Volume 16, Issue 1, 162503 (1-11)

International Journal of Industrial Chemistry (IJIC)

https://doi.org/10.57647/j.ijic.2025.1601.03

Enhanced green extraction of phytochemical compound from *Eucheuma Cottonii* using supercritical CO_2 and Sub-critical water as a solvent: Optimization and kinetics modeling

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Original Research

Received:
10 December 2024
Revised:
12 March 2025
Accepted:
12 March 2025

Abstract:

Numerous active ingredients found in *Eucheuma cottonii* have applications in the food, cosmetic, and pharmaceutical industries, among others. Extraction is one of the biomass separation procedures that yields high purity. This work used supercritical CO_2 and subcritical water as a solvent to extract phytochemical components from *Eucheuma cottonii*. Both solvents used are easily available, environmentally friendly, and affordable. Supercritical CO_2 extraction is carried out at a temperature of 60 °C and a pressure of 25 MPa, while Sub-critical water extraction (SWE) is carried out at a temperature of 120 °C − 160 °C and a pressure of 3 MPa − 5 MPa. The yields of β-carotene and linoleic acid were 0.0019 μg/g sample and 0.0841 μg/g sample. Meanwhile, the results of SWE were kappa-carrageenan and total phenolic compounds with the largest yield of 55.8% at a temperature of 160 °C and 5 MPa. The data obtained were then analyzed for optimum operating conditions using Face-centered central composite design (FCCCD), where the optimum operating conditions correspond to the largest value of the TPC yield produced. In addition, the data were analyzed using extraction kinetics modeling, where the results showed agreement with first-order kinetics and the activation energy value was 20.563 KJ/mol.

Keywords: *Eucheuma Cottonii*; FCCCD; First-order kinetics; Supercritical CO_2; Subcritical water

1. Introduction

The demand for naturally occurring chemicals produced by natural processes has sharply increased due to increased interest in functional foods. Specific drawbacks of the conventional solvent extraction methods have been demonstrated, including low recovery, flammability, toxicity, and mutagenicity. Therefore, this research uses two clean processes crucial to food technology: supercritical CO_2 extraction and subcritical water extraction (SWE). One critical step in extracting phenolic and other bioactive constituents from *Eucheuma cottonii* is solvent selection [1]. Several factors influence the efficiency of phenolic components, including solvent polarity, temperature and time. Polarity affects solubility because, generally, polar solvents dissolve polar molecules, and nonpolar solvents dissolve nonpolar molecules. The polarity of CO_2 is the primary cause of the limitations of supercritical CO_2 extraction. CO_2 is a low-polar solvent better suited for extracting nonpolar substances from *Eucheuma cottonii*, like linoleic acid and β-carotene [2]. Phenolic compounds include hydroxyl groups, so they dissolve better in polar solvents than in nonpolar ones [3].

Extraction of bioactive compounds from natural products requires environmentally friendly methods [4]. Supercritical fluid extraction satisfies these specifications. Because of this, supercritical fluid is currently used in various processes such as coatings, extractions, impregnations, particle creation, reactions, and separations [5]. which it removes from the extract after extraction. In addition, CO_2 is affordable, non-flammable, non-toxic, and environmentally friendly [6, 7]. It is possible to extract non-polar molecules from a natural product using supercritical CO_2 like β-carotene and linoleic acid in *Eucheuma cottonii* [8, 9].

Eucheuma cottonii contains phytochemical compounds that various industries can utilize. In the food and beverage sector, carrageenan is helpful as a thickening agent and product stabilizer [10–12]. The pharmaceutical industry uses linoleic acid to prevent Alzheimer's disease, control blood pressure and cholesterol, and maintain heart health [13, 14]. *Eucheuma cottonii's* β-carotene has biological properties that include anti-inflammatory, anti-cancer, anti-obesity, hepatotoprotection, and immunomodulation [15–17]. Phenolic compounds are typically involved in defence against UV radiation or hostility from pathogens, parasites, and predators. They also contribute to the colour of plants [18]. The residue of supercritical CO_2 extraction is then used as raw material for subcritical water extraction.

Subcritical water extraction (SWE) is an appropriate approach to maximise compounds' release. SWE is an emerging technique for separating chemicals from biomass at pressures of 5 to 22 MPa and temperatures of 100 to 374 °C while using water as the solvent [19]. In subcritical water extraction, pressure keeps the water in a liquid form. In contrast, the temperature of the extraction solvent is raised above the boiling point of the atmosphere. The solubility and diffusion rate of the target compounds rise as the water's viscosity and surface tension fall [20]. In comparison, the extraction procedure at room temperature is slower regarding the water's penetration into the matrix and the chemicals' transfer out of the matrix [21]. As a result, the subcritical water extraction technology achieves more efficient and quicker extraction than traditional approaches. This is the primary explanation for why extraction at higher pressures and temperatures performs better than extraction at lower atmospheric pressures and temperatures [22–24]. Water may dissolve lignocellulosic materials and extract polar organic molecules at subcritical conditions, producing essential chemicals, including saccharides and aromatic organic acids. Protein, amino acids, and phenolic compounds have all been recovered using this technique [20]. In this study, extraction with subcritical water extracts polar compounds such as carrageenan and total phenolic compounds from *Eucheuma cottonii*.

Response Surface Methodology, or RSM, is a set of statistical and mathematical tools that are helpful in modelling and evaluating situations where optimizing the desired response is contingent upon several variables [25]. In the extraction process, RSM can be used to see the effect of quantitative variables such as temperature and pressure and to determine the optimum operating conditions to obtain maximum yield [26–28]. The optimization results were then analyzed using extraction kinetics modelling, and the activation energy was calculated.

Eucheuma cottonii were restricted to polar or non-polar molecules using traditional or cutting-edge techniques. However, the purpose of this work is to use the supercritical CO_2 extraction method followed by sub-critical water extraction to extract polar compounds like carrageenan and total phenolic compounds and non-polar compounds like β-carotene and linoleic acid from *Eucheuma cottonii*. Supercritical CO_2 extraction using optimal variables that previous researchers have carried out, with operating conditions of 60 °C and 25 MPa [29]. RSM analysis was also performed to ascertain the ideal operating parameters for achieving the highest yield values. Additionally, the RSM data can be used to calculate the proper activation energy and kinetic modeling variables to aid in future scale-up procedures.

2. Materials and methods

2.1 Materials

The *Eucheuma cottonii* used was taken from Madura Island, Indonesia. The algae was dried at 60 °C for 24 hours and ground to a small size. Ethanol pro analysis and aqua dest were obtained from Smartlab Indonesia (South Tangerang, Banten). All chemicals used were analytical standards.

2.2 Supercritical CO_2 extraction

The operating conditions for extraction using supercritical CO_2 were constant at 60 °C and a pressure of 25 MPa. Before the extraction procedure began, *Eucheuma cottonii* had to be prepared with fresh water to remove any debris or salt that may have still been adhered to the seaweed. After cleaning, the seaweed was dried for 24 hours at 60 °C in an oven. After that, the dried seaweed was milled till its size decreased. This attempted to expand the contact region between the solvent and the algae extraction process. After completing this preparation step, *Eucheuma cottonii* was utilized as a raw material for supercritical CO_2 extraction. First, 12 grams of *Eucheuma cottonii* were put into the extractor. Glass beads were placed on both sides to prevent blockages and spread the solvent to all sides of the extractor as shown in figure 1 (a). Next, the extractor was installed on the heater. An HPLC (High-Performance et al.) pump was used to pump the CO_2 solvent at a 15 mL/minute rate. Conversely, ethanol was pumped using the same pump as a co-solvent at 0.25 mL/minute. Ethanol was chosen because it had safer properties than other solvents such as acetone, petroleum ether, hexane, and methanol [26]. Then, the mixture of CO_2 and ethanol was heated and flowed to the extractor. The operating pressure could be controlled using a BPR equipped with a heater. The temperature at the inlet and outlet streams of the extractor could be measured using a thermocouple. The extraction process lasted for 3 hours, with the vial being replaced every 30 minutes.

2.3 Sub-Critical water extraction

First, 1 gram of extraction CO_2 residue was inserted into the extractor between the glass beads on both sides. Then, the water was pumped using an HPLC pump at a 1 mL/minute rate. Next, the water was heated and flowed to the extractor as shown in figure 1 (b). Extraction was carried out at temperatures of 120 °C, 140 °C, and 160 °C and pressures of 3 MPa, 5 MPa, and 7 MPa. A backpressure regulator (BPR) regulates and controls the operating pressure. After the extraction process occurred, the extract and solvent were transferred to the vial. The extraction lasted 180 minutes,

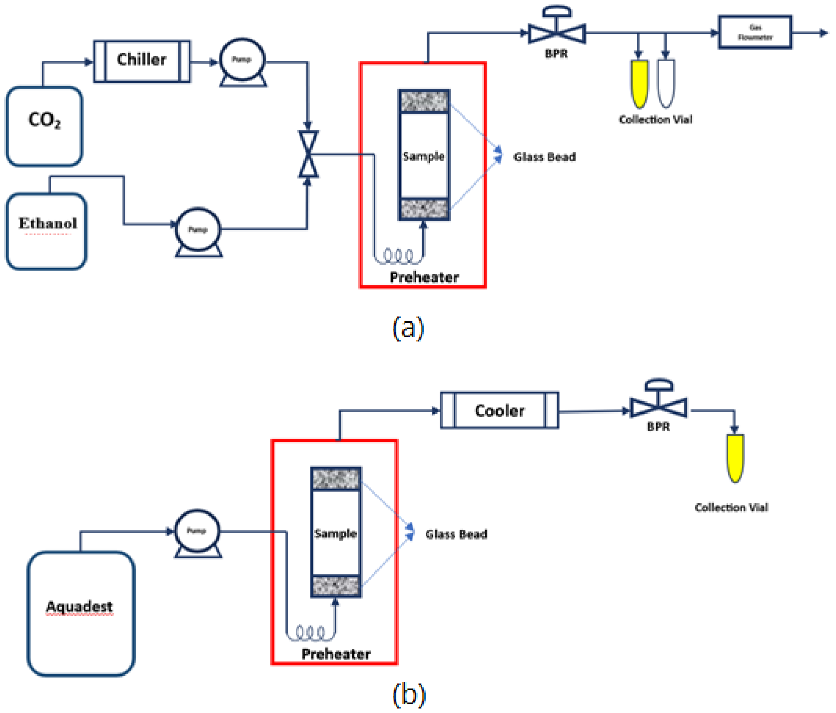

Figure 1. Extraction equipment (a) Supercritical CO_2 (b) Sub-critical water.

and the results were taken every 30 minutes.

2.4 Linoleic acid and β carotene analysis

A spectrophotometer set at 450 nm and 195 nm wavelengths was used to measure the extract's levels of β-carotene and linoleic acid, respectively. The β-carotene and linoleic acid standards (purity $> 80\%$) used to prepare the standard curve are sourced from Wako Pure Chemical Industries, Ltd., Japan. First, a calibration curve was created by measuring the concentration of linoleic acid or β-carotene in a standard solution at $100 - 500$ mg/L. Before analysis, the extract solution was diluted with n-hexane. The extract's absorbance was compared to the calibration curve to determine the presence of β-carotene and linoleic acid.

2.5 Yield analysis of carrageenan

The extract and 90% ethanol were put into an evaporator cup with a 1:2 (v/v) composition while stirring until a hydrocolloid (carrageenan fiber) was formed. Then, it was left for 30 minutes. Then, it was heated in the oven for 24 hours at 60 °C. Next, the dry carrageenan was weighed, and equation (1) was used to determine the carrageenan yield.

$$\text{Yield }(\%) = \frac{\text{weight of dry carrageenan}}{\text{weight of dry } \textit{Eucheuma cottonii}} \times 100\% \tag{1}$$

2.6 Total phenolic compound

The total content of phenolic compounds can be determined using the following procedure. First, 1 mL of extract was added with 2 mL of distilled water and 1 mL of Folin Ciocalteu reagent while stirring. Next, leave it for 5 minutes, then add 1 mL of 7% Na_2CO_3 solution. The mixture was then incubated in a dark room for 30 minutes (at room temperature). Next, the sample was analyzed using a UV-Vis spectrophotometer at a wavelength of 750 nm. The TPC content in the extract was determined based on the equation of the standard curve line for gallic acid at a concentration of $0 - 200$ mg/L.

2.7 Optimization RSM model

A faced-centered central composite design (FCCCD) was applied to determine the optimal levels of the two independent variables, namely, temperature (120 °C − 160 °C) and Pressure (3 MPa − 7 MPa). The second-order polynomial model was fitted to the data using the Design-Expert software (version 13, Stat-Ease Corporation, Minneapolis, MN, USA):

$$Y = \alpha_0 + \alpha_1 x_1 + \alpha_2 x_2 + \alpha_{12} x_1 x_2 + \alpha_{11} x_1^2 + \alpha_{22} x_2^2 \tag{2}$$

where α_{12} are the interaction coefficients; Y is the response calculated by the model (yield value); α_{11} and α_{22} are the quadratic terms ; x_1 and x_2 denote the independent variables; α_0 is the interception coefficient. The significance level for statistical analysis was set at $p < 0.05$, which is less than 0.05. R^2, adjusted-R^2, and the coefficient of variation (CV) were statistical measures used to evaluate the suitability of the generated models. This optimization method also has advantages compared to classical optimization methods that usually use a one-factor-at-a-time approach, where only one factor is varied while other variables remain constant. This classical approach tends to be expensive and takes longer [30].

2.8 Kinetic analysis

Total phenolic compound (TPC) from *Eucheuma cottonii* assisted Sub-Critical water extraction was modeled by using kinetics of order 1 and order 2 with explanation of the determinant constant values for selecting the appropriate approach model. The first-order kinetic equation can be written as in equation (3):

$$\log(C_s - C_t) = \log(C_s) - \frac{k_1}{2.303}t \tag{3}$$

Value of C_t is the TPC yield value at a certain extraction time and t is the extraction time. Value of C_s trial is carried out on the left side to get the log value $(C_s - C_t)$. A plot is made between value of log $(C_s - C_t)$ as y-axis vs value of t as x-axis. The slope value was used to obtain extraction rate (k_1) and the intercept was used to obtain the maximum extraction capacity value (C_s) [8].

A second-order mechanism model denotes two simultaneous processes involved in the extraction process. At first, the amount of extracted phytochemical substances increases quickly with time, but as the extraction process progresses, it gradually diminishes. Equation (4) can be used to characterize the phytochemical's rate of dissolution from raw material to solution.

$$\frac{t}{C_t} = \frac{t}{C_s} + \frac{1}{k_2 C_s^2} \tag{4}$$

where C_t is the concentration of TPC at any given time t (min), C_s is the extraction capacity (concentration of phytochemical compounds at saturation in (g L^{-1}), and k is the second-order extraction rate constant (L g^{-1} min^{-1}). The integrated rate law for the second-order extraction was established by considering the beginning and boundary conditions, $t = 0$ to t and $C_t = 0$ to C_t :

$$C_t = \frac{C_s^2 kt}{1 + C_s kt} \tag{5}$$

The extraction rate can be expressed as Eq. (7) by translating Eq. (6) into the linear form that is displayed in Eq. (6).

$$\frac{t}{C_t} = \frac{t}{C_s^2} + \frac{t}{C_s} \tag{6}$$

$$\frac{C_t}{t} = \frac{1}{1/C_s^2 + t/C_s} \tag{7}$$

When t approaches 0, the initial extraction rate, h, as C_t/t, can be defined as follows:

$$h = kC_s^2 \tag{8}$$

Moreover, following rearrangement, the concentration of the phytochemical's compound at any given moment can be written as:

$$C_t = \frac{t}{(1/h) + (t/C_s)} \tag{9}$$

A plot of t/C_t vs t can be used to experimentally derive the initial extraction rate (h), the extraction capacity (C_s), and the second-order extraction rate constant (k) from the slope and intercept.

2.9 Statistical analysis

The coefficient of determination R^2 and Root Mean Squared Error (RMSE) are used to assess each model's validation for total phenolic compound extraction. The coefficient determination is defined by:

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(x_i - \hat{x}_i)^2}{\sum_{i=1}^{n}(x_i - \hat{x})^2} \tag{10}$$

where x_i is the value of total phenolic compound, for observation i obtained from experiment, $\hat{x}_i$ is the mean value, $\hat{x}$ is the predicted value of citronella oil yield for observation obtained from the kinetic models and n is the total numbers of data. As for the RSME, defined by:

$$\text{RSME} = \sqrt{\frac{1}{m}\sum_{i=1}^{n}(x_i - \hat{x}_i)^2} \tag{11}$$

where m represents the number of total observations.

3. Results and discussion

3.1 SEM results

Subcritical water extraction is one of the most promising green separation and extraction techniques. Subcritical water has a temperature and pressure below its critical point of 374.15 °C and 22.1 MPa, respectively. The water pressure must be greater than the vapour pressure at a specific temperature to maintain water in its liquid state. The physical-chemical characteristics of subcritical water vary significantly as temperature rises. Water's dielectric constant drastically decreases as temperature rises, becoming less polar. This implies that temperature changes can adjust the polarity of water. As a result, water may effectively extract polar molecules at "lower" temperatures, but less polar analytes need higher temperatures to obtain a respectable extraction efficiency [20]. Therefore, SWE can extract various phytochemical compounds contained in *Eucheuma cottonii*. The samples were analysed using SEM to determine the differences in the morphology of *Eucheuma cottonii* before extraction, after extraction, and the results of carrageenan. The initial material undergoes morphological modifications as a result of the extraction procedure. This change occurs due to the first extraction mechanism, which involves the penetration of the material by the solvent. As a result of this penetration or diffusion process, the sample matrix may be damaged or ruptured. Furthermore, the solute will disperse, dissolve in the solvent, and re-emerge. Figure 2 (a) illustrates the morphological deterioration of the material; the morphology of the initial material appears smoother and more compact than figure 2 (b). The carrageenan product from subcritical water extraction is displayed in figure 2 (c). According to FTIR analyses, kappa carrageenan is the kind of carrageenan generated by SWE.

3.2 FTIR results

In the extraction process, subcritical water, operating temperature, and pressure are two crucial connected factors. Under subcritical circumstances, the level of water polarity will depend on these two factors. Figure 3 shows the difference in the results of FTIR (Fourier Transform Infrared) analysis on the starting material, supercritical extraction residue, and subcritical water extraction residue. Based on

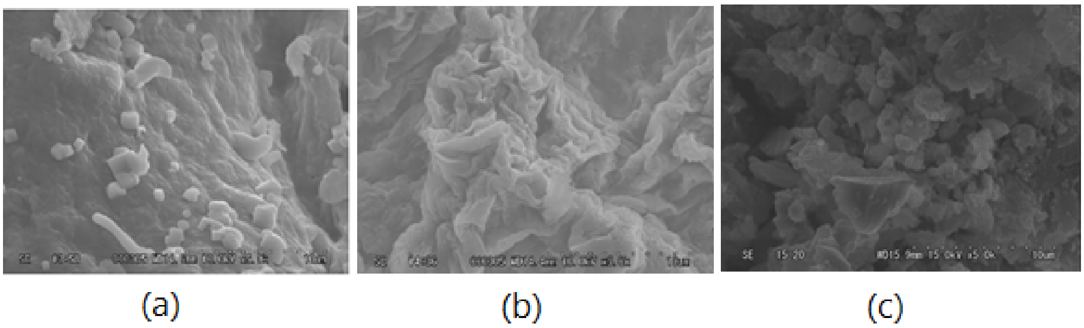

Figure 2. SEM analysis *Eucheuma Cottonii* (a) Starting material (b) Residue (c) Kappa carrageenan.

the figure, it can be seen that carrageenan has been successfully extracted because the peaks of the residue spectrum are lower than those of the starting material.

The peaks of the spectrum indicate the presence of carrageenan, namely the D-galactose-4-sulfate bond in the range of 840 − 850 cm^{-1}, the 3,6-anhydro-D-galactose bond in the range of 928 − 933 cm^{-1}, the glycosidic bond in the range of 1010 − 1080 cm^{-1}, and the sulfate ester bond (O=S=O) in the range of 1210 − 1260 cm^{-1} [31]. There are various types of carrageenan, three of which are widely used in the industrial world, namely iota (τ), kappa (κ), and lambda (λ) carrageenan. The extracted carrageenan was kappa-carrageenan, which was characterized by the presence of peaks at 932 cm^{-1} and 848 cm^{-1}.

$$A = -\log T \tag{12}$$

Based on figure 3, it can be seen that the transmittance value at each stage of the process is different. There is a significant change in transmittance value at several functional group peaks. This change indicates that the remaining functional groups in the residue have decreased. Lambert Beer's law, written in equation (12), states that the transmittance will be inversely proportional to the absorbance value [32]. so the higher the transmittance value, the smaller the level of the functional group because the functional group has been extracted.

3.3 Linoleic acid and β carotene result

The products resulting from supercritical extraction are β-carotene and linoleic acid. The standard curve is created using β-carotene as the standard. The outcome is shown in figure 4 with the line equation y = 0.0025x + 0.0008 (R^2 = 0.9923). β-carotene is a naturally occurring pigment with inherent antioxidant properties [33]. Antioxidants like (β-carotene) can stop lipids from oxidizing. Based on analysis using a UV-VIS Spectrophotometer, the results of supercritical CO_2 extraction at 60 °C and 25 MPa showed that the content of β-carotene in *Eucheuma cottonii* reached 0.0019 μg/g sample. *Eucheuma cottonii's* linoleic acid content was found to be 0,0841 μg/g sample, according to analysis performed with a UV-VIS Spectrophotometer and the results of supercritical CO_2 extraction at 60 °C and 25 MPa.

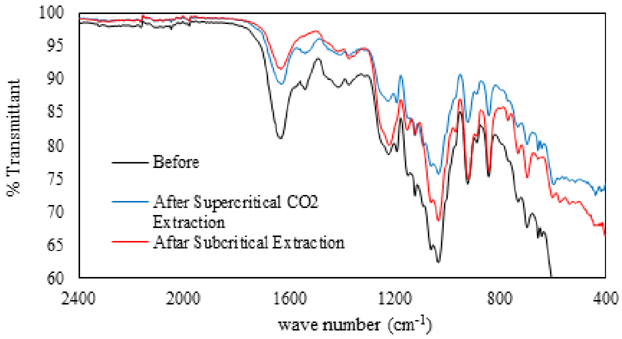

Figure 3. Results of FTIR analysis of raw material, supercritical CO_2 extraction residue and subcritical water extraction residue.

3.4 Yield of carrageenan result

The highest carrageenan yield value using SWE is 55.8%. *Eucheuma cottonii* extracted by the conventional hydrolysis method, namely at an operating temperature of 50 °C for 2 hours at pH 5, produced a yield of 38% [34]. This shows that the use of subcritical water as a solvent is better than the conventional method. It is evident from figure 5 that

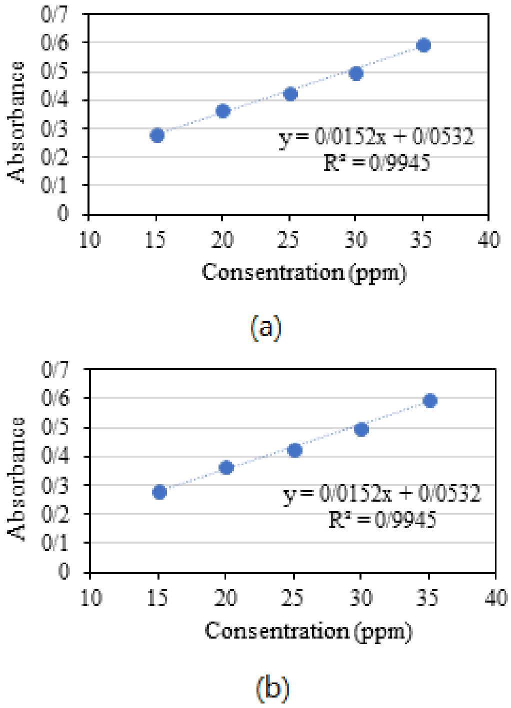

Figure 4. (a) Standard curve of β-Carotene (b) Standard curve of linoleic acid.

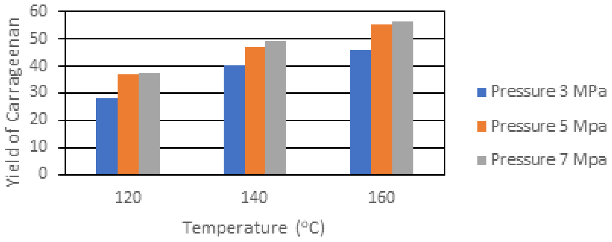

Figure 5. Carrageenan yield from sub-critical water extraction.

the yield value increases in direct proportion to increases in pressure and temperature. Although pressure is essential to maintaining the liquid state at the working temperature, it has little impact on the SWE as a variable because pressure variations below 100 MPa slightly alter the dielectric constant [35]. For instance, raising the pressure from 10 MPa to 20 MPa at 100 °C only results in a 5.2% increase in the dielectric constant. Additionally, as pressure rises, there is a proportional increase in the amount of solvent (subcritical water) per unit volume, which enhances the extract's solubility and results in a more optimum extract [36].
Since chemicals become more soluble at higher temperatures, more substances or components will be removed as the temperature rises [37]. Raising the temperature will also cause the solute's surface tension and viscosity to decrease, improving the extraction efficiency. Furthermore, in rising temperaures, carrageenan becomes more soluble in water when the temperature rises because it also increases the amount of product ions and decreases the solvent's dielectric constant.

3.5 RSM results

The ANOVA results, whose R^2 varied from 0.90 to 0.99, show the high precision of the second-order polynomial model. Based on Table 1.(a), it can be seen that the adjusted R^2 value and predicted R^2 have a difference of < 0.2. This demonstrates how the equation mode can represent actual experiments with these operational boundary conditions.
The average predicted yield displayed in equation (2) is computed using the equation in Table 1.(b). Equation in Table 1.(b) will be used to determine the expected yield value. This demonstrates that the modelling employed is suitable. With a quadratic model, the ANOVA results in Table 1.(b) are frequently significant. When the quadratic model's analysis of variance in ANOVA (Table 1.(c)) yields a p-value of less than 0.05, it is considered a logical and valid model. The model is significant, as indicated by its F-value around 119.84 – 220.19. The factors that impact the yield response include the pressure and temperature of the sub-critical water extraction process

3.5.1 Effect of temperature and pressure parameter on parameter yield

Figure 6 depicts the contour and plot of temperature against concentration to produce phytochemical substances. The temperature and pressure at which phytochemical substances are extracted from *Eucheuma cottonii* affect their

Table 1. **(a)** RSM's performance assessment.

Response	Mean	Standard Deviation	Coefficient of Variation %	R^2	Adjusted R^2	Predicted R^2
Yield (%)	44.01	0.7844	1.78	0.9973	0.9928	0.9764
TPC (mg GAE/g sample)	9.54	0.4189	4.39	0.9950	0.9867	0.9506

Table 1.(b) Mathematic equation.

Parameter response	Mathematic Equation
Yield (%)	$Y = -147.73778 + 9.46458\ x_1 + 1.90777\ x_2 + 0.009312\ x_1\ x_2 - 0.839583x_1^2 - 0.005333x_2^2$
TPC (mg GAE/g sample)	$Y = 50.22056 - 0.78625\ x_1 - 0.733104\ x_2 - 0.023062\ x_1\ x_2 - 0.322917x_1^2 - 0.003679x_2^2$

Table 1.(c) ANOVA outcomes for RSM.

Response	Source	Sum of Square	degree of freedom	Mean Square	F-value	P-value
Yield (%)	Model	667.35	5	135.47	220.19	0.0005
	A-Pressure	135.09	1	135.09	219.57	0.0007
	B-Temperature	510.05	1	510.05	829.01	<0.0001
	AB	0.555	1	0.555	0.9021	0.4123
	A^2	22.56	1	22.56	36.66	0.009
	B^2	9.10	1	9.10	14.79	0.031
TPC (mg GAE/g sample)	Model	105.17	5	105.17	119.84	0.0012
	A-Pressure	14.82	1	14.82	84.44	0.0027
	B-Temperature	79.28	1	79.28	451.69	0.0002
	AB	3.4	1	3.4	19.39	0.0217
	A^2	3.34	1	3.34	19.01	0.0223
	B^2	4.33	1	4.33	24.68	0.0157

Table 1.(d) Comparison of predicted yield and actual yield on optimal conditions.

Parameter Respons	Predicted Value	Actual Value	Residual
Pressure	5	5	-
Temperature	160	160	-
Yield (%)	54.756	55.08	0.324
TPC (mg GAE/g sample)	12.802	12.66	0.142

yield value. These two factors have a noteworthy impact. This is evident from Table 1.(c)'s *p*-values for both variables, which are less than 0.05. The rise in temperature and pressure is correlated with an increase in yield. The polarity or dielectric constant of the water reduced as the extraction temperature rose, and this decrease helped to increase the solubility of most phenolic acids in the water under subcritical circumstances [38–40]. Although it is well known that raising the temperature of subcritical water can dissolve several compounds that are comparable to organic solvents (methanol, ethanol), subcritical water, in particular, also encourages several reactions that can break down the compounds in the raw materials, including hydrolysis and decomposition [41].

Furthermore, as the pressure rises, the extract becomes more soluble in the solvent, allowing for a more significant amount of extract extraction. Additionally, as pressure rises, the solvent (subcritical water) is more soluble per unit volume, which improves the extract's solubility in the solvent and results in a more optimal extract.

3.5.2 Effect of temperature and pressure parameter on parameter TPC

Phenolic compounds are considered necessary because of their antioxidant activity. These chemicals' aromatic structure with hydroxyl substituents protects human cells from harm from oxygen and free radicals, lowering the chance of developing some diseases. Additionally, phenolic chemicals are beneficial for diabetes, Alzheimer's disease, cancer, and cardiovascular disease. The TPC yield response depends on temperature (°C) and pressure (MPa). An overview of the contour and three-dimensional graphical depiction that

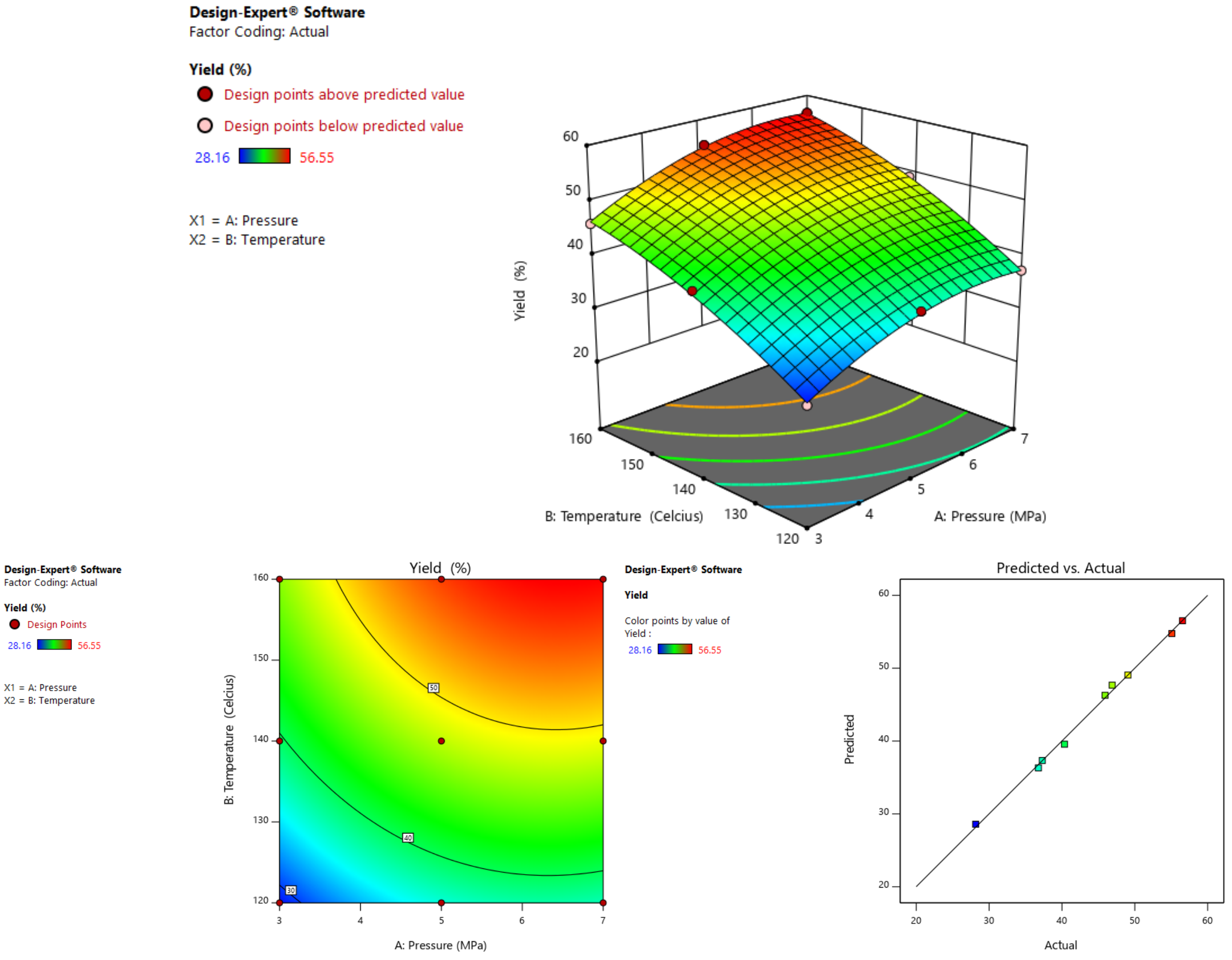

Figure 6. Effect pressure and temperature extraction on parameter yield (a) 3D-plot, (b) Contour plot and (c) Relation between actual and predicted.

illustrates how temperature and pressure relate to the TPC yield value is provided in figure 7.

Based on Table 1.(c), the temperature variable has a *p*-value of less than 0.05. This means that temperature variations significantly impact the TPC extraction procedure. There was a noticeable rise in the extracted TPC as the extraction temperature rose. It is possible because the more significant temperature variations impacted the solubility of phenolic compounds in water, which allowed the solvent (subcritical water) to extract seaweed as effectively as possible [20]. Additionally, the water's permittivity constant decreases with temperature, increasing the phenol's solubility in water [42].

In contrast, the pressure variable has a *p*-value greater than 0.05, suggesting that it has less of an impact on the response value. Because the pressure interval is too short, the impact of operating pressure on the overall amount of phenol is not very apparent. Furthermore, this can also be attributed to the polarity of water, which remains unaffected by pressure.

3.5.3 The optimization results

Figures 6 (c) and 7 (c) show the correlation between the actual results of the extraction procedure and the expected results generated using Design Expert. The straight line shows the agreement between the expected and actual yields. A more accurate representation of the results suggests that the used equation is closer to the linear line. Based on Table 1.(d), the optimal conditions for subcritical water extraction are depicted with a temperature of 160 °C and a pressure of 5 MPa. The difference between the actual and predicted values is around 0.142 − 0.508, indicating a high degree of agreement between the predicted results and reality.

3.6 Kinetics modelling

First and second-order kinetics are the kinetic models used in this investigation. The extraction process is examined using kinetic model analysis. In order to track changes in the response variable to the extraction time from 0 to 180 minutes, kinetic analysis was carried out under the ideal circumstances listed in Table 1.(d). The plots of log $(C_s - C_t)$ vs time and t/C_t vs time are displayed in figure 8. The first-order kinetic model explains a process that involves a single mechanism during the extraction of total phenolic compounds or that is only affected by one variable, in this case, temperature. According to the first-order model, solutes are dispersed evenly throughout the particles of the raw material, causing diffusion. Additionally, no chemical

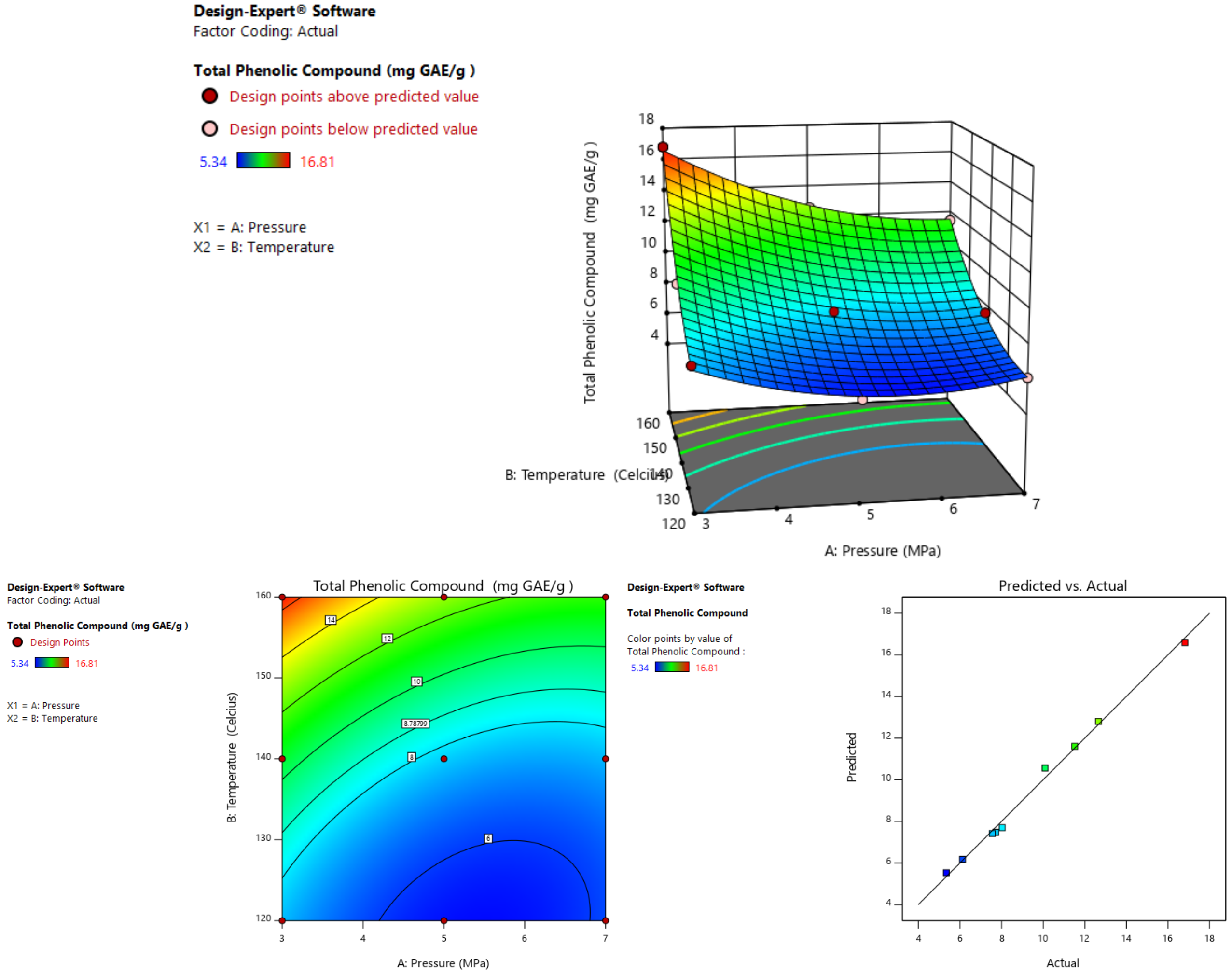

Figure 7. Effect pressure and temperature extraction on parameter total phenolic compound (TPC) (a) 3D-plot, (b) Contour plot and (c) Relation between actual and predicted.

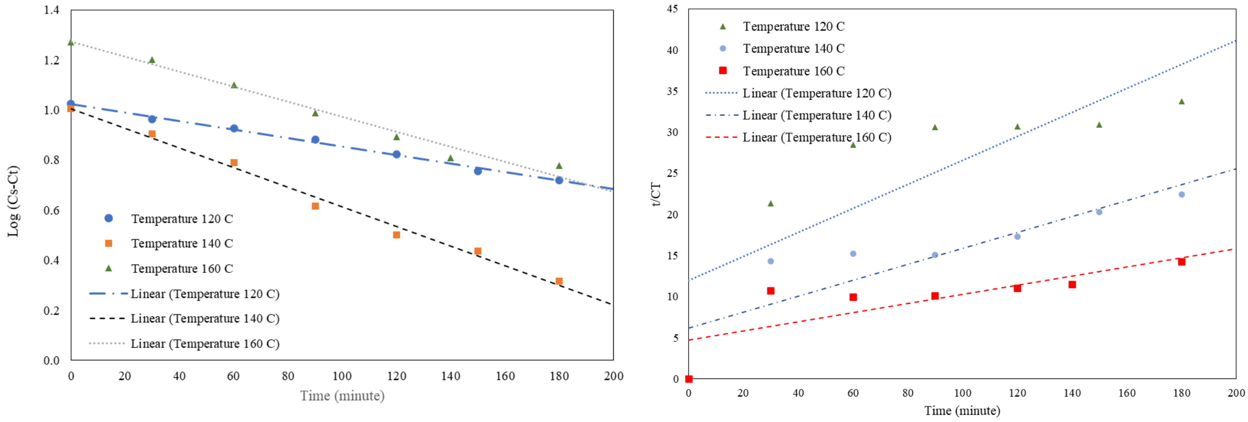

Figure 8. (a) *First-order* kinetics model (b) *Second-order* kinetics model of total phenolic compound.

reactions occur during the extraction process in batches [43]. At the same time, the second-order kinetic model describes the presence of two distinct mechanisms that occur [44]. The second-order kinetic model implies that the solute is on the surface of the material particles, and the solvent dissolves the solute rapidly at the initial stage of the extraction activity [45].

The results of the kinetics analysis of total phenolic compound extraction in *Eucheuma cottonii* showed that the first order was more appropriate than the second order. This can be seen in figure 8, which shows that the first order shows a trendline by the experiment rather than the second order. The suitability of the first-order equation with the experiment can be seen in figure 8, where the R^2 value or regression value is greater than the second order and approaches 1. The R^2 value shows a value that represents the actual experiment and is reinforced by the small RSME value shown in Table 2.

The mechanism of this first-order model is demonstrated by the *Eucheuma cottonii* phenolic component extract, which is present and increases quickly from the beginning until a specific point. The phenolic component extract then gradually increases until it reaches a stable level.

3.7 Energy activation

The Arrhenius equation can be used to calculate the activation energy of the extraction reaction, and the result is as follows:

$$k = Ae^{-\frac{Ea}{RT}} \tag{13}$$

where k is the reaction rate constant, T is the temperature, A is the Arrhenius constant, R is the universal gas constant, and Ea is the activation energy. By linearizing and simplifying the Arrhenius equation, the activation energy and the Arrhenius constant can also be graphically derived [46]. It is discovered that the linearized Arrhenius equation takes the following form:

$$\ln k = -\frac{Ea}{R}\left(\frac{1}{T}\right) + \ln A \tag{14}$$

Evidently, the equation $y = mx + c$ also takes the shape of a straight line. So that we can plot $\ln k$ against $(1/T)$, we can. This implies that the activation energy and the Arrhenius constant can be calculated from the slope and intercept, respectively, by Eq. (8). A positive value of Ea indicates the formation of a product. In contrast, a negative value indicates the degradation of the desired product [47]. The magnitude of the Ea value is proportional to the energy required to extract 1 mol of TPC. The activation energy was Ea = 20.563 kJ/mol. The activation energy required by subcritical water extraction is more petite than soxhlet. Research conducted by [48] shows the Ea value obtained to extract oil from avocado is 127.2950 kJ/mol. This shows that SWE is a better type of extraction than soxhlet.

4. Conclusion

Extraction using supercritical CO_2 followed by subcritical water, is able to maximize the separation of phytochemical compounds of *Eucheuma cottonii*, both polar and non-polar. In extraction using supercritical CO_2, the linoleic acid content value was obtained at 0.0841 μg/gsample and β-carotene at 0.0019 μg/gsample. While extraction with subcritical water produced kappa carrageenan and the best total phenolic compounds at 55.8%. By using the FCCCD method, it can be seen that the optimum operating

Table 2. Rate constant of the First order and Second order phytochemical compound extraction kinetic model.

	120 °C		140 °C		160 °C	
	Orde 1	Orde 2	Orde 1	Orde 2	Orde 1	Orde 2
Slope	-0.002	0.146	-0.004	0.097	-0.003	0.056
K	0.004	0.002	0.009	0.002	0.007	0.001
Intercept	1.024	11.995	1.005	6.224	1.271	4.718
Cs	10.572	6.863	10.113	10.334	18.678	18.010
R^2	0.994	0.649	0.991	0.635	0.995	0.521
RSME	0.138	0.510	0.236	0.627	0.479	1.005

conditions are at a temperature of 160 °C and a pressure of 5 MPa. Based on kinetic modelling, the extraction of phytochemical compounds from *Eucheuma cottonii* shows conformity with the first order modelling and the activation energy value is 20.66 kJ/mol.

Acknowledgment

We gratefully acknowledge the support of Politeknik ATI Makassar and Institut Teknologi Sepuluh Nopember for providing access to laboratory facilities and necessary materials.

Funding

This research has not received funding from any institution or external party.

Authors Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Narges Samoori, Naser foroughi Far and Alireza Khaje Amiri. The first draft of the manuscript was written by Narges Samoori and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

The datasets generated (or analyzed) during the current study are available from the corresponding author on reasonable request.

Conflict of interests

The authors have no competing interests to declare that are relevant to the content of this article.

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