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OPTIMIZATION OF CARRAGEENAN EXTRACTION FROM *KAPPAPHYCUS ALVAREZII* SEAWEED USING A FACTORIAL DESIGN STUDY

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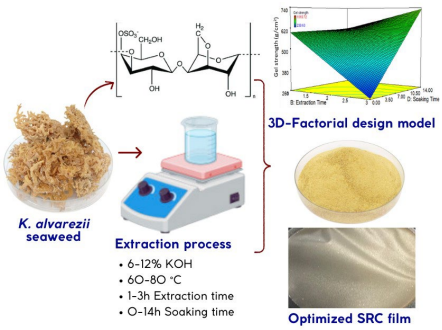
Graphical abstract



Abstract

Carrageenan is a sulfated polysaccharide extracted from seaweeds and is pivotal in various industries due to its gelling, thickening, and stabilizing properties. Despite its widespread use, the efficiency of carrageenan extraction significantly influences its quality and market value. This study aims to optimize the extraction process of semi-refined carrageenan from *Kappaphycus alvarezii* seaweed using a factorial design method, focusing on maximizing yield and film characteristics while maintaining process sustainability and cost-effectiveness. Two process parameters with optimal mechanical properties (OPT 1) and yield (OPT 2) were selected based on the predictive model generated by the factorial design analysis. The results showed that soaking time positively influences gel strength, whereas prolonged extraction time reduces gel strength. Higher extraction temperatures increase gel viscosity, whereas increased KOH concentration and extraction times lower viscosity due to structural breakdown. Besides, OPT 1 and OPT 2 film analyses show tensile strengths of 34.40 MPa and 34.52 MPa, respectively, with superior flexibility, and elongation at a break (EAB) of 31.30% outperforming commercial films (19.18%). These results highlight the films' balance of durability and adaptability, positioning them as viable and sustainable packaging alternatives.

Keywords: Seaweed, carrageenan, *Kappaphycus alvarezii*, extraction, factorial design

Abstrak

Carrageenan adalah polisakarida sulfat yang diekstrak dari rumpai laut dan sangat penting dalam pelbagai industri kerana sifatnya yang mampu menggel, memekatkan, dan menstabilkan. Walaupun penggunaannya meluas, kecekapan ekstraksi carrageenan secara signifikan mempengaruhi kualiti dan nilai pasaran. Kajian ini bertujuan untuk mengoptimumkan proses ekstraksi carrageenan dari rumpai laut *Kappaphycus alvarezii* menggunakan metodologi reka bentuk faktorial, dan fokus pada memaksimumkan ciri-ciri filem (OPT 1) dan hasil (OPT 2) sambil mengekalkan kelestarian dan proses yang menjimatkan. Dua parameter proses terbaik dengan hasil dan sifat mekanikal yang optimal dipilih berdasarkan model prediktif yang dihasilkan oleh analisis reka bentuk faktorial. Hasil kajian menunjukan masa rendaman mempengaruhi kekuatan gel secara positif, manakala masa ekstraksi yang panjang mengurangkan kekuatan gel. Suhu

ekstraksi yang lebih tinggi meningkatkan kekentalan gel, manakala peningkatan kepekatan KOH dan masa ekstraksi yang panjang menurunkan kekentalan akibat kerosakan pada struktur. Analisis filem OPT 1 dan OPT 2 menunjukkan kekuatan tegangan masing-masing 34.40 MPa dan 34.52 MPa, dengan fleksibiliti yang baik, dan pemanjangan pada titik putus (EAB) sebanyak 31.30%, mengatasi filem komersial (19.18% EAB). Hasil kajian ini menunjukan keseimbangan ketahanan dan kebolehan adaptasi filem, menjadikanya sebagai alternatif pembungkusan yang baik dan lestari.

Kata kunci: Rumpai laut, carrageenan, *Kappaphycus alvarezii*, ekstraksi, reka bentuk faktorial

1.0 INTRODUCTION

The continuous utilization of plastic materials has currently resulted in serious environmental pollution and health effects due to uncontrolled landfills with non-biodegradable plastics-based packaging [1,2]. This escalating catastrophe requires the exploration of sustainable and biodegradable alternatives to existing products. Among the bio-based polymer candidates, carrageenan made from polysaccharides derived from seaweeds emerges as an exceptionally favorable alternative due to its compatibility, biodegradability, ease of manufacture, and cost-effective film-forming properties [3–5]. Carrageenan consists of alternating copolymers of 3,6-anhydrous-galactose and D-galactose connected by α-1,3- and β-1,4-glycosidic bonds [6]. In addition to its function as a thickening, stabilizing, and gelling agent in the food industry, it exhibits an impressive diversity of applications in other industries as well, including pharmaceuticals, cosmetics, and agriculture [7–11].

Kappaphycus alvarezii, a type of red seaweed, has gained widespread recognition for its rich carrageenan content. Over the years, this seaweed has been well-studied due to its economic value and potential impact on the carrageenan industry. Compared to other carrageenan sources such as *K. striatum* and *K. malesianus*, carrageenan extracted from *K. alvarezii* stands out for its superior gel texture, gel strength, and gelling properties [5,12]. However, the full potential of carrageenan as an alternative to plastic-based materials is hindered by the intricacy of the extraction process from natural sources.

Various factors, including pH, extraction temperature, and duration, have a significant impact on both the quality and quantity of carrageenan during the extraction process. The disproportionate amount of solvent, pH, and time during the carrageenan extraction will affect its quality, structure, and dissolution process from the seaweed matrix [13]. Hence, this study employs a factorial design methodology to optimize the process conditions for SRC extraction from *K. alvarezii*. This approach allows for the simultaneous investigation of the multiple variables and their potential interactions within the extraction process. Besides, the optimized key parameters can be consistently reproduced and cost-effective, which is essential for scaling up to industrial production.

The present study highlights the optimization of the semi-refined carrageenan (SRC) extraction under alkaline treatment via a factorial design study. The effect of process conditions including extraction temperature, extraction time, soaking time, and the KOH concentration on the SRC yields and mechanical properties were investigated. Through this study, we anticipate establishing a more efficient, cost-effective, and environmentally friendly method of carrageenan extraction. This endeavor aligns with the urgent need for sustainable solutions in the face of the global plastic crisis and is expected to provide substantial benefits to industries seeking eco-friendly alternatives to conventional plastics.

2.0 METHODOLOGY

2.1 Experimental Design

The impacts of four independent variables namely extraction temperature (A), KOH concentrations (B), extraction time (C), and soaking duration (D), on the response variables of yield (Y1), gel strength (Y2), gel viscosity (Y3) and moisture content (Y4) were evaluated. The design matrix was obtained based on 2-level factorial approach by Design Expert® software (Ver. 7.0.0, Stat-Ease Inc., Minneapolis, USA). A total of 16 run experiments were generated with the high (*Hj*) and low (*Lj*) levels for each independent variable (*Xj*) were confined within a predetermined area to avoid covering the entire simplex area. The selected lower and upper limits are summarized in Table 1. The significance of coefficients between each variable and the generation of equation formula based on the selected factorial model was assessed through analysis of variance (ANOVA).

Table 1 Limits of the process parameters

Independent variable, *Xj*		Low level, *Lj*	High level, *Hj*
A	Temperature (°C)	60	80
B	KOH concentration (w/w)	6	12
C	Extraction time (h)	1	3
D	Soaking time (h)	0	14

The accuracy of the generated models was verified by selecting two optimal process parameters known as OPT 1 and OPT 2. OPT 1 was determined based on the highest viscosity value and moisture content to prioritize formulation stability, while OPT 2 was selected based on the highest yield and gel strength to optimize product performance. The discrepancy between experimentally obtained values and predicted response values was compared and quantified using the subsequent equation to calculate the residual standard error (RSE) percentage for each response variable [14]:

$$RSE\ (\%) = [(Ex - Pr)/Pr] \times 100$$

Where *Ex* is the experimental value and *Pr* is the obtained value of the response variables.

2.2 Extraction of Semi-refined Carrageenan

The extraction of semi-refined carrageenan (SRC) was conducted using a method outlined by Ganesan *et al.* [15] and Heriyanto *et al.* [16]. SRC was extracted from the red seaweed *K. alvarezii,* employing varying extraction temperature and time, KOH concentration and soaking duration as determined by the Design Expert® software. Post extraction, the samples were dried in a 70°C oven (MEMMERT, EQP004, Schwabach, Germany) for 5 hours. Next, the samples were ground using a laboratory grinder fitted with a 0.5 mm sieve (Retsch, ZM 200, Haan, Germany) to a fine SRC powder and kept at ambient temperature (25 ± 1 °C) for the subsequent fabrication of SRC-based film.

2.3 Semi-refined Carrageenan Yield Determination

The dried SRC were finely powdered before being kept in a desiccator to shield them from absorbing environmental moisture. The yield of extracted SRC was determined using the following equation [5]:

$$Yield\ (\%) = (W_c/W_{ds}) \times 100$$

Where W_c is the weight of extracted SRC and W_{ds} is the weight of dry seaweed used for extraction.

2.4 Characterization of Semi-refined Carrageenan

2.4.1 Gel Viscosity Measurement

The viscosity measurements were conducted based on the protocol outlined by Muñoz *et al*. [17]. A 1.5% (w/v) SRC solution was prepared by dissolving 1.5 g SRC powder in 100 mL of boiling distilled water, which was then maintained at 90 °C. The solution was stirred continuously for 20 minutes using a magnetic stirrer. To eliminate any bubbles formed during the heating process, the samples were kept in a water bath at temperatures between 80–90 °C for a duration of 15 minutes. The viscosity readings were taken using a Brookfield DVE Digital Viscometer, equipped with spindle No. 5 with a diameter and a length of 19 mm and 65 mm, respectively. The viscometer data were then attained after six full rotations at a speed of 30 revolutions per minute. The measurement of viscosity was performed three times and the mean was expressed in centipoises (cP).

2.4.2 Moisture Content

The moisture content was determined using the procedure described by Abd Hamid *et al.* [18]. A 2 g of SRC sample was dried for 4 hours in an oven at 105 °C ± 2 °C. The final weight of the dried sample was measured and the moisture content (Mc) was calculated using the following equation:

$$Mc\ (\%) = [(W_{in} - W_{fi})/W_{in}] \times 100$$

Where W_{in} is the initial weight of SRC and W_{fi} is the final weight of SRC.

2.4.3 Gel Strength Measurement

Approximately 1.5 g of SRC and 0.2 g of potassium chloride (KCl) were dissolved in 100 mL distilled water to produce a 1.5% (w/v) carrageenan solution containing 0.2% KCl. This solution was heated to 80 ± 2 °C for 10 minutes, then carefully poured into a sample bottle, and refrigerated for four hours to solidify. Gell strength was measured by determining the force needed to compress the carrageenan gel discs with dimensions of 7 cm in diameter and 3 cm in height. The measurements were conducted using a texture analyzer (Model CT3-1000, Brookfield, USA) equipped with a 1 cm^2 probe descending rate at 1.0 mm s^{-1}. The maximal penetration force was recorded to determine the gel's strength, and the results were expressed in g/cm^2.

2.5 Development of Semi-refined Carrageenan Film

The SRC films were prepared using the procedure outlined by Farhan and Hani [19]. Approximately 2% (w/v) of SRC was dissolved in 100 mL distilled water and stirred at 80 ± 2 °C for 10 minutes using a hot plate magnetic stirrer (C-MAG HS 7, IKA®, USA). Approximately 0.9% (v/v) of glycerol was added into the SRC solution followed by 10% (v/v) cellulose nanofibers (CNF) and subjected to heating for 10 minutes at 80 °C. Subsequently, the film-forming solution of SRC was cooled down to a temperature of 65 °C, and a volume of 80 mL was poured onto an acrylic casting plate. The fluid designated for film formation was subjected to drying at 50°C in an oven for a period of 12 hours. Subsequently, the films were conditioned in a desiccator at 25°C ± 1 °C and 50 ± 5% relative humidity (RH) using a humidity sensor (CS215-L, Campbell Scientific, United States) with sufficient desiccants before further characterization analysis.

2.6 Characterization of Semi-refined Carrageenan film

2.6.1 Tensile Strength and Elongation at Break

The tensile strength (TS) and elongation at break (EAB) of the films were measured based on the standard method of ASTM D882 [20]. The film strips with size of 10 cm x 1.5 cm were prepared and conditioned at 25 ± 1°C and 50 ± 5% RH for 48 h in a desiccator prior to testing. A tensile testing machine (AG-Xplus Series, Shimadzu, Japan) was used by attaching the film strip on the tensile grips. The film was then stretched at 50 mm/min crosshead speed. The films' TS was calculated using the following equation:

$$TS(MPa) = (F_{max}/\Phi)$$

Where F_{max} is the maximum load and Φ is the cross-sectional area of the film. The EAB of the films was then calculated using the following equation:

$$EAB\ (\%) = [(\Delta_1/ l_0)/100]$$

Where Δ_1 is the film extension n and l_0 is the initial length of the film sample. The measurements were carried out in triplicates and the mean of TS and EAB were calculated.

3.0 RESULTS AND DISCUSSION

In accordance with the generated experimental design by the Design-Expert® Software, 16 experimental runs were executed. The results of these trials are detailed in Table 2. Subsequent ANOVA analysis on each response variable indicated that the experimental data aligned well with the factorial model. This relationship is mathematically represented through equations that incorporate coded variables: A (extraction temperature), B (extraction time), C (KOH concentration), and D (soaking time). The correlation coefficient (R^2) values for the response namely yield, gel strength, viscosity and moisture content were specified that the models could predict up to 99.98%, 99.99%, 99.97% and 99.84%, respectively. These findings indicate that good-fitting factorial models were obtained.

Yield (%) = 36.40 + 0.60A – 0.066B - 1.13C – 0.40D + 0.079AB + 0.16AC – 0.026 AD – 0.20BC + 1.65BD – 0.48ABC – 0.66ABD + 0.18ACD + 0.24BCD – 0.36 ABCD

Gel strength (g/cm²) = 549.12 + 59.01A – 51.12B – 55.96C + 118.42D + 16.58AB + 14.52AD + 26.52BC + 114.71 BD + 22.17 CD – 43.47 ABC + 65.91ABD + 33.53ACD – 59.01BCD – 55.32 ABCD

Viscosity (cP) = 743.04 + 26.39A – 28.87B – 35.77C + 29.89D – 10.47AB + 80.43AC + 5.02AD – 86.83BC + 29.66CD – 60.73ABC – 31.15 ABD + 94.73ACD – 21.41BCD – 45.08 ABCD

Moisture content (%) = 10.59 + 1.08A – 0.18B – 0.083C – 0.13D + 0.12AB + 0.17AC + 0.30AD + 0.24BD + 0.075CD – 0.069ABC – 0.047ABD – 0.037ACD + 0.026BCD - 0.11ABCD

Table 2 Candidate points for a 2-level factorial design of different process parameter and response variables

Run	Independent variables				Response variables			
	A	B	C	D	Yield (%)	Gel strength (g/cm²)	Viscosity (cP)	Moisture content (%)
1	60.00	1.00	12.00	0.00	37.87	385.45	736.90	10.23
2	80.00	1.00	6.00	0.00	38.97	900.41	738.30	11.71
3	60.00	3.00	12.00	14.00	36.97	575.12	567.50	9.12
4	60.00	1.00	6.00	14.00	35.64	745.51	910.70	9.42
5	80.00	3.00	6.00	0.00	38.12	275.32	890.20	11.21
6	80.00	3.00	6.00	14.00	39.20	1019.72	775.80	12.01
7	60.00	1.00	12.00	14.00	30.87	480.29	553.00	8.75
8	60.00	3.00	12.00	0.00	32.32	299.80	544.40	8.91
9	60.00	3.00	6.00	0.00	35.10	239.63	746.20	9.81
10	80.00	3.00	12.00	14.00	35.95	754.59	685.78	11.93
11	80.00	1.00	12.00	0.00	37.82	480.29	741.00	11.81
12	60.00	3.00	6.00	14.00	38.21	575.12	934.90	9.02
13	80.00	1.00	12.00	14.00	35.56	725.02	1261.00	12.00
14	60.00	1.00	6.00	0.00	39.38	619.99	739.60	10.80
15	80.00	3.00	12.00	0.00	34.79	244.73	568.60	11.32
16	80.00	1.00	6.00	14.00	35.60	464.99	494.80	11.41

A: Extraction temperature (°C); B: Extraction time (h); C: KOH concentration (%); D: soaking time (h)

Table 3 Analysis of variance (ANOVA) of the factorial model for yield, gel strength, viscosity, and moisture content

	Yield		Gel strength		Viscosity		Moisture content	
	F-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value
Model	356.73	0.0415	3613.60	0.0130	264.11	0.0482	545.79	0.0335
A	331.51	0.0349	3388.36	0.0109	73.91	0.0737	6212.31	0.0081
B	3.92	0.2976	2542.85	0.0126	88.43	0.0674	161.98	0.0499
C	1162.42	0.0187	3047.42	0.0115	135.76	0.0545	36.00	0.1051
D	144.45	0.0528	13645.75	0.0054	94.81	0.0652	94.62	0.0652
AB	5.74	0.2517	267.37	0.0389	11.63	0.1816	73.02	0.0742
AC	22.79	0.1315	205.29	0.0444	686.37	0.0243	157.39	0.0506
AD	0.60	0.5808	684.30	0.0343	2.67	0.3496	464.21	0.0295
BC	34.88	0.1068	12804.42	0.0056	800.00	0.0225	298.35	0.0368
BD	2475.53	0.0128	478.37	0.0291	93.31	0.0657	29.75	0.1154
ABC	208.34	0.0440	1838.94	0.0148	391.35	0.0322	25.00	0.1257
ABD	402.27	0.0317	4226.60	0.0098	102.92	0.0626	11.93	0.1794
ACD	30.15	0.1147	1093.89	0.0192	952.13	0.0206	7.44	0.2237
BCD	50.60	0.0889	3388.50	0.0109	48.62	0.0907	3.64	0.3072
ABCD	120.00	0.0577	2978.39	0.0117	215.64	0.0433	65.46	0.0783

A: Extraction temperature (°C); B: Extraction time (h); C: KOH concentration (%); D: soaking time (h)

The generated mathematical models have demonstrated their significance and validity for each response, substantiated by their small *p*-values ($p < 0.05$) and large *F*-value. These findings indicate that the experimental data and factorial models correspond favorably, at a 95% confidence level. This level of confidence means that there is only a 5% chance that the observed results could occur due to error, supporting the reliability of the models in predicting or explaining the experimental outcomes [14]. Notably, a range of interactions, including AB, AC, AD, BC, BD, ABC, ABD, ACD, BCD, and ABCD, displayed significant effects ($p < 0.05$) on gel strength. Furthermore, interactions involving AC, BC, ABC, ACD, and ABCD demonstrated significant impacts on viscosity. However, only interactions involving BD, ABC, and ABD displayed significant effects on yield, while AD and BC influenced moisture content significantly. The ANOVA results for all the responses are summarized in Table 3.

As shown in Figure 1, the Pareto chart and interaction plots generated from the Design-Expert® Software illustrate the intricate dynamics of the extraction process toward the response of semi-refined carrageenan (SRC). The plots show that the interaction between extraction time and soaking time significantly impacts the SRC yield, following the effects of individual factors which is potassium hydroxide (KOH) concentration (Figure 1a). Extended extraction time correlates positively with yield (Figure 1b). Longer extraction time allows for more thorough penetration of the KOH into the seaweed biomass, facilitating the dissolution of carrageenan into the solution [21]. Conversely, an increase in soaking time leads to a reduced yield. This could be due to excessive hydration potentially leaching out some carrageenan, leading to material loss. Additionally, increasing KOH concentration from 6 to 12% was found to decrease yield from 37.5 to 35.3% (Figure 1c), possibly due to carrageenan degradation.

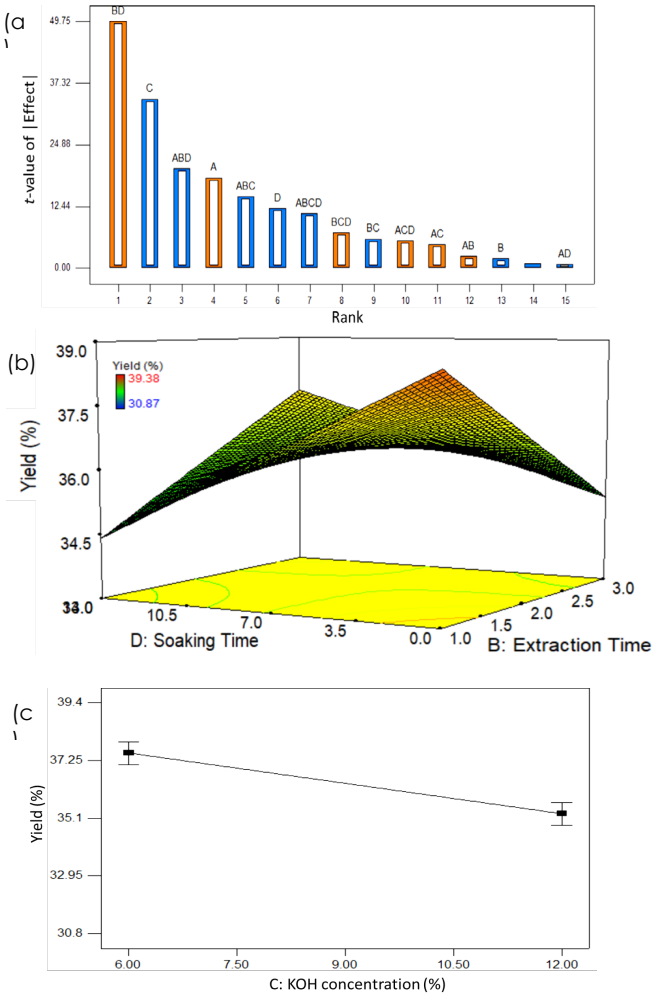


Figure 1 The interaction plots on the effect of extraction temperature (A), extraction time (B), KOH concentration (C), and soaking time (D) on the yield of semi-refined carrageenan. (a) Pareto chart for yield response, (b) 3D interaction surface plot between factor extraction and soaking time, and (c) one-factor plot of KOH concentration effect on the yield response

Figure 2 demonstrates the intricate effects of process variables on the gel strength of SRC. Notably, the Pareto chart (Figure 2a) and one-factor plot (Figure 2b) point out that soaking time significantly boosts gel strength, likely because prolonged soaking periods enhance the hydration of the carrageenan molecules, leading to stronger gel matrices through more extensive intermolecular bonding. However, the 3D interaction surface plot (Figure 2c) suggests that an increase in extraction time inversely affects gel strength. This could be due to excessive extraction times causing degradation of the carrageenan molecular structure, which can weaken the gel. These findings align with Bono *et al*. [22], reporting that prolonged extraction time weakens gel strength. Therefore, increasing soaking time while reducing extraction time helps achieve optimal gel strength by enhancing carrageenan hydration and preventing molecular degradation.

In addition, the 3D interaction surface plots, alongside the Pareto chart (Figure 3), exhibit that increasing extraction temperature from 60 to 80 °C enhances the gel viscosity of SRC, possibly due to the promotion of more effective SRC solubilization and increase in molecular interactions that contribute to viscosity. In contrast, an increase in KOH concentration slightly reduces gel viscosity, which could be attributed to a breakdown of its molecular structure and a resultant decrease in the ability to form a viscous gel [22]. Similarly, prolonged extraction time is shown to decrease viscosity due to the degradation of carrageenan chains over time, which impairs their gelling capability. A comparable trend was also reported by Illias *et al*. [5] as an extended extraction period facilitates the overprocess of carrageenan, which subsequently affects the viscosity and gel strength.

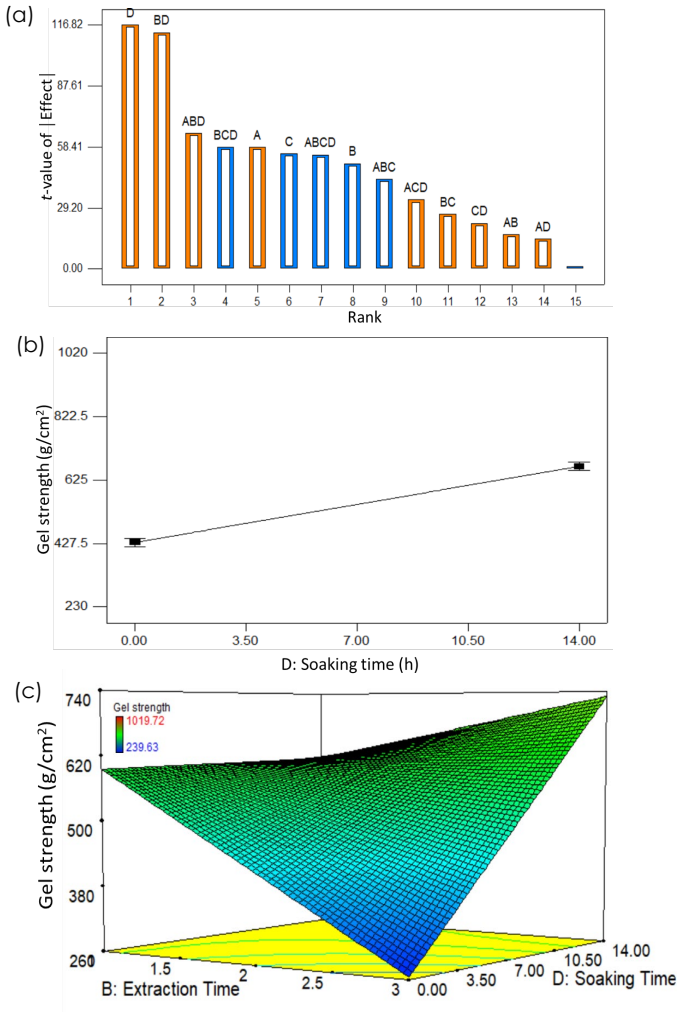


Figure 2 The interaction plots on the effect of extraction temperature (A), extraction time (B), KOH concentration (C), and soaking time (D) on the gel strength of semi-refined carrageenan. (a) Pareto chart for gel strength response, (b) one-factor plot for soaking time, and (c) 3D interaction surface plot between extraction and soaking time

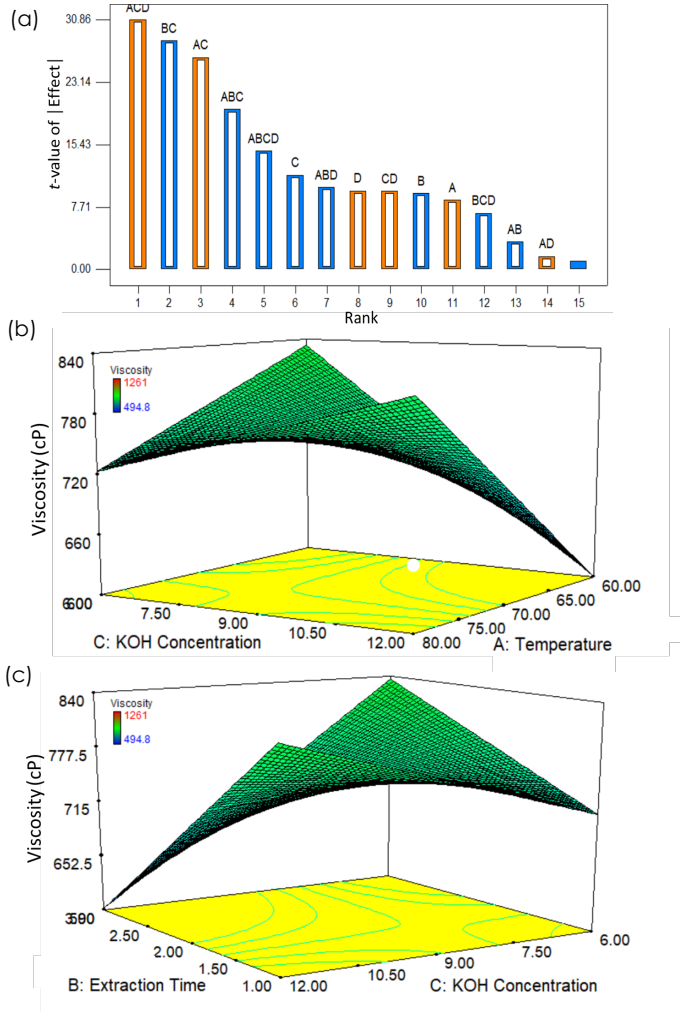


Figure 3 The interaction plots on the effect of extraction temperature (A), extraction time (B), KOH concentration (C), and soaking time (D) on the gel viscosity of semi-refined carrageenan. (a) Pareto chart, (b) 3D interaction surface plot between KOH concentration and extraction temperature, and (c) between extraction time and KOH concentration

Moreover, the extraction temperature was found to be the most significant contributor to SRC moisture content with a positive correlation (Figure 4). This could be due to higher temperatures enhancing the extraction process, allowing more water to integrate with the carrageenan. The 3D interaction surface plot between extraction temperature and soaking time further visualizes this relationship. The increase in moisture content with higher extraction temperature and longer extraction time could be attributed to the greater solubility of carrageenan or the increased hydration capacity at elevated temperatures. The extended time allows for more thorough saturation and integration of water into the carrageenan structure [16]. Therefore, achieving the optimal SRC yield and properties requires a delicate balance in optimizing temperature, extraction time and soaking time while maintaining KOH concentration at an optimal level to avoid degradation of the carrageenan polysaccharides.

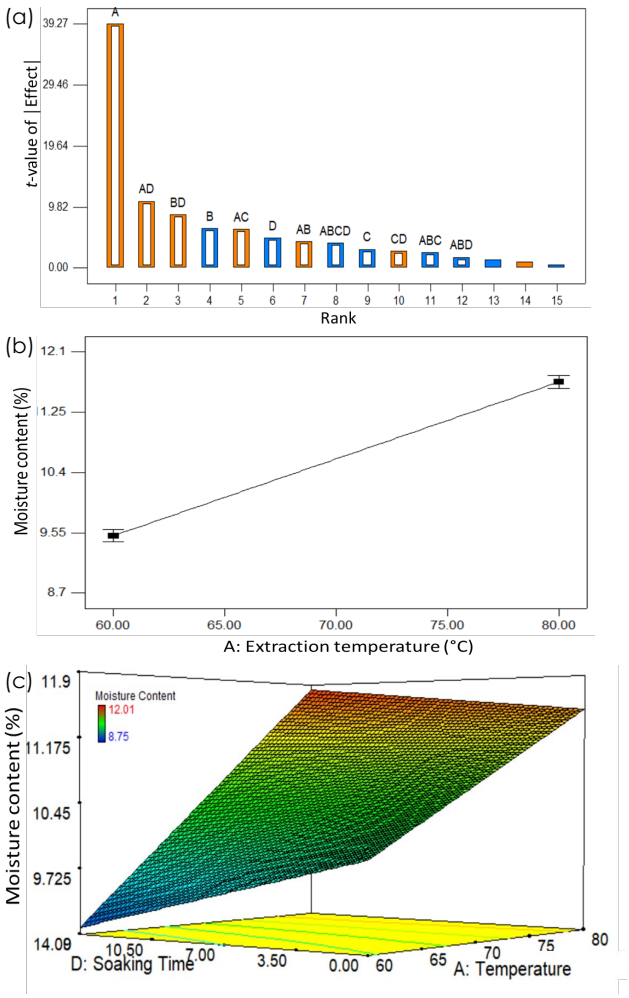


Figure 4 The interaction plots on the effect of extraction temperature (A), extraction time (B), KOH concentration (C), and soaking time (D) on the moisture content of semi-refined carrageenan. (a) Pareto chart for moisture content response, (b) one-factor plot for extraction temperature, and (c) 3D interaction surface plot between extraction temperature and soaking time

Two optimal conditions for extraction of SRC from *K. alvarezii* were selected based on the solutions given by the Design-Expert® Software to maximize SRC yield and improve its properties. The optimized process parameters were suggested based on the highest viscosity value and moisture content for OPT 1. This was achieved by setting the extraction temperature at 80 °C for 3 hours, with a KOH concentration of 6% and a soaking time of 14 hours. These specific conditions were chosen to enhance the breakdown of cellular materials, thereby facilitating the efficient release and ensuring the high quality of SRC. Conversely, the second optimization (OPT 2) aimed at maximizing the yield and gel strength of the SRC, both of which are crucial parameters for the commercial value and functional use of the extract in various applications. This involved maintaining the extraction temperature at 80 °C and soaking time at 14 hours, similar to OPT 1, but with a significant reduction in extraction time to just 1 hour and an increase in KOH concentration to 12%.

As shown in Table 4, OPT 2 was found to produce higher SRC yields and gel strength than OPT 1. OPT 2 was found to significantly enhance the efficiency of the production process, reducing energy consumption and operational costs. The increase in KOH concentration in OPT 2 to 12% aids in a more effective dissolution of cellular components, which contributes to a higher yield and stronger gel strength of SRC. This is particularly beneficial for applications requiring carrageenan with superior gel properties. The slight increase in moisture content in OPT 2 (12.01%) compared to OPT 1 (12.00%) might seem marginal, but it indicates a better hydration state of the carrageenan, which could improve its functionality and ease of use in various applications. However, a higher viscosity value in OPT 1 (1261 cP) than in OPT 2 (776 cP) provides numerous benefits in many applications. This characteristic offers better stability in formulations and able to act as an effective stabilizer.

Additionally, Table 4 demonstrated a remarkable correlation for both OPT 1 and OPT 2 across all measured response variables with the software's prediction values. The obtained values for both OPT 1 and OPT 2 on each response corresponded to the predicted values. Furthermore, the RSE percentage values were notably low, under 2%. It confirms that the software can accurately predict the impact of different process parameters on the extraction efficiency and the quality of SRC, making it a valuable tool for optimizing the extraction processes. In this study, the optimal process parameters were further used to produce SRC films. The mechanical properties of the films derived from the extracted SRC, such as tensile strength (TS) and elongation at break (EAB) were further characterized and compared with the film derived from commercial SRC (Primegum SRC, Indonesia). The SRC films have been synthesized using the identified optimal process parameters.

Table 4 Actual and predicted response values for optimal conditions of semi-refined carrageenan extraction

	OPT 1			OPT 2		
	Obt.	Pre.	RSE (%)	Obt.	Pre.	RSE%
Yields (%)	35.56	35.59	0.08	39.20	39.17	0.08
Gel strength (g/cm^2)	725.02	726.03	0.14	1019.72	1018.71	0.10
Viscosity (cP)	1261.00	1255.00	0.48	775.80	778.87	0.39
Moisture content (%)	12.00	11.88	1.01	12.01	12.05	0.33

A: Extraction temperature (°C); B: Extraction time (h); C: KOH concentration (%); D: soaking time (h); Pre: Predicted; Obt.: Obtained; RSE: Residual standard error

The mechanical properties, specifically TS and EAB, of the films were meticulously characterized and presented in Table 5. Two variants of the SRC films, designated as OPT 1 and OPT 2 films, demonstrated TS values of 34.40 MPa and 34.52 MPa respectively. These values are notably lower than that of the commercial film, which exhibited a higher tensile strength of 45.98 MPa. However, the values are still within a range considered adequate for biopolymer films (10-100 MPa), indicating that the SRC films have a respectable strength profile [23]. For EAB, the SRC films demonstrate a greater ability to stretch, with a maximum strain value of 31.30%, compared to the commercial film's 19.18%. This higher EAB suggests that the SRC films are more flexible and stretchable, which is a key characteristic for certain applications. Films with higher EAB are less prone to cracking or breaking under bending or stretching forces, which enhances their durability and resilience [24]. This property is mostly essential for films that need to be elastic, such as packaging materials.

Table 5 Mechanical properties of films derived from extracted and commercial SRC

	TS (MPa)	EAB (%)
OPT 1 film	34.40	31.30
OPT 2 film	34.52	24.44
Commercial SRC film	45.98	19.18

TS: Tensile strength; EAB: elongation at break

4.0 CONCLUSION

This study successfully optimized the extraction process of SRC from *K. alvarezii* using a factorial design methodology and identified two optimal process conditions, OPT 1 and OPT 2 achieving predictive accuracies over 99% for key properties. Mechanical testing of SRC films revealed tensile strengths close to 34.5 MPa for both OPT 1 and OPT 2 films, with exceptional flexibility (EAB of 31.30%), surpassing commercial alternatives. These findings highlight the SRC films' potential as durable, flexible, and sustainable packaging materials.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

[1] Yuriz, Y., Ismail, T. N. H. T., Mohamed, I., Hassan, N. N. M. 2021. Characteristic Properties of Plastic Wastes: Possibility of Reinforcing Material for Soil. *J. Teknol*. 83: 127–136. https://doi.org/10.11113/jurnalteknologi.v83.14676.

[2] Schmaltz, E., Melvin, E. C., Diana, Z., Gunady, E. F., Rittschof, D., Somarelli, J. A., Virdin, J., Dunphy-Daly, M. M. 2020. Plastic Pollution Solutions: Emerging Technologies to Prevent and Collect Marine Plastic Pollution. *Environ. Int*. 144: 106067. https://doi.org/10.1016/j.envint.2020.106067.

[3] Cheng, C., Chen, S., Su, J., Zhu, M., Zhou, M., Chen, T., Han, Y. 2022. Recent Advances in Carrageenan-based Films for Food Packaging Applications. *Front. Nutr*. 9: 1004588. https://doi.org/10.3389/fnut.2022.1004588.

[4] Genecya, G., Adhika, D. R., Sutrisno, W., Wungu, T. D. K. 2023. Characteristic Improvement of a Carrageenan-based Bionanocomposite Polymer Film Containing Montmorillonite as Food Packaging through the Addition of Silver and Cerium Oxide Nanoparticles. *ACS Omega*. 8(42): 39194–39202. https://doi.org/10.1021/acsomega.3c04575.

[5] Ilias, M. A., Ismail, A., Othman, R. 2017. Analysis of Carrageenan Yield and Gel Strength of *Kappaphycus* Species in Semporna Sabah. *J. Trop. Plant Physiol.* 9: 14–23.

[6] Younes, M., Aggett, P., Aguilar, F., Crebelli, R., Filipič, M., Frutos, M. J., Galtier, P., Gott, D., Gundert-Remy, U., Kuhnle, G. G., Lambré, C., Leblanc, J. C., Lillegaard, I. T., Moldeus, P., Mortensen, A., Oskarsson, A., Stankovic, I., Waalkens-Berendsen, I., Woutersen, R. A., Wright, M., Brimer, L., Lindtner, O., Mosesso, P., Christodoulidou, A., Ioannidou, S., Lodi, F., Dusemund, B. 2018. Re-evaluation of Carrageenan (E 407) and Processed Eucheuma Seaweed (E 407a) as Food Additives. *EFSA J*. 16: e05238. https://doi.org/10.2903/J.EFSA.2018.5238.

[7] Thye, K. L., Wan Abdullah, W. M. A. N., Balia Yusof, Z. N., Wee, C. Y., Ong-Abdullah, J., Loh, J. Y., Cheng, W. H., Lamasudin, D. U., Lai, K. S. 2022. λ-Carrageenan Promotes Plant Growth in Banana via Enhancement of Cellular Metabolism, Nutrient Uptake, and Cellular Homeostasis. *Sci. Rep.* 12: 1–12. https://doi.org/10.1038/s41598-022-21909-7.

[8] Shafie, M. H., Kamal, M. L., Zulkiflee, F. F., Hasan, S., Uyup, N. H., Abdullah, S., Mohamed Hussin, N. A., Tan, Y. C., Zafarina, Z. 2022. Application of Carrageenan Extract from Red Seaweed (*Rhodophyta*) in Cosmetic Products: A Review. *J. Indian Chem. Soc*. 99(9): 100613. https://doi.org/10.1016/j.jics.2022.100613.

[9] Avila, L. B., Barreto, E. R. C., Moraes, C. C., Morais, M. M., da Rosa, G. S. 2022. Promising New Material for Food Packaging: An Active and Intelligent Carrageenan Film with Natural *Jaboticaba* Additive. *Foods.* 11: 1–15. https://doi.org/10.3390/foods11060792.

[10] Gupta, N. V., Chakraborty, S., Veeranna, B., Osmani, R. A. M., Moin, A., Sastri, K. T., Sharadha, M., Ramkishan, A. 2023. Carrageenan-Based Drug Delivery Systems for Respiratory Disease. *Nat. Polym. Mater. based Drug Deliv. Syst. Lung Dis.* 381–395. https://doi.org/10.1007/978-981-19-7656-8_20/COVER.

[11] Pacheco-Quito, E. M., Ruiz-Caro, R., Veiga, M. D. 2020. Carrageenan: Drug delivery Systems and Other Biomedical Applications. *Mar. Drugs.* 18(11): 583. https://doi.org/10.3390/md18110583.

[12] Solorzano-Chavez, E. G., Paz-Cedeno, F. R., Ezequiel de Oliveira, L., Gelli, V. C., Monti, R., Conceição de Oliveira, S., Masarin, F. 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. https://doi.org/10.1016/J.BIOMBIOE.2019.105254.

[13] Ferdiansyah, R., Abdassah, M., Zainuddin, A., Rachmaniar, R., Chaerunisaa, A. Y. 2023. Effects of Alkaline Solvent Type and pH on Solid Physical Properties of Carrageenan from *Eucheuma cottonii*. *Gels*. 9(5): 397. https://doi.org/10.3390/GELS9050397.

[14] Asmawi, A. A., Salim, N., Abdulmalek, E., Abdul Rahman, M. B. 2023. Size-controlled Preparation of Docetaxel- and Curcumin-loaded Nanoemulsions for Potential Pulmonary Delivery. *Pharmaceutics.* 15(2): 652. https://doi.org/10.3390/pharmaceutics15020652.

[15] Ganesan, A. R., Munisamy, S., Bhat, R. 2018. Effect of Potassium Hydroxide on Rheological and Thermo-mechanical Properties of Semi-refined Carrageenan (SRC) Films. *Food Biosci.* 26: 104–112. https://doi.org/10.1016/J.FBIO.2018.10.003.

[16] Heriyanto, H., Kustiningsih, I., Kartika Sari, D. 2018. The Effect of Temperature and Time of Extraction on the Quality of Semi Refined Carrageenan (SRC). *MATEC Web of Conferences.* 15: 01034. https://doi.org/10.1051/matecconf/201815401034.

[17] Muñoz, J., Freile-Pelegrín, Y., Robledo, D. 2004. Mariculture of Kappaphycus alvarezii (Rhodophyta, Solieriaceae) Color Strains in Tropical Waters of Yucatán, México. *Aquaculture.* 239: 161–177. https://doi.org/10.1016/j.aquaculture.2004.05.043.

[18] Abd Hamid, K. H., Jayakumar, T., Gunasegaran, S., Mohd Azman, N. A. n.d. View of Fabrication and Characterization of Semi-refined Carrageenan Films Incorporated with TiO_2 Nanoparticles. *J. Chem. Eng. Ind. Biotechnol.* 9(1): 1–7. https://doi.org/10.15282/jceib.v9i1.9466.

[19] Farhan, A., Hani, N. M. 2017. Characterization of Edible Packaging Films based on Semi-refined Kappa-carrageenan Plasticized with Glycerol and Sorbitol. *Food Hydrocoll.* 64: 48–58. https://doi.org/10.1016/J.FOODHYD.2016.10.034.

[20] ASTM D882. 2012. D882 Standard Test Method for Tensile Properties of Thin Plastic Sheeting. https://www.astm.org/d0882-18.html.

[21] Mendes, M., Cotas, J., Gutiérrez, I. B., Gonçalves, A. M. M., Critchley, A. T., Hinaloc, L. A. R. 2024. Advanced Extraction Techniques and Physicochemical Properties of Carrageenan from a Novel *Kappaphycus alvarezii* cultivar. *Mar Drugs.* 22(11): 491. https://pmc.ncbi.nlm.nih.gov/articles/PMC11595270/.

[22] Bono, A., Anisuzzaman, S. M., Ding, O. W. 2023. Effect of Process Conditions on the Gel Viscosity and Gel Strength of Semi-refined Carrageenan (SRC) Produced from Seaweed (Kappaphycus alvarezii). *J. King Saud Univ. – Eng. Sci.* 26(1): 3–9. https://doi.org/10.1016/j.jksues.2012.06.001.

[23] Kong, U., Mohammad Rawi, N. F., Tay, G. S. 2023. The Potential Applications of Reinforced Bioplastics in Various Industries: A Review. *Polym.* 15(10): 2399. https://doi.org/10.3390/POLYM15102399.

[24] Boey, J. Y., Lee, C. K., Tay, G. S. 2022. Factors Affecting Mechanical Properties of Reinforced Bioplastics: A Review. *Polym.* 14(18): 3737. https://doi.org/10.3390/POLYM1483737.