Presented at the DLSU Research Congress 2017
De La Salle University, Manila, Philippines
June 20 to 22, 2017

An RGB Sensor – Based Chlorophyll Estimation in Carabao Mango Leaves by Multiple Regression Analysis of Hue Saturation Value Color Components

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Abstract: Measuring the chlorophyll content of a plant is usually used in estimating its nitrogen level. Destructive method such as UV-Vis Analysis and non-destructive method, such as the use of photometric features, are commonly employed in estimating the chlorophyll content of a plant. In this study, the use of Red Green Blue (RGB) sensor in comparison to digital image analysis for rapid and non-destructive measurement of chlorophyll content of Carabao mango leaves using Hue Saturation Value (HSV) color space analysis was proposed. This study introduced the utilization of an RGB sensor in estimating the chlorophyll content of plants as opposed from digital image analysis which comprises a scanner and a camera for image acquisition. Multiple regression of HSV components was used to form the mathematical models. Calibration with CM-2600D Spectrophotometer was done to standardize the readings of the TCS3200 Color Sensor. Soil Plant Analyses Development (SPAD) – 502Plus Chlorophyll Meter was used to provide the actual chlorophyll content. With respect to the predicted chlorophyll content, the correlation values were found to be – 0.9451, 0.3486 and – 0.8153 for the hue, saturation and value values of the proposed device, respectively. On the other hand, the correlation values for the hue, saturation and value values for digital image analysis were 0.8871, – 0.9941 and 0.9567, respectively. With 0.01% level of significance, the mean HSV values greatly affected the prediction of chlorophyll content for the proposed device. In contrast, only the mean saturation and value values had affected the prediction for digital image analysis. The root mean square error (RMSE) and R^2 values between the predicted chlorophyll and actual chlorophyll content were 3.2526 and 0.9403 for the proposed device, and 2.7286 and 0.9580 for digital image analysis. For this reason, the proposed device can be a practical substitute to SPAD-502Plus Chlorophyll Meter and digital image analysis.

Keywords: Proposed Device; Digital Image Analysis; HSV Color Space Analysis; Multiple Regression; Chlorophyll Content

1. INTRODUCTION

Chlorophyll is the green molecule yielded by plants which is responsible for the greenness of the plants. This pigment serves as an essential agent for their life sustenance by helping the plant to absorb energy from the sunlight which is a vital requirement in the process of photosynthesis.

It has been a proven fact that chlorophyll merely indicates the over-all health of a plant. Reinforced by studies, the chlorophyll content of a plant has shown a significant relationship with its nitrogen content. Having this fact therefore presents an implication that the chlorophyll content of any plant logically reflects its health state.

The determination of the chlorophyll content of the plant is a vital process in understanding the in-depth state of a plant. The studies have confirmed that every plant has its own optimum chlorophyll level. Thus, by obtaining the plant's chlorophyll will create a gauge advising if the plant should be attended upon or has already reached its maximum level.

There are two methods known in acquiring the chlorophyll level of a plant; the destructive method and the non-destructive one. The destructive method is the traditional way to determine chlorophyll level of a plant which requires the actual pigment to be extracted from the plant. Despite its harmful approach, this method has been confirmed to output the most accurate reading making it the benchmark in determining the amount of chlorophyll level. While the non-destructive has been the new approach in determining the chlorophyll level. Utilizing the current technology, methods have been formulated, designed, invented to analyze the chlorophyll level of a plant without causing any damage to the subject being tested.

One of the most trusted non-destructive approaches in determining the chlorophyll level of a plant is using a SPAD-502Plus chlorophyll meter. This device created by Konica Minolta has revolutionized the approach in analyzing the chlorophyll level of a plant. Studies and tests have confirmed that the readings from the UV-Vis approach, an accurate destructive method, have shown a high correlation with the readings from the SPAD-502Plus chlorophyll meter. Without requiring the plant to be subjected to damaging, the device is known to still output a reliable reading making it to be the standard instrument used in various studies and experiments. However, the downside of this device is that it is very expensive in the market.

Another method that is used is called digital image analysis, which has been used in previous works, such as the two studies of Ali et. al (2012) and "Color Sensors Information," 2016. The studies of Hu et. al (2010), Marques et. al (2011), Moghaddam (2011), and Patil (2014) utilized other methods with the same approach but used different model equations to estimate the chlorophyll content of plants. "Colour Model to Estimate Plant Chlorophyll & Nitrogen Contents" and Blackmer (1995) utilized the same method of analysis to estimate the nitrogen level content of plants. Other works even utilized the conversion of the RGB color values into HSV to estimate the chlorophyll content of plants as show in the study of Patil et.al (2014) and to identify the greenness of the plants in the work of Yang (2015). Gupta et. al (2012) and Yadav et. al (2009) have proved that digital image analysis has is highly correlated with the readings done using the SPAD-502Plus chlorophyll meter.

The method of digital image analysis may be a better and inexpensive alternative than purchasing a SPAD meter. However, it requires tedious works on photo editing to select the appropriate area to analyze and estimate the chlorophyll or nitrogen level content, therefore, the researchers proposed a device that will directly analyze the specimen and read the RGB color values then converting them into the HSV color space and estimate the chlorophyll content of the plants.

2. METHODOLOGY

2.1 Materials and Equipment Used

Fifteen leaf samples with midrib length and a cross-width of at least approximately 13cm and 5cm, respectively from each of the six Carabao mango species, namely; Lamao #1, Sweet Elena, Tanaleon, GES84, Galila, and GES77, were collected and analyzed using the proposed device, digital image analysis, and SPAD-502Plus chlorophyll meter.

The proposed device was composed of TCS3200 color sensor, Gizduino X microcontroller unit, LCD shield, and battery. TCS3200 color sensor was calibrated with Konica Minolta CM-2600D Spectrophotometer. The sensor was placed 20mm from the surface of the sample.

For digital image analysis, Canon PIXMA MP287 was used to acquire the image of the leaf sample. Adobe Photoshop CS6 was used for histogram analysis. The actual chlorophyll content was acquired using SPAD-502Plus chlorophyll meter.

For data analysis and mathematical calculations, Microsoft Excel software was used. Upon doing the statistical analysis, Stata software was operated in determining the model parameters needed in calculating the estimated chlorophyll content of a sample.

2.2 Methodology

2.2.1 Testing and Calibration of the Sensor

The TCS3200 color sensor was first programmed through the Arduino software. A white-colored paper was read through the color sensor and its values are mapped as 255, 255, 255 for the sensor's R, G, and B readings. On the other hand, a black-colored construction paper's values are mapped as 0, 0, 0 for the sensor's R, G, and B readings.

To validate the TCS3200 color sensor, the proponents followed the procedures in calibrating one in the paper entitled, "Low Cost Color Sensors for Monitoring Plant Growth in a Laboratory" by Seelye et. al (2011).

2.2.2. Prototype Assembly

Encapsulated in a 14" x 6" x 3" 3D-printed casing, a slit wherein the plant leaf can be inserted is designed so that a constant 20mm distance between the sample and the TCS3200 color sensor can be established. Powered by a 7.4-volt Samsung battery, the device can be controlled using a switch located at the side.

2.2.3 HSV Color Component Acquisition

For the proposed device, the Gizduino X was preprogramed to automatically convert the obtained RGB values to HSV color space.200 HSV readings per trial of each of the three areas per leaf samples were acquired and averaged.

For the digital image analysis, mean RGB readings of four 4 x 4 pixels per area of each leaf were obtained using Adobe histogram analysis. The mean RBG values obtained are then converted to HSV color space. (Patil, 2014)

2.2.4 Actual Chlorophyll Content Acquisition

SPAD-502Plus chlorophyll meter was used to obtain the actual chlorophyll content of the samples. Four readings per area were averaged. The average readings of each of the three areas were acquired to represent the total chlorophyll content of the leaf.

2.2.5 Model Parameter and Equation Acquisition

For both the proposed device and digital image analysis, model parameters were computed. The estimated chlorophyll content was regressed with respect to HSV values to obtain the final model equation. [10]

2.2.6 Comparative Analysis

The chlorophyll content of each of the 15-leaf sample per species were obtained using the proposed device, and digital image analysis to get the coefficients of determination and correlation with respect to the values acquired using the SPAD-502Plus chlorophyll meter.

3. RESULTS AND DISCUSSION

The TCS3200 color sensor was calibrated to make its reading closer to the actual value of colors in 8-bit map. As shown in Table 1, the calibrated RGB values were acquired utilizing the average RGB values obtained for each color sample using the TCS3200 color sensor and the CM-2600D Spectrophotometer. With the data gathered using seven diverse colors, the calibration factor for Red, Green, and Blue color components were computed as 2.77, 2.58, and 2.43, respectively.

Table 1. Average readings of the seven diverse colors using TCS3200 color sensor, calibrated and uncalibrated, and CM-2600D Spectrophotometer

Color	Uncalibrated			CM-2600d			Calibrated		
	R	G	B	R	G	B	R	G	B
White	250	249	248	254	253	254	242	240	239
Black	38	37	46	12	11	13	2	2	4
Red	230	102	112	77	16	10	192	24	35
Green	167	207	147	44	84	19	79	149	67
Light Green	225	233	219	112	149	93	181	203	177
Light Blue	195	202	225	55	65	137	122	140	189
Dark Blue	68	79	139	15	14	35	7	13	59

Table 2. Average error (0-255), percentage error and standard deviation for Red, Green, and Blue measurements of the TCS3200 color sensor, calibrated and uncalibrated, compared with CM-2600D results across seven diverse colors

	Uncalibrated			Calibrated		
	R	G	B	R	G	B
Ave Error	87.43	75	83.86	45.14	32.14	36.71
% Error	34.29	29.41	32.89	17.70	12.61	14.40
Σ	59.022	48.11	46.77	40.29	31.22	26.25

Table 2 summarizes the average error, percentage error, and standard deviation of the RGB color component readings of the uncalibrated and calibrated TCS3200 color sensor for the seven diverse colors. After calibration, the percentage error improved to 17.7031%, 12.6051%, 14.3978% for red, green, and blue, respectively.

Second calibration was performed to make the exhibited values of TCS3200 color sensor sensitive to green color since study. Fifteen RHS colors were used, twelve of which are different shades of green. As shown in Table 3, the calibrated-2 RGB values were acquired utilizing the average RGB values obtained for each color sample using the TCS3200 color sensor and the CM-2600D Spectrophotometer. With the data gathered using fifteen RHS colors, the calibration factor for Red, Green, and Blue color components were computed as 1.49, 1.58, and 1.66, respectively.

Table 3. Average readings of the 15 RHS green colors using TCS3200 color sensor, calibrated–1 and calibrated–2, and CM-2600D Spectrophotometer

RHS Color	ID	Calibrated-1			CM-2600d			Calibrated-2		
		R	G	B	R	G	B	R	G	B
Blue-Green	123A	87	161	181	49	97	119	52	124	145
Green	127C	1	14	20	13	21	23	1	3	4
Green	129C	100	165	155	46	99	72	64	129	112
Green	131C	3	20	18	15	23	20	1	5	4
Green	133C	42	72	76	32	46	44	18	35	35
Green	135C	10	49	19	18	35	14	3	19	4
Green	137C	27	46	22	30	38	22	9	18	5
Green	139C	70	93	54	45	58	28	38	52	20
Green	141C	35	81	31	30	52	17	14	42	8
Green	143C	80	96	35	52	66	21	46	55	10
Yellow-Green	145C	210	205	164	163	172	67	191	181	123
Yellow-Green	147C	104	106	71	56	62	31	68	64	31
Yellow-Green	149C	184	194	143	140	170	66	157	166	98
White	155D	240	233	230	254	253	254	233	222	215
Black	202A	20	22	30	15	14	16	6	6	8

Table 4. Average error (0-255), percentage error and standard deviation for Red, Green, and Blue measurements of the TCS3200 color sensor, calibrated-1 and calibrated-2, compared with CM-2600D results across seven diverse colors

	Uncalibrated			Calibrated		
	R	G	B	R	G	B
Ave Error	23.53	27.4	32.87	14.2	14.73	20
% Error	9.23	10.75	12.89	5.57	5.78	7.84
Σ	18.16	19.30	32.04	6.49	9.18	15.52

Table 4 summarizes the average error, percentage error, and standard deviation of the RGB color component readings of the calibrated once and calibrated twice TCS3200 color sensor across a range of RHS green colors. A vast improvement is observed across the RGB color components, with percentage errors of 5.5686%, 5.7778%, and 7.8431%, for red, green, and blue, respectively. Also, the standard deviations lowered to 6.494, 9.1844, and 15.5242 for red, green, and blue, respectively.

Using Stata, the estimated chlorophyll content was multiply regressed with the HSV values. As shown in Table 5, the final model parameters are – 209.0837, – 62.7838 and – 87.9020 for the proposed device while – 1.0422, – 53.2930 and – 41.0935 for digital image analysis.

For the proposed device, all the predictor variables, hue, saturation, and value, are statistically significant with p values less than 0.01 to the response variable, predicted chlorophyll content. While hue has no significance, for digital image analysis, both saturation and value are statistically significant with p values less than 0.01 in predicting the estimated chlorophyll content.

Compared to digital image analysis, the proposed device has a lower coefficient of determination and higher RMSE. It has a lower standard error with its hue parameter and a higher one with its saturation and value parameter. Similar with the findings of Patil (2014), the RMSE and R^2 for HSV modelling are low and high, respectively.

Table 5. Model comparison of the proposed device and digital image analysis with respect to the actual chlorophyll content

Independent Variable	Proposed Device	Digital Image analysis
Constant	143.1660*	83.2600*
Standard Error	9.8210	8.6150
Hue	-209.0837*	-1.0422
Standard Error	19.7145	24.7741
Saturation	-62.7838*	-53.2930*
Standard Error	10.4272	4.1910
Value	-87.9020*	-41.0935*
Standard Error	10.9223	10.3418
R-squared	0.9403	0.9580
RMSE	3.2526	2.7286
Number of Observation	90	90

**indicates significance at 1%*

Also, as seen in Table 5, for the proposed device and digital image analysis, the standard errors of hue are 19.7145 and 24.7741, respectively.

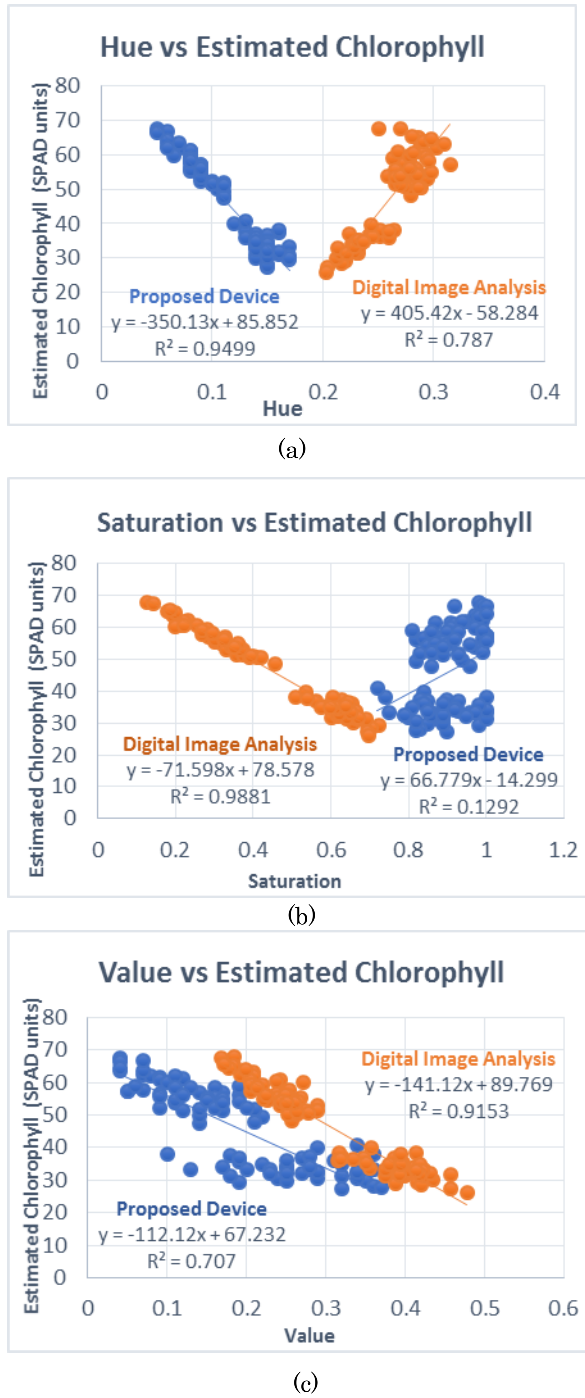

Fig.1. Scatterplot of (a) hue, (b) saturation, and (c) value with respect to the estimated chlorophyll content

Though the proposed device has lower error than digital image analysis, both methods have high values of error that means of the inaccuracy of hue readings. The standard errors of the value for the two methods are near to each other, with values of 10.9223 for the proposed device and 10.3418 for the digital image analysis, which shows that the two methods can detect the value of the specimen read with almost even accuracy. In terms of the errors in saturation values, the proposed device and digital image analysis have standard errors of 10.4272, and 4.1910, respectively. This shows that digital image analysis is more accurate in saturation reading than the proposed device.

To determine the relationship of the HSV values with respect to the estimated chlorophyll content, a scatterplot was made as shown in Fig. 1.

As shown in Fig. 1a, both the proposed device and digital image analysis attained a high value of R^2 and hence coefficients of correlation.

With respect to the estimated chlorophyll content, hue has a strong inverse linear relationship for the proposed device while direct linear correlation for digital image analysis.

As shown in Fig. 1b, digital image analysis attained a higher R^2 than the proposed device. With respect to the estimated chlorophyll content, the saturation has little correlation for the proposed device while an inverse linear correlation for digital image analysis.

As shown in Fig. 1c, despite digital analysis attaining a higher R^2 than the proposed device, both methods have their value values inversely correlated with respect to the estimated chlorophyll content.

4. CONCLUSION

In this study, the use of a color sensor to measure the chlorophyll content of Carabao mango plants through HSV color space was proposed. The goal was to develop a low-cost hand-held device that will serve as substitute to a chlorophyll estimation method known as digital image analysis.

This was achieved by first calibrating the TCS3200 color sensor of the proposed device to CM-2600D spectrophotometer to standardize and stabilize its readings and then comparing two multiply regressed models from the proposed device and digital image analysis with respect to SPAD 502-Plus chlorophyll meter. A good agreement between the predicted chlorophyll and the actual chlorophyll content was demonstrated. The root mean square error (RMSE) between predicted and actual chlorophyll measured was 3.2526 for the proposed device and 2.7286 for digital image analysis. The results indicated that HSV color space analysis provide good models for chlorophyll estimation.

The chlorophyll meter was used in the present study as the provider of the actual value because it was already an established tool for qualitative and quantitative estimation of chlorophyll content. Also, the digital image analysis method of chlorophyll estimation was used as a comparator with the proposed system because it was already a known method. However, the chlorophyll meter, despite its accuracy is costly and digital image analysis still can't provide an estimation rapidly.

The results indicate that the proposed device can be a practical substitute to digital image analysis and be used for rapid, non-destructive, and accurate, estimation of chlorophyll content in Carabao mango leaves. However, it is necessary to use the UV-Vis method of chlorophyll determination as reference and the SPAD-502Plus chlorophyll meter as the comparator to the proposed device. It is also recommended that the relationship between the SPAD units obtained from the proposed device and the estimated nitrogen level content of the leaves being considered be determined, to assure the accuracy of the proposed device.

5. ACKNOWLEDGEMENT

This research would not be possible without the help of Color Application Specialists and Philippine Rice Research Institute.

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