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The Effect of Cultivation Techniques in The Dry and Rainy Seasons on The Quality of The Seaweed *Caulerpa racemosa*

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Abstract

The seaweed *Caulerpa racemosa* has great potential to be developed because it has economic value and potential as a functional food. This seaweed contains nutrients, including protein, carbohydrates, polyunsaturated fatty acids, fiber minerals, and antioxidants. The availability of seaweed is still very dependent on nature and has not been appropriately cultivated. The planting season and appropriate cultivation methods will influence seaweed's quality/nutritional content. This research aims to analyze the nutritional content of seaweed *C. racemosa* cultivated in different growing seasons. The research method used a floating raft system with a completely randomized design and a factorial pattern carried out in two seasons, namely the dry and rainy seasons. Proximate analysis was carried out to determine the nutritional composition of seaweed C. racemosa, including protein, crude fiber, water, ash, fat, and carbohydrate (NFE). Next, it was analyzed using ANOVA with a further test with Tukey. The research results showed differences in the nutritional composition of seaweed *C. racemosa* from each treatment cultivated in the dry and rainy seasons. The nutritional composition of seaweed *C. racemosa* best treatment is found in the plant spacing of 30 cm with a depth of 50 cm in the dry season with the nutritional content namely protein (13.8%), NFE (20.75%), crude fiber (15.71%), a fluctuating fat content of 0.16-1.42%, highest ash (34.69%), water content (35%), moderate in the rainy season namely: protein (10.8%), BETN (10.78%), crude fiber (12.33%), fat content fluctuates (0.18-1.06%), highest ash (30.21%), water content (41.01%).

INTRODUCTION

Seaweed *Caulerpa* sp. is one of the seaweed commodities utilized by the community directly as food in the form of fresh vegetables. It is a potential functional

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food (Wells *et al*., 2017) since it has a delicious taste and crunchy texture (Darmawati *et al*., 2022). It contains food and nutritional values (Aroyehun *et al*., 2020; Pangestuti *et al*., 2021; Stuthmann *et al*., 2021), including protein, fiber, minerals, vitamins, polyunsaturated fatty acids (Bhuiyan *et al*., 2016) as well as bioactive antioxidants for health benefits (Kumar *et al*., 2019; Sommer *et al*., 2022). Seaweed *Caulerpa* sp. is included in Feather Seaweed (Sinurat and Fadjriah, 2019). It is reported that macroalgae consumed in fresh form have bioactive substances such as anti-bacterial, anti-fungal, and antitumor. Have a potential nutraceutical value (Syakilla *et al*., 2022).

Researchers have conducted several studies regarding the nutritional composition of seaweed *Caulerpa* sp. which is sourced from nature. Hao *et al*. (2019) found that the seaweed *Caulerpa* sp. contained total carbohydrates (71.67%), crude protein (11.39%), crude lipid (1.03%), essential amino acids (40.07%), monounsaturated fatty acids (25.72%), and polyunsaturated fatty acids (26.33%). The following researchers found that lentiviral seaweed *Caulerpa* sp. has nutritional content such as protein (14.4%), fat (0.85%), total ash (41.85%), and carbohydrates (32.95%) (Sinurat and Fadjriah, 2019). Furthermore, Tapotubun *et al*. (2020) suggested that the seaweed *Caulerpa* sp. can reduce cholesterol because it contains high fiber, omega-3 fatty acids such as eicosapentaenoic acid, and as an antioxidant, because it contains polyphenols, vitamin C, α-tocopherol, carotenoids, and selenium. The composition of these nutrients differs between species and within one species depending on the harvest age (Darmawati *et al*., 2022), species habitat, and season (Stuthmann *et al*., 2022). Therefore, the season and the planting system will affect the seaweed's nutritional content.

Proper seaweed cultivation must be accompanied by developing cultivation technology that can increase the overall quantity and quality of the seaweed. Implementing inappropriate cultivation technology will cause the quantity and quality of the nutritional content of seaweed to be less than optimal. One of the seaweed cultivation technologies is setting the planting distance and depth related to growing space and absorption of sunlight as a regulator of the photosynthesis process for growth. Besides that, each type of seaweed has characteristics and specifics related to its ability to live, adapt, and grow in certain aquatic environmental conditions (Darmawan *et al*., 2022).

Seasonal changes will have an impact on marine environmental conditions and will affect the quality and nutritional content of seaweed. Until now, there is no data and information regarding the nutritional composition aspect of seaweed C*aulerpa sp*. cultivated in different seasons, which supports its utilization as a nutritionally valuable food source. Thus, research is necessary to obtain a better nutritional composition of seaweed C*aulerpa sp*. This research is expected to provide insight into the widespread utilization of seaweed C*aulerpa sp*. is not only consumed by the community as a vegetable but can be utilized as a nutritious food product. Seaweed development will produce high economic value.

METHODOLOGY

Ethical Approval

In this study, it is noteworthy that no animals were employed. The object of this research was seaweed. Sampling was carried out carefully. Ethical approval for this research aligns with the guidelines set for scientific and responsible research. The research proposal was also reviewed and approved by the review team of the Institute for Research Development and Community Service, Muhammadiyah University of Makassar.

Place and Time

This research was carried out in April-May 2022 for the dry season and November-December 2022 for the rainy season. Cultivation of *C. racemosa* was implemented

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in the waters of the village Takalar Laguruda district, the province of South Sulawesi with a research spot with the coordinate of 05°26′07,9″ S and 119°22′29,9″ E. *C. racemosa* seaweed quality test was carried out at the Water Productivity and Quality Laboratory, Faculty of Marine and Fisheries Sciences, and the Animal Nutrition Laboratory, Faculty of Animal Husbandry, Hasanuddin University, Makassar.

Research Materials

The materials used in this research were Seaweed *C. racemosa* which came from Takalar waters, 0.3 M NaOH, HCl, n-Hexane, boric acid, 96% alcohol, bromocresol green solution, methyl red solution, and distilled water.

Research Design

This research was designed using a completely randomized design (CRD) with a factorial pattern consisting of two factors. Factor I, *C. recemosa* planting distance with 3 levels (20, 30, and 40cm), Factor II, *C. recemosa* planting depth which also consists of 3 levels (50, 100, and 150cm). This research had 9 treatment combinations, namely: A1B1 (planting distance of 20 cm with a planting depth of 50 cm from the water surface), A1B2 (planting distance of 20 cm with a planting depth of 100 cm from the water surface), A1B3 (planting distance of 20 cm with a planting depth of 150 cm from the water surface), A2B1 (planting distance of 30 cm with a planting depth of 50 cm from the water surface), A2B2 (planting distance of 30 cm with a planting depth of 100 cm from the water surface), A2B3 (planting distance of 30 cm with a planting depth of 150 cm from the water surface), A3B1 (planting distance of 40 cm with a planting depth of 50 cm from the water surface), A3B2 (planting distance of 40 cm with a planting depth of 100 cm from the water surface), A3B3 (planting distance of 40 cm with a planting depth of 150 cm from the water surface). Each treatment was repeated 3 times so that in total there were 27 experimental units.

Work Procedure
Seaweed Cultivation

Cultivation of *C. racemosa* was carried out by using a floating raft system with a floating mono-line method in the dry seasons and rainy seasons. The initial weight of the seeds is 50 grams/hill, and the spacing per clump point according to the treatment factor ware namely A1=20 cm, A2=30 cm, and A3=40 cm with a depth of B1=50 cm, B2=100 cm, and B3=150 cm from the water surface. Furthermore, it was maintained for 35 days, and then sampling was carried out to test the quality of the cultivated seaweed *C. racemosa*. The cultured seaweed samples were dried for 3 days, and then the dried seaweed was powdered and filtered. The powder seaweed was stored in desiccators at room temperature until the chemical analysis.

Proximate Analysis

The proximate analysis of protein, crude fiber, moisture, fat, ash content, and carbohydrate content (nitrogen-free extract) of seaweed *C. racemosa* was determined according to the standard method (AOAC, 2005). The protein content of *C. racemosa* was determined using the Kjeldahl apparatus and following the prescribed methods: digestion, distillation, and titration. The nitrogen content was converted to protein by multiplying by 6.25.

To measure the crude fiber, 2 g of seaweed samples were previously boiled with diluted H_2SO_4 (0.3 N). Then, the mixture was filtered and washed with 200 ml of boiling water and NaOH (0.5 N), respectively, and then re-extracted and washed with boiling water and acetone. Finally, the residual was dried at 105°C for 3 h to constant weight (Siddique *et al*., 2013a).

The moisture content of *C. recemosa* was determined by following the methods prescribed by AOAC (2005). The sample (5 g) was taken and kept in an oven at 105°C to constant weight. Then, the moisture content was calculated by subtracting the final weight from the initial weight of the sample (Sadou *et al*., 2007). The fat content of *C.*

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racemosa was extracted from the seaweed powder with petroleum ether in a soxhlet extractor. After the extraction process, petroleum ether evaporated, and the residue dried to a constant weight at 105° C.

To determine the ash content of seaweed *C. racemosa*, 5 g of the sample was kept in an oven at 100ºC overnight to release its moisture. After the moisture was removed, the sample was burned to remove the carbon particles. The ash content was obtained by calcination in a muffle furnace at 550°C for 4 h. The results were calculated as a percentage of dry matter (Sadou *et al.*, 2007). Carbohydrates were determined simply by subtracting the mean percentage of protein, lipid, fiber, moisture, and ash from 100 and expressed as nitrogen-free extract content (Siddique *et al.*, 2013b).

Water Quality Parameters

Measurement of water quality parameters started at the beginning of planting and is continued every week until the end of the maintenance period. The parameters measured included pH with a pH meter (MW102 PRO, Milwaukee), temperature with a thermometer (digital thermometer, MN Measurement), salinity with a refractometer (SainSonic Refractometer, Amazon), light intensity with a lux meter (MW700, Milwaukee), carbon dioxide with a CO_2 detector (ST302 Handheld, Newswan), current speed with a current meter, nitrate, and phosphate with the Hanna HI KIT 713.

Data Analysis

The effect of treatment (spacing and depth) on the nutritional composition of *C. racemosa* (protein, crude fiber, moisture, fat, ash content, and NFE) was analyzed using ANOVA. If there is a difference between treatments, proceed with the Tukey HSD further test (α = 0.05) confidence interval using SPSS program version 20. The relationship between water quality parameters and the nutritional content of *C. racemosa* data was analyzed using PCA (Principal Component Analysis) using XLSTAT 2017.02.44125 to make it easier and interpretable in graphical form.

RESULTS AND DISCUSSIONS

Protein Content of Seaweed *C. racemosa*

The protein content of *C. racemosa* at various combinations of spacing and depth during the dry and rainy seasons can be seen in Figure 1.

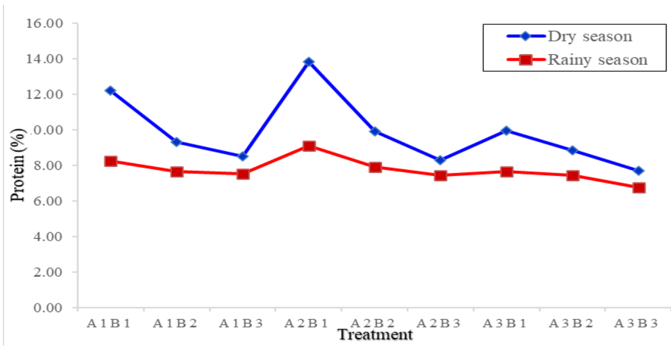

Figure 1. The protein content (%) of Seaweed *Caulerpa* sp. during the dry and rainy season.

Description: (A1B1) spacing 20cm-50cm depth; (A1B2) spacing 20cm–100cm depth; (A1B3) spacing 20cm–150cm depth; (A2B1) spacing 30cm–50cm depth; (A2B2) spacing 30cm–100cm depth; (A2B3) spacing 30cm–150cm depth; (A3B1) spacing 40cm – 50cm depth; (A3B2) spacing 40cm–100cm depth; (A3B3) spacing 40cm–150cm depth.

The protein content of *C. racemosa* cultivated in the dry season was higher than in the rainy season. *C. racemosa* cultivated in the dry season produces 7.71-13.80% protein content. Meanwhile, *C. racemosa* cultivated in the rainy season produces 6.47-

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10.80%. The highest protein content was obtained in the 30 cm spacing and 50 cm depth treatment (A2B1), because in this treatment, the nutritional adequacy and photosynthesis process went well, affecting the protein content of *C. racemosa*. It is in line with the statement by Stuthmann *et al.* (2021) and Cai *et al.* (2021), that the increase in light will affect the photosynthetic process, which will stimulate seaweed to utilize or absorb sufficient nutrients such as nitrates and phosphates, which are essential for protein preparation. With a relatively higher intensity of sunlight reaching the seaweed thallus, the process of photosynthesis and nutrient absorption takes place more effectively.

Spacing affects water traffic that carries nutrients, thallus area of seaweed exposed to sunlight and indirectly affects the photosynthesis process (Stuthmann *et al.*, 2021). The depth of the water affects the penetration of sunlight for photosynthesis which affects the protein content of *C. racemosa* (Huang *et al.*, 2020) stated that as an organism carrying out the photosynthetic process, the chemical composition of seaweed could be influenced not only by the concentration of aquatic nutrients but also the depth of the water.

The research results also showed that the protein content of Seaweed *C. racemosa* cultivated in the dry season was higher than that cultivated in the rainy season. As explained by Windarto *et al.* (2021), the quality of seaweed complying with the standards for the cultivation process is primarily determined by the season due to variations in environmental conditions such as light intensity and water nutrients. Furthermore Cai *et al.* (2021) stated that the availability of nutrients in the waters, especially the availability of nitrogen, affects seaweed protein levels. The level of nitrate content measured during the research ranged from 0.11 – 2.6 ppm, this range is suitable for seaweed cultivation according to the statement of Guo *et al.* (2015b) that good nitrate for seaweed cultivation is in the range of 0.1 – 3.5 ppm.

Crude Fiber content of Seaweed *C. racemosa*

The crude fiber content of *C. racemosa* cultivated in the dry season was higher than those cultivated in the rainy season (Figure 2), with the highest crude fiber content obtained in the treatment of plant spacing 30 cm - 50 cm depth (A2B1).

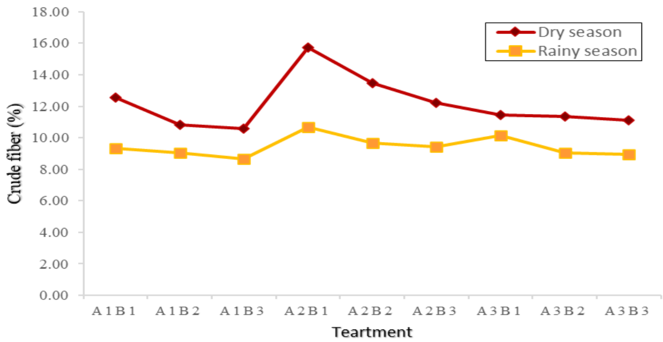

Figure 2. Crude fiber (%) of Seaweed *Caluerpa* sp. during the dry and rainy season.
Description: (A1B1) spacing 20cm-50cm depth; (A1B2) spacing 20cm–100cm depth; (A1B3) spacing 20cm–150cm depth; (A2B1) spacing 30cm–50cm depth; (A2B2) spacing 30cm–100cm depth; (A2B3) spacing 30cm–150cm depth; (A3B1) spacing 40cm – 50cm depth; (A3B2) spacing 40cm–100cm depth; (A3B3) spacing 40cm–150cm depth.

The crude fiber content of seaweed cultivated in the dry season varies between 10.57-15.71%, Meanwhile, the crude fiber of C. racemosa, which is cultivated in the rainy season between 8.91-12.33%, with the highest crude fiber content obtained in the treatment of spacing 30 cm - 50 cm depth (A2B1).

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Treatment spacing of 30cm–50cm depth gave the optimal response to the crude fiber content among all treatments. Depth affects the level of light intensity that enters the water. Changes in the intensity and quality of light penetrating the water with increasing depth affect the ability of *Caulerpa* sp. to absorb sunlight to carry out the photosynthesis process. Meanwhile, the spacing will affect the area of the seaweed thallus exposed to sunlight, so it will also affect the photosynthesis process. The photosynthesis process produces carbohydrates, especially polysaccharides which are generally in the form of fiber. Therefore, the deeper the water and the smaller the spacing, the photosynthesis process of seaweed decreases so that the carbohydrate content which is a component of crude fiber also decreases. These carbohydrate constituents are further products of photosynthesis, the levels of which are influenced by the rate of the photosynthetic process (Burfeind and Udy, 2009).

Moisture Content of Seaweed *C. racemosa*

C. racemosa cultivated in the rainy season had a higher moisture content than those cultivated in the dry season (Figure 3). The moisture content of *C. racemosa* tended to decrease in various treatment combinations (spacing depth).

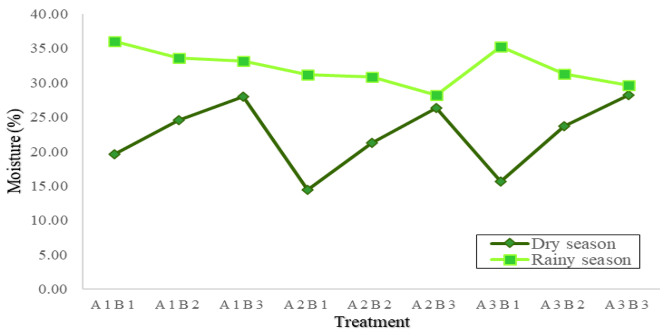

Figure 3. Moisture content (%) of Seaweed *Caluerpa* sp. during the dry and rainy season.
Description: (A1B1) spacing 20cm-50cm depth; (A1B2) spacing 20cm–100cm depth; (A1B3) spacing 20cm–150cm depth; (A2B1) spacing 30cm–50cm depth; (A2B2) spacing 30cm–100cm depth; (A2B3) spacing 30cm–150cm depth; (A3B1) spacing 40cm – 50cm depth; (A3B2) spacing 40cm–100cm depth; (A3B3) spacing 40cm–150cm depth.

The difference in moisture content of Seaweed *C. racemosa* cultivated in the dry and rainy seasons is caused by differences in the physical properties of the environment, especially salinity, which causes differences in osmoregulation processes in seaweed cells. In the rainy season, water salinity is low, so water continuously enters the seaweed thallus passively through a process of osmosis. This occurs because the salt content in the seaweed thallus is higher than that in the environment.

This situation causes the water content of seaweed in the rainy season higher than in the dry season. The water quality parameter that plays a major role in thallus formation and the morphogenetic development of seaweed is salinity because it is directly related to osmoregulation that occurs in cells (Choi *et al*., 2010).

Fat Content of Seaweed *C. racemosa*.

The fat levels of *C. racemosa* tended to fluctuate in each treatment during the dry and rainy seasons can be seen in Figure 4.

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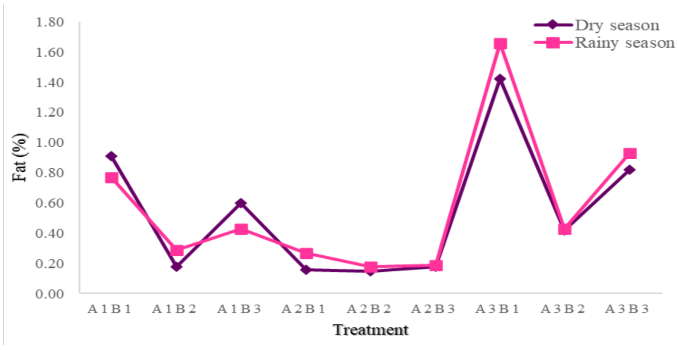

Figure 4. Fat content (%) of Seaweed *Caluerpa* sp. during the dry and rainy season.
Description: (A1B1) spacing 20cm-50cm depth; (A1B2) spacing 20cm–100cm depth; (A1B3) spacing 20cm–150cm depth; (A2B1) spacing 30cm–50cm depth; (A2B2) spacing 30cm–100cm depth; (A2B3) spacing 30cm–150cm depth; (A3B1) spacing 40cm – 50cm depth; (A3B2) spacing 40cm–100cm depth; (A3B3) spacing 40cm–150cm depth.

The fat level of *C. racemosa* at various combinations of spacing and depth in the dry season varies between 0.15-1.42%, while in the rainy season varies between 0.18-1.06%. From the research results, it was found that the lowest fat content of *C. racemosa* seaweed was obtained when the planting depth was 50 cm at each planting distance of 20 cm, 30 cm, and 40 cm, whether cultivated in the dry or rainy season. Ukabi *et al*. (2013) state that seaweed is an organism that carries out the process of photosynthesis, its nutritional composition is not only influenced by the concentration of water nutrients but also by water temperature and water depth which are also influenced by seasonal variations. Seaweed fat contains high omega-3 and omega-6 fatty acids (Rocha *et al*., 2021; Gamero-Vega *et al*., 2020). These two fatty acids are essential for the body, especially as forming brain tissue membranes.

Ash Content of Seaweed *C. racemosa*

The ash content of C. racemosa at various spacing and depth combinations varies during the dry and rainy seasons (Figure 5). During the dry season, the treatment spacing of 20 cm – 50 cm depth (A1B1) and treatment spacing of 40 cm – 50 cm depth (A3B1) tended to decrease.

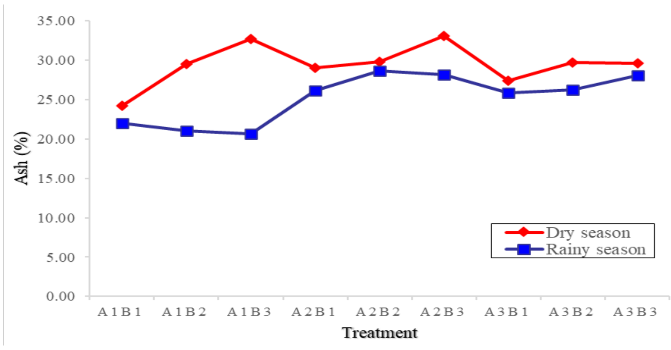

Figure 5. Ash content (%) of Seaweed *Caluerpa* sp. during the dry and rainy season.
Description: (A1B1) spacing 20cm-50cm depth; (A1B2) spacing 20cm–100cm depth; (A1B3) spacing 20cm–150cm depth; (A2B1) spacing 30cm–50cm depth; (A2B2) spacing 30cm–100cm depth; (A2B3) spacing 30cm–150cm depth; (A3B1) spacing 40cm – 50cm depth; (A3B2) spacing 40cm–100cm depth; (A3B3) spacing 40cm–150cm depth.

Observation results in Figure 5 showed that the ash content of *C. racemosa* at various spacing and depth combinations varies during the dry and rainy seasons. The increasing planting depth tended to increase the ash content of *C. racemosa*. This

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is as stated by Circuncisao *et al*. (2018) and Kasmiati *et al*. (2022), The depth of C. *racemosa* planting affects the large variations in the amount of minerals and organic components on the bottom of the water and the nature of the water depth, distance from the soil and the environment affect the amount of minerals as a component of the ash content in seaweed.

There were variations in the ash content of *C. racemosa* in each treatment combination spacing and depth both during the dry and the rainy season. The high ash content in seaweed is related to how it absorbs mineral nutrients and how it absorbs mineral nutrients. It is a form of adaptation to environmental conditions in marine waters, which contain various minerals with high concentrations. The ash content in seaweed consists of macro-minerals and trace elements, so seaweed has potential as a food source of minerals (Circuncisao *et al*., 2018; Premarathna *et al*., 2022). The high and low ash content can be related to the number of minerals in the material. The higher the mineral content, the higher the ash content.

NFE content of Seaweed *C. racemosa*

The NFE content of *C. racemosa* at various combinations of spacing and depth in the dry and rainy seasons varied in the dry season (Figure 6).

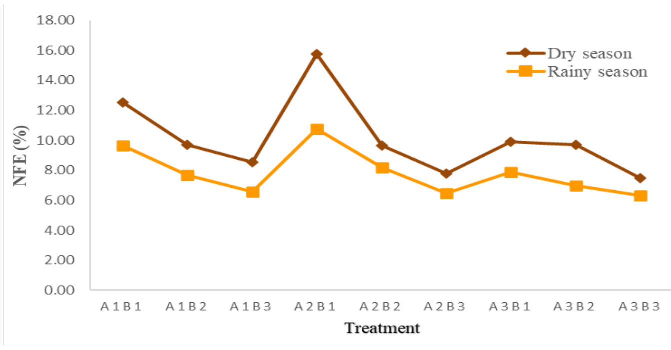

Figure 6. Carbohydrate (NFE) (%) of Seaweed *Caluerpa* sp. during the dry and rainy seasons.

Description: (A1B1) spacing 20cm-50cm depth; (A1B2) spacing 20cm–100cm depth; (A1B3) spacing 20cm–150cm depth; (A2B1) spacing 30cm–50cm depth; (A2B2) spacing 30cm–100cm depth; (A2B3) spacing 30cm–150cm depth; (A3B1) spacing 40cm – 50cm depth; (A3B2) spacing 40cm–100cm depth; (A3B3) spacing 40cm–150cm depth.

The NFE content of *C. racemosa* at various combinations of spacing and depth in the dry and rainy seasons varied (Figure 6). In the dry season, NFE levels varied between 7.52–20.75%. In the rainy season, the NFE content was 6.31-10.77%, with the highest NFE content obtained at the 30 cm-depth 50 cm spacing treatment (A2B1). In green seaweed, carbohydrates are formed from the reaction of CO_2 and H_2O with the help of sunlight through the photosynthetic process.

The increase in the content NFE of seaweed *C. racemosa* occurred in the treatment combination of 30 cm - 50 cm depth spacing during the dry and rainy seasons. This showed that 30 cm – 50 cm depth and spacing was the treatment that produced the highest increase in NFE levels.

NFE levels in the dry season are relatively higher compared to the rainy season. According to Guo *et al*. (2015a; 2015b), the carbohydrate content of seaweed is influenced by temperature, salinity, and sunlight intensity. It is stated that sufficient irradiation for seaweed will allow seaweed to carry out the photosynthesis process optimally so that carbohydrate production will be perfect for its growth. However, conditions of lack of light disrupt metabolism, causing a decrease in the rate of photosynthesis and

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carbohydrate synthesis (Pacheco *et al.*, 2021).

Relationship of Water Quality and *C. racemosa*

Water quality measurements include pH, temperature, salinity, light intensity, CO_2, current speed, nitrates, and phosphates can be seen in Table 7.

Table 1. Range of water quality parameters during the study

Environmental parameter	Range/treatment (water depth)		
	50cm	100cm	150cm
pH	7.3 – 8.3	7.4 – 7.69	7.32 – 7.63
Temperature (°C)	29.0 – 33.0	28.0 – 30.0	28.0 – 30.0
Salinity (ppt)	29.0 – 30.0	29.0 – 30.0	29.0 – 30.0
Light intensity (lux)	1413 – 3752	1320 – 3275	1295– 3130
CO_2 (ppm)	17.38 – 28.38	15.98 – 20.78	17.58 – 19.98
Current speed (cm/sec)	0.084 – 0.302	0.084 – 0.388	0.154 – 0.244
Nitrate-NO_3 (ppm)	0.11 – 0.97	0.1 – 0.8	0.11 – 2.6
Phosphate-PO_4 (ppm)	0.16 – 0.60	0.16 – 0.40	0.25 – 0.62

Table 1 shows that the water quality range measured during the research at each depth treatment is suitable for cultivating C. *racemosa*. Water quality greatly influences the life of macroalgae, such as salinity, pH, nutrient availability, and light (Herawati *et al.*, 2021) and water temperature, among the most critical factors in regulating organism metabolism (Guo *et al.*, 2015a). The water quality parameters, namely pH, temperature, salinity, light intensity, and carbon dioxide, positively affected the nutritional content of *C. racemosa*. The current velocity was very influential on the crude fiber *C. racemosa*. Phosphate and nitrate positively affected the ash content of *C. racemosa*.

CONCLUSION

Based on the research results, it can be concluded that the quality of *C. racemosa* seaweed cultivated in the dry season (April–May) is higher than in the rainy season (November – December). *C. racemosa* seaweed cultivated in the dry season contains a high nutritional value, namely protein (13.8%), carbohydrates (20.75%), and crude fiber (15.71%), while that cultivated in the rainy season contains protein (10.8%), carbohydrates (10.78%), and crude fiber (12.33%). The cultivated *C. racemosa* seaweed obtained the best protein, crude fiber, and carbohydrate (NFE) content at a planting distance of 30 cm with a depth of 50 cm. The content of protein, crude fiber, and carbohydrates decreased with the increasing planting depth, meanwhile, the content of water and ash increased with the increasing plating depth, and fat content fluctuated.

CONFLICT OF INTEREST

The authors declare no conflict of interest in writing and publishing this manuscript.

AUTHOR CONTRIBUTION

P.H.T. Soedibya directed that the manuscript can be obtained properly. Asni Anwar and Beddu Tang have been the best partners during the research.

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