Characteristics of lipid content from the analysis of structural and optical properties of brown seaweed (Sargassum polycystum) after defatting by petroleum ether: preliminary studies

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Abstract: In Indonesia, one species of brown seaweed is Sargassum polycystum, which has not been optimally utilized. Whereas their wide range of potentiality, especially their bioactive compounds. Several authors have reported that the chemical composition of seaweeds varies according to maturity, habitats, environmental conditions, treatment, and species. This work describes the structural and optical properties of Sargassum polycystum after treatment by petroleum ether to remove lipid content. The structural properties determined from the quantitative analysis of XRD spectra. The chemical bond and optical properties determined from the quantitative analysis of FTIR Spectra by applying Kramers-Kronig relation. The data XRD showed an increasing the average of the crystallite size from (23.92 nm Scherrer method; 14.35 nm Williamson-Hall method; 19.23 nm Size strain plot method) to (32.39 nm Scherrer method; 20.07 nm Williamson-Hall method; 28.06 nm method) after treatment. The analysis of FTIR showed COC, CCH, CH, and OH bonding.

1. Introduction

In the last decades, natural raw materials have been an increasing interest to human because of the sustainability and environmental friendly. The biodiversity of the marine environment provides a large pool of bioactive nutrients to be explored. One of the marine biodiversity is seaweed, abundant in species is one of the natural resources that have the potential to be explored such as bioactive compounds with diverse molecular structures and biological activities [1-3]. Seaweeds are macroscopic flora with sizes ranging from a few centimeters to 100 m in length which live around relatively shallow coastal waters. They are divided into three main classes according to their color: green (chlorophytes), red (rhodophytes), and brown (phaeophytes) [4,5]. Therefore, their relative safety is one of the main reasons behind the great chances for research and industrial development. Moreover, their bioactive compound and chemical richness as a good source for various applications not only in food and cosmetics but also in pharmacological and other products [6-8].

In Indonesia, one species of brown seaweed is Sargassum polycystum, which has not been optimally utilized. Whereas their potentiality is wide, especially their bioactive compounds. Several authors [9-12] have reported that treatment, maturity, habitats, environmental conditions, and species influenced the chemical composition of seaweeds. Many studies have shown that the treatment of seaweeds has affected the structure and chemical bonds. Ref. [13] was reported the seaweed Sargassum wightii shown
different result for antibacterial activity before and after calcinated with zirconia nanoparticles, in ref. [14] the metal–biomass interactions in the Lanthanum (III) biosorption on Sargassum sp. More study of the synthesis of zinc oxide nanoparticles using Sargassum sp. and Padina sp. extract and 0.1 M ZnCl₂ solution as a precursor in using an oven microwave also shown affected to the structural and chemical bonds [15]. Hence, the effect of treatment by chemical solvent on the structural and optical properties of Sargassum polycystum has not been experimentally investigated. In this study, we focus on the structural and optical properties of Sargassum polycystum after treatment by petroleum ether to remove lipid content as a preliminary study. The structural properties are determined by the quantitative analysis of XRD spectra. The chemical bond and optical properties are determined by the quantitative analysis of FTIR Spectra by applying Kramers-Kronig relation.

2. Materials and method
2.1 Sample collection
In this study, the material used is brown seaweed (Sargassum polycystum). The brown seaweed was collected from Lae lae Island coastal area in South Sulawesi, Indonesia. The samples were washed with seawater to eliminate the debris or epiphytes. The seaweeds were rinsed thoroughly under fresh tap water to remove the traces of salt and sand particle. After washing the seaweed it involved in the process of drying under shade room for 10 d and minced using a blender. The seaweed powder was sieved to achieve a particle size of 0.5–1.0 mm and stored in sealed plastic bags under the dark condition for further analysis.

2.2 Defatting
Take 2 g of the seaweed powder and defatted sample in 10-mL petroleum ether. Keep it on overnight at room temperature. After that filter the sample by using Whatman paper. Let it till dry.

2.3 XRD and FTIR analysis
X-ray diffraction (XRD) data (Shimadzu 7000) with radiation CuKα (λ=1.5405 Å) at range angle 10° ≤ 2θ ≤ 70° operation on 30 kV and 10 mA) was used to determine the structural properties. Fourier Transform Infra-Red (FT-IR) spectroscopy was carried out on an IR Prestige-21 FT-IR spectroscopy (Shimadzu Corp) to determine the chemical bond and optical properties of the samples. The FTIR instrument was operated in the wavenumber 550 - 4000 cm⁻¹.

3. Result and discussion
3.1 X-ray Diffraction (XRD)
X-ray diffraction patterns of all samples (before and after treatment) are shown in figure 1. The x-ray spectra presented the crystalline phase with the number joint committee diffraction pattern with a link to original entry relates to 9011972, 9012730, and 9013122 for K, C, and Cl₂ respectively.

Figure 1. XRD spectra of all samples
The phase from XRD pattern is the similarity to the result of x-ray fluorescence. The quantitative result of XRD is shown the domination of chlorine (40.22%), potassium (39.63%), and calcium (11.65%) other than that the samples have content such as sulfur and magnetite with minor content. Apart from their habitat, the ability of seaweed to absorb mineral diversity causes high mineral content [16]. Seaweeds are the great source of minerals such as Calcium (Ca), iodine (I), phosphorous (P), potassium (K), iron (Fe), and zinc (Zn), sodium (Na), magnesium (Mg) [17]. Calcium is well-known to be important in terms of health preservation due to its diverse biological roles being essential for muscle contraction, structural support, blood coagulation, and glandular secretion being the most significant consequence of the low calcium status the occurrence of osteoporosis [18]. Previous studies have shown that mineral content from Sargassum integrated as supplements in shrimp Litopenaeus vannamei feeds could improve the growth rate, survival rate, and immune system [19-20].

The structural result from XRD line broadening by using quantitative analysis for figure 2 and figure 3 is clearly shown in Table 1. Williamson Hall (WH) and Size Strain Plot (SSP) method observed by the slope and intercept this mean the structural parameters are about the average result from XRD data. According to references [21], the Scherrer equation and Williamson Hall can be written in the equation below (1):

$$D = \frac{k\lambda}{\beta \cos \theta}, \text{ where } \beta = \left(\beta_s^2 - \beta_{ins}^2\right)^{1/2}$$

(1)

Full width half maximum ($\beta$), where $\beta$ must be corrected by using instrument $\beta_{ins}$ and from the standard ($\beta_s$), $k$ is a constant and $\lambda$ is wavelength from Cu target of XRD instrument. From equation 1 we can subs the strain on the Scherrer method caused by the line broadening of x-ray spectra is convoluted by strain and size from the crystal lattice. So the Scherrer equation as explained in reference [22-23] can be written as (2-3):

$$\beta \cos \theta = \frac{k\lambda}{D} + 4\varepsilon \sin \theta$$

(2)

Where the strain is $\varepsilon = \beta / 4 \tan \theta$, equation 2 is known by Williamson Hall equation with a uniform density model (UDM). To confirm all calculations we need another way to find size and strain, as we know the recent years, size strain plot method can be presented the structural parameters from XRD data. The SSP method from reference [24-25] can be written as (4-6):

$$(d\beta_{hkl} \cos \theta)^2 = \frac{k}{D} (d^2 \beta_{hkl} \cos \theta) + \left(\frac{\varepsilon}{2}\right)^2$$

(3)

SSP has a good evaluation of the crystal-strain evaluation because the term $(d\beta_{hkl} \cos \theta)^2$ as the y-axis is plotted with $(d^2 \beta_{hkl} \cos \theta)$ as x-axis for all the peak orientation, besides that SSP method also calculate d-spacing parameters to observe the slope and intercept of the linearly fitted data.

**Figure 2.** Scherrer plot for the relationship between size and strain of each peak (left y-axis is crystallite size and right y-axis is strain)
Figure 3. Williamson Hall with UDM analysis and SSP method of all samples were indicated the extracted slope and intercept value can be converted to find D and ε value.

Table 1. Size and micro strain plot analysis from Scherrer, Williamson-Hall, and Size Strain Plot Method.

| Sample  | Scherrer | Williamson Hall | Size Strain Plot Method |
|---------|----------|-----------------|-------------------------|
|         | D (nm)   | E               | D (nm) | ε     | D (nm) | ε     |
| Before  | 13.127  | 0.0166          | 14.35  | 0.0034 | 19.23  | 0.0059 |
|         | 15.7423 | 0.0129          |        |        |        |       |
|         | 16.9991 | 0.0111          |        |        |        |       |
|         | 17.0156 | 0.0109          |        |        |        |       |
|         | 27.1314 | 0.0063          |        |        |        |       |
|         | 27.2255 | 0.0057          |        |        |        |       |
|         | 25.3507 | 0.0055          |        |        |        |       |
|         | 24.393  | 0.0057          |        |        |        |       |
|         | 27.0729 | 0.0045          |        |        |        |       |
|         | 27.7505 | 0.0039          |        |        |        |       |
| average : | 23.92 nm | 0.0097          |        |        |        |       |
|         | 19.7488 | 0.0064          |        |        |        |       |
|         | 29.4668 | 0.0056          |        |        |        |       |
|         | 30.5377 | 0.0048          |        |        |        |       |
|         | 31.401  | 0.0044          |        |        |        |       |
| After   | 29.3951 | 0.0047          | 20.07  | 0.0022 | 28.06  | 0.0072 |
|         | 30.487  | 0.004           |        |        |        |       |
|         | 32.0701 | 0.0034          |        |        |        |       |
|         | 45.8519 | 0.0021          |        |        |        |       |
|         | 34.7432 | 0.0028          |        |        |        |       |
| average : | 32.39 nm |                |        |        |        |       |

Table 1. Shows the crystallite size increasing from (23.92 nm Scherrer method; 14.35 nm WH method; 19.23 nm SSP method) before treatment to (32.39 nm Scherrer method; 20.07 nm Williamson-Hall method; 28.06 nm method) after treatment indicated the lipid content decreased. The reduced lipid
content causes the crystallite size to increase maybe some of the polysaccharides and protein may be bound together.

In general, seaweeds exhibit low-fat content ranges from 0.3% to 6% [26-30]. Additionally, the seasonal, geographical, or growth conditions cause the composition of the lipid content of seaweeds which may vary during the life cycles [30]. Moreover, the low-lipid content in macroalgae is still lack of to be a commercially interesting and valuable target [31]. However, seaweeds are rich in carbohydrates, protein, vitamin and contain a high percentage of monounsaturated and polyunsaturated fatty acids [32,33].

3.2 Fourier Transform Infrared (FTIR)
The transmittance of all samples was investigated in the range 550-4000 cm⁻¹ and we have focused on the range 1400-2000 cm⁻¹ for the next analysis to determine the optical properties. Before defatting shows peaks at 3446, 2929, 1635, 1028 cm⁻¹. The peak at 3446 cm⁻¹ corresponds to OH stretching of sugar and amino acid, 2929 cm⁻¹ corresponds to CH stretching of lipid/carbohydrate with associated compounds (Polysaccharides, proteins, glycoproteins), 1635 cm⁻¹ corresponds to COC stretching band of the carboxylic acid group with associated compounds Polysaccharides and glycoproteins, 1028 cm⁻¹ corresponds to CCH stretching consisted of mainly sugar. These results similar to a previous study for phycocolloid standards (carrageenans, agar, and alginate) which had been assigned to O–H, C–H, N–H stretching vibrations in peaks at 3280–3350 cm⁻¹ and a weaker signal peaks at 2870–2960 cm⁻¹. Peaks between 1200 and 970 cm⁻¹ are due mainly to C–C and C–O stretching bonds, common to all polysaccharides [34].

After defatting shows peaks at 3454, 2931, 1652, 1026 cm⁻¹. Based on Figure 4, the comparing spectra of the sample before and after treatment show the reduced transmittance after treatment for all ranges in wavenumber. The transmittance value after defatting is smaller than before defatting. This indicates that the absorbance value after treatment is higher than before treatment. It shows after treatment that chemical bond OH, CH, COC, and CCH are indirect may be contributed to reducing the lipid content. The phenomenon is perhaps due to the interaction with petroleum ether as a nonpolar solvent. In the case of microalgae, some neutral lipids are in the cytoplasm also forming complexes with polar lipids. These complexes are strongly linked via hydrogen bonds to proteins in the cell membrane [35]. The assignments were compared with the literature [14] that peak at 1636 cm⁻¹ corresponds to CO₂ stretching (alginate and protein), CO, CN stretching, and bending to NH in primary amides (amino acid chain). However, because of the superimposition of peaks, the assignment of several band is not easy.

![Figure 4. Comparing the FTIR spectra for all samples](image-url)
The treatment clearly shows the attenuation for transmittance COC, CCH, CH, and OH bonding that means the lipid content on the sample after treatment was indirectly reduced. Kramers Kronig (KK) relation is to analyze quantitative analysis from FTIR spectra. This method can observe the optical and electrical properties from FTIR spectra. According to references [36-39] the relation of KK can be written as:

\[ A(\omega) = 2 - \log [T(\omega)\%] \]  
\[ R(\omega) = 100 - [T(\omega) + A(\omega)] \]  

Absorption (A), reflectance (R), and transmittance (T) are dominant incidents during the interaction between infrared and matter. The refractive index (n) and the extinction coefficient (k), first the phase change \( \phi(\omega) \) can determine by:

\[ \phi(\omega) = -\frac{\omega}{\pi} \int_{-\infty}^{+\infty} \frac{\ln R' + \ln R}{\omega^2 - \omega_i^2} \]  

We have applied the K-K to solve the equation (6), and the resulting equation for simple computational:

\[ \phi(\omega_i) = -\frac{4\omega_j}{\pi} \Delta \omega \times \sum \ln \left( \frac{\sqrt{R(\omega)}}{\omega_i^2 - \omega_j^2} \right) \]  

Where, \( j \) is a series of wavenumber, if \( j \) is an odd number so then \( i \) parts is 2,4,6,8,...,\( j-1,j+1 \); and while wavenumber \( j \) is an even, \( i \) parts is 1,3,5,7,...,\( j-1,j+1 \); \( \Delta \omega = \omega_{l+1} - \omega_i \). Than for determining the refractive index (n) and extinction coefficient (k) from the relations, we can follow:

\[ n(\omega) = \frac{1 - R(\omega)}{1 + R(\omega) - 2\sqrt{R(\omega)} \cos \phi(\omega)} \]  
\[ k(\omega) = \frac{2\sqrt{R(\omega)} \sin \phi(\omega)}{1 + R(\omega) - 2\sqrt{R(\omega)} \cos \phi(\omega)} \]  

Form eq. 8 and 9 we can also observe the dielectric function \( (\varepsilon(\omega) = \varepsilon_1(\omega) + i\varepsilon_2(\omega)) \), where \( \varepsilon_1(\omega) \) the real part and \( \varepsilon_2(\omega) \) is the imaginary part determined from the relation with \( n \) and \( k \) as follows [40-42]:

\[ \varepsilon_1(\omega) = n^2(\omega) - k^2(\omega) \]  
\[ \varepsilon_2(\omega) = 2n(\omega)k(\omega) \]  

Presented by using equations (8, 9, 10, and 11) can be seen in Figure 5.

Relation with the maximum peak of the dielectric function \( (\tilde{\varepsilon}(\omega) = \varepsilon_1(\omega) + i\varepsilon_2(\omega)) \).

Based on figure 4, we have focused on the range 1400-2000 cm\(^{-1}\) for the next analysis to determine the optical properties. Figure 5 shows the \( \Delta(LO - TO) \) as distance wavenumber between two optical phonon modes where the sample before treatment is longer than after treatment. This phenomenon may due to the lipids decreased and influenced the optical properties. Then from figure 5 also shows the dielectric properties which can observe from \( \varepsilon_1 \) and \( \varepsilon_2 \) maximum peak, there is not display of the different wavenumber position due to the Sargassum polycystum as a source of the sample is a natural material with high environmental stability.
The complex refractive index $\tilde{n}(\omega) = n(\omega) + k(\omega)$ real part and imaginary part and their

**Figure 5.** The complex refractive index $\tilde{n}(\omega) = n(\omega) + k(\omega)$ real part and imaginary part and their

**4. Conclusion**

The quantitative analysis of X-ray diffraction (XRD) showed the reduced lipid content causes the crystallite size to increase because some of the polysaccharides and protein bound together. The quantitative analysis of FTIR spectra by using K–K relation showed the main peak position of the LO parts of the refractive and extinction coefficient are shifting to the higher wavenumber position for the sample before treatment is higher than the sample after treatment. The treatment clearly shows the attenuation for transmittance COC, CCH, CH, and OH bonding that means the lipid content on the sample after treatment was reduced.

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