International Journal of Food Science +Technology

Institute of Food Science +Technology ifst

Original article

An optimised low-salinity seawater decolourising method produces decolourised seaweed (*Kappaphycuz alvarezii*) as semi-refined carrageenan raw material:

Annur Ahadi Abdillah,[1,2] Mochammad Amin Alamsjah[2*] & Albert Linton Charles[1,2*]

1 Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, 1 Shuefu Road, Neipu Pingtung, 91201, Taiwan

2 Department of Marine, Faculty of Fisheries and Marine, Universitas Airlangga, Mulyorejo Surabaya, 60115, Indonesia

(Received 6 August 2020; Accepted in revised form 12 October 2020)

Abstract Semi-refined carrageenan (SRC) production from decolourised *Kappaphycuz alvarezii* treated by chemicals ($CaCO_3$) drives the search for 'greener' decolourisation methods to sustainably supply cheaper and energy efficient products. Therefore, a decolourisation method of low-salinity seawater (LSS) (3, 9 and 15 g/L) was investigated to replace $CaCO_3$. In addition, decolourised seaweed colour powder (DSP) and SRC colour, yield, viscosity and gel strength were used to screen the salinity treatments. SRC prepared from LSS (3 g/L) treatment demonstrated similar colour and physical properties to $CaCO_3$ treatments, which indicated low-salinity seawater (3 g/L) could replace $CaCO_3$ as a decolourising agent. Moreover, purity of SRC (3 g/L salinity) were confirmed by x-ray diffraction (XRD), scanning electron microscopy-energy-dispersive x-ray (SEM-EDX), and its functional group by fourier transform infrared (FTIR). In this study, waste seawater from LSS (3 g/L) exhibited radical scavenging properties. This decolourising method could be easily adopted by smallholder seaweed farmers in low- and middle-income countries.

Keywords FTIR, *Kappaphycuz alvarezii*, low-salinity seawater, natural decolourisation, SEM-EDX, semi-refined carrageenan.

Introduction

Kappaphycuz alvarezii, a seaweed farmed for its sulphated-polysaccharide kappa-carrageenan, has experienced increased cultivation in Indonesia followed by The Philippines (Bixler & Porse, 2011; Campbell & Hotchkiss, 2017). Kappa-carrageenan has been used as a thickener, an emulsifier and stabiliser in processing many food products and pet foods (Bixler & Porse, 2011; Uju *et al.*, 2019). Based on their impurities, kappa-carrageenan is grouped into refined carrageenan (RC) and semi-refined carrageenan (SRC), which uses different coding in The European Union Utilization Coding such as code E407 for RC and code E407a for SRC (Sormin *et al.*, 2018). However, processing steps and high production costs are needed to produce RC, whereas SRC is the intermediate product after alkaline treatment of seaweed. In the 1970s, the kappa-carrageenan and pet food industries started to used SRC, since it was cheaper and used lower energy to produce kappa-carrageenan, which led to increased cultivation of *K. alvarezii* and supplies of consistently high grade seaweed as SRC raw material by the seaweed farming communities (Neish *et al.*, 2017). SRC is potentially applicable to primary food packaging, which could provide both economic and environmental benefits (Sedayu *et al.*, 2018).

Kappaphycuz alvarezii is a rich source of phycoerythrin, β-carotene, cellulose and lignin (Chan *et al.*, 2013; Iskandar *et al.*, 2013). However, these pigments are eliminated during the production of kappa-carrageenan, especially during decolourising seaweed. The pigment elimination process uses decolourising agents, which are usually applied to produce decolourised kappa-carrageenan (Indriatmoko *et al.*, 2015). Decolourised seaweed could be processed as a daily food product or could be extracted to SRC and RC products. Processing SRC or RC using seaweed without a decolourising treatment produces an odorous and yellowish colour of SRC, which limits their usage in food, pharmaceutical and cosmetic industries (Anisuzzaman *et al.*, 2014).

Based on our observations, some seaweed farmers, to produce SRC and RC, use calcium carbonate

*Correspondent: E-mails: albert.lintoncharles@mail.npust.edu.tw; alamsjah@fpk.unair.ac.id

doi:10.1111/ijfs.14856

($CaCO_3$) to decolourise raw seaweed (*K. alvarezii)* materials in pre-alkaline treatments to obtain 'high-priced' decolourised seaweed. Before the alkaline treatment process, fresh seaweed is soaked in $CaCO_3$ solution to remove colour, odour and salt content (Sormin *et al.*, 2018). However, $CaCO_3$ is known to cause skin irritation and serious eye damage (Database & N.C. for B.I.P, 2020). Anisuzzaman *et al.* (2014) suggested the use of activated charcoal as an alternative decolourising method and is environmentally friendly. However, authors concluded that large volumes of activated charcoal are required to profitably decolourise seaweed and might be impractical for field operation, which might limit its use by low-income farmers. Nevertheless, producing SRC with less or without chemical agents in pre-alkaline treatment of seaweed remains a challenge to low-income farmers.

Seaweed decolourisation occurs naturally and is caused by rain and fresh water infiltration from rivers. This phenomenon was duplicated and investigated using synthetic low-salinity seawater (LSS). Therefore, this study was conducted to develop an environmentally friendly decolourising technique to produce decolourised seaweed powder (DSP) using LSS to yield SRC. Quality indicators such as colour of DSP and SRC, yield and physical properties (gel strength and viscosity) were investigated for SRC and compared with standards suggested by food regulators. SEM-EDX was used to confirm residue of calcium residues in SRC, whereas FTIR and XRD analyses were used to investigate and characterise functional groups and purity of SRC. In addition, biochemical properties evaluation of decolourised seaweed, SRC and waste-seawater product were studied to determine potential food application.

Materials and methods

Materials

Fresh seaweed (*K. alvarezii,* green variety) samples were collected from the coastal areas of Sumenep, East Java Province, Indonesia.

Decolourising treatments

Fresh ground water was used to adjust seawater salinity from high salinity (30 g/L) to low salinity (3–15 g/L). Fresh seaweed (1000 g) were soaked in the different LSS solutions (5000 mL) for 12 h at room temperature (±25 °C) to depigment samples. The low-salinity seawater treatments and soaking time were selected based on response surface methodology screening (Tables S1 and S2; Figure S1), which indicated that the lowest seawater salinity (3 g/L) showed highest colour (lightness). $CaCO_3$ (2%) was added to water then stirred for 30 minutes and stored overnight at room temperature (±25 °C) (Sormin *et al.*, 2018). The supernatant was collected, and its use as a decolourising agent was compared with LSS treatments.

Production of semi-refined carrageenan (SRC) from decolourised seaweed

Whole samples of decolourised seaweed were sun-dried for 12 h and then dried in an oven at 60 °C for 12 h to remove excess moisture. The first extraction process involved preparing alkaline solution by heating 500 mL KOH (10%) solution in a water bath at 80 °C. Dried decolourised seaweed samples (5 g) were added and boiled in the alkaline solution for 30 min to yielded SRC (Bono *et al.*, 2014). Then, the SRC samples were washed 3–4 times with distilled water. All SRC samples were dried in an oven at 60 °C for 24 h, ground and stored in a digital humidity controller (temperature: 25 °C and relative humidity: 50%) until further analysis.

Characterisation of SRC

Colour analysis

The colour of decolourised seaweed powder (DSP) and SRC samples was measured using a Chromameter CR-300 (Minolta Camera, Co. Tokyo, Japan), which was calibrated with a colour standard (white). Samples were scanned to obtain L, a^* and b^* values. The colour parameters of DSP and SRC were express as lightness percentage and were calculated using Eq. (1) in Uju *et al.*(2019):

$$\text{Lightness}\,(\%) = \left[100 - \sqrt{(100-\text{L})^2 + a^2 + b^2}\right] \quad (1)$$

Yields

Yields of SRC were determined using the Eq. (2) in Gereniu *et al.* (2017):

$$\text{Yield}\,(\%) = \left[\left(\frac{\text{Weight of dry SRC}}{\text{Weight of dry decolourised seaweed}}\right) \times 100\right] \quad (2)$$

Viscosity

SRC powder (1.5 g) was dissolved in 100 mL Mili-Q water at 90 °C, and the mixture was stabilised in a water bath at 90 °C for 15 min to eliminate air bubbles based on the method by Tavman & Turgut (2010). SRC suspension (10 mL) was poured into a sample cup until it reached the sensor. Viscosity of the SRC suspension was determined using a digital viscometer (A&D Vibro-Viscometer SV-10, Tokyo, Japan). Gel viscosity values were expressed as centipoise (cP) unit.

Gel strength

Gel strength was performed after the method by Bono *et al.* (2014) with slight modification. Gel strength was measured by dissolving 1.5 g SRC powder in 100 mL deionised (Millipore, Molsheim, France) water on a hotplate at 90 °C for 30 min and allowed to cool for 15 min. The universal Instron (The Instron Series 6654, Massachusetts, USA) was used to evaluate the gel strength of SRC. An analytical probe (12.7 mm diameter, 151 g) penetrated the gel (49 mm diameter; depth of 20 mm) at a cross-head speed of 2 mm/s. Gel Strength was expressed as g/cm^2 unit.

Scanning electron microscopy-energy-dispersive X-ray (SEM-EDX)

Impurities morphology of SRC extracted from DSP treated by low-salinity seawater (3 g/L) and $CaCO_3$ was examined by SEM-EDX (Hitachi S-300N, Tokyo, Japan) at 1000× magnification after the method in Ghani *et al.* (2019). The weight of calcium was analysed from SEM micrographs using EDX, where $CaCO_3$ standard spectrum was used to determine calcium residue in SRC. The weight of calcium was expressed as percentage (%).

Fourier transform infrared spectroscopy (FTIR)

The functional group of SRC (2 mg) extracted from DSP treated with low-salinity seawater (3 g/L) and $CaCO_3$ (mixed with potassium bromide, 200 mg) was determined using the FTIR Spectrometer (Perkin Elmer-Spectrum 100 FT-IR, Massachusetts, USA). The FTIR spectra of SRC were expressed as transmittance (%) in the range 800–4000 cm^{-1} (Gereniu *et al.*, 2017) with minor modification of the wavelength range.

X-ray diffraction (XRD)

X-ray diffraction analysis of SRC extracted from DSP treated with low-salinity seawater (3 g/L) and $CaCO_3$ was conducted using an X-ray diffractometer (Bruker D8 Advance, Karlsruhe, German) to determine impurities (from inorganic material) in the SRC. XRD settings were based on Selvakumaran & Muhamad (2015) with a step length of 0.05°, step time (1 s), the diffraction angle (2θ) from 20 to 80. The crystallinity properties of SRC were expressed as degree of peaks from different angles(θ).

Chemical properties of decolourised seaweed and SRC

Chemical analysis included protein content, which was determined based on the micro Kjeldahl distillation method; crude fibre, which was determined by acid-alkaline neutralisation method using ceramic fibre filter; ash, which was determined using a muffle furnace at 550 °C; and moisture content, which was measured using the moisture analyser (MX– 50, A & D Company Ltd, Tokyo, Japan). All chemical analyses of SRC sample extracted from DSP treated using LSS (3 g/L) and $CaCO_3$ were determined based on methods in AOAC (2000) and Charles *et al.* (2020). All results were expressed in percentage (% w/w).

Biological properties of DSP, SRC and waste-seawater product

In vitro evaluation of antioxidant capacity was determined by 2,2 diphenyl-1-picrylhydrazyl (DPPH$^\bullet$) scavenging assays and 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid (ABTS$^\bullet$) scavenging assays based on spectrophotometric methods described in Charles *et al.* (2020). In the DPPH$^\bullet$ scavenging assay, 10 mg of DSP and or SRC was mixed in 2 mL deionised water; or 2 mL waste seawater from LSS (3 g/L) was mixed with 2 mL DPPH (0.004% DPPH in methanol) All the mixtures were incubated for 30 min at ± 25 °C in the dark and then read at 517 nm using a DU 730 UV/Vis spectrophotometer (Beckman-Coulter, United States). In addition, DSP, SRC (1 mg in 0.2 mL deionised water) and waste seawater from LSS (3 g/L) (0.1 mL) samples were mixed with 5 mL of ABTS$^\bullet$ solution and read at 734 nm. The percentages of radical scavenging activities of the samples were calculated using Eq. (3) (Charles *et al.*, 2020).

Total phenolic content (TPC) and total flavonoid content (TFC) of waste seawater were analysed based on the methods in Neoh *et al.* (2016). TPC of waste seawater was expressed as mg gallic acid equivalent (mg GAE) per mL of waste seawater, and TFC was expressed as mg quercetin (QE) per mL of waste seawater.

$$\text{Inhibition (\%)} = \left[\left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{Control}}}\right) \times 100\right] \quad (3)$$

Statistical analysis

Colour, yields and physical properties parameters were analysed using analysis of variance (ANOVA) and Duncan's multiple range test. All experiments were conducted in triplicate. All statistical analyses were analysed using IBM® SPSS® statistic version 22 (SPSS Inc., New York, USA).

Results and discussion

Colour of DSP and SRC

Colour analyses indicated that lightness of DSP significantly ($P < 0.05$) increased using different LSS treatments compared with $CaCO_3$ treatment and dried seaweed powder (without decolourising

treatment) (Fig. 1). In DSP, the highest lightness percentage was found in $CaCO_3$ treatment (50.17%), followed by LSS (3, 9 and 15 g/L) (44.22, 40.13 and 38.5 %, respectively), and the lowest lightness was shown by the original seaweed powder (without decolourising treatment) (Fig. 1). Anisuzzaman *et al.*(2014) reported that decolourising seaweed requires a minimum 50 g activated charcoal for every 1 L water to soak 200 g seaweed and to obtain 60% lightness. In our study, LSS (3 g/L) treatment achieved 44% lightness, which indicated the decolourising efficiency and low-cost potential of this technique. Although the use of activated charcoal to decolourise seaweed is an attractive method, its adoption in the smaller remote islands of the Indonesian archipelago might be limited due to low forest cover, compared to larger islands, and incur import costs for seaweed farmers.

Lightness values of SRC powder extracted from DSP were significantly different, ($P < 0.05$), and the highest values were exhibited by SRC samples treated with $CaCO_3$ (70.12%) followed by LSS (3 g/L) (66.89%). The lowest lightness values of SRC powder were similar to LSS treatments (9 and 15 g/L) (62.27 and 63.81%, respectively). Lightness trends between DSP and SRC samples were similar to LSS treatments (9 and 15 g/L); however, lightness values significantly increased in LSS (3 g/L)-treated DSP and SRC samples. Colour analysis of DSP (Table S3) demonstrated significant positive correlations to SRC after the extraction process ($P < 0.01$). These findings highlighted the influence or contributions of decolourised seaweed on the final chemical composition of SRC.

In this study, decreasing salinity levels of seawater showed decreasing pigment colour, which resulted in an increase in seaweed lightness; interestingly, LSS (3 g/L) showed close lightness values with DSP treated using $CaCO_3$. The decolourising process consists of two steps; firstly, water penetrates the seaweed cell membrane during the soaking process; secondly, decolourisation continues by a dewatering process using sun drying. The authors hypothesised that different osmoticities between the solvent (low-salinity seawater, LSS) and cell membrane disrupted the lipoproteins of chloroplast membranes (Hosikian *et al.*, 2010); then, the pigment leaked as low-saline water penetrated the cell membrane, which led to depigmentation or decolourisation of the seaweed samples. According to Charles *et al.* (2020), seaweed dried using the sun-drying method exhibited more colour changes than other drying methods. Moreover, $CaCO_3$ added to water is harmful to farmers and the environment; therefore, LSS technique could safely produce decolourised seaweed powder at lower costs and sustainably adapted in low/middle-income countries (Figure S3).

Physical properties of SRC

Yields and physical properties of SRC extracted from decolourised seaweed powder (DSP) of pre-determined saline concentrations were compared with SRC extracted from decolourised seaweed prepared by $CaCO_3$. SRC yields and gel strengths were similar among the decolourising processes and showed no significant differences ($P > 0.05$) (Table 1). However, viscosity properties were significantly higher ($P < 0.05$) in $CaCO_3$-treated SRC samples (198.67 cP), followed by LSS treatments (9, 3 and 15 g/L) (142.33, 127.25 and 117.67 cP, respectively (Table 1).

Yield percentages, after processing, and physical properties such as gel strength and viscosity are reliable efficiency indicators of the extraction methods, type of solvent and seaweed culture location (Sormin *et al.*, 2018; Uju *et al.*, 2019). Solorzano-Chavez *et al.* (2019) presented similar yields of SRC treated with KOH and obtained values between 65.2 and 88.6 %. However, this study demonstrated that yields of SRC without a bleaching agent (Table 1) were higher than values reported by Uju *et al.* (2019). Gel strength values in this study agreed with Moses *et al.* (2015), who reported that SRC obtained using KOH showed gel strength values between 224 and 310 g/cm^{-2}.

In this study, colour of DSP showed significant positive correlation to viscosity of SRC ($P < 0.01$) (Table S3). Although SRC products from DSP treated with $CaCO_3$ exhibited high viscosity values, SRC obtained from DSP treated with 3 g/L LSS showed higher viscosity values compared to the market standard of 5 cP (FAO, 2007). Reportedly, the decolourising agent depolymerises carrageenan during processing and affects physical properties of kappa-carrageenan (Uju *et al.*, 2019). Furthermore, in this study, an increase in seawater saline concentration in the decolourising process was negligible to produce higher quality SRC, based on similar SRC physical properties produced among the decolourising treatments. These findings indicated LSS (3 g/L) could be used to replace $CaCO_3$ as a decolourising agent.

FTIR

Semi-refined carrageenan extracted from DSP treated by LSS (3 g/L) signalled the following spectral bands: 1227 cm^{-1}, which was characterised as ester sulphate; 924 cm^{-1} as 3,6-anhydro-D-galactose and 840 cm^{-1} as galactose-4 sulphate (Fig. 2). Moreover, SRC extracted from decolourised seaweed treated with $CaCO_3$ exhibited similar spectra bands; at 1227 cm^{-1} characterised as ester sulphate, 924 cm^{-1} as 3,6-anhydro-D-galactose and 841 cm^{-1} as galactose-4 sulphate (Fig. 2). FTIR analysis commonly uses infrared regions as fingerprint regions representative for many compounds such as carbohydrates (Balan *et al.*, 2019). Moreover, SRC transmittance

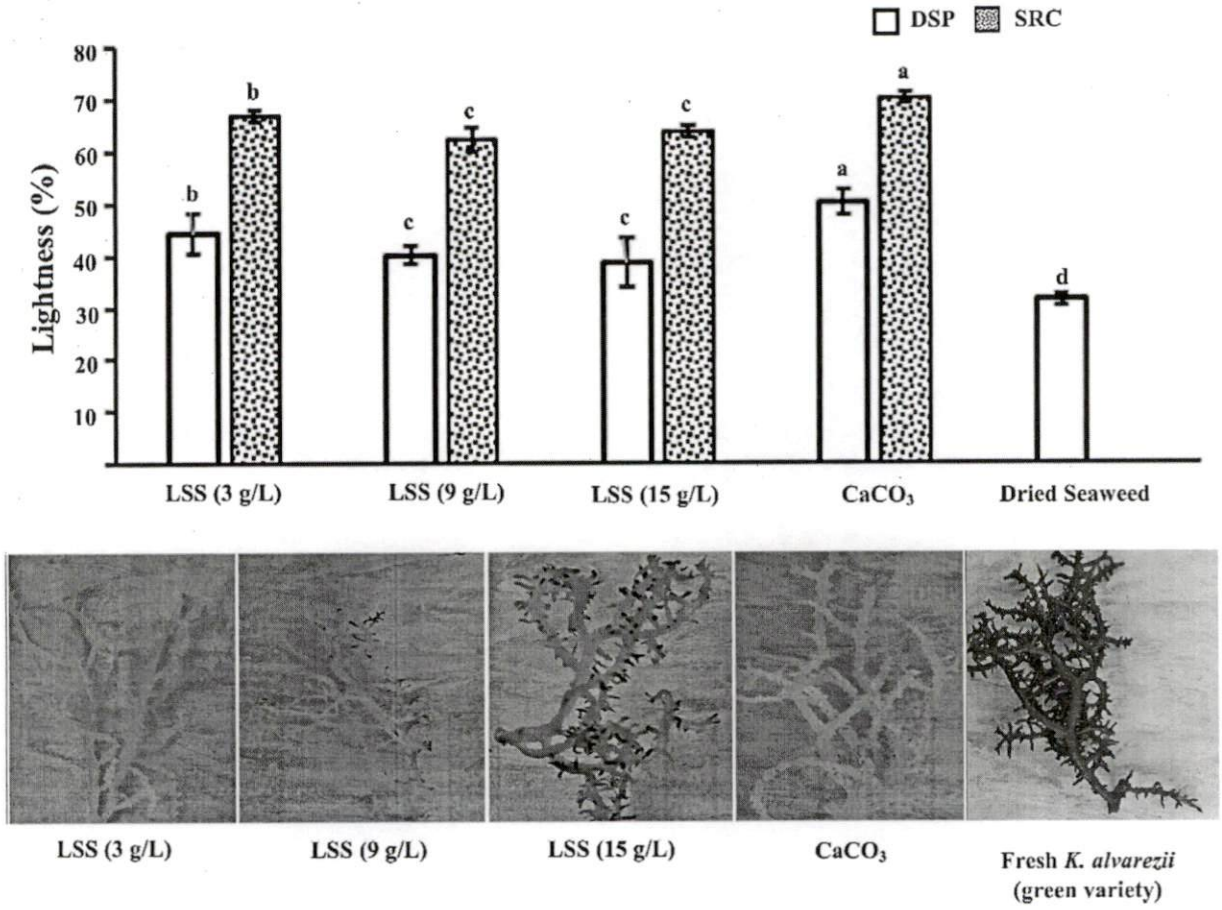

Figure 1 Colour values of decolourised seaweed powder (DSP) and Semi-refine Carrageenan (SRC) produced from DSP treated by different low-salinity seawater (LSS), and SRC produced from DSP treated by $CaCO_3$ treatment and dried seaweed (seaweed without decolourised treatment). Mean values within samples with different superscript letters (a, b, c and d) represent significant differences ($P < 0.05$).

Table 1 The effect of decolourising treatments on yields and physical properties of SRC

Parameters	LSS (3 g/L)	LSS (9 g/L)	LSS (15 g/L)	$CaCO_3$
Yield (%)	78.09 ± 6.80^{a*}	78.29 ± 5.33^{a}	77.84 ± 2.46^{a}	82.97 ± 2.32^{a}
Gel Strength (g/cm^{-2})	274.03 ± 37.28^{a}	226.76 ± 32.58^{a}	225.27 ± 49.62^{a}	277.17 ± 2.45^{a}
Viscosity (cP)	127.25 ± 3.06^{bc}	142.33 ± 5.50^{b}	117.67 ± 3.06^{c}	198.67 ± 21.50^{a}

LSS, Low-salinity Seawater; SRC, Semi-refine Carrageenan.

*The results are mean ± standard deviation (SD). Mean values with different superscript alphabets are significantly different ($P < 0.05$).

data were interpreted as SRC (kappa-carrageenan) transmittance following Moses *et al.* (2015) and Gereniu *et al.* (2017).

SEM-EDX

Scanning electron microscopy-energy-dispersive X-ray was used to investigate impurities morphology and weight percentage of oxygen, carbon, sulphur, calcium, magnesium, sodium and potassium of iota-carrageenan (Ghani *et al.*, 2019). SRC of $CaCO_3$-treated DSP exhibited higher quantities of calcium weight compared with SRC extracted from DSP treated with LSS (3 g/L) (Fig. 3). SEM images exhibited uneven surface areas, and white colour distributed on the surface of SRC in SEM micrograph

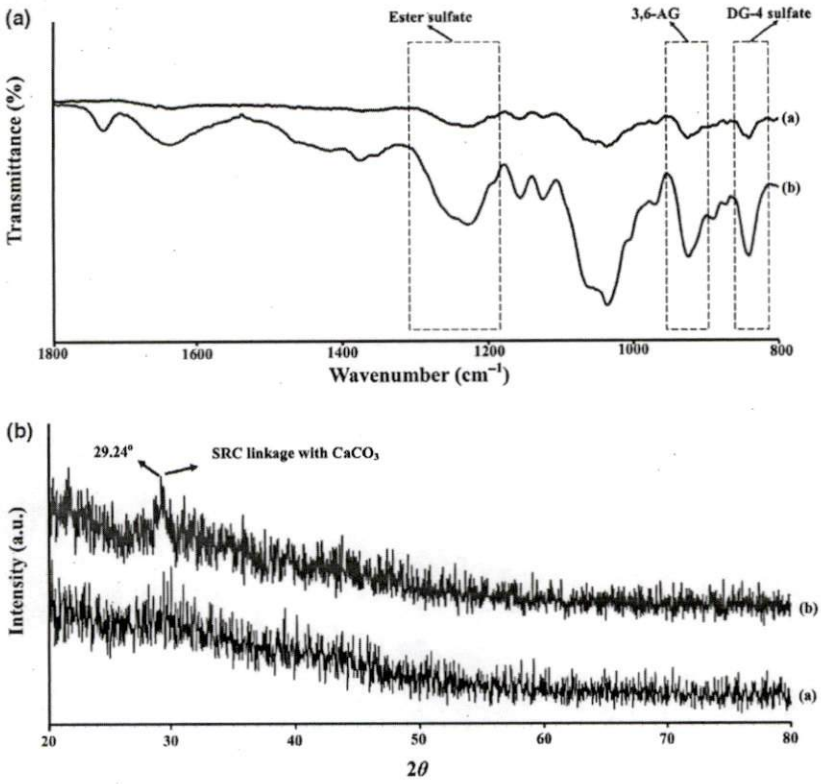

Figure 2 (a) FTIR of Semi-refine Carrageenan (SRC) produced from decolourised seaweed powder (DSP) treated by low-salinity seawater (LSS) (3 g/L) (a) compared with SRC produced from DSP treated by $CaCO_3$ (b). (b) XRD pattern of SRC produced from DSP treated by LSS (3 g/L) (a) compared with SRC produced from DSP treated by $CaCO_3$ (b).

was confirmed as $CaCO_3$ by EDX analysis (Fig. 3 and Figure S2).

XRD analysis

Selvakumaran & Muhamad (2015) reported that the level of crystallinity of kappa-carrageenan gel was enhanced by the incorporation of $CaCO_3$. Based on our study, sharp diffraction peaks were observed in XRD curve of SRC, in which one main crystalline peak at $2\theta = 29.24°$ was attributed to deposits of $CaCO_3$ in SRC treated with $CaCO_3$, and which confirmed the presence of impurities of SRC (Fig. 2). Moreover, Karthikeyan *et al.* (2017) reported sharp diffraction peaks present that indicated the presence of inorganic impurities. The amorphous structure of SRC produced from DSP presented between 25 and 30° indicated no cross-linking between SRC and $CaCO_3$ (Fig. 2).

Chemical properties of DSP and SRC

The chemical properties of DSP, SRC and $CaCO_3$ extracts are summarised in Table 2. Crude protein content of DSP treated with LSS (3 g/L) was 5.04% higher than SRC samples at 3.15%, whereas $CaCO_3$-treated samples contained 5.63 and 5.86 %, respectively. Crude fibre content of DSP was higher, but after SRC extraction, both treatments showed similar contents: low salinity (3.63%) and $CaCO_3$ (3.27%). KOH treatment is an alkaline method to extract SRC from DSP and involves eliminating protein and crude fibre (cellulose, hemicellulose and lignin) (Chan *et al.*, 2013). Decreased protein and crude fibre contents in our results showed correlation with increased colour of SRC after alkaline extraction. In another study, the low residue of protein (red protein-pigment) in SRC resulted in decreased mustard colour of SRC product prepared from the bleaching process (Uju *et al.*, 2019).

Moisture content was higher (15.66%) in LSS (3 g/L) than $CaCO_3$-treated (13.54%) decolourised seaweed powder (DSP) samples; however, SRC $CaCO_3$-treated extracts had higher moisture content (11.61%) whereas low-salinity SRC recorded 10%, which indicated SRC moisture content ranged within that (<12%) recommended by FAO (2007).

The ash content of SRC in this study was between 21 and 27%, and the ash content of SRC produced from DSP treated by $CaCO_3$ had higher values than SRC produced from DSP treated by LSS (3 g/L). Based on this phenomenon, decolourising seaweed with $CaCO_3$ contributed calcium during processing, which resulted in increasing ash content of SRC.

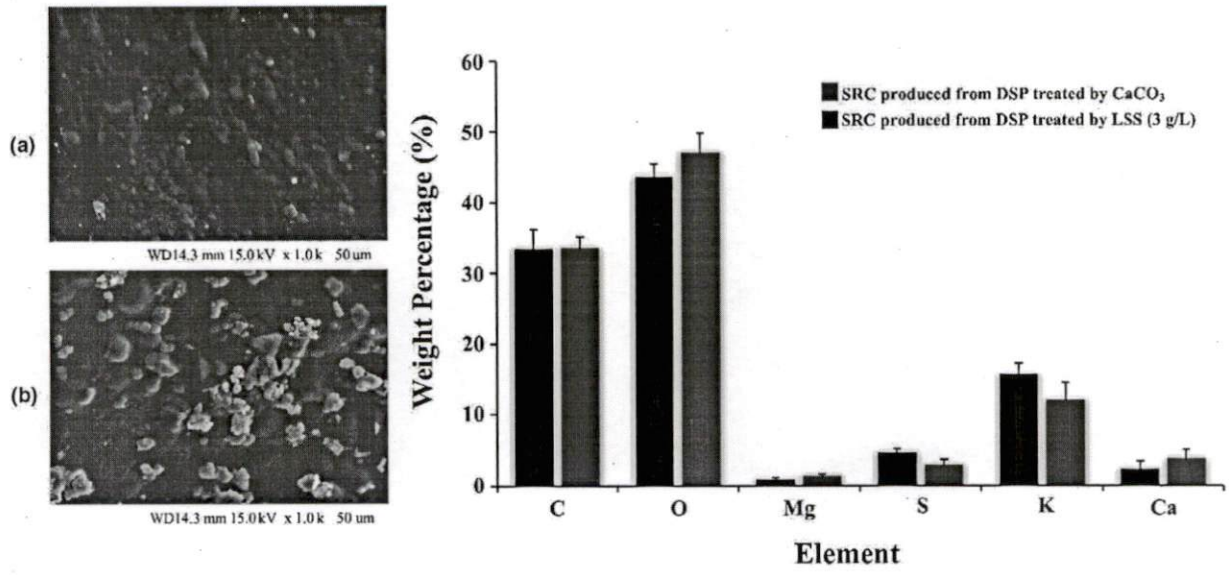

Figure 3 SEM-EDX for Semi-refine Carrageenan (SRC) produced from decolourised seaweed powder (DSP) treated by low-salinity seawater (LSS) (3 g/L) (a) compared with SRC produced from DSP treated by $CaCO_3$ (b); the white colour appointed by red arrow represents calcium deposits on SRC surface. The error bar represents as the standard deviation (SD) from mean of weight percentage of element (n = 3).

Moreover, ash content in this study was in agreement with Moses *et al.* (2015), in that SRC prepared by alkaline extraction had ash content between 26 and 29% and was below 40% benchmark suggested by McHugh (2003) and Chan *et al.* (2013).

Biological properties of DSP, SRC and waste-seawater product

In this study, low-salinity treated DSP and SRC samples exhibited scavenging activities in ABTS• (17.62 and 16.33%) and DPPH• (10.45 and 13.88%), assays, respectively (Table 2). Sun-dried *K. alvarezii* exhibited DPPH• scavenging activities below 20%, whereas DPPH• radical inhibition of edible SRC film material produced from the sun-dried *K. alvarezii* was below 10% (Charles *et al.*, 2020; Farhan & Hani, 2020), which indicated the food packaging and functional food potential of SRC.

Liquid waste from LSS (3 g/L) treatment contained TFC (0.04 mg/g) and TPC (0.09 mg/g) and was attributed to high ABTS• (94.33%) and DPPH• inhibition (56.86%) (Table 2). Chaula *et al.* (2019) reported that scavenging activities of *K. alvarezii* water extracts inhibited DPPH• by 29.67-50.56%. Plants, when stressed, tend to produce more reactive oxygen species (ROS) as by-products of aerobic metabolism, but they simultaneously employ antioxidant metabolites (Table 2) to prevent possible oxidative damage (Apel & Hirt, 2004). Therefore, authors concluded that the LSS treatment might have concentrated antioxidant

Table 2 Biochemical properties of DSP, SRC and waste-seawater product[a]

	Decolourising treatments	
Parameters	**LSS (3 g/L)**	**$CaCO_3$**
DSP		
Crude protein (%)	5.04 ± 0.54	5.63 ± 0.57
Crude fibre (%)	7.48 ± 0.41	6.51 ± 0.62
Moisture content (%)	15.66 ± 0.36	13.54 ± 0.36
Ash (%)	14.59 ± 0.13	16.43 ± 0.14
ABTS• (%)	17.62 ± 0.32	16.20 ± 0.10
DPPH• (%)	10.45 ± 0.07	8.49 ± 0.19
SRC		
Crude protein (%)	3.15 ± 2.77	5.86 ± 0.42
Crude fibre (%)	3.63 ± 0.28	3.27 ± 0.01
Moisture content (%)	10.72 ± 0.79	11.61 ± 0.32
Ash (%)	21.86 ± 2.27	27.00 ± 0.38
ABTS• (%)	16.33 ± 1.93	13.17 ± 0.26
DPPH• (%)	13.88 ± 4.11	12.94 ± 4.55
Waste seawater		
TFC (mg/g)	0.04 ± 0.005	–
TPC (mg/g)	0.09 ± 0.001	–
ABTS• (%)	94.33 ± 0.37	–
DPPH• (%)	56.86 ± 3.21	–

DSP, Decolourised Seaweed Powder; LSS, Low-salinity Seawater; SRC, Semi-refine Carrageenan.

[a]The results are expressed as mean ± standard deviation (SD) (n = 3).

metabolites in addition to decolourising, which resulted in perceived bioactivity of DSP, SRC and waste-seawater products (Table 2). However, further studies will investigate bioactive compounds contained in waste-seawater product from the decolourising process of seaweed species.

Conclusions

In this study, the low-salinity seawater (3 g/L) treatment presented high colour values of decolourised seaweed than other low-salinity seawater (9 and 15 g/L) treatments. The low-salinity seawater treatments produced raw materials with similar SRC quality compared with $CaCO_3$-treated samples; moreover, this decolourising method could substantially reduce the release of chemical pollutants ($CaCO_3$) in the environment. In addition, analysis of SRC and waste seawater from decolourisation process exhibited potential bioactive properties, which could be exploited to produce added-value functional foods and feed products. Thus, the decolourising method using low-salinity seawater promised low-cost production of SRC and facilitated sustainable adoption by smallholder seaweed farmer in low- and middle-income countries.

Acknowledgments

The authors are grateful to Tahir Professorship Endowment (UNAIR) and the MOE, Taiwan, for the fellowship to Mr. Annur Ahadi Abdillah.

Conflict of interest

The authors declare no conflict of interest.

Author contribution

Annur Ahadi Abdillah: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Writing-original draft (lead). **Mochammad Amin Alamsjah:** Project administration (supporting); Resources (supporting); Supervision (supporting); Writing-review & editing (supporting). **Albert Linton Charles:** Project administration (lead); Resources (lead); Supervision (lead); Writing-review & editing (lead).

Ethical approval

Ethics approval was not required for this research.

Peer review

The peer review history for this article is available at https://publons.com/publon/10.1111/ijfs.14856.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Anisuzzaman, S.M., Bono, A., Krishnaiah, D. & Hussin, N.A. (2014). Decolorization of low molecular compounds of seaweed by using activated carbon. *International Journal of Chemical Engineering and Applications*, **5**, 100–103.

AOAC (2000). *Official methods of analysis of AOAC international*, 17th ed. USA: AOAC International.

Apel, K. & Hirt, H. (2004). Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology*, **55**, 373–399.

Balan, V., Mihai, C.T., Cojocaru, F.D. *et al.* (2019). Vibrational spectroscopy fingerprinting in medicine: From molecular to clinical practice. *Materials*, **12**, 1–40.

Bixler, H.J. & Porse, H. (2011). A decade of change in the seaweed hydrocolloids industry. *Journal of Applied Phycology*, **23**, 321–335.

Bono, A., Anisuzzaman, S.M. & 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.

Campbell, R. & Hotchkiss, S. (2017). Carrageenan industry market overview. In: *Tropical seaweed farming trends, problems and opportunities* (edited by A.Q. Hurtado, A.T. Critchley & I.C. Neish). Pp. 193–205. 1, Cham, Switzerland: Springer International Publishing AG.

Chan, S.W., Mirhosseini, H., Taip, F.S., Ling, T.C. & 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.

Charles, A.L., Sridhar, K. & Alamsjah, M.A. (2020). Effect of drying techniques on color and bioactive potential of two commercial edible Indonesian seaweed cultivars. *Journal of Applied Phycology*, **32**, 563–572.

Chaula, D., Laswai, H., Chove, B. *et al.* (2019). Effect of clove (Syzygium aromaticum) and seaweed (Kappaphycus alvarezii) water extracts pretreatment on lipid oxidation in sun-dried sardines (Rastrineobola argentea) from Lake Victoria, Tanzania. *Food Science and Nutrition*, **7**, 1406–1416.

Database, N.C. for B.I.P (2020). *Calcium hydroxide, CID=6093208. National Center for Biotechnology Information. PubChem Database.*

FAO (2007). Carrageenan. *FAO JECFA Monographs 16*, **4**.1–6.

Farhan, A. & Hani, N.M. (2020). Active edible films based on semi-refined κ-carrageenan: Antioxidant and color properties and application in chicken breast packaging. *Food Packaging and Shelf Life*, **24**, 100476.

Gereniu, C.R.N., Saravana, P.S., Getachew, A.T. & Chun, B.S. (2017). Characteristics of functional materials recovered from Solomon Islands red seaweed (Kappaphycus alvarezii) using pressurized hot water extraction. *Journal of Applied Phycology*, **29**, 1609–1621.

Ghani, N.A.A., Othaman, R., Ahmad, A., Anuar, F.H. & Hassan, N.H. (2019). Impact of purification on iota carrageenan as solid polymer electrolyte. *Arabian Journal of Chemistry*, **12**, 370–376.

Hosikian, A., Lim, S., Halim, R. & Danquah, M.K. (2010). Chlorophyll extraction from microalgae: A review on the process engineering aspects. *International Journal of Chemical Engineering*, **2010**, 1–11.

Neish, I.C., Sepulveda, M., Hurtado, A.Q. & Critchley, A.T. (2017). Reflections on the commercial development of eucheumatoid

seaweed farming. In: *Tropical Seaweed Farming Trends, Problems and Opportunities* (edited by A.Q. Hurtado, A.T. Critchley & I.C. Neish). 1, Cham, Switzerland: Springer International Publishing AG, 1–27.

Indriatmoko, Heriyanto, Limantara, L. & Brotosudarmo, T.H.P. (2015). Composition of photosynthetic pigments in a red alga Kappaphycus alvarezi cultivated in different depths. *Procedia Chemistry*, **14**, 193–201.

Iskandar, A., Syam, R. & Trijuno, D.D. (2013). Content of carrageenan, chlorophyll a and carotenoid of Kappaphycus alvarezii cultivated in different seawater depth Laikang village, district of Mangarabombang, Takalar regency. *Journal of Applied Biotechnology*, **2**, 1.

Karthikeyan, S., Selvasekarapandian, S., Premalatha, M. *et al.* (2017). Proton-conducting I-Carrageenan-based biopolymer electrolyte for fuel cell application. *Ionics*, **23**, 2775–2780.

McHugh, D.J. (2003). *A guide to the seaweed industry FAO Fisheries Technical Paper 441.* , Rome: Food and Agriculture Organization of the United Nations. 63–64.

Moses, J., Anandhakumar, R. & Shanmugam, M. (2015). Effect of alkaline treatment on the sulfate content and quality of semi-refined carrageenan prepared from seaweed Kappaphycus alvarezii Doty (Doty) farmed in Indian waters. *African Journal of Biotechnology*, **14**, 1584–1589.

Neoh, Y.Y., Matanjun, P. & Lee, J.S. (2016). Comparative study of drying methods on chemical constituents of Malaysian red seaweed. *Drying Technology*, **34**, 1745–1751.

Sedayu, B.B., Cran, M.J. & Bigger, S.W. (2018). Characterization of semi-refined carrageenan-based film for primary Food packaging purposes. *Journal of Polymers and the Environment*, **26**, 3754–3761.

Selvakumaran, S. & Muhamad, I.I. (2015). Evaluation of kappa carrageenan as potential carrier for floating drug delivery system: Effect of cross linker. *International Journal of Pharmaceutics*, **496**, 323–331.

Solorzano-Chavez, E.G., Paz-Cedeno, F.R., Ezequiel de Oliveira, L. *et al.* (2019). Evaluation of the Kappaphycus alvarezii growth under different environmental conditions and efficiency of the enzymatic hydrolysis of the residue generated in the carrageenan processing. *Biomass and Bioenergy*, **127**, 105254.

Sormin, R.B.D., Soukotta, D., Risambessy, A. & Ferdinandus, S.J. (2018). Physico-chemical charachteristic of semi refined carrageenan from Ambon City and Southwest Maluku District. *Jurnal Pengolahan Hasil Perikanan Indonesia*, **21**, 92.

Tavman, I. & Turgut, A. (2010). An investigation on thermal conductivity and viscosity of water based nanofluids. In: *Microfluidics based microsystems. NATO Science for Peace and Security Series A Chemistry and Biology* (edited by S. Kakaç, B. Kosoy, D. Li & A. Pramuanjaroenkij). Pp. 183–202. Dordrecht: Springer.

Uju., Prasetyaningsih, E., Santoso, J., Kamiya, N. & Oshima, T. (2019). Preparation and characterization of semi-refined carrageenan from Kappaphycus alvarezii seaweed bleached by Peracetic Acid. *IOP Conference Series: Earth and Environmental Science*. Pp. 278.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

App S1.

Figure S1. Optimum color values of decolorized seaweed powder (DSP) treated by combinations of low-salinity seawater (LSS) (g/L) and time (hour).

Figure S2. CaCO3 spectrum found in SRC produced from decolorized seaweed powder.

Figure S3. SRC production step.

Table S1. Experimental results based on RSM

Table S2. Analysis of Varian (ANOVA) based on RSM (Response Surface Linear Model)

Table S3. Correlation coefficient between color of DSP, yields, and physical properties of SRC