A simple paper-based color change label using plant extracts for Ammonia gas detection

Arie Listyarini, Vivi Fauzia, Cuk Imawan*

Department of Physics, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Indonesia, Kampus Depok, Beji, Depok, Jawa Barat, Indonesia 16424

*cuk.imawan@sci.ui.ac.id

Abstract. Paper and plant extracts are materials that are cheap and easy to find everywhere, have great potential to be used as a portable, low-cost and biodegradable label for the application of chemical compounds detection. In this paper, we have explored potential methods for the label to detect ammonia gas, an indicator of meat spoilage, with the colorimetric principle. By immersing paper with plant extracts that are sensitive to ammonia, in this case, is anthocyanin from Ruellia simplex flower extract, the detection of ammonia at various concentrations can be achieved. Ammonia concentration used is 0; 0.005%; 0.025%; 0.1%, 0.25% and 0.5%. Color change images on the label when detection is scanned by a flatbed scanner. ImageJ software is used to quantify the color changes. Ammonia detection at various concentrations results in a color change from red to yellow on the paper label. Paper-based labels with discoloration that can be captured visually by the consumer's eyes are considered as promising approaches to determine the freshness of meat.

1. Introduction

Colorimetric detection of a chemical compound using paper is the simplest method. Its effectiveness testing is accessible by using low-cost and general image analysis such as Photoshop, ImageJ and devices such as digital cameras, scanners or even cell phone cameras [1]. The device labelling this indicator using paper matrix has many advantages including ease of carrying, ease of fabrication cost savings, and using fewer reagents and samples [2], [3]. Paper is a porous matrix and flexible consisting of hydrophilic cellulose fibers. Paper with intrinsic capillary properties causes fluid to flow without outside force [1]. Paper also has the advantages of inexpensive, biodegradability, ease of carrying, high availability, porous matrices and hydrophilic surfaces making the paper a suitable candidate for absorbing and loading reagents such as nanoparticle suspension and in this study natural chemicals that are sensitive to chemicals [4].

Ammonia is a compound that is released by food during the decay process. The detection of ammonia can be used as an indicator of food spoilage. The detection of ammonia using natural dyes was carried out by researchers in a previous study using Syzygium oleana seed extract with chitosan/nanocellulose as a matrix [5], curcumin in tara-gum/polyvinyl alcohol [6] and black glutinous rice extract paper label [7]. Paper as a matrix for ammonia detection labels has been made by several researchers using synthetic dyes [8] and black glutinous rice extract [7].

Ruellia simplex is a plant that is easy to grow and widely grown in the tropics. In Ruellia simplex flowers found anthocyanin which is a natural dye that changes color according to environmental pH conditions [9], [10]. Paper labels with Ruellia simplex extract can be used to detect food damage such as shrimp [11] or fish [12]. The use of Ruellia simplex as an indicator of the presence of chemical...
compounds such as ammonia is still rarely done by other researchers. In this study, we observed the response of the indicator label from paper based *Ruellia simplex* flower extract to the vapor of ammonia solution at various concentrations and exposure times. The response found was a change in label color quantified by using ImageJ software.

2. Materials and Methods

2.1. Materials
The basic material used in this observation was absolute ethanol obtained from JT Baker, Ammonium Hydroxide from Merck and Whatman filter paper No 1 Cat 1001.

2.2. Preparation of Extract from *Ruellia simplex* flowers
*Ruellia simplex* flowers obtained from the Universitas Indonesia area are weighed as much as 5 g. Then the flowers were extracted by maceration method using 70% ethanol as much as 100 mL with addition of HCl 0.1 N until the solution reaches pH 2. The maceration process was carried out for 4 x 24 hours under dark conditions and at 9 °C. The extract solution separated from *Ruellia simplex* flower residue by filtering using filter paper No.1. The extract solution was kept in the dark glass bottle and was ready to be used as an extract of the natural dyes of *Ruellia simplex*.

2.3. UV Spectroscopy of *Ruellia simplex* Flower Extract
The UV-Vis spectrum of *Ruellia simplex* flower extract was measured using the Genesys 10D Thermo UV-Vis spectrophotometer. The spectrum of extract at pH 2.0 - pH 10.0 was measured in the wavelength range of 400 - 800 nm. Previously the pH of the extract solution was adjusted as desired using a buffer solution pH 2.0 - pH 10.0.

2.4. Response test of *Ruellia simplex* flower extract to Ammonia vapor
*Ruellia simplex* flower extract in an open tube container as much as 2.0 mL each placed in a tightly closed chamber with ammonia solution with a concentration of 25% v/v. *Ruellia simplex* flower extract solution before and after exposure to ammonia vapor for 5 until 25 minutes observed for color changes. Then the UV-vis spectrum was analyzed in the wavelength range of 400 - 800 nm to confirm the difference in the intensity and maximum wavelength of the extract solution.

2.5. Preparation of paper-based color change label
Whatman paper No. 1 Cat. 1001 with a diameter of 125 mm dipped in a solution of *Ruellia simplex* flower for 30 seconds. The indicator label dried at room temperature. After thoroughly drying, the label cut with a 0.5-inch diameter paper punch. The indicator label was stored in a dry place with a closed container and protected from sunlight. The indicator label is ready to be used to detect ammonia.

2.6. Test the color change label for ammonia detection
The concentration of ammonia solution used in this experiment was 0, 0.005% v/v, 0.025% v/v, 0.05% v/v, 0.1% v/v and 0.5% v/v. Each ammonia solution at various concentrations was placed in a different tightly closed chamber which has an indicator label in it. Observation of the color change of the indicator label due to vapor exposure of ammonia solution was carried out after exposure time for 0.5 hours, 1 hour, 1.5 hours, 2 hours, and 3 hours. Changing of the color of the indicator label was observed visually and scanned using an Epson V800 flatbed scanner. The RGB spectrum was analyzed using the ImageJ program.

3. Results and Discussion

3.1. UV-Vis spectrum of *Ruellia simplex* flower extract solution in various pH ranges
The extract of *Ruellia simplex* flower at pH 2.0 produces a red color. The color of *Ruellia simplex* flower extract that has been added buffer pH 2.0 - pH 10.0 as shown as in Figure 1. (a). The initially red color of the extract turns pink at pH 4.0, colorless at pH 6.0, blue at pH 8.0 and green at pH 10.0. The red
solution indicates anthocyanin in the form of flavylium cation. Then the red color fades towards colorless due to the formation of a colorless pseudo base. Then this form of pseudo base undergoes tautomerism, the equilibrium between the keto form and the enol form results in alpha dicon which eventually forms a blue quinoidal base at pH 8.0. The extract solution starts to turn green when pH 10.0 because chalcone starts to form [13]. The UV-Vis spectrum was analyzed using a spectrophotometer at a wavelength of 400 - 800 nm. The absorption spectrum results are shown in Figure 1. (b). The UV-Vis spectrum of Ruellia simplex flower extract at pH 2.0 shows the maximum absorption peak at a wavelength of 535 nm. The absorbance peak shifts to a wavelength of 585 nm at pH 4.0 and 6.0 and subsequently shifts to wavelengths of 630 nm and 650 nm at pH 8.0 and 10.0 respectively. This similar trend of absorbance peak and its shift in the extract solution pH 2.0 - pH 10.0 were also found in the Bauhinia blakeana dunn flower extract [14], grape skin extract [15] and purple sweet potato extract [16], [17].

Figure 1. (a) Variation in color changes and (b) UV-Vis spectrum solution of Ruellia simplex flower extract at pH 2.0 - pH 10.0.

3.2. Test the response of Ruellia simplex flower extract to ammonia

The color of the Ruellia simplex flower solution when exposed to 25% v/v ammonia solution over a range of 0 – 25 minutes is shown in Figure 2. (a). The color of the Ruellia simplex extract solution changes from red to green. The decrease in the intensity of the red color of Ruellia simplex extract solution occurs within 1 - 5 minutes of exposure to ammonia vapors which then turn into slightly greenish and then dark green. This discoloration is confirmed by the maximum shift of absorption peak in the UV-Vis spectrum which can be seen in Figure 2. (b). The extract solution of Ruellia simplex flower before exposure until exposure for 5 minutes has a maximum absorption peak at a wavelength of around 522 nm. The absorbance intensity of the extract solution decreases with increasing time of exposure to ammonia vapor. After the exposure time of 8 minutes the maximum absorption peak shifts to the right to a wavelength of 650 nm and the absorbance intensity increases with increasing exposure time. The increase in pH of Ruellia simplex extract solution when exposed to ammonia vapor can be seen in Figure 2. (c). The pH was beginning changes at 5 minutes exposure time and change quickly until 20 minutes exposure time. After the exposure time of 20 minutes the pH change tends to be flat and it is assumed to have reached the pH limit of the ammonia solution.
Figure 2. (a) Change in color, (b) Uv-Vis spectrum and (c) pH solution of *Ruellia simplex* flower extract solution when exposed to the vapor of ammonia solution 25% v/v in the time range 0 - 25 minutes

3.3. Test color change label for ammonia detection

The color of the label after detecting ammonia vapor at various concentrations of ammonia solution in the range of 0 - 4 hours can be seen in figure 3. The color of the label that is initially red will turn bluish purple at low concentrations and eventually yellowish at higher concentrations. The blue color changes on the label because of alkaline conditions support the formation of a blue quinoidal base from the anthocyanin found in *Ruellia simplex* flower extract. The higher the concentration of ammonia solution and also the longer exposure time, the more the amount of ammonia vapor causes the further equilibrium to the yellow chalcone form [18]. The color produced by the indicator label paper when detecting ammonia vapor is the same as when the *Ruellia simplex* flower extract solution is exposed to ammonia vapor in figure 2 (a). This shows that the paper does not give a color change or changes the anthocyanin structure in the extract of the dye. The paper functions only as a dye binding matrix.

The color of label paper was scanned with a flatbed scanner and quantified with an RGB color system. Figure 4. (a) shows the quantification of changes in the RGB color of the label when exposed to aquabidest as blank, ammonia solution concentration of 0.025% v/v until 0.5% v/v. The figure shows a decrease in red value while the blue and green color values increase with increasing the concentration of ammonia. Likewise, the increased exposure time for the label will increase the intensity of the green color as shown in Figure 4. (b).
Figure 3. Color changes in indicator label paper at exposure to the concentration of ammonia solution 0 - 0.5% v / v for 0 - 4 hours.

Figure 4. (a) The intensity of the RGB of color change label when exposed to ammonia vapor concentration of 0 - 0.5% and (b) the intensity of the label’s green color vs. exposure time using ammonia concentration of 0.5% v/v.

4. Conclusion

Ruellia simplex flower extract contains anthocyanin compounds that can change color with changing of pH conditions. This color change can be used to detect ammonia vapor. The color change of the extract when detecting ammonia vapor is from red to green. Paper can be used as an indicator label matrix for extract of Ruellia simplex flowers. The color change produced when the indicator label paper detects ammonia as well as Ruellia simplex flower extract is from red to yellowish green. Color quantification shows a linear trend of increasing the green intensity concerning exposure time for low concentrations of ammonia solution. For the application, this color change label paper can be used as an alternative to the simple and inexpensive detection method for real-time meat freshness.

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