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Identification of Fatty Acid Content of *Sargassum* sp. and *Ulva* sp. in Different Seasonal Conditions

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Abstract

Interest in exploring seaweed as a natural component needed to produce materials that can be useful for human needs has increased in the last decade. Bioactive components such as polysaccharides, proteins, and fatty acids are responsible for the biological properties associated with seaweed. The active ingredients contained in seaweed prepare for pharmaceutical, cosmetic, and food supplement needs from seaweed *Sargassum* sp. and *Ulva* sp. in different seasonal conditions it is thought to have a contribution of specific active ingredients. The bioactive content of seaweed is largely determined by genetic factors, nutrient availability, and environmental quality conditions in the dry season and rainy season. The research aimed to determine the stability of the fatty acid content of *Sargassum* sp. and *Ulva* sp. seaweed during exploration in the dry season and rainy season. However, the fatty acid profile and morphology of the seaweed species *Sargassum* sp. obtained in the dry season and rainy season had the same results as the profile of the seaweed *Ulva* sp. which was collected in the Bangsring Beach area, Banyuwangi, Indonesia. This is thought to be due to the extreme temperature differences in the dry season and rainy season conditions that have not yet resulted in real or drastic changes in the composition and quantity of fatty acid compounds as well as the adaptability of seaweed. The presence of stable fatty acid content in the dry season and rainy season will provide advantages in the exploration of fatty acids from *Sargassum* sp. and *Ulva* sp. both for pharmaceutical, cosmetics, and food supplement purposes.

INTRODUCTION

Variations in PUFA content, especially α-linolenic acid (18:3n3), octadecatetraenoic acid (18:4n-3), arachidonic acid (20:4n-6), and eicosapentaenoic acid (20:5n3) in seaweed, which are essential compounds, are one of the considerations when exploring seaweed fatty acids to produce food supplement, pharmaceutical and cosmetic components. (Wang *et al*., 2015). PUFA content from brown seaweed *Sargassum* sp. growth and development occupies almost all coastlines in both tropical and subtropical areas (Chen *et al*., 2016). Astawan *et al*. (2005) stated that the chemical composition of seaweed differs for each individual and depends on

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differences in species, habitat, harvest age, and environmental conditions. Graeve *et al*. (2002), also stated that the chemical composition of seaweed varies greatly depending on species, habitat, age, and environmental conditions. Durmaz *et al*. (2008) also mentioned variations in the fat content of seaweed *Ulva* sp. which states that seaweed contains a total of 38.50% saturated fatty acids, 19.50% monounsaturated fatty acids, and 33.76% polyunsaturated fatty acids with the highest arachidonic acid content, namely 7.31 %.

Furthermore, the great abundance of essential minerals and biochemical content in seaweeds may vary depending on the taxonomic group, and geographical, seasonal, and physiological variations (Mabeau and Fleurence, 1993). Information regarding the active ingredient content of seaweed is important for further study because the exploration of active ingredients from seaweed that can be used as ingredients for pharmaceutical, cosmetic, and food supplement needs from seaweed *Sargassum* sp. and *Ulva* sp. in different seasonal conditions is thought to have a contribution of specific active ingredients.

METHODOLOGY

Ethical Approval

Research conducted on the identification of fatty acid content of *Sargassum* sp. and *Ulva* sp. in different seasonal conditions does not require ethical clearance because it is not related to animal clinical trials.

Place and Time

Seaweed samples (*Sargassum* sp. and *Ulva* sp.) were obtained from Bangsring Beach, Wongsorejo District, Banyuwangi, Indonesia, 8°35'34.06" South Latitude and 113°59'51.20" East Longitude (dry season in August 2006 and rainy season in November 2006). Testing was carried out at the Biochemistry Laboratory, Faculty of Fisheries, Nagasaki University, and the Anatomy Laboratory, Faculty of Fisheries and Marine Affairs, Airlangga University, Surabaya.

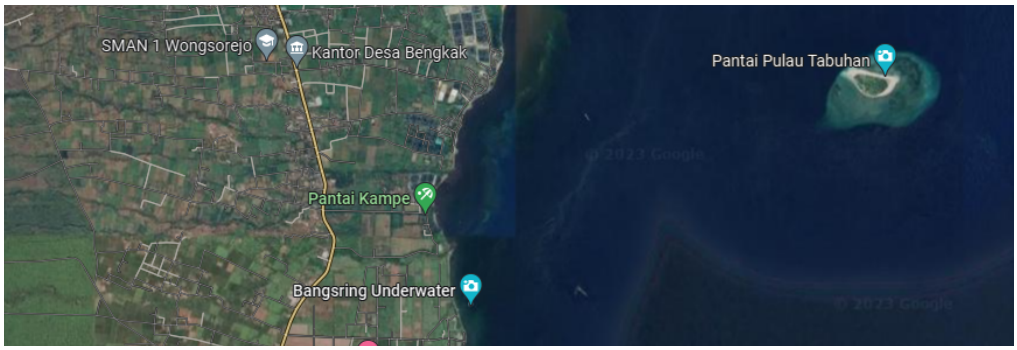

Figure 1. Bangsring Beach, Wongsorejo District, Banyuwangi, Indonesia, 8°35'34.06" South Latitude and 113°59'51.20".

Research Materials

Sargassum sp. and *Ulva* sp., Lowry's reagent, BSA, distilled water, hydrochloric acid, sodium hydroxide, buffer solution, ninhydrin solution, aquabides. Nitrogen gas, amino acid standards, nitric acid, perchloric acid, sulfuric acid, ammonium molybdate, amino naphthol sulfonate, mineral standards (Ca, Fe and P), metaphosphoric acid, acetic acid, 2,6- dichlorophenol indophenol, acetone, hexane, chloroform, trifluoroacetate, petroleum ether, boron trifluoride, methane, sodium carbonate, calcium carbonate and isopropanol.

Work Procedure

Material Preparation

Two different species were selected and based on their morphology, were classified as *Sargassum* sp. and *Ulva* sp.

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samples from each collection were placed on herbarium sheets, photographed, and maintained in the laboratory for future records. Tanks were seeded with 1–4 kg/m^3 to select a density that provided the best yield in 3 weeks. Once sufficient biomass was generated, one 100 m^2 pond was seeded with 3 kg fresh weight (FW) of each of the *Sargassum* sp. and *Ulva* sp. (i.e., 100 kg per pond) and maintained for 3 weeks before being harvested to its original density. The two species were cultured simultaneously for 6 months in the commercial ponds. The seawater was replaced twice a week (previously filtered through a 10 µm filter).

Fatty Acid Analysis

Fatty acids were analyzed based on the method from AOAC (1980), using gas chromatography. 2 g of dried seaweed, was extracted with sufficient petroleum ether. The filtrate (the part that remained) was extracted with 1 mL of 20% BF3-methane in a test tube that was tightly closed and heated in a water bath at a temperature of ± 45°C while shaking for 30 min. The solution was extracted with 2 mL of n-hexane so that 2 layers were formed. The top layer is ester and n-hexane. This layer was injected into the SHIMADZU GC-17A gas chromatograph using a 60 m long capillary column with a diameter of 0.25 mm and a flame ionization detector (FID) as a detector. The injection temperature was programmed from 180 °C for 20 min, then increased in a gradient of 10 °C/min to 220 °C for 15 min. The injector and detector temperatures used are 200 °C and 230 °C respectively with a split ratio of 1:80. Nitrogen is used as a carrier gas with a pressure flow of 0.75 mL/min.

Data Analysis

The analysis used in the early stage of research is the seaweed culture in the dry season and rainy season, namely the Wilcoxon Signed Rank Test (Kusriningrum, 2008). The results showed significant differences when the critical value of $P < 0.05$ (Hidayat and Istiadah, 2011).

RESULTS AND DISCUSSION

Fatty acids of *Sargassum* sp. are dominated by the composition of linoleic acid, palmitic acid, and oleic acid (Table 1), while the fatty acids of *Ulva* sp. are dominated by the composition of heptadecanoic acid, pentadecanoic acid and linoleic acid (Table 3). Mabeau and Fleurence (1993) determined that each seaweed species has its unique genome. Genetics plays an important role in determining the types of chemical compounds produced by seaweed. Roleda and Hurd (2019) stated that the nutrients available in the environment where seaweed grows can also influence the quality and quantity of the active ingredients produced. The availability of nutrients such as nitrogen, phosphorus, and microelements can influence the growth and metabolism of seaweed. Environmental factors such as water temperature, salinity, sunlight, and ocean currents can influence the growth and development of seaweed. Changes in environmental conditions in the dry and rainy seasons, as well as seasonal changes in temperature or pollution, can affect the chemical composition of seaweed and can change the content of its active ingredients.

Sargassum species have genetic variations in the form of subspecies or varieties. Like many other organisms, *Sargassum* can also exhibit genetic polymorphism, meaning that individuals within a species or subspecies can have genetic variations in certain traits. Genetic polymorphism in *Sargassum* sp. can appear in a variety of ways, including in the form of differences in morphology, growth, or reproductive characteristics. Genetic variation in *Sargassum* can be caused by various factors, such as genetic mutations, genetic recombination during sexual reproduction, and environmental selection pressures. Hoang *et al.* (2016) also determined seasonal growth trends in *Sargassum* sp. beds. It was found that *Sargassum* sp. biomass increased during winter and early spring, and stabilized during late spring and early summer, before decreasing during late summer and early autumn.

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This pattern of increase, stabilization, and reduction in biomass is linked to the stages of the *Sargassum* sp. lifecycle, including recruitment and growth (increase in biomass), senescence and reproduction (stabilization of the biomass), and regeneration (reduction in biomass). Anggadiredja (2017) reported that several species of green algae can not grow in high salinity and high temperatures. As a consequence of these particular circumstances, these species of green algae were not able to grow completely during the dry season. On the contrary, several species of red algae and brown algae can not grow in lower salinity and temperature, and, consequently, several species of red algae and brown algae were not able to grow during the rainy season. In such a case, the river plays an important role in lowering the water salinity of the area by contributing fresh water during the rainy season. Temperature is undoubtedly the most fundamental factor for all organisms due to its effects on molecular activities and properties and virtually all aspects of metabolism. In addition, seasonal change also affects the temperature and salinity of ocean water.

Environmental variations have a significant influence on the differences in active ingredients contained in *Sargassum* sp. Li *et al*. (2019) also stated that differences in water temperature can affect the metabolism and production of chemical compounds in seaweed. Higher temperatures tend to accelerate the growth of *Sargassum* sp. but can also affect the composition of the active compounds, thereby changing their quality. The intensity of sunlight received by *Sargassum* sp. is also very important because seaweed carries out photosynthesis. Variations in light levels can change the quality and quantity of active compounds in *Sargassum* sp. Water salinity also affects the growth and metabolism of *Sargassum* sp. *Sargassum* sp. species are more resistant to changes in salinity than others.

Changes in water salinity can affect the quality and content of active compounds in seaweed. The availability of nutrients such as nitrogen and phosphorus can also influence the growth and composition of chemical compounds in *Sargassum* sp. Nutrient-rich water can increase the production of active compounds, while pollution or changes in water quality can reduce it. Currents can also affect the stability of *Sargassum* sp. habitat and change exposure to the environment. These environmental variations can cause differences in the chemical composition and quality of active ingredients in seaweed grown in different locations. Therefore, it is important to understand environmental factors when evaluating the potential health benefits or other applications of *Sargassum* sp. as well as in the context of marine resource conservation and management.

Nutrient variations in the environment can have a major impact on the composition of the active ingredients in *Sargassum* sp. Nitrogen and phosphorus are important nutrients for the growth of marine plants, including *Sargassum* sp. sufficient availability of nitrogen and phosphorus can increase seaweed growth. *Sargassum* sp. also requires other nutrients such as potassium (K), calcium (Ca), and magnesium (Mg) for optimal growth. The availability of other macronutrients in water can influence the production of compounds such as carbohydrates, proteins, and fats in *Sargassum* sp.

These fatty acids determine the quality of the fat itself, so measuring the type and level of fatty acids is very important to determine the quality of the fat. Rocha *et al*. (2021) stated that the fatty acids contained in seaweed fat, based on the highest concentration of fatty acids, are linoleic acid, palmitic acid, oleic acid, linolenic acid, palmitoleic acid, myristic acid, and lauric acid. Meanwhile, the fatty acids included in the R group are unsaturated and have double bonds, namely palmitoleic acid, oleic acid, linolenic acid, and linoleic acid. There are 2 essential fatty acids in the thallus of *Sargassum* sp.: linolenic acid (omega 3 fatty acid) and linoleic fatty acid (omega 6 fatty acid). The

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fatty acid composition of *Sargassum* sp. in different seasons and morphological analysis of *Sargassum* sp. based on season of observation were presented in Table 1 and Table 2.

Table 1. Fatty acid composition of *Sargassum* sp. in different seasons.

Fatty acid composition of *Sargassum* sp.	Fatty acids (%)	
	Dry season	Rainy season
Lauric acid (C12:0)	1.45 ± 0.08	1.52 ± 0.01
Myristic acid (C14:0)	3.53 ± 0.11	4.11 ± 0.97
Palmitic acid (C16:0)	29.49 ± 1.48	27.99 ± 0.07
Palmitoleic acid (C16:1)	4.10 ± 0.24	4.28 ± 0.55
Oleic acid (C18:1)	13.78 ± 1.35	13.99 ± 0.92
Linoleic acid (C18:2)	33.58 ± 1.41	34.03 ± 1.04
Linolenic acid (C18:3)	5.94 ± 1.49	4.99 ± 0.85

Note: Data are the Mean ± SD from at least three independent assays. The research results did not show a significant difference when the critical value $P < 0.05$.

Table 2. Morphological analysis of *Sargassum* sp. based on season of observation.

Season of observation	Lenght of thalli (cm)	Density (individu/m^2)	DW (g/m^2)	FW (g/m^2)
Dry	49.9 ± 4.8	29 ± 3	33.0 ± 1.7	252.0 ± 15.3
Wet	52.4 ± 2.3	32 ± 1	32.8 ± 2.6	254.7 ± 12.9

Note: Temporal variation of biological variables determined in triplicates of quadrats (Means ± SD) thallus density, mean thallus length, and mean estimate of the thallus biomass in dry (DW) and fresh (FW) weights per m^2. Data are the Mean ± SD from at least three independent assays. The research results did not show a significant difference when the critical value $P < 0.05$.

Test results for fatty acid content and morphological conditions of the thallus species *Sargassum* sp. results obtained during the dry season and rainy season did not show significant differences. This is thought to be due to the extreme differences, especially in average rainfall and average daily incidence of solar energy between the conditions of the dry season (August 2006) and the rainy season (November 2006) in the Bangsring Beach area, Banyuwangi. Average weather conditions in August and November in the Bangsring area, Banyuwangi are shown in Table 5.

Hu *et al*. (2022) conducted a genetic variation study on the seaweed species Ulvaceae (Chlorophyta) which could provide important insights into the genetic diversity within the *Ulv*a genus. Xiao *et al.* (2016) stated that certain environmental factors can influence the growth, metabolism, and accumulation of certain compounds in *Ulva* sp. Water temperature is also an important factor influencing the growth and metabolism of *Ulva* sp. Changes in water temperature, average rainfall, and average daily incidence of solar energy (Table 5) can affect the rate of photosynthesis and respiration, which in turn can affect the content of pigments and photosynthetic compounds such as chlorophyll and carotenoids. The intensity of sunlight received by *Ulva* sp. affects the level of photosynthesis. Variations in light intensity can change the composition of pigments and other active compounds in *Ulva* sp. (Wan *et al*., 2022).

Water salinity also plays an important role in the growth of *Ulva* sp. (Bews *et al*., 2021; Bustomy, 2022) Changes in salinity can affect metabolism and accumulation of osmoregulatory compounds in Ulva. In water quality studies, including the availability of nutrients such as nitrogen and phosphorus, are key factors influencing the growth and composition of *Ulva* sp. Nutrient-rich water can increase the accumulation of proteins, carbohydrates, and other compounds in *Ulva*

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sp. Variations in ocean currents can influence the availability of nutrients and oxygen to *Ulva* sp.

Nutrient variations in the environment where *Ulva* sp. grows can have a significant impact on the active ingredient content in this seaweed species (Choi *et al*., 2010). Nutrition is a key factor influencing *Ulva* sp. growth and metabolism. Variations in nutrition can affect the content of various compounds in *Ulva* sp., including pigments, proteins, carbohydrates, and other compounds. Nitrogen and phosphorus are the main nutrients required by *Ulva* sp. for growth and photosynthesis.

Garcia-Poza *et al*. (2022) stated that the active ingredient content in *Ulva* sp. can vary throughout the year due to seasonal changes and environmental conditions related to the season. These nutritional variations may result in differences in the active ingredient composition of *Ulva* sp. grown in various locations and environmental conditions. Therefore, understanding the nutritional conditions at the growing site and seasonal cycles is important in utilizing *Ulva* sp. for food, health, or industrial applications. The fatty acid composition of *Ulva* sp. in different seasons and morphological analysis of *Ulva* sp. based on season of observation were presented in Table 3 and Table 4.

Table 3. Fatty acid composition of Ulva sp. in different seasons.

Fatty acid composition of *Ulva* sp.	Fatty acids (%)	
	Dry season	Rainy season
Pentadecanoic Acid (C15:0)	13.09 ± 1.03	13.01 ± 0.04
Heptadecanoic Acid (C17:0)	41.20 ± 0.09	40.22 ± 1.06
Cis-10-heptadecenoic acid (C17:1)	4.42 ± 0.18	4.43 ± 0.09
Oleic Acid (C18:1 cis-9)	1.76 ± 1.34	1.77 ± 0.21
Linolelaidic Acid (C18:2n9t)	4.28 ± 0.13	4.28 ± 0.11
Linoleic Acid (C18:2n6)	12.41 ± 0.25	12.43 ± 1.18
Arachidic Acid (C20:0)	4.83 ± 0.02	4.79 ± 1.02
g-Linolenic Acid (C18:3n6)	2.52 ± 0.03	2.71 ± 0.51
Heneicosanoic Acid (C21:0)	7.78 ± 0.28	7.69 ± 0.53
Cis-11,14-eicosedienoic acid (C20:2)	8.37 ± 0.07	8.45 ± 0.02
Cis-8,11,14-eicocetrienoic acid (C20:3n6)	1.87 ± 0.06	1.91 ± 0.17

Note: Data are the Mean ± SD from at least three independent assays. The research results did not show a significant difference when the critical value $P < 0.05$.

Table 4. Morphological analysis of *Ulva* sp. based on season of observation.

Season of observation	Lenght of thalli (cm)	Density (individu/m^2)	DW (g/m^2)	FW (g/m^2)
Dry	15.89 ± 2.79	10 ± 1	4.31 ± 2.41	77.24 ± 4.15
Wet	16.91 ± 3.33	11 ±1	3.99 ± 4.18	79.57 ± 2.91

Note: Temporal variation of biological variables determined in triplicates of quadrats (Means ± SD) thallus density, mean thallus length, and mean estimate of the thallus biomass in dry (DW) and fresh (FW) weights per m^2. Data are the Mean ± SD from at least three independent assays. The research results did not show a significant difference when the critical value $P < 0.05$.

Fluctuations in weather conditions and ranges of air temperature, water temperature, the daily incidence of solar energy, rainfall, and humidity in August and November at Bangsring Beach area, Banyuwangi, Indonesia are shown in Table 5.

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Table 5. Average weather conditions in August and November in the Bangsring Beach area, Banyuwangi, Indonesia.

Parameter	August (Dry season)	November (Rainy season)
Air temperature (°C)	23 - 31	24 - 34
Water temperature (°C)	26.5 – 27.0	28.0 – 29.0
Average daily incidence of solar energy (kWh)	6.0 – 6.7	5.1 – 6.4
Average rainfall (mm)	19-23	60-151
Average humidity (%)	97 - 98	100

Source: Meteorological, Climatological, and Geophysical Agency (BMKG) East Java

Interestingly, the fatty acid profile and morphology of the seaweed species *Ulva* sp. obtained in the dry season (August 2006) and the rainy season (November 2006) in the Bangsring Beach area, Banyuwangi had the same results as the profile of *Sargassum* sp. seaweed collected in that area. The difference in fatty acid content and morphological appearance in the dry season and rainy season in this area is also thought to be due to the adaptability of *Ulva* sp. that grows and develops in the area to maintain its optimal conditions (Wang *et al*., 2018). Order Ulvales has similar fatty acid patterns with five components palmitic (16:0), linoleic (18:2), α-linolenic (18:3), hexadecatetraenoic (16:4) and octadecatetraenoic (18:4) acids predominating. Average ratios of palmitic acid in *U. fasciata* amounted to 29.32% of all fatty acids, whereas linoleic, α-linolenic, hexadecatetraenoic, and octadecatetraenoic acids were 7.87, 17.25, 10.57 and 20.49%, respectively. Meanwhile, the average ratios of palmitic, linoleic, α-linolenic, hexadecatetraenoic, and octadecatetraenoic acids of *U. pertusa* were 27.36, 8.09, 17.96, 12.73 and 20.56%, respectively (Alamsjah *et al*., 2009).

Pohl and Zurheide (1979) explained that macroalgae have significant differences in the composition of fatty acids between the individual classes of marine algae, marine and freshwater algae, and algae (in general) and terrestrial plants. Other researchers also believed that the fatty acid composition of macroalgae was related to seasonal changes in environmental factors (Kostetsky *et al*., 2004). On the other side, Khotimchenko *et al.* (2002) determined that specific features of the fatty acid composition of marine algae do not depend on geographical location and are unrelated to seasonal changes.

Since the fatty acids from macroalgae were not significantly different based on seasonal variation, the algicidal activity of both species (e.g., *Sargassum* sp. and *Ulva* sp.) may be used as long as necessary to reduce the impact of several harmful algal bloom species problems. The existence of essential compounds that are relatively stable during the dry season and rainy season is one of the considerations for exploring seaweed fatty acids to produce food supplements, pharmaceutical, and cosmetic components.

CONCLUSION

Based on this research, it can be concluded that the thallus fatty acid levels of *Sargassum* sp. in both the dry and rainy seasons, it is dominated by the composition of linoleic acid (C18:2), palmitic acid (C16:0) and oleic acid (C18:1). Likewise, the fatty acid levels of the thallus of *Ulva* sp. in the dry season and rainy season it is dominated by the composition of heptadecanoic acid (C17:0), pentadecanoic acid (C15:0) and linoleic acid (C18:2n6). The results of the fatty acid test in the dry season and rainy season did not show significant differences. Even though there are differences in water temperature, daily incidence of solar energy, and rainfall, they do not cause interference with the metabolic process of forming fatty acid components in *Sargassum* sp. and *Ulva* sp. The same profile was also shown from the length of thalli, density, dry weight, and fresh weight of the two species, whether

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collected in the dry season or the rainy season, which did not show significant differences. This pattern remains the same from year to year so seasonal production can be predicted. Understanding the composition and amount of active ingredients, fatty acids, and other potential compounds based on seasonal and annual variations in the production of cultivated and naturally obtained seaweed, will make it easier for practitioners to explore seaweed more efficiently and effectively to fulfill food supplement components, pharmaceuticals, and cosmetic needs.

CONFLICT OF INTEREST

The author declares there is no conflict of interest.

AUTHOR CONTRIBUTION

Mochammad Amin Alamsjah: author correspondence, principal researcher, collecting data, analysis, and manuscript writing. Adibi Rahiman Bin Md Nor: co-author correspondence, research member, and data analysis.

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