Elemental composition of *Eucheuma cottonii* and *Gracillaria* sp. using scanning electron microscope - energy dispersive spectroscopic analysis

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**Abstract.** Indonesia is a major producer of the *E. cottonii* seaweed and *Gracillaria* sp. in the world. The farmers usually dry that seaweeds before sending them to plant factories to be processed into carrageenan or agar. In the present work, *E. cottonii* and *Gracillaria* sp. seaweed's chemical composition were observed using Scanning Electron Microscope-Energy Dispersive Spectroscopic (SEM-EDS) analysis, and the distribution of the main salt-minerals in the surface and the cross-section thallus of both samples were conducted. The results showed that the surface of *E. cottonii* contains the following order of elements Cl> K> Na> S> Ca> C> O> Si> Al> Mg> Fe> Mn> Zn and in the cross-section contains order of elements S> Cl> K> C > O> Na> Mg> Ca> Al> Zn> Fe> Si> Mn. Whereas on the surface of the *Gracillaria* sp. showed the presence of elements in the following order Cl> K> Si> C> O> Al> S> Fe> C> Mg> Na> Mn> Zn and on the cross-section found elements in the following order Cl> K> C> O> S> Na> Si> Al> Mg> Fe> Ca> Zn> Mn. The EDS mapping on both seaweeds surface and cross-section showed a random distribution of salt-forming elements Na, K and Mg. Furthermore, the presence of a higher amount of K, Na, adequate amount Mg, and other essential elements indicated both seaweeds' potential to be developed for salts with special functional properties.

1. Introduction

Indonesian is the largest producer of cultivated seaweed globally [1], which contributes to 38.7% of total world cultured seaweed production [2]. Carragenophytes seaweed (*Eucheuma* and *Kappaphycus*) and agarophyte (*Gracillaria*) are seaweeds that are widely cultivated in Indonesia, those seaweeds are processed to produce hydrocolloid agar and carrageenan, which are widely used in the food, beverage, chemical, textile and pharmaceutical industries [3–7].

Seaweed is cultivated in seawater and brackish water with high salinity environmental characteristics, so seaweed actively absorbs and stores certain salts and minerals to reducing free radicals and preventing disturbances in the body's metabolic system [8,9]. Therefore, seaweed accumulated the minerals that essentials for human health and have been promoted as sources of minerals, e.g., I, Fe, Ca, Mg, P, Zn,
F dan K [10]. Seaweed is also suggested to satisfy the mineral intake and fortified into food products [11,12].

The prevalence of high blood pressure, cardiovascular diseases, and obesity remained high in the European, Australian, and the US populations, as well as in the Indonesian as a result of intake of high Na containing food [13,14,15,16]. Research showed that countries that regularly consume high seaweed have lower cardiovascular-related diseases than countries that do not [17,18]. Therefore, many seaweed salts and salt fortified seaweed (gourmet salt) were developed and promoted as salt with low Na. Sea salt was fortified with dried flake seaweed Hizikia (laver) and Sargassum seaweed to increase K content [19,20]. From Ulva lactuca, Padina, Caulerpa lentillifera and Halimeda opuntia seaweeds can generate seaweed salt with low Na and health benefits [21–24].

However, the development of these salt products is only limited to the non-commercial types of seaweed, whose availability is highly dependent on nature. In this study, the elemental content of cultivated seaweed E. cottonii (marine culture) and Gracillaria sp. (brackish water) was studied. The observations were made in the surface and cross-section of thallus by using SEM-EDS (Scanning Electron Microscope- Energy Dispersive Spectroscopic).

2. Material and Method

2.1 Material
Dried E.cottonii seaweed was obtained from seaweed farmers in Kalianda (Lampung), and dried Gracillaria sp. seaweed was obtained from Karawang seaweed farmers (West Java). The seaweeds were re-dried in the oven at 40°C for 24 hours before being analyzed by SEM-EDS. Seaweed thalli were cut with a razor, and then surface and cross-section of thallus were observed.

2.2. Method
Samples were mounted on flatted stub using a carbon conductive double side tape. The microphotographs were recorded using a factory-calibrated Neoscope SEM JEOL model 6000-EDS with an accelerating voltage of 20 keV, at high vacuum mode, and Secondary Electron Image (SEI). The semi-quantification elemental analysis to identify the weight percentage of major and minor elements present in the samples was done using the Joel Energy Dispersive X-ray Spectrometer (EDS)[25]. The SEM diagrams for surfaces and cross-section of thalli were acquired with analytical position 19.0 mm, and the elements like Na, Mg, Si, P, S, Cl, K, Ca, Mn, Fe and Zn were identified. The EDS elemental analysis results were the mean of three area measurements. The EDS mapping analysis on the surfaces and cross-section thalli were performed to check the distribution of selected salt-forming elements K, Na, and Mg.

3. Results and Discussion

3.1. Surface of dried E.cottonii and Gracillaria sp.
After being harvested, the farmer dried E. cottonii and Gracillaria before transporting to the carrageenan and agar plant. The observations showed that white powder and salt were found on the surface of dried E.cottonii and Gracillaria sp. seaweed (Figure 1). Figure 1A and 1B show salt-like crystals covering the surface thallus of dried E. cottonii and the white powder covering the surface thallus dried Gracillaria sp. Scanning Electron Microscope images (Figure 1C and 1D) show that there were many fine particles on the surface of dried Gracillaria sp. and powdery layer on E. cottonii surfaces, which were thought to be salt compounds. So, it is necessary to confirm that elementals composition with EDS.
Figure 1. Dried seaweed surface picture (A) *E. cottonii*, (B) *Gracillaria* sp, SEM image of dried thallus of (C) *E. cottonii* surface, (D) *Gracillaria* sp. surfaces.

3.2. SEM-EDS Analysis of Elements of *E. cottonii* and *Gracillaria* sp.

Table 1 shows the concentration of macro and micro elements like Cl, K, Na, S, Ca, C, O, Si, Al, Mg, Fe, Mn, Zn of seaweed. *E. cottonii* (Figure 3) thallus surface contain the elements in the following order Cl>K>Na>S>Ca>Fe>Mg>Si>Al>Mn>Fe>Mn>Zn and cross-section thallus *E. cottonii* contain S>Cl>K>Na>Mg>Ca>Al>Zn>Fe>Si>Mn. The weights of different elements were given as percentage (%), in the surface of *E. cottonii* thallus maximum amount of Cl (51.87 ± 8.22%), minimum amount of Zn (0.03 ± 0.04 %) and others elements like K (23.03 ± 9.03%), Na (12.2 ± 5.55), S (5.2 ± 5.09%), Ca (2.74 ± 2.79%), C (1.72 ± 1.27%), O (1.54 ± 1.60%), Si (0.71 ± 0.69%), Al (0.40 ± 0.21%), Mg (0.32 ± 0.18%), Fe (0.19 ± 0.20%) and Mn (0.07 ± 0.01 %) were detected.

In the cross-section of *E. cottonii* thallus the maximum amount of S (26.5 ± 1.06%) and minimum amount of Si (0.11 ± 0.03%) was detected, moreover, others elements namely K (17.55 ± 1.64%), Na (7.21 ± 0.65%), S (26.50 ± 1.06%), Ca (12.1 ± 1.13%), C (16.57 ± 2.85%), O (9.69 ± 1.22%), Si (0.11 ± 0.03%), Al 0.48 ± 0.11%), Mg (2.43 ± 0.16%), Fe (0.17 ± 0.12%) were also detected in the cross-section of *E. cottonii* thallus. Most of elements that detected in the surface and cross-section of *E. cottonii* thallus were also found in seaweed sap of *E. cottonii* that analyzed using ICP-MS[26]

There were a different proportion of elements found in the surfaces and cross-section of *E. cottonii* thallus. The high content of Cl, Na and Mg on the surface of the *E. cottonii* thallus indicates a thick layer of salt covering the surfaces of seaweed thallus, meanwhile high amount of S, C, Cl, Na and K in the cross-section area indicated the high amount of sulfated hydrocolloid carrageenan and salt-forming
elements contained in the inner thallus. The carrageenan is sulfated hydrocolloid that mainly produced by *E. cottonii* and *Kappaphycus* [27,28]

**Table 1. The elemental composition of *E. cottonii* and *Gracillaria* sp. seaweed thallus.**

| Element | *E. cottonii (%)* | *Gracillaria sp (%)* |
|---------|------------------|---------------------|
|         | Surface | Cross-section | Surface | Cross-section |
| Cl      | 51.87 ± 8.22 | 17.90 ± 0.87 | 49.37 ± 4.32 | 31.78 ± 6.86 |
| K       | 23.03 ± 9.03 | 17.55 ± 1.64 | 37.70 ± 1.06 | 30.72 ± 5.65 |
| Na      | 12.2 ± 5.55  | 7.21 ± 0.65  | 0.19 ± 0.11  | 1.37 ± 0.52  |
| S       | 5.2 ± 5.09   | 26.50 ± 1.06 | 1.49 ± 0.52  | 6.53 ± 0.60  |
| Ca      | 2.74 ± 2.79  | 1.21 ± 1.13  | 0.28 ± 0.17  | 0.42 ± 0.11  |
| C       | 1.72 ± 1.27  | 16.57 ± 2.85 | 2.01 ± 0.76  | 19.55 ± 8.6  |
| O       | 1.54 ± 1.60  | 9.69 ± 1.22  | 2.00 ± 0.98  | 7.02 ± 2.96  |
| Si      | 0.71 ± 0.69  | 0.11 ± 0.03  | 3.71 ± 1.88  | 1.04 ± 0.92  |
| Al      | 0.40 ± 0.21  | 0.48 ± 0.11  | 1.64 ± 0.73  | 0.57 ± 0.44  |
| Mg      | 0.32 ± 0.18  | 2.43 ± 0.16  | 0.22 ± 0.06  | 0.46 ± 0.11  |
| Fe      | 0.19 ± 0.20  | 0.17 ± 0.12  | 1.24 ± 0.23  | 0.45 ± 0.19  |
| Mn      | 0.07 ± 0.01  | 0.00 ± 0.00  | 0.09 ± 0.08  | 0.02 ± 0.01  |
| Zn      | 0.03 ± 0.04  | 0.26 ± 0.24  | 0.05 ± 0.08  | 0.23 ± 0.00  |
| Total   | 100.02      | 100.08       | 100.00      | 100.16       |

*Gracillaria* sp. thallus surfaces showed the elements in the following order Cl>K>Si>C>O>Al>S>Fe>C>Mg>Na>Mn>Zn and in cross-section thallus Cl>K>C>Cr>Na>Si>Al>Mg>Fe>Ca>Zn>Mn respectively. Maximum amount of Cl was detected either in the thallus surface or in the cross-section thallus of *Gracillaria* sp. Minimum amount of Zn (0.05 ± 0.08%) and other elements like K (37.70 ± 1.06%), Na (0.19 ± 0.11%), S (1.49 ± 0.52%), Ca (0.28 ± 0.17%), C (2.01 ± 0.76%), O (2.00 ± 0.98%), Si (3.71 ± 1.88%), Al (1.64 ± 0.73%), Mg(0.22 ± 0.06%), Fe(1.24 ± 0.23%), Mn(0.09 ± 0.08%) were also detected in the surfaces of *Gracillaria* sp. Elements K (30.72 ± 5.65%), Na (1.37 ± 0.52%), S (6.53 ± 0.60%), Ca (0.42 ± 0.11%), C (19.55 ± 8.6%), O (7.02 ± 2.96%), Si (1.04 ± 0.92%), Al (0.57 ± 0.44%), Mg (0.46 ± 0.11%), Fe (0.45 ± 0.19%), Mn(0.02 ± 0.01%), Zn (0.23 ± 0.00%) were also find in the cross-section *Gracillaria* sp. thallus. Elements that found in *Gracillaria* sp. were also detected in the *Gracilaria corticata* [25]. Analysis of *Gracilaria* sap using ICP-MS also detected elements similar to this study [26].

The proportion of the elements found in the surfaces and cross-section of *Gracillaria* sp. thallus was a difference. A high amount of Cl and K in the thallus surfaces indicated a thick layer of salt might cover the *Gracillaria* sp. thallus's surface. Meanwhile, high amounts of S, C, Cl and K indicated the high amount of sulfated hydrocolloid agar and salt in the inner thallus. The agar is hydrocolloid that containing sulfate, where the sulfate content of the agar is depended on the extraction method and species of the seaweed [29,30].

The surface and cross-section of *E. cottonii* thallus have a higher Na content than that in *Gracillaria* sp. It is thought to be due to differences in the environmental conditions of the two algae growing. The *E. cottonii* war growth in the seawater that contains 99% of Na. Meanwhile, the *Gracillaria* sp. was growth in brackish water that has lower salinity. The proportion of K in *Gracillaria* sp. thallus was higher than in *E. cottonii* thallus. A future study is needed to understanding the mass of that element to be contained in the seaweed since EDS limited only for semiquantitative analyze of elemental composition, the SEM-EDS was suggested as a tool to measure the elements presents on the superficial layer and provides an overview of the probable elements that are present[25].
Salt on seaweed thallus surfaces is thought to come from the mixing of seaweed sap and seawater. It is presumably from a higher amount of K than Na in the seaweed surface, while sea salt has a high Na than K [31,32]. Seaweed cells lose their integrity during the drying process, so liquid quickly came out of the seaweed cells and carries minerals, which then dried on the thallus surface. Lose integrity cells were easier to release liquid that carried minerals containing in the Caulerpa sp. seaweed. [33]

Elements Na, K, and Mg are salt-forming elements in the form of NaCl, KCl, MgCl, and MgSO4. Most of the table salt contains large amounts of NaCl, which can trigger high blood pressure and other generative diseases if consumed without the intake of K, Mg and Ca, [34]. A higher proportion of K intake maintains cellular and metabolic function, attenuates high blood pressure and reduces cardiovascular disease risk. Likewise, increasing Mg intake suppresses metabolic syndrome markers and systemic inflammation and lowers blood pressure while increasing Ca intake leads to increased Na excretion and decreased blood pressure [35,36]. This study gives information about the salt-forming elements at the superficial layer of seaweed thallus, showing the prospect of tropical seaweed E.cottonii and Gracillaria sp. to produce salt with functional properties.

Salts with health claims have been widely marketed in Indonesia and the world, such as Himalayan salt and Japanese seaweed salt (Moshio). The diets salt Nutrisaline and Lososa have also been marketed. Those salts are claimed to contain a unique salt and mineral that are useful for health. Salt with low Na content, high K, and Mg have been recommended for consumers with hypertension[35]. The Indonesian National Standard has also issued a standard for dietary salt with a specificity of a maximum NaCl content of 60% and a maximum KCl of 40%[37]. Based on that ratio, we found the potential use of E.cottonii and Gracillaria sp. to be generated products that merit the standard for dietary salt.

3.3. EDS mapping of K, Na and Mg on the surface and cross-section thallus of E.cottonii and Gracillaria sp.
The distribution of K (I B-IV B), Na (I C-IV C), and Mg (I D-IV D) in the thallus of seaweed is shown in Figure 2. The results show that the distribution of K (I B) and Na (I C) in the surface of E. cottonii thallus was not evenly distributed, yet the Mg (I D) distribution was homogenous. The result of the overlay images shows the random distribution of those three elements. The distribution of K (II B), Na (II B) and Mg (II C) were homogenous in the cross-section area of E. cottonii thallus, but the distribution of Na (II C) in the edge area (close to epidermal) was slightly dense. We suspect that some seawater may evaporate in the epidermal area so that Na is denser in that area.

The distribution of Na (III, IV C) and Mg (III, IV D) on the surface and the cross-section of Gracillaria sp. were relatively homogenous. Meanwhile, the distribution of K (III, IV B) was homogenous on the surface thallus (III-B), but not evenly distributed in the cross-section thallus (IV B). The overlay of EDS images also shows the random distribution of these three elements in both surfaces, there was no specific pattern distribution of those elements in the surface. The distribution of Na in the cross-section of E. cottonii and Gracillaria sp. was a difference; in the cross-section of Gracillaria sp., there was no dense distribution of Na close to the epidermal area. The brackish water has lower salinity than seawater, so dried brackish water on the surface thallus did not generate a dense of Na on the surfaces (epidermis)Gracillaria sp.

The EDS mapping of Na, K and Mg provide the information that in the surfaces of E. cottonii thallus, some of the seawater might significantly affect the distribution and composition of elements; meanwhile, the brackish water might less affect the distribution and composition of an element in the surfaces of Gracillaria sp. The information of elements distribution is essential for product development. The inhomogeneous distribution of elements, especially in the seaweed surface thallus, needs to be considered to develop seaweed salt to obtain homogeneous products.
Figure 2. Distribution of K, Na, Mg of *E. cottonii* and *Gracillaria* sp, (I) Surface thallus of *E. cottonii*, (II) cross-section of *E. cottonii*, (III) surface thallus of *Gracillaria* sp., (IV) cross-section of *Gracillaria* sp. (A) the SEM picture of seaweed, (B) distribution of K, (C) distribution of Na, (D) Distribution of M, and (E) overlapping distribution of elements.

4. Conclusions

This study showed various composition elements on the surface and cross-section area of *E. cottonii* and *Gracillaria* sp. SEM-EDS analysis showed *E. cottonii* surface contain elements with following order Cl>K>Na>S>Ca>C>O>Si>Al>Mg>Fe>Mn>Zn were detected in *E. cottonii* thallus and cross-section thallus, meanwhile cross-section thallus contain elements with following order S>Cl>K>C>O>Na>Mg>Ca>Al>Zn>Fe>Si>Mn. The surface of the *Gracillaria* sp. showed the presence of elements in the following order Cl>K>Si>C>O>Al>S>Fe>C>Mg>Na>Mn>Zn and on the cross-section found elements in the following order Cl>K>C>O>S>Na>Si>Al>Mg>Fe>Ca>Zn>Mn. The EDS mapping of salt-forming elements Na, K and Mg showed that those elements were randomly distributed either in the surface or cross-section thallus of both seaweeds. *E. cottonii* and *Gracillaria* sp have high potency to be developed into functional salt since they contain a high amount of K, Na and adequate Mg.

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