Effect of Different Coating Materials on The Characteristics Of Chlorophyll Microcapsules from *Caulerpa racemosa*

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Abstract: The sea grape (*Caulerpa racemosa*) has a chlorophyll pigment that can be extracted using a non-polar solvent. Chlorophyll as a natural dye has unstable characteristics of temperature, pH, and light. Microencapsulation by the freeze-drying method can be used to protect chlorophyll from degradation caused by external influences where the type of coating material can affect the characteristics of the chlorophyll microcapsules. The objective of this study was to determine the characteristics of chlorophyll microcapsules with various types of coating material. Chlorophyll was microencapsulated using maltodextrin (CM), maltodextrin-alginate (CMA), and maltodextrin-fish gelatin (CMG). Chlorophyll encapsulated with maltodextrin-alginate resulting in the highest yield. The results of FTIR analysis indicated the presence of following functional groups in chlorophyll microcapsules viz., inter- and intra-molecular bonded alcohol OH, C = N stretching imine/oxime or C = O stretching conjugated ketone or alkenes, OH phenol, and CN stretching amine. CM had a particle size between 9,061 – 469.9 nm, CMA between 9,707 – 363.5 nm, and CMG between 11.49 – 433.2 nm. Based on the observation of morphology by using SEM, it showed that the all of the chlorophyll microcapsules were in the form of flake shape and porous. CM and CMA looked more fragile than CMG it can be seen from the cracks in some parts of CM and CMA. Therefore, CMG release time was longer than CM and CMA.

Keywords: Characteristics, Microcapsules Chlorophyll, Maltodextrin, Alginate, Gelatin

1. Introduction

Indonesia is one of *Caulerpa racemorsa* (sea grape) producer, especially in Jepara District, Central Java Province. By the locals, this kind of seaweed is consumed as a vegetable. *C. racemosa* categorized as Chlorophyta, a green seaweed that has a natural pigment called chlorophyll. According to Kokilam and Vasuki [1], *Caulerpa taxifolia* also categorized as Chlorophyta which taken from Pondicherry waters and Mandapam waters, India had Chlorophyll content around 0,076 ± 0,002 mg/g dry weight.

On the food industry, the request of green color is quite high. Chlorophyll is a green pigment which can be used as a colorant and natural antioxidant. However, the natural chlorophyll is not suitable...
as the food colorant as its easily degraded by enzymatic reaction or other factors such as acid, oxygen, light, and heat. Chlorophyll can be degraded and produced chlorophyll derivative such as pheophytin, pheophorbide, pyropheophytin, and pyropheophorbide [2]. Therefore, the chlorophyll microencapsulation is conducted to facilitate its handling and improve stability against oxidation and solubility.

Microencapsulation is one of the methods when active compound as core being coated in tiny particles. Microencapsulation able to create a physical barrier between core and coating materials, so able to protect the sensitive compound and control the time release of that compound. This method also aimed to repair the pigment dispersibility in water and improve the shelf-life of active compound [3, 4, 5].

Pigment microencapsulation can be conducted using the freeze-drying method, which is also known as lyophilization. Freeze-drying is one of drying method which freezes the material first followed by sublimation. Although need long period of time and expensive compared to the spray drying method, the freeze-drying method is often used to dry the materials which sensitive with high temperature [6].

Besides of the drying process on microencapsulation technology, the selection of coating material may change the physical, chemical, and biological properties of the microcapsule. Bio-polymer which often used as coating materials includes maltodextrin, alginate, and gelatin. The selection of maltodextrin as the coating materials due to its low viscosity which can form the film layer [7]. The alginate encapsulant is used to decrease the capsule permeability by improving the immunoprotective properties of microcapsule [8]. Fish skin gelatin has high solubility, white, no smell and taste, can form bio-film, so can be used as encapsulant [9]. According to the description above, the further study about the using of the various encapsulant to encapsulate the chlorophyll C. racemosa should be conducted. The aim of this study was to discover the effect of maltodextrin, maltodextrin-alginate, and maltodextrin-fish as the coating material against the characteristic of C. racemosa chlorophyll which encapsulated

2. Methods

2.1. Chlorophyll Extraction
The chlorophyll was obtained from the maceration of dried Caulerpa racemosa by using acetone 90% with ratio 1:4 (w/v) for 2 days at room temperature. Next, the filtrate was evaporated using rotary evaporator at 20°C.

2.2. Microencapsulation of Chlorophyll
The chlorophyll extract plus coating material solution 1:4 (v/v) and Tween 80 were homogenized using homogenizer (WiseTis HG-15D, Germany) for 2 minutes at 10.000 rpm. The encapsulant solution was made by dissolving the coating materials into the distilled water until its concentration becomes 10% (w/v). The coating materials used consist of maltodextrin, maltodextrin-alginate 9:1 (w/w), and maltodextrin-fish gelatin 9:1 (w/w). Then freeze-drying was performed (Heto Powerdry LL 1500, Japan) at temperature -24°C. The yield assay, Fourier Transform Infrared Spectroscopy (FTIR), morphology observation, particle size distribution, and chlorophyll time release.

2.3. Yield
The yield was determined according to the percentage of total mass of chlorophyll microcapsule produced and the total mass of solid before drying [10].

2.4. FTIR
The microcapsule of chlorophyll was determined by FTIR spectrometer (Shimadzu FTIR 8400, Japan) using the spectral resolution of 1 cm⁻¹ and the spectral profile was collected in the range between 4 000 cm⁻¹ and 400 cm⁻¹ [11].
2.5. **Morphology Observation**
The morphology of chlorophyll microcapsule was determined using Dewi et al. method with modification [12], chlorophyll microcapsule was coated by gold then observed using (SEM) (Jeol JSM 6510LA, Japan) at 15 Kv and 5000x.

2.6. **Particle Size Distribution**
The particle size distribution of chlorophyll microcapsule was determined by dispersion using a laser scattering particle size distribution analyzer (Malven Zetasizer Nano ZS ver. 6.20, UK) [13].

2.7. **Time Release**
Time release of chlorophyll was determined by modifying Belscak-Cvitanovic et al. method [14], chlorophyll microcapsule 1% (w/v) on distilled water was stirred continuously at speed 400 rpm. Next, 1 ml of solution was taken every 1 minute to analyze its chlorophyll content, the sample then spectrophotometrically measured at 646 and 663 nm by spectrophotometer UV-Vis (Shimadzu, Japan) [15]. The chlorophyll content was measured according to the equations (1), (2), and (3).

\[
\begin{align*}
\text{chlorophyll a (mg/L):} & \quad 12,25 \times A_{663} - 2,79 \times A_{646} \\
\text{chlorophyll b (mg/L):} & \quad 21,5 \times A_{646} - 5,1 \times A_{663} \\
\text{Total chlorophyll (mg/L):} & \quad 20,2 \times A_{646} + 8,02 \times A_{663}
\end{align*}
\]

2.8. **Statistical Analysis**
All experiments were conducted in triplicate and statistical analysis was carried out by using SPSS version 17.0 (International Business Machines Corporation, USA). The data were analyzed with one-way analysis of variance (ANOVA) and for mean separation using Duncan range test.

3. **Results and Discussion**

3.1. **Yield**

![Figure 1](image)

**Figure 1.** Yield of microcapsule chlorophyll with maltodextrin (CM), maltodextrin-alginate (CMA) and maltodextrin-fish gelatin (CMG) as coating materials

Note: The data was the average of triplication ± standard deviation.

Different superscript on the same column indicates significantly different (p<0.05)

The using of different coating materials with the same concentration able to affect the yield of chlorophyll which encapsulated by freeze-drying method (Figure 1). The chlorophyll which encapsulated using maltodextrin-alginate produced the highest rendement, while the lowest yield was produced from chlorophyll which encapsulated using maltodextrin. The different results showed from the previous study, the phycocyanin which encapsulated using different coating materials, such as maltodextrin, maltodextrin-alginate, and maltodextrin-carrageenan did not affect the yield of the
microcapsule. Freeze-drying was used as the microencapsulation method [16]. The result of yield was affected by the ratio of encapsulated materials and coating materials used. The yield of microcapsule also causing the thickness of the layer which wrapping the core [17].

3.2. FTIR

![FTIR Spectra](image)

**Figure 2.** The yield of microcapsule chlorophyll with maltodextrin (CM), maltodextrin-alginate (CMA) and maltodextrin-fish gelatin (CMG) as coating materials.

| Functional Groups          | CM       | CMA      | CMG      |
|----------------------------|----------|----------|----------|
| Unknown                    | 3749.62  | 3749.62  | 3749.62  |
| Intermolecular bonded      | 3387     | 3394.72  | 3387     |
| alcohol O-H stretching     |          |          |          |
| Intramolecular bonded      | 2924.09  | 2924.09  | 2924.09  |
| alcohol O-H Stretching     |          |          |          |
| C=N stretching             | 1651.07  | 1620.21  | 1651.07  |
| imine/oxime or C=O stretching conjugated ketone or alkenes |          |          |          |
| O-H banding phenol         | 1365.6   | 1373.32  | 1373.32  |
| C-N stretching amine       | 1026.13  | 1026.13  | 1026.13  |
|                            | 1080.14  | 1080.14  | 1080.14  |
|                            | 1157.29  | 1157.29  | 1149.57  |
|                            | 1242.16  | 1242.16  | 1242.16  |

**Table 1.** The functional group on chlorophyll microcapsule.

FTIR spectrophotometer used to identify the presence of chlorophyll within the microcapsule. Figure 2 indicated the same wave number showed by the third sample, either the chlorophyll microcapsule with maltodextrin, maltodextrin-alginate, or maltodextrin-gelatin as coating materials. The same wave number indicated the same functional group on maltodextrin and chlorophyll, the same wave number showed on Table 1. The results of FTIR analysis showed the presence of following functional groups in
chlorophyll microcapsule viz., inter- and intra-molecular bonded alcohol OH, C = N stretching imine/oxime or C = O stretching conjugated ketone or alkenes, OH phenol, and CN stretching amine. The same result also showed by Kumar et al. [18], which extracted the chlorophyll from the various kind of green vegetables (Hibiscus cannabinus defatted, H. sabdariffa defatted, Basella alba defatted, B. rubra defatted, Rumex vesicarius defatted, H. cannabinus native, H. sabdariffa native, B. alba native, B. rubra native, and R. vesicarius native).

3.3. SEM
According to the morphology observation using SEM, the chlorophyll microcapsule produced was a flake and porous, either the chlorophyll encapsulated by maltodextrin, maltodextrin-alginate, or maltodextrin-fish gelatin (Figure 3). On the freeze-drying method, the porous form was produced as the result of ice crystal formation during freezing and the sublimation process during drying. After drying, the formed brittle matrix can be ground into smaller pieces [19-20]. The same study also showed by Karthik and Anandharamakrishnan [21], a docosahexaenoic acid which encapsulated by freeze-drying was in the form of flake, irregular, and porous. Fish oil which encapsulated by freeze-drying also had the similar shape with our studies [22].

![Figure 3](image-url)

**Figure 3.** SEM photograph of microcapsules chlorophyll with a.) maltodextrin, b.) maltodextrin-alginate and c.) maltodextrin-fish gelatin as coating materials
3.4. Particle Size Distribution

Figure 4. Particle size distribution of microcapsules chlorophyll with a.) maltodextrin, b.) maltodextrin-alginate and c.) maltodextrin-fish gelatin as coating materials.
The chlorophyll microcapsule either encapsulated using maltodextrin, maltodextrin-alginate, or maltodextrin-fish gelatin had various size (Figure 4). According to the result, the particle size of chlorophyll which encapsulated using maltodextrin had the particle size distribution as follows: 98.3% sized 9,061 nm, 1.0% sized 58,30 nm, and 0.8% sized 469.9 nm. According to the result, the particle size of chlorophyll which encapsulated using maltodextrin-alginate had the particle size distribution as follows: 96.9% sized 9,707 nm, 1.6% sized 47,65 nm, and 1.5% sized 363.5 nm. The chlorophyll microcapsule with maltodextrin-fish gelatin as coating material had size 11,49 nm as of 73.5%, 282.7 nm as of 2.0%, and 4332 nm as of 24.5%. The variety of particle size caused by product from freeze-drying process which in the form of aggregations then fined using mortar [23].

The using of maltodextrin-alginate and maltodextrin-fish gelatin as the coating material able to produce chlorophyll microcapsule in the bigger particle size compared to chlorophyll microcapsule with maltodextrin as the coating material. The size of microcapsule particle caused by the using of more than one coating material, it can form a tissue between the coating material and led the enhancement of layer thickness which wrapped the core [24-25]. The size of the particle also often attributed to the number of active compounds which able to be encapsulated. The bigger the size, the more of active compound which can be trapped [26].

3.5. Time Release

The result showed that chlorophyll which encapsulated with maltodextrin had been released within 1 minute (Figure 5). This due to maltodextrin was water soluble. maltodextrin had high ability to absorb water. This allegedly due to maltodextrin had a high swelling capacity. The ability to absorb water was affected by the involvement of hydroxyl in order to form hydrogen chain and covalent between starch chain [27]. Therefore, chlorophyll was easily released from microcapsule.

The using of maltodextrin-alginate and maltodextrin-fish gelatin can extend the chlorophyll time release. The active compound can release from microcapsule in the controlled way and certain condition.

**Figure 5.** Time release of microcapsule chlorophyll with maltodextrin (CM), maltodextrin-alginate (CMA) and maltodextrin-fish gelatin (CMG) as coating materials

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The using of maltodextrin-alginate and maltodextrin-fish gelatin can extend the chlorophyll time release. The active compound can release from microcapsule in the controlled way and certain condition.
The release of active compound can be affected by the type of coating material used [28]. The using of two kinds of coating materials can protect the active compound, so it cannot easily release. This due to the presence of the cross-linking between two coating materials used. The result of Belsca-Cvitanovic et al. [14] research showed that the release of raspberry extract polyphenol from microcapsule using alginate and chitosan as the coating material was slower than using alginate as the coating material. This due to the cross-linking between alginate and chitosan. The maltodextrin contains polyanion while gelatin contains polycation. Polyanion only able to protect bio-active compound on the first layer and polycation able to protect the second layer after maltodextrin, it can protect the active compound well. The two kinds of coating materials, polyanion and polycation will form spiral tissue to protect Bio-active content [13]. Moreover, chlorophyll which encapsulated using maltodextrin-fish gelatin had the longest time release.

4. Conclusion

The using of maltodextrin-fish gelatin as the coating material can produce chlorophyll microcapsule with bigger yield compared to chlorophyll microcapsule with maltodextrin, but still lower compared to chlorophyll microcapsule with maltodextrin-fish gelatin as the coating material. The chlorophyll which encapsulated using maltodextrin-fish gelatin had the bigger particle size and longest chlorophyll time release compared to chlorophyll microcapsule with maltodextrin and maltodextrin-alginate as the coating material. According to FTIR analysis, indicated the presence of chlorophyll in each sample. Each chlorophyll microcapsule was in the form of flake and porous.

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