

# Research Journal of Pharmaceutical, Biological and Chemical Sciences

## Effects Of Sodium Carbonate Concentration And Temperature On The Yield And Quality Characteristics Of Alginate Extracted From Sargassum Sp.

Syharuddin Kasim\*, Asnah Marzuki, and Sumarheni Sudir.

Faculty of Pharmacy, Hasanuddin University, Makassar, Indonesia 90245.

### ABSTRACT

Individual effects of sodium carbonate concentration and temperature on the yield and quality characteristics of Alginate extracted from brown seaweed *Sargassum* sp. had been studied previously, but their combined effects have not been reported. This study investigated the effect of  $\text{Na}_2\text{CO}_3$  concentration (2%, 4%, 6% w/v) in combination with extraction temperature (50 °C, 60 °C, 70 °C) on the yield and Alginate physical-chemical properties including moisture content, pH, and viscosity. *Sargassum* sp. collected from coastal area of Takalar, South Sulawesi of Indonesia was extracted either by acid or calcium methods to isolate the Alginate sodium using those various concentration of  $\text{Na}_2\text{CO}_3$  and temperature. The result showed that the effect of different  $\text{Na}_2\text{CO}_3$  concentration by different temperature treatment was less significant toward alginate quality. The highest yields of alginate were obtained at the extraction process of 60 °C with an addition of 2% w/v  $\text{Na}_2\text{CO}_3$  both by the acid method (35.17%±4.1) and calcium method (36.55%±0.06). Identification of extracted alginate by chemical reagents showed the positive results and identification using FT-IR indicate an identical spectrum between extracted alginate and the standard.

**Keywords:** Alginate, *Sargassum* sp., sodium carbonate, temperature

*\*Corresponding author*

## INTRODUCTION

South Sulawesi, one of the provinces in Indonesia, is known to have large number of freely lived brown seaweed including *Sargassum* sp.(Phaeophyceae) on its ocean [1]. *Sargassum* sp. widely used as a source of alginate sodium production due to their high content of alginate compare to *Turbinaria* sp [2]. Alginate is widely used in the pharmaceutical industry in the form of sodium alginate for oral and topical products due to its thickening properties to form gel or coating film [3].

Alginate extracted from *Sargassum* sp vary depends on several factors such as environmental conditions of cultivation and extraction process [4]. Generally, Alginate from *Sargassum* sp was extracted using acid or calcium method with various types of solvent and condition. Different concentrations of  $\text{Na}_2\text{CO}_3$  used during extraction process have been found to affect the chemical composition and physical characteristics of alginate. Similarly, the extraction temperature gave a significant influence on the yield and characteristics of Alginate. Putrison (2013) studies proved that the concentration of  $\text{Na}_2\text{CO}_3$  gave a significant effect on the yield of extracted Alginate sodium whereas the highest yield obtained using 6% of  $\text{Na}_2\text{CO}_3$  [5]. Furthermore, Pamungkas T.A et al (2013) investigated that an increase in temperature simultantly increased the yield of alginate extracted although the water content and viscosity value of the alginate decreased [6]. There were evidences available reported that the extraction temperature of 60°C was better than 40°C, 50°C and 70°C for the extractability and quality of extracted sodium alginate [7]. However, the influence of the combination of  $\text{Na}_2\text{CO}_3$  level at a range of temperatures (40, 50, 60 and 70°C) on the yield and physicochemical properties of the alginate has not evaluated yet. Therefore, the main objective of this present work was to examine the effect of extraction conditions ( $\text{Na}_2\text{CO}_3$  concentration and extraction temperature) in extracting alginate sodium compound from *Sargassum* sp. by measuring the yield and some Quality parameters i.e moisture content, viscosity and pH.

## MATERIALS AND METHODS

### Seaweed material

Brown seaweed *Sargassum* sp. was harvested/collected from coastal area of Takalar district, South Sulawesi, Indonesia and washed thoroughly with freshwater to remove epiphytes and salt. *Sargassum* sp. were dried on direct sunlight for 2 days and placed in oven at 60 °C until the moisture content were below 10%. Then they were stored at 18°C until further analysis.

### Acid method of alginate extraction

This research was conducted at the Laboratory of Pharmaceutical Chemistry and Biofarmaka Laboratories Research Centre of Hasanuddin University, Makassar, Indonesia. Alginate extraction used general method as describe elsewhere [9] with some modification. Shortly, 10 g of dried seaweeds were ground then soaked in 300 ml HCl 0.5 N for 1 hour. Furthermore, they were washed with running water until the pH became neutral. Extraction used solution of  $\text{Na}_2\text{CO}_3$  2%, 4%, 6% (1:30) w/v and were heated at 50°C, 60°C, 70°C for 1.5 hrs on a magnetic stirrer. The extract was filtered then the filtrate was immersed in NaOCl 4% at 10% of the volume of the filtrate for 15 minutes. Further, 10% HCl was added until the formation of alginic acid. Alginic acid was separated and washed. Gel shaped of Alginic acid were added 10% NaOH to reach pH of 8-9 for sodium alginate formation. Sodium alginate on the mixture was separated using isopropyl alcohol (2:1) with constant stirring to form the fiber shape. The fibers are then dried in an oven at a temperature of 48°C for four days. Once dried, the fibers were ground and sieved with 60 mesh to obtain sodium alginate powder.

### Calcium method of alginate extraction

Alginate extraction used general method as describe elsewhere [9] with some modification. Shortly, 10 g of dried seaweeds were ground then soaked in 300 ml HCl 0.5 N for 1 hour. Furthermore, they were washed with running water until the pH became neutral. Extraction used solution of  $\text{Na}_2\text{CO}_3$  2%, 4%, 6% (1:30) w/v and were heated at 50°C, 60°C, 70°C for 1.5 hrs on a magnetic stirrer. Then filtered by the filter cloth and the filtrate was taken. The filtrate was put into a solution of 10%  $\text{CaCl}_2$  to form a precipitated calcium alginate while stirring. Calcium alginate formed is then filtered and washed with distilled water. Then sodium

hypochlorite (NaOCl) 4% of 100 mL was added to precipitate calcium alginate for 30 minutes and then filtered. Calcium Alginate was added HCl 10% slowly while stirring until alginic acid obtained characterized by deposition in the form of gel. When the conversion to alginic acid, pH was adjusted to less than 2. The precipitated alginic acid was then separated and washed. Alginic acid were added Sodium Carbonate ( $\text{Na}_2\text{CO}_3$ ), stirring until pH <9. After reaching a pH <9, then Isopropanol 95% was added to obtain a fiber Sodium Alginate while stirring until no more precipitate formed. Sodium alginate precipitate was then filtered and dried in an oven at temperature of  $\pm 50^\circ\text{C}$  until dried. Further, sodium alginate was blended to obtain Sodium Alginate powder.

### Quality Testing of Sodium Alginate

The yield of sodium alginate obtained from the extraction method of calcium and acid method were quantified. The identification of extracted alginate was tested with chemical reagents and IR spectrophotometry (Shimadzu® IRPrestige-21 FTIR-8400S). The quality properties was measured in term of moisture content (Memmert®), pH (Sartorius®), and viscosity (Viscometer Brookfield VM-BF-RV-01).

### Experimental Analysis

Data obtained from variations in temperature extraction and concentration of  $\text{Na}_2\text{CO}_3$  were statistically analyzed using two way ANOVA repeated measurement (GraphPad Prism 5.0, GraphPad Software, La Julla, California). Data are presented as mean  $\pm$  SD. P value <0.05 indicates significance.

## RESULTS AND DISCUSSION

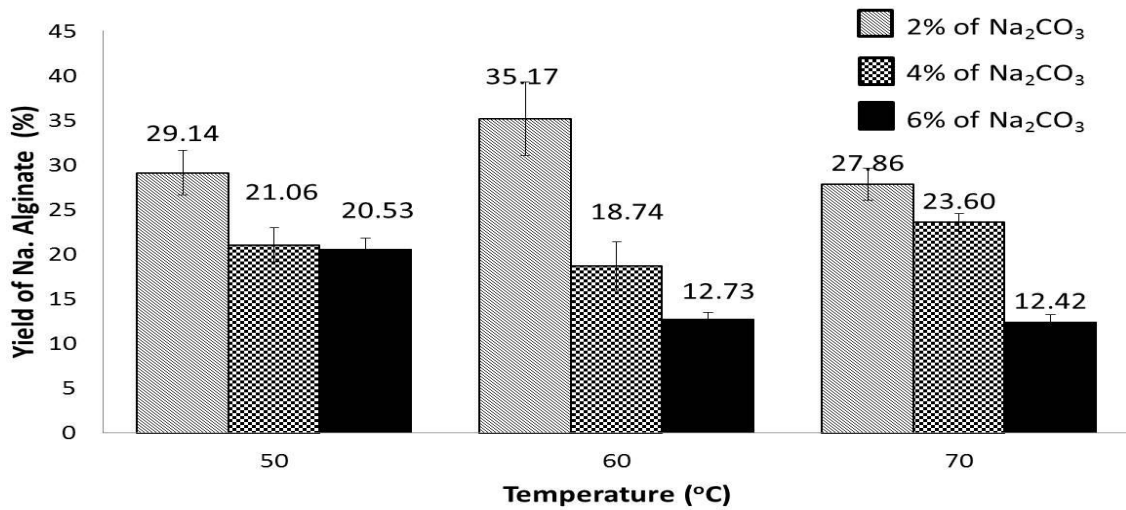
In this study, the highest yield value was obtained using acid method at a temperature of  $60^\circ\text{C}$  and concentration sodium carbonate of 2% was  $35.17\% \pm 4.1$  while the calcium method obtained  $36.55\% \pm 0.06$  of Na. Alginate (graph.1 and 2). The color of Na. alginate powder obtained were brownish yellow to brown. The water content generated in this study meets the standards by which the alginate water content <15%. The amount of moisture is allowed in the sodium alginate ranges between 5% - 20% The color and moisture content of Na. alginate were in compliance with industrial and pharmaceutical grade specifications.

In general, there was a positive correlation between high temperature of extraction and the high yield of Na. alginate produced. However, at some point of high temperature, the degradation of the alginate structure decreases the yield. This result support the study by Jayanuddin et al (2014), who obtained the greatest yield of sodium alginate used acid extraction method were at temperature of  $60^\circ\text{C}$  compare to  $50^\circ\text{C}$  and  $70^\circ\text{C}$  [10]. In contrast with temperature, higher concentration of  $\text{Na}_2\text{CO}_3$  used produced lower yield value of sodium alginate extracted. Chou and Chiang (1976) in Haerunnisa (2008) suggested that high concentrations of  $\text{Na}_2\text{CO}_3$  (3% to 5%) reduced yield and viscosity of Na. alginate because the polymer chains of alginic acid degrades into oligosaccharides [11,12]. In addition, pectin, the adhesive material between the cell walls of brown seaweed, is unstable in alkali solution. It creates a network in the cells of *Sargassum* sp. to facilitate alginate release. Therefore,  $\text{Na}_2\text{CO}_3$  is a specific solvent to extract alginates from brown seaweed. In this study, the effect of temperature extraction and concentration of sodium carbonate did not significantly affect the yield of Na. alginate. But the trend of the graph showed a difference in the group of  $\text{Na}_2\text{CO}_3$  concentration of 2% to 6%. Therefore, the Independent T Test was analyse to see any significant mean difference between the two groups in this case the average yield on  $\text{Na}_2\text{CO}_3$  concentration of 2% to 6%. The results indicates the yield (% w / w) concentration of  $\text{Na}_2\text{CO}_3$  2% vs 6%, has a significant difference (Graph. 2 & 4).

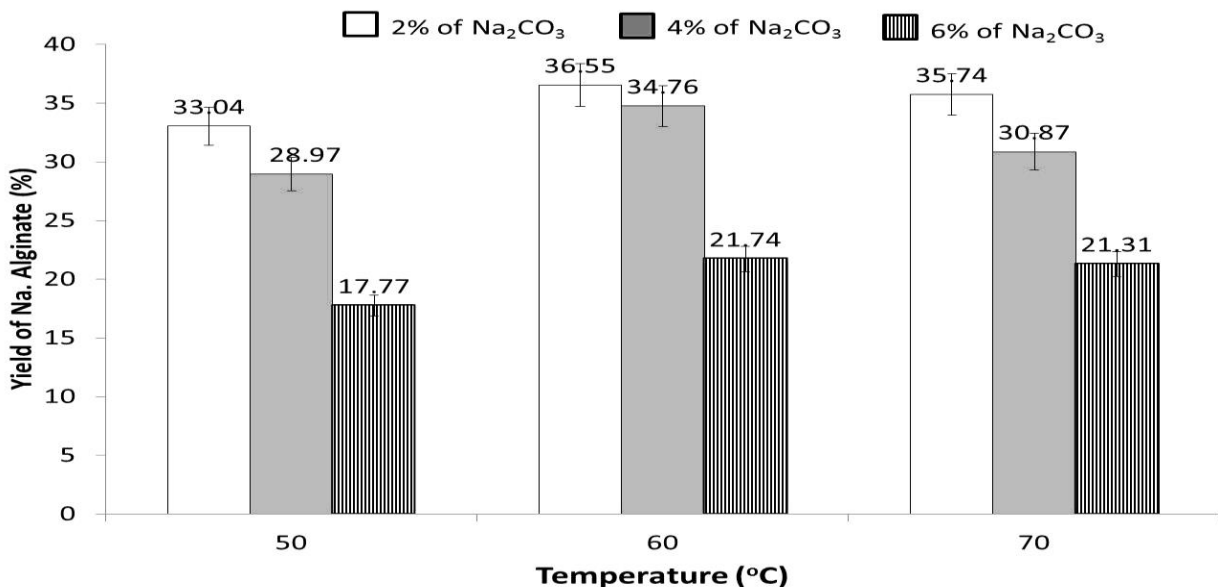
The results showed viscosity values of variation  $\text{Na}_2\text{CO}_3$  concentration of 2%, 4% and 6% (w / v) at a temperature of  $50^\circ\text{C}$  and  $60^\circ\text{C}$  extraction was 16 cP, while the concentration of 4%  $\text{Na}_2\text{CO}_3$  at  $60^\circ\text{C}$  was 18 cP. Temperature  $70^\circ\text{C}$  in combine with  $\text{Na}_2\text{CO}_3$  concentration of 4% and 6% showed viscosity of 20 cP value except at 2%  $\text{Na}_2\text{CO}_3$  concentration which resulted 16 cP. Based on the results, there was no significant effect of extraction temperature and  $\text{Na}_2\text{CO}_3$  to the viscosity grades of sodium alginate. According to McHugh (1987), the length of the polymer chain determines the quality of alginate. The longer the chain is concomitantly resulting greater molecular weight and its viscosity value. The higher the temperature of extraction process decreases the viscosity as the result of long chain polymers. These results were supported by Chou and Ciang

(1976) which states that *Sargassum* sp. provides products of alginate with low viscosity. However, the viscosity value still meet the specification of alginate in which 1% solution had a viscosity of 10-500 cP [13,15].

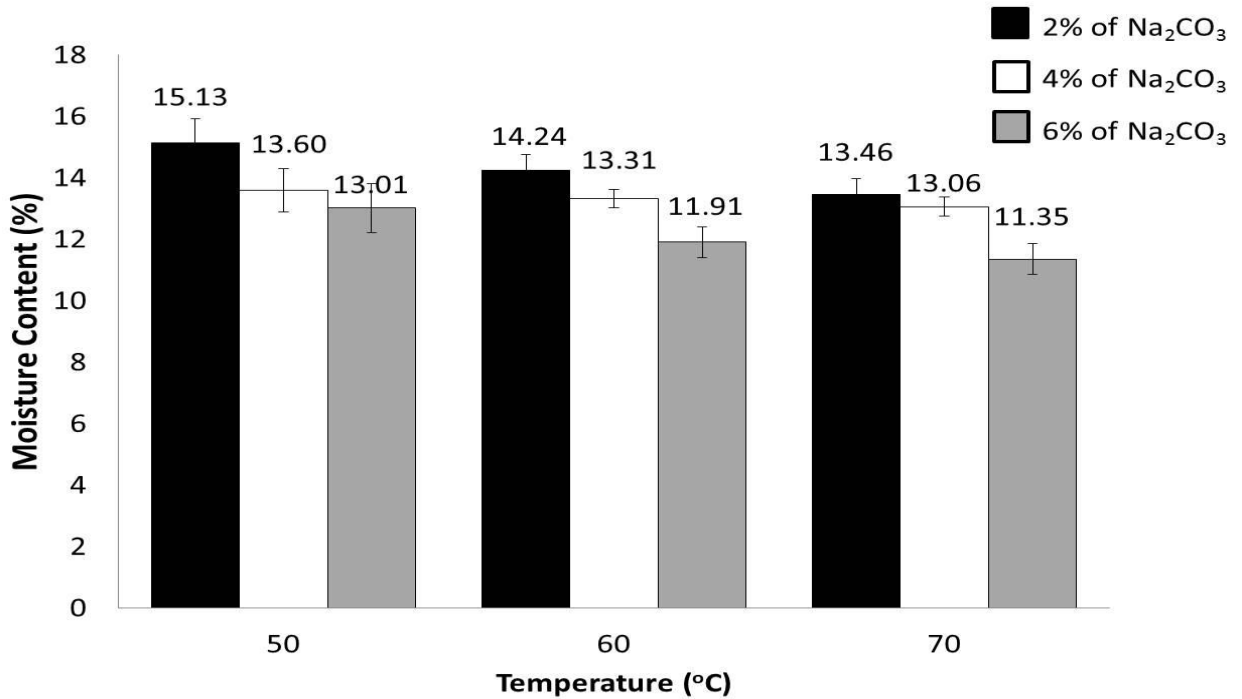
Based on the research results that pH of 7.75 to 8.59 obtained at 1% solution of Na.alginate is suitable for industrial and pharmaceutical grade (pH 3.5 to 10). pH of the current alginate apparently was influenced by the addition of sodium hydroxide during the process of conversion of alginic acid to sodium alginate. Although graph showed different pattern (Graph. 7 & 8), but the statistic analysis showed that the temperature and the sodium carbonate concentration did not affect the pH of Na. alginate.



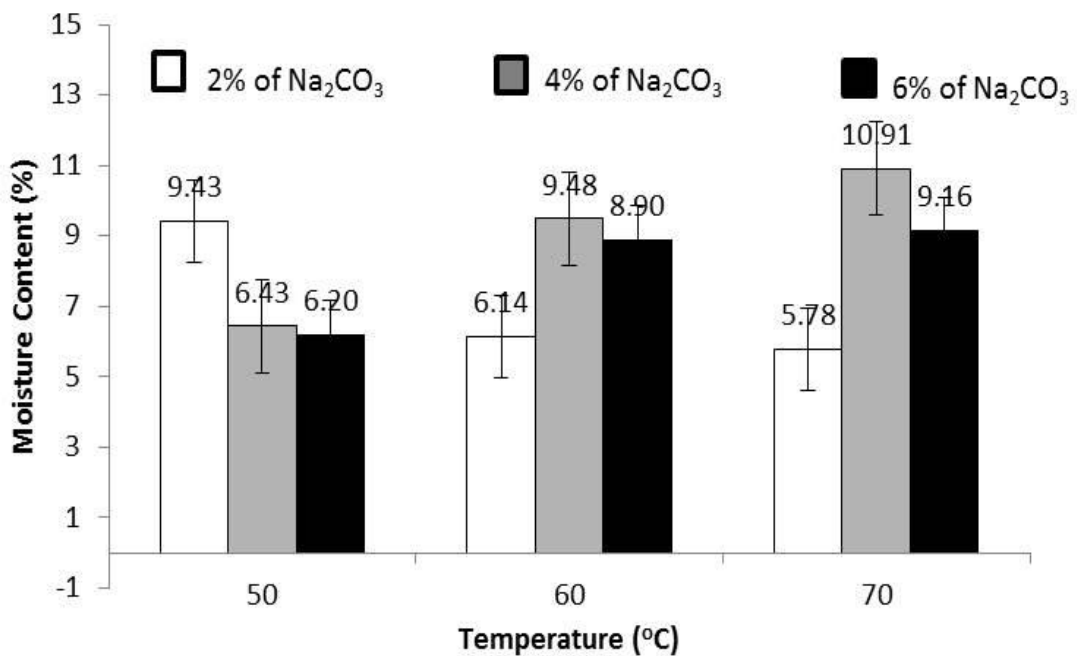
Graphic 1. The effect of temperature and Na<sub>2</sub>CO<sub>3</sub> Concentration to the yield value of Na. alginate extracted using acid method



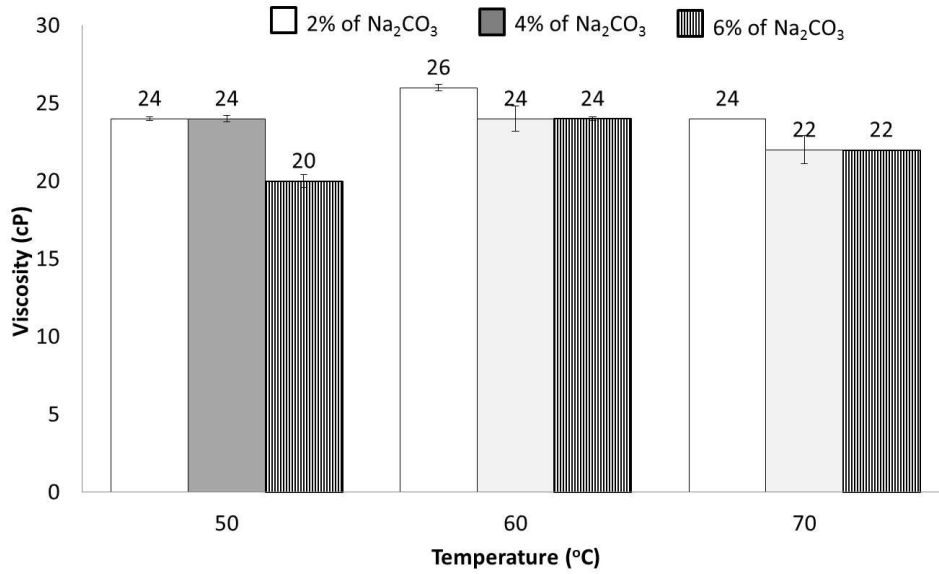
Graphic 2. The effect of temperature and Na<sub>2</sub>CO<sub>3</sub> Concentration to the yield value of Na. alginate extracted using calcium method



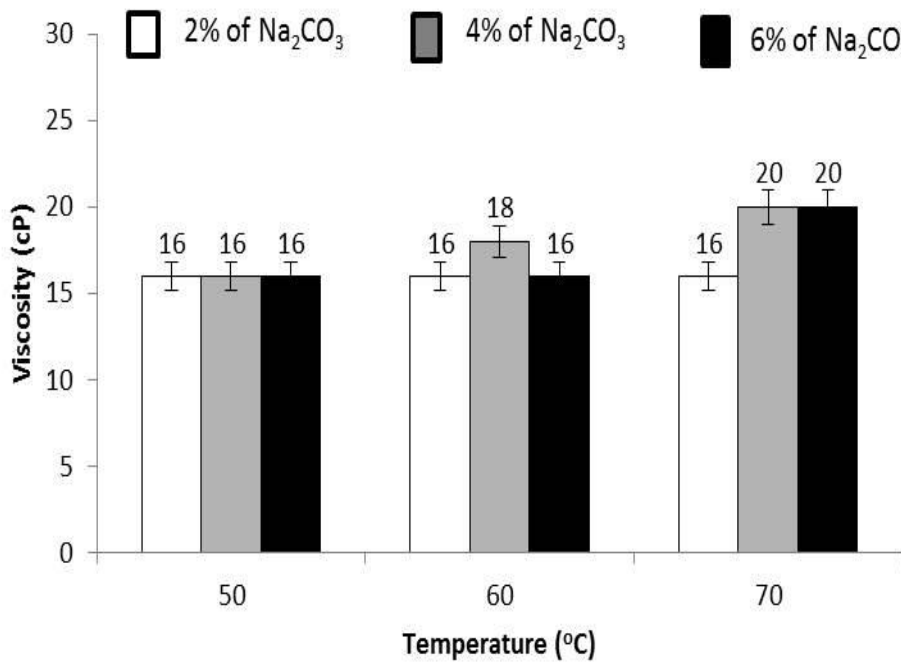
Graphic 3. The effect of temperature and Na<sub>2</sub>CO<sub>3</sub> Concentration to the Moisture content of Na. alginate extracted using acid method



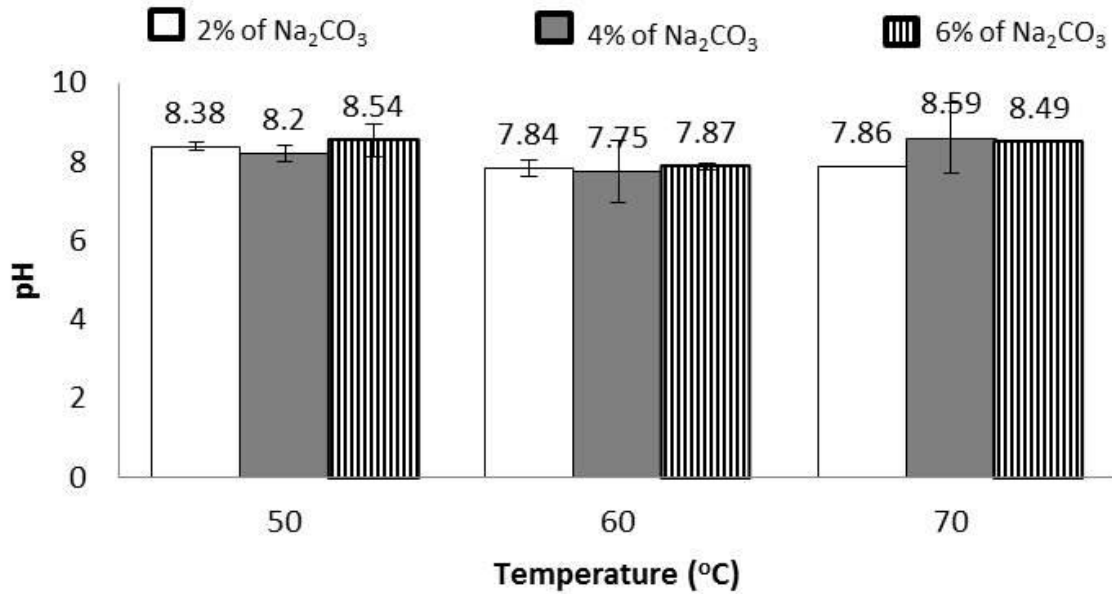
Graphic 4. The effect of temperature and Na<sub>2</sub>CO<sub>3</sub> Concentration to the Moisture content of Na. alginate extracted using calcium method



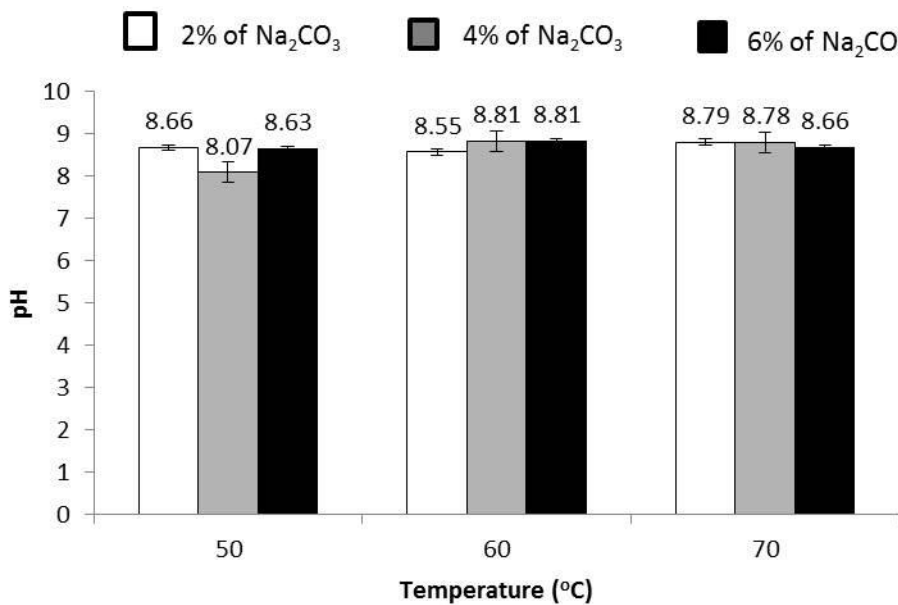
Graphic 5. The effect of temperature and Na<sub>2</sub>CO<sub>3</sub> Concentration to the Viscosity of Na. alginate extracted using acid method



Graphic 6. The effect of temperature and Na<sub>2</sub>CO<sub>3</sub> Concentration to the Viscosity of Na. alginate extracted using calcium method



Graphic 7. The effect of temperature and Na<sub>2</sub>CO<sub>3</sub> Concentration to the pH of Na. alginate extracted using acid method



Graphic 8. The effect of temperature and Na<sub>2</sub>CO<sub>3</sub> Concentration to the pH of Na. alginate extracted using calcium method

### CONCLUSIONS

- Sodium carbonate concentration had a significant effect on yield value and moisture content but had not significant effect on pH and viscosity while the extraction temperature had significant effect on water levels, but had not significant effect on yield value, pH, and viscosity of Na. alginate extracted from Sargassum sp..
- The best treatment to produce yield value and optimum viscosity is at the temperature of 60°C with concentration sodium carbonate of 2%.

## ACKNOWLEDGEMENTS

The authors are grateful to Dian **Megawati** and **Nurlislamia Zubaidah** for their great support on this research. This work used the facilities of Biofarmaka Unhas. Therefore our most appreciation to the Dean of Faculty of Pharmacy, Hasanuddin University who made this work possible.

## REFERENCES

- [1] Sofia, S.H. 16 April, 2015. Kebanggaan semu menjadi eksportir rumput laut mentah. AntaraNews [Online]. Tersedia: <http://www.antaraneews.com>. [Januari 2016].
- [2] Pemerintah Provinsi Sulawesi Selatan. 17 April, 2013. Produksi Rumput Laut Sulsel Tertinggi di Indonesia. [Online], <http://sulselprov.go.id>. Diakses pada tanggal 12 Januari 2016.
- [3] Anggariredja, J.T., Achmad, Z., Heri, P., dan Sri, I. Rumput Laut. Penebar Swadaya. Depok. 2009. Halaman 5, 6, 8, 67, 68.
- [4] Basmal, J., Tazwir, Murdinah, Thamrin, W., Bagus, S.B.U., Endar, M., dan Rinta, K. Membuat alginat dari rumput laut sargassum sp.. Penebar swadaya. Jakarta. 2013. Halaman 35-44.
- [5] Aslan, Laode M. Budidaya Rumput Laut. Penerbit Kanisius. Yogyakarta. 2006.
- [6] Rowe, R.C., Sheskey, P.J., dan Quin, M.E. Handbook of Pharmaceutical Excipient, 6th Edition. Pharmaceutical Press. London. 2009. Hal. 622
- [7] Kartini, Z., Tri, S., dan BW, S. Ekstraksi Dan Pemurnian Alginat dari Sargassum Filipendula - Kajian dari Bagian Tanaman, Lama Ekstraksi dan Konsentrasi Isopropanol. Jurnal Teknologi Pertanian. 2001 Vol. 2, No. 1.
- [8] Tambunan, A.P.M. Pengaruh Konsentrasi  $\text{Na}_2\text{CO}_3$  Terhadap Rendemen Natrium Alginat dari Sargassum cristaeifolium Asal Perairan Lemukutan. 2013. JKK, Volume 2(2), Halaman 112- 117.
- [9] Pamungkas, T.A., Ali, R., dan Sunaryo. Pengaruh Suhu Ekstraksi Terhadap Kualitas Natrium Alginat Rumput Laut Sargassum sp. 2013. Journal Of Marine Research. Volume 2, Nomor 3, Halaman 78-84.
- [10] Jayanudin, Lestari, A.Z., dan Nurbayanti, F. Pengaruh Suhu Dan Rasio Pelarut Ekstraksi Terhadap Rendemen Dan Viskositas Natrium Alginat Dari Rumput Laut Cokelat (Sargassum Sp). Jurnal Integrasi Proses. 2014. Vol. 5, No. 1, Halaman 51 – 55.
- [11] Yunizal. Teknologi Pengolahan Alginat. Pusat Riset Pengolahan dan Sosial Ekonomi Kelautan dan perikanan. Jakarta. 2004.
- [12] McHugh, D.J. Production and Utilization of Product from Commercial Seaweed [monograph on internet] Rome. FAO. Fisheries Technical Paper 288. Food and Agriculture Organization of The United Nations. 1987, [Accessed 19 Juni 2016]. Available from <http://www.fao.org/docrep/X5822E/x5822e04.htm>
- [13] Chou, H.N dan Y.M. Chiang Studies on Algin from Brown Algae of Taiwan. I. Estimation of Yield and quality of Algin. Acta Oceanographica Taiwanica. 1976. No. 6. 134-13
- [14] Food Chemical Codex. Second Supplement to the third Edition. Committee of Food Chemical Codex. National Academy Press. Washington DC. 1981.
- [15] Winarno F.G. Teknologi Pengolahan Rumput Laut. Pustaka Sinar Harapan. Jakarta. 1996.
- [16] Anonim. <http://www.ariancommerce.net/eimg/COA%20for%20Food%20-%20SODIUM%20ALGINATE.pdf>. Diakses pada tanggal 3 Juli 2016.
- [17] Moe ST, Draget KI, Skjak-Braek, dan Smidsrod O. Alginates. Dalam: A.M. Stephen (Ed 2). Food Polysaccharides and Their Applications. Marcell Dekker Inc. New York. 1996
- [18] Haerunnisa. Analisa Kualitas dan Formulasi Alginat Hasil Ekstraksi Sargassum filipendula Untuk Pembuatan Minuman Suplemen Serat Dalam Bentuk Effervescent. [Skripsi]. Jakarta. Program Studi Kimia Fakultas Sains dan Teknologi. UIN Syarif Hidayatullah. 55 halaman. 2008.
- [19] Eriningsih R, Rini M, Theresia M, Arif WS, Anna T. Eksplorasi Kandungan Pigmen dan Alginat dari Rumput Laut Coklat untuk Proses Pewarnaan Kain Sutera. Bandung: Balai Besar Tekstil. 2014.
- [20] 13. Jayanudin, Lestari, A.Z., dan Nurbayanti, F. Pengaruh Suhu Dan Rasio Pelarut Ekstraksi Terhadap Rendemen Dan Viskositas Natrium Alginat Dari Rumput Laut Cokelat (Sargassum Sp). Jurnal Integrasi Proses. 2014. Vol. 5, No. 1, Halaman 51 – 55.
- [21] Kordi, M.G. Kiat Sukses Budi Daya Rumput laut di Laut dan Tambak. ANDI. Yogyakarta. 2011. Halaman 37,49.
- [22] Yunizal. Teknologi Pengolahan Alginat. Pusat Riset Pengolahan dan Sosial Ekonomi Kelautan dan perikanan. Jakarta. 2004.
- [23] Junianto. Rendemen dan kualitas Alginat hasil ekstraksi Alga (Sargassum sp.) dari pantai selatan daerah





- Cidautan Barat. Fakultas Perikanan dan Ilmu Kelautan Universitas Padjadjaran Jatinangor. Bandung, 8 : 152-160. 2006.
- [24] Sartori, celine. The Characterisation Of Alginate Systel\Ns For Biomedical Applications . [Thesis]. Brunei University: Department of Materials Engineering. 1997.
- [25] McHugh, D.J. Production and Utilization of Product from Commercial Seaweed [monograph on internet] Rome. FAO. Fisheries Technical Paper 288. Food and Agriculture Organization of The United Nations. 1987, [Accessed 19 Juni 2016]. Available from <http://www.fao.org/docrep/X5822E/x5822e04.htm>
- [26] Sikorski, Z. E. Chemical and Functional Properties of Food Saccharides. London. CRC Press. 2004.
- [27] Anonim.[http://www.irochemical.com/product/Alginates/Sodium Alginate-I.htm](http://www.irochemical.com/product/Alginates/Sodium%20Alginate-I.htm). Diakses tanggal 16 06 2016
- [28] Chou, H.N dan Y.M.Chiang Studies on Algin from Brown Algae of Taiwan. I. Estimation of Yield and quality of Algin. Acta Oceanographica Taiwanica. 1976. No. 6. 134-13
- [29] Food Chemical Codex. Second Supplement to the third Edition. Committee of Food Chemical Codex. National Academy Press. Washington DC. 1981.
- [30] Winarno F.G. Teknologi Pengolahan Rumput Laut. Pustaka Sinar Harapan. Jakarta. 1996.
- [31] Anonim.<http://www.ariancommerce.net/eimg/COA%20for%20Food%20-20SODIUM%20ALGINATE.pdf>. Diakses pada tanggal 3 Juli 2016.