Characteristics of *Gracilaria* sp. residue of seaweed sap extraction with extraction time treatment

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**Abstract.** Seaweed sap has now been widely used as an organic fertilizer manufacturing material. The simple extraction process of seaweed sap allows the reuse of seaweed residue. This study was aimed to evaluate the effect of extraction time of *Gracilaria* sp. sap extraction to the characteristics of the residue. The extraction process was done with a variation in the extraction time of 0 (5 minutes), 2, 4, and 6 hours. Then, seaweed residue was obtained after the extraction seaweed sap was dried. The observed parameters were the yield, moisture, ash, and fat content of the seaweed residue. The results of the analysis were compared to dried *Gracilaria* sp. without seaweed sap extraction. The results show that the dried seaweed residue of all treatment had different moisture, ash, and fat content with the seaweed control. The highest yield of seaweed residue was obtained from the treatment 0 (5 minutes) hour of extraction (52.16%) with a moisture content of 10.41%, ash content of 12.39%, and fat content of 0.30%. These seaweed residues could potentially be used as biocomposite materials.

**Keywords:** extraction time, *Gracilaria* sp., seaweed sap

1. **Introduction**

*Gracilaria* sp. is a member of the genus of *Rhodophyceae* which is classified as an agar-producing group. Seaweed is grown naturally in seawater, but it has been widely cultivated in the estuarine areas and ponds. Besides agar as its main content, *Gracilaria* sp. also contains macro and micro minerals, amino acids, as well as other secondary metabolites that can be utilized in various food and non-food products.

Shah *et al* (2013) reported that the seaweed sap from *Gracilaria* sp. can be used as fertilizer on a wheat plantation. Seaweed sap is widely used as an ingredient of liquid organic fertilizer (Khan *et al* 2009; Zodape *et al* 2011, Shah *et al* 2013, Tuhy *et al* 2013, Basavaraja *et al* 2018). Seaweed sap contains many essential sea minerals required by plants, and also has a hormonal booster that has been shown to increase the growth of plants and crops (Sedayu *et al* 2013). Unlike chemical fertilizers, extracts made from seaweed can be degraded naturally, non-toxic, contamination-free, and safe against humans and animals (Dhargalkar and Pereira 2005).
Seaweed sap is contained in seaweed thallus which has a complex cell wall. Therefore, it requires exact solvent composition, temperature, and proper sap extraction time (Michalak and Chojnacka 2014). This research is a continuation of a research conducted by Nurhayati et al (2018). The previous research was extracting sap using a solution of KOH 0.3% by heating at 80°C with a variation on heating time. Seaweed residue produced from the process has not been utilized before. This time, we need to know the effect of extraction time on the characteristics of Gracilaria sp. residue after seaweed sap extraction.

2. Materials and methods

2.1. Materials
The raw material used in this research was dried Gracilaria sp. which was acquired from Lontar, Serang Province, Banten. While the extracting material used was KOH 0.3% (Merck). The equipment used include water bath, glass beaker, digital scales (HWH DJ203A, accuracy 0.01 g), mouthpiece, Cooper, Spinner, knife, bucket, stirrer, thermometer, and tray.

2.2. Methods
This research is a continuation of seaweed sap extraction from Gracilaria sp. conducted by Nurhayati et al (2018). The process of producing seaweed sap through several stages, which were washing, extraction, and filtering. Extraction was done by heating in a solution of KOH 0.3% (1:10 b/V) at 80 °C using water bath. The time of extraction was varied i.e. 0 hour (5 minutes), 2, 4 and 6 hours. After the extraction was completed, the residue was separated from filtrate using a filter cloth. The residue produced at this stage of extraction was subsequently dried directly with sunlight. The residue that had been dried then being inserted into the plastic and stored for subsequent testing. The testing parameters of seaweed residue include yield of seaweed residue, moisture content, ash content, and fat content.

2.3. Research design
This study was implemented using completely randomized design with one factor, which was extraction time; 0 (5 min), 2, 4, and 6 hours with each treatment being repeated 2 times. Analysis of data using "Analysis of Variances (ANOVA)". If there was an influence from the factor then the analysis was continued with a test Duncan Multiple Range Test (DMRT) on a 95% trust level.

3. Results and discussion

3.1. Yield
The yield was a comparison of the residues of Gracilaria sp. after extraction with the weight of the initial seaweed before extraction (table 1 and figure 1). The results of this research showed on the lowest yield in the 4 hours treatment of extraction was 32.28%. While the highest yield was obtained from the sample with 0 hour of extraction (52.16%). The result of further Duncan test showed that there was no noticeable difference among the three extraction time treatments of 2, 4 and 6 hours, but the three treatments were significantly different than 0 hour extraction. The longer extraction time caused seaweed residue to decline. Mayhoob et al (2017) reported that the yield of agar increased as the increased of extraction time to 4 hours, then decreased for a longer time. It was in accordance with the results of the research, which at 4 hours of extraction, seaweed residue has the least small yield. It signifies the agar that came out of the seaweed's thallus was greater.
Table 1. The results of dried *Gracilaria* sp. analysis without and with extraction.

| Parameters           | Seaweed before extraction | Seaweed after extraction treatment (hour) |
|----------------------|----------------------------|------------------------------------------|
|                      | 0                          | 2                                        |
|                      | 4                          | 6                                        |
| Yield (%)            | 52.16±2.28b                | 33.08±3.05a                              |
|                      | 32.28±6.79a                | 33.10±7.72a                              |
| Moisture content (%) | 15.72±0.03d                | 10.41±0.63a                              |
|                      | 11.60±0.11b                | 9.99±0.75a                               |
|                      | 13.73±0.06c                | 9.99±0.75a                               |
| Ash content (%)      | 30.59±0.18d                | 12.39±0.45c                              |
|                      | 10.06±0.26b                | 6.99±0.12a                               |
|                      | 7.14±0.67a                 | 6.99±0.12a                               |
| Fat content (%)      | 0.36±0.02d                 | 0.30±0.01c                               |
|                      | 0.28±0.05c                 | 0.10±0.01a                               |
|                      | 0.19±0.03b                 | 0.10±0.01a                               |

Figure 1. The yield of *Gracilaria* sp. residue after extraction.

3.2. Moisture content

The highest water content was found in the sample with a 6 hours treatment of 13.73%. While the lowest moisture content was found in samples with a 4 hours treatment of 9.99% extraction (figure 2). According to the Indonesian national standard regarding the quality of dried seaweed *Gracilaria* sp., maximum water content is 12% (BSN 2015). Thus, only treatments of 0, 2 and 4 hours extraction time that had met the SNI standard, while the treatment of 6 hours did not meet the SNI standard. The results of Duncan test showed that moisture content on all extraction treatments was significantly different with control. While at extraction time of 2 and 4 hours did not significantly different. The moisture content of all treatments had a lower moisture value than the initial seaweed due to the extraction residue of the seaweed had been destroyed by the heating process, resulting in a small flake that was easier to dry.

Figure 2. The moisture content of *Gracilaria* sp. residue after extraction.
3.3. Ash content
The highest ash content was found in samples with a 0 hour treatment of 12.39%. While the lowest ash content was found in the sample with a 4 hours treatment of extraction of 6.99% (figure 3). Based on the results of the print test with a 95% confidence interval it was known that there was a noticeable difference between the seaweed ash content produced from all treatments. The results of the advanced Duncan test showed that the 4 and 6 hours extraction time treatment significantly differed with the extraction time treatment of 0 and 2 hours, but the treatment between the two did not differ significantly. Similarly, the control ash levels differed markedly with all extraction time treatments. Based on table 3, it was shown that the overall sample of dried seaweed from 4 treatments had a value of ash content below the control ash content. This was because of KOH as one of the strong base and alkaline so it can attract minerals from the seaweed and caused the ash content of seaweed to be lower than the control. The longer the extraction time, made the value of the seaweed's ash content decreased due to prolonged heating affects the number of minerals attracted by KOH.

![Figure 3. Ash content of Gracilaria sp. residue after extraction.](image)

3.4. Fat content
The lowest fat content (0.10%) was found in dried seaweed samples from 4 hours extraction, while the highest fat content (0.30%) was found in the sample with 0 hour treatment (figure 4). Based on the results of the print test with a 95% confidence interval, there was a noticeable difference between the treatment to the seaweed fat content. The results of the Advanced Duncan test pointed out that the 4 hours extraction time treatment significantly differed with a 6 hours extraction time treatment, while the extraction time treatment of 0 and 2 hours did not significantly different.

![Figure 4. The fat content of Gracilaria sp. residue after extraction.](image)
Based on the results of the analysis, it is known that all treatment had a noticeable difference with the control, as shown by the values of moisture, ash, and fat content. It was shown from the decrease in the values of ash or fat levels that seaweed residue had suffered a decrease in quality. The appearance of seaweed residue was also more shattered and not intact as before. A longer extraction time treatment also caused the seaweed residue to have a value below 50%. Thus, the resulting seaweed residue cannot be used as a raw material for extraction because the warming treatment in KOH can retrace the polysaccharide inside the seaweed. It may cause the yield of agar became very low.

Muzakir (2009) stated that, the seaweed pulp contains high lignocellulose. Alkali solution can degrade lignin from seaweed through delignification mechanism so that the obtained cellulose is free of lignin. This cellulose can be used as a filler material and composite material reinforcement. Reinforced composite materials with natural fibers can produce stronger material than general materials. Thus, the seaweed residue produced in this study could potentially be used as biocomposite materials.

4. Conclusion

Dried seaweed residue of all treatments had distinct different proximate values compared with the control. The highest seaweed residue was obtained at the treatment of 0 hours extraction (52.16%) with a water content value of 10.41%, an ash content of 12.39%, and fat content of 0.30%. Based on the results it can be concluded that the seaweed residue cannot be used for further extraction. Therefore, it is recommended for further study to explore the potential of seaweed residues as biocomposite materials.

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