Available online at website: www.bbp4b.litbang.kkp.go.id/squalen-bulletin

Squalen Bulletin of Marine & Fisheries Postharvest & Biotechnology, 9 (1), 2014, 17-24

PRODUCTION OF SEMI REFINE CARRAGEENAN (SRC) FROM FRESH *Kappaphycus alvarezii* USING MODIFIED TECHNIQUE WITH MINIMUM USE OF FUEL

Produksi Semi Refine Karaginan dari Rumput Laut Kappaphycus alvarezii Segar dengan Teknik Produksi Minim Bahan Bakar

Jamal Basmal*and Diah Ikasari[1]

[1]Research and Development Center for Marine and Fisheries Product Processing and Biotechnology, Jl. K.S. Tubun Petamburan VI, Jakarta Pusat 10260, Indonesia, *Correspondence Author: jamalbasmal24@gmail.com

Article history:
Received: 20 Desember 2013; Revised: 14 Maret 2014; Accepted: 1 April 2014

ABSTRACT

Semi refine carrageenan (SRC) is generally produced by cooking dried *Kappaphycus alvarezii* into hot alkali solution at temperature of 80ºC for 2 hours. However, the temperature fluctuation during the production process affects to the increase of production cost and the reduction of the yield as well as the decrease of SRC quality. The improvement of this process can be achieved by soaking immediately fresh *K. alvarezii* into cold alkali solution, called cold alkali technique. In order to obtain its optimum condition, modification of SRC production process using cold alkali technique had been conducted. Fresh *K. alvarezii* was soaked in 8% KOH at room temperature for 10, 20, 30, 40, and 50 days. The alkalinized *K. alvarezii* was then washed with fresh water until pH reached to 7 – 8, chopped to the size of 3 – 5 mm and then dried under sun light. The SRC being produced was then evaluated for its quality. The result showed that the quality of SRC from fresh seaweed *K. alvarezii* was affected by soaking time. The highest quality of SRC was obtained by soaking *K. alvarezii* with 8% KOH solution for 50 days. This was indicated by the ester sulphate content of 6.74 %, water gel strength of 746 g/cm^2, and moisture content of 11.73 %.

Keywords: Cold alkali processing, *Kappaphycus alvarezii,* KOH solution, ester sulphate, modified technique

ABSTRAK

Umumnya *Semi Refined Carrageenan* (SRC) diperoleh dengan cara memasak *Kappaphycus alvarezii* yang sudah dikeringkan ke dalam larutan alkali panas pada suhu 80^{o}C selama 2 jam. Adanya fluktuasi suhu selama proses berlangsung berakibat pada peningkatan biaya produksi dan penurunan rendemen serta mutu produk SRC. Perbaikan proses dapat dilakukan dengan sesegera mungkin merendam *K. alvarezii* yang baru dipanen ke dalam larutan alkali dingin, yang dikenal dengan teknik alkali dingin. Untuk mengetahui kondisi optimumnya, maka modifikasi proses produksi menggunakan teknik alkali dingin ini telah dilakukan. Rumput laut *K. alvarezii* segar direndam dalam 8% larutan KOH selama 10, 20, 30, 40, dan 50 hari pada suhu ruang. *Kappaphycus alvarezii* yang telah mendapatkan perlakuan alkali tersebut kemudian dicuci dengan air tawar hingga kisaran nilai pH 7 – 8, dipotong-potong hingga berukuran 3 – 5 mm dan dikeringkan menggunakan sinar matahari. SRC yang didapatkan kemudian dievaluasi kualitasnya. Hasil penelitian menunjukkan bahwa kualitas SRC dipengaruhi oleh waktu perendaman. Hasil terbaik diperoleh dari perlakuan perendaman *K. alvarezii* dalam 80% larutan KOH selama 50 hari. Hal ini ditunjukkan oleh kandungan ester sulfat SRC yang mencapai 6,74%, kekuatan gel tanpa penambahan KCl 746 g/cm^2, dan kadar air 11,73%.

Kata Kunci: Proses alkali dingin, *Kappaphycus alvarezii* **, larutan KOH, ester sulfat, teknik produksi**

1. Introduction

Carrageenans are commercially important hydrophilic colloids (water-soluble gums) which occur as a matrix material in the cell wall and intercellular matrix of seaweed tissue. In numerous species of red seaweeds (*Rhodophyta*), they serve as a structural function, analogues to cellulose in land plants. Carrageenans have been extensively used in the food industry as thickening, gelling and protein-suspending agents, and recently has been used by the pharmaceutical industry as recipient in pills and tablets due to their half-ester sulphate moieties, The main chemical composition of carrageenans are sulphated linear polysaccharides of d-galactose and 3,6-anhydro-d-galactose. They are strongly anionic polymers and the range of ester sulphate in carrageenans is from 15% to 40% (Campo et al., 2009; Stanley, 2013; Porto, 2013). Higher level of ester sulphate means lower temperature solubility and lower gel strength. Higher sulphate content also means high risk to human consumption, therefore the sulphate content of carrageenans should be reduced before they are applied into various foods. FAO (2004) reported that sulphate should not be found in the foodstuffs. However, sulphates are commonly used as additives in the food industry. Based on the data of food consumption and reported usage of sulphates as additives, the estimated average daily intake of sulphate in food in the USA is 453 mg (FAO, 2004).

Reduction of ester sulphate in carrageenans can be conducted by using alkali solution such as potassium hydroxide solution. Based on their ester sulphate content, there are three types of carrageenan. Firstly, kappa type carrageenan, which has an ester sulphate content of about 25 to 30% and a 3,6-anhydrous galactose (AG) content of about 28 to 35%. Secondly, iota type carrageenan, which has an ester sulphate content of about 28 to 30% and a 3,6-AG content of about 25 to 30%. Thirdly, lambda type carrageenan, which has an ester sulphate content of about 32 to 39% without 3,6-AG (Porto, 2013). Kappa (κ-) carrageenan has stronger gel properties and more rigid compared to iota (α–) and lambda (λ–) carrageenans due to its lower level of ester sulphates content. The main carragenophytes of Rhodophyceae are including *Kapphaphycus alvarezii, Euchema spinosium, Chondrus chondrus* and *Gigartina* sp. *Kapphaphycus alvarezii* and *Euchema spinosium* are growing well in coastal area of Indonesia and their production are higher compared to other countries in the world (FAO, 2003). Since κ-carrageenan is mainly produced from the reaction of κ-carrageenan contained in *K. alvarezii* with potassium hydroxide, Indonesia has become potential source of κ-carrageenan.

The purity of carrageenan depends on its purification technique. Based on its purity, there are two types of carrageenans, namely semi refine carrageenan (SRC) and refine carrageenan (RC). Semi refine carrageenan (SRC) or alkali treated cottonii (ATC) is produced when seaweeds were treated with hot potassium hydroxide for several times (Basmal et al., 2005; Metha et al.,2008; Distantina et al., 2011). Meanwhile, refined carrageenan is produced by boiling further alkali treated cottonii in hot water, forming pulp which is then separated from cellulose and other materials (Basmal et al., 2005; Basmal et al., 2009a & 2009b; Chan et al., 2013).

Utilization of potassium hydroxide solution during processing of seaweeds leads to the reduction of impurities such as mud, protein, mineral and vitamin from thallus and it is mostly forming ester sulphate through the substitution of sulphate group with ion K^+ on the C_4 and possibly C_2 of carrageenan. The number of total ester sulphate is critical and mainly influenced by the concentration of KOH solution, time and temperature being used during processing. Temperature is very important during treating seaweeds with hot alkali treatment. The higher the temperature, the more carrageenan is leached out from thallus of seaweeds. Therefore, treatment using temperature must be taken carefully because increasing temperature of 1 °C will increase the reaction by 10 fold.

Normally, temperature of 75 – 80 °C is applied to obtain good quality of SRC (Basmal & Sedayu, 2011). However, the limited and inefficient used of fuel in the future will increase the production cost of SRC. Therefore, production of SRC using techniques with minimum used of fuel should be considered. One of the techniques is by soaking fresh seaweed in KOH solution for certain period to accelerate the reaction of KOH with μ-carrageenan, forming κ-carrageenan. Several advantages of this method are including minimizing the use of energy fuel (fossil), increasing the safety and reducing the accident for workers as well as reducing the waste of KOH due to its repeatable utilization to produce SRC. The aim of this study is to improve the production and the quality of SRC using modified technique with minimum use of fuel.

2. Materials and Methods

2.1. Materials

Fresh seaweed used in this study was *K. alvarezii* obtained from Serang – Banten. Other material used in the experiment was technical grade of KOH.

2.2. Methods

The experiment was carried out by using modified method of Basmal & Sedayu (2011). Normally, dried seaweed *K. alvarezii* was processed using hot alkali (70-80 °C) for 2 -3 hours with ratio of 1 : 8 between dried seaweed and KOH solution. However, in this experiment fresh *K.alvarezii* was processed directly using 8% cold alkali (KOH solution) with ratio of 1 : 8 and soaked for 10 days (treatment A), 20 days

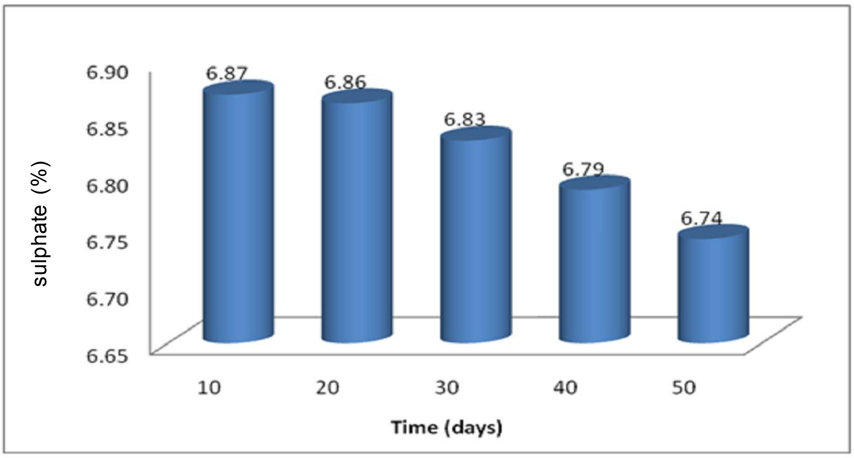


Figure. 1. Sulphate content of *K. alvarezii* after treated in 8% of KOH solution in various soaking time.

(treatment B), 30 days (treatment C), 40 days (treatment D) and 50 days (treatment E). The alkalinized *K. alvarezii* was then rinsed with tap water until reached neutral pH and dried under sun light for 3 days.

The treated SRC were then analyzed for their quality. Parameters used to evaluate the quality of SRC were gel strength value with and without addition of 1.5% KCl in carrageenan solution (Marine Colloid, 1978), moisture content (BSN, 2006b), ash content (BSN, 2006a), and sulphate content (BSN, 2004).

3. Results and Discussion

3.1. Sulphate Content

The result showed that sulphate levels of SRC being produced in the experiment were ranging from 6.74 to 6.87 % (Figure 1). The lowest sulphate level was obtained from treatment E with 6.74 %, while the highest sulphate level was gained from treatment A with 6.87%. Statistical analysis showed that there were significantly difference between treatments at level of a = 0.05, (F-test calculated 0,07). This result indicates that sulphate content of SRC was influenced by soaking time. The longer fresh *K. alvarezii* was kept in 8 % solution of KOH, the more sulphate content in SRC will be reduced. The quality degradation of sulphate content of SRC treated with different soaking time was represented by linier regression shape with equation of $Y = - 0.033 X + 6.917$ and $r^2 = 0.952$. This means that the longer fresh *K. alvarezii* soaked in 8% KOH at room temperature, the more sulphate group reacted with KOH, forming K_2SO_4 and resulted in less ester sulphate in carrageenan. This was showed by *K. alvarezii* soaked for 50 days (treatment E) with sulphate level of 6.74%. Conversely, in treatment A, ester sulphate was higher compared to other treatments. This is probably because in treatment A, *K. alvarezii* was soaked in 8% KOH only for 10 days (Fig.1).

Higher level of ester sulphate means lower solubility temperature and gel strength. Result of experiment showed that water gel strength value of treatment D was lower than other treatments. According to Metha et al., (2008) the ester sulphate content in carrageenan is difficult to determine when more impurities are present in carrageenan after extraction. Furthermore, Metha et al. (2008) reported that some molecules may have less than one sulphate ion per repeat units. Some repeat units may have no sulphate at all, while other molecules may have two or even three sulphates per repeat unit. The value of water gel strength of treatment A was only 694 g/cm^2 and increased to 1,033 g/cm^2 after addition of 1.5% KCl to carrageenan solution. This means that the addition of 1.5% KCl in carrageenan solution significantly increased gel strength by 48.85%.

Figure 2 depicts the reaction between μ-carrageenan contained in fresh *K. alvarezii* with KOH solution, forming κ-carrageenan. The quality of κ-carrageenan (repeating units) is extremely related to how much ester sulphate group that is executed by KOH to form κ-carrageenan (repeating units), in which the more ester sulphate can be executed from μ-carrageenan, the more κ-carrageenan (repeating units) is formed. It means that the gel strength value tends to increase in κ-carrageenan.

According to Metha et al. (2008) μ-carrageenan will change to κ-carrageenan with the presence of KOH by reducing some ester sulphate groups. Reducing ester sulphate groups of each repeating unit have

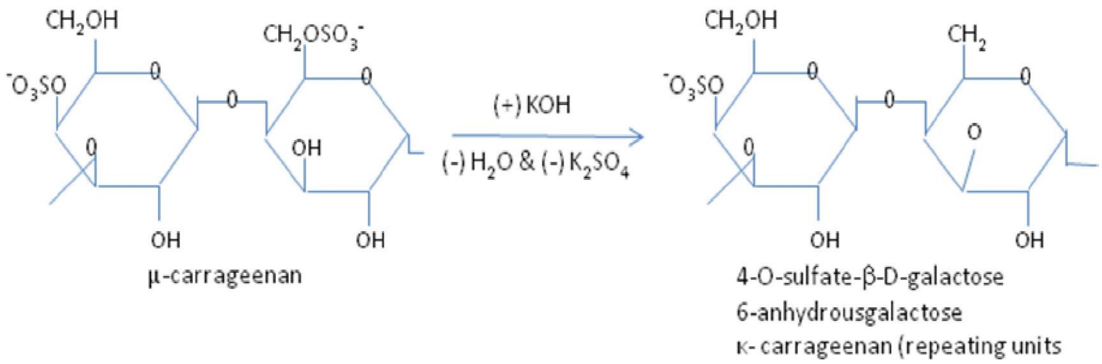


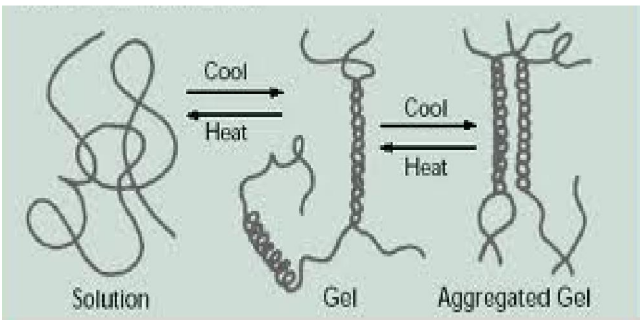
Figure 2. The conversion of μ-carrageenan to κ-carrageenan due to alkali treatment (Source: Metha et al., 2008).

different properties; some repeating units of κ-carrageenan have only one sulphate molecule while others contain more than one sulphate molecules. However, reducing ester sulphate content in repeating unit κ-carrageenan only affects to the gelling temperature.

3.2. Water Gel Strength Value

According to Campo et al. (2009) the water solubility of carrageenan depends essentially on the level of sulphate groups (very hydrophilic) and on their associated cations. Higher negative charge such as ester sulphate (OSO_3^-) will increase the value of gel strength in carrageenan when it is reacted with cation such as K^+ or Ca^{++}. The characteristic of reduced ester sulphate carrageenan, especially κ-carrageenan is having the ability to form thermo-reversible gels through cooling process. This phenomenon occurs due to the formation of a double helix structure by the carrageenan polymers (Figure 3). At temperatures above the melting point of the gel, carrageenan polymers exist in solution as random coils. During cooling process of the solution, a three-dimensional polymer network is built where double helical form the junction points of the polymer chains. Further cooling process leads to aggregation of these junction points to build a three-dimensional gel structure. Falshaw et al. (2001) reported that sulphate ester group at position-6 of a 4-linked a-D galactosyl unit which affects the overall properties of carrageenan by creating "kinks" in the polymer chain and the quantity have important effects on gelling properties. This gelling mechanism is a basic process for kappa and also iota carrageenan solutions.

Based on this experiment and evaluation, the values of water gel strength of SRC being produced were ranging from 658 to1,106 g/cm^2 (Figure 4). The highest gel strength value was obtained from 30 days soaking treatment with 1,106 g/cm^2, while the lowest was obtained from 40 days soaking treatment with 658 g/cm^2. The higher value of water gel strength at 30 days soaking treatment is possibly due to more κ-carrageenan (repeating units) was formed along with the excessive ion K^+ caused by unclean washing after treated in 8% KOH solution. The ion K^+ was accumulated amongst κ-carrageenan (repeating units) and as a result these ion K^+ together with κ-carrageenan (repeating units) were forming egg boxes structure which increases the gel strength when it was cooled.



Source : (Anonymous, 2007).
Figure 3. Characteristic of κ-carrageenan when is added by cation Ca^{++} or K^+

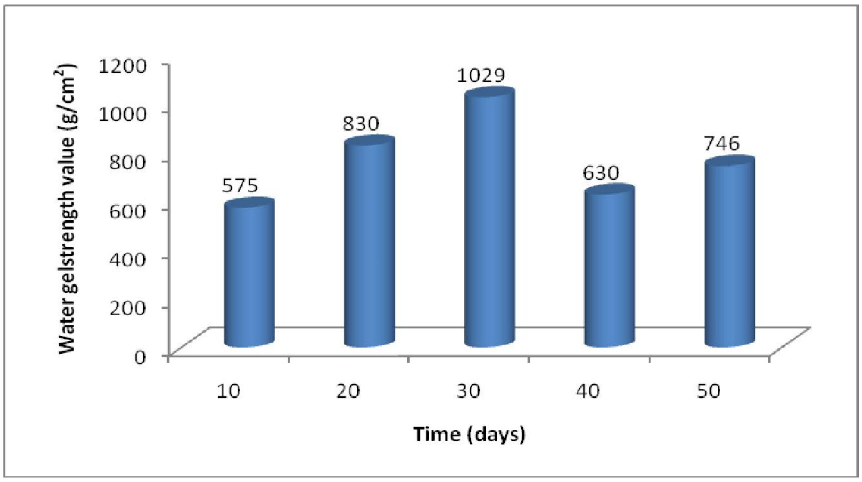


Figure 4. Water gel strength values of Semi Refined Carrageenan (SRC) produced by *K. alvarezii* treated with 8% KOH solution with various soaking time.

3.3. Gel Strength Value with Addition of KCl

When κ-carrageenan (repeating units) was added with cation such as mono or divalent ion, the value of gel strength will significantly increase. κ-carrageenans will only form gel in the presence of certain cations. Kappa carrageenan is a sensitive potassium ion and it produces rigid and brittle gel in aqueous solutions with potassium salts. The effectiveness of cation in enhancing gel strength of κ-carrageenan is described the following sequences: $K^+ > Ca^{2+} > Na^+$ (Kara et al., 2008). K^+ is more important than Na^+ in determining the gel strength of k-carrageenan gels. Results of this experiment showed that the value of gel strength increased with range of 1,033 -1,310 g/cm^2. This indicates that cation K^+ can increase the value of gel strength of each SRC being produced (Figure 5) compared to the value of gel strength without addition of KCl (Figure 4).

Figure 5 showed that the highest gel strength value was obtained from 40 days soaking treatment while the lower value was obtained from 10 days soaking treatment. From 10 to the 40 days soaking treatment, the value of gel strength increased gradually but after fresh *K. alvarezii* was soaked for 50 days in 8 % KOH solution, the gel strength value decreased at level of 1,059 g/cm^2. Comparing to the international standard (FAO, 2007; Porto, 2013), all gel strength values obtained from experiment were higher than international standard. Lower gel strength value obtained from 50 days soaking treatment (treatment E) was probably caused by the excessed cation K^+

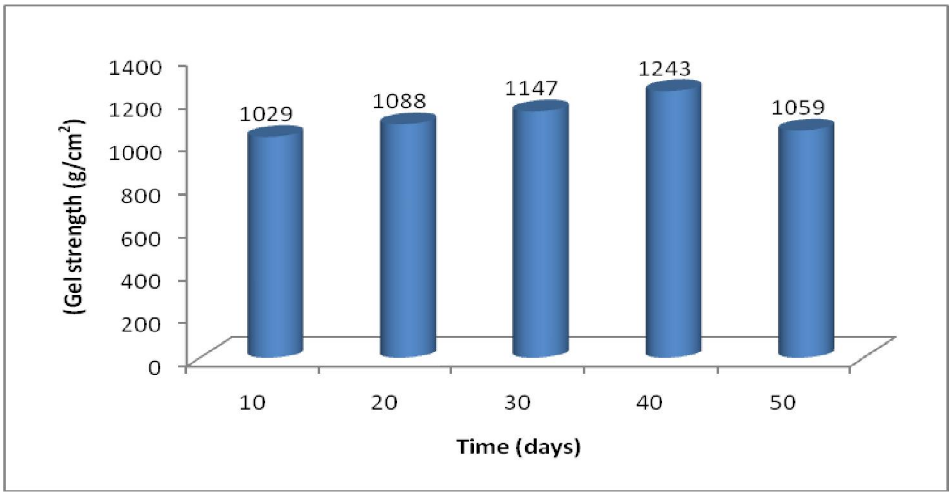


Figure 5. Gel strength value of Semi Refined Carrageenan (SRC) produced by *K.alvarezii* treated with 8% KOH solution in various soaking times.

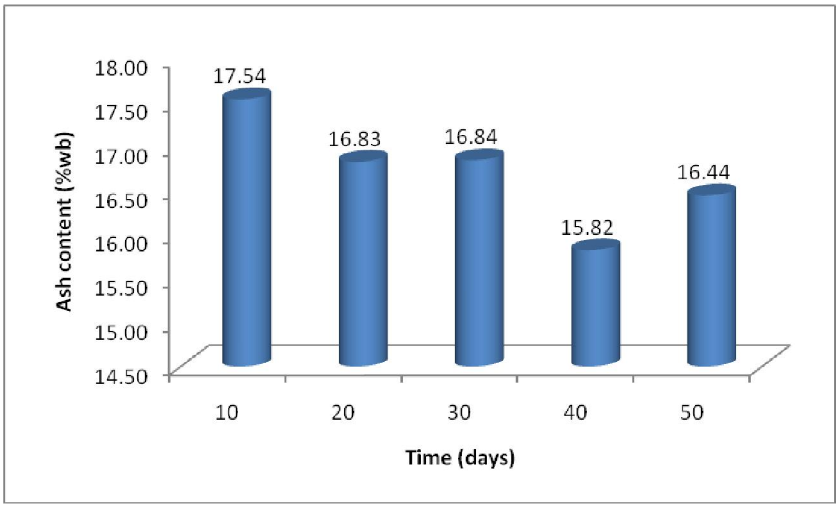


Figure 6. Ash content of SRC produced by *K.alvarezii* treated with 8% KOH solution in various soaking times.

in κ-carrageenan which contributes to the reduction of gel strength value. Porto (2013) reported that gel strength value is directly proportional to the concentration of carrageenan and cation. The excessive amount of potassium salts being induced will produce weaker gel strength and produce syneresis (spontaneous extrusion of water through the surface of gel when it is at rest), in which the more potassium concentration in solution the higher the syneresis. However, increasing concentration of cation K^+ in liquid κ-carrageenan will also reduce gelling temperature.

3.4. Ash Content

According to Basmal & Sedayu (2011) ash content is group of trace minerals such as Fe, B, Ca, Cu, Cl, K, Mg, Mn and celullose content in thallus of seaweeds. In the production of carrageenan, those minerals have to be eliminated using combination between KOH treatment and filtration. FAO (2007) reported that ash content in carrageenan should not be less than 15% and not more than 40 % on the dry basis. Results of this experiment showed that ash content in all treatments were ranging from 15.82 % to 17.54 n% (Fig. 6). The presence of mineral Ca^{++} and K^+ (excessive K^+ originally comes from unclean washing SRC after treated with 8 % KOH solution) significantly affected to the water gel strength value. This was showed by the correlation between the results of ash content and water gel values of each treatment. For example, in 30 days soaking treatment, the ash content was 17.54 % (Fig. 6) and water gel strength value was 575 g/cm^2(Fig. 4), while in 40 days treatment, the ash content was 15.82% (Fig. 6) and water gel strength value was 657.51 g/cm^2(Fig. 4).

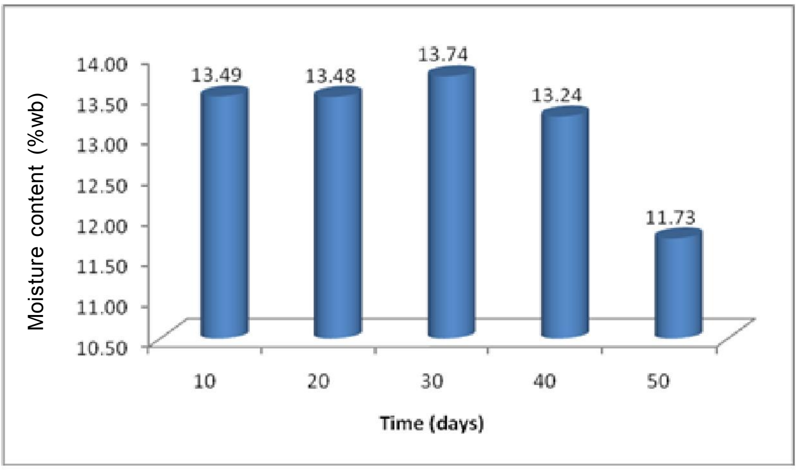


Figure 7. Moisture content of SRC after produced by *K.alvarezii* with 8% KOH solution in various soaking times.

The reduction of mineral/ash content in SRC heavily depends on the treatment including addition of KOH solution, temperature, washing system and the purity of water used to clean alkalinized *K. alvarezii*.

3.5. Moisture Content

Moisture contents of SRC were ranging from 11.73 to 13.74 %. The lowest moisture content value was obtained from 50 days soaking treatment with 11.73 %, while the highest value was resulted from 30 days soaking treatment with 13.74 % (Figure 7). Based on the statistical analysis, the moisture content of SRC was significantly influenced by soaking time, where F-test > F-table at level of 10% (0.94>0.1). The possible reason of the difference in moisture content of each treatment was due to drying method, in which products were dried using sun light. The rate of evaporation from inside to outside of the product depends on the intensity of the light, relative humidity, temperature and air velocity on the surface of the product as well as the size and width of the surface of chip product. Based on the result of this experiment, moisture content was not correlated to the value of gel strength and the reduction of sulphate content. On the other hand, the moisture content will affect the yield of product, in which increasing moisture content will increase the yield of SRC.

4. Conclusions

Based on the result of the experiment, it can be concluded that the quality of SRC that was produced from fresh seaweed *K. alvarezii* was affected by soaking time. Extending soaking time of fresh *K. alvarezii* in 8 % KOH solution at room temperature improved the quality of semi refine carrageenan (SRC) such as reducing sulphate content, increasing water gel strength and decreasing its moisture content. The best quality of SRC was obtained from fresh *K. alvarezii* soaked with 8 % KOH solution at room temperature for 50 days with ester sulphate content of 6.74 %, water gel strength of 746 g/cm^2, and moisture content of 11.73 %. All treatments were considered to comply with the FAO requirements, which states that the sulphate content in carrageenan should be less than 40% on dry basis and maximum moisture content of 12%.

References

Anonymous. (2007). *Carrageenans*. Retrieved at 19 April 2013 from *http://www.lsbu.ac.uk/water/hycar.html*

Basmal, J., Suryaningrum, T. D., & Aumelia, W. (2005). Effect of the concentration and ratio of potassium hydroxide and seaweed on the carrageenan paper quality. *Journal of Indonesian Fisheries Research*, *11*(8), 29-38.

Basmal, J., Utomo, B. S. B. & Sedayu, B. B. (2009a). The quality of Semi Refined Carrageenan (SRC) processed by waste water of re-usable SRC processing. *Journal of Marine and Fisheries Post Harverst and Biotechnology, 4*(1), 1-11.

Basmal, J., Sedayu, B. B & Utomo, B. S. B. (2009b). Effect of KCL concentration on the precipitation of carrageenan from *E. cottoni* extract. *Journal of Marine and Fisheries Post Harverst and Biotechnology*, *4* (Special Edition), 73-80.

Basmal, J. & Sedayu, B. B. (2011). Effect of KOH and KCL combination on the quality of semi refined carrageenan (SRC). *Proceeding of National Innovation Product Processing Technology and Biotechnology Seminar*, 27-36.

Campo, V. L., Kawano, D. F., Silva Júnior, D. B. & Ivone Carvalho, I. (2009). Carrageenans: biological properties, chemical modifications and structural analysis, *Carbohydrate Polymers*, *77*, 167-180.

Chan, S. W., Mirhosseini, H., Taip, F. S, Ling, T. C. & Tan, C. P. (2013). Comparative study on the physicochemical properties of k-carrageenan extracted from *Kappaphycus alvarezii* (doty) doty ex Silva in Tawau, Sabah, Malaysia and commercial k-carrageenans. *Food Hydrocolloids, 30*, 581-588.

Distantina, S., Wiratni, Fahrurrozi, M., & Rochmadi. (2011). Carrageenan properties extracted from *Eucheuma cottonii*, Indonesia. *World Academy of Science, Engineering and Technology,* 54, 5p.

Falshaw, R., Bixlerb, H. J., & Johndrob, K. (2001). Structure and performance of commercial kappa-2 carrageenan extracts. I. Structure analysis. *Food hydrocolloid, 15*, 441-442.

FAO. (2003). *A Guide to The Seaweed Industry*. FAO Fisheries Technical Paper, 441. Rome,105p.

FAO. (2004). *Sulfate in Drinking Water*. International Satndard for Dringking Water. Background Documents for Development of WHO Guidelines for Dringking Water Quality. WHO/SDE/WSH/03.04/114. World Health Organization. *http://www.who.int/water_sanitation_health/dwq/chemicals/sulfate.pdf.*

FAO. (2007). Carrageenan. *Prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007), superseding specifications prepared at the 57th*.

Kara, S., Tamerler, C., Bermek, H., & Pekcan, O. (2003). Cation effects on sol-gel and gel-sol phase transitions of k-carrageenan-water system. *International Journal of Biological Macromolecules*, *31*, 177-185.

Lee, J. S., Lo, Y. L., & Chye, F. Y. (2008). Effect of Kþ, Ca2þ and Naþ on gelling properties of *Eucheuma cottonii*. *Sains Malaysiana*, *37*, 71-77.

Metha. A.S., Mody, K.H., Iyer, A. & Ghosh, P. K. (2008). Preparation of semi refine kappa-carrageenan recycling of alkali solution and recovery of alkali from spent liquor. *Indian Journal of Chemical Technology, 15*, 45-52.

Marine Colloid, FMC. (1978). Raw material test laboratory standard practice. *Marine Colloids.* Div. Corp. Springfield. New Jersey, USA. 53pp.

National Standardization Bureau (BSN). (2004). *Analysis of sulphate SO42 by turbidimetri method*. *BSN*- SNI 06-6989.20-2004. ICS-13.060.50.

National Standardization Bureau (BSN). (2006a). Procedure of chemical analysis Part *1: Determination of ash content on the fisheries products.* BSN- SNI 01-2354.1-2006.ICS-67.120.30.

National Standardization Bureau (BSN). (2006b). Procedure of chemical analysis Part *2: Determination of water content on the fisheries products*. BSN- SNI 01-2354.2-2006.ICS-67.120.30.

Porto, S. (2013). *General Properties and Spesification of Carrageenan*. Retrieved at August 1st, 20013 from *http://www.agargel.com.br/carrageenan-tec.html.*

Stanley, N. (2013). *Chapter 3 : Production, properties and uses of carrageenan*. FMC Corporation, Marine Colloids Division. Rockland, Maine, USA. Retrieved at July 27, 2013 from *http://www.fao.org/docrep/x5822e/x5822e05.htm.*