Characterization and physicochemical properties of chlorophyll extract from *Spirulina* sp.

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**Abstract.** *Spirulina* sp. is a group of microalgae which has a number of pigments with diverse bioactivities. Chlorophyll is one of the dominant pigments in *Spirulina* sp. that play an important role in capturing light as a crucial element for photosynthesis. This study aims at investigating the characteristic of chlorophyll extract from *Spirulina* sp. and its physicochemical properties. The effects of light exposure (white, yellow, and UV-C) and metal ions (Sn2+, K+, and Fe3+) on chlorophyll stability were investigated. The green spots with RF 0.45 and 0.37 values were observed in the TLC experiment, indicating the chlorophyll a and b, respectively. The UV-Vis analysis was employed to identify the presence of chlorophyll and FTIR was utilized to detect the typical absorbance of characteristics functional groups for chlorophyll. The results show the chlorophyll was less resistance to all kinds of light (white, yellow, and UV-C). Metal ions of Sn2+, K+, and Fe3+ exhibited no obvious effect on the chlorophyll stability in the dark condition, but some metal ions gave a noteworthy effect in the presence of light.

1. Introduction

*Spirulina* sp. is a filamentous cyanobacteria which enable to convert solar energy into chemical energy through photosynthesis reaction. *Spirulina* sp. has been widely used as nutritious food since it has high protein content. This microalgae are also used to feed many animals [1]. The functional compounds that can be extracted from *Spirulina* are very abundant and diverse and have been proposes to treat a large number of diseases [1]. Instead of rich protein, they also contain have many diverse bioactive compounds, including pigments such chlorophyll, phycocyanin, and carotene [2].

The use of synthetic colorants are quite common in industries [2]. However, some studies found evidences linked the synthetic colorants to a number of potential health problem, most notably certain types of cancer and hyperactivity [3]. Therefore, exploitation variative of colorants from microalgae including *Spirulina* have been carried out for replacing the synthetic colorants by their natural counterparts. Chlorophyll is one of the most dominant pigments that can be isolated from *Spirulina* biomass. This bioactive pigment is used as natural food coloring and has antioxidant and anti mutagenetic activities as well [4-5]. This effect implies another value-added of using natural pigment. Chlorophyll and its derivat products have been largely used in pharmaceutical products. This pigment has been found to be hasten the wound healing process by more than 25% by trigerring tissue growth and prevents the advancement of bacteria infectious [6]. Chlorophyll pigment is used as natural green colour to replace artificial coloring. In manufactures, coloring is essential for their customers.
Chlorophylls are a group of molecules found in abundance in nature. In photosynthesis, chlorophyll acts as light harvesting in reaction center (RC) in photosystem I and II [7]. The chemical structure of chlorophyll contains a porphyrin ring with one reduced pyroline ring with a Mg$^{2+}$ ion coordinated and a long non polar phytol chain that increases the hydrophobicity of the molecule (Fig.1)[8].

![Figure 1. Chemical structure of chlorophyll.](image)

As can be seen in the Fig 1, when chlorophyll extracted from chloroplast, the magnesium ion become unstable and may easily be displaced by a weak acid [4],[9]. The intended functional application of chlorophyll as natural colorants from *Spirulina* are both to enhance the appearance of foods, beverages, and cosmetics, and to added value benefits from its bioactive compounds. Various physicochemical parameters influence the stability of chlorophyll, such as pH of the product and temperature have been studied and found that chlorophyll is less stable compared to the synthetic colorants [8], [10]. Here, we describe the characteristics of chlorophyll extract from *Spirulina* sp., and its physicochemical properties to light (white, yellow, and UV-C) and metal ions (Sn$^{2+}$, K$^+$, and Fe$^{3+}$). The results were expected to provide an information for a proper method to preserve the chlorophyll pigment.

2. Methods

2.1. Raw material

*Spirulina* sp. were obtained from Biology Department, Universitas Padjadjaran. Acetone (p.a. grade) for chlorophyll extraction was purchased from Merck (Darmstadt, Germany). The metal salts used are KCl, SnCl$_2$.2H$_2$O, and FeCl$_3$, and were purchased from Bratachem, Indonesia. All tech grade raw materials were used after further purification.

2.2. Extraction and characterization of chlorophyll extract

Extraction as carried out based on the principle and procedure developed by Lichtenthaler et al. [11] with some modification. Extraction was conducted firstly by mixing biomass-solvent at ratio 0.03 gmL$^{-1}$. The cold acetone was used as a solvent extraction. Cell disruption was carried out by sonication the mixture for 15 minutes at room temperature and dark condition. After that, the mixture was centrifuged at 2000 rpm for 10 minutes. The next is diluted the pellet by 15 mL cold acetone. The extract was kept at 4$^\circ$C and dark conditions for further analysis. The chlorophyll concentration was measured (Equation 1) using the method developed by Lichtenthaler et al.[11-12]. The extract then concentrated for further characterization. Qualitative characterization was evaluated using thin layer chromatography (TLC) method using n-hexane:acetone (7:3) as an eluent phase. The chlorophyll extract was also evaluated by FTIR analysis using Shimadzu UV-Mini 1240 (Japan) to confirm the data.
Chlorophyll concentration (mg/L) = 25.5×_{A650} + 4×_{A665} \tag{1}

2.3. Photostability of Chlorophyll
The photostability of chlorophyll from *Spirulina sp.* was studied by incubating the samples at different variation light (white, yellow, and UV-C) at room 25°C for 7 days observations.

2.4. Stability of Chlorophyll on ion metal
The stability of chlorophyll on metal ions was analyzed by incubating the samples at different solution contained metal ions of Sn^{2+}, K^+, and Fe^{3+} at room temperature with light and without light conditions as a control.

3. Results and Discussion

3.1. Characteristic of Chlorophyll from *Spirulina sp.*
The characteristics of chlorophyll were determined by evaluating the absorption spectrum of the samples using UV-Vis spectrophotometer (Fig. 3.1). Extract chlorophyll has green color and shows peaks absorbance at 662 nm, 616 nm, 580 nm, 477 nm and 429 nm. The peak of chlorophyll found was in line with previous research [13]. It is shown that chlorophylls from *Spirulina sp.* have peak absorbance at 665 and 430 nm for chlorophyll a, and 620 and 470 nm for chlorophyll b (Table 1). Chlorophyll a also is found much more abundant that the chlorophyll b. This finding supports the important role of chlorophyll a as an antenna for capturing light required for photosynthesis [14].

The chlorophyll extract was also evaluated and separated using TLC technique. It was observed the green spots with retention factor (RF) value of 0.45 and 0.37, indicating the proper RF for chlorophyll a and b, respectively (Fig. 3.2.a) as has been reported in the previous research [15].

![Figure 2. Absorption spectra of chlorophyll extract that were recorded from 300-800 nm. All spectra were measured at room temperature.](image)

**Table 1.** The peaks are observed in the spectra of chlorophyll of *Spirulina sp.*

| Peak | Wave Length (nm) | Absorbance | Compounds       |
|------|-----------------|------------|-----------------|
| 1    | 662             | 0.546      | Chlorophyll a   |
| 2    | 616             | 0.105      | Chlorophyll b   |
| 3    | 580             | 0.062      | Unknown         |
| 4    | 477             | 0.273      | Chlorophyll b   |
| 5    | 429             | 0.869      | Chlorophyll a   |
The qualitative detection of chlorophyll then further confirmed using FTIR at the wave number ranges of 500 cm\(^{-1}\) to 4000 cm\(^{-1}\) (Fig. 3.2.c). The IR was detected the functional group of CH\(_3\) SP\(^3\) at 2900 cm\(^{-1}\), absorbance spectra of C=O at 1750 cm\(^{-1}\), and spectra absorbance of C=C at 1600 cm\(^{-1}\). These spectras are specific for chlorophyll structure [16]. The results confirmed that the chlorophyll pigment has been successfully obtained using acetone as a solvent.

\[\text{Figure 3.} \quad \text{Chlorophyll extract characterization using (a) TLC of acetone extract from Spirulina sp., (b) references [15], (c) FTIR Shimadzu UV-Mini 1240 (Japan)}\]

3.2. Stability of Chlorophyll on light and metal ions

The stability of chlorophyll to lights was investigated under varieties of lights (white, yellow, and UV-C) and darkness conditions. As shown in Fig. 3.3, in the lightness conditions, the residual rate concentration of chlorophyll decreased significantly over a day to all kinds of lights. In contrast, no significant degradation of chlorophyll under the darkness conditions over 7 days, indicating that long-term storage of chlorophyll would be better to avoid light.

\[\text{Figure 4. The effect of lights on the residual concentration of chlorophyll.}\]

The effect of metal ions on chlorophyll was evaluated by incubating chlorophyll in the solution contained metal ions of Sn\(^{2+}\), K\(^+\), and Fe\(^{3+}\) at room temperature with light and without light conditions as a control. Ion Sn\(^{2+}\), K\(^+\), and Fe\(^{3+}\) showed no obvious effect in the dark conditions (Fig. 3.4). In contrast, the addition of Sn\(^{2+}\), K\(^+\), and Fe\(^{3+}\) ions with lightness made the concentration decrease significantly and the largest degrees of decreasing was led by Fe\(^{3+}\), suggesting to avoid contacting the chlorophyll solution with Fe\(^{3+}\).
4. Conclusion

From the results it can be concluded that the residual concentration of chlorophyll rapidly decreased over 1 day in the brightness conditions and to all kinds of lights, but kept stable in darkness which indicated that long-term storage for chlorophyll would be better to avoid light. Upon additions of metal ions of Sn$^{2+}$, K$^+$, and Fe$^{3+}$, there was no obvious change on the chlorophyll concentrations in the dark condition, indicating its stability under dark condition. However, in the presence of light, the metal ions induced the instability of the chlorophyll indicated by the decreases of concentration. The Fe$^{3+}$ ions gave significant effect in reducing the stability chlorophyll both in dark and under the light conditions. This indicated the incompatibility of the Fe$^{3+}$ ions with chlorophyll and that the storage of chlorophyll in ferrum vessel must be avoided. Additionally, the results suggest that for longer term preservation of chlorophyll, it should be kept in the darkness.

5. References

[1] García J L, de Vicente M and Galán B 2017 Microalgae, old sustainable food and fashion nutraceuticals Microb Biotechnol 2783
[2] Begum H, Yusoff F M D, Banerjee S, Khatoon H and Shariff M 2016 Availability and Utilization of Pigments from Microalgae Crit. Rev. Food Sci. Nutr 56 13 2209–2222
[3] Wrolstad R and Culver C 2012 Alternatives to Those Artificial FD&C Food Colorants Annu. Rev. Food Sci. Technol 31 59–77
[4] Halim R, Hosikian A, Lim S and Danquah M K 2010 Chlorophyll extraction from microalgae: A review on the process engineering aspects Int. J. Chem. Eng.
[5] De Tecnologia D, De Maringá U E and Paulo S 2011 Growth and content of 362–373.
[6] Wrolstad R E 2004 Symposium 12: Interaction of Natural Colors with Other ingredients. Anthocyanin Pigments — Bioactivity and Coloring Properties J. Food Sci 69 5 C419–C425.
[7] Andrade L M 2018 Chlorella and Spirulina Microalgae as Sources of Functional Foods, Nutraceuticals, and Food Supplements; an Overview MOJ Food Process. Technol 6 2
[8] Chen X B, et al. 2006 Hydrogen peroxide-induced chlorophyll a bleaching in the cytochrome b 6f complex: A simple and effective assay for stability of the complex in detergent solutions Photosynth. Res. 90 3 205–214, 2006.
[9] Saha S and Murray P 2018 Exploitation of Microalgae Species for Nutraceutical Purposes: Cultivation Aspects Fermentation 4 2 46.
[10] Nicoletti M 2016 Microalgae Nutraceuticals Foods 5 3 54.
[11] Lichtenháler H K and Buschmann C 2005 Extraction of Photosynthetic Tissues: Chlorophylls And Carotenoids Handb. Food Anal. Chem. 2 2 165–170.
[12] Kristopo H and Limantara L 2008 Photodegradation and antioxidant activity of chlorophyll a from spirulina (spirulina sp.) Powder Indo. J. Chem 8 2 236–241.

[13] Cinque G, Croce R, Holzwarth A and R. Bassi 2002 Energy transfer among CP29 chlorophylls: calculated Förster rates and experimental Biophys. J. 79 1706–1717.

[14] Fiedor L, Kania A, Myśliwa-Kurdziel B, Orzel L and Stochel G 2008 Understanding chlorophylls: Central magnesium ion and phytol as structural determinants Biochim Biophys. Acta - Bioenerg 1777 12 1491–1500.

[15] Quach H T, Steeper R L and Griffin G W 2004 An Improved Method for the Extraction and Thin-Layer Chromatography of Chlorophyll a and b from Spinach J. Chem. Educ. 81 3 385

[16] Chang H, Kao M J, Chen T L, Chen C H, Cho K C and Lai X R 2013 Characterization of natural dye extracted from wormwood and purple cabbage for dye-sensitized solar cells Int. J. Photoenergy

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