Carrageenan Extraction of *Kappaphycus alvarezii* Seaweed from Nusa Lembongan Waters Using Different Alkaline Treatments

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Abstract

Kappaphycus alvarezii is a carrageenan-producing red seaweed that is widely cultivated in Nusa Lembongan waters, Bali, Indonesia. Carrageenan is generally extracted using an alkaline base~~,~~. ~~i~~In this study three different types of alkali were used to extract carrageenan originating from Nusa Lembongan Waters. This study aims to determine the quality of the extraction. The three alkalis used were KOH, NaOH, $Ca(OH)_2$, and distilled water as a control. The 20 g dried seaweed was weighed, washed with the tap running water, and immersed in 0.15% alkaline solution (KOH, NaOH, $Ca(OH)_2$) as well as distilled water for 24 hrs. Followed by rinsing with running water until pH was neutral. The solution was soaked and heated at 100°C for 2 hours with a ratio of seaweed and water 1 kg in 20 L, and filtered. The extract was mixed with 1.25% KCl. The carrageenan precipitate was put in the oven at 60-80°C until dry for 48 hours. The yield test results showed that the use of alkaline $Ca(OH)_2$ resulted in a yield of 29.28% better than alkaline KOH (28.18%) and NaOH (27.7%). Based on the analysis of Fourier Transform Infrared Spectroscopy (FTIR), extraction using alkaline $Ca(OH)_2$ showed functional groups characteristic of iota-carrageenan, whereas using alkaline KOH and NaOH showed functional groups characteristic of kappa-carrageenan.

Keywords: Alkaline, Carrageenan, FTIR, Kappaphycus alvarezii

INTRODUCTION

Globally, seaweed is one of the most important marine resource sectors either biologically or economically. Seaweed cultivation is one of the fast–growing aquaculture sectors (Mariot *et al*., 2021). Indonesia is one of the largest superior seaweed producers in the world with a production value of up to 9.9 million tons (FAO, 2021). The Province of Bali contributed 149 tons of seaweed production (KKP, 2020), most of which was produced from the waters of Nusa Lembongan.

Kappaphycus alvarezii seaweed cultivation has developed very rapidly since the demand for carrageenan from industry has increased in many countries, including Indonesia (Kasim & Mustafa, 2017). *K. alvarezii* is one of the seaweeds that is widely cultivated by the people of Nusa Lembongan because it has a fast growth rate (about 4.5% per day) (Rudke *et al*., 2022). In spite of that, *K. alvarezii* also has a high amount of polysaccharides in the cell wall so this can be a potential source of carrageenan (Manuhara *et al*., 2016; Riyaz *et al*., 2022). Carrageenan is a hydrocolloid compound extracted from red seaweed species namely *K. alvarezii*, *Chondrus crispus*, and *Eucheuma spinosum* (Azevedo *et al*., 2015; Bouanati *et al*., 2020; Arun *et al*., 2021). Carrageenan can be used as a stabilizer, emulsifier, thickener, food, cosmetics, biostimulants for plants and animals, as well as immunostimulant (Mariot *et al*., 2021; Vaghela *et al*., 2022). Carrageenan acts as a stabilizer because it contains sulfate groups which are negatively charged along the polymer chain and are hydrophilic which can bind water and other hydroxyl groups (Supriyantini *et al*., 2017).

Carrageenan extraction from *K. alvarezii* is generally carried out using an alkaline base (KOH) (Bono *et al*., 2014; Solorzano *et al*., 2019). The function of KOH is to help the extraction of polysaccharides effectively and to accelerate the elimination process of 6-sulfate to 3,6-anhydro-D-galactose thereby increasing the quality of the carrageenan produced (Azevedo *et al*., 2015). However, several other researchers used different alkaline bases such as NaOH and $Ca(OH)_2$ as carrageenan extracting compounds (Das *et al*., 2021; Ganesan *et al*., 2018). According to research

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conducted by Panggabean, *et al* ~~in~~ (2018), it is known that differences in concentration of alkali can affect yield results. The higher of alkali concentration during the alkalization process takes place, resulting the higher pH, and this will enhance the ability of alkali to extract.

Research on the extraction of carrageenan using different alkaline methods from seaweed cultivation in Nusa Lembongan has never been explored before. There is still a lack of information, so, it is important to provide information about the chemical-based carrageenan data of *K. alvarezii* and the potency utilization of *K. alvarezii* seaweed, especially in Nusa Lembongan, Bali.

MATERIALS AND METHODS

The seaweed used in the study was *K. alvarezii* which was obtained from a seaweed cultivation area in Nusa Lembongan Waters, Bali. Dried seaweed was extracted at the Diponegoro University, Biology Laboratory, Central Java. The research method used in this study was laboratory experimental, to investigate cause-and-effect relationships by giving one or several treatments (Sunaryo *et al.,* 2015). The obtained data were analyzed using descriptive methods.

The process of extracting carrageenan from *K. alvarezii* was carried out according to the protocol of Erjanan *et al*. (2017) with a few modifications. The dried seaweed was weighed as much as 20 g, then the seaweed was cut into small pieces (± 1 cm), then the seaweed was washed with running water until clean. Immersion in 0.15% alkaline solution (KOH, NaOH, $Ca(OH)_2$) and distilled water was done. After 24 hours the seaweed was then washed again with running water until a neutral pH was obtained, then the seaweed was soaked and heated at 100°C for 2 hours with a ratio of seaweed and water 1 kg in 20 L, so the water needed was 400 ml. After the heating process, filtering is carried out with a filter cloth in a hot state. The filtered filtrate is then mixed with 1.25% KCl in each treatment, and let stand for 30 mins. KCl solution is used for the carrageenan precipitation process. The carrageenan precipitate is put in the oven at 60-80°C until dry for 2 days (48 hours). After finishing in the oven, the carrageenan is crushed into carrageenan powder and then weighed for the analysis process. Yield analysis was carried out by comparing the weight of carrageenan with the weight of the dried seaweed used. Yield is calculated based on the formula:

Yield (%) = (Carrageenan weight)/(Dry seaweed weight) x 100% (Leksono *et al*., 2018)

Carrageenan Composition Test (FT-IR)

The functional group produced by carrageenan was tested using an infrared spectrophotometer. The infrared spectrum is used to determine the presence of several chemical bonds in organic compounds. The functional group test followed the method of Silverstein & Bassler (1962). The sample is weighed as much as 1 mg, then mixed with 100 mg KBr and pressed for 10 minutes at a pressure of 8-10 psi until a thin pellet is obtained, then the pellet is inserted into the cell holder and the spectra are made.

RESULTS AND DISCUSSION

Yield is a comparison of the dry weight of the extract with the amount of raw material before extraction process. Yield is one of the important parameters to determine the effectiveness of the extraction process (Sinurat, 2017). The percentage yield of carrageenan resulting from *K. alvarezii* extraction with three different alkaline treatments is shown in Figure 1. It shows that the extraction using alkaline $Ca(OH)_2$ resulted in the highest average percentage of 29.28% compared to other alkaline treatments. Whereas extraction using distilled water produces the lowest average yield percentage, which is equal to 23%. The average yield percentage from the KOH alkaline treatment was 28.18% and the NaOH alkaline treatment was 27.7%. Giving alkali in the extraction process can accelerate the process of cell wall plasmolysis thereby facilitating the separation process between the cell wall and cytoplasm of seaweed cells so that the ability to extract is greater than using aqueous water (Wulandari *et al*., 2019).

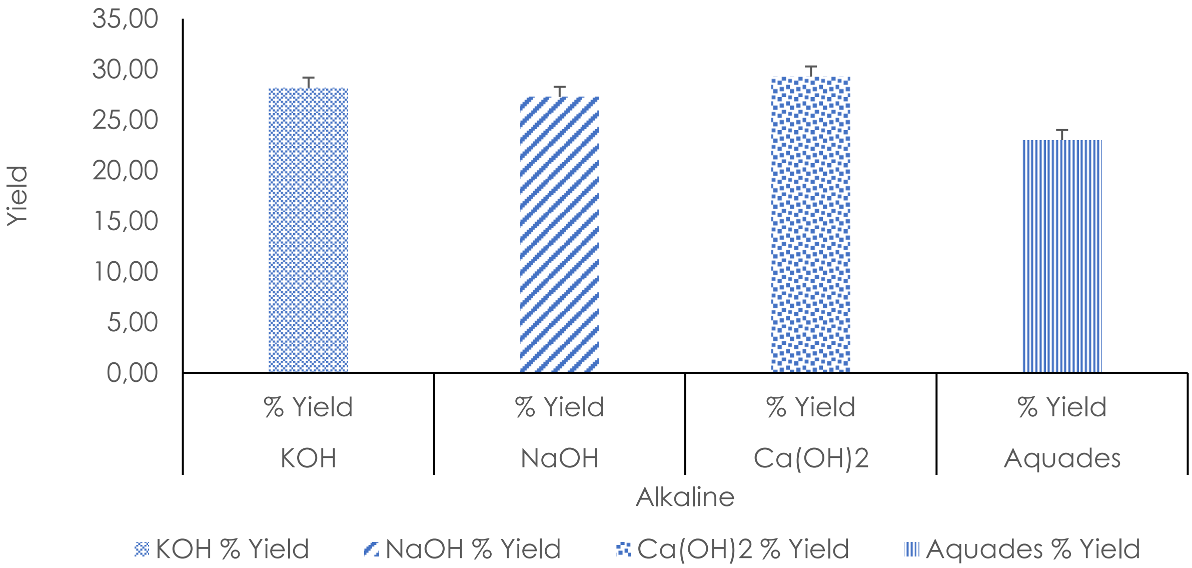

Figure 1. Yield from carrageenan extraction using 4 different alkaline treatments namely (KOH, NaOH, $Ca(OH)_2$, and distilled water.

The yield from the study showed that alkaline treatment could increase the yield ie. 25%. This results is in accordance with FAO standards (2007). On the other hand, treatment without alkali (distilled water) produced a yield value of less than 25%. Alkali treatment resulted in a higher yield value allegedly because the alkaline solution was able to accelerate the process of forming 3,6 anhydro-galactose. Later, most of the sodium, magnesium and calcium content in carrageenan will be bound to the ester group and the 3,6 anhydro-galactose capopolymer so that the extraction process can increase the value of the yield (Wulandari *et al*., 2019). This statement is supported by (Gerung et. al, 2019) that the administration of an alkaline/base solution can break down the cell wall thereby increasing the solubility of carrageenan in water and preventing the hydrolysis reaction of the glycosidic bond in the carrageenan molecule.

The yield values of the three types of alkaline treatment, namely the treatment with $Ca(OH)_2$, had the highest yield value of 29.28% compared to other alkaline treatments, namely KOH ~~of~~ (28.18%) and NaOH (27.7%). This difference occurs because in the extraction stage besides dissolving carrageenan, the reaction between carrageenan and alkaline solvents also takes place. It is estimated that the reaction that took place involved the exchange of ions, that is, cations in dissolution replaced sulfate ions in carrageenan (Distantina *et al*., 2013). The calcium cation in $Ca(OH)_2$ has the largest molecular weight of 74.093 g/mol (Winaya *et al.,* 2020) compared to the molecular weight of the potassium cation in KOH of 56.11 g/mol (Park *et al.,* 201) and the sodium cation in NaOH of 39.997 g/mol (Winaya *et al.,* 2020). The $Ca(OH)_2$ yield result was the highest than KOH, NaOH, and distilled water extraction results.

FT-IR Spectrum Analysis of Carrageenan in Three Different Treatments

Samples suspected of carrageenan compounds can be proven by identifying them using an infrared spectrophotometer or FTIR. FTIR analysis is a technique used to determine the functional group of a compound. Based on Figure 2 and Table 1, the results of carrageenan extraction using different alkalis, namely KOH, NaOH, and $Ca(OH)_2$, show that the functional groups of glycosidic bonds and sulfate esters are present in all carrageenan with four different alkaline treatments. The ester sulfate and 3,6-anhydrogalactose functional groups from the use of alkali $Ca(OH)_2$ have a wave number that is almost close to the wave number of commercial carrageenan (Sham, 2022), while the use of alkali $Ca(OH)_2$ also produces functional group 3, 6 anhydrogalactose 2 sulfates. On the other side, the use of alkaline KOH, NaOH, and distilled water did not produce this functional group. The four alkaline treatments produced the O-H hydroxyl functional groups with the resulting wave numbers not approaching the commercial carrageenan wave numbers. Furthermore the glycosidic linkage produced in each treatment was pointed from different wave numbers. KOH is pointed from 1073.270 cm^{-1}, NaOH (1071.31cm^{-1}), $Ca(OH)_2$ (1075.81cm^{-1}), and distilled water (1072.74cm-1), respectively.

FTIR test results are used to identify the functional groups and compounds that have been extracted. The FTIR spectra of the extracted carrageenans were compared to one another, based on the differences in the alkali used. The wave range observed in this FTIR test is 4000-400 cm^{-1}. FTIR test results of *K. alvarezii* extraction using 3 different alkaline treatments showed several important absorption peaks (wavelengths) indicating functional groups that are generally found in carrageenan.

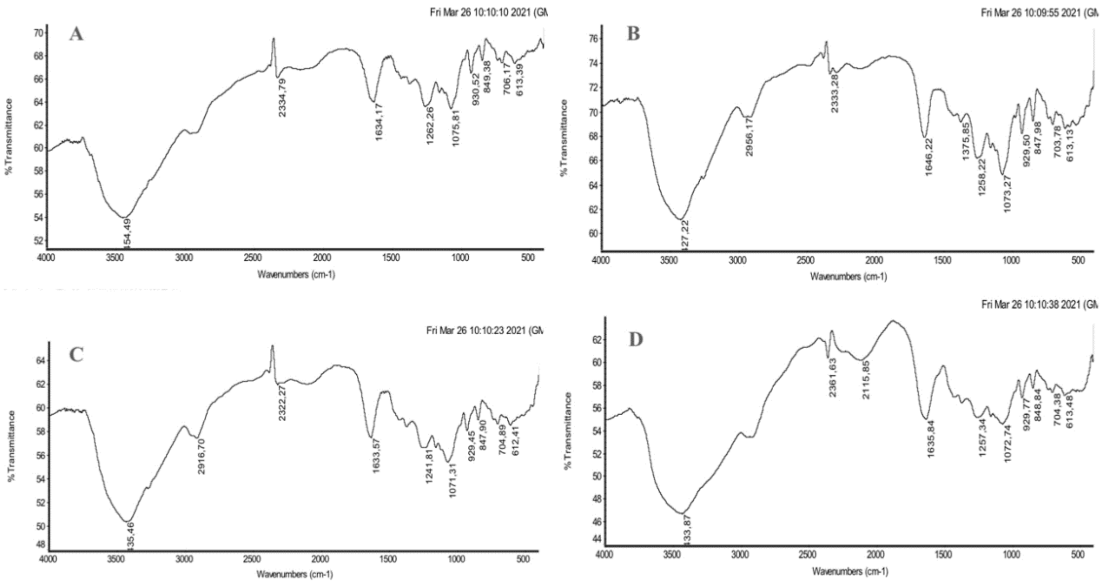

Figure 2. The FTIR test results analysis of carrageenan extracted using alkali (A) NaOH, (B) KOH, (C) $Ca(OH)_2$, and (D) distilled water.

Table 1. Wavelength analysis on carrageenan bonds based on four alkaline treatments (NaOH, KOH, $Ca(OH)_2$, and distilled water)

Wave number (cm^{-1})	Functional groups	Commercial Carrageenan (Syam, 2022)	Alkaline Treatments			
			KOH	NaOH	$Ca(OH)_2$	Distilled Water
3200-3600	*O-H*	3410,15	3427,22	3435,46	3454,49	3433,87
1220-1265	*Ester sulfate*	1261,45	1258,22	1241,81	1262,26	1257,34
928-933	*3,6-anhydrogalactose*	931,62	929,5	929,45	930,52	929,77
840-870	*3,6-anhidrogalactose-4-sulfate*	864,11	847,98	847,9	849,38	848,84
800-805	*3,6 anhydrogalactose-2sulfate*				408,46	
1010-1080	*Glikosidic linkage*		1073,27	1071,31	1075,81	1072,74

FTIR test results of carrageenan extraction with alkaline KOH treatment had a wavelength peak in the 3427 cm^{-1} region which indicated the O-H functional group due to the presence of hydrogen bonds in the carrageenan structure which caused a shift towards shorter wave numbers. The peak wavelength in the area around 1258 cm^{-1} indicates absorption of sulfate esters in the carrageenan extraction results using alkaline KOH, this is supported by Balqis *et al*., 2017) indicating that the wave number of sulfate ester absorption obtained is approx. at 1228.928 cm^{-1}. The results of other studies on carrageenan stated that sulfate esters were shown at a wave number of 1240-1260 cm^{-1} (Chopin and Whalen, 1993), as well as research by Tanusorn *et al*. (2018) noted that sulfated esters were shown at a wave number of 1210 and 1260 cm^{-1}.

The FTIR test value of the alkaline NaOH treatment showed the same functional group as O-H at a wave number of 3435 cm^{-1} and the S=O ester sulfate functional group a wave number of 1241cm^{-1}. It is shown that the sulfate ester content in the alkaline NaOH treatment was compared to the alkaline KOH treatment. Meanwhile, the wave number at 929.25 cm^{-1} indicates the presence of the C-O-C functional group. In the 3,6-anhydrogalactose structure, the C-O-C content of 3,6-anhydrogalactose from the alkaline NaOH treatment is slightly higher than the alkaline KOH treatment. The peak at a wave number of 847.9 indicates the presence of the 3,6-anhydrogalactose-4-sulfate functional group which has a strong intensity, the same thing was also found in the KOH and distilled water treatment.

Based on the results of the FTIR test for the $Ca(OH)_2$ treatment, it was shown that there were 2 functional groups 3,6-anhydrogalactose-4-sulfate and 3,5-anhydrogalactose-2-sulfate with respective wave number of 849.38 cm^{-1} and 408.48 cm^{-1}. The functional group 3,5-anhydrogalactose-2-sulfate was only found in the alkaline $Ca(OH)_2$ treatment and was not found in other treatments. The alkaline treatment $Ca(OH)_2$ also found several other groups such as O-H with a wave number of 3454.49 cm^{-1}, a sulfate ester group with a wave number of 1262.26 cm^{-1} as the same as the other treatments.

From the results of identification with infrared spectroscopy and the description of the wavelength, it can be concluded that the carrageenan produced by $Ca(OH)_2$ is of the Iota type. Iota type is characterized by the presence of galactose 2-sulfate and 4-sulfate, 3,6-anhydrogalactose, and sulfate ester groups (Villanueva *et al*., 2004; Campo *et al*., 2009). Other treatments using alkaline KOH, NaOH, and distilled water shows the chemical structure of carrageenan from kappa-carrageenan type, ~~this is~~ based on the functional groups found such as (ester sulfate), (3,6-anhydrogalactose), and (galactose-4-sulfate) (Campo *et al* ., 2009;Verma *et al*., 2014).

CONCLUSION

The application of alkaline $Ca(OH)_2$ in extraction methods produces the highest amount of carrageenan yield compared to the application of alkaline KOH, NaOH, or distilled water. Based on the FTIR analysis, $Ca(OH)_2$ produced iota-carrageenan type. KOH, NaOH, and distilled water extraction methods produced kappa-carrageenan type.

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