



Original Paper

## Physical And Chemical Characteristics Of Cassava Leaf Chlorophyll As Natural Dye Powder (*Manihot esculenta Crantz*) With Tween 80 And Alkaline Type Variations

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**Abstract**— Cassava leaves are a good source of minerals Ca, Mg, Fe, Mn, Zn, vitamins A and B2 (riboflavin). In addition, cassava leaves contain bioactive compounds that are beneficial to the body, namely chlorophyll. Chlorophyll is a natural green dye that is generally found in plants whose composition is contained in the chloroplasts. The amount of chlorophyll in cassava leaves is classified as good, namely 18.141 mg/l. This is one of the potentials to utilize cassava leaves as natural dyes. Production of cassava leaf coloring powder using foam mat drying. Foam mat drying is a method of foaming technique by adding foam. The foaming agent used is tween 80 which can produce foam or froth and speed up drying. Chlorophyll can be easily degraded due to exposure to heat (temperature), acids, light and alcohol. The use of NaHCO<sub>3</sub> can increase the levels of chlorophyll in the leaves. In addition, the use of MgCO<sub>3</sub> can maintain the green color of leaf chlorophyll. The purpose of this study was to determine the interaction of tween 80 variations and types of bases on the physical and chemical profiles of cassava leaf natural dye powder. The research design used a laboratory experimental method with two variations, namely tween 80 and base type. In this study there were four treatment combinations. This study used several test parameters including physical and chemical tests, namely dissolving time, color (L, a, b), total dissolved solids, pH, water content, and chlorophyll content. The results showed that the variation of tween 80, the type of base and the interaction of the two had a significant effect on the b color value and the L color value in cassava leaf natural dye powder.

**Keywords**— natural dyes, cassava leaves, chlorophyll

### I. INTRODUCTION

Cassava leaves contain bioactive compounds that are beneficial to the body in the form of chlorophyll. Chlorophyll is a natural green dye that is generally found in plants whose composition is contained in the chloroplasts. According to [1] the amount of chlorophyll in cassava leaves is quite good, namely 18.141 mg/l. This is one of the potentials to utilize cassava leaves as natural dyes. Natural dyes are environmentally friendly dyes. This is because natural dyes contain natural components that have relatively low pollution

load values, are easily biodegradable and non-toxic.

The drying method used is foam mat drying. Foam mat drying is a method of foaming technique by adding foam. The foaming agent used is *tween* 80 which can produce foam or froth and speed up drying. Chlorophyll can be obtained through the dissolution process. The solvent is the substance that is present in more quantity than the other substances in a homogeneous mixture. Alkaline conditions are usually applied to the blanching process of green leafy vegetables to prevent the degradation of chlorophyll into pheophytin which is green-brown in color. The bases that can be used are NaHCO<sub>3</sub> and MgCO<sub>3</sub>. NaHCO<sub>3</sub> and MgCO<sub>3</sub> are alkaline salts. According to [2], the use of NaHCO<sub>3</sub> can increase chlorophyll levels in suji leaves. In addition, based on research by [3] showed that the use of MgCO<sub>3</sub> can maintain the green color of the chlorophyll of suji leaves. Based on this description, the purpose of this study was to determine the interaction effect of *tween* 80 variations and base types on the physical and chemical profiles of the natural dye powder of cassava leaf chlorophyll.

### II. MATERIALS AND METHODS

#### A. Tools and Materials

The tools used in this study were a pH meter, knife, Philips HR2115 blender, oven, cutting board, basin, *beaker glass*, measuring cup, dropping pipette, digital balance, analytical balance, glass funnel, stove, boiler, gas, pan, basin, aluminum foil, scissors, ruler, filter cloth, filter paper, *General colorimeter*-AMT 507r, cuvette, refractometer, *microwave*, UV-Vis SP-3000 spectrophotometer, *hand mixer*, laptop, *software* SPSS

The materials used in this study were cassava leaves aged ± 3 months and taken from the third to fifth order from the top of the leaf which is the main ingredient. Other supporting materials are distilled water, maltodextrin, buffer solution 4, buffer solution 8, *tween* 80, NaHCO<sub>3</sub>, MgCO<sub>3</sub>, and 80% acetone.

## B. Research

Design The experimental design used was Completely Randomized Design (CRD) which was arranged with two factors. The first factor is the variation in the addition of *tween 80* % and the second factor is the different types of bases in the form and  $MgCO_3$  of 0.5%. The treatment combinations can be seen in Table 1.

Table 1. Treatment combinations

Tween 80 concentration (A)	Type of base (B)	
	$NaHCO_3$ (0,5%) (B1)	$MgCO_3$ (0,5%) (B2)
0,75% (A1)	A1B1	A1B2
1% (A2)	A2B1	A2B2

## C. Research Stages

### Raw Material Preparation

The raw material used in this research is cassava leaves. Cassava leaves that have been prepared are sorted to separate the bones and leaves. After that, wash it with running water to clean the dirt on the cassava leaves. Then it is drained and the raw material is ready to be used for further processing.

### Extraction

Leaves Cassava leaves that have been *trimmed* are then weighed as much as 200 grams and cut into small pieces to increase their surface area. After that, the destruction process was carried out using a blender using distilled water in a ratio of 1: 3 or as much as 600 mL. Then filtered to separate the dregs and filtrate of cassava leaves. After that, the addition of bases in the form<sub>3</sub> and  $MgCO_3$  of 0.5% Then deposition was carried out for 24 hours. Then the cassava leaf extract was separated to separate the chlorophyll extraction results from the cassava leaves and the precipitate so that the chlorophyll extraction results from cassava leaves were obtained.

### Making Natural Dyes Powder

The drying method used for making natural dyes powders is using the foam mat drying with the addition of 5% maltodextrin as filler and *tween* 0.75% and 1%. Then do the stirring with a *mixer* until foamy. After that, drying was carried out using a microwave for 30 minutes with stirring every 1 minute. After that, natural dye powder is obtained which is then crushed using a blender so that the resulting dye powder is smoother.

## D. Analysis Procedure

### Dissolving

Time Dissolving time is the time when all powders dissolve completely in water. Solubility analysis was carried out to determine the speed of solubility of instant drink powder in water when consumed. Dissolving time is calculated based on the time the powder dissolves in seconds (s). The higher the solubility value obtained, the better the quality of the product produced [4]. Determination of dissolution time is carried out by dissolving 2 g of dye powder with 20 ml of distilled water or 1: 10 and stirring is carried out.

## Color

Determination of color values in cassava leaf coloring powder using the *Color Reader*. In this tool it can be seen that the value of  $L^*$  has a value of 0 (black) to 100 (white) which states that reflected light produces achromatic colors of white, gray and black. Positive  $a^*$  values (0 to 100) indicate a red color. Meanwhile, a negative value of  $a^*$  (0 to -100) indicates a green color. For the notation  $b^*$  is the notation of a mixed chromatic color from blue to yellow.

### Total Dissolved Solids

Testing for total dissolved solids content begins with hand-refractometer calibration using distilled water, then 1-2 ml of sample is dripped onto a refractometer prism at 25°C then the degree of Brix is measured. °Brix measured indicates the dissolved solids content in the solution [5].

### pH

Measurement is carried out by dipping the cathode tip in a thick dye sample and every time you want to measure the pH of another sample, the probe is cleaned using distilled water first and dried with a *tissue*. The pH value read is the value when the pH meter is stable or constant.

### Moisture content

The method for determining the water content by drying is that a sample of 3-5 grams is weighed and put into a cup that has been dried and the weight is known. Then the sample and cup were dried in an oven at 105°C for 6 hours. The cup is cooled and weighed, then dried again until a constant weight is obtained.

Water content can be calculated using the following formula:

$$\text{Moisture content (\%bk)} = \frac{(a+b)-c}{c-a} \times 100$$

### Chlorophyll content

Testing the total chlorophyll content was carried out by weighing the sample as much as 0.1 g, then adding 10 mL of 80% acetone. The solution is filtered with filter paper. The filtrate obtained was measured for its absorbance using a spectrometer with a wavelength of 645 (A) and 663 (B). Calculation of chlorophyll content was carried out using the formula:

$$\text{Chlorophyll a (ppm)} = 12.7B - 2.69 A$$

$$\text{Chlorophyll b (ppm)} = 22.9 A - 4.68 B$$

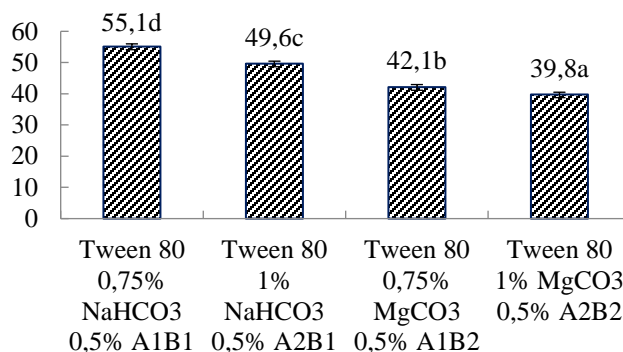
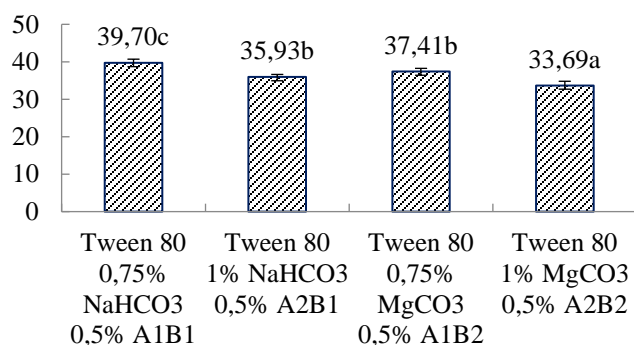
$$\text{Total chlorophyll} = 8.02 B + 20.2 A$$

(source : [6])

## III. RESULT AND DISCUSSION

### A. Dissolving

Time is done to determine the time the sample dissolves in water. Dissolving time is done by dissolving the sample in distilled water and calculating the time until the sample dissolves in the distilled water. Analysis of the calculation of the dissolution time can be seen in Figure 1.



Results of fingerprint analysis the variance indicates that the variation factor of the addition of *tween* 80 has a significant effect on the dissolution time value. The addition *tween* 80 affects the dissolving time, namely the more concentration of *tween* 80 used, the faster the resulting dissolution time. According to [7] the concentration of *tween* 80 can accelerate the solubility of a powder because *tween* 80 has properties that can dissolve in water so that it will increase the speed of dissolving the powder in water. The higher the addition of *tween* 80 to the material, the porosity of the material will increase so that it is easier to absorb water and dissolves more quickly in water.

The results of the analysis of variance showed that the factor of adding base had a significant effect on the value of dissolution time. The difference in bases in the form of NaHCO<sub>3</sub> and MgCO<sub>3</sub> has different water content values. The water content of the NaHCO<sub>3</sub> has a higher water content than the MgCO<sub>3</sub>. The water content affects the soluble time value of the material. This is supported by [8] stating that the greater the value of the water content, the longer the water absorption process will take which causes the resulting soluble time to increase or be higher. The high moisture content of the material makes it difficult for the material to spread or disperse in water because the material tends to be sticky, thus no pores are formed and the material is unable to absorb large amounts of water.

## B. Color

### Value (L)

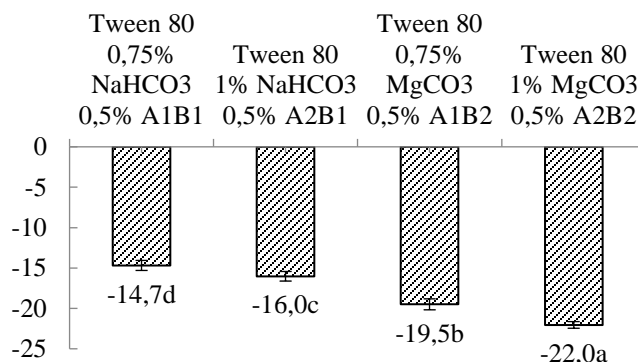
The value of L or *lightness* is a parameter to identify the brightness value in the sample. The higher the L value, the higher the brightness value produced by the natural coloring powder of cassava leaves. The L color value test was carried out using a *colorimeter*. The resulting value is a number from 0 to 100 which states that reflected light produces black to white. The calculation of the L color value can be seen in Figure 2.

The results of the analysis of variance showed that the factor of variation in the addition of *tween* 80 is significantly different from the value of L in natural dye powder. The addition *tween* 80 to the manufacture of cassava leaf natural coloring powder has an effect, namely the higher the concentration of *tween* 80 given, the lower the resulting L value, this is because *tween* 80 has foaming properties that can maintain color and minimize damage when heated. This is supported by [9], namely that *tween* 80 has hydrophilic and hydrophobic properties in one molecule which is capable of forming foam. The foam produced can strengthen the protective layer on the material in the foam system so that the color of the powder does not fade or be damaged due to the drying process. the addition of the type of base and the interaction between the two significantly affected the value of the color L in the natural coloring powder of cassava leaves.

The results of the analysis of variance showed that the factor of adding the type of base had a significant effect on the L value of the natural dye powder. The addition of bases in the form of NaHCO<sub>3</sub> and MgCO<sub>3</sub> had a significant effect on the color value of L, this was due to the fact that NaHCO<sub>3</sub> and MgCO<sub>3</sub> are alkaline materials so that they act as stabilizers in chlorophyll color. The addition *tween* 80 also affects the color value of L because *tween* 80 is a surfactant that can suppress the degradation of chlorophyll to pheophytin which can affect the brightness of the cassava leaf coloring powder.

### Value a

Value color a is a parameter to measure the color red – green. The more negative the value of a, the greener the resulting color will be and the more positive the value of a, the redder the resulting color will be. The calculation of the color value a can be seen in Figure 3.



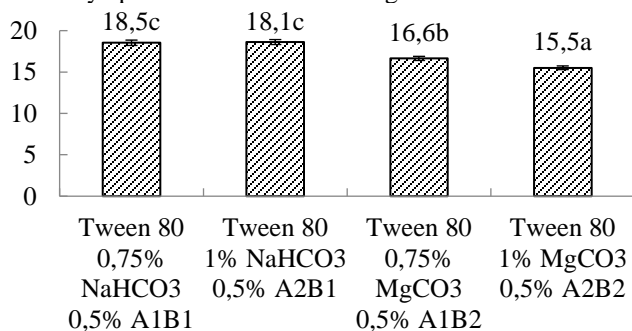
The results of the analysis of variance showed that the

variation factor of the addition of tween 80 had a significant effect on the value of a color. The addition tween 80 to the production of natural coloring powder for cassava leaves has an effect, namely the higher the concentration of *tween* 80 given, the higher the value of a produced, this is because *tween* 80 has foaming properties that can maintain color and minimize damage when heated. This is supported by [9], namely that *tween* 80 has hydrophilic and hydrophobic properties in one molecule which is capable of forming foam. The foam produced can strengthen the protective layer on the material in the foam system so that the color of the powder does not fade or be damaged due to the drying process. In addition, the value of a is influenced by the total chlorophyll content contained in the material. The higher the chlorophyll content produced, the higher the a value produced. According to [2] *Tween* 80 is a non-ionic detergent which suppresses the formation of pheophytin in chlorophyll. To stabilize the green color in plants, you can change chlorophyll to chlorophyllide, giving surfactants can create an alkaline environment so that it can increase the activity of chlorophyllase enzymes converting chlorophyll to chlorophyllid, surfactants or non-ionic detergents can protect the green color by creating an alkaline atmosphere.

The results of the analysis of variance showed that the factor of adding base type a had a significant effect on the value of color a. addition of bases in the form of  $\text{NaHCO}_3$  and  $\text{MgCO}_3$  has different characteristics. On the addition of  $\text{NaHCO}_3$  the resulting color is yellowish green, this is because the addition of  $\text{NaHCO}_3$  has a lower pH compared to  $\text{MgCO}_3$  which causes a pheophytin reaction, namely a green color change to yellowish green or brown. According to [3] this natural green color can change to green-brown and may turn brown due to the substitution of magnesium by hydrogen to form pheophytin. The reaction can proceed more slowly in a solution with alkaline conditions.

#### Value b

Value b is a color that identifies blue and yellow. The more positive the value of b, the more yellow the resulting color, while the more negative the value of b, the bluer the resulting color. The results of calculating the value of b in cassava leaf natural dye powder can be seen in Figure 4.



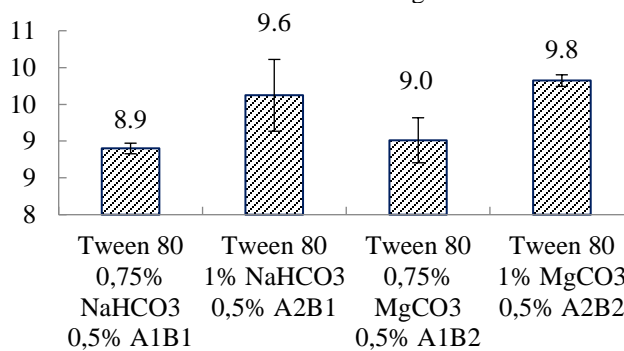
The results of the analysis of variance showed that the factor variations in the addition of *tween* 80 had a significant effect on the value of color b in cassava leaf natural dye powder. The addition *tween* 80 to the manufacture of cassava leaf natural coloring powder has an effect, namely the higher the concentration of *tween* 80 given, the lower the resulting L value, this is because *tween* 80 has foaming properties that can maintain color and minimize damage when heated. This is

supported by [9], namely that *tween* 80 has hydrophilic and hydrophobic properties in one molecule which is capable of forming foam. The foam produced can strengthen the protective layer on the material in the foam system so that the color of the powder does not fade or be damaged due to the drying process. The use of *tween* 80 can suppress the formation of pheophytin by creating an alkaline atmosphere, so that the higher the concentration of *tween* 80, the higher the b value in the sample.

The results of the analysis of variance showed that the factor of adding the type of base had a significant effect on the value of color b in the cassava leaf natural dye powder. The addition of base types in the form of  $\text{MgCO}_3$  and  $\text{NaHCO}_3$  have alkaline properties so that they are able to maintain the green color of the material. addition of  $\text{NaHCO}_3$  shows a high b value, this is because the addition of  $\text{NaHCO}_3$  occurs a pheophytinization reaction, besides that the  $\text{NaHCO}_3$  has a lower pH compared to the sample with the addition of  $\text{MgCO}_3$ , so the chlorophyll is unstable during the heating process. Unstable chlorophyll will change into its derivative compounds so that it is degraded. During heating, organic acids will be released from the tissues which will affect the formation of pheophytin [10]. The interaction of the two has a significant effect on the value of b. The combination of an increasingly negative a value and an increasingly positive b value correlates with a good visual appearance.

#### C. Total Dissolved Solids

Total dissolved solids value shows the percentage of dissolved material in a solution which is generally expressed in units of % sugar or °Brix. Most of the components contained consist of water-soluble components such as glucose, fructose, sucrose and protein. Calculation of the total dissolved solids value can be seen in Figure 5.

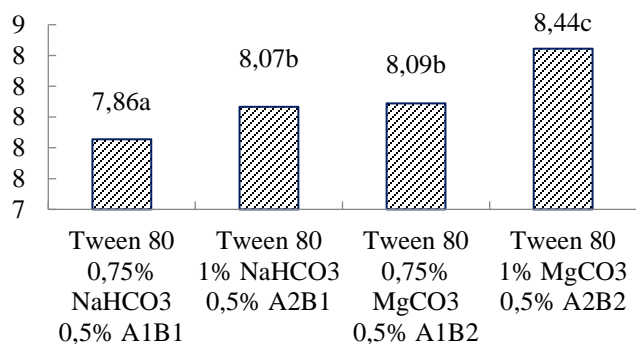


The results of the analysis of variance showed that the variation factor of the addition of *tween* 80, the difference in the addition of the type of base and the interaction between the two did not significantly affect the total dissolved solids value in natural cassava leaf dye powder. The research data shows that the more the concentration *tween* 80 is given, the higher the total dissolved solids produced. This is supported by [11] *Tween* 80 has hydrophilic properties which easily binds to water so that it can increase the solubility and total dissolved solids of a material. This is supported by [12], namely *Tween* 80 has an HLB (Hydrophilic Lipophilic Balance) of 15.0 which is high (> 11) where the HLB value describes the solubility properties of a material. A material that has a high HLB value means that it has many hydrophilic groups which dissolve easily in water and hydrophobic groups which do not dissolve easily in water, the presence of hydrophobic groups

in *tween* 80 causes the total dissolved solids to increase due to the addition of *tween* 80.

#### D. pH

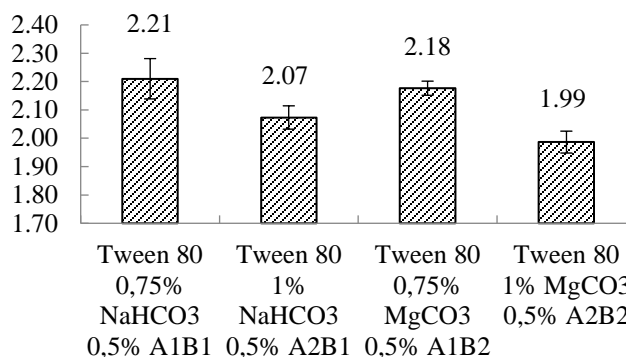
pH is one of the acid indicator in a substance. This pH measurement is carried out using a pH meter. The results of the pH value of cassava leaf coloring powder with variations in the addition of *tween* 80 and the addition of different bases can be seen in Figure 6.



The results of the analysis of variance showed that the variation factor of the addition of *tween* 80 had a significant effect on the pH value. The addition *tween* 80 can cause the pH to increase, this is supported by [13] The pH of *tween* 80 ranges from 6 – 8. The addition *tween* 80 has an impact on the pH of natural dye powders, namely the more *tween* 80 added, the higher it will be The pH of the dye powder. The results of the analysis of variance showed that the base addition factor had a significant effect on the pH value of natural cassava leaf coloring powder. According to [14] that the pH value of chlorophyll is affected by the pH of the extracting solution. This indicates that the higher the pH value of the extracting solution, the higher the pH value of the chlorophyll extract. The addition of MgCO<sub>3</sub> and NaHCO<sub>3</sub> is intended to maintain the degree of acidity of the chlorophyll extract so that it becomes alkaline in an atmosphere so that degradation due to acidic pH which can trigger the formation of pheophytin can be inhibited. This is supported by [15] the main function of adding a stabilizer is to inhibit the formation of pheophytin by conditioning the degree of acidity (pH), so that it becomes alkaline. The difference in the pH value in the addition of NaHCO<sub>3</sub> and MgCO<sub>3</sub> is caused by the difference in the degree of dissociation of the base used, causing the pH value of the natural coloring powder of cassava leaves to be different [16].

#### E. Moisture Content

Moisture content is one of the most important components in determining the shelf life of a material. The higher the water content produced, the product's shelf life will be shorter or not last long. This is due to the presence of microbial growth caused by the high water content in the material. The results of the analysis of water content with the difference in the addition of NaHCO<sub>3</sub> and MgCO<sub>3</sub> and variations in the addition of *tween* 80 can be seen in Figure 7.



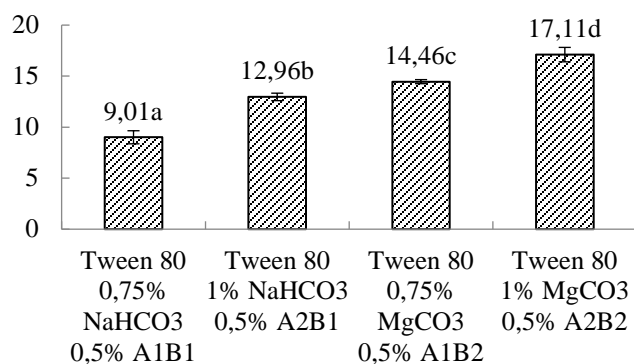
The water content value of natural dye powder ranges from 1.99 to 2.21%, this is in accordance with SNI 01 -4320-1996, that is, the water content for instant drink powder products is  $\geq 5\%$ . The results of the analysis of variance showed that the variation factor by adding *tween* 80 had a significant effect on water content, while the addition of different bases and the interaction between the two factors had no significant effect on water content of natural dye powders. The addition *tween* 80 concentration affects the water content, the more *tween* 80 concentration added, the lower the ka of the water produced. This is supported by [11] that water bound together with maltodextrin and *tween* 80 will evaporate more easily so it can reduce the water content of the resulting product. The addition *tween* 80 causes a lot of water in the material to be bound by *tween* 80 during the foaming process. *Tween* 80 is a non-ionic surfactant which has hydrophilic and hydrophobic sides in one molecule.

The stirring process causes the movement of the hydrophobic groups preventing contact with water and leads to the air so that the hydrophobic groups attract with the air, while the hydrophilic groups of the surfactant molecules attract with water in the material so that there will be gas or air adsorbed in the thin layer [17]. In samples A1B2 and A2B2 with the addition of base in the form of MgCO<sub>3</sub>, the water content value was lower than in samples A1B1 and A2B1, namely the addition of base in the form of NaHCO<sub>3</sub>. This is due to the drying process of the dye powder at the same time, samples A1B1 and A2B1 have a more sticky texture so that the water content is higher than samples A1B2 and A2B2 with the addition of base MgCO<sub>3</sub>. This is supported by [18] sodium bicarbonate (NaHCO<sub>3</sub>) has the ability to bind water, so that food that is soaked or added to NaHCO<sub>3</sub> will cause a high water content in the material.

#### F. Chlorophyll content

Calculation of chlorophyll content is carried out by dissolving the sample in acetone and then measuring the absorbance using wavelengths 645 and 663. The value of chlorophyll content is used to determine how much chlorophyll content is in the material 8.





The results of the analysis of variance showed that the variation factor of the addition of *tween* 80 had a significant effect on the value of chlorophyll content. The addition *tween* 80 can cause the chlorophyll value to increase, the higher the concentration of *tween* 80 added, the chlorophyll content will increase. This is supported by [9] namely *tween* 80 can bind to maltodextrin which acts as a protective layer because of *tween* 80's ability to form foam thereby expanding the surface of the material and making it easier for water to evaporate so that it can accelerate drying and can prevent damage to bioactive components such as chlorophyll in heating process. Increasing the concentration of *tween* 80 can thicken the protective layer contained in the foam system, so that components present in the foam system such as chlorophyll can be protected from oxidative damage during the drying process.

The results of the analysis of variance showed that the factor of adding the type of base had a significant effect on the value of chlorophyll content. The addition of NaHCO<sub>3</sub> and MgCO<sub>3</sub> has alkaline properties so that it can suppress the reaction for the formation of pheophytin, according to [19] the addition of salts such as sodium, magnesium or calcium can reduce pheophytinization because an electrostatic coating of the salt will occur. In samples A1B1 and A2B1, namely the addition of base in the form of 0.5% NaHCO<sub>3</sub>, the results showed that the value of chlorophyll content was lower than the total value of chlorophyll content in samples A1B2 and A2B2. This is because the color produced by the dye with the addition of NaHCO<sub>3</sub> yellower is than the sample with the addition of MgCO<sub>3</sub>. The greenish yellow color of the dye is caused by the degradation of chlorophyll due to the heating process. This is supported by [20] that unstable chlorophyll will change into its derivative compounds so that they can be degraded. The pheophytinization process is a reaction for the formation of pheophytin which turns green in color from yellow to green-brown which causes the chlorophyll content in the material to be damaged. One of the causes of the damage to chlorophyll is due to heating. The pheophytin reaction occurs because the Mg ion in the center of the chlorophyll molecule is released and replaced by H ions. Denaturation of the protective protein causes magnesium ions to be easily released and replaced by hydrogen ions to form pheophytin. According to [21] the use of a base in the form of MgCO<sub>3</sub> will produce a more stable dye compared to the addition of base NaHCO<sub>3</sub> dyes.

#### IV. CONCLUSION

The variation *tween* 80, the type of base and the interaction between the two have a significant effect on the value of color

and color L in the natural dye powder of cassava leaves, that is, the higher the concentration of *tween* 80 and the addition of base in the form of MgCO<sub>3</sub>, the greener the color will be.

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