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Effect of Extraction Time and Na_2CO_3 Concentration on The Characteristics of Alginate Extracted from *Sargassum* sp.

Erliza Hambali[1,2], Satya Candra Wibawa Sakti[3], Mochamad Zakki Fahmi[3], Febri Eko Wahyudianto[4], Nuryono[5], Yessi Permana[6], Mohamad Yani[2], Ellya Sinurat[7], Borneo Satria Pratama[8]

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Abstract. Alginate is a polysaccharide extracted from cell walls and intracellular matrix of brown seaweed, and has been widely used for industrial purpose due to gelling capabilities in the presence of divalent cations. However, the characteristics of alginate extracted are depend on alginate gel strength and viscosity. The objective of this research was to investigate the effect of extraction time and Na_2CO_3 concentration on the characteristics of alginates extracted from *Sargassum* sp. by acid method. Alginate extractions were conducted by varying the extraction time for 1.5, 2.0 and 2.5 hours; and varying the Na_2CO_3 concentration of 1, 2 and 3 %. The characteristics observed are alginate yield, viscosity, pH, and water content. The alginates extracted using various Na_2CO_3 concentration and extraction time resulted alginates with low, medium and high viscosity. Furthermore, the yield, pH and water content of alginates extracted in this research had fulfilled the standard of Food Chemical Codex.

1. Introduction

Alginate is an anionic polysaccharide which is isolated from cell walls and intracellular matrix of brown seaweed. Alginate serves to maintain the mechanical strength of seaweeds to survive in the oceans, and naturally binds to the salt cations that occur in the oceans, such as Na, Ca and Mg [1]. Alginate is homopolysaccharide of α-L-Guluronic acid (G), β-D-Manuronic acid (M), or

heteropolysaccharide consisting of both acids which arranged in crosslink (MG). The ratio of M blocks, G blocks and MG blocks of aliphate extracted is determined by species of extracted brown seaweeds; and will affect the gel strength of the alginate solution. The general formula of alginate molecules is $(C_6H_7O_6Na)n$ [2]. Alginates are collected from macroalgae of sub-tropical seas, such as species of Macrocystis from North America and Australia; species of Laminaria from Norwegia, France, and Japan; and species of Sargassum and Turbinaria around tropical seas of Malaysia, Phillipine and Indonesia [3]. Alginate molecular structure is illustrated on Figure 1 [19].

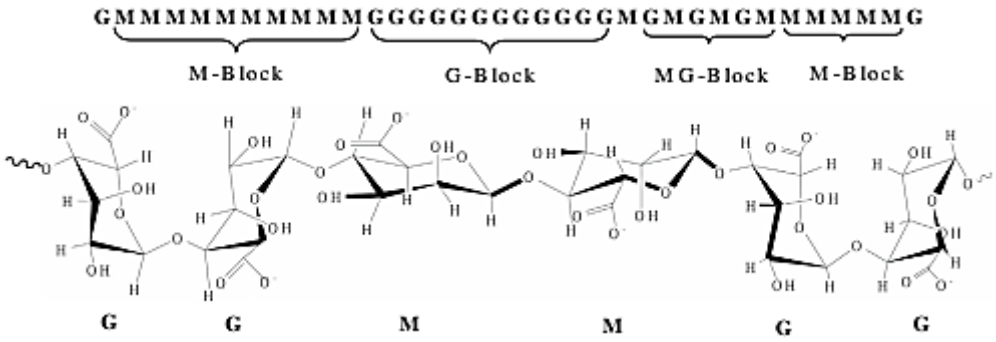

Figure 1. Alginate molecular structure.

Alginate has been applied by various industries for different purposes. Alginate in the cosmetics industries is used for the manufacturing of hand lotions and creams, whereas in the food industries, alginate is used for thickening, gelling, water binders, emulsion stabilizers, salad emulsifier and ice cream stabilizer [4]. One of the most important properties of alginates is their ability to form gel through cross linking reaction in the presence of divalent cations, i.e. Ca^{2+}, by involving ionic-bonding interaction between carboxyl groups present in alginate and cations (Alginate gel formation). Important physical properties which affect the gelling capability of alginates are gel strength and viscosity. Gel strength is mainly dependent on the content and length of the guluronic acid (G) in the alginate. Alginates rich in guluronic acid are known to form strong gels but fragile, whereas those rich in mannuronic acid or mixed sequences form weaker gels but flexible. Whereas, the viscosity of an alginate solution is directly determined by the alginate concentration and the chain length of the alginate polymer, which is proportional to its molecular weight [5].

The alginate extraction method is classified into two based on different intermediates formed (calcium alginate or alginic acid). Formation of calcium alginate is performed by immersing the material in $CaCl_2$ solution, whereas the formation of alginic acid is done by soaking the material in HCl or H_2SO_4 solution [1]. Research conducted by Laksanawati *et al*. [6] showed that the cost of alginate production with calcium method is cheaper than the acid method, based on the difference of reagents used in both methods. However, alginates extracted by acid method had higher viscosity due to the higher purity Na-alginates compared to calcium methods. Therefore, this research was conducted to find out the effects of extraction time and Na_2CO_3 concentration to the characteristics of alginate extracted by acid method.

2. Research Methodology

2.1. Materials and Equipments

The raw material used was*Sargassum* sp. obtained from local farmers on the coast of Binuangeun, West Java. The materials used for alginate extraction were distilled water, HCl, Na_2CO_3, and isopropyl alcohol. The equipments used for alginate extraction were big cormorant with capacity of 20 L, small cormorant with capacity of 10 L, beaker glass with capacity of 5 L, pH meter, analytical balance,

grinding machine, Planton net, thermometer, milling machine, and a instrument of pH meter and BrookField Viscometer.

2.2. Alginate Extraction Methods

The Na-alginate extraction process was carried out by using the Sinurat*et al*. [7] method with a modification with no decolorization process. 100 g of *Sargassum* sp. wasrinsed in clean water and then soaked with 1% HCl at a ratio of 1: 2 for 60 minutes. Then, it was rinsed with aquadest and extracted using 1, 2 and 3 of % Na_2CO_3 solution at a ratio of 1:30 (w/v) at 65±5°C for 90, 120 and 150 minutes. Following this, filtration was done by using a plankton net.

Then, 10% HCl solution was added with ratio of 1:1 and allowed to form white or ivory alginic acid to a pH of 2-3 for 30 minutes. Subsequently, any excessive pH was neutralized by the addition of dilute Na_2CO_3, while stirring to ensure homogenization of the solution, until a pH of 7-8 was achieved. Isoprophyl alcohol (IPA) was added when the alginate had reached a neutral pH, to obtain the Na-alginate fiber. Following this, obtained fibers were dried to form an Na-alginate.

2.3. Alginate Yield

The Na-alginate yield obtained from the *Sargassum* sp. extraction process was calculated, based on the weight of Na-alginate after drying, as compared with the dry weight of the feedstock. Calculation of Na-alginate yield content was performed using the following formula:

$$\text{Yield (\%)} = \frac{B-(\%WC\ x\ B)}{A} \text{ x } 100\%$$

Where A is the initial weight of *Sargassum* sp.used for extraction, B is the weight of alginate obtained from extraction process, and %WC is the percentage of water content in the alginate.

2.4. Alginate Viscosity

The viscosity analysis of the alginate was conducted in accordance with the method referred in Sinurat *et al*. [7]. Observations were performed at a 1-5% solid concentration to determine the relationship between concentration and the viscosity of the solution. 1.5 g of Na-alginate will be weighed out. Then, 98.5 g of aquadest weighed in a 100 mL glass beaker. The alginate which was weighted before will be added into the aquadest, so the total weight of Na-alginate and aquadest will reach 100 g. The mixture was stirred gradually and heated to a temperature of 75°C and stirred until the temperature is constant, and kept at that temperature for 10 minutes. The top of the beaker glasswas covered with aluminum foil to prevent a loss of water in the heating process. The viscosity of the solution was measured, by using an BrookField viscometer. The viscosity will be expressed in centipoise (cP).

2.5. Alginate pH

The pH of alginate was determined bymethod of Sinurat *et al*. [7]. 3 g of Na-alginat was weighed and inserted into beaker glass with capacity of 300 mL 197 g of water was added to the beaker glass, so the total weight of sample was 200 g. The sample was heated and stirred until it dissolved into water at 60-80°C. The electrode of pH meter was dipped into the solution, and the pH value was displayed on the screen.

2.6. Alginate Water Content

The water content analysis was conducted according to the SNI 01-2891-1992 method. 2 g of Na-alginate was weighed into an aluminum cup which the dry weight was recorded, then dried in an oven at 105°C for 16-24 hours. The sample will be cooled in a desiccator for 30 minutes and then weighed. The water content was calculated using the following equation:

$$\text{Water content (\%)} = \frac{A-B}{A} \text{ x } 100\%$$

Where A is the weight of initial sample and B is the weight of sample after drying.

3. Results and Discussions

3.1. Alginate Yield

Alginate yield obtained from various extraction time and Na_2CO_3 concentration is described in Figure 2.The yield of alginates extracted by 1% Na_2CO_3 for 90, 120 and 150 minutes were 19.65%, 20.5% and 23.75%; the yield of alginates extracted by 2% Na_2CO_3 for 90, 120 and 150 minutes were 19.25%, 23.25% and 26%; and the yield of alginates extracted by 3% Na_2CO_3 for 90, 120 and 150 minutes were 21.7%, 23.25% and 27.25%, respectively. Overall, the alginates extracted by 3% Na_2CO_3achieved higher yield compared to alginate extracted by 2% and 1% Na_2CO_3. Similar result was reported by previous study [8]. During the extraction process, Na_2CO_3 will enable the separation process of alginate which contained in alginofit cell walls from cellulose. Therefore, the higher Na_2CO_3 concentration used in extraction process, the higher yield will be obtained [9][10].

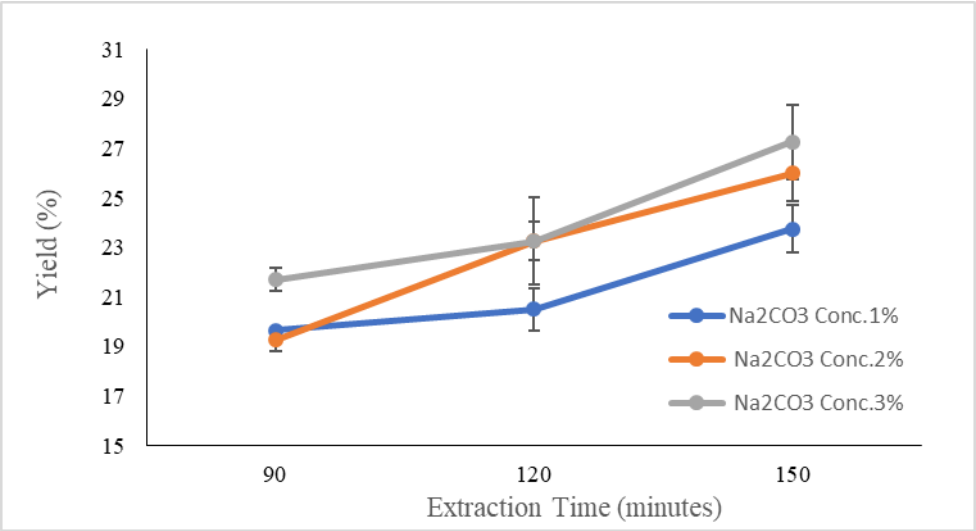

Figure2. Alginate yield obtained from various extractions time and Na_2CO_3 concentrations.

On the other hand, the longer extraction times of alginate by 1%, 2% and 3% Na_2CO_3 gave positive effect to the alginate yield. This might be due to the more seaweed cells wall broken, which facilitated the alginate extraction [11]. Furthermore, the softening of algal tissue at high extraction temperature may increase the solubility of alginates and improves the diffusion rate of Na_2CO_3, thus giving a higher extraction yield [12]. The industrial standard of alginate extraction yield based on Food Chemical Codex (FCC) is 18% [20]. Therefore, the alginates yield obtained from all variables had fulfill the FCC standard.

3.2. Alginate Viscosity

Alginate can be characterized from its viscosity in the form of a solution. Viscosity of alginate is affected by gel strength, temperature, alginate concentration, alginate molecular weight, and the ratio of units M and G from alginate molecules [13]. An increase in the proportion of G units produces more viscous alginate solution. Furthermore, ratios of M and G units also affect the rate of formation of hydrogels its characteristics. Alginate which contains high manuronic acid (M units) the has a non-

rigid structure and more flexibility, whereas alginate which contains high guluronic acid (G units) tends to have rigid structure and high porosity [14].

Alginate viscosity obtained from various extraction time and Na_2CO_3 concentration is presented in Figure3. The viscosity of alginates extracted by 1% Na_2CO_3 for 90, 120 and 150 minutes were 82.15 cP, 67.60 cP and 31.65 cP; the viscosity of alginates extracted by 2% Na_2CO_3 for 90, 120 and 150 minutes were 84.12 cP, 78.45 cP and 46.66 cP; and the viscosity of alginates extracted by 3% Na_2CO_3 for 90, 120 and 150 minutes 165.50 cP, 82.25cP and 61.40 cP, respectively. However, the overall viscosity of alginates extracted by 1%, 2% and 3% Na_2CO_3 was decreased by longer extraction time. This phenomenon was similar with previous research [12][15]. At high temperature, longer extraction time will increase the yield, but decrease the viscosity of the alginate. This is due to the depolymerization of the polymeric chain of alginate [9]. Alginate molecular weight is one of the parameters which affect the alginate viscosity. Alginates with higher viscosity has higher molecular weight compared to alginates with lower viscosity [8]. Therefore, depolymerization of alginate polymeric chain will decrease the molecular weight of alginate and decrease the viscosity.

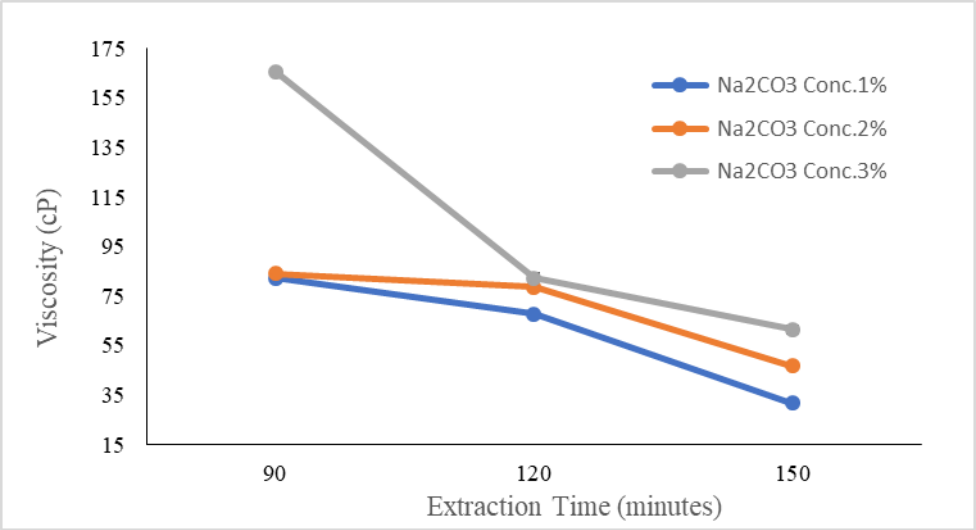

Figure3. Alginate viscosity obtained from various extraction times and Na_2CO_3 concentrations.

Based on the viscosity, alginate is classified into 3 levels, i.e. low viscosity (<60 cP), medium viscosity (60-110 cP) and high viscosity (110-800 cP) [7]. Therefore, this research generated alginates with low, medium and high viscosity. The highest viscosity in this researchwas obtained by alginate extraction using 3% Na_2CO_3 for 90 minutes. Alginates with high viscosity are used to obtain more resistant gels and applicable for food and cosmetic industries, whereas alginates with low viscosity are more useful to obtained polyelectrolite complexes, for the paper industries, or for the production of micro- and nano-particles of drugs [15]. The specific viscosity for certain applications can be achieved by choosing certain viscosity grades of alginates and/or by adjusting the alginate concentration. Increasing alginate concentration will increase the viscosity of the solution. Another method is by adding a small amount of calcium salt into the solution, by cross-linking reaction of alginate molecules to increase the molecular weight and viscosity [16].

3.3. Alginate pH

The data of alginate pH obtained from various extraction time and Na_2CO_3 concentration is describedin Table 1. The pH of alginates extracted was in the range of 7.55-8.05, whereas the alginate

pH standard based on FCC was in the range of 3.5-10 [20]. Therefore, all of the alginate extracted had fulfilled the FCC standard of pH. The pH beyond the range will affect alginate solution. At higher pH (>10), the alginate is not stable due to the degradation occurs by a mechanism of β-elimination, whereas at lower pH (<3.5), the sodium alginate will be precipitated [17][18].

Table 1. Alginate pH obtaned from various extraction time and Na_2CO_3 concentrations.

Na_2CO_3 concentrations	Extraction time (min)		
	90	120	150
1	7.65	7.95	8.05
2	8	7.9	8
3	7.55	7.60	7.85

3.4. Alginate Water Content

Water content can affect storability of food product. The food product which have low water content usually have longer shelf life compared to the product which have high water content. The data of alginate water content obtained from various extraction time and Na_2CO_3 concentration is presented inTable 2.The water content of alginates extracted was in the range of 7.5-12.25%, whereas the alginate water content based on FCC was below 15% [20]. Therefore, all of the alginate extracted had fulfilled the FCC standard of water content.

Table 2. Alginate water content obtaned from various extraction time and Na_2CO_3 concentrations.

Na_2CO_3 concentrations	Extraction time (min)		
	90	120	150
1	11.5	11.9	12.25
2	9.5	11	12.25
3	7.5	11	12.25

The water content of the alginates extracted in this research was relatively low compared to water content of alginate from previous studies [22][23] by the same acid extraction method. This was due to the usage of isopropanol rather than ethanol for alginate precipitation process. Previous study showed that isopropanol had better performance to precipitate alginate compared to ethanol. Moreover, at higher concentration of isopropanol, the number of hydroxyl groups is increased, thus have stronger bond to the water [24].

4. Conclusions

This research was conducted to investigate the effect of Na_2CO_3 concentration and extraction time towards the characteristics of alginate extracted from *Sargassum* sp. by acid method. The experiments were performed by using 1%, 2% and 3% of Na_2CO_3 concentrations and 90, 120 and 150 minutes of extraction times. Increasing time and concentration of Na_2CO_3 had positive effects on yield of alginate, due to the improvement of Na_2CO_3 diffusion rate. However, the viscosity of alginate was decreased at longer extraction time. This was due to the depolymerization of the polymeric chain of alginate. The alginates extracted from various Na_2CO_3 concentration and extraction time generated alginates with low, medium and high viscosity. Furthermore, the yield, pH and water content of alginates extracted from this research had fulfilled the standard of Food Chemical Codex.

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