Fat, water and ash content in *Chlorophyceae*, *Rhodophyceae* and *Phaeophyceae* macroalgae at Sepanjang Beach, Yogyakarta, Indonesia

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Abstract. Communities in Sepanjang Beach have utilized the macroalgae as main ingredients in various special processed products. The objective of this study was to analyze the content of fat, water, and ash in *Chlorophyceae*, *Rhodophyceae*, and *Phaeophyceae* macroalgae at Sepanjang Beach, Yogyakarta, Indonesia to support optimization of macroalgae processed products. This research was conducted at Sepanjang Beach, Gunungkidul District, Yogyakarta, Indonesia in August 2020. A method of descriptive exploratory was used in this research. Analysis of fat content, water, and ash content was carried out on *Ulva lactuta*, *Palmaria palmata*, *Sargassum crassifolium*, *Gelidium spinosum*, *Gelidiella acerosa*, and *Gracilaria verrucosa* quantitatively using Soxhlet method for content of fat, the thermogravimetric method for content of water, and gravimetric method for content of ash. The results showed that the highest fat, water, and ash content was found in *U. lactuta* in the central of Sepanjang Beach 0.174%, *S. crassifolium* in the eastern 89.460%, and *U. lactuta* in the eastern 8.715% respectively.

Keywords: ash; fat; macroalgae; Sepanjang Beach; water; Yogyakarta

1. Background

Sepanjang Beach is one of the beaches located in Gunungkidul and is the habitat of various kinds of macroalgae including *Chlorophyceae*, *Rhodophyceae*, and *Phaeophyceae*. These kinds of macroalgae are used by the community in Sepanjang Beach for direct consumption, processed to be typically processed products or sold in dried condition. It is not only beneficial in increasing the added value of macroalgae, it also increases the income of the surrounding community.

Various kinds of macroalgae have been utilized by Asian communities, particularly Japanese, China, and Korea consume macroalgae as daily food [1]. The highest consumption rate is *Phaeophyceae* that reaches 66.5%, followed by *Rhodophyceae* 33%, and *Chlorophyceae* 5% [1]. The value of macroalgae in the 2015 global market also has reached $ 10.4 billion and is projected to reach $ 14.7 billion by 2021 [2]. This links to the natural nutrition content in macroalgae and the utilization of macroalgae as “food as pharma” in health [2].

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The high percentage and utilization value of macroalgae are supported by the components contained in macroalgae. Several main components in macroalgae are polysaccharides, minerals, protein, a little fat, polyphenols, and pigment [3]. Macroalgae like Phaeophyceae are also known to have the accumulation of specific metabolites such as alginic acid polysaccharides, laminarin and fucoidan, phlorotannin phenolic compounds, high fucoxanthin carotenoid, and iodine with low Na/K ratio so that it can be used as functional ingredients [4]. Furthermore, the utilization of various kinds of macroalgae as food sources associated with low-calorie content, and high polysaccharide content, vitamin, protein, mineral, various food fibers, and bioactive compounds that are potential of becoming antioxidants that are beneficial to human health [5].

The utilization of macroalgae is not limited to food and health, it is also used in the industrial field. Phaeophyceae and Rhodophyceae are frequently used in the industrial field as stabilizers and emulsifiers, meanwhile, Chlorophyceae tends to be used traditionally [3]. The utilization is associated with polysaccharide content, particularly fucoidan, alginate, and laminarin in Phaeophyceae, carrageenan, and agar in Rhodophyceae, and the sulfated or carboxylated polysaccharides in Chlorophyceae [3].

Information regarding the fat, water, and ash content in various kinds of macroalgae in Sepanjang Beach is indispensable to picture macroalgae potentials as vital food for local and wider communities. Several studies have reviewed the fat, water, and ash content in macroalgae at Gunungkidul waters, such as fat and water content in Sargassum hystrix dry powder at Sepanjang Beach [6], fat, water, and ash content in Ulva sp. at Kukup Beach [7], and fat and ash content in Ulva sp. at Sepanjang Beach [5]. Based on these results, the relationship between the location and the fat, water, and ash content of each species of macroalgae is also unknown. This is important to know because environmental factors can affect the survival of macroalgae, including the fat, water, and ash content in macroalgae.

Overall, the fat, water, and ash content in various kinds of macroalgae at Sepanjang Beach have not been identified entirely. This study is needed as the basis of potential mapping of various kinds of macroalgae at Sepanjang Beach as essential foodstuffs that can be developed to typically processed products with economic value. This study aims to analyze the fat, water, and ash content in macroalgae at Sepanjang Beach, Gunungkidul, Yogyakarta.

2. Materials and method
This study was conducted at Sepanjang Beach, Gunungkidul, Yogyakarta, Indonesia in August 2020. The analysis of fat, water, and ash content in macroalgae was conducted at the Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, State University of Semarang, Semarang, Indonesia.

2.1. Materials
The materials of this study are various kinds of macroalgae such as Chlorophyceae (Ulva lactuta), Rhodophyceae (Palmaria palmata, Gelidium spinosum, Gelidiella acerosa, and Gracilaria verrucosa), and Phaeophyceae (Sargassum crassifolium) which were found as the study took place. The differences of various kinds of macroalgae at Sepanjang Beach were carried out on the morphological characteristics and the natural color of macroalgae.

2.2. Sampling method
The method of this study is descriptive-explorative. This method provides a more detailed illustration of circumstances with the exploratory study [8]. The macroalgae sample was obtained by purposive sampling technique using line-transect in 0-10 m from the coastline in eastern (110°34’9.65”E - 110°34’9.726”E and 8°8’14.791”S - 8°8’15.16”S), central (110°34’6.383”E - 110°34’6.885”E and 8°8’14.750”S - 8°8’15.155”S), and western (110°34’2.210”E - 110°34’2.199”E and 8°8’14.349”S - 8°8’14.770”S) area of Sepanjang Beach at low tide.
2.3. Macroalgae sample preparation
The fresh macroalgae samples taken are being washed and cleansed with fresh water. Then the fresh weight of each macroalgae sample is being weighed until they reach 250 grams per sample. Each sample is mashed, then put into labeled containers and kept in a cool box to further be analyzed in the laboratory.

2.4. Fat content analysis
The analysis of fat content uses the Soxhlet method. This refers to SNI 01-2354.3-2006 [9, 10]. Weighing 2 grams of each macroalgae sample then mix it with 8 grams of annealed sand in each sample, then wrap it with filter paper and put it into a Soxhlet extraction tube. In this method, petroleum ether (PE) or diethyl ether (DE) solvent is sufficiently used. The next step is stringing the tools and connecting them with a water hose, heated for 4 hours. Then the PE or DE that contains oil is moved into a clean bottle in which weight has been identified. The analysis is continued with evaporating ether by a water bath until it becomes a little concentrated, then dried in an oven with 100°C temperature until the weight is constant. The last step is calculating the percentage of fat content using the equation as follows (SNI 01-2354.3-2006):

\[
\text{Fat content (\%)} = \frac{\text{residual weight and extraction tube (g)} - \text{weight of the empty tube (g)}}{\text{weight of the sample (g)}} \times 100\% \quad (1)
\]

2.5. Water content analysis
The analysis of water content uses the thermogravimetry method. This refers to SNI 01-2354.2-2006 [9]. Weighing 1 gram of each macroalgae sample that has been mashed using porcelain dish which weight has been identified. The next step is drying the sample in an oven at 100°C temperature for 3-5 hours, then chilled in a desiccator and weighed. Then re-heating the sample in the oven for 30 minutes, chilled in a desiccator, and weighed again. The treatment is repeated until a constant dish and sample weight is obtained with less than 0.2 mg or 0.0002 g difference. And then the percentage of the water content is calculated using the following equation (SNI 01-2354.2-2006):

\[
\text{Water content (\%)} = \frac{\text{weight of the dish and initial sample (g)} - \text{weight of the dish and dried sample (g)}}{\text{weight of the dish and initial sample (g)} - \text{weight of the empty dish (g)}} \times 100\% \quad (2)
\]

2.6. Ash content analysis
The analysis of ash content uses the gravimetry method. This refers to SNI 01-2354.1-2006 [9, 10]. Each cleaned macroalgae sample is mashed and put in an oven at 80°C temperature until dry. Then 1 gram of each macroalgae sample is weighed. The next step is putting the fresh sample and dried sample into a porcelain crucible. The analysis is continued by annealing the sample to muffle at 500°C temperature until the color becomes white. Then putting crucible into desiccator to chill, weighing the obtained ash, then comparing the ash content in fresh sample and processed sample using the following equation (SNI 01-2354.1-2006):

\[
\text{Ash content (\%)} = \frac{\text{weight of the dish and processed sample (g)} - \text{weight of the empty dish (g)}}{\text{weight of the fresh sample (g)}} \times 100\% \quad (3)
\]

3. Result and discussion
3.1. Result
The various kinds of macroalgae are found in the hard substrates such as rocks or dead corals of Sepanjang Beach, especially U. lactuta and S. crassifolium (figure 1). U. lactuta is found at the eastern, central, and western area of Sepanjang Beach. P. palmata and S. crassifolium are only found at the eastern area of Sepanjang Beach. G. spinosum is only found at the central area of Sepanjang Beach. G. acerosa is found at the central and western area of Sepanjang Beach. G. verrucosa is found the western area of Sepanjang Beach.
The result of the analysis of fat, water, and ash content in Chlorophyceae, Rhodophyceae, and Phaeophyceae macroalgae is shown in table 1. Based on the table, it can be seen that the fat, water, and ash content in various kinds of macroalgae at Sepanjang Beach, each range between 0.100-0.174%; 71.910-89.460%; and 5.513-8.715%.

The highest fat content is in U. lactuta (Chlorophyceae) at the central area of Sepanjang Beach (figure 3.), and the lowest is in G. verrucosa (Rhodophyceae) at the western area of Sepanjang Beach (figure 4.). The highest water content is in S. crassifolium (Phaeophyceae) at the eastern area of Sepanjang Beach (figure 2.), and the lowest is in G. spinosum (Rhodophyceae) at the central area of Sepanjang Beach (figure 3.). The highest ash content is in U. lactuta (Chlorophyceae) at the eastern area of Sepanjang Beach (figure 2.), and the lowest is in S. crassifolium (Phaeophyceae) at the eastern area of Sepanjang Beach too (figure 2.). The results are also shown the relationship between species macroalgae, sampling location, and fat, water, and ash content. The eastern area of Sepanjang Beach is characterized by the presence of a trench that causes the wave energy to be more dynamic. The central area of Sepanjang Beach is tending a lot of basins in the dead coral which protects the growth of macroalgae. The western area of Sepanjang Beach is dominated by dead coral and rarely basins so it tends to be dry. This condition can affect the physiological processes of macroalgae.

Figure 1. U. lactuta and S. crassifolium at Sepanjang Beach.
Table 1. Fat, water, and ash content in *Chlorophyceae*, *Rhodophyceae*, and *Phaeophyceae* macroalgae at Sepanjang Beach.

| Species                  | Contents (%) |
|--------------------------|--------------|
|                          | Fat          | Water        | Ash           |
| **Eastern Area of Sepanjang Beach** |              |              |               |
| *Ulva lactuta*           | 0.103        | 80.630       | 8.715         |
| *Palmaria palmata*       | 0.160        | 85.840       | 4.423         |
| *Sargassum crassifolium* | 0.129        | 89.460       | 4.210         |
| **Central Area of Sepanjang Beach** |              |              |               |
| *Gelidium spinosum*      | 0.114        | 71.910       | 5.513         |
| *Gelidiella acerosa*     | 0.142        | 79.350       | 5.669         |
| *Ulva lactuta*           | 0.174        | 83.720       | 6.752         |
| **Western Area of Sepanjang Beach** |              |              |               |
| *Gracilaria verrucosa*   | 0.100        | 79.960       | 7.568         |
| *Gelidiella acerosa*     | 0.106        | 77.990       | 6.263         |
| *Ulva lactuta*           | 0.165        | 85.470       | 5.837         |

Figure 2. Fat, water, and ash content in *Chlorophyceae*, *Rhodophyceae*, and *Phaeophyceae* macroalgae at the eastern area of Sepanjang Beach.
Figure 3. Fat, water, and ash content in Chlorophyceae, Rhodophyceae, and Phaeophyceae macroalgae at the central area of Sepanjang Beach.

Figure 4. Fat, water, and ash content in Chlorophyceae, Rhodophyceae, and Phaeophyceae macroalgae at the western area of Sepanjang Beach.

3.2. Discussion
The result of this study is different from other similar studies conducted in Gunungkidul. The fat content in this study is higher than the fat content in Lailatussifa et al. [6] which results in 0.05±0.02%, but lower than the fat content in da Costa et al. [7] which results in 5.17%, and 0.38% in Jatmiko et al. [5].
Different in the water content, the water content in this study is higher than the water content in Lailatussifia et al. [6] which results in 13.43±0.15%, and in da Costa et al. [7] which results in 11.53%. The ash content in this study is higher than in da Costa et al. [7] which results in 2.94%, but lower than in Jatmiko et al. [5] which results in 31.4%.

Overall, the variation of nutrition content in various kinds of macroalgae is affected by internal and external factors, such as geographical location and age of thallus [7], seasons [11], environmental conditions, and sampling method [12]. So the variation of fat, water, and ash content in various kinds of macroalgae are also affected by the characteristics of each species, the topographic conditions of the beach, and the type of substrate. This is proved by several studies, such as in Ma’ruf et al. [13] which shows that the fat, water, and ash content in Caulerpa racemosa that comes from waters in Jepara are 8.681±0.964%, 92.375±0.027%, and 20.910±1.290% respectively, while the fat, water, and ash content in Gracilaria verrucosa obtained from the pond with the muddy substrate are 3.322±0.109%, 80.701±0.239%, and 19.575±1.614% respectively, different from G. verrucosa in a sandy substrate which are 2.902±0.034%, 79.348±0.307%, and 21.852±1.229% respectively. The result of the study in Gazali et al. [14], shows that the fat, water, and ash content in Sargassum sp. at Lhok Beach waters, West Aceh, are 0.79±0.04%, 10.54±0.25%, and 52.74±0.53% respectively. The result of the study in Manteu et al. [15], shows that the fat, water, and ash content in Sargassum polycystum and Padina minor from Lemito Beach waters, Pohuwato, are 0.50±0.11%, 17.69±0.03%, and 24.51±0.13% respectively.

The result of the study in Saloso [10], shows that the fat, water, and ash content in four Sargassum macroalgae (Sargassum crassifolium, S. cristaefolium, S. polycystum, and Sargassum sp.) at Kupang bay waters are 0.94-1.99%, 16.19-19.64%, and 22.56-24.11% respectively. The result of the study in Dewinta et al. [9], also shows that the fat, water, and ash content in Sargassum cristaefolium, and S. crassifolium at Pane islands waters, Centre Tapanuli, are 0.25% and 0.30%, 41.28% and 41.52% respectively.

The fat content in macroalgae consists of saturated and unsaturated fats. Saturated fats in macroalgae involve palmitic and myristic acid, while unsaturated fats particularly polyunsaturated involves fatty acid (n-3, or omega-3) such as docosahexaenoic and eicosapentaenoic acid [2]. The result of the study in Polat and Ozogul [16], also shows that the composition of saturated fatty acid (SFA), monounsaturated fats (MUFA's), polyunsaturated fats (PUFA's) in Rhodophyceae macroalgae (Jania rubens, Laurencia papillosa, Spyridia filamentosum, and Dasya rigidula), and Phaeophyceae macroalgae (Padina pavonia, and Stypodium schimperi) each ranges 28.86-60.43%, 12.52-32.94%, and 5.54-41.42%.

The low-fat content in this study is related to the sunlight intensity and photosynthesis process in each species. The result of the study in Dewinta et al. [9], shows that the low-fat content in Sargassum sp., is probably due to the species' need for higher light intensity for the photosynthesis process compared to other macroalgae species. The fat content in macroalgae is also affected by seasons. This is proved by the result of the study in Polat and Ozogul [16], which states that the highest fat content is found in S. schimperi (2.16%) in summer, while the lowest fat content is found in S. filamentosum (0.08%) in spring.

The water content in various kinds of macroalgae in this study is still compatible with the range stated in Herrmann et al. [17], around 74-89%. The high water content in macroalgae particularly Phaeophyceae may result in issues in the utilization of macroalgae as the source of fuel so that a reduction in the water content is needed [18]. The water content in macroalgae is affected by harvest time. The result of the study in Gallagher et al. [18], shows that the highest water content is found in Laminaria digitata (Phaeophyceae) sample, taken in January and April, while the lowest is in July. The water content in macroalgae is also affected by the kind of macroalgae. Furthermore, the difference of water content in Chlorophyceae, Rhodophyceae and Phaeophyceae ranges 780-920 g/kg, 720-910 g/kg, and 610-940 g/kg respectively [19]. The difference in light intensity and temperature used in the drying process can also affect the water content in macroalgae [20]. The drying method used will affect the nutrition content in macroalgae including the water content in Sargassum polycystum [20].
The ash content in macroalgae samples can depict the total mineral content in it [5]. The ash content in macroalgae is closely related to the level of mineral absorbance occurring through the surface of thallus, and as the adaptation of macroalgae towards the marine water environment with high mineral content [9]. Generally, the highest mineral contents in macroalgae are iron and iodine, but those compositions may be fluctuated even in the same species because of the endogenous and exogenous factors [21]. Based on the result of the study in Jatmiko et al. [5], it is identified that Ulva sp. at Sepanjang Beach contains Na, Mg, S, Cl, I, Fe minerals 6.86%, 7.92%, 11.05%, 11.58%, 4.46%, 1.95%, 0.27%, and 0.86% respectively. The ash content in macroalgae is also affected by seasons. The result of the study in Polat and Ozogul [16], shows that the highest ash content is in J. rubens (51.63%) in spring, and the lowest is in D. rigudula (3.00%) in autumn.

4. Conclusion
The highest fat, water, and ash content sequentially are 0.174% in U. lactuta (Chlorophyceae) at the central area of Sepanjang Beach, 89.460% in S. crassifolium (Phaeophyceae) at the eastern area of Sepanjang Beach, and 8.715% in U. lactuta (Chlorophyceae) at the eastern area of Sepanjang Beach. Chlorophyceae, Rhodophyceae, and Phaeophyceae macroalgae at Sepanjang Beach are the potential to be developed as foodstuffs through a further study on mineral contents and the effect of macroalgae consumption on health.

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