Compared Potential of Smartphone Camera and Spectrophotometer To Measure Beef Blood Content

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Abstract: Beef consumption increases every year in Indonesia. However, beef must be considered safe and halal. Complete bleeding during slaughter will cause the quality of the meat to be good. One way to determine this is to test the blood content in beef. In general, meat is obtained from the process with or without stunning before slaughter. This study compares the potential of a smartphone camera with a spectrophotometer to measure the blood content in beef with and without stunning before slaughter at the slaughterhouse and at the site of slaughter of sacrificial animals. This study used 12 local cattle for each treatment. The green solution of 0.1% beef extract and Malachite Green was reacted with the addition of H_2O_2 solution. If there is no hemoglobin in the meat extract, the mixed solution changes color gradually from green to blue according to the hemoglobin content in the meat extract. The gradation of this color change was measured using a spectrophotometer as the gold standard and the image processing technique produced by a smartphone camera using ImageJ software. The results obtained were converted into concentrations using the Lambert-Bier formula. The blood concentration in beef obtained by stunning was lower than that obtained without stunning before slaughtering using either spectrophotometer A, spectrophotometer B, or smartphone camera devices but not significantly different after T test using SPSS ($P>0.05$). The correlation test of the tool produces a regression equation $y=1.161x-0.000$ between spectrophotometers A and B and $y=0.5613x-0.0005$ between spectrophotometer A and camera devices with correlation coefficients of 0.9962 (very strong) and 0.7431 (strong) so that mobile cameras have the potential to measures the concentration of blood in meat.

Keyword: Beef, Stunning, Slaughter, Smartphone Camera

Introduction

Beef is one of the foodstuffs of animal origin that is very important to meet the nutritional needs of the community because beef is one of the foodstuffs of animal origin which is rich in protein, iron and several important vitamins, especially vitamin B. very strategic economy. In addition to its nutritional value, people judge meat from its characteristics such as tenderness, taste, aroma, color and preferred oil essence. In the last decade, the average consumption of beef in Indonesia has reached 2.37 kg per capita per year and tends to increase and is expected to continue to increase in line with public awareness of the importance of animal protein, population growth, and increasing people's purchasing power (Agustina) et al., 2016). For 2020, the national demand for beef is 717.15 thousand tons, but the production capacity is only 515.63 thousand tons or 71.9%. As a result, the deficit of 201.52 thousand tons or 28.1 percent

of the national beef demand still has to be met through imports. The average consumption of beef reached 0.008 kg per week per capita in Indonesia in 2015 and 2016 and increased to 0.009 kg per week per capita in 2017 and 2018. The demand for beef in Indonesia continues to increase so that production and consumption continues to grow. As a result, the price of beef in the domestic market continues to increase (BPS 2019).

Beef is obtained through slaughter. Slaughter cows must pay attention to animal welfare to reduce animal suffering during the slaughter process. Suffering in animals can increase the occurrence of stress (Amanda et al. 2017). There are two types of treatment before slaughter, namely stunning and non stunning (Pisestyani et al. 2015). This handling minimizes accidents and ensures safe, healthy, whole and halal meat quality (Mandala et al. 2016). Rough and unkind treatment that occurs during handling can increase stress and reduce meat quality (Pisestyani et al. 2015). Cattle will also be stressed through transportation such as preparation for transportation, loading or loading, and transportation carried out using trucks, ships, or other transportation and handling during transportation is not good enough (Bulitta et al. 2015; Anton et al. 2016). Excessive stress will cause the muscle glycogen content to be low and will affect the quality of the meat produced (Lukman et al. 2012). Stress can reduce levels of glycogen which is a substrate for lactate production, so that at postmortem the pH of meat will decrease (Lonergan 2010).

Slaughtered cattle should be examined to ensure that the animal is completely dead by observing the corneal reflex and complete bleeding through cessation of blood flow after slaughter (Amanda et al. 2017). Completeness of bleeding can be characterized by analysis with the Malachite Green test (Indriarti et al. 2021). Sabow et al. (2016) stated that the remaining hemoglobin in the carcass was the result of lower blood loss and was driven by stress levels. Handling before slaughtering livestock can use two ways, namely by stunning (stunning) and without stunning (non stunning). Food of animal origin must require food guarantees that are safe, healthy, whole, and halal (ASUH). One of the several factors that can determine its safety and halalness is the perfection of bleeding. In addition to determining the halal status, meat with imperfect blood flow will easily rot. Complete expulsion of the blood indicates that the slaughtering process does not cause excessive stress. Animals that are stressed will drain a lot of blood to the brain and muscles. Excessive stress will cause the muscle glycogen content to be low and will affect the quality of the meat produced (Lukman et al. 2012).

The perfection of bleeding can be tested by using malachite green with meat extract added. The amount of blood left on the meat will cause hemoglobin oxidation so that the bronze green remains green. Meanwhile, in meat that does not contain a lot of blood, malachite green will be oxidized to a blue color (Supratikno et al. 2014). However, the reading results will be subjective if the observations are made visually, so testing is carried out using a spectrophotometer with the colorimetric method. However, spectrophotometers are quite difficult to use in remote areas because they are quite expensive and require experts to use them. A tool that has the potential to be used as an alternative for checking blood levels in beef is a cellphone camera.

Methods

1. Preparing meat extract samples

This study used 24 pieces of beef longissimus dorsi or tenderloin as much as 5 grams/piece. The meat comes from 12 cows slaughtered stunningly and 12 cows slaughtered without stunning. The beef was chopped into small pieces, soaked in distilled water for 30 minutes, and then centrifuged at 3000 rpm for 10 minutes. The supernatant was stored frozen in an Eppendorf tube until use.

2. Preparing of standard solutions

Solutions are prepared as shown in Table 1. The standard solution will be used to make a standard curve for colorimetric analysis.

Table 1. Composition of standard solutions in mL

Solution	Tubes (mL)									
	1	2	3	4	5	6	7	8	9	10
$FeCl_3$ 0.1%	-	-	-	-	-	-	-	-	-	*0.4*
$FeCl_3$ 0.01%	*0.1*	*0.2*	*0.4*	*0.6*	*0.8*	*1.0*	*1.2*	*1.6*	*2.0*	-
HCl 0.1 N	*1.9*	*1.8*	*1.6*	*1.4*	*1.2*	*1.0*	*0.8*	*0.4*	-	*1.6*
Malachite Green (MG) 0.1%	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
H_2O_2 3%	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
$KMnO_4$ 0.3%	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

The solution that was put into the tube was 0.1% FeCl3 in tube 10. Then 0.01% FeCl3 was put in tube 1 to tube 9. Then 0.1N HCl was added to each tube except tube 9. A total of 0.1 mL malachite green 0.1% and 0.1 ml H2O2 3 0.1 mL of % was added to all tubes, then homogenized using a vortex and allowed to stand for 20 minutes for the reaction to take place completely. A total of 0.1 mL of 0.3% KMnO4 was added and then homogenized again with a vortex to stop the reaction.

3. Sample Reaction A

Total of 0.1 mL of 0.1 N HCl was added 0.1 mL of meat extract in a test tube. Then, 0.1% malachite green and 3% H2O2 were added, 0.1 mL each. The sample solution was homogenized using a vortex and allowed to stand for 20 minutes for the reaction to take place completely. Then 0.1 mL of 0.3% KMnO4 was added and homogenized again using a vortex to stop the reaction.

4. Colorimetric Analysis

A series of standard solutions and 2 mL of sample solution were transferred in a cuvette and the transmission was measured using a BOECO® S-220 UV-VIS and Vernier spectrophotometer at a wavelength of 430 nm. Then 0.2 mL of the standard solution and the sample reaction solution were transferred using a micropipette into the microplate well. The microplate containing the solution was placed on top of the HP©XPA as a light source. The top of the microplate is covered with a modified wooden box with a hole in the top to house the Samsung® Galaxy Note 8 camera phone. The scanned image saved in the Joint Photographic Experts Group (JPEG) format was processed using ImageJ software to perform color index analysis. Then the color index is divided into red, green and blue (RGB) and then proceed with image selection on wells of the same size. After the wells are selected, then proceed with calculations to display the results of the analysis in the form of numbers of color intensity. The results obtained are converted into absorbance values using the Lambert-Beer equation

A = absorbance value

I = intensity of the red, green, and blue components

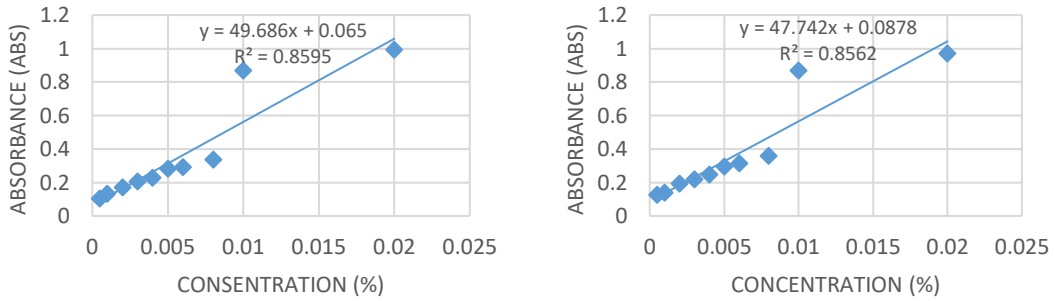
I_0 = maximum value of a pixel (Mäntele and Deniz 2017)

Then the absorbance value is entered into a linear equation (y = ax + b) from the standard curve obtained so that the concentration of blood in the meat is known. The letter y is the absorbance value and x is the concentration value. Quantitative data analysis was used by the Microsoft® Excel 2019 program to create regression test graphs and calibration curves. Paired T-test analysis used the IBM® SPSS Statistics 20 program. Correlation test was used to measure the strength of the relationship between the spectrophotometer and the camera phone. Paired T-test was used to compare the results of measuring blood concentrations from a spectrophotometer with a cell phone camera.

Result

1. Measurement of Blood Concentration Using a UV-Vis Spectrophotometer

Measurement of blood concentration in meat was obtained based on a standard solution curve. The standard solution was analyzed colorimetrically using a UV-Vis spectrophotometer Boeco® S-220 as a reference system so that the absorbance value of each concentration of the standard solution was obtained based on the Lambert and Beer equation. Then the linearity test was carried out between the concentration and absorbance of the standard solution to obtain a curve and a linear equation along with the coefficient of determination as shown in Figure 1. The standard solution is a series of solutions made by diluting 0.01% FeCl3 mother liquor with 0.1 N HCl and several other compounds as can be found. in Table 1. The results of the linearity test between the absorbance obtained and the concentration of the standard solution produced a linear curve y = 49.68x + 0.065 with a coefficient of determination (R2) of = 0.859 using a Boeco® S-220 UV-Vis spectrophotometer and y = 47.74x + 0.087 with a coefficient of determination (R2) of = 0.856 using a Vernier spectrophotometer.



Spectrophotometer UV-Vis Boeco S220 Spectrophotometer Vernier

Figure 1. Standard solution calibration curve using a spectrophotometer with a wavelength of 430 nm

The results of taking the image of the standard solution and the sample reaction solution can be seen in Figure 2. The image obtained using the Samsung® Galaxy Note 8 was processed using the application ImageJ to get the color intensity value. The ImageJ application will divide the pixels in the standard solution image and sample reaction into three components, namely the red (red), green (green), and blue (blue) channels (Soldat et al. 2009).

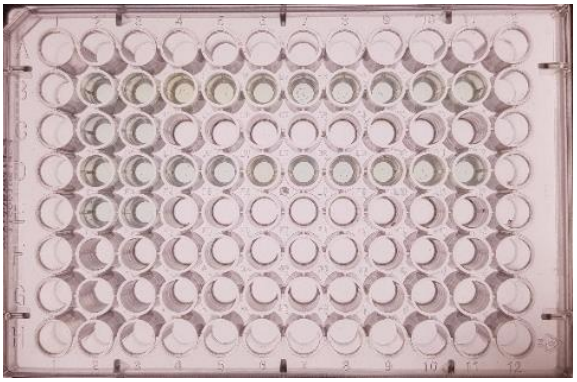


Figure 2. The microplate containing the buffer solution and the reaction of the sample captured by the Samsung® Galaxy Note 8 camera

The RGB channel intensity is selected using the ROI manager. The value from RGB channel processing is then converted using the Lambert-Beer formula into absorbance values (Ostergaard 2016). The absorbance values processed into a standard curve with three channels: red (red), green (green) and blue (blue) as shown in Figure 3.

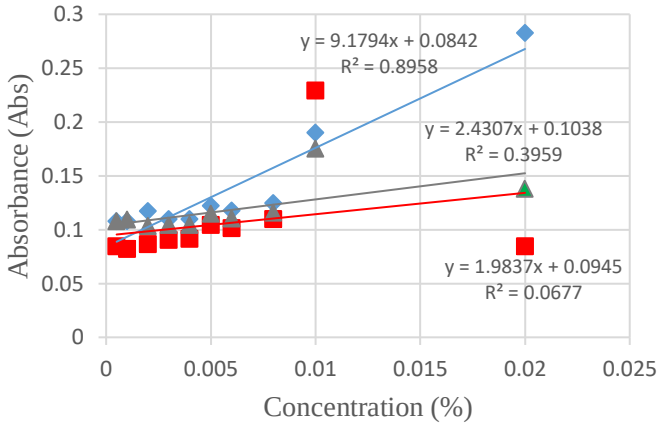


Figure 3. Calibration curve standard solution using a camera phone with canal ♦ Blue (y = 9.1794x + 0.0842); ■ Red (y = 1.9837x + 0.0945); ▲ Green (y = 2.4307x + 0.1038);

The steepness of the three channels is different. The steepness of the curve indicates the sensitivity of the canal to changes in blood concentration in the meat extract. The steepness of the curve with a coefficient of determination close to 1 indicates the higher the sensitivity of the channel to analyze changes in blood concentration (De Morais and De Lima 2014). The channel used to measure blood concentration is the blue channel because the blue channel has the highest steepness with a coefficient of determination (R2) 0.8958 and the linear equation y = 9.1794x + 0.0842. Thus the blue channel linear regression curve is used as a calibration curve to determine the absorbance value of the sample extract solution based on the results of processing the color intensity of the image using ImageJ.

Table 2. Comparison of the results of measuring blood concentrations in slaughtered meat with and without stunning using 3 tools (1 x 10^{-3} gram/dL)

Treatmens	Beaco® S-220 Spectrophotometer	Vernier Spectrophotometer	Cellphone Camera
Stunning	2.2 ± 1.14[a]	2.1 ± 1.28[a]	0.9 ± 0.66[a]
Non Stunning	2.7 ± 1.54[a]	2.8 ± 1.82[a]	1.6 ± 0.96[a]

Note:letters *superscript* Differentin the same column indicate significant differences ($p<0.05$)

The absorbance value obtained will be converted into concentration using a standard solution linear regression equation so that the blood concentration in the meat extract solution is obtained (Table). Based on the measurement results using the Beaco® S-220 spectrophotometer, the blood concentration in beef slaughtered with stunning is lower than beef slaughtered without stunning. However, based on paired T-test this difference was not significant ($P>0.05$). The same thing was also obtained in the paired T test of the measurement results with the Vernier spectrophotometer and the results of image processing produced by

cellphone using ImageJ when compared to the UV-Vis spectrophotometer Boeco S220, which was no different (P>0.05) as shown in Table 2.

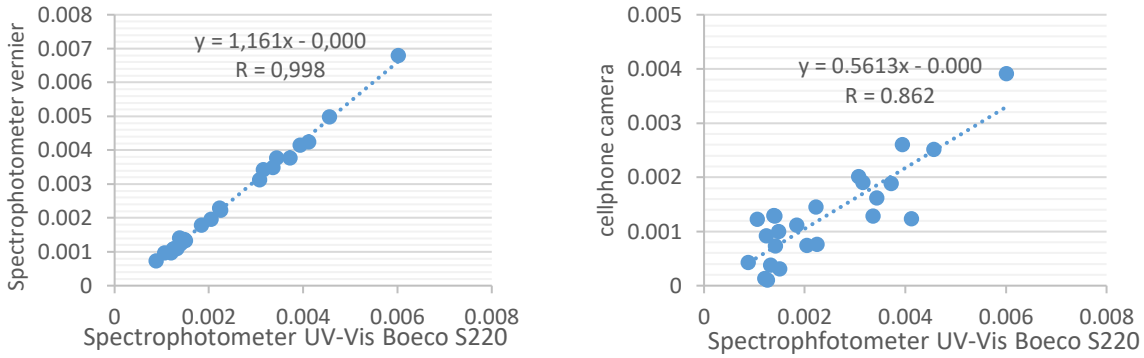


Figure 4. Correlation between UV-Vis spectrophotometer Boeco S220 with Vernier spectrophotometer and UV-Vis spectrophotometer Boeco S220 with image processing obtained with a cellphone camera using Image

The performance of the Vernier spectrophotometer and image processing produced by cellphone using ImageJ needs to be analyzed for the potential to perform the function of the Boeco S220 UV-Vis spectrophotometer in colorimetric analysis. Therefore, a potential comparison analysis was carried out using a correlation test. Based on the correlation test using the measurement results of the standard solution, it was found that the regression equation of the Beaco® UV-Vis S-220 Spectrophotometer with the Vernier Spectrophotometer was y = 1.161x - 0.000 and the correlation coefficient R = 0.998, while the correlation test using the measurement results of the standard solution was found that the regression equation The Beaco® S-220 spectrophotometer with a cell phone camera is y = 0.5613x - 0.000 and the correlation coefficient is 0.862.

Discussion

Beef is one of the foodstuffs of animal origin that has nutritional value in the form of protein containing a complete and balanced amino acid composition (Nurwantoro et al., 2012). The quality of beef is influenced by two factors, namely before slaughter such as genetics, species, breed, type of livestock, age, feed, stress and after slaughter such as withering methods, electrical stimulation, cooking methods, carcasses and meat, additives including meat tenderizing enzymes, hormones and antibiotics, intramuscular fat or marbling, storage methods, types of meat muscles, and location in a flesh muscle. Slaughter process must be done to get beef. The purpose of slaughter is to bleed and kill the animal by stopping the oxygen supply to the brain, arteries and veins, esophagus and trachea (Gregory, 1998). Bleeding is the main requirement of meat to be consumed and also to maintain the quality of the meat. Meat with imperfect blood flow will have an unattractive appearance and also easily become a good growth medium for microorganisms (Lawrie, 1995). The presence of blood around the meat can cause the quality of the beef to decrease and the meat to rot quickly. The degree of

completeness of blood discharge is a factor that affects the chance and level of contamination of microorganisms in carcasses (Lowrie, 2003). Most of the components of blood bodies are erythrocytes. Hemoglobin is an erythrocyte pigment. Hemoglobin is a determinant in measuring blood concentrations in beef (Soeparno, 2005; Warriss 2010). Hemoglobin in beef comes from incomplete bleeding caused by ineffective slaughtering methods. The remaining blood concentration affects the quality and safety of beef. Therefore, a method is needed to determine the quality of meat based on the blood content in meat quantitatively.

This study uses a modified Malachite Green test to detect blood concentrations in beef slaughtered with and without stunning as a treatment with the assumption that there is no difference in blood content in beef due to differences in the perfection of blood loss after slaughter. MG will compete with Hb for binding to oxygen derived from H2O2. Hb has a higher affinity than MG, so Hb will bind to oxygen first. If the bleeding is not perfect then the mixed solution of MG, Hb and H2O2 will still lead to a green color, but if the bleeding is perfect then the mixed solution will turn green-blue (Indriarti *et al* 2021). To ensure that the solution color changes quantitatively, a colorimetric test was carried out using a UV-Vis spectrophotometer Boeco S220 as a reference. This study used two spectrophotometers from different sales service providers. The second spectrophotometer is Vernier's Go Direct spectrophotometer.

The working principle of a spectrophotometer is that the amount of light absorbed by the particles in a solution depends on the type and number of particles. This spectrophotometer is used as a reference for the other two devices. Its working principle is based on the Lambert-Beer law which states that the amount of light absorbed is directly proportional to the concentration of the solution (Star 2018). Based on previous research, the visible light used for the modified MG test is visible light with a wavelength of 430 nm. If radiation or white light is passed through a colored solution, radiation with a certain wavelength will be absorbed selectively and other light radiation will be transmitted. The maximum absorbance of the colored solution occurs in the color region that is opposite to the observed color, for example a red solution will absorb maximum radiation in the green color region. In other words, the absorbed color is the complementary color of the observed color (Suharta, 2005).

The results obtained will provide absorbance values using the Lambert-Beer equation. The absorbance value obtained will be easily converted into a concentration value if a calibration curve is available from the colorimetric test series of standard solutions. Absorbance is the ratio of the intensity of the absorbed light to the intensity of the incident light. This absorbance value will depend on the concentration of the substance, the higher the concentration of the substance in a solution, the more molecules that will absorb light at a certain wavelength so that the absorbance value will be even greater. The absorbance value will be directly proportional to the concentration of the substance (Neldawati et al. 2013). The spectrophotometer's accessibility constraint becomes a challenge to find alternative devices that are cheaper and easier to obtain. The choice is set on a device capable of producing digital images for easy image processing, namely a cellular phone camera. The method used for processing the mind is public-owned software and is often used in the medical world, namely ImageJ. ImageJ is

software developed by Wayne Rasband at NIH in 1987. This software is Windows-based and public property so it can be developed by anyone who is interested and then stored in the Plugin Folder provided (Schneider et al. 2012).

Colorimetric tests using a spectrophotometer require the preparation of a series of standard solutions to produce a calibration curve that is important in the quantitative analysis of colorimetric tests using a spectrophotometer. The series of standard solutions of this study can be seen in Table 1. Calibration curve is a method used to determine the concentration of a substance in a sample using a series of standard solutions whose concentration is known. The concentration of the standard solution that forms a series was analyzed using a spectrophotometer with the same wavelength as the sample measurement, namely 430 nm. The results of the absorption of the standard series that form a straight line (linear) which states the relationship between the concentration of substances in the standard solution and the absorption response of the instrument. Linear relationship between the concentration of the standard solution by absorbance will establish the following equation y = ax + b, where y = absorbance, x = concentration of the analyte, a = slope*(slope)*,b = intercept (intercept).

The intercept is the response value of the instrument to the blank with the ideal value being zero. The slope is the sensitivity value of this test method, the greater the slope value, the greater the sensitivity value of this method. Based on this research, the standard solution spectrophotometer calibration curve A shows the linear equation y = 49,686x + 0.065 with a coefficient of determination ($R^{2)}$of 0.8595. This shows that the concentration value can explain the absorbance value of 85.95%, while the standard calibration curve of the spectrophotometer B shows a linear equation y = 47.742x + 0.0878 with a coefficient of determination (R2) of 0.8562. The concentration value explains the absorbance value of 85.62% (Figure 1). The value of the coefficient of determination ranges from 0 to 1. The value of the coefficient of determination that is close to one indicates the independent variable that provides almost all the information needed to predict the variation of the dependent variable, meaning that the two variables have a stronger relationship (Kurniawan and Yuniarto 2016). FeCl3 solution is a representation of blood hemoglobin. Blood hemoglobin contains the element Fe. In the standard solution there will be a competitive reaction between FeCl3 and MG.

In this spectrophotometry,light is used as a source of light/energy*visible*. Visible light is part of the electromagnetic spectrum that the human eye can perceive. The wavelength of visible light is 380 to 750 nm. All rays can be seen by the eye then the light is included in the visible light*(visible).*The visible light source commonly used in the visible spectro is a tungsten lamp. Samples that can be analyzed with this method are only samples that have color. This is a distinct weakness of the visible spectrophotometric method. Therefore, for samples that do not have color, they must first be colored using specific reagents. Therefore, the use of MG becomes suitable for use in this method. The light coming from the lamp is passed through the lens to the monochromator, then the light will be converted to light which was originally polychromatic into monochromatic light (single) according to the desired wavelength. A beam of light is passed through a sample containing a substance with a certain concentration. Some of the light that is formed is absorbed (absorbed) and some is passed. The light that is passed

is then received by the sensor. The light received is calculated to determine the light absorbed by the sample. The light absorbed is proportional to the concentration of the substance contained in the sample, so that the concentration of the substance in the sample will be quantitatively known (Triyati, 1985). The main advantage of the spectrophotometric method is that it provides a simple way to determine small quantities of a substance. In addition, the results obtained are quite accurate, where the numbers that are read are directly recorded by the detector and printed in the form of digital numbers or graphs that have been regressed (Yahya S, 2013).

The results of this study indicate that, based on the colorimetric test, meat from cattle slaughtered with stunning has less blood content when compared to meat from cattle slaughtered without stunning but based on the T test this difference is not significant (P>0.05) . The results were obtained based on measurements using the UV-Vis spectrophotometer Boeco S-220, GoDirect spectrophotometer Vernier and image processing captured by the Samsung® Galaxy Note 8 using ImageJ. Based on this research, it is also shown that the correlation test between devices shows a very strong correlation between the results obtained using a UV-Vis spectrophotometer and the image processing obtained from a cell phone camera using ImageJ.

Conclusion

The use of colorimetric analysis can be used to measure the blood content in meat quantitatively. Meat from cows that have been stunned before being slaughtered contains less blood than meat from cows that have been slaughtered without being stunned. The measurement results of standard solutions using image processing obtained with a cellular phone camera are strongly correlated with the results of measurements using a UV-Vis spectrophotometer. To strengthen the conclusions of this study, it is necessary to use this method with a larger number of samples and be used on other beef cattle.

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