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Characteristics of seaweed caraginan *Kappaphycus alvarezii* on cultivation system with different seed weight

Karakteristik karaginan rumput laut *Kappaphycus alvarezii* pada sistem budidaya dan berat bibit yang berbeda

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ABSTRACT

Seaweed (*Kappaphycus alvarezii*) could be a source of carrageenan needed for industrial sector. The purpose of this study to analyze the characteristics of carrageenan seaweed cultivated with bag and non-bag cultivation systems and different seed weights. The research was conducted in the waters of Bone Bay and the Laboratory of the Indonesian Center for Brackish Water Cultivation and Fisheries Extension in Maros. This study used a factorial completely randomized design (CRD) consisting of factor A (using bags and non-bags), and factor B (seed weight 15, 50, 75, 100, and 125 g). The results showed that the cultivation method factor, seed weight factor, and their interaction had an effect on carrageenan yield, gel strength, ash content and water content ($P<0.05$). Different cultivation systems with different initial seed weight combinations showed different best results for each parameter. The highest value of yield of carrageenan was observed in the bag culture system with a seed weight of 100 g, namely 29.18 ± 1.10%. The highest value of gel strength was observed in the non-bagged cultivation system with a seed weight of 75 g, namely 1344.69 ± 18.43 g/cm^2. The highest value of ash content was found in the non-bagged cultivation system with 125 g of seed weight, namely 30.02 ± 0.13%. The highest value of water content was found in the bag culture system at a seed weight of 15 g, namely 38.63 ± 0.26%. Different cultivation methods and seed weight resulted in other carrageenan characteristics of seaweed for each parameter.

Keywords: gel strength, ash content, moisture content, yield

ABSTRAK

Rumput laut (*Kappaphycus alvarezii*) memiliki potensi sebagai sumber karaginan yang banyak dibutuhkan untuk bidang industri. Penelitian ini bertujuan untuk menganalisis karakteristik karaginan rumput laut yang dibudidayakan dengan sistem budidaya kantong dan non kantong serta berat bibit yang berbeda. Penelitian dilaksanakan di perairan Teluk Bone dan Laboratorium Balai Riset Budidaya Air Payau dan Penyuluhan Peikanan Maros. Percobaan penelitian dilakukan melalui rancangan acak lengkap (RAL) faktorial yang terdiri faktor A (memakai kantong dan non kantong), faktor B (berat bibit 15, 50, 75, 100, dan 125 g). Hasil penelitian menunjukkan bahwa faktor metode budidaya, berat bibit dan interaksi keduanya berpengaruh terhadap rendeman karaginan, kekuatan gel, kadar air, kadar abu ($P<0,05$). Penggunaan sistem budidaya yang berbeda dengan kombinasi berat awal bibit berbeda menunjukkan hasil terbaik yang tidak sama pada tiap parameter. Nilai tertinggi rendeman keraginan teramati pada sistem budidaya kantong dengan berat bibit 100 g yakni 29,18 ± 1,10%. Nilai tertinggi kekuatan gel teramati pada sistem budidaya non kantong dengan berat bibit 50 g yaitu 1344,69 ± 18,43 g/cm^2. Nilai kadar abu tertinggi pada sistem budidaya non kantong dengan berat bibit 125 g yaitu 30,02 ± 0,13%. Nilai kadar air tertinggi pada sistem budidaya kantong dengan berat bibit 15 g yaitu 38,63 ± 0,26%. Penggunaan metode budidaya dan berat bibit yang berbeda menghasilkan karakteristik karaginan rumput laut yang berbeda pada tiap parameter.

Kata kunci: kekuatan gel, kadar abu, kadar air, rendemen

INTRODUCTION

One of the aquaculture commodities that has become mainstay of country's foreign exchange earner due to its production keep increasing each year is seaweed. Nowadays, Indonesia is the biggest producer of seaweed after China with productions of up to 9.1 million in 2021 (KKP, 2022). One of the species of seaweed that usually cultivated is *Kappaphycus alvarezii.* This species of seaweed has some excellences such as it is easy to be cultivated, the cost required is relatively lower for its management, and the time for its rearing is shorter (Ikhsan *et al*., 2022).

K. alvarezii is one of *Carragenophytes,* a seaweed as carrageenan source that widely use for pharmaceutical, industrial, food, as thickener, gelling agent, stabilizing agent, pill packaging material, cosmetics, printing and textile industry (Basiroh *et al*., 2016; Sormin *et al*., 2018). The increasing of population and industrial growth, and the tendency of people to return to natural products are driving to the increasing need towards carrageenan (Harun *et al*., 2013). According to the benefits and needs towards carrageenan in various sector, more efforts are needed to increase the production volume and quality product of *K. alvarezii* as carrageenan source and as country's foreign exchange earner. The quality of carrageenan in seaweed are affected by various factors, such as the location of cultivation, type of seed, planting season (Afandi *et al*., 2015; Simatupang *et al*., 2021), cultivation method, harvesting technique, handling of post-harvesting, and methods that used for extraction (Hiariey *et al*., 2021).

Implementing inappropriate cultivation method can cause the seaweed growth is less optimal, therefore the product quality of carrageenan is below the commercial standards (Saputra *et al*., 2021). Usually, the cultivation of seaweed is carried out by surface method, off-bottom, and bottom method (Fernando *et al*., 2021), floating raft, and longline (Sunarpi *et al*., 2020), and horizontal and vertical method (Ariyati *et al*., 2016; Wiyanto *et al*., 2019). The use of bags in seaweed cultivation is considered to be very effective for enhancing the growth of seaweed to prevent disease attacks (Dewi & Suryaningtyas, 2020; Kusuma *et al*., 2021). Some previous study showed that the growth of seaweed is better when used bags. The growth of *Eucheuma spinosum* reached 5.13-6.01% a day by using net bag method (Hendri, 2020).

The growth of *K. alvarezii* seaweed that cutltivated by using net bag off-bottom method could reach 4.95 ± 0.70 %/day. As for the growth of *K. alvarezii* seaweed that cutltivated by using longnet and horinet method reached only -3.30%-3.77%/day (Chayani *et al*., 2021). The use of bag in seaweed cultivation has tendency that can increase the growth of seaweed. The study by Periyasamy and Rao (2017), showed that seaweed cultivation using monoline netbag method had better growth performance than using monoline tubular method thus it enabling seaweed cultivator to gain bigger profit. Hendri (2020), stated seaweed that grow optimally can produce high quality carrageenan.

High quality carrageenan is suitable with standards required by the industry (Veeragurunathan *et al*., 2016). Therefore, carrageenan content is one of parameters to determine seaweed quality (Simatupang *et al*., 2021). This study aims to analyze the characteristic of carrageenan from different seaweed cultivation system and seed weight.

RESEARCH METHOD

This study was carried out by two steps, seaweed cultivation and carrageenan characteristic extraction. The seaweed reared using longline method with bag and non-bag cultivation systems, the seeds weight was varied (15, 50, 75, 100, and 125 g). The study used factorial completely randomized design, which is A factor was cultivation method (bag and non-bag cultivation systems) and B factor was seaweed seeds weight. The seaweed cultivation construction design can be seen in Figure 1.

The measured water quality parameter such as salinity, temperature, pH, water brightness, and water flow speed were carried out in situ, meanwhile nitrate, phosphate, and carrageenan content were analyzed in the laboratory of Balai Riset Budidaya Air Payau dan Penyuluhan Perikanan Maros. The carrageenan analysis included carrageenan yield, ash content, and water content were done by using gravimetric method, while gel strengths test using texture analyzer that referred to AOAC (1995). As for the condition of seaweed in bag can be seen in Figure 2.

Carrageenan yield

The carrageenan extraction method used in this study was referred to Yong *et al*. (2014), method. The seaweed was washed first using freshwater until the talus is free from dust, then the seaweed was dried using drying cabinet at temperature of 40°C for three to five days. Dry seaweed was measured as much as 15 g then 90 mL of KOH 0.6% was added into it, and boiled in waterbath for two hours at temperature of 60°C. Seaweed was washed using flowing freshwater with netral pH.

Seaweed was boiled with aquades 1:20 (v/v) in waterbath for two hours in temperature of 90°C. The seaweed extract then was filtered using filter with mesh size of 40 and the seaweed was pressed. Seaweed was stored in freezer for 24 hours. Carrageenan extract was thawed and dried using an ovem at temperature of 50°C to reduce water content in seaweed. The seaweed extract was precipitated using propanol, then was filtered using a filter, and the carrageenan was dried in an oven with temperature of 50°C. Carrageenan yield was calculated referring method AOAC (1995).

$$\text{Carrageenan yield (\%)} = \frac{\text{Carrageenan weight}}{\text{Dry seaweed weight}} \times 100$$

Gel strength

As much as 1.5 g of carrageenan flour was added to 100 mL of aquades, then it was boiled at a hot plate with stirrer and KCl 0.5% solution was added into the mixture placed in a standard bottle, and chilled until springy. The gel strength was measured by using texture analyzer referred to AOAC (1995), with this following formula:

$$\text{Gel strength (g/cm}^2\text{)} = \frac{\text{Probe weight (g)}}{\text{Contact surface area of the probe}} \times \text{calibration value}$$

$$\text{Calibration value} = \frac{\text{bProbe weight (g)}}{\text{Distance between probe and gel (cm)}}$$

Water content

Water content was measured by using Gravimetric method. Empty cup was sterilized in oven at 105°C for two hours, then was chilled to room temperature by transferring it into the desiccator for approximately 30 minutes. Empty cup was measured (A g), and as much as 2 g of samples was put into the cup (B g). Cup was put into the oven for 16–24 hours in 105°C. Cup was

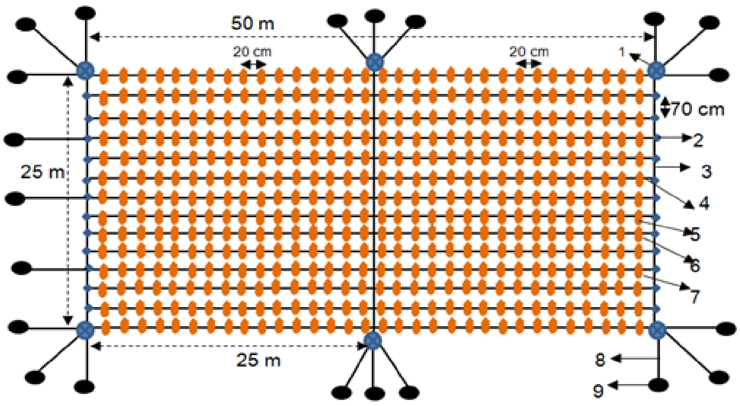

Figure 1. The seaweed cultivation construction design.

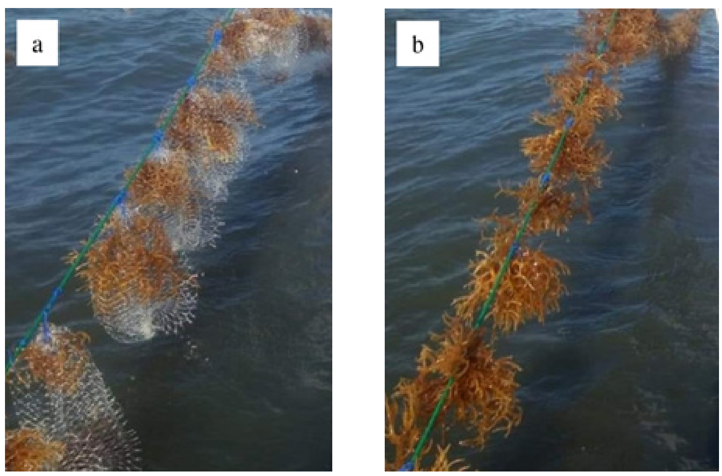

Figure 2. The condition of seaweed in stretch rope, bag (a) and non bag method.

placed into the desiccator and left for ± 30 minutes then weighed (C g). The extraction method was referred to procedure of SNI 01-2345.2-2006 (BSN, 2006a), using this following formula:

$$\text{Water content (\%)} = \frac{Bc - Ck}{Bc - Ak} \times 100$$

Note:
Ak = Empty cup weight (g),
Bk = Cup weight + seaweed weight (g),
Ck = Cup weight + dry seaweed (g)

Ash content

Ash content was determined referring to Gravimetric method. Ash content can be known from weight of mineral residu resulted from combustion. The sterilization of empty cup was carried out by using ashing furnace in temperature that gradually increased until it reached 550°C ± 5°C. The temperature was maintained for two hours, then it was gradually lowered until the temperature reached 40°C.

The cup was put into desiccator and cooled for 30 minutes, then weighed (A g). Sample that has been weighed as much as 2 g was put into cup and heated using an oven at temperature of 105°C for two hours. The cup was moved into ashing furnace and the temperature is gradually increased until 550°C for three to four hours, so as the white ash is produced, then the temperature is gradually lowered until 40°C. Cup was put into the desiccator and rested for 30 minutes, then the sample was weighed (B g). The calculation method is referred to SNI 01-2354.1-2006 (BSN, 2006b), with this following formula:

$$\text{Ash content (\%)} = \frac{Bc - Ap}{\text{Seaweed weight}} \times 100$$

Note:
Ap = Weight of porcelain cup (g)
Bc = Cup weight + seaweed weight (g)

Water quality

The water quality measurement in this study includes temperature, pH, water salinity, water brightness, water flow speed every two days, nitrate and phosphate measurements every seven days. Temperature measurements used thermometer YSI Pro20I, salinity measurement utilized a refractometer, pH measurement used a pH meter, water brightness used secchi disk and water flow speed used current meter. Nitrate and phosphate were analyzed in laboratory used titration method. The result of water quality measurement were temperature 27.75-29.50°C, pH 7.0-8.5, salinity 15-32 g/L, water brightness 42-225 cm, water flow speed 14.73-39.84 cm/second, nitrate 0.0427-0.4696 mg/L, phosphate 0.0079–0.0738 mg/L.

Data analysis

This study used factorial completely randomized design (CRD) consisted of two factors. First factor was A factor (bag and non-bag system) and second factor was B factor (seed weight of 15, 50, 75, 100, and 125 g). Total experimental units in this study were three replications of each treatment. Carrageenan yield, gel strength, water content, and ash content were analyzed using analysis of variance (two way-ANOVA) by SPSS ver.16 in 95% of confidence level. The further test was carried out by using Tukey test, meanwhile the result of water quality measurement is presented descriptively.

RESULTS AND DISCUSSION

Carrageenan yield

Carrageenan yield is the percentage of carrageenan weight from extracted seaweed (Failu *et al*., 2016). This study showed that there was effect from bag and non bag factor treatment, seed weight, and the interaction between both factors towards carrageenan yield of *K. alvarezii* ($P<0.05$) (Table 1). The carrageenan yield using bag cultivation system showed significantly different result ($P<0.05$). The highest carrageenan yield from observed seaweed were in seed weight of 15, 50, 100 and 125 g and the lowest carrageenan yield found in seed weight of 75 g.

The carrageenan yield using non bag cultivation system also showed significantly different result ($P<0.05$). The highest carrageenan yield found in seed weight of 75, 100, and 125 g, meanwhile the lowest carrageenan yield found in seed weight of 50 g. Significantly different interaction levels among bag and non bag cultivation system were found seed weight of 15 g, 50 g, 75 g, and 100 g. Overall, the produced carrageenan yield value in this study complies with the minimal commercial standards from Ministry of Trade, it is 25% (Failu *et al*., 2016). This is expected due to harvest age reached 45 days, as Harun *et al*. (2013) stated that polysaccharide content is directly straight proportional with harvest age of seaweed, followed by the carrageenan yield component is also higher.

Carrageenan yield value in treatment A_1B_3 (bag cultivation system, seed weight is 75 g) was 23.70 ± 0.62 g, in treatment A_2B_1 (non bag cultivation system, seed weight is 15 g) was 23.06 ± 0.80 g, and in treatment A_2B_2 (non bag cultivation system, seed weight is 50 g) was 18.07 ± 0.68 g. The carrageenan yield in all those treatments has not reached minimum standard, which is 23%. This is expected due to a disease attack called ice-ice, and also there were epiphytes found in thallus of seaweed that could be one of the factors effected on seaweed growth during cultivation. This results was in line with previous study by Bunga *et al.* (2013), ice-ice, a disease that usually attacked seaweed during cultivation was found at day 30, expected causing the content of carrageenan yield is reduced as with increasing the age of seaweed.

Gel strength

Gel strength is a parameter showing the ability of carrageenan to form a gel. Therefore, the gel strength become main characteristic of carrageenan (Ega *et al*., 2016). According to this study the A factor (bag and non bag cultivation system), B factor (seed weight), and the interaction between both factors are influential on gel strength of *K. alvarezii* seaweed (P<0.05) (Table 2). The gel strength in bag cultivation system showed highest value in seed weight of 50, 75, 100 g, while the lowest was 125 g.

The gel strength of seaweed cultivated in non bag cultivation system showed the significant highest value in seed weight of 15, 50, and 100 g, meanwhile the lowest found in seed weight of 75 g dan 125 g. The interaction between gel strength among bag and non bag cultivation system that showed significantly different results were seed weight of 15 g and 50 g. The value of gel strength extracted from *K. alvarezii* were 742.62–1344.69 g/cm^2. This value is already met as the high value of gel strength found in this study, the carrageenan yield value was also remained high.

Husna *et al.* (2016), stated that whether the value of gel strength is high or low, it is influenced by various factors and one of them is carrageenan concentration. The gel strength that influenced by cultivation system was found in seed weight of 15 and 50 g. Using bag cultivation system can actually increase the value of carrageenan yield which ultimately increasing gel strength. Besides the cultivation system, the longer harvest age of seaweed until 45 days is also one of the factors that can improve gel strength of seaweed.

This is in line with previous study by Harun *et al.* (2013), the gel strength is influenced by harvest age of seaweed, the longer the seaweed is harvested, the value of gel strength is higher. Bunga *et al.* (2013), stated that as longer as the harvest age of seaweed, 3.6-anhydrogalactose is increased therefore the double helix is also increased and

Table 1. Carrageenan yield.

A Factor (Cultivation system)	B Factor (Seed weight) (g)				
	15 (B_1)	50 (B_2)	75 (B_3)	100 (B_4)	125 (B_5)
Bag (A_1)	27.50 ± 0.49^{a*} (A_1B_1)	29.14 ± 0.73^{a*} (A_1B_2)	23.70 ± 0.62^{b*} (A_1B_3)	29.18 ± 1.10^{a*} (A_1B_4)	27.60 ± 0.94^{a*} (A_1B_5)
Non bag (A_2)	23.06 ± 0.80^{b**} (A_2B_1)	18.07 ± 0.68^{c**} (A_2B_2)	26.15 ± 0.90^{a**} (A_2B_3)	25.65 ± 0.67^{a**} (A_2B_4)	27.43 ± 0.96^{a*} (A_2B_5)

Note: (a,b,c) different superscript in horizontal lines is significantly different (P<0.05); meanwhile different star sign (*,**) in vertical lines is significantly different (P<0.5).

Table 2. The gel strength of seaweed.

A Factor (Cultivation system)	B Factor (Seed weight) (g)				
	15 (B_1)	50 (B_2)	75 (B_3)	100 (B_4)	125 (B_5)
Bag (A_1)	786.62 ± 48.93^{b*} (A_1B_1)	876.08 ± 61.49^{ab*} (A_1B_2)	940.45 ± 14.72^{ab*} (A_1B_3)	1060.33 ± 55.68^{a*} (A_1B_4)	742.62 ± 140.03^{b*} (A_1B_5)
Non bag (A_2)	1244.59 ± 47.57^{a**} (A_2B_1)	1344.69 ± 18.43^{a**} (A_2B_2)	839.17 ± 84.18^{b*} (A_2B_3)	1042.91 ± 165.32^{ab*} (A_2B_4)	822.34 ± 171.17^{b*} (A_2B_5)

Note: (a,b,c) different superscript in horizontal lines is significantly different (P<0.05); meanwhile different star sign (*,**) in vertical lines is significantly different (P<0.05).

gel forming occurs faster. Otherwise, Supriyantini *et al*. (2017), stated that the gel strength is also influenced by water flow factor, as the water flow is stronger, the good nutrient in water is plenteous for the growth of seaweed. The water flow in this study was between 14.73-39.84 cm/s.

Ash content

The residu from the minerals that are not burned in carrageenan burning by high temperature ashing process is called ash content (Bunga *et al*., 2013). The result of this study showed that cultivation system, seed weight, and the interaction between both factors effected on ash content of *K. alvarezii* seaweed (P<0.05) (Table 3). Ash content in bag cultivation system showed significantly different result (P<0.05). It is known that the highest weight seed was 75 g and the lowest was 50 g.

The ash content in seed weight of 15 g, 100 g, and 125 g did not significantly different. The ash content in seaweed that cultivated in non bag cultivation system showed significantly different result (P<0.05). The highest value found in seed weight of 15 g and 125 g, meanwhile the lowest found in seed weight of 50 g. The ash content in seed weight of 75 g and 100 g did not significantly different. The interaction among group of bag and non bag cultivation system that had significantly different results found in seed weight of 15 g, 50 g, 100 g, and 125 g.

The ash content of carrageenan in this study is in accordance with standards set by FAO, it is between 15–40 %. The high level of ash content of carrageenan probably is caused by the optimum water salinity value. Water salinity value in this study was between 21-29 g/L. This result was in line with Zainuddin (2016), the seaweed that lives in high water salinity contains lot of minerals, such as Na, Ca, Mg, and K because seaweed can absorb minerals from their environment when they grow.

Water content

Water content can be an indicator of product's shelf life including seaweed, which seaweed should store at low water content. If the seaweed stores in high water content, it can cause the growth of other harmful organism during product storage process. This study found that cultivation system (bag and non bag), seed weight, and the interaction between those factors had no significantly different (P<0.05) effect towards the water content of *K. alvarezii* seaweed (Table 4). The water content in bag cultivation system did show significant different result (P<0.05).

The highest water content found in seed weight of 15 g and 50 g, meanwhile the lowest were in seed weight of 100 g and 125 g. The water content from the seaweed cultivated using non bag cultivation system showed significant different result (P<0.05). Significantly, the highest water

Table 3. Ash content of seaweed.

A Factor (Cultivation system)	B Factor (Seed weight) (g)				
	15 (B_1)	50 (B_2)	75 (B_3)	100 (B_4)	125 (B_5)
Bag (A_1)	27.08 ± 0.22^{b*} (A_1B_1)	25.58 ± 0.51^{c*} (A_1B_2)	28.81 ± 0.25^{a*} (A_1B_3)	27.41 ± 0.18^{b*} (A_1B_4)	27.29 ± 0.60^{b*} (A_1B_5)
Non bag (A_2)	29.92 ± 0.15^{a**} (A_2B_1)	27.46 ± 0.37^{c**} (A_2B_2)	28.34 ± 0.38^{b*} (A_2B_3)	28.75 ± 0.31^{b**} (A_2B_4)	30.02 ± 0.13^{a**} (A_2B_5)

Note: (a,b,c) different superscript in horizontal lines is significantly different (P<0.05); meanwhile different star sign (*,**) in vertical lines is significantly different (P<0.05).

Table 4. Water content.

A Factor (Cultivation system)	B Factor (Seed weight) (g)				
	15 (B_1)	50 (B_2)	75 (B_3)	100 (B_4)	125 (B_5)
Bag (A_1)	38.63 ± 0.26^{a*} (A_1B_1)	38.60 ± 0.22^{a*} (A_1B_2)	36.54 ± 0.27^{b*} (A_1B_3)	36.55 ± 0.21^{b*} (A_1B_4)	36.01 ± 0.80^{b*} (A_1B_5)
Non bag (A_2)	35.27 ± 0.30^{b**} (A_2B_1)	37.00 ± 0.10^{a**} (A_2B_2)	34.15 ± 0.15^{c**} (A_2B_3)	34.58 ± 0.27^{c**} (A_2B_4)	36.41 ± 0.20^{a*} (A_2B_5)

Note: (a,b,c) different superscript in horizontal lines is significantly different (P<0.05); meanwhile different star sign (*,**) in vertical lines is significantly different (P<0.05).

content found in seed weight of 50 g and 125 g, then the the lowest were in seed weight of 75 g and 100 g. The interaction among groups of bag and non bag cultivation system that significant different (P<0.05) were found in seed weight of 15 g, 50 g, 75 g, and 100 g.

Overall, the water content of carrageenan in this study is sufficient high compared to FAO standard which is only 12% for maximal water content. The water content of carrageenan in seaweed is influenced by harvest age, the longer seaweed is harvested, the water content is higher (Harun *et al*., 2013; Asikin & Kusumaningrum, 2019). Different opinion stated by Bunga *et al*. (2013), the longer seaweed is cultivated, the water content is lower. This is expected due to the longer seaweed is harivestedm the free water content is higher, therefore the evaporation during drying shows larger causing lower water content.

CONCLUSION

This study conclude that different cultivation method and different seed weight of seaweed allow different carrageenan content in seaweed, The use of bag in this study provided an increasing of carrageenan content in seed weight of 15 to 100 g. The carrageenan content in seaweed that cultivated using this bag cultivation system is also followed by the increase of gel strength, ash content, and water content.

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