

PAPER • OPEN ACCESS

Preparation and characterization of semi-refined carrageenan from *Kappaphycus alvarezii* seaweed bleached by Peracetic Acid

To cite this article: Uju *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **278** 012077

View the [article online](#) for updates and enhancements.

Preparation and characterization of semi-refined carrageenan from *Kappaphycus alvarezii* seaweed bleached by Peracetic Acid

Uju^{1,2*}, E Prasetyaningsih¹, J Santoso¹, N Kamiya^{3,4} and T Oshima⁵

¹Department of Aquatic Product Technology, Bogor Agricultural University, Bogor, Indonesia

²Surfactant and Bioenergy Research Center, Bogor Agricultural University, Bogor, Indonesia

³Department of Applied Chemistry, Graduate School of Engineering, Kyushu University Motooka, Nishi-ku, Fukuoka, Japan

⁴Division of Biotechnology, Center for Future Chemistry, Kyushu University, Motooka, Nishi-ku, Fukuoka, Japan

⁵Advanced Business Development Division, Business Strategy Group, Mitsubishi Gas Chemical Company, Inc., Mitsubishi building, 5-2, Marunouchi 2-chome, Chiyoda-ku, Tokyo 100-8324, Japan

*E-mail: ujusadi@ipb.ac.id

Abstract. Semi-refined carrageenan (SRC) is one of the products from *Kappaphycus alvarezii*, which has the potential to be developed in Indonesia. However, unbleached seaweed will produce SRC with light brown colour due to natural seaweed pigments. Peracetic acid (PAA) is a strong oxidizing agent that has the potential to be used as a bleaching agent in the SRC production. This research aimed to study the effects of the PAA as a bleaching agent on the characteristics of SRC from *K. alvarezii*. Chopped seaweed was heated in 10% KOH at 80°C for 30 minutes. Bleaching was carried out at room temperature for 90 minutes using PAA at a concentration of 0.5%, 1.5%, and 2.5% (w/w) or using 1.5% (w/w) of sodium hypochlorite. Bleaching using PAA produced SRC whiter than without bleaching and bleaching using sodium hypochlorite. SRC brightness increased with increasing concentrations of PAA and while the yield reduced, as well as the viscosity, gel strength, sulfate content and ash content. The best concentration of PAA was 0.5%. At best, PAA concentration bleaching produced white SRC flour with a lightness value of 80.46±0.01; yield of 23.50%; viscosity 99.33 cP, gel strength 307.63 g/cm², sulfate 13.29% (w/w), ash 8.33% (w/w) and acid-insoluble ash 1.33% (w/w).

Keywords: bleaching, concentration, *Kappaphycus alvarezii*, peracetic acid, SRC

1. Introduction

Carrageenan is an important marine hydrocolloid obtained from red seaweed (Rhodophyta). Some species of red seaweed that produce carrageenan known as carrageenophyte were reported by some previous reports [1–3]. *Kappaphycus alvarezii*, with the commercial name cottonii, is a largely cultivated seaweed species in Indonesia [3, 4]. Since 2000, the global production of seaweed grew exponentially, and after 2006, Indonesia became the largest producer of carrageenophyte seaweed [3].



Carrageenan is composed on a disaccharide backbone of alternating 3-linked β -D-galactopyranose (G) and 4-linked α -D-galactopyranose (D). According to the position of sulfate/s (S) in the disaccharide repeating unit, cyclization of the D units forming an anhydro ring (DA), and presence of pyruvate (P) on G units, carrageenan are classified as kappa (κ), iota (ι) and lamda (λ) carrageenan. Kappa and iota carrageenans have capability to form gel whereas the iota carrageenan is characterized as only a thickener agent [1, 4]. Based on their characteristics, carrageenan is used as a gelling, thickening, stabilising, and water-binding agent in various food products, such as instant products, desserts, sauces, milk, yoghurt, and meats.

Based on the purity of carrageenan, the productions of carrageenan are grouped into two different methods [2], i.e. refined carrageenan (RC) and Semi Refined Carrageenan (SRC). The process of SRC is simpler than RC. RC is extracted into an aqueous solution followed by a filtration step to remove residues, whereas SRC is obtained by the soaking of seaweed in the alkali solution. Cleaned and washed seaweed as a raw material of SRC are heated in the hot alkali solution for a few hours. The hydroxide part of the reagent penetrates the seaweed and reduces the amount of sulfate in the carrageenan. As a result, the 3,6-AG and the gel strength of the carrageenan increase [2]. To obtain a white colour SRC, the alkali-treated seaweed is bleached with a solution of oxidation such as calcium or sodium hypochlorite.

One of the materials able to work as an oxidizing agent is peracetic acid (PAA). PAA is a strong oxidizing agent synthesized from hydrogen peroxide with acetic acid and sulfuric acid as a catalyst [7]. As a bleaching agent, PAA has some advantages such as more environmentally friendly, low corrosive, and more degradable [8]. The pre-treatment of seaweed waste in the carrageenan industry using PAA 1.9% was able to produce whiter waste [9]. To the best of our knowledge, there is limited information available on the use of PAA for SRC bleaching on the physicochemical properties of SRC. This research was conducted with the aim to study the effect of PAA concentration as a bleaching agent on the quality of SRC produced from *K. alvarezii* seaweed.

2. Materials and Methods

The main material used in the production of SRC in this study was *K. alvarezii* seaweed taken from CV Babad Serang Utara, Serang, Banten. Peracetic acid was obtained from PT. Peroksida Indonesia Pratama, Cikampek, Karawang Jawa Barat-Indonesia as a Branch of Mitsubishi Gas Chemical Company, Inc., Japan.

2.1. SRC preparation

K. alvarezii seaweed was washed with running water to remove some impurities such as sand and salt. The seaweed was dried and cut into a shorter size. The dried seaweed (50 g) was heated at 80 °C for 30 minutes in a 10% KOH solution. The ratio of seaweed to KOH was 1:10. The heated seaweed was soaked in distilled water for 4 hours then rinsed and soaked again in distilled water overnight. The process was repeated until the pH was around 7. The neutral SRC was bleached with PAA 0.5%, 1.5% or 2.5% (w/w). For control treatments, we used $\text{Ca}(\text{ClO})_2$ 1.5% (w/w) as a bleaching agent and SRC without bleaching process. The bleached SRC was neutralized with distilled water. The neutral SRC was dried using freezer dryer for 48 hours and it was characterized. The treatment steps and SRC characterizations are presented in figure 1.

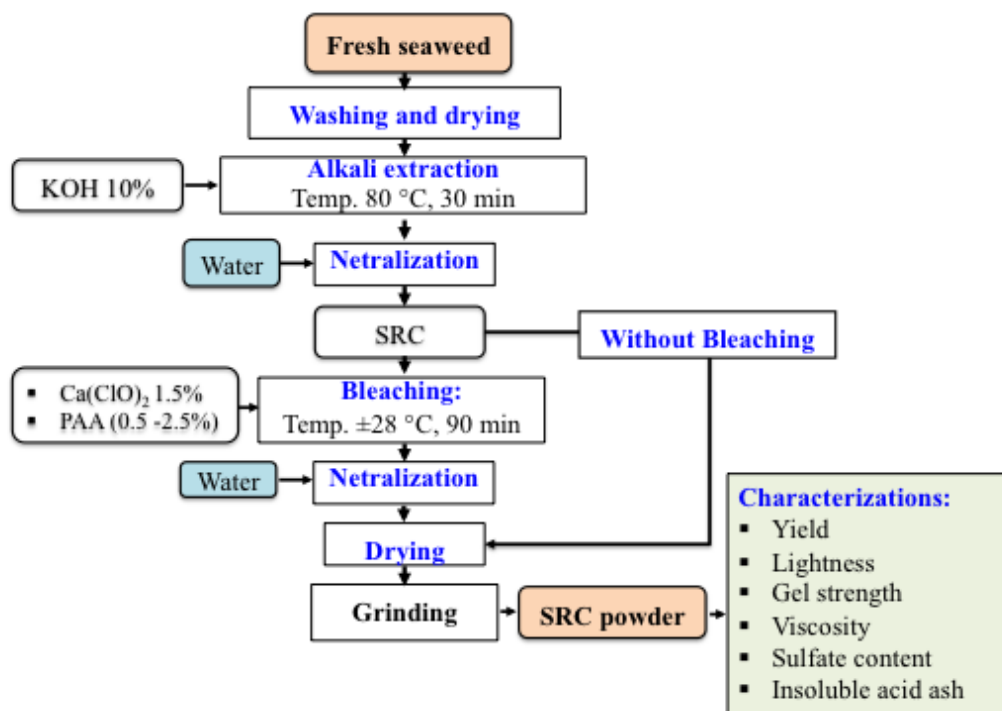


Figure 1. Treatment steps and characterization of seaweed and SRC powder.

2.2. Analysis of SRC

Colour measurement was done using the Chromameter CR-300 (Minolta Camera, Co. Japan). Before using this tool, chromameter was calibrated with white colour standard. Scanning of a sample will obtain the data L , a^* and b^* which known as Hunter colour notation. The a^* notation states the chromatic colour of the red-green mixture with a $+a$ (positive) value from 0 to +100 for red and a $-a$ (negative) from 0 to -80 for green. The b^* notation states the blue-yellow mixture chromatic colour with the $+b$ (positive) value from 0 to +70 for yellow and $-b$ (negative) from 0 to -80 for blue. The L (Lightness) notation ranges from 0 (black) to 100 (white). The formula to calculate L is as follows:

$$L(\%) = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \quad (1)$$

Yield of SRC is calculated as a ratio of SRC produced by the initial dried seaweed. The gel strength [10], viscosity and sulfate content [11], ash content [12], and acid insoluble ash content [13] were analyzed.

2.3. Statistical analysis

All data experiments were carried out in triplicates and the measurements of data were presented as a mean and standard deviation. To evaluate the effect of treatments of parameter, the data were analyzed by analysis of variance (ANOVA). Significant differences at $P < 0.05$ between the treatments were determined by Duncan's analysis for multiple comparison tests.

3. Results and Discussion

3.1. Colour of SRC

The colour of SRC without bleaching treatment was a mustard yellow. The colour was similar with the fresh seaweed (figure 1a). Bleaching treatments using 1.5% calcium hypochlorite ($\text{Ca}(\text{ClO})_2$) and PAA could produce SRC with whiter colours. Furthermore, we also measured the lightness of SRCs (figure 1b). It was clear that bleaching could significantly produce a lightness of SRC ($P < 0.05$). In addition, the lightness of SRC increased with the increase in PAA concentration. At lower

concentrations (0.5%), PAA bleaching produced higher lightness than that produced by 1.5% $\text{Ca}(\text{ClO})_2$.

K. alvarezii is a red seaweed which has a red colour pigment dominated by phycoerythrin. As a result, SRC without bleaching will produce a mustard colour SRC indicated by the colour pigment residues on the SRC. In addition, the mustard colour of SRC also was caused by some retained cellulose [14]. The bleaching process could remove the colour pigment of seaweed and, as a result, it produced a clean and white SRC. The use of blanching materials will damage the chromophore [15].

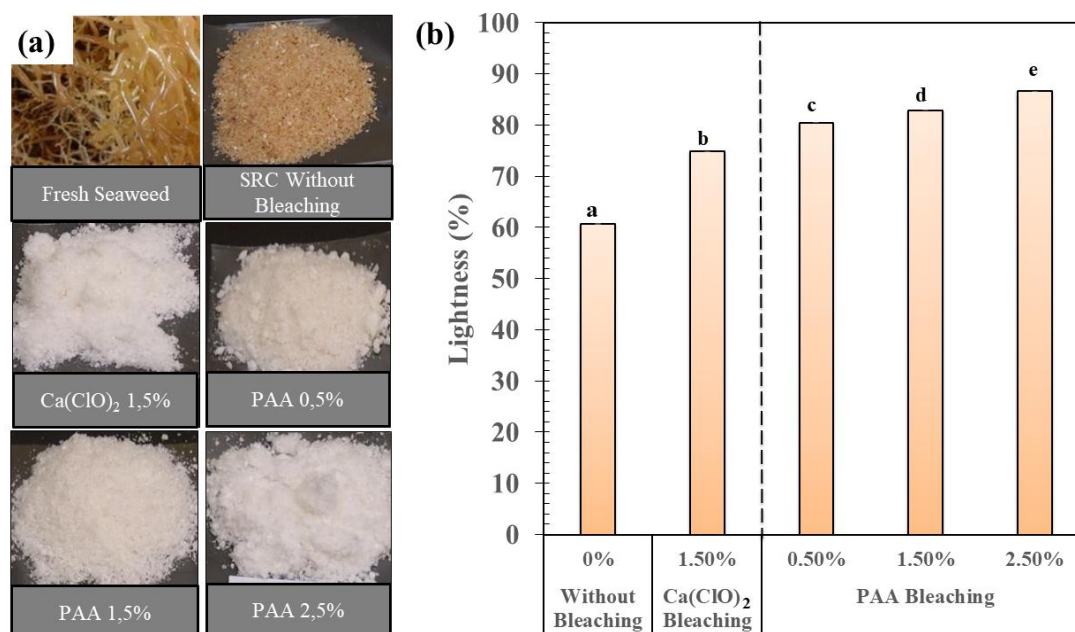


Figure 2. The effect of bleaching methods on the appearance of seaweed and dried SRCs (a), and SRC lightness (b). The different letters on the bar of chart indicated significant different ($P < 0.05$).

3.2. Yield of SRC

Yield is an indicator of the efficiency and effectivity of a method. The highest yield (55.02%) was obtained from SRC without bleaching. Yield of SRC bleached by 1.5% of $\text{Ca}(\text{ClO})_2$ was higher than 1.5% PAA ($P < 0.05$). Furthermore, increasing the PAA concentration produced lower SRC yield. This, presumably, was caused by the hydrolysis phenomenon. The hydrolysis causes the resulting SRC yield to be lower due to the loss of pigment and cellulose-carrying chromophore.

3.3. Physical and chemical characteristics of SRC

SRC without bleaching produced the highest gel strength (1122 g/cm²) and the lowest gel strength was obtained from the treatment using $\text{Ca}(\text{ClO})_2$ (table 1). The gel strength of SRC was higher than the gel strength of the SRC reported by a previous study [16]. In addition, the increase of PAA concentration caused the gel strength to decrease. The decrease of gel strength presumably was caused by the ability of PAA to depolymerize carrageenan [17]. Furthermore, the depolymerization effect increases as PAA concentration is increased.

Sulfate content is an important parameter of seaweed hydrocolloid. The reduction of sulfate content is expected to improve the gel strength of carrageenan [5]. As shown in table 1, the highest sulfate content was found at SRC without bleaching treatment and the lowest sulfate content was obtained from SRC bleached by $\text{Ca}(\text{ClO})_2$. In addition, sulfate content reduced with the increase of PAA concentration. The results indicated that $\text{Ca}(\text{ClO})_2$ and PAA could help remove sulfate content.

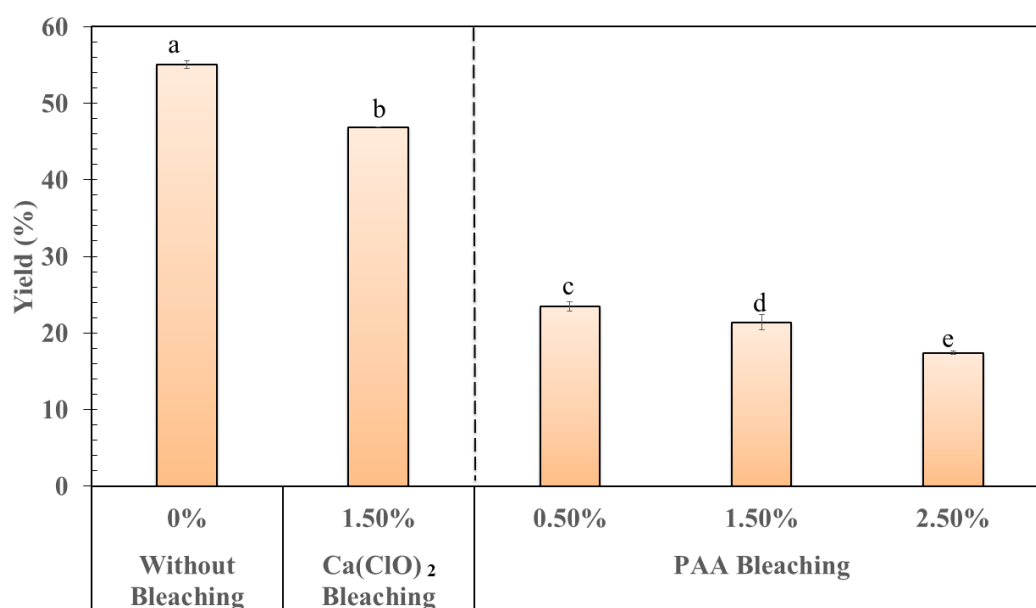


Figure 3. The effect of bleaching methods on yield of SRC. The different letters on the bar of chart indicated significant different at $P < 0.05$.

Table 1. Physical and chemical characteristics of SRC at various bleaching treatments.

Parameter	Without bleaching	Ca(ClO) ₂ bleaching	PAA bleaching		
	0%	1.5%	0.5	1.5%	2.5%
GS (g/cm ²)	1122±95 ^a	55±20 ^b	308±32 ^c	330±19 ^c	123±6 ^d
Sulfate (%)	15.91±0.04 ^a	8.59±0.07 ^b	13.29±0.17 ^c	12.35±0.38 ^d	10.98±0.34 ^e
Viscosity (cP)	249.0±22.2 ^a	12.56±2.34 ^b	99.3±11.3 ^c	71.8±5.8 ^d	70.00±2.7 ^d
Ash (%)	13.63±0.1 ^a	21.10±0.26 ^b	8.33±2.02 ^{bc}	10.12±0.09 ^c	7.84±0.52 ^d
AIA (%)	1.46± 0.37 ^{ab}	1.79±0.11 ^{ab}	1.33±0.44 ^{ab}	1.40±0.12 ^a	2.31± 0.51 ^b

Notes : GS = Gel strength

AIA = Acid insoluble ash

The superscript letters in the same row indicated significant different at $P < 0.05$.

Regarding the data on sulfate content and gel strength, we find that the opposite phenomenon occurred. In theory, SRC with lower sulfate content should produce a higher gel strength but in practice we obtained the opposite results. To explain this phenomenon, the viscosity of SRCs was checked. The trend data of gel strength and viscosity were identical. This phenomenon indicated that the values of gel strength was determined mainly by the length of the carrageenan chain, whereas the effect of reducing the sulfate content was too small compared to the length of the carrageenan chain. Furthermore, the reduction in the viscosity of SRC and increase in the PAA concentration is a strong evidence that PAA has the ability to depolymerize carrageenan.

The ash content and acid insoluble ash (AIA) are two important factors that indicate the purity of a carrageenan product. Ash content in carrageenan can derive from macro and micro minerals [18] which was absorbed or retained in seaweed as a material carrageenan. The total ash content of unbleached SRC was higher than the SRCs bleached by PAA. However, there were no significant effects in the differences ($P > 0.05$) among the PAA concentration bleaching. This indicated that PAA bleaching could reduce the ash content of *K. alvarezii* seaweed and the 0.5% of Ca(ClO)₂ was effective in the bleaching of SRC. In addition, SRC bleached by Ca(ClO)₂ had a higher concentration

of ash. This might presumably occur precipitated salt and could be removed mineral during processing of SRC.

AIA indicated the presence of contamination or impurities during the carrageenan processing, which might come from water or chemical residue. As can be seen in table 1, the value of AIA of bleached SRCs was not significantly different than that of unbleached SRC ($P>0.05$), except than that of SRCs bleached using 2.5% PAA. This indicates that a little residue of the bleaching agent was absorbed in the final SRC.

4. Conclusions

Peracetic acid (PAA) can be used as a bleaching agent in SRC processing. PAA bleaching improves the colour appearance and chemical characteristics of SRC. However, PAA bleaching may reduce the yield, gel strength, and viscosity of SRC. The best concentration of bleaching using PAA is 0.5%.

Acknowledgments

We would like to thank the Ministry of Research, Technology and Higher Education of the Republic of Indonesia for the financial support and we are also thankful for PT. Peroksida Indonesia Pratama, Cikampek, Karawang, Jawa Barat-Indonesia for the PAA reagent.

References

- [1] Van de Velde F and de Ruiter G A, 2002 "Carrageenan" in Biopolymers, A. Steinbuchel, S. de Baets, and E. J. VanDamme, Eds., " vol. 6 of Polysaccharides II: Polysaccharides from Eukaryotes (Weinheim: Wiley-VCH)
- [2] McHugh D J 2003 A guide to the seaweed industry. FAO Fisheries Technical Paper-T441 (Rome: FAO)
- [3] Valderrama D, Cai J, Hishamunda N and Ridler N 2013 Social and economic dimensions of carrageenan seaweed farming: a global synthesis. FAO Fisheries and Aquaculture Technical Paper No. 580 (Rome: FAO)
- [4] Bixler H J and Porse H, 2011 A decade of change in the seaweed hydrocolloids industry *J. Appl. Phycol.* **23** 321–335
- [5] Campo V L, Kawano D F, da Silva D B, and Carvalho I, 2009 Carrageenans: Biological properties, chemical modifications and structural analysis - A review, *Carbohydr. Polym.* **77** 167–180
- [6] Mehta S, Mody K H, Iyer A and Ghosh P K 2008 Preparation of semi-refined K-carrageenan: Recycling of alkali solution and recovery of alkali from spent liquor *Indian J. Chem. Technol.* **15** 45–52
- [7] Zhao X, Wang L and Liu D 2008 Peracetic acid pretreatment of sugarcane bagasse for enzymatic hydrolysis : a continued work *J. Chem. Technol. Biotechnol.* 956
- [8] Jolhe P D, Bhanvase B A, Patil V S and Sonawane S H 2015 Sonochemical synthesis of peracetic acid in a continuous flow micro-structured reactor *J. Chem. Eng.* **276** 91–96
- [9] Uju, Wijayanta A T, Goto M, and Kamiya N 2015 Great potency of seaweed waste biomass from the carrageenan industry for bioethanol production by peracetic acid–ionic liquid pretreatment *Biomass and Bioenergy* **81** 63–69
- [10] Food Marine Colloids Corp (FMC Corp) 1977 *Carrageenan. Marine colloid monograph number one*, (New Jersey (US): US Marine Colloids Division FMC Corporation)
- [11] Food and Agriculture Organisation (FAO) 2007 *Compendium of Food Additive Specification, FAO JECFA Monographs 4* (Rome: FAO)
- [12] Association of Official Analytical Chemists (AOAC) 2005 *Official Methods of Analysis of The Association of Chemist* (Virginia: AOAC)

- [13] Badan Standardisasi Nasional (BSN) 2010 *Cara Uji Kimia-Bagian 1: Penentuan Kadar Abu dan Abu Tak Larut Asam pada Produk Perikanan*. SNI 2354.1: 2010 (Jakarta: BSN)
- [14] Imeson A P 2000. *Handbook of Hydrocolloids: Carrageenan*, ed Philips GO and Williams PA (England: Woodhead Publishing Limited)
- [15] Baross D P, Silva V L, Hamalianen H and Colodette J L, 2010 Effect of last stage bleaching with peracetic acid on brightness development and properties of eucalyptus pulp *Bioresources* **5** 881-898
- [16] Bono A, Anisuzzaman S M and Ding O W, 2014 Effect of process conditions on the gel viscosity and gel strength of semi-refined carrageenan (SRC) produced from seaweed (*Kappaphycus alvarezii*) *Journal of King Saud University-Engineering Sciences* **26** 3-9
- [17] Sandria N, Uju and Suptijah P, 2017 The depolymerization of kappa carrageenan using peracetic acid *Jurnal Pengolahan Hasil Perikanan Indonesia*. **20** 524-535
- [18] Chan S W, Mirhosseini H, Taip F S, Ling, T C and Tan C P 2013 Comparative study on the physicochemical properties of κ -carrageenan extracted from *Kappaphycus alvarezii* (Doty) Doty ex Silva in Tawau, Sabah, Malaysia and commercial κ -carrageenans. *Food Hydrocolloids* **30** 581-588