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Improvement Quality Of Agarose From *Glacilaria Verrucosa* Red Algae By Using NaOH And EDTA

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Abstract: Agar consisted of two component is agarose and agaropectin, that could be isolated from group *Rhodophyta* of seaweeds, such as *Glacilaria verrucosa*. Agarose is neutral polymer, while agaropectin is polymer that contain sulphate, so agarose can be used as gel electrophoresis. The aim of this research to improve quality of isolate agarosa from *Glacilaria* verrucosa with use NaOH and EDTA solution . Treatment of using NaOH solution to hydrolysis agar to break banding of agarose and agaropectin, where as EDTA solution bond the covalent ionic in agaropectin. Result of agarose which was obtained giving specific peak in spectra IR with wavenumber in the region 930 cm^{-1} and 890 cm^{-1}, indicated 3,6-anhydro-l-galactose. Using NaOH 10% and EDTA solution caused drawback sulphate and ash content, but increase constant melted temperature, gel temperature and gel strength. The characteristics of agarose from this isolation were ash content 1,7180% (w/w), sulphate content 0,4897% (w/w), melted temperature 87 ^oC, gel temperature 40 ^oC, and gel strength 938,9 gram/cm^2.
Key word : Agar, Agarose, NaOH, EDTA, *Glacilaria verrucosa.*

Introduction

Agar is consist of two polysaccharides as agarose and agaropectine. Agarose can be separated from agaropectin. Where Agarose contain low sulphate value and high 3,6- anhydrogalactose. It is important criterion to ionic character of agarose, and agaropectin contain unsaturated chemical bonds in the sulphate and pyruvate. Its bonds bestow high UV absorption in agarose gels and interfere with the detection of nucleic acids after electrophoresis[1,2].

Agarose can be acquired by way isolate from seaweeds, one of that is red algae *Glacilaria* sp. Red algae is one of the great marine resource cultivation in Indonesian. Agarose possess ability to shape the strong gel with electic charge near neutral, so it is always used in the biotechnology field[3], such as : agarose gel of electrophoresis and agarose stationary fase of chromatography, beside that agar and agarose can be used to medicine, cosmetic, tissue technique, encapsulation sel, immunology, and microorganism culturs[2].

Several way could be done to abtain agarose from algae, such as : using NaOH to hidrolyse agar from red algae by way elimination of the unstable sulphate group on C-6 L-galactose unit when the hydroxyl group on C-3 had been ionised. It can give improvement stability by shape 3,6-anhidro-L-galaktosa [4]. And using EDTA to give khelat effect to bivalent cation agaropectin[2]. The combination of that mathodes were be hoped to separate agarose from agaropectin.

By virtue of that, to employ the marine resource of Indonesian is red algae *Glacilaria verrucosa* and to obtain the better quality of agarose, so this research would be done by using combination of NaOH and EDTA

to isolate agarose from red algae *Glacilaria verrucosa* with determinate of its characteristics were ash and sulphate content, melting point, gel point, and gel strength.

Experimental Section

Materials.

Agarose (pa., Sigma), chloride acid (pa., Merck), sulphate acid (pa., Merck), barium chloride (pa., Merck), ethanol (pa., Merck), isopropanol (pa., Merck), potassium bromide (pa., Merck), calcium hypochlorite/ caporite (tehnis, Tjiwi kimia), ethylenediamine tetraacetate (pa., Merck), sodium hydroxide (pa., Sigma), Red Algae (*Glacilaria verrucosa)*, and distilled water.

Tools

Spectrophotometre FT-IR Spectrum one (Perkin Elmer), Texture Analyzer (TA XT Plus), oven (Venticell), furnace ((Thermolyne 48010-33), thermometre (Safety), waterbath (Thermostart HH-6).

Mathods

Sample Preparation

The red algae (*Glacilaria verrucosa*) was harvested from one of *Glacilaria verrucosa* dike in Palopo city.

Bleaching Sample

The red algae was washed out and soaked in caporite 1% for 0,5 hour, furthermore it was rinsed with tap water until caporite odor is lose, after that cutted into bits and dried[3].

Determination of Moisture Content of *Glacilaria verrucosa*

The dryed algae was weighed approximate 3 gram in tare a porcelain crucible, then it was dried at 105 ^{o}C for 5 hours, weighed, furthermore done drying and weighing with ranges 1 hour until the different of two weighing respectively was not more 0,25 %. Moisture content is counted with formula as follow[5,6]:

$$\text{Moisture content} = \frac{A - (B - C)}{A} \times 100\ \%$$

Where, A is First weight of algae, B is weight of crucible and end weight of algae, and C is weight of crucible.

Isolation Agar from *Glacilaria verrucosa*

The dryed *Glacillaria verrucosa* algae was weighed approximate 10 gram and soaked in tap water for 1 hour at room temperature, furthermore that algae was boiled for 3 hours in 700 ml distilled water and then filtered hot under pressure. The filtrate was frozen at -20 ^{o}C and thawed, after that removing gel agar from the thawed liquid and dryed in oven at 50 ^{o}C until obtained dry agar[4,7].

Isolation Agarose from *Glacilaria verrucosa*

The dryed *Glacillaria verrucosa* algae was weighed approximate 10 gram and soaked in tap water for 1 hour at room temperature, then cooked in NaOH 10% at 85 ^{o}C for 2 hours, after that soaked in H_2SO_4 0,025 % for 1 hour, then washed with tap water until neutral. Furthermore the algae was boiled for 3 hours in 700 ml distilled water and then filtered hot under pressure. The filtrate was frozen at -20 ^{o}C and thawed, then removing gel agarose from the thawed liquid. The gel agarose was soaked in 0,03 M EDTA solution at 50 ^{o}C for 12 hours, and then it was soaked again in 0,015 M EDTA solution at 50 ^{o}C for 12 hours. After that the gel agarose was separated from EDTA solution. The gel agarose was solved in 20 ml distilled water with heating, then cooled until at 70 ^{o}C and added isopropanol 30 ml, at the same time stired until homogeneous, after that cooled at room temperature in a night to getted complete precipitate. The Precipitate was filtered then soaked in

isopropanol 25 ml for 1 hour, filtered and soaked again in isopropanol 25 ml for 1 hour, filtered and pressed. The precipitate was dried in oven at 50 ^{o}C and obtained dry agarose[2,4,7,8].

Determination of Spectra Infra Red of Agarose

Agarose were prepared, entered in mortal and added KBr sufficiently, after that mixed until homogeneous, then pressed to result dry pellet, furthermore it was determined its spectra by using spectrum one FT-IR (Fourier Trasform Infra Red) spectrophotometer.

Determination of Ash Content of Agarose

Agarose was weighed approximate 1 gram accurately in a tare porcelain crucible, and burned at 700 ^{o}C for 3 hour until free carbon and obtained a constant weight, then determined ash content with formula as follow[5] :

$$\text{Ash content} = \frac{B - C}{A} \times 100\ \%$$

Where, A is weight of agar or agarose, B is weight of crucible and weight of ash, and C is weight of crucible.

Determination of Sulphate Content of Agarose

Approximate 0,5 gram of agarose was hydrolyzed with added 50 ml HCl 0,5 N, then boiled for 15 minute, furthermore determinated sulphate content with way : product of hydrolyze was added 5 ml $BaCl_2$ 10%, at the same time stired for 5 minute. It was cooled at room temperature for 5 hours, poured through filter paper ash free, washed with boiled water until chloride ion free. The filtrate was dried and burned at 700 ^{o}C for 1 hour, weighed as $BaSO_4$ and for sulphate content was counted with formula[6] :

$$\text{Sulphate content} = \frac{\text{Weight of } BaSO_4 \times 0{,}4116}{\text{Weight of agarose}} \times 100\ \%$$

Determination of Melting and Gel Temperature of Agarose

The 1,5% agarose solution was prepared and 3 ml entered in glass tube and cooled in a night with vertical position at room temperature. A stick (0,1618 gram) was put on surface gel agarose and entered thermometer in that glass tube. The glass tube was entered in the beaker that consist water, then cooked. Melting temperature gel agarose was noted when the stick on the surface gel move down until reach base glass tube. Whereas gel temperature was determined with allowing the agarose solution to be cool at room temperature, and gel temperature was noted when the glass tube was moved oblique and the agarose solution did not flow again[9,10].

Determination of Gel Strenght of agarose

Agarose 1% was prepared in container with size 4 cm^2 and height 1 cm, then allowed shape gel in a night, after that determined its gel strength by using instrument texture analyser (TA XT Plus) in g/cm^2.

Stick probe stressor with diameter 3,5 cm, it is determined be such that its rate pre test 1 mm/s, rate test 1 mm/s, and rate post test 2 mm/s, with range probe to surface of gel agarose was 7 mm and force was 3,6 gram. After that instrument texture analyser was activated so stick stressor press surface of gel until broken. Data was obtained through recorder graphics[8].

Results and Discussion

Mean of moisture content was obtained from *Glacilaria verrucosa* algae that used this research was 15,09%(w/w). The organoleptic's test of agar and agarose product of isolation could be seen in table 1, and the pictures could be seen in figure 1, whereas it's characteristics determination could be seen in table 2.

Table 1 : The organoleptic's test of agar dan agarose product of isolation from *Glacilaria verrucosa* algae

Material test	The organoleptic test			
	Color	**Taste**	**Odor**	**Shape**
Agar product isolation	yellow	tasteless	odorless	thin sheet*
Agarose product isolation	white yellow	tasteless	odorless	hank*
Agarose standart	white	tasteless	odorless	powder

* = Shape of agar and agarose product isolation are not powdered

A **B** **C**

Figure 1 : (A) Agar product isolation, (B) Agarose product isolation, and (C) Agarose standar(sigma)

Table 2 : Characteristics of agar dan agarose product of isolation from *Glacilaria verrucosa* algae

Materials test	Randemen	Ash content (%)	Sulphate content (%)	Melting temperature (^oC)	Gel temperature (^oC)	Gel strenght (gram/cm^2)
Agar product isolation	9,5218	5,8579	2,1066	70	31	10,93
Agarose product isolation	8,6010	1,7180	0,4897	87	40	938,9
Agarose standar	-	0,3261	0,03	93	36	1363

Beside the characteristics, spectra Infra Red (IR) of agar and agarose result of isolation from this research were too determined. It to determine its similarity with standart agarose (figure 2), where from each spectra IR, there were absorption band, as follow ; Spectra IR in region of function group (4000 – 1500 cm^{-1}), such as : absorption band in around wavenumber region 3400 cm^{-1}, showed there was hydroxyl group that shape hydrogen bond, absorption band in around wavenumber region 2900, showed there was alkane group (CH_3 or CH_2)[11], and absorption band in around wavenumber region 1600 cm^{-1}, showed there was aldehide group vibration (-CHO in galactose compound)[12]. Spectra IR in finger print region (1500 – 400 cm^{-1}), there was absorption band in around wavenumber region 1370 cm^{-1}, showed there was ether group (C-O-C), absorption band in around wavenumber region 1070 cm^{-1}, showed structure design of galactose compound, absorption band in around wavenumber region 930 cm^{-1}, showed specific vibration of 3,6-anhydro-L-galactose, absorption band in around wavenumber region 890 cm^{-1}, it was specific absorption band of 1,3-β-D-galactose that differentiate spectra IR of agar and agarose to carragenan, absorption band in around wavenumber region 860 cm^{-1}, showed there was L-galactose-6-sulphate, absorption band in around wavenumber region 830 cm^{-1}, showed there was D-galactose-2-sulphate, absorption band in around wavenumber region 740 cm^{-1}, showed there was galactose bond[1,13,14,15].

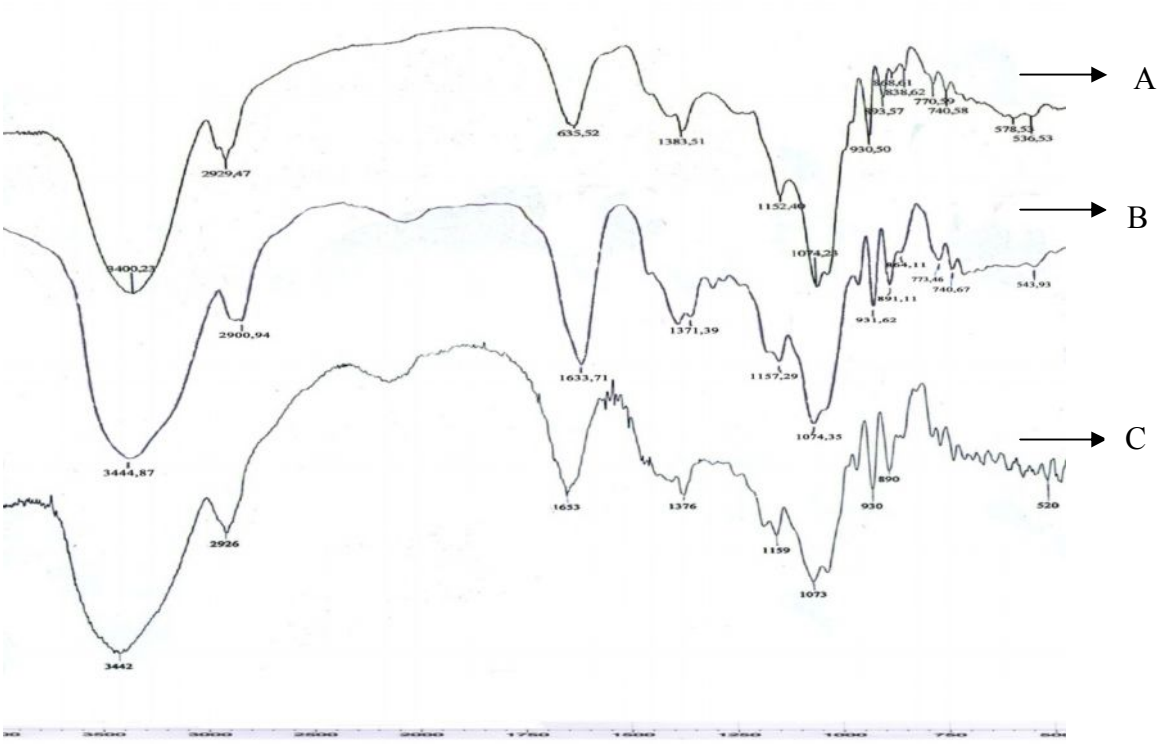

Figure 2 : Spectra IR (A) Agar result of isolation, (B) Agarose result of isolation, and (C) Standart Agarose was used (Sigma)

Conclusion

Based on the results of this study showed that, using NaOH 10% and EDTA solution caused drawback sulphate and ash content, but increase constant melted temperature, gel temperature and gel strength. The characteristics of agarose product from this isolation were ash content 1,7180% (w/w), sulphate content 0,4897% (w/w), melted temperature 87 ^{o}C, gel temperature 40 ^{o}C, and gel strength 938,9 gram/cm^{2}.

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