Characteristic of semi refined and refined carrageenan flours used in the making of biofilm (bioplastic)

P Wullandari, B B Sedayu, T D Novianto and A W Prasetyo

Indonesian Research Institute for Fisheries Post-harvest Mechanization, Ministry of Marine and Fisheries, Special Region of Yogyakarta, Indonesia
E-mail: utides@gmail.com

Abstract. The physical, microbiological, and thermal properties of the raw material, semi refined and refined carrageenan derived from *Eucheuma cottonii*, were performed in purpose of preliminary research for biofilms (bioplastics) production. The physical properties i.e. particle size, pH, water content, gel strength and viscosity were tested. Meanwhile, the microbiological analysis were performed for the Total Plate Count, mold, and yeast. The thermal properties were performed using Differential Scanning Calorimetry (DSC). The results showed that the Semi Refined Carrageenan (SRC) and Refined Carrageenan (RC) flour have respectively a 60 and 80 mesh of particle size, an 8 and 7 of pH, a 7.9 and 8 % of the moisture content, a 560 and 1140 g / cm² of gel strength, and an 80 and 35 mPa·s of viscosity. The overall results of the microbiological tested showed that the SRC and RC were recognized as safe to be used for food application in accordance to the regulation, and the thermal analysis showed that the melting points of SRC and RC flour were respectively 168 °C and 175 °C.

1. Introduction
Production and consumption of plastics are currently increasing in line with the increasing use of plastics as packaging in industries and households [1]. Plastic produced from petroleum processing has a low level of biodegradability so that it can survive in the environment for a long time. This can be a burden on the environment [2]. An alternative solution that can be done is to replace the use of plastics derived from petroleum with bioplastics [3].

Bioplastics are plastics made from polymers derived from biomass. Glucose-based polymers such as starch are often used as materials for making bioplastics. This is because starch has advantages over other ingredients such as abundance, low price and biodegradability. However, the environmental virtue of its production is increasingly questioned, as current starch production systems rely on the same crucial biomass that staple food markets (cereals, corn, potatoes) [4].

Seaweed is a material that has the potential to be used for bioplastic materials. Seaweed has the advantage that it is abundant, low cost, and does not compete with other staple foodstuffs [5]. One of the seaweed derivatives is carrageenan. Carrageenan is a polysaccharide extracted from *Rhodophyceae* seaweed, which has a lot of hydrocolloid content after starch and gelatin [6]. Carrageenan is formed by repeating units of d-galactose and 3,6-anhydro-galactose which bind to α-1,3 and β-1,4-glycosidic bonds [7]. Carrageenan is commonly used for coagulants and emulsifiers [8]. Commercially,
carrageenan is divided into three main groups based on sulfate groups and their position, namely kappa (κ), iota (ι), and lambda (λ) [9]. Meanwhile, based on the level of purity, carrageenan is grouped into two, namely RC (refined carrageenan) and SRC (semi refined carrageenan). SRC has a lower purity level than RC because it still contains a small amount of cellulose which also settles with carrageenan. This study aimed to compare the physicochemical and microbiological properties between RC and SRC in the manufacture of bioplastics.

The physicochemical and microbiological properties that are obtained from this research are served as initial information for the next research, which is the making of bioplastic. The making of bioplastic will be conducted using an extrusion process with an extruder that needs crucial information like melting point, gel strength, viscosity, moisture content, etc. The research about bioplastic from seaweed or its derivative products using the extrusion method is still relatively new and very promising because Indonesia has abundant seaweed and the bioplastic can be mass-produced through this extrusion process.

2. Materials and Methods
The material used in this research is semi refined carrageenan (SRC) flour and refined carrageenan (RC) flour from CV. Karagen Indonesia, and other materials that used for the analysis. SRC flour is obtained from red seaweed (*Eucheuma cottonii*) cultivated in Karimun Jawa Islands, has yellowish-white color, can form a gel so it plays a role in the food industry and medical field including as stabilizers, materials thickeners and emulsifiers [10]. RC flour also obtained from *Eucheuma cottonii* that cultivated in Karimun Jawa Islands, has white color, has higher purity than SRC flour, and already meets the food-grade standards, made through washing, alkali treatment for carrageenan extraction, neutralization, filtration and precipitation [11].

The SRC and RC flour was analyzed for their characteristics, i.e. particle size, pH, moisture content, gel strength, viscosity, Total Plate Count (TPC), mold, yeast, and thermal properties. Particle size was measured using sifting. Degree of acidity (pH) was measured by a sample of 0.2 grams was weighed and dispersed into 20 ml of distilled water at a temperature of 80°C. The sample was homogenized with a magnetic stirrer, then the degree of acidity was measured at room temperature with a pH meter. Moisture Content refers to the method [12], where a sample of 2 grams flour was put into a fat-free cup then weighed and recorded, then the cup containing the sample was put into the oven at 105 °C for 4 hours. The cup is then weighted. Gel Strength was measured with the method [12], where a sample of 0.90 grams flour was added 0.18 g KCl and then added 80 ml of distilled water in a beaker, then heated to 80°C while continuing to stir. The hot solution is then poured into a cup to be compacted and stored at 20°C for 1 hour. Gel strength was measured using TA.XT plus Texture Analyzer, Viscosity was measured from a 1.5% (w/w) solution of carrageenan at 75°C using a Viscometer Model BM (Ogawa Seiki, CO Japan). Total Plate Count was measured using [13] method. Yeast and mold counts were enumerated on Potato Dextrose Agar (PDA) (Merck HG00C100) with chloramphenicol (2%) (Spread plating). The plates were incubated at 25 °C for 7 days [13]. Thermal properties was analyzed using DSC (Differential Scanning Calorimetry).

3. Results and Discussion
Many parameters can describe the quality of Semi Refined Carrageenan and Carrageenan Flour. The quality requirements for Refined Carrageenan are written in National Standard of Indonesia (SNI) number 8391-1:2017 (Table 1), meanwhile, the quality requirements for Semi Refined Carrageenan are written in Draft Philippine National Standards (DPNS) 601: 2008 (Table 2).
Table 1. Quality requirements of refined carrageenan [14].

| No | Kind of analysis | Unit  | Requirements |
|----|------------------|-------|--------------|
| 1  | Chemical         |       |              |
|    | a. Moisture content | %     | Maximal 12  |
|    | b. Ash content   | %     | 15 – 40     |
|    | c. Acid insoluble ash | %  | Maximal 1  |
|    | d. Acid insoluble matter | % | Maximal 2  |
|    | e. Sulphate      | %     | 15 – 40     |
| 2  | Microbial contamination |     |             |
|    | a. Total Plate Count | Colony/gram | Maximal 5000 |
|    | b. *E. coli*     |       | Negative    |
|    | c. *Salmonella*  | Per 25 g | Negative    |
|    | d. Mold and yeast | Colony/gram | Maximal 5000 |
| 3  | Metal contamination |     |             |
|    | a. Arsen (As)    | mg/kg | Maximal 3  |
|    | b. Cadmium (Cd)  | mg/kg | Maximal 2  |
|    | c. Mercury (Hg)  | mg/kg | Maximal 1  |
|    | d. Lead (Pb)     | mg/kg | Maximal 5  |
| 4  | Physical         |       |             |
|    | a. Gel strength (water gel, 1.5% at 10°C) | g/cm² | Minimal 700 |
|    | b. Viscosity (1.5% concentration, 75°C) | cPs  | Minimal 5  |

Table 2. Quality requirements of Semi Refined Carrageenan [15].

| No | Physical/microbiological characteristics | Specification               |
|----|----------------------------------------|------------------------------|
| 1  | pH value                               | 8 - 11                       |
| 2  | Moisture content                       | Max. 12%                     |
| 3  | Viscosity                              | 5 mPa.s                      |
| 4  | Total Plate Count                      | Max. 5000 cfu/g              |
| 5  | Mold and yeast                         | 300 cfu/g                    |
| 6  | *E. coli*                              | Negative in 1 g              |
| 7  | *Salmonella sp.*                       | Negative in 25 g             |

The appearance of semi refined carrageenan (SRC) and refined carrageenan (RC) flour are shown in Figure 1. SRC flour has a yellow to tan color, meanwhile, the RC has white color, and both flours have neutral odor and taste. The RC flour has a softer texture than SRC flour.

![Image of SRC and RC flour](image_url)

*Figure 1. The appearance of semi refined carrageenan (SRC) and refined carrageenan (RC) flour [16].*
3.1. Particle size
SRC flour has a bigger particle size than RC flour, 60 mesh compared to 80 mesh. According to [17], semi refined carrageenan is carrageenan with a low purity level because it still contains a little amount of cellulose which also settles along with the carrageenan. Meanwhile, refined carrageenan is carrageenan which should not contain cellulose anymore because it has been through the precipitation process [17]. This precipitation process by 2 propanol or other alcohol aimed to get the higher quality of carrageenan thus made its particle size smaller.

3.2. pH
SRC flour has pH 8 because there are KOH remnants on the wastewater originating from seaweed [17]. It has met the quality requirements from DPNS 601 : 2008, where the pH value of SRC flour is 8 – 11. Meanwhile, RC flour has pH 7, because it experienced more washing treatment to eliminate the remaining alkali contained in seaweed. The level of microbial growth is related to different moisture content and pH values. The optimum condition for most bacteria to grow is at about pH 7, and they can’t grow at pH below 4 [13].

3.3. Moisture content
Moisture content in carrageenan flour will affect its shelf life because it is related to microbial activity [19]. The moisture content of refined carrageenan flour has met the requirement of National Standard of Indonesia (SNI) number 8391-1:2017, where the maximal moisture content for refined carrageenan is 12%. The moisture content of semi refined carrageenan flour also has met the requirement of DPNS 601:2008, where the maximal moisture content for semi refined carrageenan is 12%. The moisture content of semi refined carrageenan flour is slightly lower than the moisture content of refined carrageenan flour (Figure 4) because both types of flour are being dried using a hot air dryer.

3.4. Gel strength
Gel strength of RC flour is higher (1140 g/cm²) than strength of SRC flour (560 g/cm²) because RC flour has higher purity than SRC flour. *Eucheuma cottonii* is a good source of kappa carrageenan, which can
form strong gel. The 3,6 Anhydro-D-galactose unit of kappa carrageenan allows a helical secondary structure, that is essential for gel forming properties [20] (Figure 5). Higher gel strength values indicate that the product has better gel strength. Good gel quality comes from the solid emulsion system because gel is an semi-solid emulsion that is formed from half solid particles and half liquid [22]. Gel strength value of RC flour has met the requirement of National Standard of Indonesia (SNI) number 8391-1:2017, where the minimal gel strength value is 700 g/cm².

3.5. Viscosity
The viscosity of SRC flour is higher than the viscosity of RC flour. The viscosity of SRC flour has met the requirement of National Standard of Indonesia (SNI) number 8391-1:2017, where the minimal viscosity value is 5 mPa.s. Meanwhile, the viscosity of RC flour also has met the requirement of DPNS 601:2008, where the minimal viscosity value is 5 mPa.s.

Gel viscosity increased with cooking time at lower KOH concentration, whereas at higher KOH concentration, the gel viscosity decreased [21]. Carrageenan solution's viscosity is determined by the nature of carrageenan as a polyelectrolyte. The force of repulsion between negative charges along the polymer chain i.e. sulfate group results in the molecular chain stiffens. The polymer surrounded by water molecules immobilized due to its hydrophilic nature, thus causing a carrageenan solution to become viscous [21].

3.6. Microbiological characteristic
Microbiological characteristics (Total Plate Count, mold and yeast) for SRC and RC flour are shown in Figure 7.
3.7. Total plate count

Although SRC and RC flour have low moisture content, there is still a chance for contamination from pathogenic and non-pathogenic microorganisms during processing [13]. Total Plate Count (TPC) analysis is aimed at showing the number of microbes contained in a product by counting bacterial colonies grown on agar media. The principle of this method is if living microbial cells are grown on the medium so that these cells will develop multiply and form colonies that can be seen directly without using a microscope [22]. The total plate count (TPC) value of SRC flour is higher than those of RC flour, it means that the number of microbes in SRC flour is higher than those in RC flour. This is caused by SRC flour still contains not only carbohydrates but also xylose, glucose and uronic acids, other cations such as ammonium, calcium, magnesium, potassium and sodium are present as galactose esther. Glucose is an available C source that is essential for microbial activity [23]. According to National Standard of Indonesia (SNI) number 8391-1:2017, the TPC value for refined carrageenan is maximal 5000 colony per gram. Therefore, the TPC value of RC flour has met the requirements of National Standard of Indonesia (SNI). Meanwhile, TPC value of SRC flour also has met the requirements of DPNS 601:2008, where the maximal TPC value is 5000 colony per gram.

3.8. Mold

The mold content of SRC flour is slightly higher than the mold content of RC flour, and both types of flour have met the requirements of the National Standard of Indonesia (SNI) number 8391-1:2017 and DPNS 601 : 2008, where mold content is maximal 5000 colony per gram. Yeasts and molds grow better in low pH food products (below 4) where bacteria cannot compete [13]. Flour with high moisture content will be more vulnerable to mold. Although RC flour has slightly higher moisture content than SRC flour (8 to 7.9) but mold is likely to grow in SRC flour, because SRC flour contains glucose that serves as nutrient for mold’s growth [24].

3.9. Yeast

The yeast content of RC flour is higher than the yeast content of SRC flour because SRC flour has pH of 8 (alkaline). Yeast prefers an acidic environment to grow because if it is in an alkaline environment, yeast will compete with other microbes to grow and can be contaminated [25]. Both types of flour have met the requirements of the National Standard of Indonesia (SNI) number 8391-1:2017 and DPNS 601:2008, where yeast content is maximal 5000 colony per gram.

3.10. Thermal characteristic

Thermal characteristics of SRC and RC flour are analyzed using DSC (Differential Scanning Calorimetry). DSC detects the increase of heat changes during exothermic transformation and heat absorption during endothermic transformation [26]. The results are shown in the figures below.
The DSC thermographs of SRC and RC powder exhibited a quite similar pattern with a remarkable endothermic peak followed by a broad exothermic peak at higher temperature. However, the endothermic peaks of each samples, which was associated with their melting temperature ($T_m$) were observed slightly different whereby the $T_m$ of RC was higher than those of SRC i.e. 175°C and 168°C, respectively. This might be due to the existing residual compounds in SRC particles such as insoluble aromatic compounds [26] that caused a lower melting temperature of the SRC powder. Moreover, this might also be attributed to the lower crystallinity degree of SRC polymer in compar [5]. Furthermore, a broad exothermic peak occurred at higher heating temperature of 230–245 °C was attributed to the thermal decomposition of carrageenan molecular structure from the both materials, also the decomposition of residual cellulose that was naturally contained in SRC particles [5].

![Figure 8. DSC analysis result of SRC flour.](image1)

![Figure 9. DSC analysis result of RC flour.](image2)
4. Conclusions
SRC flour showed higher pH and viscosity than those of RC flour, the moisture content of SRC flour was almost equal to SRC flour. Meanwhile, RC flour had higher gel strength than SRC flour. As for the microbiological characteristics, SRC flour had higher Total Plate Count and mold content than RC flour. Meanwhile, RC flour had higher yeast content than SRC flour. The melting temperature of RC flour was higher than those of SRC flour. Both SRC and RC flour has met the requirements of National Standard of Indonesia (SNI) number 8391-1:2017 and DPNS 601:2008. Therefore, both of the materials are potential to be used in the making of bioplastic.

References
[1] Ahmed T, Shahid M, Azeem F, Rasul I, Shah AA, Noman, Muhammad S 2018 Biodegradation of plastics: current scenario and future prospects for environmental safety Environ. Sci. Pollut. Res. 25 8 7287-7298
[2] DiGregorio B E 2009 Biobased performance bioplastic: Mirel Chem. Biol. 16 1 1-2
[3] Russo I, Confente I, Scarpi D, Hazen B T 2019 From trash to treasure: The impact of consumer perception of bio-waste products in closed-loop supply chains J. Clean. Prod. 218 966-974
[4] Mülhaupt R 2013 Green polymer chemistry and bio-based plastics: dreams and reality Macromol. Chem. Phys. 214 2 159-174
[5] Sedayu B B, Cran M J, Bigger S W 2020 Reinforcement of Refined and Semi-Refined Carrageenan Film with Nanocellulose Polymers 12 5 1145
[6] Mustapha S, Chandar H, Abidin Z Z, Saghravani R, Harun M Y 2011 Production of semi-refined carrageenan from Eucheuma cottonii J Sci. Ind. Res. 70 10 865-870
[7] Rusli, A, Metusalach S, Tahir M M 2017 Characterization of Carrageenan Edible films Plasticized with Glycerol Jurnal Pengolahan Hasil Perikanan Indonesia 20 2 219-229
[8] Sun G, Liang T, Tan W, Wang L 2018 Rheological behaviors and physical properties of plasticized hydrogel films developed from κ-carrageenan incorporating hydroxypropyl methylcellulose Food Hydrocol. 85 61-68
[9] Oakenfull D, Naden J, Paterson J 2000 Solvent structure and the influence of anions on the gelation of κ-carrageenan and its synergistic interaction with locust bean gum Gums and Stabilisers for the Food Industry 10 ed Williams A P, Phillips O G (Cambridge: Woodhead Publishing) pp 221-228
[10] Sumarni N K, Sulastri E 2017 Ekstraksi dan Karakterisasi SRC dari Rumput Laut Jenis Eucheuma cottonii (SRC Extraction and Characterization of Eucheuma cottonii Seaweed). Prosiding Seminar Nasional Kimia UNY 2017 Sinergi Penelitian Dan Pembelajaran Untuk Mendukung Pengembangan Literasi Kimia Pada Era Global Oktober 2017 361–366 [In Indonesian]
[11] Sedayu B B, Cran M J, Bigger S W 2020 Reinforcement of refined and semi-refined carrageenan film with nanocellulose Polymers 12 5
[12] Association of Official Analytical Chemistry (AOAC) 2000 Official Method of Analysis of the Association of Official Analytical Chemistry 17th Edition ed Sydney W (Arlington: AOAC)
[13] Ntuli V, Mekibib S B, Molebatsi N, Makoto M, Chatanga P, Asita O A 2013 Microbial and Physicochemical Characterization of Maize and Wheat Flour from a Milling Company, Lesotho Microbial and Physicochemical Characterization of Maize and Wheat Internet Journal of Food Safety 15 11–19
[14] Direktorat Pengolahan dan Bina Mutu Direktorat Jenderal Penguatan Duya Saing Produk Kelautan dan Perikanan Kementerian Kelautan dan Perikanan 2018 SNI Produk Perikanan Non Pangan (SNI for Non-Food Fishery Products) [In Indonesian]
[15] Anonim 2008 DPNS 601 : 2008 Carrageenan – Food Grade – Spesifikasi
[16] Wullandari P 2020 Memahami Perbedaan antara Tepung Semi Refined Carrageenan (SRC) dan Tepung Refined Carrageenan (RC) (Understanding the Difference between Semi Refined Carrageenan (SRC) Flour and Refined Carrageenan (RC) Flour) [In Indonesian]
[17] Panggabean J E, Dotulong V, Montolalu R I, Damongilala L J, Harikedua S D, Makapedua D M
2018 Ekstraksi karaginan rumput laut merah (Kappaphycus alvarezii) dengan perlakuan perendaman dalam larutan basa (Red seaweed (Kappaphycus alvarezii) carrageenan extraction by immersion in alkaline solution) Media Teknologi Hasil Perikanan 6 3 65–70 [In Indonesian]

[18] Sedayu B B, Basmal J, Bandol U B S 2008 Optimalisasi Penggunaan Air pada Proses Pembuatan Semi-Refined Carrageenan (SRC) (Optimization of water utilization in Semi-Refined Carrageenan (SRC) processing) Jurnal Pascapanen Dan Bioteknologi Kelautan Dan Perikanan 3 2 183 [In Indonesian]

[19] Wenno M R, Thenu J L, Cristina L C G 2012 Karakteristik Kappa Karaginan dari Kappaphycus alvarezii Pada Berbagai Umur Panen (Characteristics of Kappa Carrageenan from Kappaphycus alvarezii at Different Harvesting Times) Jurnal Pascapanen Dan Bioteknologi Kelautan Dan Perikanan 7 1 61–67 [In Indonesian]

[20] Distantina S, Wiratni, Fahirrozi M., Rochmadi 2011 Carrageenan properties extracted from Eucheuma cottonii, Indonesia World Academy of Science, Engineering and Technology 78 738–742

[21] Sitompul R, Darmanto Y S, Romadhon 2017 Aplikasi karagenan terhadap kekuatan gel pada produk kamaboko dari ikan yang berbeda (Application of carrageenan to gel strength in kamaboko products from different fish) Jurnal Pengolahan dan Bioteknologi Hasil Perikanan 6 1 38–46 [In Indonesian]

[22] Yunita M, Hendrawan Y, Yulianingsih R 2015 Analisis Kuantitatif Mikrobiologi pada Makanan Penerbangan (Aerofood ACS) Garuda Indonesia Berdasarkan TPC (Total Plate Count) dengan Metode Pour Plate (Quantitative Analysis of Food Microbiology in Flight (Aerofood ACS) Garuda Indonesia Based on the TPC (Total Plate Count) with the Pour Plate Method) Jurnal Keteknikan Pertanian Tropis Dan Biosistem 3 3 237–248 [In Indonesian]

[23] Reischke S, Kumar M G K, Bååth E 2015 Threshold concentration of glucose for bacterial growth in soil Soil Biol. Biochem. 80 218–223

[24] Parrott K 2009 Mold Basics (Petersburg: Virginia Tech)

[25] Wahidah N 2016 Viabilitas Mikroba, pH, Kadar Alkohol, Daya Kembang dan Volume Gas Adonan Roti Manis pada Proses Pembuatan Yeast Segar Berbahan Dasar Sirsak (Annona muricata Linn.) (Microbes Viability, pH, Alcohol Level, Volubility and Sweet Bread Dough Gas Volume in Fresh Yeast Production Used Soursop as Raw Material (Annona muricata Linn.). Skripsi Program Studi S1 Teknologi Pangan, Universitas Diponegoro, Semarang (Final Report) [In Indonesian]

[26] Masarin F, Cedeno F R, Chavez E G, de Oliveira L E, Gelli V C, Monti R 2016 Chemical analysis and biorefinery of red algae Kappaphycus alvarezii for efficient production of glucose from residue of carrageenan extraction process Biotechnol. Biofuels 9 122