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Isolation of Carrageenan from *Eucheuma cottonii* by Variying Alkaline Solutions

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Abstract

The carrageenan can be isolated by combining alkaline KOH, NaOH, and $Ca(OH)_2$ solutions. Alkaline solutions of KOH: NaOH, KOH: $Ca(OH)_2$, and NaOH: $Ca(OH)_2$ were combined to extract carrageenan. The amount of the solution was varied, the carrageenan groups were characterized using FTIR, and the carrageenan's gel strength was tested. The extraction results obtained the highest yield in the combination of alkaline solutions $Ca(OH)_2$ 80%+KOH 20%, which is equal to 70.18% FTIR test results for carrageenan produced from the extraction of *Eucheuma cottonii* seaweed showed absorption showing the type of kappa (κ) carrageenan. The highest gel strength test results were obtained in an alkaline solution of $Ca(OH)_2$ 80%: NaOH 20%, which was 252.5 g bloom, and the lowest was made in a solution of $Ca(OH)_2$ 80%: KOH 20%, which was 21.0 g bloom.

Keywords: Alkaline solutions, Carrageenan, Gel strength, Eucheuma cottonii

INTRODUCTION

Indonesia has abundant natural wealth, including Maluku Province, West Seram Regency, West Seram District, Piru Village, and Wael Hamlet, which has much natural wealth both on land and at sea. These natural resources have many benefits, but their utilization could have been better in the community, especially those from the sea, such as microalgae and macroalgae. Macroalgae are plants that live in the sea, usually called algae, have a size from a few centimetres to several meters, chlorophyll, and simple reproductive organs. Macroalgae have indistinguishable parts, such as the roots, stems, and leaves; all of these are called thallus, so macroalgae are included in the class of low-level plants (Jannah, 2020).

Macroalgae usually attach to rocks, wood, muddy sand, mollusc shells, and other macroalgae, so they need a substrate to attach or stick (Jannah, 2020). Macroalgae are generally divided into three divisions, namely *chlorophyta* (green algae), *phacophyta* (brown algae), and *Rhodophyta* (red algae) (Guiry, 1997). *Rhodophyceae* family macroalgae, such as *Euchema spinosum* and *Euchema cottonii*, usually used as pollutan indicator (Yusuf et al., 2016; Teheni et al., 2016) and can be used as carrageenan. According to Craige (1978) in (Peranginangin et al., 2013), in the cell wall or intracellular matrix of seaweed, there is carrageenan in it, and most of the components making up the dry weight of seaweed are carrageenan, compared to other components.

Based on Samodra & Rokhmah (2024) research, *Eucheuma cottonii* seaweed has a relatively high kappa carrageenan content of around 53.19% dry weight and has disease resistance. The economic value of Kappa carrageenan is also very high, around 10 to 20 times the price of regular seaweed. Thus, *Eucheuma cottonii* has the potential to become carrageenan. Carrageenan is a polysaccharide compound composed of 3,6 anhydrous galactose compounds; carrageenan can be produced by extracting red seaweed using hot water or an alkaline solution at high temperatures (Murdiningsih & Hasan, 2017). Carrageenan is a natural additive that benefits various industries, such as food and cosmetics. *Eucheuma cottonii*, if made with alkaline-treated carrageenan (ATC), will produce cosmetic ingredients. If it is made with Semi Refined Carrageenan (SRC), it will produce food products such as jelly. SRC is one of the carrageenan products with a lower purity level than pure carrageenan. Crude carrageenan contains some cellulose, which precipitates along with carrageenan. Commercially, *Eucheuma cottonii* seaweed can be produced into crude carrageenan by extraction using an alkaline KOH solution (Jaya et al., 2019).

In comparison to other KOH concentrations (6% and 10% KOH) and cooking times (30 and 45 minutes), the results of Rizal et al. (2016) study on the optimization of crude carrageenan produced from

seaweed (*Eucheuma cottonii*) were the highest yield of 33.83%. The seaweed was extracted by varying the concentration of KOH solution, made at a KOH concentration of 8% for 60 minutes. Chayo et al.'s research (2018) extracted carrageenan from green algae Chlorophyceae by varying the concentration of NaOH solution of 0.1, 0.2, and 0.3 N with an extraction time of 35 minutes and a constant temperature of 100˚C. HCl solution was added to the resulting filtrate until the pH was neutral. Carrageenan was characterized by FTIR to produce kappa carrageenan. The extraction results were NaOH with a concentration of 0.3 N, the highest yield value of 45.483%, and the resulting gel strength of 25.44 g/cm^2.

Liu et al. (2022), the research results from *Eucheuma cottonii*, were extracted with $Ca(OH)_2$ and CO_2 for neutralization. The extract was precipitated in 96% ethanol, dried, and crushed. The results obtained were 36.2% ± 0.8% yield. Alkaline solutions of KOH, NaOH, and $Ca(OH)_2$ have been used to extract carrageenan from several types of macroalgae. The research of carrageenan extraction from *Eucheuma cottonii* by combining alkaline KOH, NaOH, and $Ca(OH)_2$ solutions must be done to determine the effect on the yield and the resulting gel strength.

METHODOLOGY

Materials and Instrumentals

The equipment used in this study is an analytical balance (Ohaus), oven (Lab Shell), knife, glassware set, hot plate (Cimarec), water bath (Buchi), magnetic stirrer, plastic containers, digital pH (Seveneasy), and FTIR Spectrophotometer (Shimadzu). The materials used in this study are Seaweed (*Eucheuma cottonii*), potassium hydroxide (KOH) 0.3 N, sodium hydroxide (NaOH) 0.3 N, calcium hydroxide ($Ca(OH)_2$) 0.3 N, aquades, filter paper, and pH paper.

Methods

Preparation of Sample

Eucheuma cottonii seaweed from Wael Village is cleaned and washed under running water. Furthermore, the seaweed is dried in the sun for 4 days.

Carrageenan Extraction (Rizal et al., 2016)

Carrageenan was extracted using an alkaline solution of 0.3 N KOH, 0.3 N NaOH, and 0.3 N $Ca(OH)_2$. The solutions of KOH:NaOH, KOH:$Ca(OH)_2$, and NaOH:$Ca(OH)_2$ were combined, and the volume of each solution was varied, as indicated in Table 1, to complete the extraction.

Table 1. Volume variations of alkaline solution (1) KOH:NaOH; (2) KOH:$Ca(OH)_2$; and (3) NaOH:$Ca(OH)_2$

NaOH/ $Ca(OH)_2$ of 0.3 N \ (KOH/ NaOH) of 0.3 N	V (mL)	0 %	20 %	40 %	50 %	60 %	80 %	100 %
0 %								200:0
20 %							160:40	
40 %						120:80		
50 %					100:100			
60 %				80:120				
80 %			40:160					
100 %		0:200						

5 g of dried seaweed was weighed, and put into a 250 mL Erlenmeyer, then added 200 mL of 100% KOH:0% NaOH was, covered with a plastic clip and tied with a rubber band; extraction was carried out in a water bath at 60˚C for 3 hours. After that, it was filtered, and the residue was taken and washed with distilled water until the pH of the sample was neutral. They were then dried in the oven for 18 hours at 60˚C. The crude carrageenan was weighed, and the yield was determined (Jaya et al., 2019). The same is done for other variations of alkaline solutions. The 100% of KOH, NaOH, and $Ca(OH)_2$ were used for comparison. After calculating the yield, the carrageenan was mashed to form a powder, and the characterization was continued using the FTIR instrument for the six samples with the highest yield from each combination of alkaline solutions. The obtained carrageenan was mashed to form a powder and then characterized using the FTIR instrument for the six samples with the highest yield from each combination of alkaline solutions. The carrageenan yield (Rizal et al., 2016) can be calculated based on the mass of carrageenan produced compared to dried seaweed. The equation for calculating carrageenan yield is:

$$\text{Yield (\%)} = \frac{\text{Mass of crude carrageenan (g)}}{\text{Mass of dried seaweed (g)}} \times 100\%$$

Characterization of Carrageenan with FTIR

The obtained carrageenan was characterized for its functional group structure using the FTIR instrument to determine the type of functional groups formed in it. The characterization results are in the form of spectrum readings.

Determination of Gel Strength

Gel strength can be measured using a Texture Analyzer. Seaweed flour used is flour in the form of coarse carrageenan. The gel was prepared with 0.5 g of coarse carrageenan powder dissolved in 100 ml of distilled water. The mixture was gradually stirred while

heated at 80 °C. The dissolved carrageenan and formed gel were poured into a plastic container with a height of 3 cm, then left at room temperature and cooled in an air-conditioned room at 10 °C for four days. After that, the gel strength was measured using the TA-XT Plus Texture Analyzer with a cylindrical probe (L= 35mm, D= 12.7 mm) (Jaya et al., 2019).

RESULTS AND DISCUSSION

Preparation of Sample

The seaweed used is *Eucheuma cottonii*, which has irregular branches, sharp edges, smooth surface, and brown. Seaweed is first cleaned and washed with fresh water 3 to 4 times to remove salt and adhering dirt (Figure 1.a), then dried in the sun (Figure 1.b) to reduce the water content in the seaweed so that it dries faster because the high water content in seaweed raw materials can prevent the absorption of alkaline solutions to extract (separate) carrageenan from other components (Rizal et al., 2016). The time needed for seaweed to dry is 4 days in summer conditions.

(a) (b)

Figure 1. (a) Seaweed before drying (b) Seaweed after drying

Before drying, seaweed has a soft physical shape, a slightly fishy aroma, and a brown colour. After drying, the characteristics change; the physical form is complex, has a pale yellow and blackish-red colour, and still has a slightly fishy aroma. After the seaweed was dried, carrageenan was extracted.

Carrageenan Extraction

Carrageenan extraction using a water bath and different quantities of an alkaline solution, dried seaweed was extracted for three hours at 60 ºC. Extraction was done using an alkaline solution to separate the carrageenan compounds from other components. The alkaline solution serves to help separate polysaccharides from seaweed and accelerate the elimination of the 6-sulfate group from the monomer form 3,6-anhydro-D-galactose (Malle et al., 2014; Sormin et al., 2019).

The extracted seaweed is then filtered to separate the residue and the filtrate. The residue was washed using distilled water to neutralize the pH and measured using pH paper. Furthermore, the residue was dried in the oven for 18 hours. After drying, the residue was weighed, and the % rendition was calculated. Rendering is one of the critical parameters in assessing the effectiveness of the process of making carrageenan flour. The % yield of carrageenan and the pH of various alkaline solutions can be seen in Figures 2–5.

The carrageenan can be extracted by using various alkaline solutions. The effect of pH on carrageenan yield can be seen in Figure 2 to Figure 5. From these data, it can be seen that the pH of the solution is inversely proportional to the amount of soaking. Even though the pH of 100% $Ca(OH)_2$ solution was smaller, it produced a high yield compared to 100% KOH or 100% NaOH solutions. When the alkaline solution is combined using $Ca(OH)_2$, it has the highest yield of each combination compared to the other combinations. Based on these results, relatively small changes in the pH value of each alkaline solution do not affect increasing carrageenan yield.

Based on the yield obtained in this study, the highest yield in each combination of alkaline solutions $Ca(OH)_2$:KOH, $Ca(OH)_2$:NaOH, and KOH:NaOH, was produced in varying solution volumes of Ek_{C+K1}, Ek_{C+N1}, and Ek_{K+N1} are 70.18, 57.52, and 55.83% respectively. Meanwhile, as comparisons, Ek_K, Ek_N, and Ek_C have yields of 49.52, 20.25, and 58.24%, respectively. The result of EkK was higher than research by (Distantina et al., (2011), a yield of 38.22% was extracted with 0.3 N KOH solvent. The yield results of Ek_N were lower than the results of the research by Chayo et al. (2018) who found that the yield obtained from extraction with 0.3 N NaOH solvent was 45.483%. The harvest age of the seaweed can influence differences in the high or low yields of carrageenan yield used because the polysaccharide

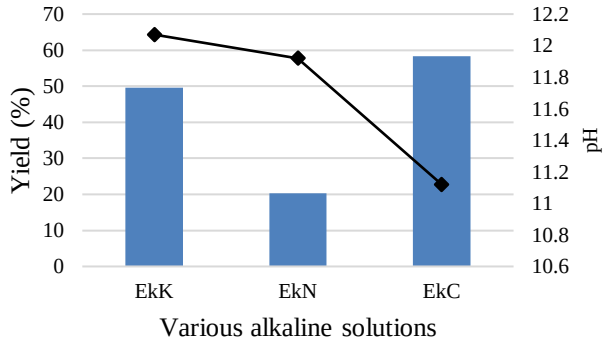

Figure 2. Histogram of the yield of carrageenan and pH variations of KOH, NaOH and $Ca(OH)_2$ alkaline solutions

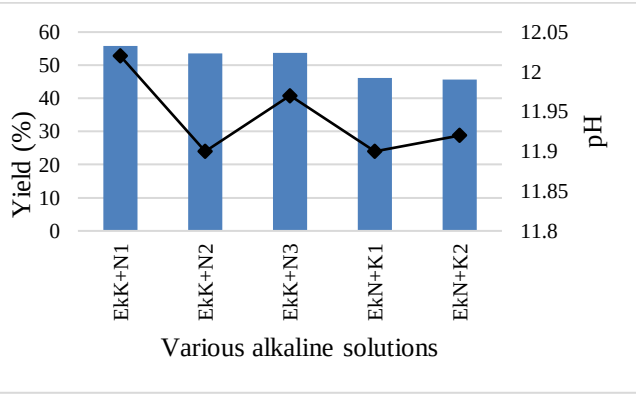

Figure 3. Histogram of the yield of carrageenan and pH variations of KOH:NaOH alkaline solution

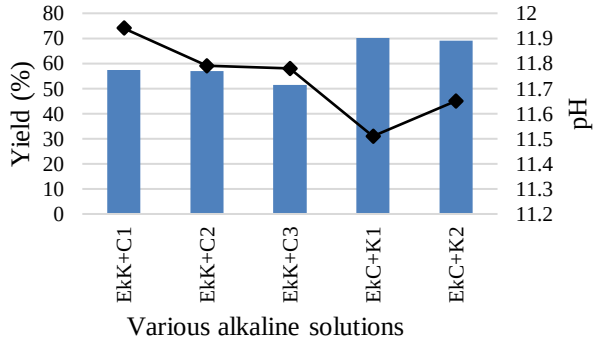

Figure 4. Histogram of % soaking of carrageenan and pH variations of KOH+$Ca(OH)_2$ alkaline solution

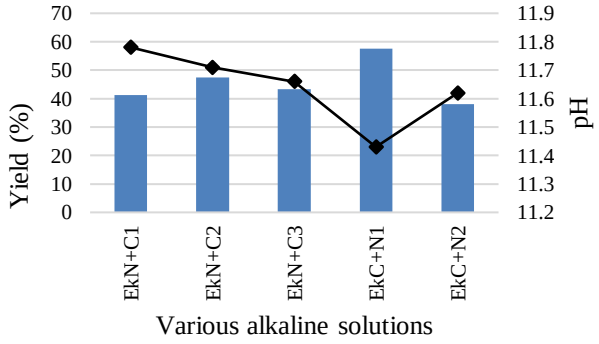

Figure 5. Histogram of % soaking of carrageenan and pH variations of NaOH+$Ca(OH)_2$ alkaline solution

compounds contained in it are different at each harvest age (Sari & Kasim, 2024). Differences in extraction methods, length of time, and high extraction temperatures can influence thealkaline solvent's interaction with the polymer chains in carrageenan, thus affecting the resulting yield (Desiana & Hendrawati, 2015).

Based on these results, the $Ca(OH)_2$ alkaline solution increases the carrageenan yield because each variation using the $Ca(OH)_2$ alkaline solution produces a higher yield than other alkaline solutions. This is because a reaction occurs between carrageenan and the alkaline solvent used, either KOH, NaOH, or $Ca(OH)_2$, where the reaction occurs as an ion exchange reaction. Cations in alkaline solvents replace the H^+ ions in the hydrogen sulfate groups. The Ca atomic weight in the $Ca(OH)_2$ base is greater than the K and Na atomic weights in the KOH and NaOH bases, so the yield produced by $Ca(OH)_2$ is higher than KOH and NaOH (Ferdiansyah et. al., 2023). Potassium ions have a specific effect and bind to the polymer randomly. Calcium ions also have a larger binding target because they are divalent cations in contrast to sodium ions, which can only induce conformational changes in carrageenan at high salt concentrations and low temperatures (Liu et al., 2022). This phenomenon causes the extracted carrageenan to be higher than potassium and sodium by using calcium.

The combination of the alkaline solution $Ca(OH)_2$ 80%:KOH 20% or Ek_{C+K1} yields the highest when compared to an alkaline solution alone or without being coupled with another alkaline solution without comparison. According to the Quality Specifications for Carrageenan (SNI 01-2690-1998), the carrageenan produced in this research has the quality requirements with a yield of more than 25%, except for Ek_N (NaOH 100%), which still has a yield of less than 25%.

Characterization of Carrageenan using FTIR

Tests were carried out using FTIR to prove the presence of functional groups in the carrageenan compound. The FTIR test was carried out for samples with the highest yield from each combination of alkaline solutions. Three samples had the highest yield, that is for Ek_{C+K1}, Ek_{C+N1}, and Ek_{K+N1}, as well as

for three samples as a comparison, Ek_K, Ek_N, and Ek_C, so there were six samples tested to determine the presence of functional groups in the carrageenan compound. The resulting IR spectrum is the absorption of various chemical components in the carrageenan extract. The spectrum of FTIR analysis results of the carrageenan sample can be seen in Figure 6, and the characteristics of the carrageenan functional groups can be seen in Table 6.

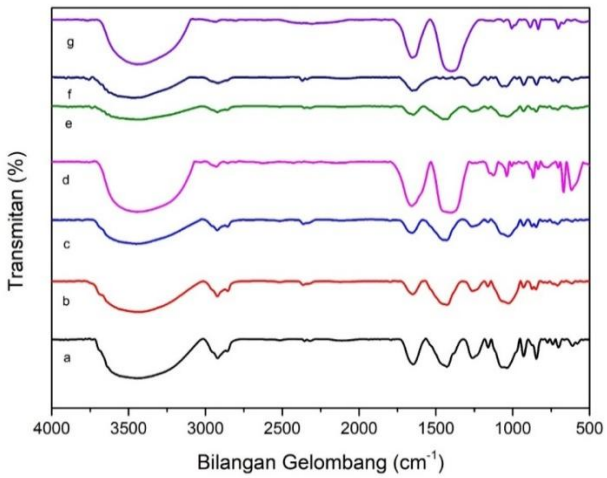

Figure 6. Spectrum of FTIR analysis results (a) KOH 100%, (b) NaOH 100%, (c) $Ca(OH)_2$ 100%, (d) KOH 80%:NaOH 20%, (e) $Ca(OH)_2$ 80%:KOH 20%, (f) $Ca(OH)_2$ 80%:NaOH 20%, and (g) Seaweed powder

According to Ferdiansyah et al. (2023), the spectrum that appears shows strong absorption at wave numbers 1210–1260 cm^{-1}, which is absorption from sulfate ester bonds (S=O) for all types of carrageenan. The absorption in the wave number range of 1250–1370 cm^{-1} is the absorption for the asymmetric −S=O stretching vibration of sulfate esters. Absorption in the 1010–1080 cm^{-1} region indicates absorption from glycosidic bonds. Another chemical group is characteristic of the carrageenan type, namely the 928–933 cm^{-1} absorption, which shows the presence of 3,6-anhydro-D-galactose bonds. Wave numbers in the range 840–850 cm^{-1} indicate the presence of galactose-4-sulfate bonds, while in the range 800–805 cm^{-1} show absorption of 3,6-anhydrogalactose-2-sulfate. The characteristic absorption appearance of the 3,6-anhydrogalactose-2-sulfate bond can be used to differentiate iota carrageenan from kappa carrageenan (Liu et al., 2022).

Sulfate ester bonds (S=O) are evident in the FTIR analysis spectrum data in this study, as evidenced by the development of a signal or absorption at wave numbers 1263.37 cm^{-1} for samples Ek_C, Ek_{C+K1}, Ek_{C+N1}, and at 1261.45 cm^{-1} for samples Ek_K, Ek_N, and Ek_{K+N1}. On the absorption spectrum 1028.06–1068.56 cm^{-1}, which appears in all samples) indicates the presence of glycosidic bonds. The galactose-4-sulfate bond is shown in the region 846.75–875.68 cm^{-1}, and the 3,6-anhydro-D-galactose bond in the area 928–933 cm^{-1}, which appears in the spectrum of each sample. Absorption in the 800–805 cm^{-1} area does not appear in any samples, thus indicating that the samples do not contain bonds of 3,6-anhydrogalactose-2-sulfate. Based on the FTIR spectrum, the carrageenan produced from the extraction of the seaweed *Eucheuma cottonii* is a type of kappa (κ) carrageenan.

The analysis data in Table 6 showed that the wave spectrum produced from extraction results uses a combination of alkaline solutions with varying volumes, that KOH 100%, NaOH 100%, $Ca(OH)_2$ 100%, KOH 80% : NaOH 20%, $Ca(OH)_2$ 80% : KOH 20% and $Ca(OH)_2$ 80% : NaOH 20% are following the literature, although only slight differences in wavenumber shifts occur between the use of various alkaline solutions.

Table 6. Characteristics of *Eucheuma cottonii* seaweed carrageenan functional groups

Carrageenan absorption wave number	Carrageenan absorption wave number (cm^{-1})						
	KOH 100%	NaOH 100%	$Ca(OH)_2$ 100%	KOH 80% : NaOH 20%	$Ca(OH)_2$ 80% : KOH 20%	$Ca(OH)_2$ 80% : NaOH 20%	Powder Seaweed
Sulfate esters (−S=O)	1261.45	1261.45	1263.37	1261.45	1261.45	1263.37	-
Galactose-4-sulfate (C−O−SO_3 on C4)	846.75	848.68	846.75	835.18	844.82	846.75	835.18
3.6-anhydrogalactose-2-sulfate (C−O−SO_3 on C2)	-	-	-	-	-	-	-
Glikosidik (C−O−C)	1037.7	1028.06	1029.99	1039.63	1068.56	1037.7	1058.92
3.6-anhydro-D-galactose (C−O)	929.69	929.69	929.69	931.62	929.69	929.69	943.19

Determination of Gel Strength

Carrageenan's unique property is its ability to convert liquid form to solid and sol form to reversible

gel, widely used in the food and pharmaceutical industries (Sormin et al., 2019). The gel-formed research result can be seen in Figure 7. Gel strength is the main parameter used to indicate carrageenan quality in forming gels (Desiana & Hendrawati, 2015). Gel strength states the strength of an object against compressive forces without experiencing shape deformation (Azevedo et al., 2015).

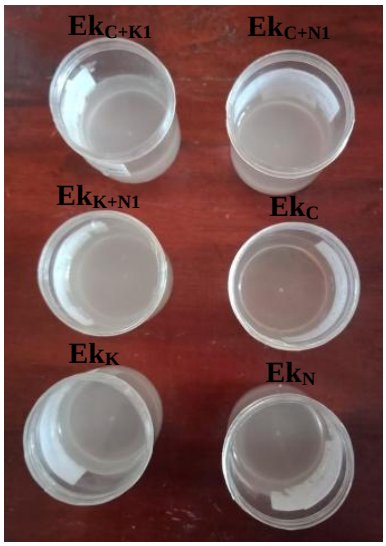

Figure 7. Variation of gel formed

Table 7. Gel strength results

No.	Sample	Gel strength (g bloom)
1.	Carrageenan Standard	100 –1200
2.	Ek_{C+K1}	21.0
3.	Ek_{C+N1}	252.5
4.	Ek_{K+N1}	123.0
5.	Ek_{C}	68.0
6.	Ek_{K}	35.5
7.	Ek_{N}	ND

ND : *Not detected*

Based on Table 7, the gel strength ranges from 21.0–252.5 g bloom. The highest gel strength value was produced in the combined Ek_{C+N1} alkaline solution, 252.5 g bloom, and the lowest gel strength value was produced in Ek_{C+K1}, 21.0 g bloom. As for the Ek_N sample, the gel strength was not detected. The general mechanism of the carrageenan gel formation process consists of two stages, namely, the transition from fiber form to strand form during cooling and the aggregation process between strands, which depends on the presence of cations (Azevedo et al., 2015). Ferdiansyah et al. (2023) have examined the effect of K^+ and Na^+ ions on the gel formation properties of kappa carrageenan. In the presence of Na^+ ions, kappa carrageenan is found as coils at room temperature. In contrast, in the presence of K^+ ions, kappa carrageenan can be found in the form of coils or helices and Ca^{2+} ions have greater binding capacity (divalent ions), and K^+ ions have specific targets compared to Na^+ ions. The gel strength produced using Ca^{2+} and K^+ ions is higher than that of Na^+ ions. The helix chain's crosslinking close to the sulfate affects the gel that forms on carrageenan. The gel formation process is also influenced by the molecular weight of calcium, potassium, and sodium ions (Bhernama, 2019). This is possible because the physical form of the carrageenan produced affects the strength of the gel.

The gel strength value produced in Ek_{C+N1} was higher than the results of research by (Supriyantini et al., 2017), who compared the strength of carrageenan gel based on sampling location, namely 61.86 g bloom and 78.57 g bloom. Meanwhile, the gel strength results in this study were still low compared to research by Wenno et al. (2012) of 330 g bloom. The quality standard for carrageenan gel strength set by the FAO (Food Agriculture Organization) is >500 g bloom. Based on the research results showed that the gel strength value does not meet FAO quality standards.

CONCLUSION

Based on the research conducted, it can be concluded that soaking for all combinations of alkaline solutions with variations in solution volume produced a soaking of > 25%, which had a value range from 38.04–70.18%. The highest soaking was produced in a combination of intermediate alkaline solutions $Ca(OH)_2$ 80%:KOH 20% or Ek_{C+K1} is 70.18% and the lowest soaking value is produced in an alkaline solution that is not combined with other alkaline solutions, that 100% NaOH of 20.25%. Characterization of carrageenan using FTIR resulted in the presence of functional groups in carrageenan with the appearance of absorption at 1261.37–1263.37 cm^{-1} for sulfate ester bonds, absorption at 1028.06–1068.56 cm^{-1} for glycosidic bonds, absorption at 846.75–875.68 cm^{-1} for the galactose-4-sulfate bond, absorption at 928–933 for the 3,6-anhydro-D-galactose bond and no absorption appears in the 800-805 cm^{-1} area indicating the absence 3,6-anhydrogalactose-2-sulfate bonds thus indicating that the carrageenan from Eucheuma cottonii is a type of kappa (κ) carrageenan. The strength value of the resulting gel has a value ranging from 21.0–252.5 g bloom. The highest gel strength value was produced

in the combination of Ek_{C+N1} alkaline solution, which was 252.5 g bloom and the lowest gel strength value was produced in Ek_{C+K1}, which was 21.0 g bloom.

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