DOI: http://dx.doi.org/10.15578/iaj.19.2.2024.167-178 *Indonesian Aquaculture Journal, 19 (2), 2024, 167-178*

Available online at: http://ejournal-balitbang.kkp.go.id/index.php/iaj

GENETIC DIVERSITY, GROWTH AND CARRAGEENAN QUALITY OF KOTONI (RED SEAWEED) ACROSS THREE CULTIVATION SITES IN EASTERN INDONESIA

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(Received: September 9, 2024; Final revision: November 11, 2024; Accepted: November 11, 2024)

ABSTRACT

*Indonesia is a leading global producer of seaweed, with kotoni seaweed highly valued for its high-quality carrageenan, an essential ingredient in various industries. This study aimed to evaluate the growth, carrageenan quality, and genetic diversity of kotoni seaweed cultivated at three distinct sites in eastern Indonesia: Banggai, West Halmahera, and Biak. The research involved cultivating kotoni seaweed over a 45-day period using the long-line technique, with growth monitoring conducted every five days and water quality parameters measured concurrently. Additionally, ex situ analyses of nitrate, phosphate, and ammonia were performed every 15 days. Carrageenan quality was assessed by measuring carrageenan content, gel strength, and viscosity, in addition to proximate composition analysis. Genetic diversity was evaluated using the cytochrome oxidase subunit 1 (COI) gene, involving DNA extraction, PCR amplification, and sequencing to determine genetic similarity across the cultivation sites. Significant differences in growth rates and carrageenan quality were observed across the three sites. West Halmahera exhibited the highest growth rate, making it the most favorable site for large-scale seaweed cultivation. Although Biak had a lower growth rate, it produced carrageenan with superior gel strength and viscosity, indicating higher product quality. Genetic analysis confirmed 100% similarity among the samples across sites. These findings underscore the importance of site-specific cultivation practices to optimize both yield and carrageenan quality, supporting the sustainability and economic viability of kotoni seaweed cultivation in Indone*sia.

KEYWORDS: Carrageenan; cultivation sites; genetic diversity; kotoni; seaweed cultivation

INTRODUCTION

Indonesia is a leading global producer of seaweed, with its cultivation providing essential livelihoods for numerous coastal communities. The cultivation of seaweed, particularly high-value species like Kotoni, contributes significantly to the economy while offering environmental benefits such as carbon sequestration, water purification, and marine habitat creation (Rimmer *et al*., 2021). Kotoni seaweed, prized for its high-quality carrageenan, is in strong global demand due to its applications across various industries, including food, pharmaceuticals, and cosmetics. Its robust growth and adaptability to different marine environments make it especially favorable for cultivation (García-Poza *et al*., 2020). Indonesia's focus on cultivating species like *Kappaphycus* and *Eucheuma*, with millions of tons produced annually, highlights the nation's dominance in the global seaweed industry (Kim *et al*., 2017). In contrast to countries like China, Korea, and the Philippines, where large corporations often control seaweed farms, Indonesia's seaweed industry is more community-centered, directly engaging local populations. This approach benefits households economically and socially, particularly empowering women and enhancing community well-being (Larson *et al*., 2021). The Indonesian government is also working to diversify the species cultivated and advance aquaculture techniques, aiming to further enhance the industry (Zuccarello & Paul, 2019).

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Despite these advances, the seaweed industry in Indonesia faces challenges impacting the growth, quality, and sustainability of production. Environmental variability - such as fluctuations in water temperature, salinity, and light - affects both growth performance and product quality (García-Poza *et al*., 2020). Additionally, monoculture practices and limited genetic strains increase susceptibility to diseases and environmental stresses, raising concerns about genetic erosion and loss of diversity (Biris-Dorhoi *et al*., 2020). Variations in carrageenan content and texture across seaweed crops contribute to inconsistent product quality, affecting market value and consumer confidence. This highlights a need for research into how cultivation conditions and genetic factors influence product characteristics (Roleda & Hurd, 2019). Variability in quality can often be attributed to both environmental conditions and genetic differences across species (Biris-Dorhoi *et al*., 2020). Understanding the impact of environmental changes, such as rising temperatures, on seaweed growth is crucial to sustainable cultivation practices (Kumar *et al*., 2020).

Genetic diversity is essential for the resilience of seaweed populations against diseases, pests, and environmental changes. Research has shown varying levels of genetic diversity in seaweed species, including Kotoni, across different cultivation regions, underscoring the importance of understanding how genetic variation affects growth performance and product quality in diverse environments (Rimmer *et al*., 2021). For instance, geographic location has a significant influence on genetic variation within Kotoni seaweed populations (Risjani & Abidin, 2020).

Assessing Kotoni seaweed quality involves examining factors like carrageenan content, gel strength, viscosity, and nutritional profile, as these parameters determine its market value and suitability for industrial processing. The quality of cultivated seaweed is influenced by factors such as water quality, temperature, and harvest timing, which impact the concentration of valuable compounds like carrageenan (Rimmer *et al*., 2021).

There remains limited data on the genetic diversity of Kotoni seaweed across different cultivation sites. Comprehensive studies examining the relationship between genetic variation, growth performance, and product quality are needed. Comparative studies across diverse environments would provide valuable insights into the interactions between genetic diversity, environmental factors, and cultivation practices. Through in-depth analysis at multiple cultivation sites, this study aims to contribute to developing sustainable and optimized cultivation practices for Kotoni seaweed in Indonesia. The primary objective is to evaluate the growth performance of Kotoni seaweed in three distinct cultivation sites in eastern Indonesia, analyze product quality at these sites, and assess the genetic diversity of Kotoni seaweed.

MATERIALS AND METHODS

Kotoni seaweed source and research sites

The materials employed in this study included Kotoni seaweed seeds sourced from local farmers across three distinct cultivation sites: Biak, Banggai, and West Halmahera (Table 1). These seeds were selected based on their adaptation to local environmental conditions over a period of at least two years, thereby ensuring their suitability for the experimental conditions.

Seaweed cultivation trial

The seaweed cultivation trials utilized the longline cultivation technique over a single rearing cycle of 45 days. Initial seedlings, each weighing approximately 50 g, were tied to cultivation ropes using polyethylene rope (1.5 mm diameter) at intervals of 15 cm along each cultivation rope. The cultivation ropes, constructed of polyethylene (4 mm diameter) and measuring 25 m in length, were deployed with 20 ropes per cultivation site. The planting depth for the seedlings was maintained at 25–30 cm from the water surface.

Table 1. Seaweed cultivation sites

Cultivation Sites	Geograhical Coordinates
Padaido, Biak, Papua	S 2° 42’ 53.23”
	E 136° 24’ 22.91”
Pagimana, Banggai, Central Sulawesi	S 0°47’42”
	E 122°38’52”
Jailolo, West Halmahera, North Maluku	N 1°2’58”
	E 127°26’49”

Growth monitoring was conducted every five days by weighing marked seaweed clumps to assess their development. Due to logistical constraints, only 30 seaweed clumps were weighed at each cultivation site. Simultaneously, water quality was monitored in situ at the same interval, assessing parameters such as temperature, pH, and salinity. Additionally, ex situ water quality measurements were conducted every 15 days to analyze nutrient parameters, including nitrate, phosphate, total ammonia nitrogen (TAN), and total suspended solids (TSS). Water samples, collected using 500 ml polyethylene bottles, were analyzed at the Water Quality Laboratory, Research Institute for Coastal Aquaculture and Fisheries Extension (RICAFE), Maros, South Sulawesi. Nitrate concentrations were determined using the cadmium reduction method, while phosphate levels were measured via the ascorbic acid method (APHA, 2012). TAN was quantified through the phenate method, and TSS was assessed using the gravimetric method (APHA, 2012).

Carrageenan quality assessment

The evaluation of carrageenan quality included several key parameters: carrageenan content, gel strength, viscosity, and proximate composition. Initially, the seaweed samples were dried before being used for further analysis. Approximately 50 g of dried seaweed was cleaned with distilled water and subsequently subjected to a series of treatments.

The seaweed was first boiled in a 0.6% KOH solution at a 1:6 ratio for two hours, maintaining a temperature between 60 and 65°C. After this treatment, the seaweed was thoroughly rinsed until the pH approached neutral (approximately pH 7). Next, the seaweed was boiled in distilled water at a 1:18 ratio for two hours at 90°C. The resulting filtrate was then filtered and frozen overnight. Upon thawing, the filtrate was precipitated with 2-propanol, resulting in carrageenan fiber formation, which was subsequently dried in sunlight and weighed.

To assess the gel strength of the carrageenan, 1.5 grams of ground carrageenan were dissolved in 100 mL of a 0.5% KCl solution. This mixture was heated to 80°C with continuous stirring for 15 minutes. The solution was then poured into a glass cylinder (9 cm height, 7 cm diameter) and covered with aluminum foil. The gel was allowed to solidify at room temperature for 12 to 15 hours, after which the gel strength was measured using a Gel Strength Apparatus (CT3 Texture Analyzer).

Viscosity measurements were performed in the Hasanuddin University laboratory using a rotary viscometer (Brookfield RVT, USA). Viscosity was assessed using a 1.5% carrageenan solution at 75°C. For this, a 7.5 g sample of carrageenan powder was dissolved in 500 mL of distilled water with continuous agitation for 20 minutes. The solution was then heated on a hotplate for 20–30 minutes until reaching 80°C, while maintaining constant stirring. Viscosity, expressed in centipoise (cP), equivalent to one millipascal second (mPa·s), was measured using a viscometer with spindle number 2 at a speed of 60 rpm.

Proximate composition analysis was conducted at RICAFE Maros following the standardized procedures of AOAC (1990). Moisture content was determined by drying samples at 105°C for 16 hours in an oven (Memmert, Germany). Ash content was measured using a muffle furnace at 550°C (Barnstead, Thermolyne, CA, USA). Crude protein was quantified through the micro-Kjeldahl method, which includes digestion, distillation, and titration. Lipid content was measured via extraction using a solvent mixture of chloroform and methanol.

Genetic identification of seaweed

Thallus samples were collected for genetic analysis using clean tools, knives, or by hand while wearing gloves. The apical tip of the thallus, the youngest and typically the cleanest part of the plant, was selected for sampling. For preservation, samples were dried using silica gel for 12 hours or until fully desiccated, ensuring that the silica gel retained its color. If the gel became damp, it was replaced with fresh, dry gel to complete the drying process (Zuccarello & Paul, 2019). DNA extraction was conducted on 0.5 grams of the apical tip using a modified Chelex method (Zuccarello & Lokhorst, 2005; Zuccarello & Paul, 2019). The seaweed samples were lysed in a CTAB extraction buffer containing 2% CTAB, 0.1 M Tris-HCl (pH 8.0), 1.4 M NaCl, 20 mM EDTA, and 1% PVP.

Following DNA extraction, amplification was performed with a PCR solution. The PCR reaction mixture had a total volume of 20 µL and comprised: 1x buffer, 2.5 mM MgCl, 0.2 mM dNTPs, 0.5% bovine serum albumin (BSA), 1 U Taq polymerase (Vivantis, Selangor Darul Ehsan, Malaysia), and 7.5 pmoles of each COI primer GazF1 (5'-TCAACAAATCATAAAGATATTGG-3') and GazR1 (5'-ACTTCTGGATGTCCAAAAAAYCA-3') (Saunders, 2005).

PCR cycling conditions were set as follows: an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 45°C for 1 minute, and extension at 72°C for 1 minute, with a final extension at 72°C for 5 minutes (Zuccarello & Paul, 2019). The quality and quantity of PCR products were assessed using 1% agarose gel electrophoresis, and the PCR products were subsequently sent to 1st BASE (Singapore) for

sequencing.

After sequencing, results from both forward and reverse primers were aligned to ensure accuracy in determining the correct nucleotide sequence. COI gene sequences from various haplotypes (genetically distinct variants within a species) were retrieved from the NCBI database (www.ncbi.nlm.nih.gov) and imported into MEGA X software. The sequencing results and retrieved haplotypes were aligned using ClustalW within MEGA X for sequence alignment analysis.

To evaluate DNA diversity and phylogeny, the Neighbor-Joining method was applied using IQ-tree software (Trifinopoulos *et al*., 2016). IQ-tree was used to select molecular evolution models for each codon position (ModelFinder) and to construct a phylogenetic tree using the Neighbor-Joining (NJ) method with 500 bootstrap replicates and the Kimura 2-parameter model. Base pair differences among samples were calculated in MEGA X (Tamura *et al*., 2013).

RESULTS AND DISCUSSION

Growth and Production

The growth of Kotoni seaweed at the three study sites exhibited significant differences, with an overall increasing trend observed at varying rates ($p<0.05$). The highest growth rate was recorded in seaweed cultivated in West Halmahera, whereas the lowest growth was noted in seaweed from Biak (Figure 1). This variation may be attributable to several favorable environmental factors unique to the West Halmahera site, alongside potentially superior genetic quality of seaweed in this region compared to the other two locations. West Halmahera, with its remarkable growth performance, emerged as a prime candidate for large-scale seaweed cultivation. Banggai, though less productive than West Halmahera, showed promising results that could be further optimized with targeted cultivation practices. In contrast, Biak's lower growth performance raised concerns regarding the viability of seaweed cultivation in this area. This suggests the need for a comprehensive investigation into local environmental factors that may limit seaweed growth and an evaluation of alternative species or varieties more suited to Biak's conditions.

Water quality, substrate type, and seasonal conditions interact to create environments that either support or hinder seaweed growth. Higher seaweed coverage is typically observed closer to the shore, where sandy substrates, shallow waters, and lower turbulence create suitable growth conditions. Additionally, areas with seagrass beds, which stabilize sediment, tend to provide a favorable environment for certain seaweed species (Johan *et al*., 2015). The performance of seaweed cultivation is heavily influenced by seasonal changes. Factors such as monsoons, changes in water temperature, light availability, and nutrient fluxes vary with the seasons and can significantly impact seaweed growth (Erlania & Radiarta, 2014).

The research findings indicated that the cultivation sites in Banggai and Halmahera yielded approximately 1.4 tons of Kotoni seaweed, while the Biak site produced less than half this quantity (Figure 2). This variation in production may be attributed to differences in environmental conditions at each site, as well as possible genetic variations in the seaweed strains being cultivated.

Over a 45-day cultivation period, several water quality parameters were measured, including pH, salinity, temperature, TSS, nitrate, phosphate, and ammonia. These parameters exhibited fluctuations that could potentially affect seaweed growth. Acidity (pH) fluctuations were observed at all sites, ranging from 7.55 to 8.60 (Figure 3), within the optimal pH range

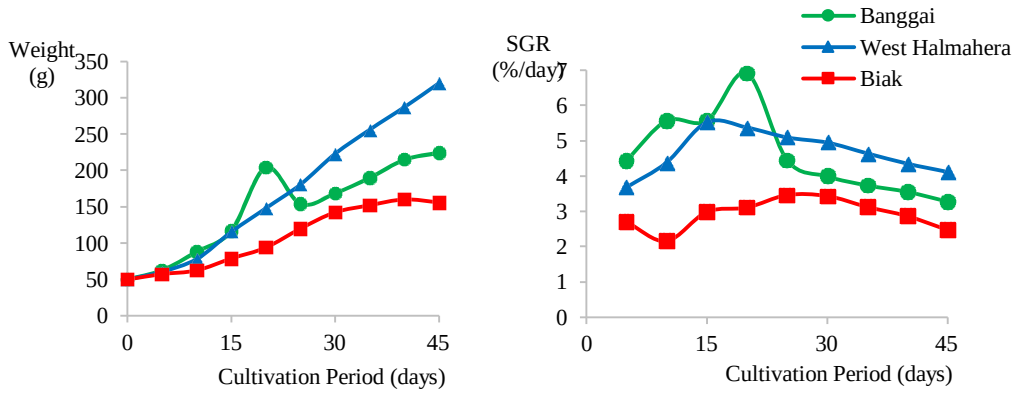

Figure 1. Weight and specific growth rate (SGR) of Kotoni seaweed cultivated over 45 days across three distinct cultivation sites. Different letters at the ends of the curves indicate statistically significant differences ($p<0.05$).

for seaweed growth as established by previous studies, which indicate that seaweeds thrive at pH levels between 7.5 and 8.5 (Andayani & Pamungkas, 2018; Bohari & Musbir, 2022). Notably, Biak exhibited slightly elevated pH levels at the onset of the cultivation period, potentially due to local environmental conditions or anthropogenic influences.

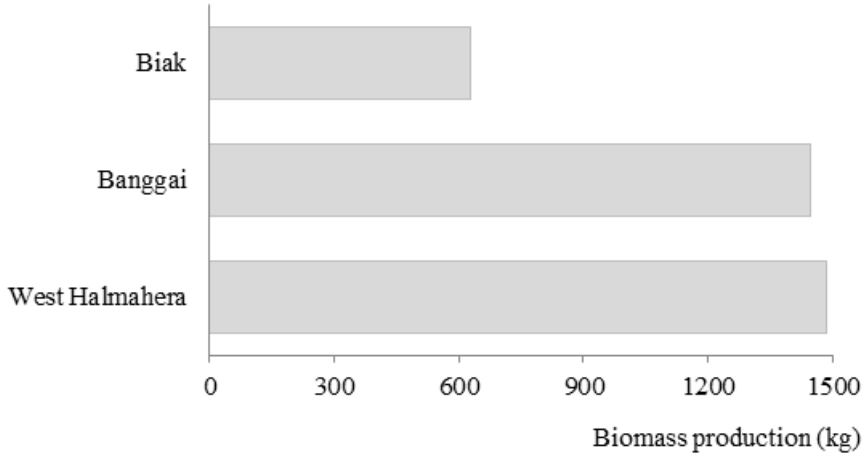

Figure 2. Production of Kotoni seaweed across three cultivation sites.

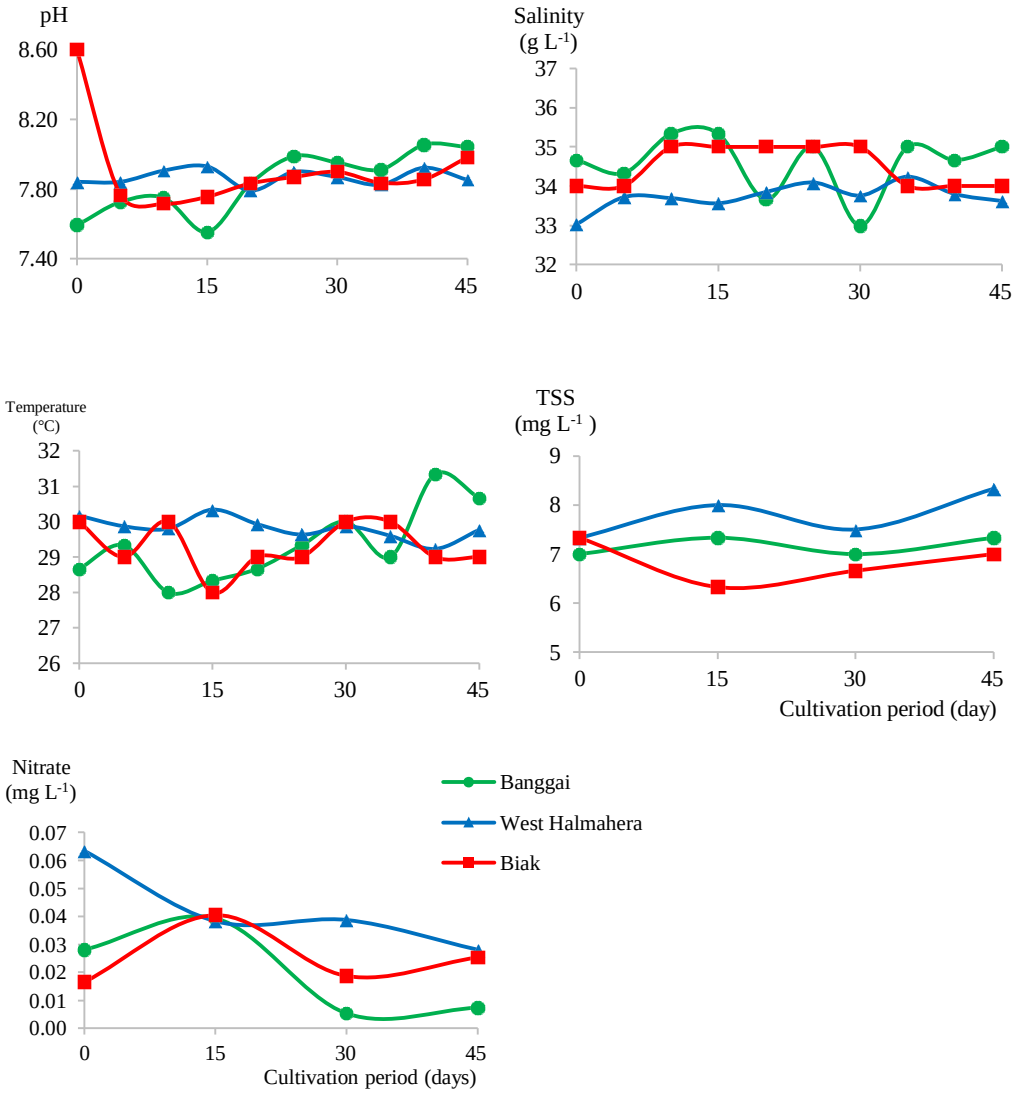

Figure 3. Water acidity (pH), salinity, temperature, total suspended solids (TSS) and nitrate levels at three cultivation sites over 45-day cultivation period.

Salinity was identified as another critical factor, with measurements ranging from 33-35 g L^{-1} across all sites, indicating relatively stable conditions favorable for seaweed growth. Research suggests that the optimal salinity range for various seaweed species, including *Gracilariopsis heteroclada* and *Gracilaria edulis*, is between 25-35 g L^{-1} (Andayani & Pamungkas, 2018; Bohari & Musbir, 2022).

Water temperatures ranged from 27-31°C (Figure 3), with West Halmahera displaying smaller fluctuations compared to the other two sites. Studies have shown that stable and suitable temperatures are crucial for maximizing growth rates and physiological performance in seaweeds (Kumar *et al*., 2020). The ideal temperature for seaweed growth is typically between 25°C and 30°C (Andayani & Pamungkas, 2018). TSS values ranged from 6.3-8.3 mg L^{-1}, indicating relatively low and sufficiently clean water conditions conducive to cultivation. Elevated TSS levels can reduce light penetration, hindering photosynthesis and thus negatively impacting growth (Roleda & Hurd, 2019).

Comparative studies from other regions have reported similar environmental conditions conducive to seaweed cultivation. For example, Kotoni cultivation in the Philippines has been shown to thrive under comparable temperature and salinity conditions, with optimal growth occurring at salinities of 30-35 g L^{-1} and temperatures around 28-30°C (Hurtado *et al*., 2015). Research in Madagascar further emphasizes that environmental factors such as temperature and salinity significantly influence the growth and health of cultivated seaweeds, underscoring the importance of maintaining optimal conditions (Ateweberhan *et al*., 2014).

Nutrient dynamics in seaweed cultivation, particularly regarding phosphate, nitrate, and ammonia concentrations, are critical for optimizing growth conditions and ensuring compliance with water quality standards. The availability of nitrates and phosphates is essential, with optimal levels supporting healthy growth and productivity (Andayani & Pamungkas, 2018). Among the study sites, West Halmahera consistently showed the highest nitrate levels, suggesting a higher nutrient load compared to Banggai and Biak (Figure 3). However, all three sites exhibited nitrate concentrations below the optimal growth range (0.5-1.0 mg L^{-1}) as recommended by Roleda & Hurd (2019), indicating potential limitations for supporting optimal seaweed growth. Nitrate is a crucial nutrient for seaweed, providing a nitrogen source essential for the synthesis of proteins and other vital compound (Makmur *et al*., 2016).

Phosphate concentrations varied significantly across the three cultivation sites, with Banggai displaying the highest levels and the most fluctuation over time (Table 2). In contrast, West Halmahera and Biak maintained stable yet low phosphate levels. Although Banggai recorded higher phosphate levels compared to the other sites, concentrations remained below the ideal range for seaweed growth (0.1-0.5 mg L^{-1}) as outlined by Simatupang *et al*. (2021). Phosphate plays a vital role in energy transfer and cellular processes in seaweed, making it a limiting nutrient for growth (Makmur *et al*., 2016).

Table 2. Phosphate and ammonia content in the waters at three cultivation sites over 45-day cultivation period

Days	Phosphate (mg L^{-1})			Ammonia* (mg L^{-1})		
	Banggai	West Halmahera	Biak	Banggai	West Halmahera	Biak
0	0.0373	<0.0019	<0.0019	<0.0017	<0.0017	<0.0017
15	0.0544	<0.0019	<0.0019	<0.0017	<0.0017	<0.0017
30	0.0347	<0.0019	<0.0019	<0.0017	0.0132	0.0969
45	0.0334	<0.0019	<0.0019	<0.0017	0.0081	<0.0017

*Ammonia in the form of TAN

Ammonia levels remained consistently low in Banggai throughout the observation period, indicating a stable ecosystem (Table 2). West Halmahera showed a slight increase in ammonia on days 30 and 45, while Biak experienced a notable spike in ammonia levels on day 30. According to Roleda & Hurd (2019), the acceptable ammonia concentration for seaweed cultivation is below 0.1 mg L^{-1}, and the observed ammonia concentrations in this study mostly fell within this range across all sites.

The overall environmental factors (pH, salinity, temperature, TSS levels, and ammonia) at all three sites were generally suitable for seaweed cultivation; however, nitrate and phosphate levels were too low to support optimal seaweed growth. The growth and health of seaweed are significantly influenced by water quality parameters, including nutrient levels (such as nitrogen and phosphorus), salinity, temperature, and the presence of pollutants (Radiarta & Erlania, 2015). Nutrient dynamics can vary substantially based

on local environmental conditions and anthropogenic influences. For example, seaweed cultivation sites near river mouths often experience higher nutrient influx due to runoff, which can enhance growth rates but also poses risks of nutrient overload and eutrophication (Bohari & Musbir, 2022). In contrast, sites located further from freshwater sources may exhibit lower nutrient levels, though they maintain stable growth conditions (Simatupang *et al*., 2021).

While environmental factors undoubtedly play a critical role in influencing seaweed growth, genetic factors are equally important. Genetic diversity among Kotoni seaweed strains significantly impacts growth rates and yields, with different strains exhibiting variations due to inherent genetic differences, even when these differences are not visibly evident in morphological traits (Ratnawati *et al*., 2020). The interaction between genotype and environment is crucial, as different genotypes may respond uniquely to the same environmental conditions, highlighting the need for targeted breeding programs that account for both genetic and environmental factors (Simatupang *et al*., 2021). Advances in genetic engineering and molecular techniques offer potential for enhancing traits such as growth rates and stress resilience, thus improving aquaculture practices in challenging environments (Charrier *et al*., 2015; Maili *et al*., 2016).

Product Quality of Kotoni

The results revealed significant differences in carrageenan content and gel strength among the three sites. Notably, Banggai demonstrated a consistent increase in carrageenan content throughout the cultivation period, reaching up to 30% by day 45 (Figure 4). This suggests that the environmental conditions in Banggai may be particularly conducive to carrageenan accumulation. Previous studies have shown that the growth rate and chemical composition of Kotoni seaweed are significantly influenced by environmental parameters such as salinity and nutrient levels (Ateweberhan *et al*., 2014; Kumar *et al*., 2014).

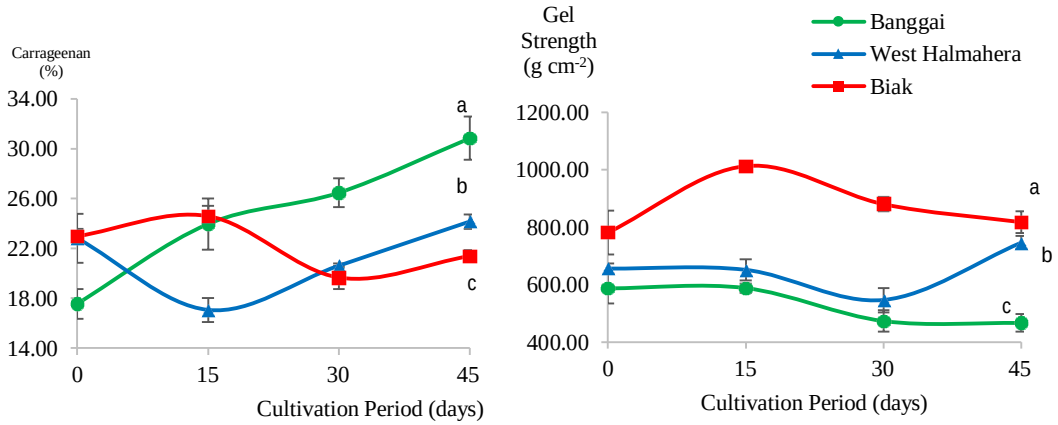

Figure 4. Carrageenan content and gel strength of Kotoni seaweed at three distinct cultivation sites over 45-day cultivation period. Different letters at the ends of the curves indicate statistically significant differences ($p < 0.05$).

Despite the increase in carrageenan content, gel strength fluctuated across all sites, with Biak consistently producing the highest gel strength. This variability in gel strength suggests that other factors, possibly environmental, may influence the mechanical properties of carrageenan. The mechanical properties of carrageenan depend on various factors, including molecular weight and degree of sulfation, both of which can be affected by local environmental conditions and cultivation practices (Rupert *et al*., 2022; Usuldin *et al*., 2017). The superior gel strength observed in Biak implies that seaweed grown there may possess a more robust and stable carrageenan structure, making it particularly suitable for industrial applications that require strong gels. Results from West Halmahera, although not as high as Biak, still demonstrated competitive quality, indicating potential for producing high-quality carrageenan as well (Figure 4).

The study also identified significant variations in carrageenan viscosity among the sites. Once again, Biak stood out, producing carrageenan with the highest viscosity—a crucial parameter for applications requiring thickening agents (Figure 5). Viscosity, which is influenced by the molecular weight and sulfate content of carrageenan, is essential for applications as a thickening agent (Luhan *et al*., 2022; Norhazariah *et al*., 2018). The higher viscosity observed in Biak's carrageenan could be attributed to a favorable balance of these factors in the local seaweed, potentially due to unique environmental conditions at the site. This finding aligns with previous research indicating that carrageenan viscosity can be significantly affected by the cultivation environment and the specific strains of Kotoni used (Rimmer *et al*., 2021; Simatupang *et al*., 2021).

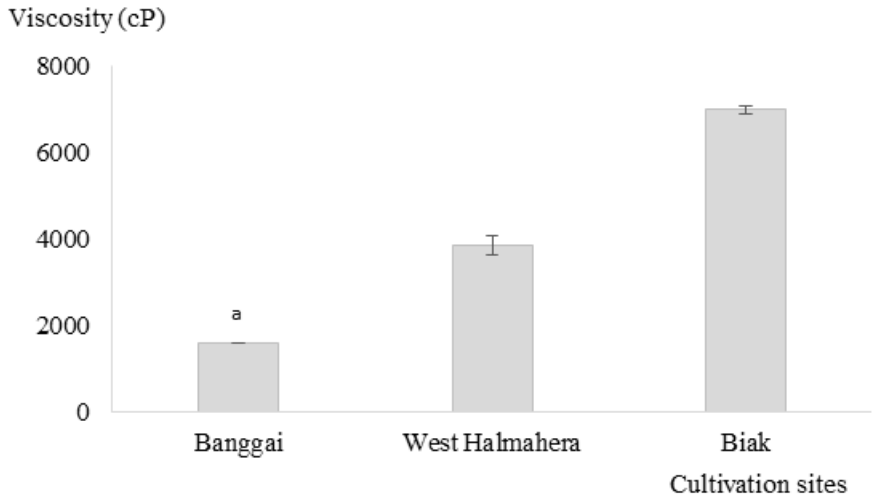

Figure 5. Viscosity of Kotoni seaweed at three distinct cultivation sites over 45-day cultivation period. Different letters above the bars indicate statistically significant differences ($p < 0.05$).

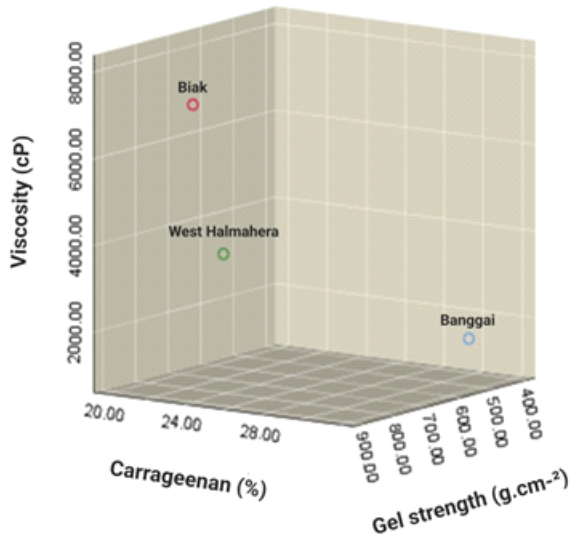

Figure 6. Three-dimensional mean values of product quality variables (carrageenan, gel strength, and viscosity) of Kotoni seaweed across three distinct cultivation sites.

When assessing the overall quality of carrageenan, which includes both gel strength and viscosity, Biak emerged as the site with the highest carrageenan quality (Figure 6). In contrast, despite Banggai's high carrageenan yield, its overall product quality was lower due to reduced gel strength and viscosity. This finding suggests that, although Banggai seaweed may produce a greater quantity of carrageenan, the functional properties of this carrageenan are inferior to those from Biak. Research indicates that environmental factors such as nutrient availability, water quality, and temperature are crucial in determining carrageenan quality. For instance, studies have shown that variations in these factors across cultivation sites can result in significant differences in the biochemical composition of Kotoni (Kumar *et al.*, 2014; Maradhy *et al.*, 2021). Furthermore, the genetic diversity of seaweed species across locations may also contribute to variations in carrageenan quality, as certain strains are better adapted to specific environmental conditions (Lim *et al.*, 2014; Tan *et al.*, 2022).

Interestingly, the proximate composition of Kotoni seaweed showed minimal variation across the three sites, with carbohydrate content being the highest (~60%), followed by ash (~30%) and fiber (~10%) (Figure 7). This consistency in proximate composition suggests that the nutritional profile of the seaweed remains relatively stable across different environments, though minor variations in components such as ash and fiber content were observed. However, these variations did not appear to significantly correlate with the functional properties of carrageenan, as indicated in the quality assessments.

The study highlights that Biak exhibits a distinct advantage in producing high-quality carrageenan, char-

acterized by superior gel strength and viscosity. These findings emphasize the importance of site selection in seaweed cultivation, particularly for industries relying on high-quality carrageenan for diverse applications. Future research should focus on exploring specific environmental factors and potentially unique Kotoni strains in Biak that may contribute to the production of higher-quality carrageenan. Identifying and replicating these favorable conditions at other cultivation sites could enhance carrageenan quality across regions, ultimately improving production efficiency and increasing the final product's overall value.

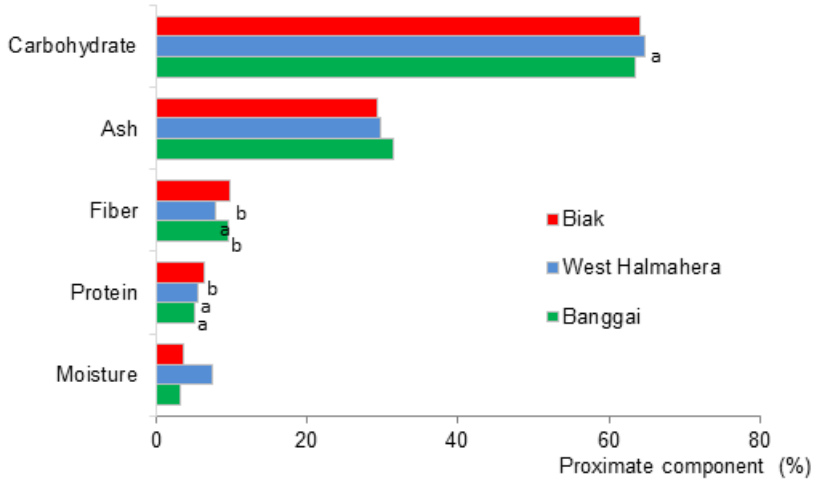

Figure 7. Proximate composition of Kotoni seaweed at three cultivation sites after 45-day cultivation period. Different letters following the bars for each proximate component indicate statistically significant differences ($p < 0.05$).

Genetic identification of seaweed

The study utilized the mitochondrial marker gene, cytochrome oxidase subunit 1 (COI), to genetically identify seaweed samples from three distinct sites in Indonesia. Analysis revealed that all samples across these locations were of the species *Kappaphycus alvarezii*, with a 100% similarity match. This finding builds on prior research by Ratnawati *et al.* (2020), which also identified *K. alvarezii* across 14 seaweed cultivation sites spanning from Sumatra (West Indonesia) to Maluku (East Indonesia) using the COI gene. The confirmation of a uniform species across various geographic regions is significant for standardizing quality and performance in seaweed production.

Phylogenetic analysis, represented by a neighbor-joining tree, visually confirmed the genetic identification (Figure 8). The tree placed samples from the study alongside other *Kappaphycus* and *Eucheuma* genera sequences obtained from the NCBI GenBank, further validating the identification results. This consistency with known genetic database sequences not only supports the accuracy of the identification but also underscores the reliability of COI as a genetic marker for seaweed species identification. COI genes have been successfully applied in identifying multiple Rhodophyta seaweed species (Dumilag *et al.*, 2018; Iha *et al.*, 2015; Ratnawati *et al.*, 2020).

While current cultivation practices effectively yield large quantities of seaweed, their sustainability may be compromised without strategies to enhance genetic diversity. Low genetic variation in large-scale aquaculture poses potential risks, as the observed phenotypic diversity appears primarily influenced by environmental conditions rather than genetic differences (Ratnawati *et al.*, 2020).

The confirmation that all samples belong to *K. alvarezii* has practical implications for seaweed cultivation, as it ensures that farmers are cultivating a species renowned for its high carrageenan content, which is in demand across various industries. However, this uniformity also implies that variations in product quality are more likely due to differences in cultivation practices or environmental factors than genetic factors. This insight is valuable for guiding efforts to optimize cultivation practices to maximize both carrageenan yield and quality.

CONCLUSIONS

The study concludes that Kotoni seaweed cultivated in West Halmahera exhibited high growth rates and productivity. Notably, while seaweed from Banggai displayed a higher carrageenan content, seaweed from Biak demonstrated superior gel strength and viscosity. Genetic analysis indicated 100% genetic similarity among Kotoni samples from the three cultivation sites, confirming their uniform classification as *Kappaphycus alvarezii*.

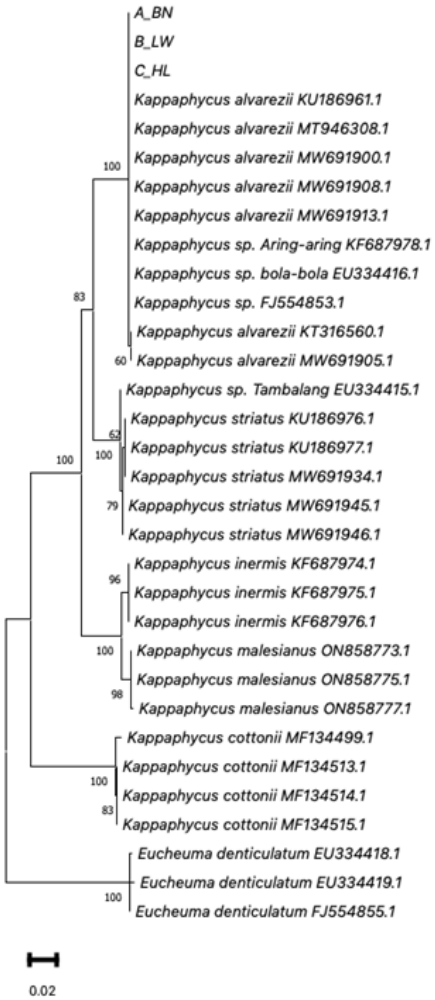

Figure 8. Neighbor-joining phylogenetic tree based on the DNA sequences of samples and selected genera of *Kappaphycus* and *Eucheuma* obtained from NCBI GenBank. BN = Biak, LW = Banggai, HL = West Halmahera.

ACKNOWLEDGEMENTS

This research was supported by funding from the Budget Implementation List provided by the Research Institute for Seaweed Culture, Ministry of Marine Affairs and Fisheries, Indonesia. The authors would like to acknowledge the valuable contributions of the technicians who assisted with the fieldwork.

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