



Utilization of red cabbage (*Brassica oleracea* var. *Capitata* L.) anthocyanins as a sensor for nitrite detection in domestic wastewater

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Abstract

Monitoring wastewater quality is increasingly essential for enhancing wastewater treatment procedures. Domestic wastewater, produced by residential and commercial operations, contains considerable organic, inorganic, and biological pollutants, including nitrite ions, which provide serious health hazards. This research develops a nitrite detection sensor utilizing anthocyanins derived from red cabbage (*Brassica oleracea* var. *Capitata* L.). The extraction utilized ultrasonic-assisted extraction (UAE) using a solvent combination of 96% ethanol, distilled water, and 80% acetic acid, resulting in the maximum anthocyanin content of 7.653 mg/g dry weight. The sensor's performance was assessed in terms of stability, selectivity, and sensitivity. The results demonstrated temperature stability at 30°C, with a retention rate of 98.92%, and selectivity for nitrite was verified in the presence of several possible interfering chemicals. Sensitivity tests indicated a detection threshold of 250 ppm for nitrite. The sensor exhibited a shelf life of 19 hours at ambient temperature (25°C) and 11 days at 5°C.

INTRODUCTION

Monitoring the safety of wastewater is an increasingly significant worldwide issue. This monitoring is essential for the development, optimization, management, and enhancement of understanding wastewater treatment plant processes (Modin et al., 2022). Domestic wastewater is a category of wastewater produced by human activities in residential areas, restaurants, workplaces, and marketplaces (Yulirohyami et al., 2022). The existence of these activities necessitates an efficient residential waste management system to avert environmental degradation. Examples are tofu wastewater and domestic wastewater.

Domestic wastewater comprises substantial quantities of organic and inorganic constituents, together with biological contaminants (Koul et al.,

2022). Nitrogen constitutes a significant contaminant in residential wastewater. Alongside ammonium and nitrate ions, nitrogen constituents like nitrite ions significantly influence the quality of residential wastewater. Nitrite contamination varied from 0.05 to 0.93 mg/L in smoked fish industrial wastewater, 0.0295 to 0.7249 mg/L in agricultural wastewater, 1.9155 mg/L in household sewage, and 0.0002 to 0.3130 mg/L in urban wastewater (Artiningsih and Triastianti, 2018; Lumunon et al., 2021; Mutiah et al., 2022; Yulianti et al., 2022). As per Government Regulation of the Republic of Indonesia number 82 in PP RI (2001), regarding Water Quality Management and Water Pollution Control, the permissible limit of nitrite is 0.06 mg/L.

Nitrite are intermediate compounds between ammonium and nitrates. Ammonium oxidizes into nitrite and then oxidizes nitrite into nitrate (Manahan,

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2001). Nitrite are generally found in small amounts because they are unstable and will undergo oxidation into nitrates through the nitrification process (Yulianti et al., 2022). However, nitrite have an impact on health, including skin irritation, pulmonary edema, seizures, respiratory disorder, threatening the balance of acid-base in the blood, stimulation of the central nervous system, and damage to the digestive tract (Lumunon et al., 2021). Detection of excess nitrite needs to be done to avoid potential danger. Nitrite ions pose a major threat to human health, requiring rapid and reliable detection of their presence in water (Ludmerczki et al., 2021). The nitrite ion is unstable and has a low concentration, so it is rarely detected properly in water tests (Du et al., 2016).

Some common techniques for detecting nitrite are fluorophores (Unnikrishnan et al., 2014), ion chromatography (Berardi et al., 2021), frequency spectrum electrodes (Ludmerczki et al., 2021), UV spectrophotometry (Drolc and Vrtovšek, 2010), and the Griess reaction (Numan et al., 2021). These methods have disadvantages, such as the requirement for specialized expertise and advanced, complicated and relatively expensive equipment, making it less suitable for routine analysis that requires low costs. Furthermore, the procedure employs enormous quantities of chemicals. The use of high amounts of chemicals complicates the treatment of laboratory wastewater (Gill et al., 2019). To detect nitrite in home wastewater, a simple, easy-to-use, low-cost, and efficient sensor with high sensitivity and selectivity is required.

The nitrite detection sensor developed is based on bioactive compounds. Red cabbage (*Brassica oleracea* var. *Capitata* L.) contains anthocyanin, a bioactive compound that is used to detect nitrite (Tan et al., 2023). Anthocyanin color is used as a sensor to detect nitrite because it causes a color change in the colorimetric reaction. Color changes on this sensor are an important indicator of its success in detecting targets. The level of sensitivity of a sensor to certain concentrations of target compounds, such as anthocyanins to nitrites, is a key factor in accurate detection (Wulandari et al., 2018). Therefore, this research aimed to develop a sensor with red cabbage anthocyanin to detect nitrite with good stability, selectivity, and sensitivity.

MATERIALS AND METHODS

This research was carried out on January 22–May 17, 2024 at the Chemistry Laboratory, Vocational School of IPB University. The tools used in this research include a knife, analytical balance (Quattro FH 300), ultrasonic cell disrupter (BIOBASE UCD-250), oven (Mettler UN 55 5300), hot plate (IKA C-MAG HS 4), cuvette, spectrophotometer UV-VIS (Genesys 30 Thermo), refrigerator (Panasonic NR-AF171), and glassware commonly used in laboratories. The materials used in this research include red cabbage from supermarkets in Bogor, ethanol 96%, distilled water, CH₃COOH 99% (MERCK), aluminum foil, Whatman filter paper, KCl (MERCK), CH₃COONa (MERCK), HCl, NaOH, citric acid (MERCK), sodium citrate (MERCK), boric acid, 40% formalin, sodium benzoate (MERCK), NaCl, monosodium glutamate (MSG), PbCl₂ (MERCK), ZnCl₂ (MERCK), MgSO₄ (MERCK), and NaNO₃ (MERCK), NaNO₂ (MERCK), tofu wastewater samples (the liquid byproduct generated during tofu production), and home wastewater samples (water from daily household activities, such as washing dishes, which may also contain residues from food preparation).

The extraction of anthocyanins from red cabbage was carried out by weighing 100 grams of fresh red cabbage and extracting them with a solvent mixture consisting of 161.30 mL of 96% ethanol, 32.30 mL of distilled water, and 6.40 mL of acetic acid in a 25:5:1 ratio, as adapted from Wulandari et al. (2018). In Wulandari's study, the extraction method involving this specific solvent mixture was used for extracting anthocyanins from other plant materials, which was purple sweet potato. The red cabbage mixture was extracted using the ultrasound-assisted extraction method with an extraction time of 90 minutes, ultrasonic output power of 100 W, pulse duration of 30 seconds on: 30 seconds off, and temperature of 30°C (Ravanfar et al., 2018). The filtrate was then filtered and stored in a dark bottle in a refrigerator at 5°C. Extraction was carried out 3 times each with varying concentrations of acetic acid in Table 1.

Determination of the total anthocyanin concentration was carried out with a UV-VIS spectrophotometer using the differential pH technique (Tan et al., 2021). The extract was dissolved in potassium chloride buffer

Table 1. Variations in extraction solvents for total red cabbage anthocyanins

Code	Solvent (% w/v)		
	Ethanol	Distilled water	Acetic acid
A	96	-	20
B	96	-	40
C	96	-	60
D	96	-	80
E	96	-	99

solution (pH 1.0), and sodium acetate buffer (pH 4.5) was used for the second sample. The absorbance was recorded at 510 nm and 700 nm. The total anthocyanin concentration was calculated using equations (1), (2), and (3) as follows.

$$A = (A_{\lambda 510} - A_{\lambda 700})_{\text{pH 1.0}} - (A_{\lambda 510} - A_{\lambda 700})_{\text{pH 4.5}} \dots\dots\dots(1)$$

$$\text{Total anthocyanin concentration (mg/L)} = \frac{A \times \text{DF} \times \text{MW} \times 1,000}{\epsilon \times l} \dots\dots\dots(2)$$

$$\text{Total anthocyanin concentration (mg/g DW)} = \frac{\text{mg total anthocyanin}}{\text{gram dry weight}} \dots\dots\dots(3)$$

Remarks: A = absorbance obtained from the equation; mw = molecular weight of anthocyanin (449.2 g.mol⁻¹); DF = dilution factor; ϵ = molar absorptivity coefficient (26,900 L.cm⁻¹.mol⁻¹); l = cuvette thickness (1 cm).

Temperature stability was determined by adding 5 mL of anthocyanin extract that has been diluted 10 times into a test tube then heated in a water bath at temperatures of 30°C, 40°C, and 50°C for 90 minutes. Every 30 minutes, the color intensity was observed using a UV-VIS spectrophotometer at a wavelength of 540 nm (Wulandari et al., 2021). Temperature stability was calculated using equations (4) and (5) as follows.

$$\text{Degradation (\%)} = \frac{A_0 - A_t}{A_0} \times 100\% \dots\dots\dots(4)$$

$$\text{Stability (\%)} = 100\% - \% \text{ degradation} \dots\dots\dots(5)$$

Remarks: A₀ = Absorbance of anthocyanins at time-0; A_t = Absorbance of anthocyanins at time-t.

The effect of temperature on the color intensity of anthocyanins was analyzed for reaction kinetics as a function of time by referring to (Loypimai et al., 2016) and (El Seoud et al., 2016) with the following equations.

$$\text{Pseudo zero order reaction: } A_t = A_0 - kt \dots\dots\dots(6)$$

$$\text{Pseudo first order reaction: } A_t = A_0 \cdot e^{-kt} \dots\dots\dots(7)$$

$$\text{Pseudo second order reaction: } \frac{1}{A_t} = \frac{1}{A_0} + kt \dots\dots\dots(8)$$

$$\text{Half-life (t}_{1/2}\text{) zero order: } \frac{A_0}{2k} \dots\dots\dots(9)$$

$$\text{Half-life (t}_{1/2}\text{) first order: } \frac{\ln(2)}{k} \dots\dots\dots(10)$$

$$\text{Half-life (t}_{1/2}\text{) second order: } \frac{1}{k \times A_0} \dots\dots\dots(11)$$

$$\text{Shelf life (t}_{90}\text{) zero order: } \frac{0.1 \times A_0}{k} \dots\dots\dots(12)$$

$$\text{Shelf life (t}_{90}\text{) first order: } \frac{\ln(1.111)}{k} \dots\dots\dots(13)$$

$$\text{Shelf life (t}_{90}\text{) second order: } \frac{0.111}{k \times A_0} \dots\dots\dots(14)$$

The shelf life of anthocyanin extract was determined based on the effect of temperature on the degradation in anthocyanin color intensity by calculating the activation energy using the Arrhenius equation (El Seoud et al., 2016) with the following equation.

$$k = A \cdot e^{-E_a/RT} \dots\dots\dots(15)$$

Remarks: k = reaction rate coefficient; t = time (minutes); E_a = activation energy (kJ/mol); R = ideal gas constant (8.314 J/mol K); T = temperature (Kelvin). The activation energy and reaction rate coefficient were determined from the slope and intercept between each linear regression result; between ln(k) and 1/T (Li et al., 2013).

Material interference by anthocyanin extract and its selectivity were carried out for NO₂⁻, NO₃⁻, Pb²⁺, Zn²⁺, boric acid, Mg²⁺, NaCl, sodium benzoate, formalin, and MSG with each concentration of 10,000 ppm. The selectivity test was carried out by applying 1 mL of anthocyanin extract to 4 mL (1:4) of the material to be tested, and the color change was observed.

The sensitivity of the anthocyanin extract was tested against nitrite ions with a concentration of 10; 25; 50; 125; 250; 500; 750; 1,000; 2,500; 5,000; 7,500; and 10,000 ppm. The response time was determined using a stopwatch to measure the time until the color difference between the blank and the test solution appeared. The color response scale of anthocyanin extracts for various concentrations of nitrite ions was observed and represented in concentration trajectories.

Nitrite concentration in a sample was determined by adding 1 mL of anthocyanin extract into a test tube containing 4 mL of the sample to be tested (1:4), and the color change was observed (Wulandari et al., 2018).

RESULTS AND DISCUSSION

Determination of total anthocyanin concentration in red cabbage

The total concentration of red cabbage anthocyanins was measured to evaluate the levels used as a nitrite detection sensor. Ultrasonic assisted extraction (UAE) was used to extract red cabbage anthocyanin, which was then measured using a UV-VIS spectrophotometer differential pH method. Ultrasonic-assisted extraction is classified as an environmentally friendly extraction technique because of its high performance with less solvent and time consumption, and it is suitable for heat-sensitive compounds (Yusoff et al., 2022). The highest concentration of anthocyanins in red cabbage was obtained using the UAE method compared to the conventional percolation method (Ravanfar et al., 2018). According to Oancea (2021), anthocyanins have low stability at high temperatures, so extraction at low temperatures is needed to obtain large concentrations of anthocyanins. The total concentration of anthocyanins in dry weight samples changes

according to the amount of acetic acid used as a solvent during the extraction process. Based on Table 1, there are codes A, B, C, D, and E. Code D with 96% ethanol solvent and 80% acetic acid has the highest total anthocyanin concentration among the other codes, which can be seen in Table 2. The code D extract with the highest total anthocyanin concentration was then further analyzed.

Temperature stability and shelf life of anthocyanin extract

Temperature plays an important role in the anthocyanin storage process. Anthocyanins are classified as bioactive compounds, which are natural compounds that are easily damaged at certain temperatures. Temperature stability analysis needs to be carried out to determine the damage to anthocyanins as bioactive compounds that will be developed into nitrite sensors. Determination of the temperature stability of anthocyanin extract was carried out at temperatures of 30, 40, and 50°C with heating times of 30, 60, and 90 minutes. The results of determining the temperature stability of anthocyanin extract are shown in Figure 1. The degradation in absorbance values in percentage reduction in anthocyanin color intensity is shown in Table 3.

The absorbance of the anthocyanin extract degradation during the heating time of 0–90 minutes. According to Figure 1, anthocyanin experienced a

Table 2. Determination of total anthocyanin concentration

Extract	Total anthocyanin concentration (mg/g DW)
A	6.121±0.2157
B	6.602±0.0508
C	7.468±0.0105
D	7.653±0.0457
E	7.464±0.0105

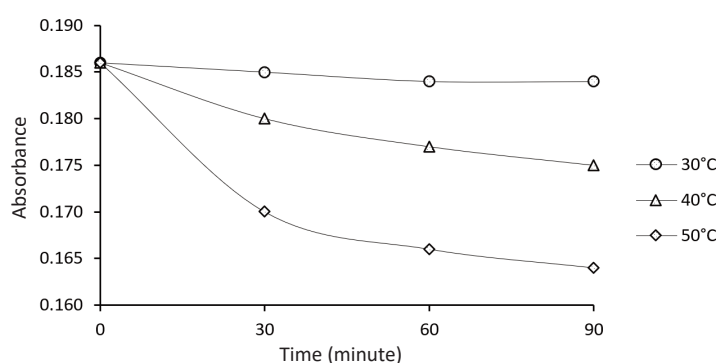
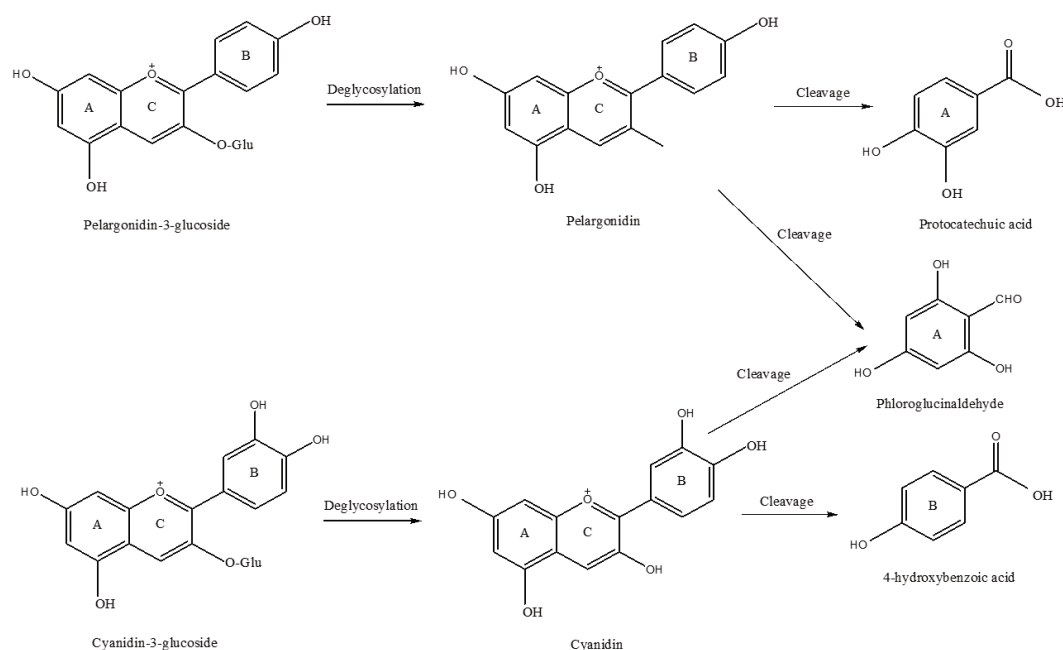


Figure 1. Temperature stability of anthocyanin extracts

Table 3. Percentage of degradation in anthocyanin color as affected by temperature

Temperature (°C)	Degradation anthocyanin color intensity (%)			Stability (%)
	Time (minute)			
	30	60	90	
30	0.54	1.08	1.08	98.92
40	3.23	4.84	5.91	94.09
50	8.60	10.75	11.83	88.17

**Figure 2.** Thermal degradation mechanisms of two common anthocyanins (Patras et al., 2010)

degradation in absorbance along with the increase in temperature shown. There was a significant degradation in absorbance at temperatures of 40°C and 50°C, whereas at 30°C, the degradation in absorbance tended to be stable. According to Table 3, the absorbance of anthocyanins experienced degradation by 1.08% at temperature of 30°C. This shows that red cabbage anthocyanin tends to be stable at 30°C with a stability of 98.92%. At temperatures of 40°C and 50°C, the absorbance experienced degradation along with the heating time. The highest degradation in absorbance occurred at 50°C for 90 minutes, namely 11.83% with stability of 88.17%. Degradation of anthocyanins due to the influence of temperature has been proven through previous research results, including in grapes (Reyes and Cisneros-Zevallos, 2007), rosella flowers (Aurelio et al., 2008), frambozen (Verbeyst et al., 2011), and purple sweet potato (Wulandari et al., 2021). The thermal degradation mechanisms of two common anthocyanins are shown in Figure 2.

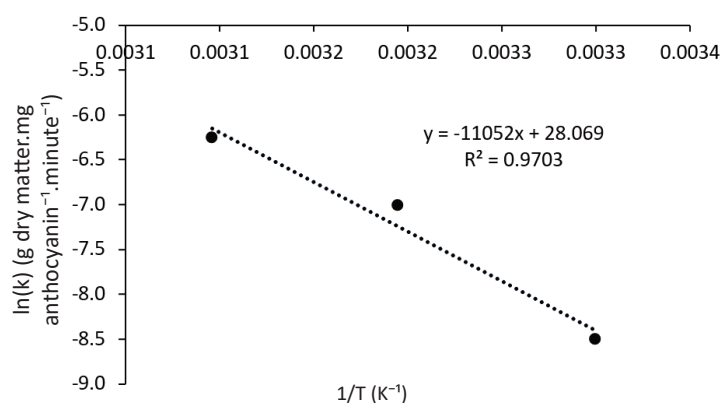
The thermal degradation mechanism of two common anthocyanins, namely pelargonidin-3-glucoside and cyanidin-3-glucoside, is shown in Figure 2. Both anthocyanins undergo deglycosylation, which is the removal of the glucose group to produce pelargonidin and cyanidin, respectively. Pelargonidin then undergoes bond cleavage to produce protocatechuic acid and fluoroglucinol aldehyde. Meanwhile, cyanidin undergoes bond cleavage to produce 4-hydroxybenzoic acid and fluoroglucinol aldehyde. This degradation process shows how heating can affect the stability of anthocyanins.

Table 4 shows the kinetics of thermal degradation of anthocyanins at temperatures of 30, 40, and 50°C. The data show that anthocyanin degradation follows second order reaction kinetics with a higher coefficient of determination (R^2) compared to zero order and first order. Previous research results showed that the kinetics of thermal degradation of anthocyanins from Barbados cherries (Mercali et al., 2013), black rice (Loypimai et al., 2016), and purple corn (Slavu et

Table 4. Thermal degradation kinetics of anthocyanins

Temperature (°C)	Pseudo zero order			Pseudo first order			Pseudo second order		
	k	R ²	t _{1/2} (hours)	k	R ²	t _{1/2} (hours)	k	R ²	t _{1/2} (hours)
30	0.0001	0.8909	63	0.0001	0.8912	115	0.0002	0.8914	63
40	0.0005	0.9391	760	0.0007	0.9431	990	0.0009	0.9469	845
50	0.0010	0.8194	374	0.0013	0.8290	533	0.0019	0.8386	393

Remarks: Zero order unit; k = mg anthocyanin.g dry weight⁻¹.minute⁻¹. First order unit; k= minute⁻¹. Second order units; k = g dry matter.mg anthocyanin⁻¹.min⁻¹

**Figure 3.** Arrhenius plot of red cabbage anthocyanin extract

al., 2020) followed first order kinetic modeling. First order in the literature and second order in experiments for thermal degradation of anthocyanins can be caused by inaccurate temperatures used in the system, causing the anthocyanin concentration to be lower.

A higher system temperature causes the reaction rate to increase and the half-life ($t_{1/2}$) to experience degradation. The values of the second order reaction rate (k) are 0.0002; 0.0009; and 0.0019 for temperatures of 30, 40, and 50°C, respectively, with half-lives of 63, 845, and 393 hours. The higher the system temperature, the higher the reaction rate and the shorter the anthocyanin degradation half-life, with the highest reaction rate constant value achieved at a temperature of 50°C, namely 0.0019.

The temperature stability data obtained was then used to analyze the shelf life of red cabbage anthocyanin extract using Arrhenius acceleration. Arrhenius equation describes the relationship between the chemical reaction rate constant, reaction temperature, and activation energy (Ebrahim et al., 2021). The equation obtained is $y = -11052x + 28.069$ with an R^2 of 0.9703 (Figure 3). According to Wibisono and Bintoro (2022), the value of the determination coefficient (R^2) that is close to the value of one (1) means that the ability of the free variable to cause

the existence of the bound variable is getting stronger. According to Li et al. (2013), in an Arrhenius plot, $\ln(k)$ is plotted against $1/T$, and the resulting straight line shows the logarithmic relationship between reaction rate and the inverse of temperature. The second order reaction rate constant at the temperature range of 30–50°C is used to determine shelf life (Table 4). The results of the linearity plot of red cabbage anthocyanin extract based on the Arrhenius equation are shown in Figure 3.

According to Figure 3, the plot illustrates the dependence of the reaction rate constant on temperature. The coefficient of determination (R^2) on an Arrhenius plot provides an indication of the extent to which the mathematical model fits the experimental data. A high R^2 value indicates that changes in the reaction rate constant $\ln(k)$ can be significantly explained by changes in temperature ($1/T$) to predict reaction kinetics. The shelf life of red cabbage anthocyanin extract was determined using the Arrhenius equation, which was obtained for 19 hours at room temperature (25°C) and 11 days at 5°C storage temperature.

Selectivity of anthocyanin extracts

Selectivity is the ability to distinguish targets from interference molecules and display specific responses

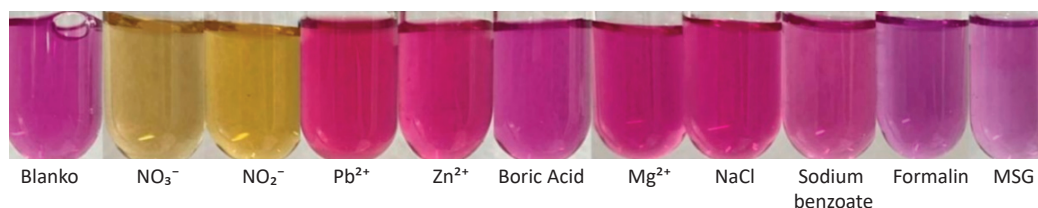


Figure 4. Anthocyanin selectivity towards several chemical components with a concentration of 10,000 ppm

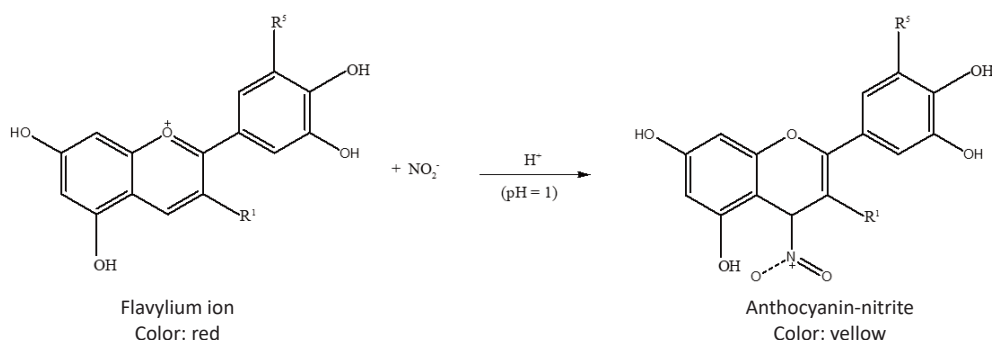


Figure 5. Reaction of nitrite ion with anthocyanin, where: $R^5 = \text{H}$ (Cyanidin) and OH (Delphinidin); $R^1 = \text{Sambubiosi}$ (Galán-Vidal et al., 2014)

to target compounds (Wusiman and Taghipour, 2022). According to Wulandari et al. (2021), the selectivity of anthocyanin extract is determined using NO_3^- , Pb^{2+} , Zn^{2+} , boric acid, Mg^{2+} , NaCl , sodium benzoate, formalin, and MSG. Some components contained in domestic wastewater can interfere with the performance of nitrite (NO_2^-) detection by anthocyanins, so it is necessary to carry out selectivity tests on several of these chemical components. The anthocyanin extract was analyzed for its selectivity towards nitrite and other chemicals with a concentration of 10,000 ppm each. The results of selectivity are shown in Figure 4.

According to Figure 4, the test results show that anthocyanins have good selectivity towards nitrite at a concentration of 10,000 ppm, which is characterized by a significant color change from reddish to yellowish. Other chemicals (NO_3^- , Pb^{2+} , Zn^{2+} , boric acid, Mg^{2+} , NaCl , sodium benzoate, formalin, and MSG) do not react with anthocyanins as indicated by no color change, where these chemicals are relatively stable at a reddish color and brown. The reaction between nitrite and anthocyanin is shown in Figure 5.

According to Galán-Vidal et al., (2014), anthocyanins have a specific reaction to nitrite, where this reaction changes color from red to yellow. The color change in anthocyanins occurs because the anthocyanin flavylium ion that gives the red color has been

substituted by the nucleophilic nitrite ion to form anthocyanin-nitrite. The formation of anthocyanin-nitrite induces the flavylium cation (red) to change to chalcone (yellow) in an acidic condition (Galán-Vidal et al., 2014).

Sensitivity and response time of anthocyanin extracts to nitrite

Anthocyanin extracts are sensitive to nitrites at certain concentrations. The reaction of nitrite with anthocyanin at a concentration of 10–10,000 ppm was performed to determine the sensitivity range. The results of the interaction between anthocyanin extract and various nitrite concentrations are shown in Figure 6. According to figure 6, there is a change in the color of anthocyanin from red to yellow. The color of the solution begins to fade at a nitrite concentration of 250 ppm so the sensor's visual detection limit is 250 ppm. The color change of anthocyanin to yellow increases from a concentration of 250–10,000 ppm.

To determine the reaction time of anthocyanin with nitrite at a concentration of 10–10,000 ppm, the response time must be known. According to Government Regulation of the Republic of Indonesia number 82 in PP RI (2001), concerning Water Quality Management and Water Pollution Control, the maximum

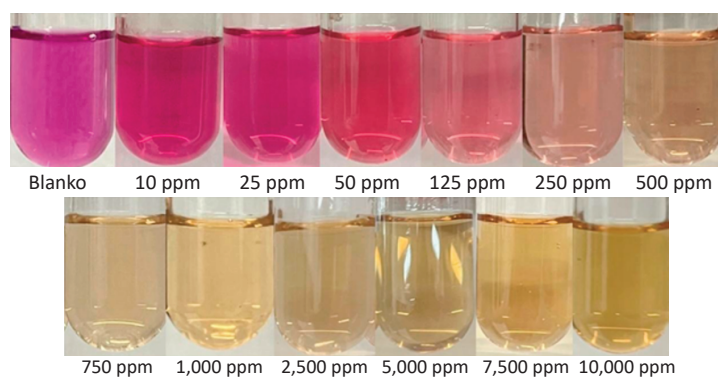


Figure 6. Sensitivity of anthocyanins to nitrites

Table 5. Response time of anthocyanin extracts to nitrite

Nitrite concentration (ppm)	Change time (minutes)
10	-
25	-
50	-
125	-
250	120.20
500	59.20
750	29.12
1,000	18.30
2,500	6.36
5,000	5.55
7,500	4.39
10,000	3.48

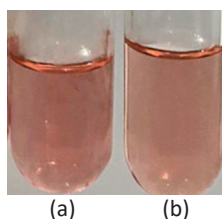


Figure 7. Nitrite concentration in samples of (a) tofu wastewater and (b) household wastewater

concentration of nitrite parameters is 0.06 mg/L; so any concentration above this can harm both life and the ecosystem. Based on Table 5, the relationship between nitrite concentration and response time is negatively correlated, where the higher the nitrite concentration, the faster the time required to react with anthocyanins. Nitrite with a concentration of 10; 25; 50; and 125 ppm does not change color to yellow. Nitrites with a concentration of 250; 500; 750; 1,000; 2,500; 5,000; 7,500; and 10,000 ppm. The use of anthocyanin extract in detecting nitrites is

only effective at concentrations above 250 ppm. This is due to the weak strength of the reaction between nitrites and anthocyanins at low concentrations; thus, the visible discoloration only appears at higher nitrite concentrations.

Determination of nitrite concentration in samples

Anthocyanin extract was used as a nitrite detection sensor in both tofu and household wastewater samples. Tofu and household wastewater samples must be filtered first to eliminate coarse particles

and suspended contaminants that may interfere with the analysis process. The results of samples of tofu wastewater and household wastewater after adding anthocyanin extract are shown in Figure 7. According to Figure 7, both tofu and household wastewater have a yellowish pink color with a concentration similar to 250 ppm as in Figure 6. Determination of nitrite concentration in a sample is only based on the color range obtained in sensitivity determination so that it is less accurate in describing the nitrite concentration in the sample.

CONCLUSIONS

Red cabbage anthocyanin extract using the ultrasonic assisted extraction (UAE) method with a mixed solvent of 96% ethanol: distilled water: 80% acetic acid had the largest anthocyanin concentration with a concentration of 7.653 mg/g DW. The nitrite sensor from red cabbage anthocyanin has the highest temperature stability of 98.92% at 30°C, is selective for nitrite with the smallest sensitivity of 250 ppm, and has a shelf life of room temperature (25°C) for 19 hours and at 5°C for 11 days.

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