A colorimetric label for detecting acetic acid vapor using *Ipomoea pes-caprae* flower extract as a functional dye

M Syintia and C Imawan*

Physics Department, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Indonesia

*Corresponding authors: cuk.imawan@sci.ui.ac.id

Abstract. Colorimetric labels used to detect acetic acid vapor are becoming very popular as they are useful for directly monitoring the freshness of dairy products. In this paper, an investigation of a colorimetric label made of tracing paper dyed with *Ipomoea pes-caprae* flower extract to detect acetic acid vapor was reported. For extraction of *Ipomoea pes-caprae* flowers have been used maceration method. The absorbance spectrum as a function of the pH of the extract solution has been measured with UV-Vis spectrophotometer equipment. These labels were tested for detecting of acetic acid vapor produced by the solutions of various concentrations for 12 hours. The labels were then scanned with a flatbed scanner, and then its image has been analysed using ImageJ software to obtain its RGB color component. The label was originally green and after detecting acetic acid vapor the color changed to pink. The greater concentration of acetic acid solution provides a change from the color of the labels more quickly. The red components of the RGB show the highest sensitivity to acetic acid vapor. Results of the stability against the temperature and humidity of the label color test indicate that the color is more stable when stored at 10 °C and 55% relative humidity. Based on these results, this colorimetric paper label can be used as a monitoring label for freshness of dairy products.

1. Introduction
Various methods have been developed to detect and determine the gas content of a food product. Gas measurement can be carried out by an electrochemical or optical method. In the food industry, optical methods are preferred because it does not damage the sample, has a smaller size, and does not require electrical power [1]. Optical methods depend on the type of dye used, which are luminescent dye or dye that works as a pH indicator (colorimetric method). The use of fluorescent dyes requires additional devices to excite electrons and detect luminescence, making it less practical than the colorimetric method[2]. The colorimetric method is based on discoloration due to changes in pH. Considering that the colorimetric method is more food grade, easily applied and the color change of the indicator after detecting gas can be observed directly, this method is chosen for detecting acetic acid vapor.

Colorimetric labels require dyes that change its color when interacting with the target compound. The dyes used can be natural or synthetic dyes. Some synthetic dyes that have been used for colorimetric labels are bromothymol blue, methyl red[3], bromocresol green, phenol red-purple[4], dimethyl yellow, and chlorophenol red [5]. Compared to synthetic dyes, natural dyes are considered safer and environmentally friendly. Natural dyes that are widely developed in colorimetric labels are anthocyanin and betalain [6]. Anthocyanin has the best response to pH compared to other natural dyes.
The anthocyanin color range includes almost the entire color spectrum of visible light. The anthocyanin properties are non-toxic, easily extracted, soluble in water and other organic solvents, and are contained in almost all plants, making this compound suitable for use as a dye.

A matrix is needed to immobilize dyes on a colorimetric label. The compounds that are widely developed as label matrices are cellulose[7], chitosan[8], polyvinyl alcohol[9], and starch[10]. Cellulose has several advantages compared to other matrix compounds. This compound is odorless, has good mechanical properties, is biodegradable, and easy to form sheets.

In this study, the anthocyanin was obtained from the extract of Ipomoea pes-caprae flower. Cellulose paper was used as a matrix. The prepared label was tested to detect acetic acid vapor and the color change from the label was analyzed quantitatively by counting RGB value. This color is used to set the standard color of the label used as a reference when the label is used as an indicator.

2. Methods

2.1. Materials

*Ipomoea pes-caprae* was obtained from local plants. The solvents in the anthocyanin extraction process were hydrochloric acid (HCl) and ethanol, ordered from Mallinckrodt and J. T. Baker respectively. Whatman No.1 filter paper was used to filter the extract solution. Tracing paper used as a label matrix were obtained from the local market. Glacial acetic acid (99.9% CH\(_3\)COOH), Magnesium Chloride (MgCl\(_2\)), Magnesium Nitrate (Mg(NO\(_3\))\(_2\)), Sodium Chloride (NaCl), and Potassium Nitrate (KNO\(_3\)) ordered from Merck.

2.2. Extraction of *Ipomoea pes-caprae* flowers and label fabrication

The solvent used in the extraction process was ethanol-HCl 1.5 M (85:15 v/v). Ipomoea pes-caprae were immersed in solvents with a solid-liquid ratio of 4: 5 (w/v). During the maceration process, the container was wrapped in aluminum foil. This solution was stored for 24 hours at 5 °C. After immersion, the solution was filtered using Whatman No. 1 filter paper and stored at 10 °C, before being used for further experiments.

The extracts obtained were conditioned at pH 2-13 by adding a 0.5M NaOH solution gradually until they reached the desired pH value of the solution. The absorbance of the solution was measured by the UV-visible spectrophotometer - Genesys 10S from Thermo Scientific. The results of the analysis of optical properties led to the selection of a solution with pH 9 as a dye solution for the detection label of acetic acid.

Label fabrication was done by cutting tracing paper into a circle with a diameter of 1.5 cm using a hole punched paper. The paper was then immersed in an extract solution of pH 9 for 1 hour, then allowed to dry for 5 hours at 25 °C.

2.3. Characterization and acetic acid sensing testing of the label

The color characterization of labels before and after detecting acetic acid vapor was carried out using a flatbed scanner from the Epson Perfection V800. The scanning results were then analyzed by ImageJ software to obtain RGB values. The sensing label properties of acetic acid were tested by placing labels in bottles containing 30mL acetic acid solution with varying concentrations of 20%, 40%, 60%, 80%, and 100%. The distance between the label and the surface of the acetic acid solution was kept about 10 cm. Observations for discoloration of labels were carried out at each interval of exposure to 15, 30, 60, 120 and 240 minutes.

2.4. Temperature and Relative Humidity Stability test of the label

The labels were tested at 100, 250 and 400 C for one week. Testing the color stability of the label for relative humidity (RH) was carried out in airtight containers for five days. The relative humidity was conditioned and varied using saturated salts, using MgCl\(_2\) (33%), Mg(NO\(_3\))\(_2\) (55%), NaCl (75%), and KNO\(_3\) (94%). Color label observations in both tests were carried out every 24 hours by scanning labels using a flatbed scanner.
3. Result and Discussion

3.1. The color of the extract solution at various pH

The extract solutions have various colors for pH 2-13, as seen in Figure 1. The color of the solution at pH 2-3 is red. The solution is transparent for a pH range of 4-6 and has a light brown color at pH 7, light blue at pH 8, and is green at pH 9. After being conditioned at pH > 10, the solutions become yellowish.

The color change of the solution after pH conditioning is due to a shift in the anthocyanin acid-base equilibrium in the solution. Anthocyanin in a solution can be in the form of flavylum cation, carbinol pseudo base, quinoidal base, or chalcone. Flavylum cation can be orange, red or purple, depending on the type of aglycone [11]. This species is dominant at pH 1-3. The equilibrium will shift towards carbinol pseudo base at pH 4-6. Because this species is colorless, the color of the solution will appear more transparent. The solution will turn bluish when the pH is conditioned in the pH range 7-9. This is due to a shift in the equilibrium towards the quinoidal base [12]. In the base condition, the chalcone species will be more dominant and cause the color of the solution to get yellow [13].

![Figure 1. The color of the Ipomoea pes-caprae extract solution at pH 2-13](image)

3.2. The absorbance spectrum of the extract solution

The results of the absorbance measurement for the pH 2-13 solution are shown in Figure 2. The extract solutions with pH 2 and pH 3 had an absorbance peak at a wavelength of 533 nm. This area absorbs green, so the solution looks purplish red. The peak of absorbance at pH 2 and pH 3 was 0.5910 and 0.3863 respectively. This decrease in absorbance shows the red color of the extract solution pH 3 is weaker than pH 2. There is no absorbance peak for the extract solution pH 4-7. The intensity of absorbance at 533 nm for this pH range dropped dramatically, where pH 4, 5, 6 and 7 respectively had intensities of 0.1202, 0.1250, 0.0959 and 0.1445. Therefore, the color of the solution looks transparent. The absorbance peak shifted to a wavelength of 596 and 612 nm for pH 8 and 9 respectively. This area absorbs orange and shows a blue-green color in the solution. There was a slight increase in absorbance in the wavelength range of 675-725 nm for pH 9 extract solution. Because this area absorbs red, the extract solution pH 9 has a greener color than pH 8. The absorbance peak shifts to wavelength 380, 383, 388, and 381 nm for solutions conditioned at pH 10, 11, 12 and 13 respectively. The colors absorbed in the 380-400 nm wavelength range are purple, which has a complementary yellow-green color. Therefore, the extract solution at pH 10-13 has a yellow color.

![Figure 2. The absorbance spectrum of extract solution at pH 2-13](image)
3.3. Effect of substrate on label response
Testing labels of two different types of paper against glacial acetic acid have been carried out. The papers used were tracing paper and Whatman No.1 filter paper. The response of the two types of labels is shown in Figure 3. The difference in label responses from the two is due to the morphology of each paper and the comparison between the total anthocyanin immobilized on paper with the amount of anthocyanin acting with acetic acid. Based on the results of testing, the label of tracing paper looks to have a greater degree of color change than the label made from Whatman filter paper. This is because the fibers in tracing paper are denser so the amount of anthocyanin absorbed is less than Whatman's paper.

![Figure 3. The response of two different labels against the solution of 100% acetic acid](image)

3.4. The response of the labels against acetic acid solution
The difference in label color changes to the vapor concentration of acetic acid after 12 hours of exposure is shown in Figure 4. It can be seen that the red color change is greater than the green and blue components. This indicates two things: first, there is an increase in the total RGB intensity which causes the color to get weaker; second, the red color will be more dominant for the label that is exposed to the greater acetic acid vapor concentration. The label color will change from green to red as the acetic acid solution concentration increases.

![Figure 4. Plotting of the acetic acid concentration against changes in RGB values after 12 hours](image)

The fitting results of the RGB change against concentration are used to determine the standard color of the label. Examples of label designs based on fitting results are shown in Figure 5. Through these standard colors, the amount of acetic acid vapor can be known by matching the color of the label to the standard color.
3.5. The color stability test against temperature

The measurement results of the RGB intensity of the label stored at 10 °C, 25 °C, and 40 °C show the same pattern. Figure 6a shows the color of the label monitored for 7 days when stored at 10 °C. There was an increase in RGB label intensity on the first day, then it decreased again on the second day. This pattern is repeated until the 7th day. This can be caused by other unregulated factors, such as the relative humidity of the room during the day.

Changes in RGB intensity are greater for higher temperatures. As shown in figure 6b, the label that has the fewest changes is the label stored at 10 °C. This occurs because the thermal degradation of anthocyanins occurs more rapidly for higher temperatures [14]. In addition, at all three storage temperatures, it was seen that the green components have the least change in the RGB value.

3.6. The color stability test against relative humidity

The results of measuring the RGB intensity in various relative humidity are shown in Figure 7. The red, blue and green colors have the same pattern. This indicates that the color of the label fades somewhat, but does not change color from one color to another. The changes in the color due to relative humidity are also smaller than changes in color due to acetic acid vapor. Therefore, the label can still be used as an indicator of acetic acid vapor. The measurement results will be more accurate after taking into account the correction factor of the label stability testing against relative humidity. Referring to the graph shown in the inset of Figure 7, the label is well stored at 55% RH.
4. Conclusion
The colorimetric indicator label can be used to detect acetic acid vapor. The color of the label will change from green to red, with the intensity of red increasing and faster for the higher concentration of acetic acid vapor. The change in color from green to red is caused by a decrease in the label pH of the environment due to acetate vapor. Because the initial condition of the label is pH 9 and is green, when the acetic acid vapor increases, the environment will become more acidic and cause the dye to become red. Labels are best stored at cooler temperatures (10 °C) and at 55% relative humidity.

References
[1] Puligundla P, Jung J and Ko S 2012 Food Control 25 328
[2] Saliu F and Pergola R D 2018 Sens. Actuators B: Chem. 258 1117
[3] Kuswandi B and Nurfawaidi A 2017 Food Control 82 91
[4] Rukchon C, Nopwinyuwong A, Trevanich S, Jinkarn T and Suppakul P 2014 Talanta 130 547
[5] Zhang Y and Lim L-T 2018 Sens. Actuators B: Chem. 255 3216
[6] Calogero G, Yum J-H S A, Marco G D, Gratzel M and Nazeeruddin M K 2012 Sol. Energy 86 1563
[7] Devarayan K and Kim B-S 2015 Sens. Actuators B: Chem. 209 281
[8] Yoshida C M P, Maciel V B V, Mendonça M E D and Franco T T 2014 LWT - Food Sci. Technol. 55 83
[9] Pereira Jr V A, Arruda I N Q D and Stefani R 2015 Food Hydrocoll. 43 180
[10] Silva-Pereira M C, Teixeira J A, Pereira-Júnior V A and Stefani R 2015 LWT - Food Sci. Technol. 61 258
[11] Martin J, Navas M J, Jiménez-Moreno A M and Asuero A G 2007 Anthocyanin Pigments: Importance, Sample, Preparation and Extraction," in Phenolic Compounds - Natural Sources, Importance and Applications, M. Soto-Hernandez, Ed. (London: Intech)
[12] Zhang Y, Butelli E and Martin C 2014 Curr. Opin. Plant Biol. 19 81
[13] Ananga A, Georgiev V, Ochienj J, Phills B and Tsolova V 2013 Production of Anthocyanins in Grape Cell Cultures: A Potential Source of Raw Material for Pharmaceutical, Food, and Cosmetic Industries," in The Mediterranean Genetic Code - Grapevine and Olive (London: Intech)
[14] Askar K A, Alsawad Z H and Khalaf M N 2015 Beni-Suef Univ. J. Basic Appl. Sci. 4 262