International Journal on Advanced Science Engineering Information Technology

Vol.10 (2020) No. 4
ISSN: 2088-5334

Effect of Seed Type and Harvest Time of Seaweed (Eucheuma Cottonii) on The Quality of Alkali Treated Cottonii

Yuli Wibowo[a], Ahmad Nafi[b,1], Rizqi Ridha Jawara[b,2]

[a] *Department of Agro-industrial Technology, Faculty of Agricultural Technology, University of Jember, Indonesia*
E-mail: yuliwibowo.ftp@unej.ac.id

[b] *Department of Agricultural Product Technology, Faculty of Agricultural Technology, University of Jember, Indonesia*
E-mail: [1]ama_nafi.ftp@unej.ac.id; [2]rizqijawara@gmail.com

Abstract **– Seaweed is a strategic commodity in Indonesia. The most cultivated species of seaweed in Indonesia is *Eucheuma cottonii*. To increase added value, *Eucheuma cottonii* is processed into various products, including Alkali Treated Cottonii (ATC). ATC is a semi-finished product with high value-added and can be used in both the food and non-food industries. The quality of ATC is strongly influenced by the quality of the seaweed used, which itself is strongly determined by the seed and harvest time of the seaweed. This research aimed to analyze the effect of the seed type and harvest time of seaweed on the quality of the ATC produced. The study was carried out in two stages. The first stage involved cultivating seaweed from different seaweed seeds (Lampung culture and Sumenep culture) and different harvesting times (40, 45, 50 days). The seaweed cultivation was carried out in the waters of Sumenep Regency, East Java, Indonesia. The second stage was to produce ATC from seaweed that had been harvested according to the relevant treatments. This research used a completely randomized design comprising two factors (seaweed seed and harvest time). The quality of seaweed was measured based on the parameters of water content, ash content, sulfate content, and gel strength. The data obtained were processed statistically using ANOVA. The results of the research show that the type of seaweed seed significantly affected the water content, sulfate content, and gel strength but did not significantly affect the ash content. The results also showed that the harvest time significantly affected the water content and gel strength but did not significantly affect the ash content and sulfate content. The best quality seaweed was obtained from the Sumenep type of culture seeds with a harvest time of 40 days after planting. The ATC produced was characterized by an ash content of 13.27%, water level of 13.79%, sulfate content of 1.17%, and gel strength of 1,372.74 gf/cm^2.**

Keywords **— seaweed seed; harvest time, *Euchema cottonii*; alkali treated cottonii; quality.**

I. Introduction

Seaweed is a polycellular form of algae (macro-algae) that lives in the sea or a brackish water environment [1]. While seaweed can grow naturally in the sea (wild seaweed), a lot of seaweed is currently cultivated [2]. The seaweed that is widely cultivated in Indonesia is a type of carrageenan-producing seaweed (karaginofit) that is both well-known and widely cultivated in Indonesia. It originates from the genus *Eucheuma*, especially from type E. Cottonii [3].

Carrageenan is a hydrocolloid extracted from red seaweed which is a complex compound of polysaccharides built by a number of galactose units and 3,6-anhydro-galactose both containing sulfate and not with α-1,3-D-galactose and β-1, -3,6-anhydro-galactose bonds [4]. Carrageenan has a very important role as a stabilizer, thickener, gelatin and emulsifier. These properties can be widely used in the food, pharmaceutical, cosmetics, toothpaste, and other important industries [5], [6]. In the food industry, carrageenan is widely used because of its ability to modify the texture and the taste associated with softness and crispness [7]. In the health sector, carrageenan has the potential to be an anti-virus in various diseases such as herpes, hepatitis A, genital human papillomaviruses (HPV), and blocking HIV virus in women [8].

In the world of commerce, carrageenan can be obtained in the form of refine or semi-refine carrageenan. Refine carrageenan has high gel strength and yield which is generally produced from semi refine carrageenan in the form of *alkali treated cottonii* (ATC) or *alkali treated cottonii chips* (ATCC) [2]. The ATC industry is currently increasingly in demand along with the success of seaweed cultivation in several regions in Indonesia [9]. The processing of seaweed (*Eucheuma*) into ATC in principle is very simple and does not require the application of high technology [10].

Besides being determined by the processing process, the quality of ATC can be influenced by the quality of seaweed used. The quality of seaweed is influenced by several factors such as the condition of the water, the process of cultivation, harvesting, and post-harvest handling [11]. Seaweed harvest age has a real effect on the quality of carrageenan produced [12]. The right harvest time affects the quality of seaweed produced [13]. Harvesting seaweed could be done at the earliest 45 days after planting, but in different areas it showed that the best harvesting was at the age of seaweed aged 50 days after planting [14]. Harvest age had a significant effect on the strength of carrageenan gel. The older the harvest age, the strength of the gel produced tended to increase, and decreased after reaching the peak of growth [15].

The levels of carrageenan in each species of *Eucheuma* seaweed vary depending on the type of species and place of growth [3]. This shows that the type of seaweed and the conditions of cultivation can affect the quality of ATC produced [11]. The quality of seaweed is largely determined by the quality of the seeds used [13]. A good standard for selecting seaweed seedlings is from seaweed plants that grow well, are still fresh, have no spots, are homogeneous in color, and are not easily broken [16].

This research was conducted to improve the quality of seaweed in Sumenep Regency as a center for seaweed production in Indonesia, especially in East Java Province. Seaweed seeds used in the study were local seaweed seeds from Sumenep Regency, East Java, Indonesia, and seaweed seeds imported from Lampung Marine Aquaculture Center.

This research aimed to determine the effect of seaweed seeds and harvest age on the quality of ATC produced. The results of the research were expected to be able to find the right type of seaweed seeds to be cultivated in Sumenep Regency with optimal harvest age so that seaweed with good quality was obtained as a raw material for the manufacture of ATC.

II. Material And Method

The main raw materials used in the research were two types of seaweed (*Eucheuma cottonii*), namely local seaweed in Sumenep Regency and seaweed obtained from Lampung (Lampung Culture). Research supporting materials were KOH and other chemicals and non-chemical for the needs of analysis.

The design of this research used a complete randomized design with 2 factors covering the type of seaweed seeds (Factor A) and the harvest age (Factor B). The combination of research treatments can be seen in Table 1.

TABLE I
The Combination of Research Treatments

Harvest Age	Seaweed Seeds Types	
	Local Sumenep (B_1)	Lampung Culture (B_2)
40 day (A_1)	A_1B_1	A_1B_2
45 day (A_2)	A_2B_1	A_2B_2
50 day (A_3)	A_3B_1	A_3B_2

The process of seaweed cultivation in this research was carried out in the territorial water of Bluto Subdistrict, Sumenep Regency, East Java Province, Indonesia. Seaweed cultivation used raft method. The characteristic of water conditions in the Bluto Sub-district can be seen in Table 2 [18]. Seaweed that had been harvested were used as raw material for the manufacture of ATC. Before processing, the seaweed must be prepared first. The process of preparing seaweed is presented in Fig. 1.

TABLE II
Characteristic Water Conditions in the Bluto Sub-district

No.	Parameter	Unit	Value
1	Bottom of the water	-	-
2	Depth at the lowest ebb	m	0.95
3	Current speed	cm/sec	17.90
4	Surface temperature	°C	31.77
5	Salinity	‰	30.55
6	pH	-	7.71
7	Brightness	%	61.06
8	Dissolved oxygen	mg/l	6.50
9	Nitrate	mg/l	0.14
10	Ammonium	mg/l	0.35

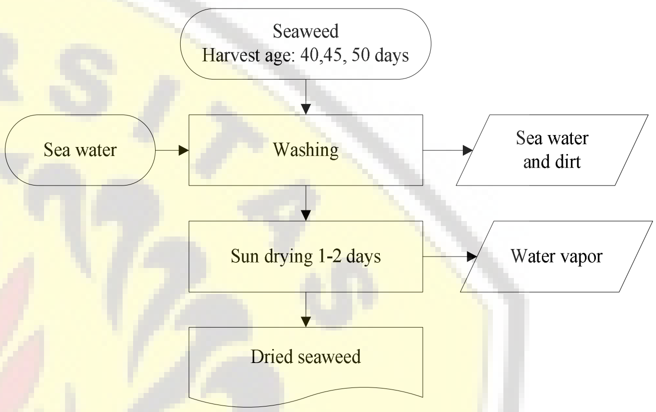

Fig. 1 Preparing seaweed for ATC raw material

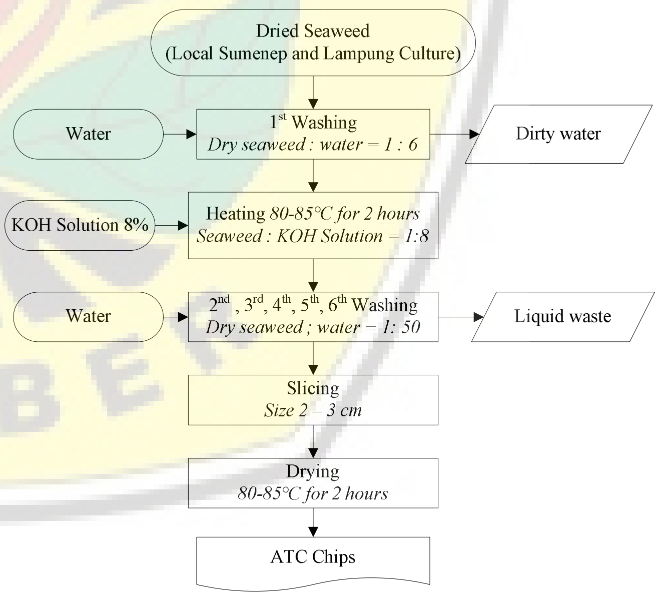

Fig. 2 Flow diagram of processing ATC

ATC manufacturing was done at the Faculty of Agricultural Technology, University of Jember. The method of making ATC referred to research conducted by Supriyantini *et al.* with modification [17]. The harvested seaweed was dried first, then washed thoroughly and drained. Seaweed was boiled in 8% KOH solution at 80-85°C for 2 hours. Boiled seaweed was then washed by using clean water until it got neutral. Seaweed was then cut into 2-3 cm lengths, then dried using an oven at 60°C for 20 hours, so that ATC was obtained in the form of chips. The flow diagram of the ATC manufacturing process can be seen in Fig. 2.

Variables of research observations included gel strength [25], sulfate content [26], water content [27] and ash content [27]. The research data were processed statistically by using ANOVA at the level of 5%, followed by the DUNCAN test if the result was significantly different. The data was presented in the form of a table and histogram.

III. Results and Discussion

A. Ash Content

The analysis of ash content was intended to determine the mineral content contained in ATC. The results of ATC ash content analysis on the treatment of seaweed seedlings and harvest age ranged from 13.27 to 16.29%. The results showed that the lowest ATC ash content was produced in the treatment of Sumenep-type local seaweed with harvest age of 40 days, while the highest ATC ash content was obtained in the treatment of Lampung culture seaweed with harvest age of 40 days. The ATC ash content obtained in this research was still in accordance with the maximum standards required by Chemical Food Codex, which was 14 - 40%.

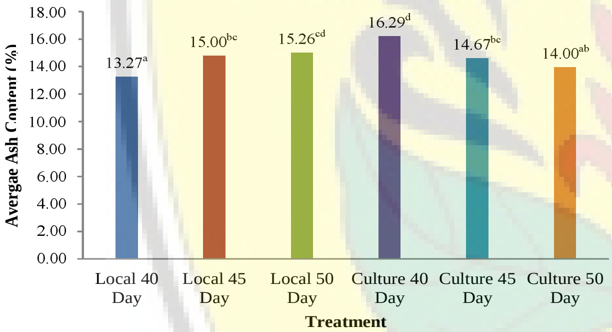

Fig. 3 Ash content test of Alkali Treated Cottonii

The result of statistical analysis showed that the treatment of seaweed seedlings and harvest age resulted in ATC ash content was not significantly different ($p < 0.01$). This was due to seaweed used as raw material for making ATC, including Sumenep local seaweed seeds and Lampung culture seaweed seeds, grown in the same waters in Sumenep Regency. The ash content in seaweed was usually affected by the condition of the waters where the seaweed was grown, including salinity. Waters with high salinity caused seaweed to contain lot of mineral salts, while high and low ash contents were influenced by the presence of other mineral salts attached to seaweed such as sodium and calcium [16] [17]. Salinity levels in the waters of Sumenep Regency was 30.5‰ in average [18].

B. Water content

ATC water content testing aimed at determining how much water content was still being lagged as this was related to the quality of ATC. The results of ATC water content analysis on the treatment of seaweed seedlings and harvest age in the research ranged from 10.89 to 27.94%. The highest ATC water content value was produced from the treatment of Sumenep local seaweed seedlings with harvest age of 50 days, while the lowest ATC water content value was obtained from the treatment of Lampung culture seaweed with harvest age of 50 days.

The results of ATC water content testing on the type of Sumenep local seaweed and Lampung culture showed a tendency for data patterns to differ from the longer harvest age in the treatment. The longer the harvest age of Sumenep local seaweed was, the higher the ATC water content produced would be. On the other hand, the longer the harvest age of Lampung culture seaweed was, the lower the water content of ATC produced would be.

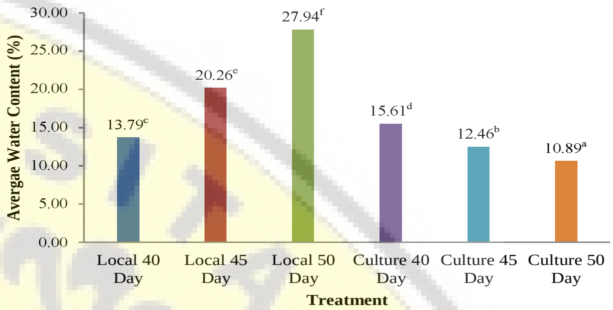

Fig. 4 Water content test of *Alkali Treated Cottonii*

The results of the statistical analysis showed that the treatment of the types of seaweed seeds and harvest age showed a significant effect on ATC water content ($p < 0.01$). The interaction between the two treatment factors also gave results that significantly affected the ATC water content produced.

The water content of ATC in the type of Sumenep local seaweed seedlings rose along with the increase of harvest age. The older the age of seaweed was, the higher the water content of ATC produced would be. The increase of ATC water content by increasing the harvest age of seaweed on the treatment of Sumenep local seaweed, with increasing harvest age was claimed to be related to the increasing sulfate content of ATC which affected the binding capacity of water. The sulfate group had hydrophilic properties (likes water) [19]. The higher the sulfate group contained in seaweed, the greater the water bound by the sulfate group so that caused the water content to be high [20].

In contrast to the types of Sumenep local seaweed, ATC water content obtained from Lampung seaweed culture decreased along with the increasing harvest age. The older the age of Lampung seaweed culture, the lower the ATC water content produced. The decrease in water content of Lampung culture seaweed species was also thought to be related to the sulfate content of ATC in this type, which decreased so that affected the binding capacity of water. If the sulfate content in seaweed was high, it would have bound much water at the time of extraction, and likewise, if the sulfate content was low then the water binding capacity at extraction was low [21].

The difference in the results of the ATC water content test on the Sumenep local seaweed and Lampung culture was indicated to be caused by differences in the age of the peak types of seaweed that affected the sulfate content. The longer the harvest age of seaweed, the higher the water content. This was presumably due to the hydrophilic property of the sulfate group, which caused seaweed to absorb quite a lot of water. However, after going through the process of extraction and drying, the free water on seaweed may have evaporated, and what remained was bound water, especially chemically bound. Factors that influenced the water content in ATC included the drying system, the innate properties of the product such as the presence of hygroscopic ions and the presence of treatment factors in the process of ATC making [5].

C. Sulfate Content

Sulfate content was one of the parameters used for various types of polysaccharides found in red algae. Sulfate content was one of the important parameters for determining ATC quality. The sulfate content of carrageenan kappa affected physical properties such as gel strength and 3.6 an-hydro galactose content [19].

The results of sulfate content analysis of ATC in the treatment of seaweed seedlings and harvest age ranged from 0.75 to 1.65%. The lowest sulfate content value was obtained from the treatment of Lampung culture seaweed seeds with a harvest age of 50 days, while the highest sulfate content was obtained from the treatment of Sumenep local seaweed seeds with a harvest period of 50 days.

The results of ATC sulfate content testing on Sumenep local seaweed species and Lampung culture at various ages of seaweed harvest showed the similar pattern as ATC water content testing. The longer the harvest age of Sumenep local seaweed, the higher the ATC sulfate content produced. Conversely, the longer the harvest age of Lampung culture seaweed, the lower the sulfate content of ATC produced.

The results of statistical analysis showed that the treatment of seaweed seedlings and the treatment of seaweed harvest age had no a significant effect on the ATC sulfate content produced. The different types of seaweed seeds, both Sumenep local seaweed seeds and Lampung culture seaweed seeds, did not have a significant effect on the ATC content produced. Similarly, the differences in the age of seaweed harvesting, including 40, 45 and 50 days, also did not have a significant effect on the amount of ATC sulfate content produced. The interaction between the two treatments also revealed the results that were not significantly different from the contents of ATC sulfate produced.

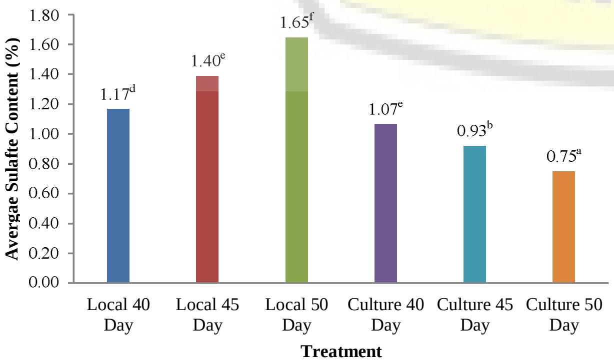

Fig. 5 Sulfate content test of *Alkali Treated Cottonii*

The difference data pattern of ATC sulfate content in Sumenep local seaweed and Lampung culture were caused by the peak different ages of the two types of seaweed. The peak age of Sumenep local seaweed was assumed to be reached at 40 days after planting, while in Lampung seaweed culture was reached up to 50 days after planting. The difference in age of these peaks was also caused by the type of Lampung seaweed seedlings which still needed an adaptation process in the new environment in the territorial waters of Sumenep Regency. The process of adaptation of seaweed in new waters was able to slow down the growth and affected the content of seaweed, which caused the peak age, especially in Lampung culture seaweed was quite long [22].

The sulfate content of ATC continuously decreased along with the age of seaweed up to the peak of seaweed growth. After reaching the peak age and the older the harvest age, the sulfate content will be higher [23]. This difference was also supported by the different water content test results of the two types of seaweed. Sulfate had hygroscopic properties that were binding on water, so the higher the water content, the higher the sulfate content in ATC [24]. The sulfate content obtained in this research is still in accordance with the maximum standards required by FAO of 15-40%.

D. Gel Strength

Gel strength is one of the important physical properties to determine the quality of ATC produced. Gel strength is the resistance of the material to rupture due to the pressure given. Gel strength is the main physical property of carrageenan because the gel strength shows the ability of carrageenan in gel formation.

The analysis results of ATC gel strength on the treatment of seaweed seedlings at various harvest ages ranged from 355.67 - 1,372.74 gf/cm^2. The highest value of ATC gel strength was produced in the treatment of the type of Sumenep local seaweed seedlings with harvest age of 40 days. While the lowest ATC gel strength value was obtained in the treatment of Lampung culture seaweed with a harvest age of 40 days.

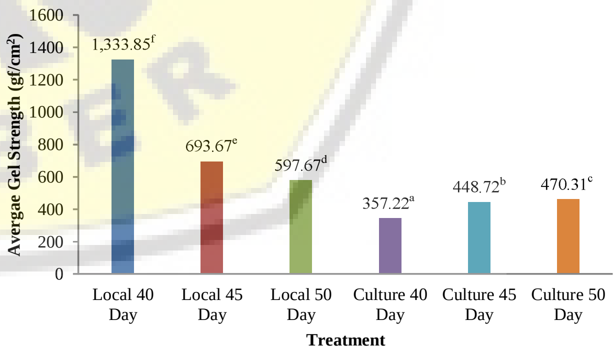

Fig. 6 Gel strength test of *Alkali Treated Cottonii*

The results of statistical test revealed that the treatment of types of seaweed and the treatment of harvest age of seaweed had a significant difference on the ATC gel strength produced. The interaction between two treatments also revealed the significant different results on the ATC gel strength gel produced.

Based on the result of the test on the strength of ATC gel produced, the difference of seaweed type in various harvest ages showed a different data pattern to the two seaweeds used in the research. The strength of ATC gel obtained from the Sumenep local seaweed decreased along with the increase of the harvest age. The older the Sumenep local seaweed age, the lower the strength of ATC gel produced. Whereas, to the seaweed type of Lampung culture, the older the seaweed age, the higher the value of ATC gel strength produced.

In Sumenep local seaweed, the decrease of the gel strength was caused by the peak age of the Sumenep local seaweed that was assumed to be in the harvest age of 40 days old after cultivation. The older the seaweed age, the strength of ATC gel produced tend to increase up to its peak age, and then decrease after rjeaching the growth peak. The results of the analysis showed the increase of sulfate content along with the increase of the harvest age. The high content of sulfate affected the binding capacity toward water which influenced the gel strength. This was in line with the statement [15] that the increase of gel strength directly proportional with 3.6- anhydrogalactose and inversely proportional with sulfate content. The higher the sulfate content, the lower the value of the gel strength.

In culture Lampung seaweed, the strength of ATC gel produced by the seaweed increased along with the increase of the harvest age. The older the seaweed age, the higher the ATC gel strength produced. The increase of the gel strength was caused by the peak age of the Lampung culture seaweed was different from the Sumenep local seaweed. The peak age of the Lampung culture seaweed was assumed to be in the harvest age of 50 days after cultivation. The results of the analysis showed the decrease of sulfate content along with the increase of the harvest age. The sulfate content decreased during the increase of the harvest age and it was inversely proportional with 3.6- anhydro galactose that was increase up to the growth peak.

The difference of gel strength between Sumenep local seaweed and Lampung culture seaweed was caused by different peak age of the two seaweeds. Sumenep seaweed's peak age was on the 40^{th} days after cultivation while Lampung culture seaweed's peak age was on the 50^{th} days after cultivation. This difference was because Lampung culture seaweed was still in the process of adaptation with new waters in Sumenep region. The chemical change on the seaweed was the plant's physiology response toward the need of carrageenan as tissue constituent compound.

The water content in ATC affected the value of the ATC gel strength. The older the harvest age of the Sumenep local seaweed, the higher the water content produced in ATC. In Lampung culture seaweed, the water content of ATC decreased along with the increase of the harvest age. The high-water content on the ingredient caused the high-water content on the gel. With the high content of water in the gel, it caused the gel soft and fragile so the gel strength decreased.

Besides being caused by the influence of sulfate content, the high strength of the gel in ATC was also influenced by the content of 3,6-anhydrogalactose group contained in seaweed. The work mechanism of KOH solution eased the release of 6-sulfate group from its polymer to be 3,6-anhydrogalactose that could increase the gel carrageenan [19]. The higher the content of 3,6-anhydrogalactose, the higher the value of the gel strength and the lower the sulfate content.

E. Effectiveness Test

Effectiveness test was used to know which treatment gave the highest effect as the best treatment to all the parameter combinations that had been analyzed. Effectiveness test helped in determining the best treatment from the observed treatments. The results of the effectiveness test showed that the treatment of different seaweed types on various harvest ages resulted in the effectiveness value ranged from 0.15-0.84.

TABLE III
THE EFFECTIVENESS VALUE OF HARVEST AGE VARIATION AND SEAWEED TYPES TOWARD THE ATC PRODUCED

Treatment	Effectiveness Index
Local 40 Day	0.84
Local 45 Day	0.36
Local 50 Day	0.15
Culture 40 Day	0.33
Culture 45 Day	0.56
Culture 50 Day	0.69

The result of effectiveness test showed that the best treatment was ATC obtained from the Sumenep local seaweed with the harvest age of 40 days. The treatment was considered as the best treatment because it had the value of ash content of 13.27%; water content of 13.79%; sulfate content of 1.17%; and gel strength value of 1,372.74 gf/cm2.

IV. CONCLUSION

The result of statistical test showed that the harvest age of seaweed gave significant effect on the water content and gel strength, however, it gave no significant effect on the ash and sulfate contents, with significance value of 95% or $p<0.05$. The type of seaweed seed *Euchema cottonii* gave significant effect on the water content, sulfate content and gel strength but it gave no significant effect on the ash content with significance value of 95% or $p<0.05$. The result of effectiveness test showed that the best quality of ATC obtained from the Sumenep local seaweed with the harvest age of 40 days after cultivation.

REFERENCES

[1] V.K. Dhargalkar and D. Kavlekar. *Seaweed – a field manual. National Institute of Oceanography*, Dona Paula. Goa. 2004.

[2] D.J. McHugh. *A Guide to the Seaweed Industry*. Food and Agriculture Organization of the United Nations. FAO FisheriesTechnical Paper 441. 2003.

[3] J.T. Anggadiredja, A. Zatnika, H. Purwanto H, and S. Istini. *Seaweed: Cultivation, Processing and Marketing of Potential Fishery Commodities*. Penebar Swadaya. Jakarta. 2006.

[4] A. Soegiarto, Sulistijo, W. Atmaja, and H. Mubarak. *Seaweed (Algae): Benefits, Potential and Cultivation Effort. SDE 45*. National Oceanology Institute. SDE 45. Lembaga Oseanologi Nasional. LIPI. Jakarta. 1978.

[5] J. Basmal. *Prospects of Seaweed Industry (Euchema sp) Producing Semi Refine Carrageenan and Refine Carrageenan*. Installation of the Marine Fisheries Research Institute, Center for Fisheries Research and Development Agency for Agriculture, Jakarta. 2000.

[6] C. Rolin, R. Inkster, J. Laing, J. Hedges, and L. McEvoy. Seaweed Cultivation Manual. *Shetland Seaweed Growers Project 2014-16.* Coastal Cummunity Fund. NAFC Marine Centre University of Highlands and Islands. 2014.

[7] G.R. Sanderse, "Polysaccharides in food," *J. Food Technol*. 35 (7). p.50-59. 1981.

[8] V.L. Campo, D.F. Kawano, D.B. da Silva Jr., and I. Carvalho. "Carrageenans: Biological properties, chemical modifications and structural analysis – A review," *Carbohydrate Polymers* 77:167-180. 2009.

[9] A. Kustantiny, C. Sarwanto, Bernhard, H. Hadiastuty, S. Wahyuni. *Profile of Seaweed Business and Investment Opportunities*. Editor: Nikijuluw V. Ministry of Maritime Affairs and Fisheries. Jakarta. 2009.

[10] FFA Salasa. *Fish and Seaweed Processing Technology*. Fisheries Education and Training Center of the Ministry of Maritime Affairs and Fisheries. Jakarta. 2002.

[11] B. Setha, M.N. Mailoa, and F.F. Gaspersz. "Analysis of Quality Sheet Carrageenan of *Euchema cottonii*," *International Journal of ChemTech Research* Vol.9 No.01: 92-94. 2016.

[12] J.W. Marseno, M.S. Medho, and Haryadi, "Effect of Harvest Time of Seaweed Eucheuma Cottonii on Physical, Chemical and Fungsional Properties of Carrageenan," *AGRITECH*, Vol. 30, No.4: 212-217. 2010.

[13] S. Sulaeman, "Seaweed Commodity Agribusiness Development Through Business Cluster Model," *Infokop* No.28 Tahun XXII, 2006.

[14] M.R. Wenno, J.L. Thenu, and C.G.C. Lopulalan. "Characteristics of Kappa Carrageenan from *Kappaphycus alvarezii* at various harvest ages," *JPB Fisheries* 8(1): 61-67. 2012.

[15] S. Widyastuti, "Physical and Chemical Characeristics of Carrageenan Extracted from Seaweed Euchema cottonii and E. spinosum at Different Harvest Age," *Agroteksos* 20(1). 2010.

[16] J. Santoso, N. Sukri, and Uju, "Characteristics of Alkaline Treated Cottonii (ATC) at various harvest ages," *Journal of Fisheries and Marine Sciences Research*. 6(2): 85–90. 2007.

[17] E. Supriyantini, G.W. Santosa, and A. Dermawan, "Quality of Carrageenan Extract from Kappaphycus alvarezii Seaweed Cultivated in the waters of Kartini Beach and Kemojan Karimunjawa Island, Jepara Regency," *Marina Oceanographic Buletin*. Vol 6 No 2:88-93. 2017.

[18] Y. Wibowo, M.S. Ma'arif, A.M. Fauzi, L. and Adrianto, "Diagnosis of Feasibility of Sustainable Seaweed Industry Clusters," Agrointek 5(1): 33-34. 2016.

[19] J. Basmal, Syarifudin, and W.F. Ma'ruf, "Effect of Concentration of Potassium Hydroxide Solution on Quality of Kappa Carrageenan Extracted from Eucheuma cottonii," *Indonesian Fisheries Research Journal* 9 (5): 95 – 103. 2003.

[20] Th.D. Suryaningrum, Murdinah and M.D. Erlina, "Effect of Alkali Treatment and Volume of Extracting Solutions on the Quality of Carrageenan from Euchema cottonii Seaweed," *Indonesian Fisheries Research Journal*. 9(5): 65-76. 2003.

[21] A.P. Romenda, R. Pramesti, and A.B. Susanto, "Effect of Different Types and Concentrations of Alkaline Solutions on Gel Strength and Viscosity of Carrageenan Kappaphycus alvarezii, Doty." *Journal of Marine Research* 2(1): 127-133. 2013.

[22] A.R. Erpin and Ruslaini, "Effect of Harvest Age and Seed Weight on Growth and Content of Carrageenan Seaweed (Euchema spinosum) Using Long Line," *Mina Laut Indonesia Journal*.Vol.03 No.12. 2013.

[23] S. Lewerissa, *Effect of Eucheuma cottonii Harvest Age on Characteristics of Carrageenan and Edible Film Produced*. Postgraduate Thesis Program, Gadjah Mada University. Yogyakarta. 2005.

[24] V.D. Loupatty and A. Moniharapon, "Efficient Use of NaOH Alkali Solution in Processing Eucheuma Seaweed into Semi Carrageenan. *Biam Magazine* 10(1): 1-7. 2014.

[25] N.K. Sumarni and E. Sulastri, "Extraction and Characterization of Semi Refine Carrageenan from Seaweed (Eucheuma cottonii)," *Proceedings of the National Chemistry Seminar*. Yogyakarta State University. 2017.

[26] S. Distantina, Wiranti, M. Fahrurrozi, and Rochmadi, "Carrageenan Properties Extracted from *Euchema cottonii*, Indonesia" *World Academy of Science, Enginering and Technology International Jurnal of Chemical and Molecular Engineering.* Vol:5, No:6. 2010.

[27] Association of Official Analytical Chemist (AOAC). *Official Methods of Analysis.* USA: Association of Official Analytical Chamist Inc Mayland. 2005.