JKPK (JURNAL KIMIA DAN PENDIDIKAN KIMIA), Vol. 11, No. 2, 2026 pp. 220-232
Chemistry Education Study Program, Universitas Sebelas Maret ISSN 2503-4146
https://jurnal.uns.ac.id/jkpk ISSN 2503-4154 (online)

A Natural Paper-Based Colorimetric Indicator from Red Dragon Fruit for Detecting Fish Freshness Using RGB Analysis

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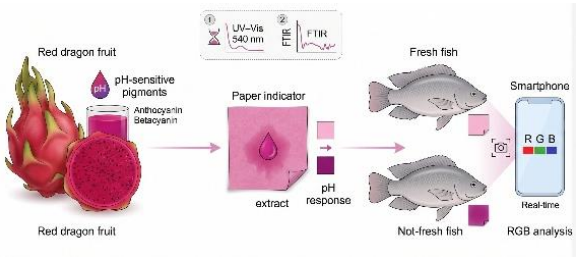
ABSTRACT



Red dragon fruit is known to contain anthocyanin and betalain pigments that can serve as practical colorimetric indicators for fish freshness because of their sensitivity to changes in pH. This study presents a preliminary proof-of-concept that confirms the presence of these pigments through UV–Vis spectrophotometry and FTIR analysis and evaluates the performance of a natural colorimetric indicator derived from red dragon fruit extract for detecting the freshness of Nile tilapia using RGB analysis. An absorbance peak at 540 nm indicated the presence of betacyanin. The FTIR spectrum confirmed the characteristic functional groups of the pigments, with a band around 2900 cm^{-1} attributed to C–H stretching, a small peak at 1642 cm^{-1} corresponding to C=C stretching, a C–H vibration at 1384 cm^{-1}, C–O stretching at 1275–1269 cm^{-1}, a sharp peak at 1043 cm^{-1} assigned to C–O–C stretching, and C–H bending at 876 cm^{-1}. The practical performance of the natural colorimetric indicator was evaluated by placing the paper-based indicator in contact with Nile tilapia samples and observing the color response at 15-min intervals for 1 h. Fish freshness was assessed through RGB analysis using the Color Meter app installed on an Android smartphone. The obtained RGB values were subsequently visualized for data analysis and interpretation. The results showed that the RGB values changed in response to changes associated with fish freshness over time, indicating that the developed natural colorimetric indicator has potential for further development as a simple tool for fish freshness detection.

Keywords: *biosensor; fish freshness; red dragon fruit; real-time analysis.*
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How to cite: L. D. Assaat, C. S. Parameswary, N. Fatmasari, I. Affifah, I. Irhamni, and I. B. Rachman, "A Natural Paper-Based Colorimetric Indicator from Red Dragon Fruit for Detecting Fish Freshness Using RGB Analysis," *Jurnal Kimia dan Pendidikan Kimia (JKPK)*, vol. 11, no. 2, pp. 220–232, 2026. [Online]. Available: https://doi.org/10.20961/jkpk.v11i1.118520
Received: 2025-05-08 *Accepted: 2026-08-16* *Published: 2026-08-31*

INTRODUCTION

Detection of fish freshness plays a vital role in ensuring that fish quality is properly maintained during transportation and that the fish remains safe for consumption. Various technologies and analytical instruments for fish freshness detection have been extensively investigated in recent years [1], ranging from computer modelling [2] and Artificial Intelligence-based approaches [3], [4], which enable the assessment of fish quality based on visual indicators such as eye clarity and gill condition [2], to integrated methods involving robotic arms and fisheye analysis [5]. The degradation of fresh fish

occurs through several mechanisms, including autolytic enzymatic spoilage, lipid hydrolysis, oxidative spoilage, and microbial activity. These processes result in the formation of Total Volatile Base Nitrogen (TVB-N), trimethylamine-nitrogen (TMA-N), and dimethylamine (DMA) dehydrogenase [6]. Protein breakdown in spoiled fish also results in an increase in pH, as consistently reported for various fish species [7].

Sensory assessment using organoleptic scoring has been employed as an alternative approach for evaluating the deterioration of fish over time. The deterioration of Nile tilapia can be characterized by changes in gill color, softening of the abdominal and dorsal regions, increased convexity of the eyes, increased viscosity of gill mucus, and the development of a sour odor [8]. A significant decrease in the organoleptic score for the flesh texture of Nile tilapia was observed earlier than changes in gill color and odor, indicating that fish freshness at room temperature, without any preservative treatment, deteriorates drastically after 6–8 hours of exposure to ambient conditions [8]. Changes in pigmentation may result from biochemical transformations such as oxidation, while changes in texture can be attributed to glycolytic processes, and changes in odor are associated with bacterial growth. In addition, changes in odor and texture may also result from lipid oxidation, particularly the oxidation of polyunsaturated fatty acids into aldehydes [9].

Visual examination as an approach to assessing color changes may provide slower or less precise detection than instrumental color measurement [10], as discoloration occurs as a result of various chemical reactions in deteriorating fish tissue [11], some of which may occur within milliseconds to seconds [12]. The duration of the detection process is also susceptible to temperature changes, which can affect the durability of natural colorimetric biosensors [13] and may result in less reliable data acquisition. Direct RGB-based colorimetric analysis offers a real-time observation method that can reduce subjectivity in visual assessment, minimize bias in color interpretation caused by lighting variations when indicators are photographed and analyzed at a later stage, and provide a faster and more practical detection method by reducing the need to transport indicators to analytical instruments.

One potential natural source of pigments that can be used as a colorimetric indicator is red dragon fruit (*Hylocereus costaricensis*), which contains betacyanins and anthocyanins in both its pulp and peel [14]. The coexistence of anthocyanins in betalain-pigmented species, including those containing betacyanin, has been a subject of discussion [15]. Nevertheless, the presence of anthocyanin and betacyanin in red pitaya pulp has been confirmed through UV–Vis spectrophotometric analysis at a wavelength of 538 nm, with a reported Total Betanin Content (TBC) of 30.15 ± 0.03 mg/g sample and Total Anthocyanin Content (TAC) of 15.16 ± 0.02 mg/g sample [14]. The UV–Vis spectrum of anthocyanins consists of two main wavelength regions, namely 260–280 nm in the UV region and 490–550 nm in the

visible region, with two additional humps between these regions indicating the presence of substitution groups [16]. Although anthocyanin content has been reported to be approximately half that of betacyanin, anthocyanins are more sensitive to changes in pH than betacyanins [17]. The colorimetric response of anthocyanins is demonstrated by observable color transitions from reddish hues under acidic conditions to pink as the pH increases [18]. This characteristic supports the potential use of anthocyanins as natural acid–base colorimetric indicators [19]. Quantifying the color response of a detector, such as the natural colorimetric indicator used in this study, can provide more consistent data interpretation than qualitative visual assessment alone.

Indicators containing mixtures of dyes such as cresol red, bromocresol purple, Breathrom film, and eco-DEHCH have previously been used to monitor fish quality during storage [20]. The use of automated seafood freshness detection based on machine learning and paper-based pH sensors containing a mixture of methyl red and bromocresol purple has also been investigated [21]. However, the systems used in these studies require relatively sophisticated technology and complex indicator preparation, which may limit their practical implementation in remote areas. Therefore, the present study adapts the paper-based pH sensor concept by incorporating red dragon fruit extract as a natural pigment-based acid–base indicator for detecting fish freshness. To the best of the authors' knowledge, the direct determination of RGB values using the Color Meter application for fish freshness detection with a red dragon fruit-based biosensor has not yet been explored. The data were acquired in real time and subsequently visualized to facilitate accurate measurement and minimize bias in color interpretation associated with the time interval between data acquisition and analysis. The detection kit developed in this study provides a simple approach to fish freshness detection using readily available natural resources and has the potential to be applied in areas where access to advanced analytical technology is limited.

METHODS

1. Preparation of Natural Colorimetric Indicator

Red dragon fruit (*Hylocereus costaricensis*) was purchased from a local market in Serang City. The fruits were peeled, and the pulp was ground. The pulp extract was then separated from the seeds through gravimetric filtration, and the liquid pulp extract was collected. From 100 g of pulp, 8 g of pulp extract was obtained, yielding an 8% mass extract. The extract was diluted with distilled water at a ratio of 1:10 v/v (1 mL of extract was diluted to 10 mL by the addition of distilled water), and the pH of the extract was then adjusted to pH 2 using 0.1 M HCl, as verified using a universal pH indicator. A substrate for the natural colorimetric indicator was prepared from Whatman Grade 41 paper with a particle retention range of 20–25 µm. The paper was cut into 2 × 2 cm pieces and immersed in the

diluted extract for 5 min. Drying was performed by natural air exposure at room temperature. The paper-based colorimetric indicator was then stored in a closed container to prevent thermal degradation of the pigments.

2. Characterization of Red Dragon Fruit Extract

The analysis and characterization of the biosensor extract were conducted using UV–Vis spectroscopy with a PG Instrument T60 equipped with 10 mm path-length quartz cuvettes over a scanning range of 400–650 nm. Instrumental analysis using IR spectroscopy was performed with a Bruker Alpha II IR spectrometer over a spectral scanning range of 4000–500 cm^{-1} at a resolution of 4 (medium resolution). A blank procedure was conducted using the small diamond tub, which had been cleaned consecutively with ethanol, water, and ethanol again, and was then analyzed by selecting the “blank sample” option in the software.

3. Fish Sample Preparation and Examination

The fish species used in the examination was Nile tilapia. The fish was transported alive to the laboratory and slaughtered immediately before the examination procedure. The post-mortem time of the fish was recorded at 7:15 AM, after which the fish flesh was cut and crushed. Fish sample preparation and examination were conducted at room temperature (25 °C). The prepared fish was then labeled as “fresh fish.” The sample labeled as “not-fresh fish” was prepared one day before the examination using the same procedure and stored overnight in a refrigerator at 1–5 °C. It was then removed from the refrigerator and kept at room temperature until the time of examination. The biosensors were placed at the bottom of two bottles. During the examination, 10 g of fresh fish and 10 g of not-fresh fish were placed in the respective bottles and allowed to come into contact with the colorimetric indicators. Observations were performed every 15 min for 1 h by directing the device toward the samples to acquire and record the RGB data.

4. RGB Data Acquisition and Revisualization

Red, Green, and Blue (RGB) data from the natural colorimetric indicator after exposure to fresh and not-fresh fish were analyzed using the Color Meter app version 4.5.0, developed by Contechity AB and released in 2023. The application was installed on an Oppo A16 smartphone running the Android operating system and equipped with a 13-megapixel rear camera. The Color Meter app allows direct RGB measurement through live camera sampling by directing the camera toward the observed object without capturing an image, as in conventional picture-taking mode. The object was measured at a distance of 5 cm from the region of interest to the smartphone. When using the Color Meter app, the auto-focus/auto-exposure (AE/AF) feature was not applied because no image capture took place. Ambient daylight was used as the illumination source. The Color Meter app allows direct measurement of RGB values

and supports automatic calibration using a white reference to compensate for variations in lighting conditions. White paper was placed around the objects, which in this study were bottles containing the samples to be observed, to minimize light diffusion that might interfere with RGB data acquisition. The RGB values were subsequently visualized using the RGB Color Picker website for data interpretation and further analysis.

RESULTS AND DISCUSSION

1. Extraction of Red Dragon Fruit as the Raw Material for Natural Colorimetric Indicator

The material was selected according to the following criteria: shiny skin, fresh appearance, no signs of dryness, not fully ripened, pinkish-red skin, and no brownish discoloration [22]. The obtained red dragon fruit pulp extract was evaluated at different pH values prior to the experimental procedure. It was confirmed that, at lower pH values, the color of the extract ranged from pinkish to colorless, whereas at higher pH values, the extract appeared violet. The color changes observed in this study are consistent with the pH-dependent color changes of anthocyanins resulting from structural transformations. Under weakly acidic conditions, anthocyanins may appear colorless because they exist in the pseudobase form; under acidic conditions, they appear red due to the predominance of the flavylium ion form; and under weakly acidic or neutral conditions, they appear violet in the anhydrobase form [23]. In addition to anthocyanins, red dragon fruit pulp also contains betacyanin, which belongs to the betalain pigment group and may change to a yellow color as a result of the formation of betalamic acid and betaxanthins [24], [25]. Although the observed color changes supported the presence of anthocyanins, the absence of betacyanin could not be confirmed without further chemical analysis.

Pigment extraction from red dragon fruit was conducted using a maceration process. Solvent selection was based on several considerations. Red dragon fruit extract is known to contain anthocyanins and betacyanins, both of which are readily soluble in polar solvents [26], [27]. Water was selected as the solvent for preparing the natural colorimetric indicator in consideration of green chemistry principles. For analytical purposes, ethanol was used as the solvent because of its better solubility performance [28]. In this study, the RGB characteristics of red dragon fruit pulp extracts dissolved in ethanol and water were also compared.

A previous study compared ethanol and water as extraction solvents, followed by acidification with citric acid to obtain extracts at pH 2 [19]. The study reported UV–Vis spectrophotometric absorbance values for anthocyanins of 0.8 when ethanol was used as the solvent and 0.75 when water was used [19]. These findings suggest that both water and ethanol can be used as solvents in this study. The extraction solvent should therefore be selected carefully to optimize the extraction process while also considering green chemistry principles. Water was preferred as the solvent for anthocyanin

extraction because of its availability, practicality, and safety. Pigment identification in this study was conducted by analyzing the RGB (Red, Green, and Blue) values of the samples. Lighter-colored samples generally produce higher total RGB values, whereas more concentrated pigmentation results in lower RGB values. RGB analysis was performed using a real-time analysis method with the Color Meter application on a smartphone, which can directly measure the Red, Green, and Blue channel values as well as the RGB values regardless of internet availability. The detection kit and procedure were designed for practical implementation in public places or schools; therefore, under actual observation conditions, data acquisition might not always follow fully standardized procedures, and statistical analysis may be limited. Lighting conditions were adjusted by placing white paper around the objects to minimize interference from variations in illumination. Despite the limited statistical analysis, this preliminary investigation provided useful data and findings that may support further related research.

Table 1. RGB value comparison of Red Dragon Fruit ethanol extract and water extract

	Ethanol	Water
R value	255	254
G value	102	192
B value	180	245
Color Interpretation		

Table 1 shows the difference of RGB value and the color interpretation of red dragon fruit extracted with ethanol and water as solvent. A difference in the RGB value and color interpretation between ethanol-red dragon extract and water-red dragon extract was quantitatively measured and visually observed. The ethanol-red dragon extract has a darker and more vivid color, rather than the water-red dragon extract which appears to be lighter and more transparent. Based on the data, the ethanol extracted RGB data has a higher red component and a comparatively low green component, but water extracted RGB data has less saturated pink, implying a shift in chromatic intensity. This is one of the indications that extraction of anthocyanin was better conducted using ethanol, than using water as the solvent. This result is in accordance with the findings that anthocyanin extraction was best performed using ethanol-based solvent in which the ethanol content was above 70%, and this is due to its strong affinity for anthocyanins [29]. In the structure of anthocyanin, the non-polar groups are more dominant than the polar group, indicating that higher ethanol concentration as anthocyanin's solvent is preferred. Based on this consideration, further characterization of red dragon fruit extract using UV-Vis and IR spectroscopy were conducted using ethanol-based extraction methods.

2. UV-Vis Spectrophotometer Analysis

UV-Vis spectrophotometer was used to confirm the pigment in red dragon pulp extract at pH 2. According to Figure 1, it can be observed that the red dragon pulp extract spectrum was at maximum absorbance at wavelength of 540 nm at pH 2. At pH 5, the maximum absorbance could not be clearly determined that might due to the limitation of

measurement that was not fully covered the UV-Vis wavelength range and/or to the instability of the solution that is caused by the absence of buffer [30], [31]. UV Vis maximum absorbance was reported at 510 nm for anthocyanin [32]. Absorbance at wavelengths of 300 nm and 530 m indicates the presence of betacyanins [33]. According to the data and reference of UV Vis, the presence of betacyanin as the pigment in red dragon pulp extract is more supported, rather than anthocyanin.

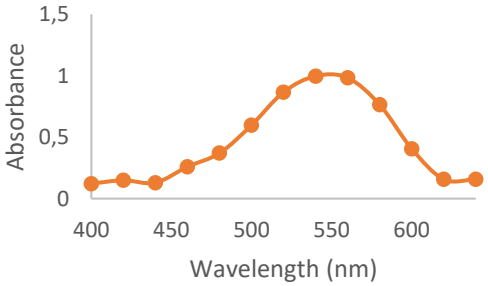

Figure 1. UV-Vis Spectra of Red Dragon Pulp Extract at pH 2

3. FTIR Result of Red Dragon Fruit

The FTIR spectra obtained in this study are shown in Figure 2 and are consistent with the characteristic functional-group spectra reported for red dragon fruit extract [34]. The broad FTIR band observed at approximately 3277–3281 cm^{-1} corresponds to O–H stretching vibrations of hydrogen-bonded hydroxyl groups [34]. A small peak observed at 2977–2981 cm^{-1} was attributed to aliphatic C–H stretching, while a characteristic C=C stretching band associated with aromatic rings was observed at 1642 cm^{-1} [35]. The absorption band at 1275–1269 cm^{-1} was assigned to C–O stretching [36], whereas the band at 1384 cm^{-1} was attributed to C–H vibrations [37]. A sharp peak corresponding to C–O–C stretching was observed at 1043 cm^{-1} [38], while the C–H bending band at 876 cm^{-1} indicated the presence of aromatic rings, which typically exhibit absorption bands within the 600–1000 cm^{-1} region [39], [40].

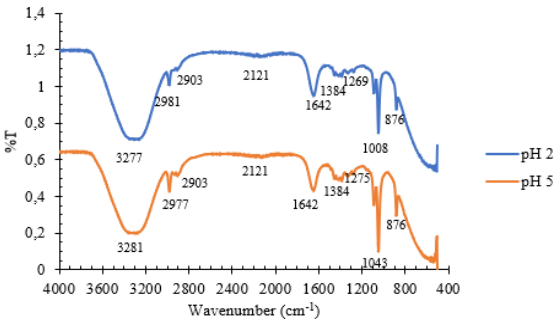

Figure 2. FTIR Spectra of Red Dragon Fruit Extract

The effect of pH to red dragon pulp was studied using FTIR spectra and as can be observed in Figure 2, an incremental difference between spectra of pH 2 and pH 5 red dragon pulp extract was hardly noticeable. A slight shift on several functional group bands like O-H stretching at 3281 cm^{-1} which shifts to 3277 cm^{-1} and C-O-C stretching at 1043 cm^{-1} to 1008 cm^{-1} might be associated with the structural change of anthocyanin, as flavylium ion favors in more acidic environment [23], in which might affect the stretching of C-O-C group due to the formation of positive charge on C-O-C group. A little shift on the typical O-H stretching absorption band at around 3200 cm^{-1} might also be attributed to the effect of different water content which affect the hydrogen bonding.

4. Natural Colorimetric Indicator Response to Fish Spoilage

Previous reports have indicated that ethanol performs better than water as a

solvent for red dragon fruit extraction. However, for the preparation of the natural colorimetric indicator, water was selected as the solvent because of its availability, environmental compatibility, and safety. Changes in the RGB values were recorded in real time at 15-min intervals for 1 h using the Color Meter app. Table 2 presents the RGB values of the natural colorimetric indicator obtained from the application after exposure to fresh fish (FF) and not-fresh fish (NF) across three samples. The RGB value was then analyzed and the color was regenerated using RGB color code by inputting the value for better visualization. Figure 3 shows the data visualization of RGB value for biosensor for detecting fish freshness.

It can be observed that exposure to fish for an hour, both with the fresh fish and not-fresh fish, depicts a subtle color change, according to the RGB value shown in the Table 2 and RGB color interpretation from Figure 3. As in fresh fish, the vivid color has become disappeared and appeared to be lighter at 60 minutes observation, while in not-fresh fish, the change of the color was even more significant, that it appeared to be darker and changed to be violet at 60 minutes observation rather than pinkish as at the initial condition. It appears that the color change of the natural colorimetric indicator has just started to show at 60 minutes observation, and this might be coming in response to the deterioration effect of fish freshness to the pH values. An initial increase of pH on post-mortem fish was due to the glycogen in the muscle that has been metabolized to lactic acid and further increase in pH values reflected the production and activity of alkaline bacterial microbes [41]. However, change in color after an hour contact might also be associated to the natural light-induced oxidation of red dragon pigments that might occur during observation [42]. White balance has been corrected automatically by the apps, consistent Red value at 255 for most of the samples might indicate camera saturation and this might reduce analytical sensitivity.

Table 2. RGB value comparison of biosensor after exposure to Fresh Fish (FF) and Not-Fresh Fish (NF) every 15 minutes for 1 hour Observation

n	Sample	Time (min)	R	G	B
1	FF	0	255	185	227
1	FF	15	255	214	240
1	FF	30	255	212	237
1	FF	45	255	215	243
1	FF	60	255	212	237
1	NF	0	255	175	240
1	NF	15	255	157	211
1	NF	30	255	172	212
1	NF	45	255	174	216
1	NF	60	217	162	176
2	FF	0	195	163	165
2	FF	15	227	172	183
2	FF	30	251	207	220
2	FF	45	247	208	215
2	FF	60	221	182	182
2	NF	0	200	161	156
2	NF	15	226	179	178
2	NF	30	254	194	206
2	NF	45	251	191	191
2	NF	60	232	194	198
3	FF	0	255	225	254
3	FF	15	238	201	222
3	FF	30	247	207	219
3	FF	45	229	190	206
3	FF	60	186	162	161
3	NF	0	235	189	212
3	NF	15	225	186	188
3	NF	30	220	193	197
3	NF	45	206	184	188
3	NF	60	222	210	215

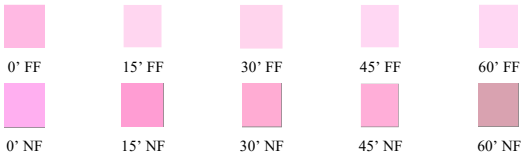

Figure 3. Change of Biosensor Color After Exposure to Fish for 1 hour

International Commission on Illumination (CIE) recommended color space called CIELAB (or CIE76 L*a*b*) that is based on the opponent color theory of color vision which implies that two colors cannot be both green and red, nor blue and yellow at the same time. The CIELAB units included the asterisk (*) to differentiate the CIELAB [system from the units of other color systems [43]. ΔE*ab calculation provides information about the difference between two colors designated as two points in the L, a, b color space in which L represents darkness to lightness, a represents greenness to redness, and b represents blueness to yellowness [44]. ΔE* value greater than one indicates that color difference is observable by the human eye [45]. The basic Euclidean distance formula ΔE (CIE76) is written as follows:

$$\Delta E^*_{ab} = \sqrt{(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2}$$

In this work, the RGB value was converted to Lab value by an online conversion website rgbatohex.com that is based on MATLAB code and implement Python OpenCV for calculation in RGB conversion. The conversion formula and mathematical equation used in this program uses the following steps: RGB to XYZ conversion, which includes gamma correction formula and RGB to XYZ matric transformation, then XYZ to LAB conversion as detailed in Table 3.

Table 3. Conversion Steps of RGB to LAB

Step 1 RGB to XYZ Conversion

Gamma Correction Formula
// RGB Gamma Correction
if (R <= 0.04045) R = R / 12.92
else R = pow((R + 0.055) / 1.055, 2.4)
 	
if (G <= 0.04045) G = G / 12.92
else G = pow((G + 0.055) / 1.055, 2.4)
 	
if (B <= 0.04045) B = B / 12.92
else B = pow((B + 0.055) / 1.055, 2.4)
 	
RGB to XYZ Matrix Transformation
X = 0.4124564 * R + 0.3575761 * G + 0.1804375 * B
Y = 0.2126729 * R + 0.7151522 * G + 0.0721750 * B
Z = 0.0193339 * R + 0.1191920 * G + 0.9503041 * B

Step 2 XYZ to LAB Conversion

LAB Calculation Formula
// Normalize XYZ values
Xn = X / 95.047 // D65 illuminant
Yn = Y / 100.000
Zn = Z / 108.883
 	
// Apply LAB function
if (t > 0.008856) t = pow(t, 1/3)
else t = (7.787 * t) + (16/116)
 	
L = 116 * fy - 16
a = 500 * (fx - fy)
b = 200 * (fy - fz)

The obtained Lab value was then used to calculate ΔE of the fresh fish and not-fresh fish, with three replicates and the calculations were shown in Table 4. Delta E values are associated with human perception level and ΔE value of 3-10 indicates that two colors are perceptible at glance while ΔE value of 11-49 indicates that colors are more similar than opposite [46]. According to Table 4, it can be observed that ΔE values ranged from 4.88 to 28.90. Table 4 summarizes the ΔE value and 53.33% of ΔE value is within 11-49 range that looks more similar, while the rest 46.67% of ΔE

value is in 3-10 range indicating that two colors are perceptible at glance. The standard deviation data are large relative to the means that might corelates with biological or measurement variability in the experiment procedures

Table 4. Calculation of Standard Deviation (SD) of ΔE

Time (min)	n	ΔE_1	ΔE_2	ΔE_3	Mean ΔE_1	SD
0	3	12.31	4.96	13.47	10.25	4.62
15	3	28.90	6.99	11.53	15.80	11.57
30	3	19.61	7.14	8.83	11.86	6.76
45	3	19.85	10.43	9.63	13.30	5.68
60	3	18.92	4.88	17.65	13.82	7.77

CONCLUSION

A preliminary study of a natural colorimetric indicator derived from red dragon fruit extract and analyzed using RGB values was successfully conducted. The extract was characterized using FTIR and UV–Vis spectroscopy to identify its functional groups and pigment-related spectral features. The colorimetric indicator exposed to not-fresh fish showed a darker color compared with that exposed to fresh fish, indicating that changes in RGB values occurred in response to differences in fish freshness. These findings demonstrate the potential of the proposed detection method for practical applications in non-laboratory settings, such as schools, markets, and other public places. However, further studies involving independent replication and more comprehensive statistical analyses are required to validate the method and improve the consistency and reliability of data interpretation.

Author Contributions: L. D. Assaat served as the first and corresponding author, responsible for conceptualizing the study, developing the methodology, designing the experiments, supervising the research process, analyzing data, interpreting the results, and preparing the manuscript; C. S. Parameswary contributed to laboratory investigation, data collection, pigment characterization, and manuscript revision; N. Fatmasari contributed to experimental procedures, data validation, data analysis, and manuscript improvement; I. Affifah contributed to laboratory analysis, data processing, and manuscript review; Irhamni contributed to data interpretation, critical evaluation, and manuscript refinement; I. B. Rachman contributed to supervision, scientific discussion, critical review, and manuscript editing. All authors reviewed and approved the final version of the manuscript.

Conflict of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this manuscript.

Declaration of Generative AI and AI-Assisted Technologies: Generative AI tools were used to assist in preparing the graphical abstract and proofreading the manuscript. All

AI-assisted outputs were reviewed and edited by the authors, who are responsible for the final content.

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