

Improving the Detection of Blood on Poultry Meat Based Colorimetry Using a Camera

Koekoeh Santoso^{1*}, Hadri Latif¹, La Jumadin², and Putri Gusfani Wida¹

¹ School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor 16680, Indonesia

² Department of Biology Education, Faculty of Teacher Training and Education, HaluOleo University, Kendari 93231, Indonesia

*e-mail: koekoehipb@gmail.com

Abstract. The purpose of this study was to measure blood levels in quail meat with a cellphone camera using a spectrophotometer as a standard. Samples were obtained from 9 pieces of quail meat cut by hanging, 9 pieces of quail meat cut without hanging, 9 pieces of quail carcass dead for 4 h, and 9 pieces of quail carcass dead for 8 h. The relative concentration of blood in the meat of the quail carcass with or without hanging measured on 430 nm with spectrophotometer after 4 h and 8 h were 0.0149%, 0.0165%, 0.0173%, and 0.0177%, respectively. Since the absorption coefficient of the main blood absorber namely hemoglobin differs significantly in the violet and red spectral regions, the time dependence of the absorption intensity obtained by spectrophotometer was compared with a similar dependence of the blue channel of a smartphone, measured using ImageJ. It has been shown that measurements by the smartphone have a similar tendency as the measurements of the spectrophotometer, and they can be used as indicators of blood concentration threshold in quail meat, and, as a consequence, determine the duration of this procedure. © 2024 Journal of Biomedical Photonics & Engineering.

Keywords: carrion; colorimetry; digital image; ImageJ; quail.

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1 Introduction

Quail are livestock that produce meat and eggs as a source of animal protein [1]. The perfect bleeding process can be seen by looking at the hemoglobin parameters in the carcass. Hemoglobin levels can be determined using the spectrophotometer method.

The UV-Vis spectrophotometry method has the advantage that the results obtained are quantitative so that the results can be more accurate [2]. The spectrophotometric method uses monochromatic light to observe changes in the clarity of the blood-Ethylen Diamine Tetra Acetic Acid (blood-EDTA) boundary at one point, namely the top part, which is recorded in the form of absorbance values. Light absorbance values that gradually change indicate changes in the concentration in blood plasma [3]. The light absorbance spectrum stores cellular interaction information that describes the erythrocyte aggregation process. The absorbance

spectrum can be analyzed using the Principal Component Analysis (PCA) method, which is a calculation used for data analysis in multidimensional data sets, especially in the field of graphics. PCA reduces information without having to eliminate the main information by finding eigenvalues and eigenvectors to obtain covariance and correlation values between dimensions in data [4]. The spectrophotometer uses serum or plasma as an object of registration, so it is not influenced by blood cells like in whole blood samples [5]. The use of a spectrophotometer requires preparation of serum samples, so it requires more blood and the process takes a long time.

There is an alternative method to determine the color intensity in a solution, namely the digital imaging method using a camera [6]. The camera measures electromagnetic radiation from colored zones via the camera sensor [7]. Digital imaging methods with cameras are capable of detecting objects, and role of camera sensors is to capture the color of an object and convert

visual data into digital data, which can be processed [8]. The algorithm of the camera processes information to produce output values red (R'), green (G'), and blue (B') from the actual red (R), green (G), and blue (B) values [7]. The color resulting from the malachite green test then are captured by the camera and visualized. The resulting camera image is then processed using an image processor, namely ImageJ [9]. This research is expected to be able to show the potential of cameras to measure blood concentration in quail meat based on calorimetry carried out with a spectrophotometer as the standard.

2 Materials and Methods

Equipment and sample preparation were carried out at the Physiology Laboratory, Department of Anatomy, Physiology, and Pharmacology, School of Veterinary Medicine and Biomedical, Bogor Agricultural University. Quail meat samples were obtained from the Observation Laboratory of the Department of Anatomy, Physiology, and Pharmacology, School of Veterinary Medicine and Biomedical Medicine, Bogor Agricultural University.

This study used a completely randomized design with factors of quail slaughtered by hanging, not hanging, and quail carcasses which were kept for 4 h and 8 h. The number of quail was calculated using the Federer formula $(t-1)(n-1) \geq 15$. We used 36 quails: 9 slaughtered by hanging process, 9 slaughtered without hanging process, 9 carcasses that were kept for 4 h, and 9 carcasses that were kept for 8 h.

The difference in the treatment of the carcasses that were kept for 4 h and 8 h before measuring blood concentrations, was based on the lag time required between slaughtering and selling poultry in the market which usually takes 4 to 8 h.

Before examination of the sample, a standard curve is made as a reference for the results. The standard curve was prepared from FeCl_3 in distilled water, then diluted again in HCl.

Quails that were slaughtered by hanging and not hanging were taken from the thigh, then cut into small pieces and soaked in distilled water for 30 min. The extract was centrifuged at 3000 rpm for 10 min, then the precipitate and liquid were separated. Quail carcasses were kept for 4 h and 8 h, then subjected to the same treatment as quails that were slaughtered by hanging or not hanging. The extract used is from the liquid part.

The meat extract was reacted with malachite green and H_2O_2 then homogenized using a vortex and left for 20 min so that the reaction took place perfectly. After 20 min KMnO_4 was added to stop the reaction and then it was homogenized again using a vortex.

The results were registered with a spectrophotometer after adding KMnO_4 . Scanning is also performed using a digital camera in the microplate. Digital camera calibration begins with extracting feature points in the video, then tracing them throughout the video. The movement of these points is then minimized using the objective function and changing the camera parameters, and in this way, a set of calibrated parameters is

obtained [9]. The sample to which KMnO_4 had been added was measured using a spectrophotometer, then the sample was transferred to a microplate using a micropipette at 0.2 mL/well. The microplate was placed on the scanner and closed using a mobile camera Scanjet XPA. Image scanning using a scanner is carried out from under the microplate. The microplate when using a mobile camera is placed on top of the mobile camera Scanjet XPA and then covered with a wooden box. Taking images using a mobile camera is done from above the microplate. Images that have been scanned with a scanner are saved in Tagged Image File Format (TIFF) format and images taken by mobile cameras are saved in Joint Photographic Experts Group (JPEG) format.

The images were processed using image processing software ImageJ obtained from the plate wells. ImageJ represents the color component in pixels and converts it to numbers in the RGB color component. The intensity of the color obtained is translated into absorbance by the Lambert-Beer law. Next, the absorbance value is entered into the equation of the standard curve obtained so that the concentration of blood in the meat is known.

The UV-VIS Spectrophotometer calibration procedure refers to the procedures of the 2020 National Directorate of Thermoelectric and Chemical Measurement Units. The calibration procedure consists of 3 stages, namely initial inspection, equipment preparation, and calibration. The initial inspection stage begins with carrying out a visual inspection, checking the stray radiant energy (SRE) value using an SRE filter in the 200 nm to 450 nm area, measuring baseline flatness and noise from the instrument, as well as monitoring and recording the environmental temperature around the spectrophotometer. The equipment preparation stage is carried out by cleaning the standard filters used either using an air blower or with a lint-free cloth (microfiber). The calibration stage begins with turning on the instrument for 30–60 min, setting the wavelength scanning range from 240 nm to 650 nm, setting the slit size, paying attention to the wavelength scanning distance (interval/step), and repeating the measurement 9 times.

Using ImageJ for particle analysis requires the definition of the part of the image which is an object/particle and the part of the image which is the background. It is referred to as image segmentation. This can be done using Image > Adjust > Threshold. By setting the Threshold level, you can adjust the brightness level of the image for the definition of objects/particles and their background. After the Threshold adjustment is made, the measurement of the size/area of the intended particle can be carried out by the particle analysis feature in ImageJ. Particle analysis in ImageJ can be done by clicking Analyze > Set Measurements to determine what output you want in particle analysis. Then particle analysis is done by clicking Analyze > Analyze Particles.

Data were analyzed quantitatively using Microsoft Excel 2010 to obtain concentration. The results of the concentrations were analyzed statistically by comparing blood concentrations in meat slaughtered by hanging, not

hanging, carcass meat which was kept for 4 h and 8 h using a spectrophotometer and a camera, then analyzed using the descriptive one-way ANOVA (analysis of variance)) method.

3 Results and Discussion

3.1 Quail Blood Concentration Measurement with a Spectrophotometer

Measurement of the concentration of quail blood samples was carried out using a spectrophotometer. The standard spectrophotometer calibration curve is obtained by measuring the absorbance of a standard solution at a wavelength of 430 nm. Based on the measurement results of the standard spectrophotometer solution, the standard spectrophotometer calibration curve is obtained as shown in Fig. 1.

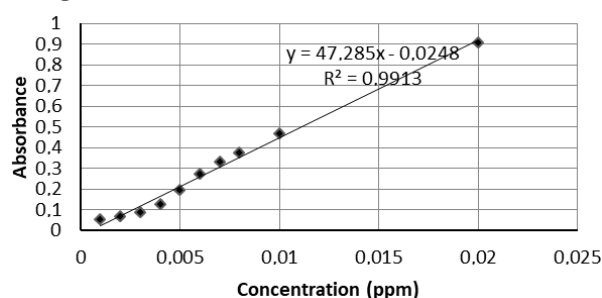


Fig. 1 Spectrophotometer standard calibration curve at an absorbance wavelength of 430 nm.

The standard curve shows the relationship between the concentration of the solution (x-axis) and the absorbance of the solution (y-axis). The regression line equation serves to determine the mathematical model used to predict one variable from another. While the correlation coefficient shows the relationship between concentration and absorbance. Based on the calibration curve above, the regression line equation is $y = 47.285x - 0.0248$ with a correlation coefficient of 0.9913. This shows that there is a positive correlation between concentration and absorbance. The concentration increases, the absorbance value also increases [10]. The correlation value is considered good if it is more than 80% ($R^2 \geq 0.80$) [11]. The largest R^2 value is 1, and the smallest is -1 , so that it can be written as $-1 \leq R^2 \leq 1$. For $R = 1$, it is called a perfect positive relationship and the direct linear relationship is very high.

3.2 Image Processing Program for Measuring Quail Blood Concentration with a Smartphone Camera Using ImageJ

Mobile phone cameras can capture color, which in principle is a derivative of the colorimetric analysis method. Images taken using a cellphone camera are saved in the JPEG format, which is an image with subtle color changes [6]. The results of taking images with the camera can be seen in Fig. 2.

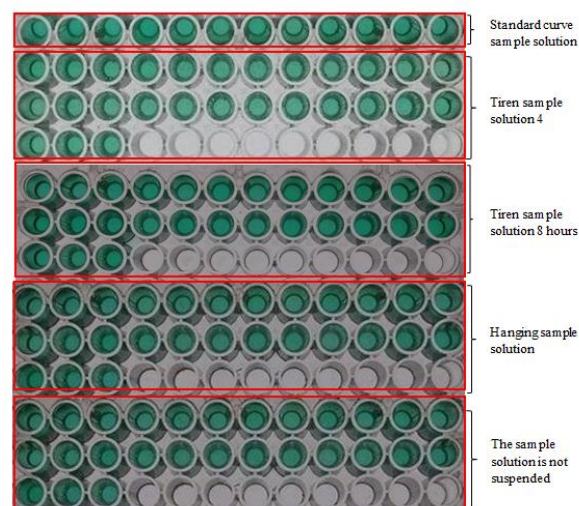


Fig. 2 Camera results from the microplate.

The compiled image processing program can determine the image parameters of objects analyzed through color images, namely the RGB color index. The RGB color index is analyzed using the help of ImageJ software and collected using Microsoft Office Excel 2013 to obtain the distribution of three indices. The ImageJ program automatically calculate, and the resulting data may be selected through the Regions of Interests (ROI) manager. The ROI manager contains a list of analyzes and the ROI manager function can help in selecting the quality of the calculated results. The standard camera calibration curve is described in Fig. 3.

The cellphone camera calibration curve (see Fig. 2) has different tilt angles for R, G, B. Of course, hemoglobin has a different absorption coefficient at different wavelengths, but it remains constant. Therefore, the calibration lines R, G, B can be different, but the angle of inclination must be the same. The fact that the angles are different means that the measurement scheme and spectral device characteristics have a great influence on the results.

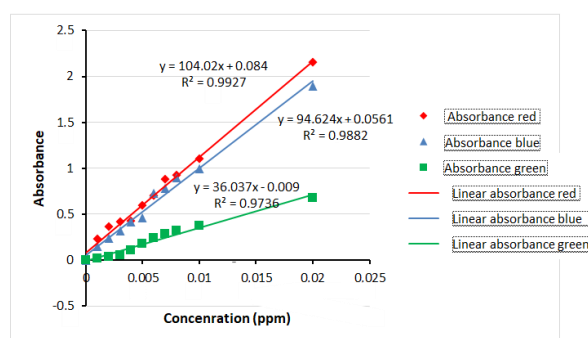


Fig. 3 Cellphone camera calibration curve.

Based on the calibration curve above, it was shown that the RGB channel produces different regression value equations and correlation coefficients. The correlation coefficients of each RGB channel are $y = 104.02x + 0.0084$, $y = 94.624x + 0.0561$, and

$y = 36.037x - 0.009$ with correlation coefficients $R^2 = 0.9927$, $R^2 = 0.9882$, and $R^2 = 0.9736$, respectively. This shows that there is a positive correlation between concentration and absorbance. The curve that has the steepest slope indicates that the curve is most sensitive to changes in concentration. Based on the calibration curve (see Fig. 3), the red curve was chosen for further calculations because of the larger correlation coefficient with better linearity which is reflected in the steeper shape of the curve. This is because when the sample solution is exposed to light, the camera will capture more light reflected by the red complement so that the absorbed color has the highest value. Sample solution and sample concentration have a linear relationship with absorbance. Absorbance is directly proportional to the concentration according to the application of the Beer-Lambert law in absorbance measurements.

3.3 Comparison of Quail Blood Concentration Measurements Using Spectrophotometers and Mobile Cameras

In this study, colorimetric measurements using spectrophotometry and image processing using cellphone cameras have the same measurement principle, namely measuring the wavelengths reflected by the sample. However, in the process of measuring these two tools have differences. The color value obtained from the capture of the wavelength by the spectrophotometer is the wavelength reflected by one surface area. Meanwhile, the color value obtained with the help of ImageJ is the color value captured by a digital camera from the reflection of the wavelength by the cellphone camera from a small portion of the surface area. A comparison of blood concentration in the carcass and non-carcass quail meat can be seen in Table 1.

The results obtained using the spectrophotometer show a significant difference ($p < 0.05$) between the slaughtered quail meat group and the carcass quail meat group. The value of blood concentration in carcass meat was greater than in the group of slaughtered quails hanging and slaughtered not hanging. The carcass sample smells bad, so the sample has produced H_2S [12]. The myoglobin content of the carcass samples was more than that of the quail samples which were slaughtered by

hanging or not, so the concentration numbers were lower than those of the live samples. This is caused by several factors which are the disadvantages of digital imaging. First, the lighting system consists of lighting coming from the scanner which affects the intensity of the light produced. If the resulting color is weaker or stronger, it will affect the subtracting number for calculating the difference in absorbance in the quail meat solution. The absorption value in the solution obtained for carcass and non-carcass quail meat depends not only on blood concentrations, but also on other products. The turbidity of the solution increased over time, indicating an increase in other components in the solution. In this case, the results shown in Table 1 are not only interpreted as changes due to changes in blood concentration, but as changes due to an increase in the amount of other impurities. Indirectly this is evidenced by the presence of smell.

Blood concentration and absorbance are the ratios of the intensity of the absorbed light to the intensity of the incident light. This absorbance value depends on the levels of substances contained in a sample, the more molecules absorb light at a certain wavelength so that the absorbance value is greater, or in other words, the absorbance is directly proportional to the concentration of the substance contained in a sample. The graph of the relationship between the absorbance values obtained using the spectrophotometer and using a cellphone camera is presented in Fig. 4.

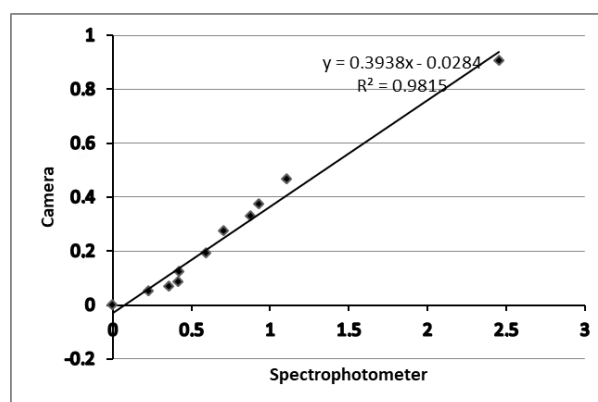


Fig. 4 The relationship between the absorbance value of the spectrophotometer and digital image processing.

Table 1 Concentration of blood in quail meat (%).

Treatment	Spectrophotometer	Mobile Camera
Slaughter hanged	0.0149 ± 0.00108^a	0.0022 ± 0.00007^a
Slaughter is not hung	0.0165 ± 0.00078^b	0.0051 ± 0.00064^b
Carcass 4 h	0.0173 ± 0.00135^c	0.0062 ± 0.00065^c
Carcass 8 h	0.0177 ± 0.00183^c	0.0067 ± 0.00043^d

Note: Different superscript letters (a, b, c, d) in the same column indicate a significant difference.

From the graph on Fig. 4, the regression equation $y = 0.3938x - 0.0284$ is obtained with a correlation coefficient value of 0.9815. The absorbance value of solution obtained using the standard spectrophotometer with the measurement results of digital image processing has a high positive correlation with the absorbance value of a digital camera with a correlation value of more than 0.9. This shows a very strong relationship. This regression value can explain the changes in the value of the variable y (actual lab value). The absorption of hemoglobin in the violet region of the spectrum (430 nm) differs significantly (by two orders of magnitude) from the absorption in the red region. Therefore, it will be correct to compare the dependencies obtained from the

spectrophotometer (430 nm) with the smartphone calibration curve in the blue channel. As can be seen from the comparison in Figs. 2 and 3, they differ significantly.

4 Conclusions

Measurements using smartphone are indicative, namely, they allow one to determine the duration for a certain threshold of relative blood concentration in the meat of suspended quails.

Disclosures

The authors declare that they have no conflict of interest.

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