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Nutrient Enrichment and Postharvest Culture to Enhance Production and Quality Performance of *Gracilaria verrucosa*

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Abstract: The present study was aimed to analyze the use of N, P and Fe elements as macro and micro nutrients, respectively, followed by postharvest treatments, in response to production and quality of *G. verrucosa*. The experiment was a complete randomized design with four treatments and in triplicate: non-enriched; enriched with N; enriched with N+P and enriched with N+P+Fe. After harvesting from various enrichment media (0d), the seaweed were subjected to postharvest culture in the dark for 3, 8 and 12 days (3D, 8D, 12D) or left on light condition as similar to growing condition for 8 and 12 days, then continue to 3 days dark treatment (8L3D; 12L3D), respectively. The highest biomass was obtained when the seaweed culture medium enriched with combination of N+P+Fe and differ significantly with other treatments. Postharvest culture of 3D (N+P enriched) had begin elevated the agar content, while in other nutrient treatments it appeared at 8D. The highest agar content was in 8L3D (N enriched) and significantly different compare to others ($p < 0.05$). Gel strength increased in 3D (non-enriched); 3D, 8D, 8L3D (N enriched) and 8D, 8L3D (N+P+Fe enriched), whereas the best gel strength was in 8L3D (N enriched). This suggest, enrichment with macro and micro nutrients enhanced the production and the application of postharvest culture was effective both in increasing agar content and gel strength of *G. verrucosa*. Especially, N enriched followed by the application of postharvest culture led the best quality of agar.

Key words: Nutrient, production, quality, agar content, gel strength, *Gracilaria verrucosa*

INTRODUCTION

The economic important of *Gracilaria verrucosa* (Hudson) Papenfuss is undoubted as its being the raw material world-wide in agar production. In Indonesia, cultivation of this species has been employed broadly, with the production reach to 6, 5 million tones in 2012, which increased about three folds from the year of 2008 (2, 1 million tones) (source: Ministry Affairs and Fisheries Republic of Indonesia). This magnitude growth unfortunately merely due to expansion of farm area, while previous studies mentioned that productivity are primarily controlled by nutrients (Lobban and Wynne, 1981; Macler, 1986; Cole and Sheath, 1990; Lobban and Harrison, 1997), in spite of light, temperature and salinity (Parker, 1982; Xu *et al.*, 2001; Raikar *et al.*, 2001; Bunsom and Prathep, 2012).

Most farmers in Indonesia only rely on available water surrounding the pond, while some have added N (nitrogen) or N+P (nitrogen+phosphate) or NPK (nitrogen+phosphate+potassium) before farming activities to increase the growth of *Gracilaria*. In spite those macro elements, in fact the seaweed also need the micro elements, however the use of mineral such as iron (Fe) together with the macro nutrients have not been applied yet. Liu *et al.* (2000), as well as Kakita and Kamishima (2007) found Fe elements was important in cell growth of red algae.

The quantities production of seaweed must be consistent with the quality of agar produced (Armisen, 1995), such as the agar content or gel strength. However, technological expertise of agar content and gel strength is only dominated by industry. Application of traditional culture methods and lack of knowledge on agar quality by farmers are a constraint, in which may lead to unstable price of seaweed.

Based on the situation, this study was addressed to analyze the use of N, P and Fe elements as macro and micro nutrients respectively, followed by postharvest treatments, in response to production and quality of *G. verrucosa*. We applied N, P, Fe enrichment as De Boer (1981) stated that these elements concentration differ based on biology process in water column and become a limiting factor for seaweed growth. Further, the harvesting seaweed from variety nutrient enrichments continue to treat in *postharvest culture*. Such condition subject to enhance the quality of agar, in this case agar content and gel strengths. The methods supposed to be applicable for farmers for better quantity and quality of *Gracilaria* production.

MATERIALS AND METHODS

Plant material: *Gracilaria verrucosa* used in this study, were collected from traditional farm in Muara Gembong Village, Bekasi, East Java, Indonesia. Experimental

studies were conducted at nearby farm of Ministry of Marine and Fisheries Affair for Estuarine and Marine Culture Development at Pusakajaya Utara district, Cilebar, Karawang, East Java, Indonesia. Before released to the tank, the seaweed were acclimated for 24 h.

Gracilaria verrucosa cultivation: Twelve 2x1.5x0.80 (m) compartments were constructed using bamboo poles and covered by polyethylene blue plastics over sided and based, prepared for cultivation. Light was maintained by putting transparent plastic roof 1.5 m above the compartments to ensure sunlight entering the media and prevent from the rain. The plants were cultivated in broadcast method at initial density of 15 kg ww per compartment for 2 weeks using constant aeration and filled with water to about 1.5 m³ (stocking density equal to 10 kg/m³). The experiment was a complete randomized design with four treatments and in triplicate: Treatment 1: Non-enriched, Treatment 2: Enriched with N, Treatment 3: Enriched with N+P and Treatment 4: Enriched with N+P+Fe.

Nutrient enrichments were applied weekly, following full water exchange. Every 3 days after enrichment, there was also 50 % water exchange to ensure the stability of water quality similar the field condition as described by Truno (1988). Inorganic N and P were supplied in the form of urea (contain 46% N) and SP (Super Phosphate, contain 36% P₂O₅), while Fe was in the form of FeCl_{3.6}H₂O. Each concentration of nutrients were 50, 5 and 2 ppm for N, P, Fe, respectively as based on preliminary experiment results. Culture condition was checked for salinity, temperature, Dissolve oxygen and pH.

After harvesting, *Gracilaria* were weighted, fresh amounts of 1 kg ww of seaweed were taken for drying (2 days) and further analyzed for the agar quality (referred to as 0d). The remaining algae, each of 1 kg were then used for postharvest culture periods.

Postharvest culture period condition: *Gracilaria* postharvest cultivation were applied at a density of 10 times from previous culture period (1.5 kg/15/L). Rounded green plastic PE were prepared and completely covered with black plastic film for dark treatment, supplied with continuous aeration. Plant materials were then cultivated in those compartments with following treatments: (1) Dark conditions (3, 8 and 12 days dark = 3D, 8D, 12D) with no nutrient added and (2) Left on light condition as similar to growing condition for 8 and 12 days and continue to 3 days dark treatment (8L3D; 12L3D) without any nutrient addition (the detail of these methods were presented in (Fig. 1). Afterward, each treatment was harvested at each certain postharvest culture time for agar content and gel strength analysis.

Sample analysis: Biomass production of seaweed was obtained at the end of harvest time. For agar extraction, a minimum of 50 g of dry algae were used. Dry algae were pre-treated in 0.1% H₂SO₄. The seaweed was extracted by adding 1500 mL distilled water at boiling temperature for 2 h and filtered through a muslin cloth, left at room temperature and dried. The dried agar was then weighted for agar content. The gel strength was determined with a Nikansui gel tester using 1 cm² plunger at 20°C for 1.5% filtrate agar.

Statistical analysis: Data were tested for normality (Kolmogorov-Smirnov) and homogeneity (Brownforsythe Welch) and subjected to one-way Analysis of Variance (ANOVA) at 5% level of significance to determine differences in the treatment effect. Where significant differences occurred, a Duncan's Multiple Range Test for *posthoc comparison* was performed.

RESULTS

Nutrient treatments had a significant effect on biomass production of *G. verrucosa* over the growing period (p<0.05). *Posthoc comparison* (Table 1) reveals that N+P+Fe enriched affect significantly higher on production of *G. verrucosa* and significantly different with other treatments (p<0.05).

The agar content and gel strength performance of *G. verrucosa* over cultivation and postharvest culture showed differences between treatments. Three days of dark (N+P enriched) had begin elevated the agar content, while in other nutrient treatments it condition appeared after 8 days culture in dark (Fig. 2). However, all 8L3D agar content was better in each group of nutrient enrichment. The highest agar content was in 8L3D (N enriched) and significantly different compare to others (p<0.05).

After the postharvest cultivation, gel strength increased in 3D (non-enriched); 3D, 8D, 8L3D (N enriched) and 8D, 8L3D (N+P+Fe enriched). As shown in Fig. 3, the best gel strength was in 8L3D (N enriched), that was not different with 3D and 8D on the same enrichment group (p>0.05), but significantly different compared to other enrichment treatments (p<0.05).

DISCUSSION

Overall, the water quality during the study supported *Gracilaria* growth (DO: 3.3-3.8, pH: 7.7-8.3, temperature: 25-30°C, salinity: 18-20°C). Nutrient enrichment with

Table 1: Seaweed production after various enriched treatments. Superscript with different letter indicate significant different (p<0.05)

Treatments	Seaweed production (kg)
Non-enriched	17.30±0.20 ^a
N	18.77±1.56 ^b
N+P	21.30±1.31 ^b
N+P+Fe	23.40±0.61 ^c

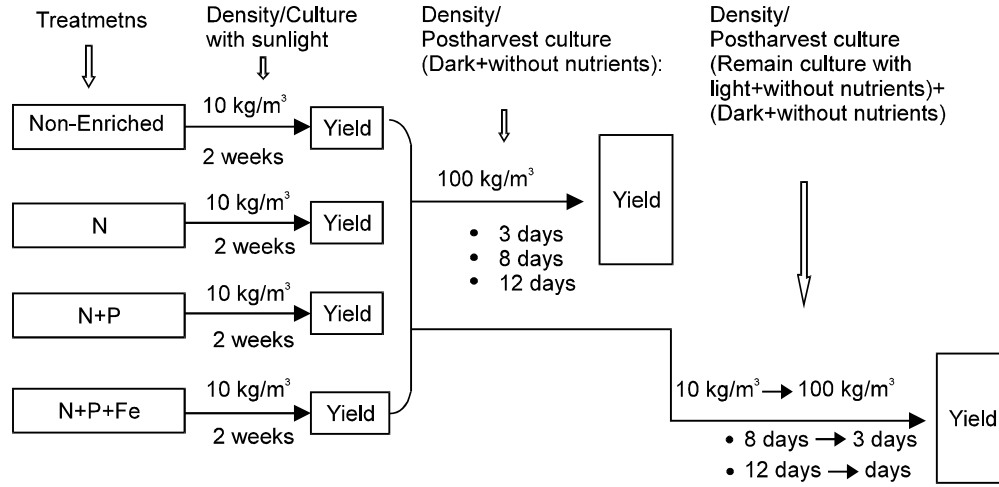


Fig. 1: Diagram of method used for culture and postharvest culture periods of *Gracilaria verrucosa*. Rosyida, E. Nutrient enrichment and dark treatments into cultured media to enhance production and quality performance of *gracilaria verrucosa*

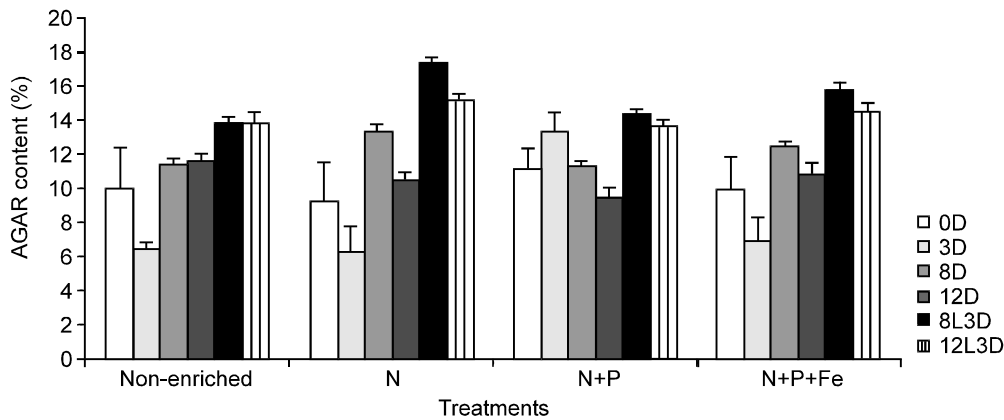


Fig. 2: Agar content of *G. gracilaria verrucosa* in various treatments of culture and postharvest culture condition (Mean±SD). Rosyida, E. Nutrient enrichment and dark treatments into cultured media to enhance production and quality performance of *gracilaria verrucosa*

sufficient concentration may provide maximum growth rates of *Gracilaria*, however very high concentration, especially ammonium and phosphorus have been proved to deteriorate this macro algae (Briggs and Funge-Smith, 1993). In this study, the growth rate of *G. verrucosa* has been found to increase significantly after the culture medium enriched with combination of macro (N, P) and micro element (Fe). Fe addition to N and P may stimulated enzyme activity in photosynthesis reaction, which could attribute to the good growth of the algae. As De Boer (1981) explained Fe functions not only as a structural component, but also as a co-factor for enzymatic reactions for the seaweed. In addition, various studies emphasized effect of iron on marine algal growth for nitrogen utilization and carbon assimilation (Liu *et al.*, 2000; Cordover, 2007). Effect of iron enriched on *G. verrucosa* growth in this study agrees with other *Gracilaria* species reported by Liu *et al.* (2000) and also

Kakita and Kamishima (2007). Moreover, these results may extends previous finding regarding Fe regulation on macro algae.

The agar content and gel strength of *G. verrucosa* generally showed improvement after dark treatment on postharvest cultivation. The effect of dark treatment on enhancing content and gel strength of agar as shown in our results, has been supported by previous studies. Macler (1986) judged that in the dark, carbon (C) was degraded from storage products (*floridean starch* dan *floridoside*) by enzyme phosphorylase dan α -galactosidase, to provide C for agar biosynthesis. In addition, the elevation of enzyme activity in dark condition (Rincones *et al.*, 1993) may eliminate sulphate and stimulate precursor of 3, 6 Anhydrogalactose (3, 6 AG) chain, that in turn, generate the gel strength (Hemmingson and Furneaux, 2003; Villanueva *et al.*, 2009).

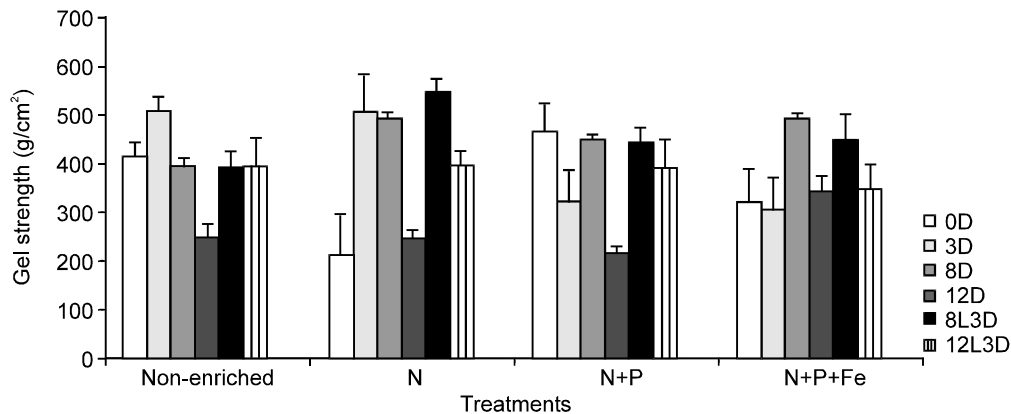


Fig. 3: Agar gel strength of *Gracilaria verrucosa* in various treatments of culture and postharvest culture condition (Mean±SD). Rosyida, E. nutrient enrichment and dark treatments into cultured media to enhance production and quality performance of *Gracilaria verrucosa*

During the postharvest culture period, the thalli of *Gracilaria* did not show any deterioration and paling of color until 8 days of dark treatments, but turn little to a yellowish at 12 days in such absent of light condition. Villanueva *et al.* (2009) experienced good performance of Rhodophyta, *Chondrus crispus* when cultured in the dark up to 10 days. Meanwhile, thalli appeared degeneration in *G. chilensis* after 3 weeks stored in the dark (Hemmingson and Furneaux, 2000). In general, all treatments at 12 days dark in this study showed remained good agar content, however it has been inversely correlated with the gel strength, except for the algae that treated previously with N+P+Fe. The regulation of Fe element may be important in this situation, as one of the mineral function in seaweed is to avoid physical stress (Mtolera, 2003). However, studies are needed to evaluate the effect of Fe on *Gracilaria* agar quality, as this is not understood yet.

It was observed in this study that *Gracilaria* left culture without nutrient addition after being enriched and continue to treat in dark for 3 days (8L3d), overall had higher agar content compare to that treated enriched and directly culture in dark. This condition, especially after N enriched, also performed the highest gel strength. Macler (1986) pointed that when N limited in Rodophyta, they tend to losses the pigments of photosynthetic and cellular protein, but in the same situation according to Lapointe and Duke (1984) the seaweed may accumulate carbohydrate. Macler (1986) identified more carbon in starved algae and its devoted to the formation of agar. In addition, dark condition led C in storage products that yielded from photosynthesis, also used for agar biosynthesis. This suggests that the starvation after being enriched, coupled with dark treatment in this study supported the potential to increase agar content.

Conclusion: In conclusion, nutrient enrichment with macro (N, P) and micro elements (Fe) increased production of *Gracilaria verrucosa* significantly. The application of postharvest culture was effective both in increasing agar content and gel strength, especially N enriched followed by the application of postharvest culture led the best quality of agar.

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