Correlation Analysis between Total Chlorophyll Content and Color Intensity in *Bambu duri (Bambusa blumeana)* Leaf Extract

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Abstract: *Bambu duri* leaves are known to contain many bioactive compounds, one of which is chlorophyll. Chlorophyll is a green pigment found in leaves which is often used as a natural food colorant. This study aims to: (i) determine the effect of temperature and maceration time on total chlorophyll content and color intensity of *bambu duri* leaf extract, (ii) determine the correlation between total chlorophyll content and color intensity (L*a*b*). This study used a factorial randomized block design with two factors. The first factor is the maceration temperature consisting of 30, 45, and 60°C. The second factor is the maceration time consisting of 24, 36, and 48 hours. Data were analyzed by analysis of variance and continued with the Tukey's test. Correlation analysis using Pearson correlation analysis. The results showed that the interaction between temperature and maceration time had a very significant effect on the total chlorophyll content and color intensity (L*a*b*) of the *bambu duri* leaf extract. Correlation analysis between total chlorophyll content and color intensity (L*a*b*) showed $r = -0.989$, $r = -0.983$, and $r = 0.981$. These results indicate that there is a very strong relationship between total chlorophyll content and color intensity (L*a*b*) of *bambu duri* leaf extract.

1 INTRODUCTION

Food coloring is a type of food additive that is often added to food products to improve product quality. Types of food coloring based on the source can be divided into two, there are synthetic and natural dyes (Winarno, 1992). Synthetic dyes are obtained through chemical reactions (sulfuric acid or nitric acid), while natural dyes are obtained from plants, animals, and minerals that have color pigments. Color pigments in plants can be found in the roots, rhizomes, wood, fruit, seeds, flowers, and leaves.

Bambu duri (Bambusa blumeana) leaves are part of the bamboo plant which has the characteristics of small leaves (9.5-15 cm long and 2.5-4.5 cm wide) and green. The green color of the leaves indicates that *bambu duri* leaves contain a green pigment called chlorophyll and has the potential to be used as a source of natural dyes. According to Aryanti *et al.* (2016), chlorophyll is a green pigment found in chloroplasts together with carotene and xanthophyll in all living things capable of photosynthesis. Regulation of the Head of National Food and Drug Administration of Republic Indonesia Number 37 of 2013 about the Maximum Limit for Use of Colored Food Additives, states that chlorophyll and its derivative compounds are included as natural food additives so that they are safe for consumption. Apart from being a natural dye, chlorophyll compounds can also be used as potential antioxidants because they have effective activity in fighting lipid peroxidation, DNA degradation, and overcoming cases of anemia (Banu and Devi, 2015 and Vankova *et al.,* 2018).

The potential chlorophyll content of *bambu duri* leaves to be used as a natural dye can be determined from the level of color intensity. According to Lukitasari *et al.* (2017), color intensity shows the strength of the color when the color contained in the extract is applied to the product. Chlorophyll content can be obtained through solvent extraction, which is then analyzed using a spectrophotometer, while the color intensity level can be analyzed using the CIELAB color system which consists of three variables, namely L* (brightness), a* (redness), and b* (yellowish).

The maceration extraction method is a method that can be used to obtain the chlorophyll content contained in *bambu duri* leaves. The maceration

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method was chosen because it is a simple method, can produce the maximum amount of extract, and did not damage the compounds contained due to the use of high temperatures. Chlorophyll is a compounds that is easily degraded due to certain conditions, such as heat, light, oxygen, and acidic conditions (Heaton and Marangoni, 1996). The color of the extract produced can affect the main characteristics in determining product acceptance, therefore it is important to prevent and reduce chlorophyll degradation during the extraction process. Optimization of the use of temperature and time during the extraction process is an effort to reduce chlorophyll degradation and increase the recovery of color pigments. Based on the explanation above, it was necessary to do a research about the effect of temperature and maceration time on total chlorophyll content and color intensity of *bambu duri* leaf extracts and also correlation analysis between total chlorophyll content and color intensity.

2 MATERIALS AND METHODS

2.1 Materials

Bambu duri (Bambusa blumeana) leaves obtained from Mengwi, Badung, Bali. Some characteristics of *bamboo duri* leaves that being used are young leaves in 1-3 positions which are counted from the shoots, have green color, and have a measurement of ± 9 cm × 2 cm. Chemicals that being used are acetone pa (Merck), 96 percent technical ethanol (Bratachem), and aquades (One Med).

2.2 Equipments

Oven (Blue M), blender (Philips), analytical balance (Shimadzu), vacuum rotary evaporator (IKA RV 10 digital), spectrophotometer (Biochrome SN 133467), vortex (Barnstead Thermolyne Maxi Mix II), micropipette (Socorex), sieve 60 mesh (Retsch), color reader (Accuprobe HH-06), and glassware.

2.3 Preparation of Materials

Bambu duri leaves that have been sorted are cleaned first, then dried in an oven at a temperature of 50 ± 2°C for 6 hours or until the leaves are easily crushed. Then the dried leaves are cut into pieces and blend until smooth. The finely powdered *bambu duri* leaves are then sieved with a 60 mesh sieve. Materials that did not pass the sieve are blended back to pass the 60 mesh sieve. The water content of *bambu duri* leaf powder is around 11.06%.

Bambu buri leaf sample extraction was carried out using the maceration method. A total of 25 grams of *bambu duri* leaf powder is put into a dark glass bottle, then 250 mL of 96 percent ethanol solvent is added. The comparison of *bambu duri* leaf powder with ethanol solvent is 1:10 (w /v). The maceration process was carried out at temperatures (30 ± 2˚C, 45 ± 2 ° C, and 60 ± 2˚C) and time (24 hours, 36 hours, and 48 hours) according to the treatment. During the maceration process, the shaking process is carried out manually every 6 hours for 5 minutes. After the maceration process, the filtering process was carried out using filter paper twice. The first filtering used coarse filter paper which then produces filtrate I and pulp. The dregs were then added with 50 mL of solvent, shaken for 5 minutes, and then filtered using coarse filter paper to produce filtrate II. Filtrates I and II were then mixed and filtered again using Whatman filter paper no.1. The filtrate is then evaporated with a vacuum rotary evaporator at a temperature of 40˚C with a speed of 100 rpm and a pressure of 100 mBar until all the ethanol evaporated.

2.4 Chlorophyll Analysis

Chlorophyll analysis in this study used a modified method according to Nollet (2004). The sample of *bambu duri* leaf extract was weighed as much as 0.01 grams, then diluted to 5 mL with 80 percent acetone. Then from the dilution as much as 0.5 mL was taken and placed in a 5 mL measuring flask. Furthermore, 80 percent of acetone is added up to the mark limit. Chlorophyll content was calculated by measuring the absorbance at 645 and 663 nm. Calculation of chlorophyll content is carried out with the formula:

Total chlorophyll (ppm) = 20.2 A_{645} nm + 8.02 A_{663} nm

Chlorophyll a (ppm) = 12.7 A_{663} nm - 2.69 A_{645} nm

Chlorophyll b (ppm) = 22.9 A_{645} nm - 4.68 A_{663} nm

2.5 Color Intensity Analysis

The color intensity analysis in this study used the Weaver method (1996). The measurement of the color intensity of the *bambu duri* leaf extract was carried out using the L*a*b* parameter. The extract sample was placed on a petri dish then the color reader was turned on and calibrated. The parameter L* represents the level of brightness and changes between 0 (black) to 100 (white), parameter a* represents the level of greenness (-a*) or redness (+a*), and parameter b* represents the level of blueness (-b*) and yellowish (+b*).

2.6 Statistic Analysis

The experimental data were analyzed by analysis of variance (ANOVA) and if the treatment was influential, it would be continued with the Tukey's test using Minitab 17 software. The correlation value between chlorophyll content and color intensity was analyzed using the Pearson correlation method in the IBM Statistic SPSS 26 software.

3 RESULTS AND DISCUSSION

3.1 Total Chlorophyll in *Bambu duri* Leaf Extract

The results showed that the treatment of temperature and time of maceration and their interactions had a very significant effect (P≤0.01) on the total chlorophyll of *bambu duri* leaf extract. The average value of total chlorophyll of *bambu duri* leaf extract can be seen in Table 1.

In Table 1, the highest total chlorophyll value (ppm) of *bambu duri* leaf extract was found in the maceration temperature treatment of 60°C and 36 hours of maceration time of 80,625.74 ± 436.94 ppm and the lowest total chlorophyll was found at 30°C maceration temperature treatment and 24 hours maceration time as much as 49,296.76 ± 359.54 ppm. These results indicated that the higher temperature used, namely 60°C and the longer maceration time up to 36 hours, the more total chlorophyll produced. Changes in temperature during the extraction process can affect the solubility of a compound due to the influence of density (density is very sensitive to temperature changes), so that the higher temperature in the extraction process can accelerate mass transfer and increase the extraction yield (Bimakr *et al.,* 2011).

Chlorophyll content in the *bambu duri* leaf extract increased and achieved maximum results at a treatment temperature of 60°C and maceration time of 36 hours. It can be seen that in every 48 hours of maceration time treatment, the total chlorophyll content contained in the extract decreased. This decrease in total chlorophyll content occurs due to high-temperature treatment and long extraction times which can result in a pheophytin reaction.

The pheophytin reaction is a reaction that occurs because chlorophyll was damaged and becomes its derivative, namely pheophytin. The existence of high-temperature treatment for a long time can cause the protein molecules that bind to chlorophyll to experience denaturation so that the chlorophyll will be released. The free chlorophyll released was unstable, so the magnesium ion (Mg^{2+}) contained in it can be easily replaced by hydrogen ions (Fitria, 2015). The change of chlorophyll to pheophytin causes discoloration of the extract, from originally green to brownish-green. Beside, an increase in temperature and the length time of maceration can also increase the occurrence of oxidation reactions, so that chlorophyll degrades (Hanum, 2000). The chlorophyll oxidation reaction occurs in its functional group, namely the isocyclic ring which forms agglomerated chlorophyll and the rupture of the tetrapyrrole ring to form a colorless product (Prasetyo *et al.,* 2012). The results of this study are in accordance with the statement of Aryanti *et al.* (2016), which states that chlorophyll dye is a compound that is very easy to change (degrade) into its derivatives after processing (the effect of the extraction factor).

3.2 Color Intensity

3.2.1 Brightness Level (L*)

The results of the analysis of diversity showed the treatment of temperature and time of maceration and their interactions had a very significant effect (P≤0.01) on the brightness (L*) of *bambu duri* leaf extract. The L* value represents the dark to light levels in the range of 0-100. The average brightness level (L) of *bambu duri* leaf extract can be seen in Table 2.

Table 1: Average value of total chlorophyll (ppm) of *bambu duri* leaf extract at temperature and time of maceration treatment.

Maceration temperature (°C)	Maceration time (Hours)		
	(24)	(36)	(48)
(30±2)	49,296.76±359.54^{i}	55,799.06±277.29^{g}	54,062.67±397.77^{h}
(45±2)	60,309.28±480.19^{f}	63,010.78±670.58^{d}	61,398.53±639.20^{e}
(60±2)	70,383.91±396.35^{c}	80,625.74±436.94^{a}	71,043.78±548.93^{b}

Note: Different letters behind the mean value indicate a significant difference at the 5% error rate (P⩽0.05). The data are mean of two groups in each treatment.

Table 2: Value of brightness level (L*) of *bambu duri* leaf extract at treatment temperature and time of maceration.

Maceration temperature (°C)	Maceration time (Hours)		
	24	36	48
30±2	20.10±0.06[a]	19.35±0.14[c]	19.77±0.15[b]
45±2	18.90±0.08[d]	18.31±0.12[f]	18.71±0.06[e]
60±2	17.78±0.11[g]	16.89±0.05[i]	17.54±0.06[h]

Note: Different letters behind the mean value indicate a significant difference at the 5% error rate (P⩽0.05). The data are mean of two groups in each treatment.

Table 3: The value of redness (a*) of *bambu duri* leaf extract at treatment temperature and maceration time.

Maceration temperature (°C)	Maceration time (Hours)		
	24	36	48
30±2	-7.74±0.13[a]	-8.64±0.16[c]	-8.22±0.18[b]
45±2	-8.97±0.11[d]	-9.75±0.10[f]	-9.34±0.18[e]
60±2	-10.06±0.06[g]	-11.25±0.18[i]	-10.66±0.15[h]

Note: Different letters behind the mean value indicate a significant difference at the 5% error rate (P⩽0.05). The data are mean of two groups in each treatment.

Table 2 showed the highest brightness (L*) value of *bambu duri* leaf extract was found at a temperature treatment of 30°C with the maceration time of 24 hours, which was 20.10 ± 0.06 and the lowest brightness (L*) was found at temperature treatment of 60°C with a maceration time of 36 hours as much as 16.89 ± 0.05. These results indicated that the higher temperature and maceration time used, the lower level of brightness (L*) produced. The resulting brightness level (L*) was inversely proportional to the chlorophyll content in the extract. Putri *et al.* (2012) stated that chlorophyll is a green pigment that tends to be dark, therefore the measurement results of the brightness level will be inversely proportional to the color intensity of chlorophyll. These results are in line with the research of Manasika and Widjanarko (2015) which states that the high content of extracted pigments can cause the color of the extract to get darker, so that it can reduce the brightness level (L*). The low-level of brightness (L*) on the use of high temperatures and long maceration times was also caused by the pheophytin reaction. In this reaction, the chlorophyll was damaged so that the color of the extract which was originally green turns into greenish-brown (dark). This is consistent with the statements of Sajilata and Singhal (2006) and Gross (1991) which state that color changes in pigments indicate degradation due to exposure to temperature and light with high intensity for a long time. This causes the measurement result of the brightness level (L*) to decrease.

3.2.2 Redness Level (a*)

The results of the analysis of diversity showed the treatment of temperature and time of maceration and their interactions had a very significant effect (P≤0.01) on the level of redness (a*) of *bambu duri* leaf extract. The value (a*) represents the green to red color level in the range of -100 to +100. The average value of redness (a*) in the *bambu duri* leaf extract can be seen in Table 3.

Table 3 showed the highest value of redness (a*) in the *bambu duri* leaf extract was at 30°C maceration temperature and 24 hours maceration time, which is -7.74 ± 0.13 and the lowest redness (a*) contained at temperature treatment of 60°C and maceration time of 36 hours as much as -11.25 ± 0.18. These results indicate that the use of higher temperature and longer maceration time can reduce the redness level (a*). The degree of redness shows the color intensity from green to red and is related to the amount of color pigment contained in the extract. Aryanti *et al.* (2016) stated that the level of redness (a*) is related to the solubility of chlorophyll pigments, the lower chlorophyll content in the extract, the higher level of redness and conversely the higher chlorophyll content, the lower redness value, and the resulting color will be more green.

3.2.3 Yellowish Level (b*)

The results showed the treatment of temperature and time of maceration and their interactions had a very significant effect (P≤0.01) on the yellowing level (b*)

Table 4: Value of yellowish level (b *) of *bambu duri* leaf extract at temperature and time of maceration treatment.

Maceration temperature (°C)	Maceration Time (Hours)		
	24	36	48
30±2	3.09±0.05^{i}	3.71±0.10^{g}	3.21±0.05^{h}
45±2	3.86±0.04^{f}	4.46±0.09^{d}	4.17±0.06^{e}
60±2	4.83±0.06^{c}	5.48±0.07^{a}	4.95±0.09^{b}

Note: Different letters behind the mean value indicate a significant difference at the 5% error rate (P≤0.05). The data are mean of two groups in each treatment.

of *bambu duri* leaf extract. The value (b*) represents the blue to yellow color level in the range of -100 to +100. The average value of yellowish level (b*) of *bambu duri* leaf extract can be seen in Table 4.

Table 4 showed the average value of yellowish level (b*) of the highest *bambu duri* leaf extract was found in the maceration temperature treatment of 60°C and 36 hours of maceration time, namely 5.48 ± 0.07 and the lowest yellowish level (b*) was found at a temperature of 30°C and a 24 hour maceration time of 3.09 ± 0.05. These results indicated that the higher temperature and the longer of maceration time used, the greater degree of yellowish (b*) produced. The value of yellowish (b*) in this study produced positive results, it showed that the *bambu duri* leaf extract has a yellow pigment. The presence of a yellow pigment in the extract was probably due to the degradation of chlorophyll compounds due to the use of high temperatures for a long time. The same thing was stated by Du *et al.* (2014) who states that chlorophyll was a very sensitive compound, chlorophyll will be very easily degraded on exposure to temperature and light, so it will change its color to yellowish.

In addition, the yellowish color of the *bambu duri* leaf extract was also caused by the ethanol solvent used. Based on Prasetyo *et al.* (2012) research, extracted *suji* leaves using ethanol solvent 95% tend to be yellowish-green. The yellowish-green color comes from chlorophyll b, xanthophyll, and other polar compounds (Gross, 1991).

3.3 Correlation between Total Chlorophyll Content and Color Intensity (L*a*b*)

Pearson's correlation coefficient was used to evaluate the relationship between total chlorophyll content and color intensity (L*a*b*) in *bambu duri* leaf extract. Based on the data in Table 5, the correlation coefficient value between total chlorophyll content and color intensity showed a very strong relationship. Sugiyono (2007) states that the very strong relationship category is indicated by the correlation coefficient value which is in the range of 0.80-1.00. The highest correlation coefficient *(r = -0,989)* was in the relationship between the total chlorophyll content and the brightness level (L *), followed by the correlation value *(r = -0,983)* which is in the relationship between the total chlorophyll content and the redness level (a*), and the lowest *(r = -0,981)* was in the relationship between the total chlorophyll content and the yellowish level (b*). The results of this studies are almost the same as the research conducted by Agarwal and Gupta (2018) which received a correlation value *(r = - 0.822)* on the relationship between the chlorophyll content of spinach leaves and brightness (L*) and research by Mazza and Oomah (1994) which obtained the result of the correlation value *(r = -0.873)* on the relationship between the chlorophyll content of peas and the degree of redness (a*).

The resulting correlation coefficient between total chlorophyll with brightness (L*) and redness (a*) was negative. This indicates that the higher total chlorophyll content, the lower the brightness (L*) and redness (a*) levels of the extract produced. This result was in accordance with the statement of Putri *et al.* (2012) which states that chlorophyll is a green pigment that tends to be dark, so that the measurement results of the brightness level will be inversely proportional to the color intensity of chlorophyll. Meanwhile, Aryanti *et al.* (2016) also stated that the level of redness was related to the solubility of chlorophyll pigments, the lower chlorophyll content in the extract, the higher level of redness, and conversely the higher chlorophyll content, the lower level of redness, and the greener resulting color. The results of this study were not much different from those of Putri *et al.* (2012) which resulted in a correlation coefficient between brightness (L*) and total chlorophyll of *(r = -0.996).*

The resulting correlation coefficient between total chlorophyll and yellowish level (b*) was positive. This means that the higher total chlorophyll content, the yellowish level (b*) of the extract will also increase. The increase between the total chlorophyll

Table 5: Pearson correlation coefficient between total chlorophyll and color intensity (L*a*b*).

	Total chlorophyll	Brightness level (L*)	Redness level (a*)	Yellowish level (b*)
Total chlorophyll	1	-0.989**	-0.983**	0.981**
Brightness level (L*)	-0.989**	1	0.996**	-0.997**
Redness level (a*)	-0.983**	0.996**	1	-0.993**
Yellowish level (b*)	0.981**	-0.997**	-0.993**	1

Note: ** Significant correlation at level P <0.01

content and the yellowish level (b*) occurs due to the influence of temperature treatment and maceration time which then results in the degradation of the chlorophyll pigment. This was consistent with the statement of Du *et al.* (2014) who states that chlorophyll is a very sensitive compound, chlorophyll will be very easily degraded on exposure to temperature and light, so it will change its color to yellowish.

There are two types of chlorophyll in plants, there are chlorophyll a and chlorophyll b. Chlorophyll a has a characteristic dark green (green-blue) color while chlorophyll b has a light green (green-yellow) color. Based on the analysis of chlorophyll a and b content in the *bambu duri* leaf extract, it was found that the average chlorophyll a content was higher (47%) than chlorophyll b. According to Indrasti *et al.* (2019), the amount or high levels of chlorophyll, especially chlorophyll a, has implications for the appearance of a green color in plants. The high chlorophyll a content can also affect the rate of degradation of chlorophyll. Schwartz *et al.* (2008) stated that the degradation of chlorophyll to pheophytin in chlorophyll a can take place 2.5-10 times faster than chlorophyll b. Chlorophyll a degrades to pheophytin a which is gray in color, while chlorophyll b undergoes degradation to pheophytin b which is brown in color (Indrasti *et al.,* 2019). This explanation is in accordance with the results of the research obtained, where the highest correlation value between chlorophyll content and color intensity is at the brightness level (L*). This indicates that the high chlorophyll a content can reduce the brightness (L*) of the extract because chlorophyll a is easily degraded at high temperatures to a darker color. In addition, the lowest correlation value obtained between the relationship between total chlorophyll and yellowish level (b*) can also indicate that the amount of chlorophyll b content is less than chlorophyll a so that the resulting correlation value is lower than the other color intensities.

4 CONCLUSIONS

The treatment of temperature and maceration time had a very significant effect on the total chlorophyll content and color intensity (L*a*b*) of *bambu duri* leaf extract. Increasing the maceration temperature to 60°C and the maceration time of up to 36 hours can increase the total chlorophyll content and the yellowish level (b*), as well as decrease the brightness (L*) and redness (a*) values. At a temperature of 60°C and a maceration time of 48 hours, the total chlorophyll content, brightness (L*) and redness (a*) values decreased, while the yellowish level (b*) values increased. Pearson correlation analysis between total chlorophyll content and color intensity (L*a*b*) of *bambu duri* leaf extract had a very strong relationship. The correlation value between total chlorophyll content and brightness level (L*) was *(r = -0.989)*, total chlorophyll content with redness (a*) was *(r = -0.983)*, and total chlorophyll content with a yellowish level (b*) was *(r = -0,981)*.

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