Effect of Paper Matrix on the Properties of the Ammonia Gas Indicator Label

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Abstract. Indicator labels for detecting ammonia gas are useful for monitoring the freshness of foods such as meat, shrimp and fish because of its simple detection methods. In this research, paper labels made using black glutinous rice extract are used as ammonia gas indicator. The indicator labels were tested by exposing them to ammonia gas obtained from an ammonia solution with a concentration of 0.005%, 0.025%, 0.05%, 0.1%, and 0.5%. The color of the label was scanned using the color scanner equipment and its image was analysed using ImageJ to obtain the RGB component values. The UV-vis spectra of the extract solution at pH 2 and 5 show the absorbance peak at 531 nm wavelength, then shifted to 499 nm for pH 7, 8, and 13. The color of these labels is determined by the pH value of the matrix paper. Once exposed with ammonia gas, the color of the label from Whatman paper changes from red to purplish brown and then to yellow. While the label using HVS paper changed from greyish brown to dark brown. Based on the RGB analysis, the red color component indicates better sensitivity to ammonia gas than the green and blue components. Storage conditions of the labels at low temperatures and in dark show better color stability. These results indicate that the labels of the black glutinous rice extract on Whatman paper matrix can be used as a good indicator of ammonia gas and has the potential to be applied in the monitoring of freshness of meat.

Keywords: Indicator label; black glutinous rice extract; ammonia gas

1. Introduction

Recently, several researchers have developed a visual sensing method known as an indicator label. This sensing method is simple, safe, and easy to use. One of the interesting and widely researched applications of the visual sensing is currently as an intelligent packaging that can monitor the quality and safety of food products [1]. It uses the concept of colorimetry by utilizing the changes that occur in dyes used when detecting a given stimulus. Previous researchers have used natural dyes containing anthocyanins from red cabbage [2] and rosella flowers [3] extracts as a functional material. Anthocyanins are widely chosen because they are environmentally sensitive such as to pH, temperature and light. In addition, anthocyanins can be obtained easily in nature in abundant quantities.

Indicator labels are made by immobilizing natural dyes in the matrix. The commonly used matrices include PVA [3], tara gum [4], and cellulose [5]. Cellulose as a matrix from the indicator label is in the form of paper. This type of matrix is easy to prepare and low-cost so it is often used.
This work reports the effect of paper matrix used as a matrix for anthocyanin from black glutinous rice extract as a sensitive dye for indicator labeling on the sensing properties of ammonia gas. The paper matrix used for the indicator labels is Whatman and Wood-free (HVS) paper.

2. Materials and Methods

2.1 Black Glutinous Rice Extraction
Black glutinous rice (*Oryza sativa* L.) obtained from the local market. HCl and ethanol solutions used as extraction solvent, purchased from Mallinckrodt and J.T. Baker, respectively. Ammonia solution was obtained from Emsure. The extraction was prepared by maceration of 80 grams of black glutinous rice in a solvent consisting of 15% HCl and 85% ethanol. Then it was stored in a dark condition for 24 hours. The produced extract had dark red color with a pH of 0.85. A qualitative anthocyanin test contained in the extract was carried out for pH values 2, 5, 7, 8, and 13 using 0.5 M NaOH.

2.2 Indicator Label Preparation
The indicator label was prepared using paper matrix cutted in square with dimension of 6x6 cm$^2$ which immersed in 10 ml of pH 2.0 of black glutinous rice extract. There were two types of paper used, 220 gram HVS paper and Whatman no.1 paper. The immersing time for the paper in extract solution were 10, 20, 30, 60, and 120 minutes. After that the dyed paper was dried at room temperature for 10 minutes. The dyed paper labels were cutted in circle with diameter of 1.25 mm for further characterization and testing.

2.3 Characterization and Testing
The extract solution was characterized using Thermo Scientific GENESYS 10S to obtain absorption spectrum in range of 200-800 nm. This characterization was carried out for the extract with pH of 2, 5, 7, 8, and 13.

Ammonia sensing properties of the indicator label were tested by putting the label inside a glass jar contained of ammonia solution with various concentration for 3 hours. The concentrations of ammonia used were 0%, 0.005%, 0.025%, 0.05%, 0.1%, and 0.5%. Stability of the label color against temperature was investigated by storing them in temperature conditions of 8°C, 24°C, and 40°C for 3 hours. Furthermore, the stability of the label color against lighting was carried out by placing them in dry cabinet with controlled relative humidity of 85% and temperature of 24°C for 3 days in dark and light conditions.

For quantization of the label color, the label was scanned using Epson Perfection v800 Photo Scanner and then the resulted images were analyzed with imageJ software. This procedure resulted in the value of color component intensities i.e red, green and blue.

3. Results and Discussion

3.1 Color and UV-Vis Spectrum of Black Glutinous Rice Extract
Colors and UV-vis spectrum of the black glutinous rice extract at various pH values are shown in Figure 1. The color of the extract is red at pH 2 and changed to clear red when the pH value is increased to 5. The red color appeared in the extract because anthocyanin is in the form of flavylum cation, while at pH 5 the concentration of flavylum cation decreased making the extract color seems faded. At pH 7-8 the color of the extract is brown and became yellow at pH 13. The yellow color at pH 13 is caused by anthocyanins changing its form to chalcone. These color changes have also been observed in *vitis amurensis husk* [6] and purple sweet potato [7] extracts. The extract at pH 2 and 5 show the peak of absorbance at a wavelength of 531 nm. The absorbance peaks shifts toward a lower value of 499 nm when the extract pH adjusted to 7, 8, and 13.
3.2 Effect of Paper Matrix on Label Color

The colors of the indicator label obtained by immersing the paper for various times into the extract of pH 2 are shown in Figure 2 (a). The HVS paper made the color of the label appeared to be grayish brown. The color of this label is caused by HVS paper having a base value of pH of 8.8, so that anthocyanins are conditioned at the pH of the alkaline environment. On the contrary, the Whatman paper used as the matrix produced a red label. The most effective immersion time in making the indicator label is 30 minutes because it caused no significant changes to the properties of the label paper.

The response of the indicator label towards ammonia gas measured for 10 minutes with ammonia solution of 0.5% in concentration as shown in Figure 2 (b). Labels using HVS paper matrix did not experience significant color changes because after exposure to ammonia gas only shifted from grayish brown to dark brown. This is because the anthocyanins on HVS paper have been conditioned at alkaline pH, after detecting ammonia gas becomes more alkaline. Conversely, the label of the Whatman paper matrix undergoes a very noticeable color change, namely red to purplish brown. The initial condition when anthocyanin in the Whatman paper matrix is acidic so the color is red, when the anthocyanin detects ammonia gas, the environment becomes more alkaline, causing it to change to a purplish brown color. Thus, the Whatman paper matrix label is good to use as an indicator label because the color changes are very visually observed.

Figure 2. Effects of paper matrices to the labels color at different immersion time (a) and the color changes after exposure to ammonia gas for 10 minutes (b).

3.3 Ammonia Sensing Properties of the Labels
The color change of the indicator label from the black glutinous rice extract using Whatman paper matrix when detecting ammonia gas is shown in Figure 3. The color of the label changes from red to purple after being exposed to ammonia gas for two hours. This shows that there is a chemical reaction between anthocyanin and ammonia gas so that the original anthocyanin structure of the flavylium cation when in the acidic environment becomes a quinoid base after exposure to ammonia [8]. Figure 3 shows the intensities of Red, Green, and Blue components of the label before and after ammonia exposure. The red color component intensity decreased as the ammonia gas concentration increased until 0.1%. There are no further changes of the red intensity for ammonia gas concentration above 0.1%. Changes in the intensity are also found for the blue and green components but they have a pattern that initially increases in intensity until the concentration of ammonia is 0.025% and after that it decreases to a concentration of 0.1%. The results show that changes in the intensity of the red component can be used to determine the color changes experienced by the indicator label when sensing ammonia gas.

![Figure 3](image.png)

**Figure 3.** Changes in RGB values for different ammonia concentrations after being exposed for two hours.

Figure 4 shows the label response time when exposed to ammonia gas at different concentrations. The color change of the label from red to purplish brown to 0.005% concentration occurs after exposure to ammonia gas for 1 hour. In contrast to this, at larger ammonia gas concentrations, the color change of the label has occurred when the exposure time is 30 minutes. A rather typical phenomenon is observed at high concentrations of 0.1% and 0.5% where the color change of the label occurs very rapidly from red to purplish brown and then becomes yellow.
3.4 Effect of Temperature and Storage Conditions on Label Color Stability

Figure 5 (a) shows changes in the intensity value of the red component for storage conditions at various temperatures. At a low temperature of 8°C, the intensity of red decreases a little until the storage time is 24 hours, then the value is constant up to 60 hours. Whereas the labels were stored at 24°C and 40°C, the intensity of the red color decreased sharply at the beginning of 6 hours of storage time, after which the red intensity did not change significantly for up to 60 hours. These results indicate that temperature affects the stability of anthocyanin colors. Increased ambient temperature has a strong influence on anthocyanin degradation due to oxidation reactions [9]. Besides being caused by oxidation reactions, anthocyanin color degradation can also be caused by a deglicolization reaction that removes sugar groups from the anthocyanin structure [10].

Figure 5. Changes of red intensities in different temperature (a) and light conditions (b)

Based on the results shown in Figure 5 (b), the intensity of the red component of the indicator label stored in dark conditions for 60 hours did not experience significant changes. While in light conditions, the intensity of red decreases sharply from a value of 156 to 128 for the initial storage time of 6 hours, after this time there is no further change of up to 60 hours. These experimental results show that the labels stored in dark conditions have more color stability when compared to light.
conditions. These results are consistent with the results of previous studies using rosella flower extract [10]. Light affects the stability of anthocyanins which can accelerate color degradation.

4. Conclusion

The indicator label for sensing ammonia gas has been successfully prepared using black glutinous rice extract and paper matrix. This type of paper, especially the pH of the paper, greatly influences the color and sensing properties of anthocyanins. This label can detect ammonia gas with a concentration range from 0% to 0.5%. The label changes color from red to purple and then becomes yellow when the ammonia gas concentration detected is high. Labels have good stability when stored in low temperatures and dark conditions.

Acknowledgments

This research was financially supported by Hibah Publikasi Internasional Terindeks untuk Tugas Akhir Mahasiswa (PITTA) 2018, with the project 2244/UN2.R3.1/HKP.05.00/2018 from Universitas Indonesia.

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