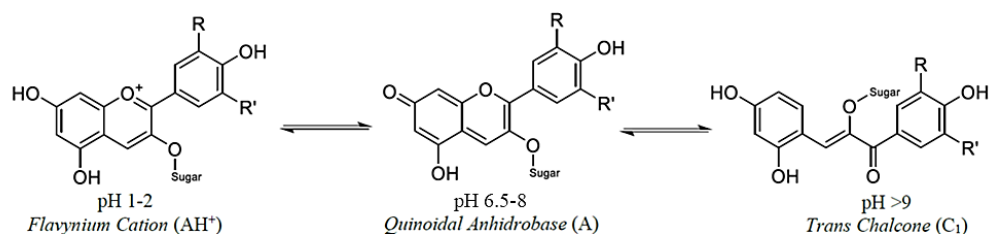


Figure 7. Structural changes of anthocyanins due to differences in pH [29]

Table 2. Stability of anthocyanin strips stored for 10 days





	Before the addition of alkaline solution (Anthocyanin phytochemical indicators)	After the addition of alkaline solution (Anthocyanin phytochemical indicators)
Dark room		
Light room		

Table 2 shows that after 10 days of storage, the color of the anthocyanin strips did not change, which was pink. The stability of the anthocyanins strip, both in the dark and light room, was tested by phytochemicals. The addition of the base causes a color change to light green, indicating the presence of anthocyanins.

3.4. Anthocyanin Strip Test on Tuna

Anthocyanin strip test for the freshness of tuna has been performed by placing the anthocyanins strip directly on the meat and the extract of tuna. Tuna meat and extract were stored at different temperatures (0 and 27°C). Tests were also conducted by adding anthocyanin solution directly to the tuna extract. The test was carried out with variations in storage time ranging from 3, 6, 12,

and 24 hours. The color changes on the anthocyanin strips are shown in Table 5.





































Table 3. Organoleptic results of tuna at 0°C and 27°C as a function of time

Storage temperature (°C)	Storage time (hour)				
	0	3	6	12	24
0	The shape did not change, bright pink color, had a fresh smell, the texture was firm and dense	fresh smell, reddish in color, had a firm texture	fresh smell, reddish in color, a soft texture	a fishy odor, the color turned brown, a soft texture	Smell little fishy, had a red color, a soft texture
27		fresh smell, the color changed to brownish-red, had a firm texture	a fishy odor, the color was brownish-red, had a firm texture	Strong fishy odor, had a brown color, soft texture	Overly fishy odor, had a brown color, soft texture

Table 4. pH of tuna extract

Storage temperature (°C)	Storage time (hour)				
	0	3	6	12	24
0	5.7	5.8	5.9	6.1	6.3
27	5.7	5.8	5.9	6.6	6.9

Table 5. Color change of anthocyanin strips and anthocyanin solution

Storage temperature (°C)	Initial	Storage time (hour)				
		0	3	6	12	24
Tuna meat						
0						
27						
Tuna extract						
0						
27						
A direct test of anthocyanin solution on extract						
0						
27						

Based on the organoleptic observations by the panelists in Table 3 and the color change of the anthocyanin strips in Table 5, it can be concluded that the tuna freshness at a storage temperature of 27°C and storage time of 12 and 24 hours was diminished. While at 0°C for 24 hours, the tuna was still relatively fresh. Anthocyanin strips showed color changes at each storage time. Anthocyanin strips tested directly on tuna meat showed a color change from pink to green to blackish gray, while anthocyanin strips tested directly on the tuna extract showed a color change from pink to greenish pink. This showed that the color change of the anthocyanin strips tested directly on tuna meat resulted in a better color change. The color changes that occur are related to the pH of the tuna meat. The degree of acidity of tuna fish will increase when fish are stored for a relatively long time and at high temperatures (Table 4). This pH value is related to the number of biogenic amines such as histamine. This is also supported by TVB-N data in Figure 8.

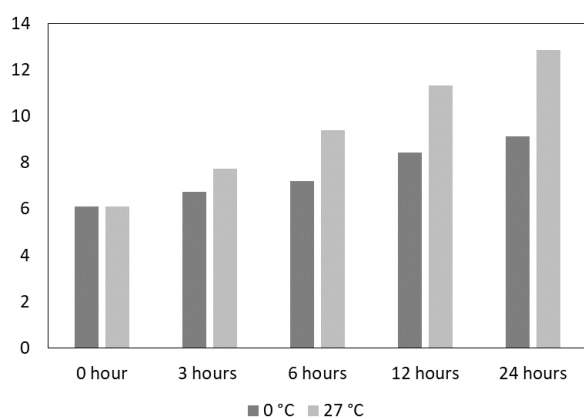


Figure 8. TVB-N analysis of tuna

Based on Figure 8, the volatile basic nitrogen content in tuna meat reached 11.3011 and 12.8445 mg/100 g at storage times of 12 and 24 hours at 27°C. TVB-N values of fish meat above 10 mg/100 g indicated the fish is not in fresh condition [31]. Hence, the tuna meat was not in the best quality when stored for 12 and 24 hours at 27°C.

The histamine biogenic amine showed a different pattern of increase at each storage temperature. Storage temperature affects the formation of histamine [32]. Histamine formation occurs rapidly at higher temperatures. The concentration of histamine in tuna at 20°C in 24 hours increased ten times from the initial concentration. This causes tuna to spoil more quickly when the storage temperature is increased [33]. Tuna stored at room temperature will spoil and be unacceptable for consumption after being stored for 14 hours [34]. Fish spoilage can be caused by microorganisms, which can usually be detected by the smell of fish and due to the decomposition of trimethylamine oxide (TMAO), which is naturally present in the living tissues of marine fish. The amount of TMA is related to the level of fish spoilage; thus, TMA is used as an indicator of the freshness level of fish [32].

3.5. FTIR-ATR Spectra

Structural changes of anthocyanin due to interaction with biogenic amines were analyzed by comparing the FTIR-ATR spectra before and after the interaction. The FTIR-ATR spectra in Figure 9 show a change in absorbance 1600 cm⁻¹ wavenumbers against the storage time of the fish meat. Fish stored for a long time leads to increasing absorbance in 1600 cm⁻¹ the area that corresponds to the ketone compounds [35]. Changes in the anthocyanin structure occur because the interaction with biogenic amines forms ketone compounds. The increase in absorbance shows an increase in the concentration of ketone in anthocyanin strips as fish storage time increases.

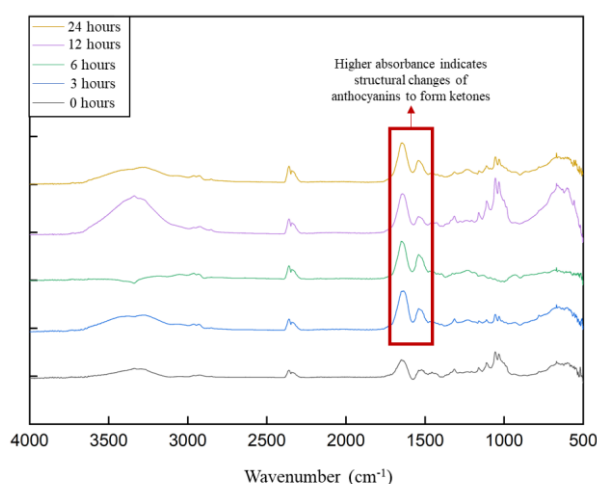


Figure 9. FTIR-ATR spectra of anthocyanin strip

4. Conclusion

Based on the results, the anthocyanin content in the Kepok banana bracts varied from 1.26 mg/100 g to 5.08 mg/100 g with maximum absorption of anthocyanin solution (pH 1) at 513 nm, indicating a cyanidin-3-rutinoside compound. The application of anthocyanin extract to detect the freshness of tuna using a cellulose-based strip indicator. The color change of the anthocyanin strip paper occurred from pink to green, then changed to colorless and blackish gray, which can express the quality of tuna from fresh to not fresh, respectively.

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