Molecular functional group and optical analysis on chlorophyll of green choy sum and cassava leaves extracts

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Abstract. Recently, chlorophyll can improve the optical characteristics of thin films. The role of chlorophyll is a dopant in the thin film. Chlorophyll is very good in transferring electron which is strongly related to energy. If the energy gap of the film is low, the characteristics of the film are better because it requires less energy to jump one electron from the valence band to the conduction band. However, an important factor to consider in this case is the chlorophyll content. The more chlorophyll content will improve the light absorption rate. This study examined the characterization of chlorophyll of green choy sum and cassava leaves extracts. The analysis was carried out based on the Fourier Transform Infra-Red (FTIR) and UV-Vis Spectrophotometer. The results of the analysis were molecular functional groups, vibration frequencies, bond force constants between molecules, optical characteristics and chlorophyll content on both of chlorophyll extracts.

1. Introduction

The development of semiconductor materials, especially thin films, is progressing more rapidly. These are developments in fabrication methods, the use of primary materials, and the use of doping materials. The addition of dopant to the thin film material helps to obtain the material characteristics because the addition of dopant can change the physical properties, such as electronic structure, core level, and dielectric properties of the film [1]. Usually, dopant materials that used in ferroelectric applications are light sensors. Dopant material determines that the fabricated transistor is a PNP or NPN transistor. However, recently, natural materials in the form of chlorophyll are known to have very competent abilities in the electron transfer that began to be applied in thin film solar cells. The use of chlorophyll as a dopant of thin films has been started since the last decade, especially on how to fabricate and apply chlorophyll thin films as Dye-Sensitized Solar Cells (DSSCs), Cell Chlorophyll Photovoltaics, and Luminescent Solar Concentrators [2-5]. Chlorophyll in the literature is extracted from marigold leaves, noni leaves and guava leaves (long-lived plants). The optimum chlorophyll content is owned by plants that receive sufficient sunlight intensity and short-leaf-age [6-7]. One of the short-lived plants is vegetables, where vegetable plants have a relatively fast leaf age and harvest age around 25-30 days after planting day. The investigation of the chlorophyll content of ten species of...
green leafy vegetables that are commonly consumed by Indonesian people in-vitro that have been done [8], vegetable green choy sum and cassava are interesting for further investigation. In this work, we extracted chlorophyll from green choy sum leaves and cassava leaves. The optical properties and functional groups of the molecule were then characterized. From the characterizations at least we determined the highest chlorophyll content of the extract, so it is more effective to be applied and integrated.

2. Experimental methods

2.1 Chlorophyll extraction

Extraction is a diffusional process of one or several materials from solids or liquids using solvent media. Leaves were released from the bones of the leaves, weighed 100 g each, then disposed under running water, blended with 200 ml of aquadest, filtered, and continued with the blanching process or the solution was heated to separate chlorophyll. Chlorophyll was filtered and rinsed with distilled water, then dried in Yenaco digital oven at 50 °C for 1 hour. Dry solids were crushed using mortar, then filtered with 200 mesh filters [9-10]. Blending aimed to destroy the leaves so that they were small. Blanching at 60 °C for 10 minutes was carried out to inhibit the work of the chlorophyllase enzyme, thereby reducing the possibility of color degradation or even a decrease in the quality of chlorophyll. Besides, blanching can also kill plant cells so that the chloroplast organelles were destroyed and chlorophyll comes out easily. Furthermore, drying was done to keep the sample obtained not easily damaged and can be stored for a long time [11].

2.2 Characterization of chlorophyll

Chlorophyll is a green pigment that found in chloroplasts which plays a role in photosynthesis. Chlorophyll consists of chlorophyll a and chlorophyll b. Chlorophyll a is chlorophyll which absorbs light with a wavelength of 600 nm to 700 nm, while chlorophyll b absorbs light at a wavelength of 500 nm to 600 nm. The physical characteristic of chlorophyll is receiving and or reflecting light with different waves (fluorescent or fluorescent). Chlorophyll absorbs light with wavelengths in intervals of 400 nm to 700 nm. The chemical characteristic of chlorophyll is soluble in more polar organic solvents such as ethanol.

The measurement of chlorophyll quantity was performed using the UV-Vis spectrophotometers, then the quality of chlorophyll was analyzed using FTIR. The optical characterization of chlorophyll was started by weighing 0.1 g of chlorophyll extract, then dissolved the extract in 10 ml ethanol. The intensity of absorbance was measured from 350 nm to 700 nm [12] on the UV-Vis spectrum. Measurement of chlorophyll content was calculated based on equations (1) [13].

\[
Ch \alpha = 16.29 A_{665} - 8.54 A_{652}
\]

Where 16.29 and 8.54 are the constants then \( A_{665} \) and \( A_{652} \) are the intensity of absorbance at a wavelength of 665 nm and 652 nm.

FTIR characterization was started with taking chlorophyll sample 0.5 mg-1 mg, the sample was crushed together with 100 mg KBr 99% using mortar. The mixture of materials was formed into pellets using a pressure device. The transmittance of the trailer was taken from 400 cm\(^{-1}\) to 4,000 cm\(^{-1}\) [14].

The atoms in a molecule cannot move, but it is vibrating. Chemical bonds that connect two atoms can be assumed as two balls connected by springs. When infrared radiation is passed through a material, the molecules absorb energy and there is a transition between the ground vibration and excited states. Absorption of energy at various frequencies can be detected by an infrared spectrophotometer which plots the amount of infrared radiation that is transmitted as a function of the radiation frequency. The plot provides information on molecular functional groups. The greater the bond force constant will result greater vibrational frequency, distance, and energy between the quantum vibrational levels. The force constants for single, double, and triple bonds are \( 5 \times 10^{5} \) dyne/ cm, \( 10 \times 10^{5} \) dyne/ cm, and
15 x 10^5 dyne/cm, respectively. Hooke's law can estimate the vibrational region that occurs according to equation (2) [15].

\[ v = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}} \]  

(2)

Frequency analysis and molecular bond force constants in the FTIR spectrum are described in equations (3-6) [15].

\[ E = \frac{hc}{\lambda} \]  

(3)

\[ \lambda = \frac{1}{v} \]  

(4)

\[ f = \frac{c}{\lambda} \]  

(5)

\[ k = f^2 \times 4\pi^2 \times \mu \]  

(6)

Where \( \lambda \) = wavelength (nm), \( v \) = wavenumber (cm\(^{-1}\)), \( f \) = vibration frequency (s\(^{-1}\)), \( k \) = bond force constants (dyne/cm), and \( \mu \) = reduced mass (g)

3. Results and Discussion

3.1 Optical characteristics

Chlorophyll is an active pigment that absorbs light. When optics are subjected to chlorophyll, there is an interaction between light and matter that causes electron excitation to higher energy level. The ability of the material to absorb light can be affected by excitation. The more electrons are excited, the more electrons flow. Therefore, the material that has the most chlorophyll content will have the ability to absorb light and produce optimal electricity. Measurement of chlorophyll using UV-Vis spectroscopic method [16] showed that chlorophyll \( a \) had two absorbance peaks, they were at wavelengths of 430 nm and 662 nm. Using the equation (1), the chlorophyll content obtained was 5.118 mg/L. In the characterization of chlorophyll in green choy sum and cassava (Figure 1), the two absorbance peaks were located at wavelengths of 460 nm and 660 nm. This result can be claimed as chlorophyll \( a \), because of the presence of a very sharp peak at 660 nm. The peak at 460 nm is the peak of chlorophyll \( a \) which has been affected by the presence of protein so that peak has a shift. Proteins have very high absorbance intensity in the UV spectrum [12]. Chlorophyll content of green choy sum leaves and cassava leaves based on calculations using equations (1) were 5.358 mg/L and 4.029 mg/L.

![Figure 1. UV-Vis Spectrum of chlorophyll a in green choy sum and cassava leaves extract](image-url)
3.2 Chlorophyll molecular group

Infrared spectroscopy is a method of observing molecular interactions with electromagnetic radiation in wavenumber of 400 cm\(^{-1}\) to 4,000 cm\(^{-1}\). Absorption of electromagnetic waves can cause excitation of energy levels in molecules. The ability of compounds to absorb electromagnetic radiation in the infrared spectrum is related to the phenomenon of molecular vibrations. Figure 2 shows the result of FTIR characterization of chlorophyll of green choy sum and cassava which shows 5 peaks on each sample. Based on the references [17], the chlorophyll of green choy sum and cassava form stretching vibrations of hydrogen atoms (O-H) at wavenumbers 3,310 cm\(^{-1}\) and 3,436 cm\(^{-1}\). Hydrogen bonding causes the peak to widen and a shift towards shorter wavenumbers. The afliliatic of C-H vibration was formed at 2,930 cm\(^{-1}\) and 2,934 cm\(^{-1}\). The change in the structure of C-H caused the peak to shift towards the maximum. In addition, there was a peak in the double bond area around the wavenumber 1,650 cm\(^{-1}\) and 1,550 cm\(^{-1}\) which was a stretching vibration of C = O and C = C or amide II protein, as well as CO at wavenumber 1,300 cm\(^{-1}\) which was a carboxylic acid. The existence of a C-O vibration was included as a molecular fingerprint because it is in the absorption area between 1,500 cm\(^{-1}\) - 400 cm\(^{-1}\). Table 1 shows the wavenumber and vibration frequency, while the molecular bond force constant is shown in Table 2.

Table 1. Wavenumber and vibration frequency that assume the form of symmetrical stretching of the molecule bond of O-H, C-H, C=O, C=C, and C-O

| Molecule | Wavenumber (cm\(^{-1}\)) | Wavenumber (cm\(^{-1}\)) | Vibration frequency (Hz) | Vibration frequency (Hz) |
|----------|-------------------------|-------------------------|-------------------------|-------------------------|
|          | Experiment | Literature [17] | Experiment | Literature [17] |
| O-H      | green choy sum     | 3310          | 3029-3639    | 9.93 x 10\(^{13}\) | 1.115 x 10\(^{14}\) |
|          | cassava            | 3436          |              | 1.03 x 10\(^{14}\) |
|          | green choy sum     | 2930          | 2809-3019    | 8.79 x 10\(^{13}\) | 9.513 x 10\(^{13}\) |
|          | cassava            | 2934          |              | 8.80 x 10\(^{13}\) |
| C=O      | green choy sum     | 1651          | 1712-1763    | 4.95 x 10\(^{13}\) | 5.190 x 10\(^{13}\) |
|          | cassava            | 1656          |              | 4.97 x 10\(^{13}\) |
| C=C      | green choy sum     | 1542          | 1500-1675    | 4.63 x 10\(^{13}\) | 4.760 x 10\(^{13}\) |
|          | cassava            | 1541          |              | 4.62 x 10\(^{13}\) |
| C-O      | green choy sum     | 1300          | 1357-1423    | 3.90 x 10\(^{13}\) | 3.338 x 10\(^{13}\) |
|          | cassava            | 1331          |              | 3.96 x 10\(^{13}\) |
Table 2. Wavenumber and bond force constant that assume the form of symmetrical stretching of the molecule bond of O-H, C-H, C=O, C=C, and C-O

| Molecule | Wavenumber (cm⁻¹) | Bond force constant (dyne/cm) |
|----------|-------------------|------------------------------|
|          | Experiment        | Literature [17]              | Experiment        | Literature [17] |
| O-H      | green choy sum    | 3310                         | 3029-3639         | 6.12 x 10⁵     | 7.7 x 10⁵      |
|          | cassava           | 3436                         | 6.59 x 10⁵        | 6.59 x 10⁵     | 5.1 x 10⁵      |
| C-H      | green choy sum    | 2930                         | 2809-3019         | 4.69 x 10⁵     | 6.59 x 10⁵     |
|          | cassava           | 2934                         | 6.10 x 10⁵        | 5.1 x 10⁵      | 5.1 x 10⁵      |
| C=O      | green choy sum    | 1651                         | 1712-1763         | 11.01 x 10⁵    | 12.1 x 10⁵     |
|          | cassava           | 1656                         | 11.07 x 10⁵       | 9.6 x 10⁵      | 9.6 x 10⁵      |
| C=C      | green choy sum    | 1542                         | 1500-1675         | 8.40 x 10⁵     | 9.6 x 10⁵      |
|          | cassava           | 1541                         | 8.39 x 10⁵        | 9.6 x 10⁵      | 9.6 x 10⁵      |
| C-O      | green choy sum    | 1300                         | 1357-1423         | 6.82 x 10⁵     | 7.05 x 10⁵     |
|          | cassava           | 1331                         | 5.8 x 10⁵         | 5.8 x 10⁵      | 5.8 x 10⁵      |

In addition to being able to identify molecular groups, data from FTIR characterization can also be used to calculate the vibration frequency and the bond force constant between atoms by assuming the bond as a simple harmonic oscillation motion. This calculation was carried out based on equation (3-6) which is available in Table 1-2. Although the difference in values is not significant, from the results of this calculation it can be concluded that molecules with large bond constants will have large energy to cause molecules to vibrate, causing a large frequency of molecular vibrations. The size of the bond force constant depends on the type of bond of the molecule, a molecule that has a double bond will produce a bonding force stronger than a molecule that has a single bond [15].

The atoms in a compound bind each other to form molecules. When a molecule receives the force or energy from the outside, the molecular state is disrupted therefore an atomic vibration occurs. Vibration energy can estimate the magnitude of the bonding force between these atoms [15]. Table 1 and Table 2 represent of wavenumber, vibrational frequencies, and constants of bonding forces of O-H, C-H, C = O, C = C, and C = O molecules from green choy sum chlorophyll and cassava chlorophyll. Based on the table, the molecules contained in green choy sum chlorophyll have a lower vibration frequency and bond force constants than the molecules that found in cassava chlorophyll. A molecule that has a stronger bond indicates that the bond between the atoms is stable, when it reaches a stable state, the molecule does not accept or cannot bind to other atoms. So it can be concluded that green choy sum chlorophyll has more advantages than cassava chlorophyll. In addition, the content of chlorophyll a in the green choy sum extract was greater, and the green choy sum extract also has a lower bonding force constant. This suggests that the bond in the mixture of green choy sum chlorophyll with another molecule is more likely to occur than cassava chlorophyll.

4. Conclusion

Extraction and characterization of chlorophyll using UV-Vis and FTIR were successfully carried out. Chlorophylls were extracted from green choy sum leaves and cassava leaves using the method of blend, blanch, and dry at low-temperature. Chlorophyll extracts were characterized using FTIR and UV-Vis. The characterization results showed that the extraction method carried out can remove chlorophyll a from leaves with greater content of chlorophyll a in green choy sum extract compared to cassava extract.

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