Pak. J. Biotechnol. Vol. 12(1) 1- 5 (2015)
www.pjbt.org

ISSN Print: 1812-1837
ISSN Online: 2312-7791

THE QUALITY ENHANCEMENT OF AGAR EXTRACTED FROM *GRACILARIA VERRUCOSA* CULTURED IN VARIOUS CONDITIONS OF POSTHARVEST PERIODS

Eka Rosyida[1,2], Enang H. Surawidjaja[2*], Sugeng H. Suseno[2], and Eddy Supriyono[2]

[1] Faculty of Animal Husbandry&Fisheries, Tadulako University, Palu, Indonesia; [2] Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor, Indonesia. Email[*]: enang_harris@yahoo.com

Article received September 6, 2014; revised November 28, 2014; accepted Decermber 4, 2014

ABSTRACT

This study was aimed to evaluate the quality of agar extracted from the red seaweed, *Gracilaria verrucosa* upon postharvest culture treatments. After harvesting from the culture medium (0d), the seaweed were subjected to postharvest culture in the dark in salinity of 20 % (ambient) and 30% (salinity increased) for 3, 8 and 12 days respectively. Each treatment was in triplicate. Results indicated that 8d (20 %) and 3d (30 %) treatments had improved the agar content and reached approximately 40% more than harvested algae before postharvest treatments (0d). The gel strength had a significant increase in 3d and 8d of both 20% and 30%. The sulphate and ash content of the agar was lower in 3d (20%) and 8d (30 %) than others in each group and all treatments carried out in dark with salinity of 30% produced agar with lower water content when compare to dark with salinity of 20%. It indicates that postharvest treatments in general may perform good agar properties. Hence, the application of postharvest culture in the dark, either in ambient or increased salinity may reasonable to enhance the quality of *G. verrucosa*.

Key words: dark treatment, postharvest culture, agar content, gel strength, agar production

INTRODUCTION

Gracilaria verrucosa has recently been recognized as a great potential source of agar in Indonesia. For comer-cial purposes, this agar is widely used in food application, pharmacology and dentistry. Broadly, the culture technique of this species has increased the produc-tion through cutting methods, ferti-lizing, even hormone application (FAO Coorp.Doc.Rep. 2014). However, presently the quantities production of raw material is in consistent with the quality of agar produced.

Biochemically, agar consists of 3,6-anhydro-galactose and D-galactose units. However, in agar biosynthesis, those units may be substituted by sulphate, methyl and pyruvate group that associated with agar gel strength. The amount of substi-tuents, environmental factors during culture periods and also the extraction of agar procedures are considered to be a relevant factor against the agar quality (Freille-Pelegrin and Murano, 2002).

Sulphate content of agar is especially very important in relation to the quality of agar. In agar industry, *desulphation* is applied since it may enhance the quality of agar. Several studies had conducted to perform a better quality of agar by *desulphation*, especially from *Gracilaria* spp. (Rath and Adhykary, 2004; Freile-Pelegrin et al., 2005; Praiboon *et al*., 2006). Beside the sulphate content elimination, Armisen (1995) explained that such *desulphation* may also reduce the ash content of agar. On the other hand, research on aquaculture technology has been conducted to manipulate the sulphate content during the growth of the seaweed. A technique by post-harvest culture in the dark has been posed to improve both the agar content and the gel strength of agar (Ekman *et al*., 1991; Rincones *et al*., 1993; Hemmingson and Furneaux, 2003). In addition, the dark treatments together with altered salinity at postharvest culture also showed a better quality of agar, particularly the agar content (Ekman *et al*., 1991; Freille-Pelegrin and Murano, 2002).

The aimed of this study was to evaluate the agar quality of *G.verrucosa* resulted from post harvest culture condition with various dark treatments periods in ambient and increased salinity. The study was supposed to be an applicable method for enhancing the quality of agar.

MATERIALS AND METHODS

Cultivation of Gracilaria verrucosa: *Gracilaria verrucosa* used in the present study were collected from traditional farm. The seaweeds were acclimated for 24 hours before released to the tank. Cultivation was prepared using twelve 2 x 1.5 x 0.80 m compartments constructed from bamboo poles and covered by polyethylene blue plastics over sided and based. Light was maintained by putting transparent plastic roof 1.5m above the compartments to ensure sunlight

entering the media and prevent from the rain. Broadcast method was applied in algal culture with initial density of 15kg/1.5m^3 (equal to 10kg/m^3). Over 2 week's periods, the seaweed was grown using constant aeration and ambient salinity (20%). Nutrient enrichments were applied weekly using 50ppm of inorganic N (urea, contain 46% N) 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). Culture condition was checked for Salinity, temperature,Dissolve oxygen and pH.

Two weeks of cultivation had resulted in DGR of 1.6% d^{-1}. After harvesting, fresh amounts of 1 kg of seaweed were taken for drying (2 days) and further analysed for the agar quality controlled (referred to as 0day=0d). The remainning algae, each of 1.5 kg were then used for the postharvest treatments.

Postharvest culture condition: *Gracilaria verrucosa* postharvest cultivation was applied at a density of 10 times from previous culture period (1.5kg/15 L). Rounded green plastic PE were prepared for postharvest culture and completely covered with black plastic film for dark treatment supplied with continuous aeration. The post-harvest experiment was a complete randomized design with three dark treatments (in triplicate) runs for 3, 8 and 12days respectively, each at salinity of 20% (ambient) and 30%. The latter salinity was achieved with the addition of natural sea salt.

Sample analysis: Seaweed production was obtained at the end of each harvest time, and the agar production was calcu-lated from seaweed production times each agar content of various treatments.

A minimum of 50 g of dry algae were used for extraction process for agar content. Dry algae were pretreated in 1 L of 2% NaOH for 2 hours at 90^{o}C to soften the cell wall. The algae were then washed with fresh water to remove excess NaOH. The seaweed was extracted by adding 1500 mL distilled water at boiling tempera-ture for 2 hours and filtered through a muslin cloth, left at room temperature and dried. The dried agar was then weighted for yield (agar content) calculation. The gel strength was determined with a Nikansui gel tester using 1 cm^2 plunger at 20^{o}C for 1.5% filtrate agar.

The extraction of agar for ash, sulphate and water content as well as viscosity was prepared with acid pretreatment (0.1% H_2SO_4) to prevent hydrolysis of yield. The ash content was determined following the standard methods of AOAC (2000). Residual samples (whitish color) were weighted after heating at 600^{o}C. The sulphate content was analysed using 1.0g of sample hydrolysed in 50mL 0.2N HCl for 6 hours. The hydrolyte was transferred and mixed with $BaCl_2$ 10% and then boiled for 2 hours. The super-natant was filtered with whattman No. 1 filter paper, washed with distilled water and boiled in a water bath to remove excess chloride. Filter paper was oven dried at 100^{o}C, cooled in a dessicator and weighted for sulphate content calculation. Viscosity determination was conducted at 75^{o}C (1.5% of agar solution) using *Brookfield viscometer*. The agar water content was analysed at 100-102^{o}C using 5 g of samples that oven dried for 6-16 hours. All samples were pooled per treatment to ensure the appropriate amount to be tested.

Statistical analysis: Data were subjected to one-way Analysis of Variance (Anova) at 5% level of significance to determine differences in the treatment effect. Later, the postharvest comparison was performed by Duncan's Multiple Range Test.

RESULTS

In general, postharvest treatments had a significant effect on agar content and gel strength of *G.verrucosa* over dark periods both in salinity of 20% and 30% (p<0.05) respectively. The increase of agar content was significant for seaweed under 8d (20 %) and 3d (30%) treatments compare to the control (p<0.05) (Figure 1).

The results on gel strength showed a signi-ficant increase after 3 and 8 days either in 20% or 30% group (Figure 2). Postharvest comparison revealed that these treatments also significantly different with others in each group of treatment (p< 0.05).

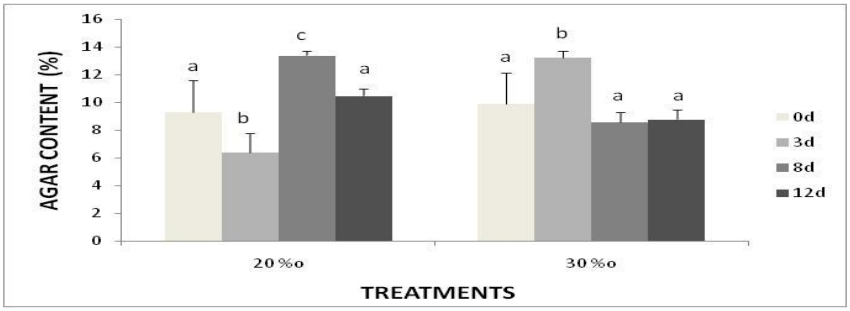

Figure-1: Agar content of *Gracilaria verrucosa* in different time of dark at salinity f 20% and 30% (mean±sd). Bar with different superscript letters are significant different ($p<0.05$). All control (0d) was from harvested algae at ambient salinity (20%).

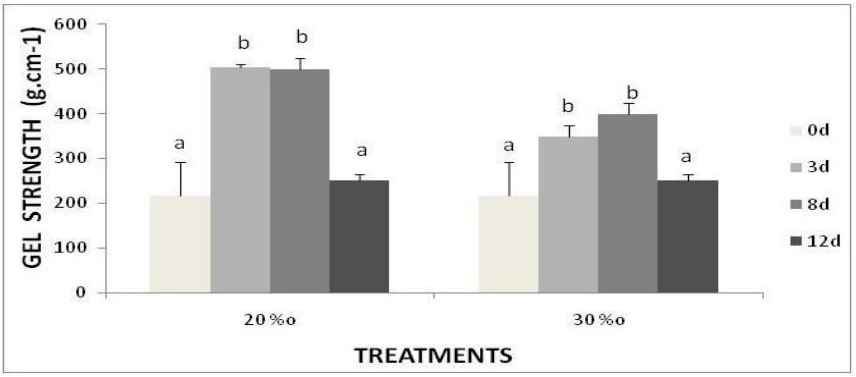

Figure - 2: Agar gel strength of *Gracilaria verrucosa* in different time of dark at salinity of 20% and 30% (mean±sd). Bar with different superscript letters are significant different ($p<0.05$). All controls (0d) were from harvested algae at ambient salinity (20%).

The seaweed production, and also the agar production of various post-harvest culture treatments with dark in salinity of 20% and 30% are summarized in Table 1. The results indicated that the agar extracted from 3d (30 %) treatment exhibited high production, followed by 8d (20%) treatment.

The physical and chemical properties of agar analysis showed that the sulphate and ash content of the agar was lower in 3d either in 20% or in 30% than others in each group (Tabel 2). Meanwhile, all treatments in higher salinity (30%) produced agar with lower water content when compare to treat-ments in lower salinity (20%).

Table-1: The seaweed production, agar content and agar production of *Gracilaria errucosa* treated in various time of dark at salinity of 20% and 30% (mean ±sd). All controls (0d) were from harvested algae at ambient salinity (20%)

DARK (DAYS)	SEAWEED PRODUCTION (kg)		AGAR CONTENT (%)		AGAR PRODUCTION (kg)	
	20%	30%	20%	30%	20%	30%
0	18.77±1.56		9.33±2.25		1.751	1.751
	18.77±1.56		9.33±2.25			
3	18.98±0.07		6.40±1.40		1.215	2.515
	18.98±0.07		13.25±0.30			
8	18.21±0.19		13.40±0.30		2.440	1.537
	17.87±0.19		8.60±0.70			
12	16.92±0.29		10.50±0.50		1.777	1.399
	15.90±0.30		8.80±0.70			

Table -2: Ash content, sulphate content, viscosity and water content of agar *Gracilaria verrucosa* after dark treatments at 20‰ and 30‰. All controls (0d) were from harvested algae at ambient salinity (20‰). Values presented were from pooled samples.

Dark treatments (days)	Ash content (%)		Sulphate content (%)		Viscosity (cP)		Water content (%)	
	20 %	30%	20%	30%	20%	30%	20%	30%
0	4.75		6.80		4.22		19.28	
	4.75		6.80		4.22		19.28	
3	4.34		3.95		4.16		22.74	
	3.74		1.91		3.82		21.71	
8	4.25		6.05		3.88		23.07	
	4.62		4.05		4.90		18.29	
12	5.91		6.23		4.07		18.08	
	4.52		6.00		4.12		15.38	

DISCUSSION

Several studies had been pointed out to aquaculture technology for sulphate content manipulation in *Rhodophyta* species (Ekman *et al*., 1991; Rincones *et al*., 1993; Freille-Pelegrin and Murano, 2002; Hemmingson and Furneaux, 2003; Villanueva *et al*., 2009). However, those studies held in laboratory condition with a very limited sample (in weight) of algal taken, even in unialgal culture (±2-3g ww), and the seaweed used were harvested from natural stocks. In the present study evoked to introduce such post-harvest culture methods especially in Indonesia with some modifications. We used algae that mostly cultured from farm in a larger density than previous studies, as in fact the extraction process needs a minimum of 50 g dry weight of algae. This condition supported by Armisen and Galatas (2011) who suggested to enhance the quantities of seaweed to be tested in the study to obtain good results that similar to industrial process condition that in turn may provide accurate data for massive cultivation in the field. In this study, we also used an inorganic nutrient that was generally applied by the farmer. In addition, the water quality during the study periods were: DO: 3.3-3.8, pH: 7.7-8.3, temperature: 25-30 ^{o}C, and ambient salinity: 20%, that in a range of water quality which may support *Gracilaria verrucosa* growth (Raikar *et al*., 2001; Cirik *et al*., 2010).

This study proved similar results as before that the postharvest culture treatment may improve the quality of agar, either the agar content or the gel strength. This dark condition according to Macler (1986) could activate the agar biosynthesis because at the same time the storage products of algae (*floridean starch* dan *floridoside*) was degraded by certain enzymes and in turn provide carbon for agar biosynthesis. In other words, dark condition released carbon in torage products that yielded from photosynthesis and it was used for agar bioshynthesis. Moreover, the increasing of enzyme activity in dark condition may eliminate sulphate and stimulate precursor of 3,6-Anhydrogalactose (3,6 AG) chain, that could generate the gel strength (Hemmingson and Furneaux, 2003; Villanueva *et al*., 2009).

The postharvest culture treated in this study led to an increase of agar content more than 40% both in 8d (20%) and 3d (30%), respectively when comp-ared to the control treatment (0d). In spite the different methods used, this finding was higher than in *G. cornea* treated in dark and altered salinity for 8 days (4days dark in salinity 50%, follo-wed by 4 days dark in salinity 25%) in which obtained a 26% increase in agar content (Freille-Pelegrin and Murano, 2002). Our results from dark and salinity treatments also showed a shorter time (only 3 days in salinity 30%) to achieve higher agar content than previous study. In terms of gel strength performance, a signi-ficant increase of >100% was evident after 3 and 8days either in 20% or in 30% group. This is a good value since the similar study remains very limitted to be referred. The previous finding in quite long ago (Rincones *et al*., 1993) obtained only less than 20% elevation of gel strength in *G. lemaneiformis* after 10 and 30 days of dark cultivation.

The higher agar production obtained in the present study indicates that dark treatment combined with increased Salinity (10%) may reliable to *G. verrucosa* culture. Elevation of agar content after dark and salinity treatments allocated a direct effect to the agar production as it is the major consideration why *Gracilaria* being cultured. There is no information exists in relation to agar content coupled with agar production from the whole seaweed production

as we did in this study. Therefore, more studies are needed in this area to get the complete data for both culture and economic prespective.

The physical and chemical properties indicate the further important for this red algae quality assessment that also observed to support the application of these dark and increased salinity methods. A low value of sulphate and ash content of agar as well as the low water content of agar derived from post har-vest treatments (particularly in 3d in 30%) suggest that this treatment may perform good quality of agar from various aspects of physico-chemical of agar. In general, the value obtained in this treatment covered a good range that required for ash 4%, sulphate 5% and water 20% content of agar (FAO.Coorp.Doc.Rep. 2014).

CONCLUSION

Postharvest culture with dark condition either in ambient or increased salinity performed better quality of agar, however in general the best quality of agar showed in the 3days dark treatment at salinity of 30% Hence, these methods of postharvest culture supposed to be applicable in *G.verrucosa* culture technique in the future.

REFERENCES

AOAC (Association of Official Analytical Chemists). Official Methods of Analysis, 17th Edition. Association of Official Analytical Chemists, Inc. Gaithersburg, Maryland, USA (2000).

Armisen, R., World-Wide Use and Important of *Gracilaria*. Journal of Applied Phycology **7:** 231-243 (1995).

Armisen,R. and F.Galatas, Production, Properties and Uses of Agar (Chapter-1). *In* Production an utili-zation of products from commercial seaweeds. Available from FAO Coorp. Doc. Rep. Pp. 32 (2011).

Cirik, S., Z. Cetin, I. Ak, S. Cirik and T. Göksan, Green House Cultivation of *Gracilaria verrucosa* (Hudson) Papenfuss and Determination of Chemical Composition. Turk. J. Fish.Aquat.Sci. **10**: 559-564 (2010).

Ekman, P., S.Yu and M. Pedersen, Effects of altered salinity, darkness and algal nutrient status on florido-side and starch content, α-galac-tosidase and agar yield of cultivated *Gracilaria sordida*. British Phycological Journal **26**:123-131(1991).

FAO.Coorp.Doc.Rep.Training manual on Gracilaria culture and seaweed processing in China. Produced by Fisheries and Aquaculture Department. Available from http://www.fao.org/ do crep/field/003/ab730e/AB730E00.htm (2014).

Freile-Pelegrin,Y., D.Robledo,M.Pedersen, E.Bruno and J. Rönnqvist, Effect of dark and salinity treatment in the yield and quality of agar from *Gracilaria cornea* (Rhodophyceae). Ciencias Marinas **28**(3):289-296 (2002).

Freile-Pelegrin,Y. and E.Murano, Agar from the three species of Gracilaria (Rhodophyta) from Yucatan Peninsula. Bioresource Tehnology **96**:295-302 (2005).

Hemmingson, J.A. and R.H. Furneaux, Variation of native agar gel strength in light deprived *Gracilaria chilensis* Bird, McLachlan *et* Oliveira. Botanica Marina **46**: 307-314 (2003).

Macler, B.A., Regulation of Carbon Flow by Nitrogen and Light in the Red Algae, *Gelidium coulteri*. Plant Physiology **82**:136-141 (1986).

Praiboon,J.,A.Chirapart,Y.Akakabe, O.Bhumibhamon and T.Kajiwara, Physical and chemical characterization of agar polysaccharides extracted frm the Thai and Japanese species of *Gracilaria*. ScienceAsia 32, supplement **1:** 11-17 (2006).

Raikar, S.V., M. Iima and Y. Fujita, Effect of temperature, salinity and light intensity on the growth of *Gracilaria* spp. (Gracilariales, Rhodo -phyta) from Japan, Malaysia and India. Indian Journal of Marine Sciences **30**: 98-104 (2001).

Rath,J. and S.P.Adhykary, Effect of alkali treatment on the yield and quality of agar from red alga Gracilaria verrucosa (Rhodophyta, Gracilariales) occurring at different salinity gradient of Chilaka lake. Indian Journal of Marine Sciences **33**(2):202-205 (2004).

Rincones, R.E., S.Yu and M. Pedersen, Effect of dark treatment on the starch degradation and the agar quality of cultivated *Gracilariopsis lemaneiformis* (Rhodophyta, Graci-lariales) from Venezuela. Hydrobiologia **260/261**: 633-640 (1993).

Truno, G.C., Manual on Seaweed Culture, "Pond culture of Caulerpa and Pond culture of *Gracilaria*. ASEAN/SF/1988/Manual No.3. Available from http://www.fao.org/ docrep/ field/003/ac417e/ac417e00.htm (accessed 20 Nov 2012) (1988).

Villanueva,R.D., L.Hilliou and I.Sousa-Pinto, Postharvest culture in the dark: An eco-friendly alternative to alkali treatment for enhancing the gel quality of k/t- hybrid carra-geenan from *Chondrus crispus* (Gigartinales, Rhodophyta). Biore-source Technology **100**: 2633-2638 (2009)