Fatty acid map of various species seagrasses on the Donggala Beach

by Paulina Taba

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Abstract. Quantitative identification of seagrass fatty acids has been carried out from the species of Thalassodendron ciliatum, Cymodocea rotundata, Cymodocea serrulata, Thalassia hemprichii, and Enhalus acoroides. Fat analysis samples taken in Donggala regency aquatic is done by the Soxhlet method and analysis of fatty acids using by the GC method. It was found that variations in fat content were between 0.27% - 1.01%, while the saturated fatty acid concentrations found varied between 4.39% - 8.03%, unsaturated fatty acids varied between 4.48% - 18.39% , and omega 3 (EPA) varies between 0.27% - 1.24%. If seagrass fatty acid data is combined with the results of protein, phosphate, and minerals analysis data, it can be the basis for estimating the fertility status of seagrass and the healthy environment of a region, especially the coastal aquatic of Donggala regency.

1. Introduction

The characteristics of coastal areas have two characteristics, namely the promising quality of life and high biodiversity. However, excessive utilization and not paying attention to environmental management rules can cause ecosystem degradation in the area. Increasing the number of inorganic nutrients such as ammonia, nitrate, and phosphate in the waters can increase the amount of phytoplankton biomass, but at the same time consume large amounts of oxygen and cause massive mortality of other organisms and can damage ecosystems in the form of shifts towards a new equilibrium, eliminating previously existing species. One of the main sources is excessive fertilizer use for agricultural land. This excess can be carried over into the irrigation water then continues into the marine environment. In addition, human activities due to urbanization are also an important source of these nutrients. Fisheries and aquaculture that use nitrogen and phosphate fertilizers in the culture can contribute nutrients to the marine and coastal environment to trigger an imbalance of ecological systems in marine and coastal waters [11, 17].

Seagrass, which is one of the ecological systems in the sea or coastal region, is a flowering plant (angiosperms) that has a true rhizome of leaves and roots that live submerged in the sea, colonizing an area through the dioecious distribution of fruit (propagule) [10]. According to Den [4] this plant has several properties that allow life in the marine environment, namely (a) able to live in salt water

media, (b) capable of functioning normally in a setting state, (c) having a well-developed anchor root system and (d) able to carry out pollination and generative cycles in a state of sunset. Seagrass has a real difference with plants that live in other seas such as macroalgae or seaweeds. Seagrasses also have a real root system, leaves, internal transportation systems for gases and nutrients, and stomata that function in gas exchange. Roots in seagrasses do not function important in water extraction, because the leaves can absorb nutrients directly from the sea water and nitrogen fixation through the root hood. To keep the body so that it stays afloat in the water pool of this plant is equipped with air space.

Seagrass leaves and rhizomes have a high nitrogen content, so dugongs prefer them. Nonetheless, it remains unknown how much of the carrying capacity of a seagrass against dugong population in the waters. Seagrass ecosystems function as energy suppliers in both the benthic and pelagic zones. Seagrass detritus of old leaves decomposed by a collection of microorganisms (such as sea cucumbers, clams, crab and bacteria) to produce organic material either suspended or dissolved in the form of nutrients. Nutrients are not only beneficial for seagrass plants but also useful for the growth of phytoplankton and subsequently zooplankton and juvenile fish/shrimp [7].

Growth, morphology, abundance and primary production of seagrass in water are generally determined by the availability of phosphate nutrients, nitrates, and ammonium which play an important role in determining the function of seagrass beds [5, 12]. The adequacy of nutrients in seagrass waters can act as a limiting factor for their growth so that the efficiency of the nutrient cycle in seagrass ecosystems will be very important to maintain the primary productivity of seagrass and autotrophic organisms that live in them. [5, 12].

Seagrasses thrive, especially in open tidal areas and coastal waters or global which are mud, sand, gravel and dead coral fractures with depths of up to four meters. In very clear waters, some species of seagrass are even found growing to depths of 8-15 m and 40 m [6]. Seagrass beds can be in the form of single vegetation, composed of one type of seagrass that grows into a dense field, while mixed vegetation consists of two to 12 types of seagrass that grow together on one substrate. Seagrass species that usually grow with single vegetation are *Thallasia hemprichii*, *Enhalus acoroides*, *Halophila ovalis*, *Uninervis Halodule*, *Serrulate Cymodeceae*, and *Thalassodendron ciliatum* [3].

On the muddy substrate in mangrove area towards the sea, often found seagrass beds of single species associated high. While seagrass mixed vegetation forms in lower intertidal areas and shallow subsidies. Seagrass grows well in a sheltered area, and the sand substrate is stable and close to the sediment that moves horizontally [9]. Seagrass growth is strongly influenced by internal factors such as physiological and metabolic conditions and external factors such as nutrients (nutrients) and water fertility.

Litter produced by seagrass is a food source for the lives of various communities of organisms in seagrass ecosystems such as the Crustacea community, small fish, rock shrimp, and large fish, one type of fish that is highly dependent on seagrass is dugong and green turtles. Seagrass can produce 65-85% organic matter in the form of detritus and donated to aquatic as much as 10-20% [2].

Seagrass leaves and rhizomes have a high nitrogen content, so dugongs prefer them. However, it is not yet known how much power a seagrass supports a dugong population in water. Seagrass ecosystems function as energy suppliers in both the benthic and pelagic zones. Old seagrass leaf detritus is decomposed by a group of microorganisms (such as sea cucumbers, shells, crabs and bacteria) so that organic matter is either suspended or dissolved in the form of nutrients. Nutrients are not only beneficial for seagrass plants but also useful for the growth of phytoplankton and subsequently zooplankton and juvenile fish/shrimp [7].

This research was carried out in an effort to collect as much information as possible about the nutritional aspects of seagrass ecology that grew on the mangrove average of coastal ecosystems in Donggala district. The focus of this study aims to determine the availability of fatty acid nutrients in seagrass, specifically the type and composition of fatty acids.

2. Research Method

2.1. Research Implementation

The research location is the coastal waters of Donggala district. Seagrass plants that focus on the analysis are *Thalassodendron ciliatum*, *Cymodocea rotundata*, *Cymodocea serrulata*, *Thalassia hemprichii*, and *Enhalus acoroides,* which are dominant seagrass species in the study area. A sampling of seagrass plants was carried out in the mangrove line and carried out by purposive sampling. The implementation of the study, which included preliminary research and sample preparation, was carried out in the Tadulako University chemistry laboratory. While the analysis of samples for fatty acid determination was carried out in an integrated laboratory, Bogor Agricultural Institute.

2.2. Tools and Materials

The tools used in this research include; freeze dryer, scissors, threaded tube, mortar, water bath, bottle, rotary evaporator, centrifuge, GC equipment Shimadzu brand model 17.A, NaOH 0.5N, BF_3 18%, saturated NaCl, hexane, Na_2SO_4 anhydrous, chloroform, methanol, KCl 0.88%, and distilled water.

2.3. Work Procedures

In the analysis carried out two (2) phases, namely collection and preparation sample and analysis of samples.

2.3.1. Collection and Preparation Sample. Each seagrass species is collected randomly. Washed thoroughly, then cut into small pieces, then stored in a freeze dryer for approximately 24 hours, then ground until smooth.

2.3.2. Samples Analysis. The sample analysis was divided into 2 (two) parts, namely: fat analysis, and hydrolysis and esterification

Fat analysis. Accurately weighed ± 30 mg each seagrass species which has been finely ground, then transferred to 250 mL Erlenmeyer. 75 mL of chloroform-methanol (2 : 1) was added, shaken for 30 minutes. Separated fluid (filtrate 1), repeated extraction by adding 75 mL of chloroform-methanol (2 : 1), shaken for 3 minutes and then separated the liquid (filtrate 2). Filtrates 1 and 2 are inserted into a 250 mL beaten squash and added 25 mL of KCl 0.88%, shaken well and left until the chloroform layer is separated. The chloroform layer is transferred to the rotary evaporator, then dried. In the rotary evaporator containing crude fat, 5 mL of hexane is added, shaken for 3 minutes and the solution is transferred into a centrifuge tube and run at a speed of 3500 rpm for 15 minutes. Transfer the clear solution into the test tube whose weight is known, then dry it with freeze dryer. Fat weight can be calculated.

Hydrolysis, Esterification, and Injection. Accurately weighed fat each seagrass species was 15.9 mg, 17.1 mg, 16.0 mg, 13.3 mg, and 17.44 mg into Teflon-covered tubes. 1 mL of NaOH 0.5N was added in methanol and heated in a water bath for 20 minutes. Next, add 2 mL BF_3 16% and reheat it in a water bath for 20 minutes. Lifted the tube and cooled to room temperature, then added 2 mL of saturated NaCl and 1 mL of hexane and shaken well for 2 minutes. The hexane layer is transferred with a dropper pipette into a small bottle containing 0.1 gram of Na_2SO_4 anhydrous, left for 15 minutes. Then injected into gas chromatography. Both standard and sample volumes are injected with 2 microliters.

2.4. Data Analysis

In determining the type and composition of fatty acids in this study, qualitative and quantitative analyses were carried out from the chromatogram data obtained. Qualitative analysis is done by comparing the retention time of sample fatty acids with certain standard fatty acid retention times,

where the same retention time shows the same fatty acid. Quantitative analysis was carried out based on the calculation of sample fatty acids compared to standard fatty acids, as follows:

$$\%\ AL = \frac{\left(\frac{A_{sp}}{A_{st}}\right)\ x\ C_{st}\ x\left(\frac{V}{100}\right)\ x\ 100}{W_{sp}}$$

Where: % *AL* = percent fatty acid level, A_{sp} = Area of sample fatty acids, A_{st} = Area of standard fatty acids, W_{sp} = Weight of sample, C_{st} = Concentration of standard fatty acids, and V = volume.

3. Results And Discussion

This section presents the results of the analysis of seagrass fat, especially the species *Thalassodendron ciliatum*, *Cymodocea rotundata*, *Cymodocea serrulata*, *Thalassia hemprichii*, and *Enhalus acoroides*. The general description of each type of seagrass analyzed can be seen in Figure 1. Furthermore, as the main parameters in this study, the types, and composition of fatty acids contained in seagrass fat (seagrass) were carried out using gas chromatography. Gas chromatography conditions were as follows: column = sp - 2330 10% chromosorb W AW 100/120 mesh, length = 6 feet x 1/8 inch, gas N_2 rate flow = 20 mL/min, gas H_2 rate flow = 30 mL/min, air flow rate = 250 mL/min, injection temperature = 200°C, temperature detector FID = 250°C, the temperature of the column (temperature program) = initial temperature of 150°C was held for 5 minutes and a final temperature of 190°C was held for 25 minutes and the temperature rises column 10°C/min.

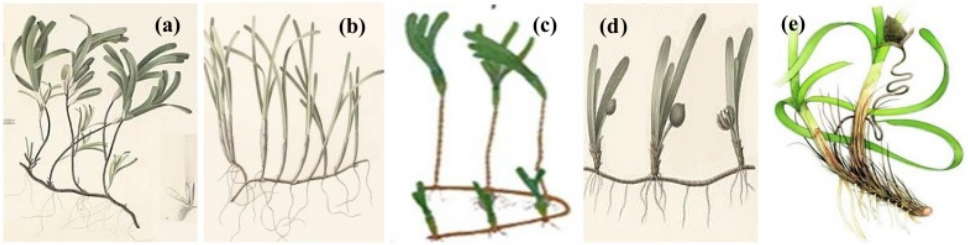
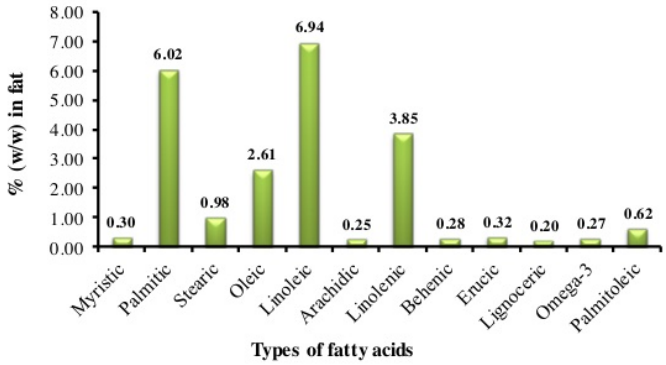


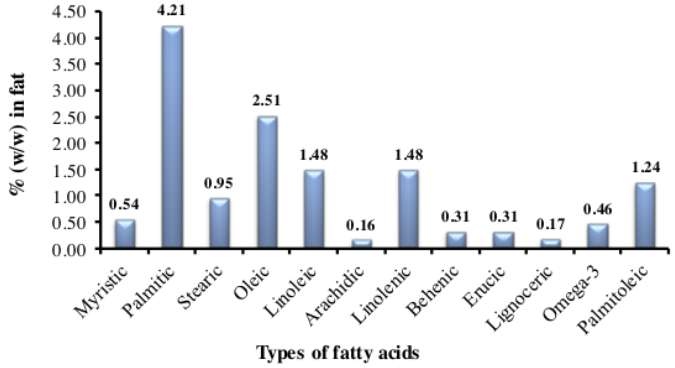
Figure 1. (a) *Thalassodendron ciliatum*, (b) *Cymodocea rotundata*, (c) *Cymodocea serrulata*, (d) *Thalassia hemprichii*, and (e) *Enhalus acoroides*

The results of the *Thalassia hemprichii* species analysis with 0.39% fat content; myristic fatty acids 0.30%, palmitic 6.02%, stearic 0.98%, oleic 2.61%, linoleic 6.94%, arachidic 0.25%, linolenic 3.85%, behenic 0.28%, erucic 0.32%, lignoseric 0.20%, omega-3 (EPA) 0.27%, palmitoleic 0.62% (Graph 1).



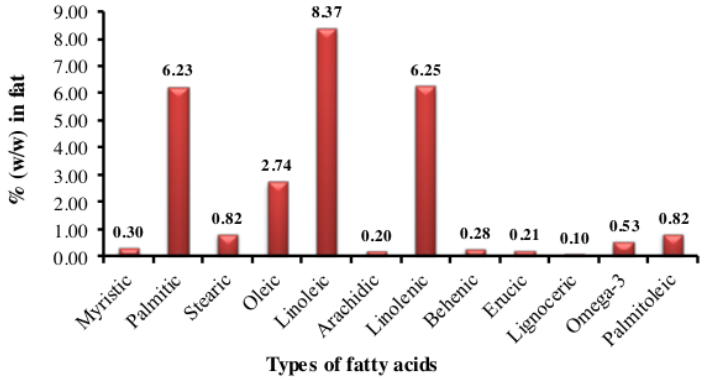
Graph 1. The fatty acid content in *Thalassia hemprichii* species

The results of the *Cymodocea rotundata* species analysis with 0.36% fat content; myristic fatty acid 0.54%, palmitic 4.21%, stearic 0.95%, oleic 2.51%, linoleic 1.48%, arachidic 0.16%, linolenic 1.48%, behenic 0.31%, erucic 0.31%, lignoseric 0.17%, omega-3 (EPA) 0.46%, palmitoleic 1.24% (Graph 2).



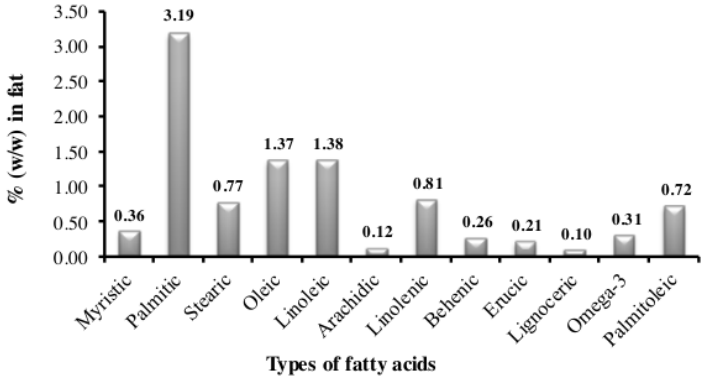
Graph 2. The fatty acid content in *Cymodocea rotundata* species

The results of the *Cymodocea serrulata* species analysis with 1.01% fat content; myristic fatty acid 0.30%, palmitic 6.23%, stearic 0.82%, oleic 2.74%, linoleic 8.37%, arachidic 0.20%, linolenic 6.25%, behenic 0.28%, erucic 0.21%, lignoseric 0.10%, omega-3 (EPA) 0.53%, palmitoleic 0.82% (Graph 3).



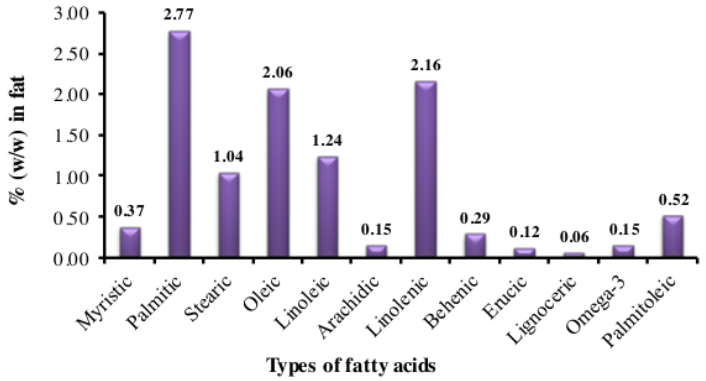
Graph 3. The fatty acid content in *Cymodocea serrulata* species

The results of the *Thalassia hemprichii* species analysis with 0.38% fat content; myristic fatty acid 0.36%, palmitic 3.19%, stearic 0.77%, oleic 1.37%, linoleic 1.38%, arachidic 0.12%, linolenic 0.81%, behenic 0.26%, erucic 0.21%, lignoseric **0.10**%, omega-3 (EPA) 0.31%, palmitoleic 0.72% (Graph 4).



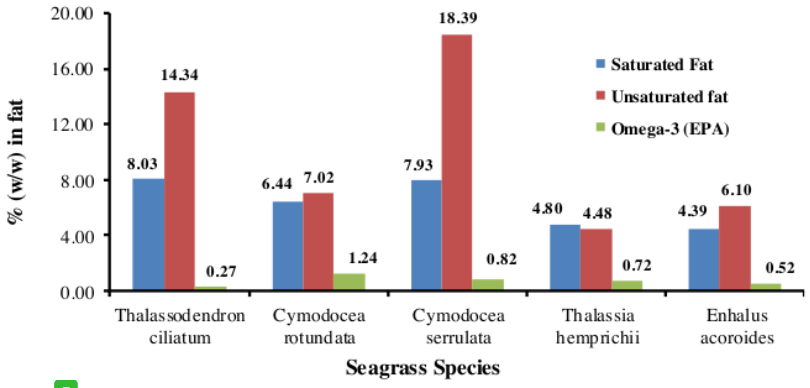
Graph 4. The fatty acid content in *Thalassia hemprichii* species

The results of the *Enhalus acoroides* species analysis with 0.27% fat content; myristic fatty acid 0.37%, palmitic 2.77%, stearic 1.04%, oleic 2.06%, linoleic 1.24%, arachidic 0.15%, linolenic 2.16%, behenic 0.29%, erucic 0.12%, lignoseric 0.06%, omega-3 (EPA) 0.15%, palmitoleic 0.52% (Graph 5).



Graph 5. The fatty acid content in *Enhalus acoroides* species

The saturated fatty acid content of five seagrass species (*Thalassodendron ciliatum*, *Cymodocea rotundata*, *Cymodocea serrulata*, *Thalassia hemprichii*, and *Enhalus acoroides*) is lower than unsaturated fatty acids except in the *Thalassia hemprichii* species (Graph 6).



Graph 6. The content of saturated fatty acids, unsaturated fatty acids, and omega-3 in seagrass fat

Palmitic is a saturated fatty acid contained by the highest of the five species of seagrass, respectively; 6.02%, 4.21%, 6.23%, 3.19%, and 2.77%. The highest unsaturated fatty acid content in the fat of the five seagrass species is linolenic, which is 3.85%, 1.48%, and 2.16% except for *Cymodocea serrulata* and *Thalassia hemprichii* species which are linoleic, ie 8.37% and 1.38%. Whereas omega-3 fatty acids (EPA) were highest in *Cymodocea rotundata* species (Graph 6).

Identified fatty acids are the result of analysis of seagrass using alkaline solutions. The results of this analysis show that of the 11 (eleven) identified fatty acids (except omega-3) all contain even C numbers of both saturated fatty acids and unsaturated fatty acids. This is consistent with the presence of fatty acids in nature which generally contain even C atoms because they are the synthesis of 2-carbon units [13, 15, 16]. In addition, the results of the analysis also show that the highest concentration of fatty acids is palmitate where these fatty acids are commonly found in animal and plant fats, including in marine animals and plants.

The most important unsaturated fatty acids in the metabolism of plants and marine animals are polyenoic fatty acids (C_{20}, C_{22}, and C_{24}), the results of this analysis also show that these three important polyenoic fatty acids are also found in seagrass fats which varies (Graphs 1, 2, 3, 4, and 5).

Seagrass Fertility in aquatic is very important to be realized and maintained because it can be a measure of the environmental health of coastal waters. Seagrass growth is caused by the availability of sufficient seagrasses nutrients, for example; phosphate, nitrogen, carbonate, iron, copper, manganese and others as well as a hygienic aquatic environment so that the photosynthesis process in seagrasses goes well [1, 6, 14]. Thus the seagrass fat is obtained from the synthesis that is a healthy aquatic environment to produce polyenoic fatty acids.

4. Conclusion

Seagrass, especially the Thalassodendron ciliatum species, Cymodocea rotundata, Cymodocea serrulata, Thalassia hemprichii, and Enhalus acoroides obtained from the coastal waters of Donggala district based on the results of the analysis using GC (Gase Chromatography) having five results. At first, analysis results of *Thalassia hemprichii* species with 0.39% fat content, containing myristic fatty acid 0.30%, palmitic 6.02%, stearic 0.98%, oleic 2.61%, linoleic 6.94%, arachidic 0.25%, linolenic 3.85%, behenic 0.28%, erucic 0.32%, lignosenic 0.20%, omega-3 (EPA) 0.27%, and palmitoleic 0.62%. At second, analysis results of *Cymodocea rotundata* species with 0,36% fat content, containing myristic fatty acid 0.54%, palmitic 4.21%, stearic 0.95%, oleic 2.51%, linoleic 1.48%, arachidic 0.16%, linolenic 1.48%, behenic 0.31%, erucic 0.32%, lignosenic 0.17%, omega-3 (EPA) 0.46%, and palmitoleic 1.24%. Third, analysis results of *Cymodocea serrulata* species with 1,01% fat content, containing myristic fatty acid 0.30%, palmitic 6.23%, stearic 0.82%, oleic 2.74%, linoleic 8.37%, arachidic 0.20%, linolenic 6.25%, behenic 0.28%, erucic 0.21%, lignosenic 0.10%, omega-3

(EPA) 0.53%, and palmitoleic 0.82%. Next, analysis results of *Thalassia hemprichii* species with 0,38% fat content, containing myristic fatty acid 0.36%, palmitic 3.19%, stearic 0.77%, oleic 1.37%, linoleic 1.38%, arachidic 0.12%, linolenic 0.81%, behenic 0.26%, erucic 0.21%, lignosenic 0.10%, omega-3 (EPA) 0.31%, and palmitoleic 0.72%. At last, analysis results of *Enhalus acoroides* species with 0,27% fat content, containing myristic fatty acid 0.37%, palmitic 2.77%, stearic 1.04%, oleic 2.06%, linoleic 1.24%, arachidic 0.15%, linolenic 2.16%, behenic 0.29%, erucic 0.12%, lignosenic 0.06%, omega-3 (EPA) 0.15%, and palmitoleic 0.52%.

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