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Characteristics of Rapid Visco Analyzer Carrageenan Extract with Enzymatic Pretreatment of *Kappaphycus striatum*

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Abstract. Carrageenan is a polysaccharide compound extracted from red seaweed and is widely used by food, cosmetic, and advanced materials industries because of its good properties as an environmentally friendly stabilizer. Carrageenan extraction generally uses alkaline treatment for one full day, where the treatment is to obtain carrageenan quality with good gel characteristics. The use of cellulase enzymes is thought to accelerate the desulfuration process of seaweed, where cellulase enzymes are used to break down cellulose in seaweed cell walls. By using a rapid visco analyzer (RVA), carrageenan was tested to see the pattern and viscosity value. This study aims to determine the effect of enzymatic pretreatment on the profile of carrageenan with a shorter alkalization process compared to the alkalization commonly used by the industry. The results showed that enzymatic treatment before KOH alkalization would produce a carrageenan profile with a viscosity value of 272-360 cP, whereas the NaOH alkalization only reached 19-24 cP. The results of the test using RVA showed that the addition of an enzymatic process could change the physicochemical properties, such as viscosity and gel point of the carrageenan alkalinized with KOH. However, there was no significant difference in the properties when treated by alkalization using NaOH, which can be described from the value of the gelling point of carrageenan treated by cellulose enzyme. Adding enzymes to KOH will accelerate the gelation process, which occurs at an average temperature of 42.78°C. Meanwhile, carrageenan without enzymatic addition has an average gelation value of 37.48°C.

Keywords: Carrageenan, Extraction, Enzyme, Rapid Visco Analyzer

INTRODUCTION

Carrageenan is a polysaccharide compound extracted from red seaweed. Many are produced from the type of seaweed *Kappaphycus alvarezii* and *Kappaphycus striatum* (Laksono et al., 2021). Currently, the carrageenan industry uses alkaline compounds as the main ingredient in extracting carrageenan (Rhein-Knudsen, 2015). The use of this alkali has the aim that the extract obtained will have a low sulfate content so that the gel strength of the carrageenan will be better. The alkaline process in question is carried out for a fairly long time, up to one day (Tasende, 2016).

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Currently, carrageenan extraction using enzymes is still under development. Research conducted by Naseri et al. (2020) performed multi-extraction of carrageenan from commercial spinosum, which is expected to produce high-purity carrageenan without any protein impurities. Then there is also a study by Jiang et al. (2022), who extracted iota carrageenan using CO_2 and assisted by enzymes to help reduce the alkaline level used. The literature reports multiple cases of the enzymatic extraction of carrageenans from red seaweed; Blanco-Pascual et al. (2014) used an alcalase to extract a hybrid from Mastocarpus stellatus. Papain was used by De Araújo et al., (2012) to extract lambda carrageenan from Soliera filiformis. Cellulase, Aspergillus niger, and conventional boiling extraction of carrageenan from Eucheuma cottonii have been contrasted by Varadarajan et al. (2009).

Enzymes are natural biocatalysts generally used to speed up or stimulate reactions. The hypothesis in this study is that carrying out an enzymatic process before the alkaline process can accelerate the desulfurization process of seaweed so that alkaline treatment does not need to be carried out for too long to produce carrageenan with good gel strength (Tarman, 2020).

The enzymatic mechanism here is hydrolyzing cellulose tissue, breaking -1,4 glycosidic bonds into oligosaccharides and simple sugars from seaweed so that the hydrocolloid components can come out easily (Ejaz et al., 2021). The enzyme specifically used for this task is the cellulase enzyme (Rosyidah et al., 2019). The profile and viscosity value can be seen using the Rapid Visco Analyzer to determine the gelation characterization of carrageenan (Diharmi et al., 2019).

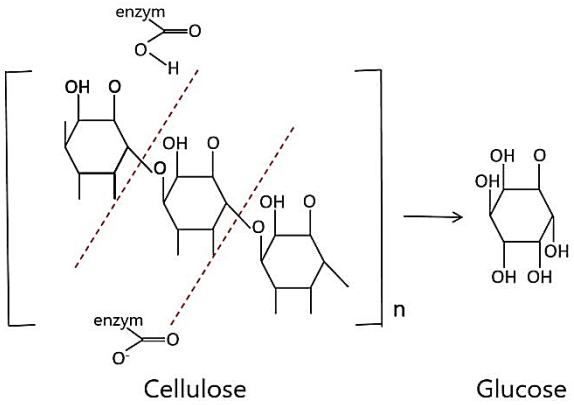

Fig. 1: Mechanism of cellulolysis (Hannan et al., 2014)

The Rapid Visco Analyzer (RVA) is an instrument that is used to measure physiochemical properties such as gel point and viscosity in the function of time related to starches and gums (Balet et al., 2019), one of which is carrageenan. By looking at the RVA profile of the carrageenan sample, the quality of the carrageenan can be known so that its utilization and application in specific fields can be determined, for example, whether the material is suitable for use in the food or material industry. Understanding the hydrocolloid gelling behavior in the food sector helps with product development and standardization.

This study aimed to see the viscosity profile, whether there is a very significant difference with some of the extraction process treatments, which were pretreated with cellulase enzyme in which the alkaline process was only carried out for a few hours, and to compare the character of the carrageenan extracted purely with alkali according to industrial procedures.

MATERIALS AND METHODS

Tools and Materials

The main material used in the production of carrageenan in this study was *Kappaphycus*

striatum seaweed taken from PT Indoflora Cipta Mandiri, West Java. Other chemicals used in this study were KOH for analysis (Supelco, Germany), NaOH for analysis (Supelco, Germany), KCl for analysis (Supelco, Germany), and Celluclast® (cellulase enzymes by Novozymes, Denmark). The equipment used in this research is a water bath, hotplate, overhead stirrer, centrifuge, oven, rapid visco analyzer (RVA 4500, Perten instruments), and several other glassware and plasticware.

Raw Material Treatment

Dried seaweed was washed using fresh water to remove salt and dirt. Next, the seaweed was dried in a refrigerated room at 20°C to avoid more brownish discoloration (Pralisa Putri et al., 2018), and the moisture content was measured periodically with referred to AOAC (1995) until water content reached 12%. The clean and dry seaweed is packaged using plastic and ready for processing.

Enzymatic Process

The enzymatic process refers to the research of Varadarajan (2009) with a slight modification where the sample used is still whole seaweed. The use of whole seaweed is intended to see the effect of the extraction time in which the cellulose breakdown process on the seaweed cell wall affects the concentration of the enzymes used. Namely, 1 gram of cellulase enzyme is dissolved in 1 L of distilled water to make 0,1% cellulase solution and heated in a water bath shaker at a temperature of 60^{o}C. The dried seaweed is then washed in running water for about 20 minutes. Seaweed was put into the enzyme solution with a shaker setting of 160 shakes/minute for 15 minutes. After 15 minutes, the seaweed is drained.

Extraction Process

The dried seaweed was washed in running water, then put into a 2.5% alkaline solution (KOH and NaOH) (6 grams in 240 mL of water), and then heated in a water bath at a temperature of 70°C for 24, 16, 2, and 1 hour. After draining the seaweed from the samples pretreated with enzymes, the samples were immediately dissolved in an alkaline solution.

After the specified time, the seaweed was drained and washed with running water for approximately 1 hour, assuming the pH was close to neutral (7-8). Afterward, the seaweed is added to 1.2 L of water and agitated with an overhead stirrer until the seaweed is pulverized. This seaweed solution is heated at a temperature of 70-80°C for 2 hours.

The solution was centrifuged after the water extraction, and the supernatant was collected. 10% KCl was as much as 6.25% of the total supernatant. The solution was molded and dried in an oven at 50°C, ground, and ready for viscosity testing using a rapid Visco analyzer. The treatment was modified from the research of Rhein-Knudsen (2018) by adjusting the process conditions in the industry (PT Indoflora Cipta Mandiri).

RVA Profile Measurement of Carrageenan

The viscosity profile of the carrageenan solution was determined by measuring the viscosity using a Rapid Visco Analyzer (RVA 4500, Perten instruments). The test was carried out by inserting 0.28 g of hydrocolloid sample and 28 g of distilled water (1% w/w aqueous solution) into the sample can. Use a non-stick stirrer to dissolve the gum thoroughly in the water before starting the test. Measurement results can be determined by looking at the viscosity value during a decrease in temperature from 80 to 20°C for 70 minutes with 160 rpm rotation (Young et

al., 2003 and 2007) so that the viscosity profile obtained is a function of time and temperature. Viscosity profiles were measured based on the use of enzymes during pretreatment before extraction and the duration of their alkalization. The viscosity data obtained were then analyzed descriptively.

RESULTS AND DISCUSSION

Viscosity Profile

Viscosity measurement using RVA was performed to determine the differences in gelation properties of all treatments, including enzyme pretreatment and alkaline solvents (Freile-Pelegrín, 1997). By comparing several profiles, the tendency of the carrageenan properties produced can be seen, and later it can be applied according to these properties. The profile of carrageenan with alkalization using KOH and NaOH is shown in Figures 1 and 2, and changes in viscosity with a function of time can be seen in tables 1 and 2.

Table 1. Carrageenan viscosity at a specific time of KOH alkalization

Temp	Viscosity (cP)		
(°C)	1 hour	2 hours	24 hours
77,1	11	14	15
80	112	41	23
70	46	42	27
60	49	46	29
50	57	50	33
40	66	57	40
30	433	410	269
25,55	401	359	295

Figures 2 and 3 show the profile of carrageenan against the alkalization time used. The profile pattern is by the measurements made by Chen et al. (2002), where the viscosity at the beginning of the measurement is low and starts to increase when the temperature begins to drop.

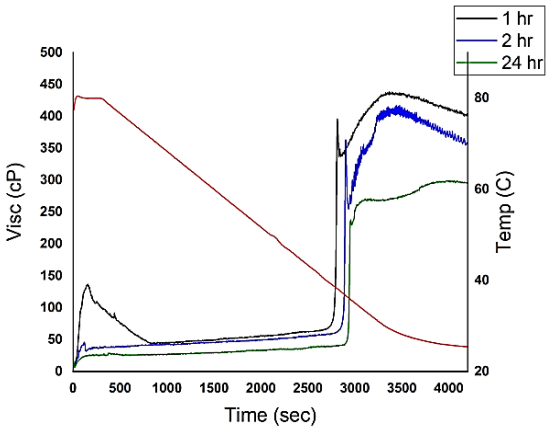

Fig. 2: RVA profile for carrageenan extract with different KOH alkalization time

Table 2. Carrageenan viscosity at a specific time of NaOH alkalization

Temp	Viscosity (cP)		
(°C)	1 hour	2 hours	24 hours
77,3	2	3	11
80	5	3	8
70	5	4	6
60	3	5	4
50	23	30	11
40	24	23	13
30	29	25	13
24,45	32	29	16

The two alkali treatments showed that the longer the alkalization, the lower the viscosity value obtained. In addition, using KOH produces carrageenan with a higher viscosity value where the viscosity value can reach ten times. The NaOH alkalization for 24 hours resulted in a three times lower carrageenan viscosity than the 2-hour alkalization. Meanwhile, from the decrease in temperature in Tables 1 and 2, gelation occurs faster in using NaOH than KOH.

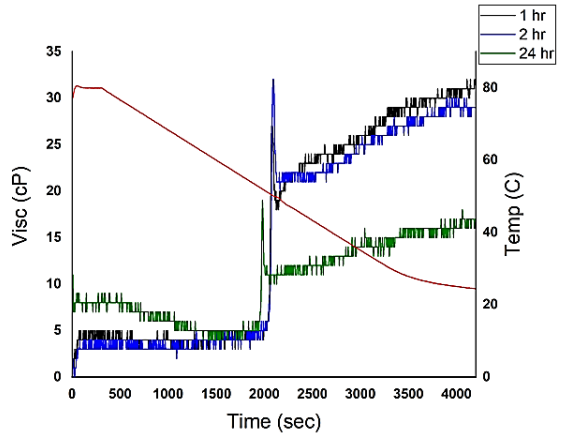

Fig. 3: RVA profile for carrageenan extract with different NaOH alkalization time

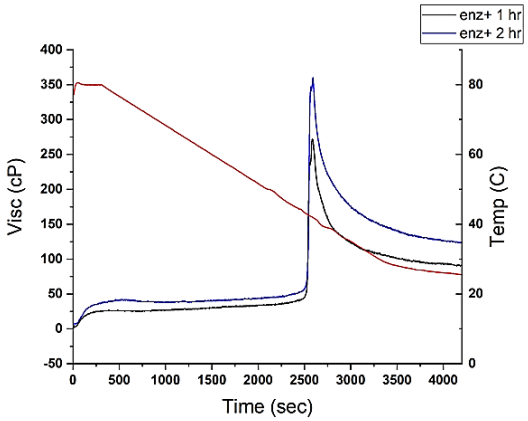

Fig. 4: RVA profile for carrageenan extract with cellulase enzyme pretreatment with different KOH alkalization time

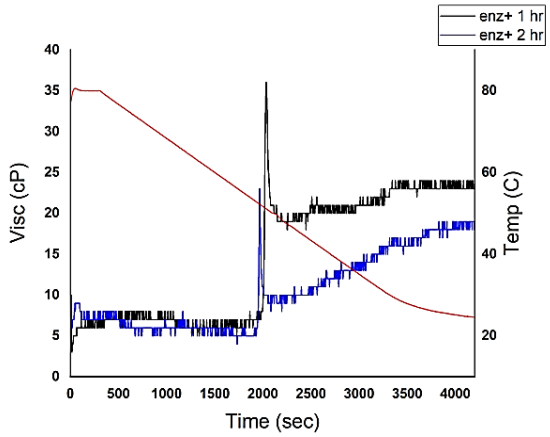

Fig. 5: RVA profile for carrageenan extract with cellulase enzyme pretreatment with different NaOH alkalization time

Table 3. Carrageenan viscosity at a specific time of KOH alkalization with enzymatic pretreatment

Temp (°C)	Viscosity (cP)	
	1 hour	2 hours
77,11	4	8
80	15	19
70	26	38
60	30	41
50	35	44
40	191	254
30	108	150
25,55	93	122

Table 4. Carrageenan viscosity at a specific time of NaOH alkalization with enzymatic pretreatment

Temp (°C)	Viscosity (cP)	
	1 hour	2 hours
77,3	9	10
80	6	8
70	7	6
60	6	6
50	19	10
40	20	14
30	24	18
24,45	22	18

The difference in the carrageenan profile with enzymatic pretreatment is shown in Figures 4 and 5. In KOH alkalization, there was a drastic decrease after the gelation process reached peak viscosity; this occurred in both alkali treatment times. Whereas NaOH's pattern measured was similar to the treatment without enzymatic pretreatment. Besides that, when viewed from the decrease in temperature in Tables 3 and 4, gelation occurred faster in the use of NaOH compared to KOH, the same as in Tables 2 and 3. So it can be said that the enzyme had no

significant effect on the gelation of the resulting carrageenan extract.

According to Young et al. (2007), the RVA pattern for carrageenan itself has several types. From the RVA pattern, it is possible to determine the type of carrageenan, whether the carrageenan is of the kappa, iota, or hybrid type, as shown in Figure 6. It can be used to determine the type of carrageenan with an unknown source. From this test, it can be concluded that carrageenan extracted using alkali and enzymatic pretreatment on Kappaphycus striatum seaweed will produce kappa-type carrageenan.

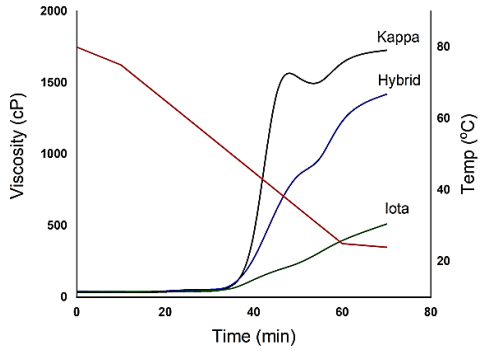

Fig. 6: RVA profile for several type of carrageenan (Young et al. (2007))

Effect of Alkalization Time

The low viscosity value of carrageenan, which was alkalized for 24 hours, was due to the long heating effect. Continued heating resulted in smaller particle sizes (Saputra et al., 2021). In addition, Desiana et al. (2015) added that the viscous and hydrophilic properties of carrageenan are influenced by the extraction time, which causes negative charges along the carrageenan polymer chain to repel each other, which makes water molecules immobilized around them.

Effect of Alkali Solvent

KOH and NaOH are alkaline compounds commonly used by industry in the carrageenan extraction process. One factor that affects the viscosity value is the sulfate content. Oliveira et al. (2020) stated that one of the functions of alkaline solutions in the carrageenan extraction process is to catalyze the loss of the 6-sulfate group from its monomer unit, which forms 3,6-anhydrogalactosam and according to Meiyasa et al. (2018), the alkalization process is needed to remove sulfate component of the 6-sulfate monomer to 3,6-anhydrous-D-galactose. The same thing was also stated by Distantina (2007); the removal of sulfate groups caused an increase in gel strength properties. Uy et al. (2005) stated that the gel formation process carried out by alkali occurs by transforming the sulfate group to the galactose group by K^+ and Na^+ ions by forming a salt compound. The second is the dehydration process of water molecules to form anhydrous galactose polymer, where alkali reacts with H atoms to produce carrageenan compounds and water. the conversion scheme of carrageenan precursors to carrageenan can be seen in Figure 7.

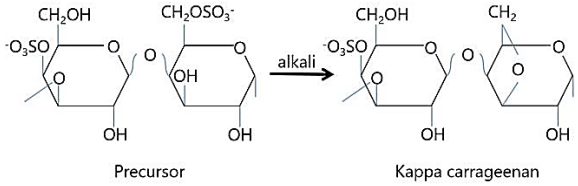

Fig. 7: Scheme of alkali solution on kappa carrageenan extraction (Flashaw, 2001)

From the results of the alkalization between KOH and NaOH, the final and highest viscosity values in KOH alkalization were much higher than NaOH. According to Bhernama (2019), the results of carrageenan sulfate levels formed from the alkalization of NaOH have a smaller value than KOH. It was also confirmed by Hidayah (2013) that carrageenan produced from NaOH alkalization could not form a gel quickly.

Effect of an Enzymatic Process

As previously explained, enzymes are used as biocatalysts to speed up reactions. In carrageenan extraction, the use of enzymes is carried out before alkalization by KOH or NaOH. The enzymes here are intended to degrade the outer skin tissue of the *Kappaphycus striatum* so that the hydrogel component (precursors of carrageenan) in the seaweed can be easily contacted with alkali to speed up the extraction process. The addition of the enzyme significantly changed the carrageenan profile from the data obtained by comparing the results of 2 hours of alkalization with and without enzyme treatment.

Figures 7 and 8 have similarities where carrageenan with enzymatic pretreatment will produce lower viscosity values. It may be because there is still a lot of cellulase enzyme in the outer tissue of carrageenan, which causes the sulfate binding process with potassium or sodium ions to be disrupted. Meanwhile, according to Varadarajan et al. (2019), the cell wall tissue of seaweed is not completely degraded, lowering the viscosity value compared to carrageenan extracted without enzyme pretreatment. It is explained by the presence of impurity bonds in carrageenan.

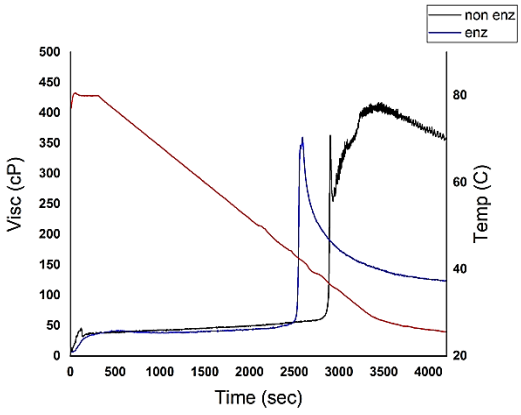

Fig. 7: RVA profile for extract carrageenan with and without enzyme cellulase pretreatment with KOH alkalization

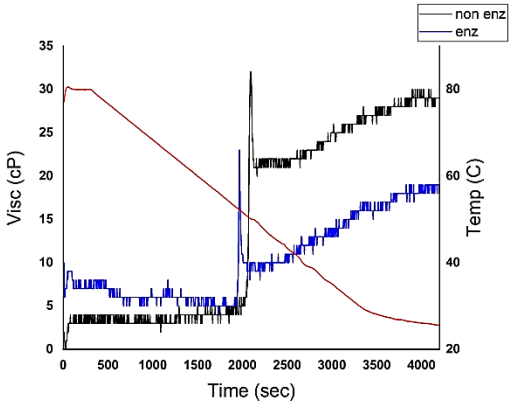

Fig. 8: RVA profile for extract carrageenan with and without enzyme cellulase pretreatment with NaOH alkalization

Another research conducted by Sulistiawati et al. (2019) used another seaweed, Kappaphycus alvarezii. It is explained that the cellulase enzyme slightly increased the viscosity value but decreased with extraction using an excess enzyme concentration. This difference could be due to the absence of alkali in the extraction process. Extraction using only enzymes will increase the viscosity value and enzyme concentration.

Gelation

Gelation is the method of constructing a gel out of a system of polymers (Oliviera, 2008; Ahmed. 2015). Links between the chains created by branched polymers can produce ever-larger polymers. At a particular point in the reaction, linkages between the polymer result in the development of a single macroscopic molecule. As the linking process continues, larger branching polymers are produced. The reaction reaches what is known as the gel point, at which point the system loses fluidity and viscosity increases significantly (Odian, 2004). The gelation mechanism in carrageenan can be seen in the following figure.

The gel point can be known from the

temperature at which the viscosity increases drastically in the RVA profile, which means the carrageenan starts to form a double helix formation, as shown in Figure 9. Gel point values of carrageenan taken from the measured RVA profile are shown in Table 5.

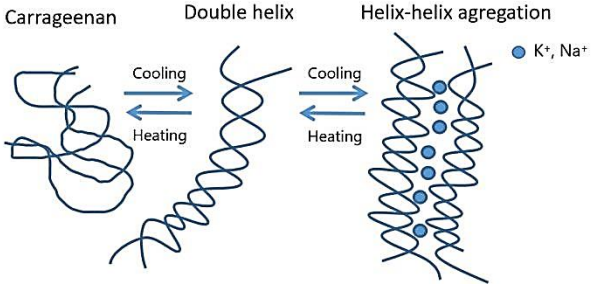

Fig. 9: Carrageenan gelation mechanism (Pacheco-Quito et al., 2020)

Table 5. Carrageenan gel point value for each alkalization treatment

Alkali	Cellulase (%)	Alkalization (hr)	GelPoint (°C)
KOH	0,1%	1	42,8
		2	42,75
	0%	1	38,95
		2	37,4
		24	36,1
NaOH	0,1%	1	51,25
		2	52,4
	0%	1	50,4
		2	50,3
		24	52

By knowing the gel point of carrageenan, we can determine the required temperature for the gel that has been in the form of helix-helix aggregation into double helix formation or in the form of carrageenan again, so that energy efficiency can be obtained, especially in industrial applications.

Table 5 shows no appreciable differences in the alkalinized samples with NaOH, as shown by the readable gelling point. Enzymes can also speed up the gelation of KOH, which happens at a temperature of 42,78°C on average. Carrageenan's average gelation temperature without enzymatic addition is 37,48°C.

CONCLUSIONS

Rapid Visco analyzer can be used as a qualitative test of samples of various polysaccharide materials, one of which is carrageenan. Under the reference from Young et al. (2003 and 2007), the RVA results of carrageenan compounds have a typical pattern, and it was found that *Kappaphycus striatum* produces kappa-type carrageenan.

In the enzymatic pretreatment of the KOH alkalization sample, there was a significant difference in the curve. It indicated that the cellulase enzyme could affect the quality and properties of the carrageenan extract obtained. Adding an enzymatic process can open new opportunities for carrageenan applications in the future. In contrast to the enzymatic pretreatment with NaOH alkalization, the results obtained are similar, so the use of enzymes in NaOH alkalization produces carrageenan with properties that are not much different from the process without enzymatic pretreatment. KOH has quite good results from the two solvents used, judging from the high viscosity and gel point values and the interaction between KOH and cellulase enzymes that produce carrageenan with new properties.

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