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Subcritical Water Extraction of Epigallocatechin Gallate from *Camellia sinensis* and Optimization Study Using Response Surface Methodology

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Abstract: Background: Camellia sinensis is a plant whose leaves and buds are used to produce tea. With many medicinal activities already found, green tea has been consumed widely in the world. **Methods**: The subcritical water extraction (SWE) of epigallocatechin gallate (EGCG) from green tea leaves and the effect of the different extraction conditions are investigated by response surface methodology (RSM). Furthermore, the model of the extraction processes is reviewed for application at the industrial scale. **Results**: Based on the RSM data, the maximum yield of extraction is determined via optimizing different parameters of the extraction processes. Optimal conditions are as follows: extraction time of 6 min, extraction temperature at 120 °C, and a sample/solvent ratio of 1:40 g/mL. Under such conditions, the best yield of EGCG is 4.665%. Moreover, the model of the extraction processes, which can be utilized for industry scale purpose, indicates a good correlation with the experimental data. **Conclusions**: Overall, SWE is competent and environmental-friendly, and it is also a highly selective and fast method. SWE is a promising method to take the place of organic solvents used in the extraction of weak polar and even non-polar natural compounds. Further studies on the scale-up of the extraction processes are ongoing.

Keywords: *Camellia sinensis*; subcritical water extraction; response surface methodology; modeling

1. Introduction

Camellia sinensis is a perennial green tree that naturally grows in Asia. Nowadays, it is widely cultivated throughout the world, mainly in tropical and subtropical regions. The leaves of *Camellia sinensis* have been used and named as green tea, a popular beverage in the world. In addition, green tea has been found to exhibit various medicinal activities such as anti-cancer, anti-cardiovascular diseases, anti-diabetes, and anti-aging [1]. Polyphenols, especially catechins, are the most crucial components that contribute to the beneficial effects of green tea [2]. Catechins (flavan-3-ols) are natural flavonols originally derived from catechu, which is the liquid extract of *Acacia catechu* [3]. Epigallocatechin, epicatechin gallate, epicatechin, and epigallocatechin gallate (EGCG) are four main catechins found in green tea [4]. Among them, EGCG generally presents with the highest content, which can make up to 15% of the total catechins content. During extraction and storage, EGCG can be degraded, and this degradation was shown to depend on many factors including temperature and duration [4,5]. According to Friedman, the average content of EGCG decreased by 28% after six months

of storage [5]. Previous studies have indicated that EGCG undergoes oxidation at temperatures below 44 °C, while epimerization ordinarily exists at higher temperatures [1].

Extraction is the first step to recover the desired natural products from the plant materials. In general, heating [6] and Soxhlet extraction [7] are the most popular traditional techniques for extraction of green tea, which show several disadvantages like time and solvents consumption, tedium, low selectivity, and/or low efficiency [8]. Recently, more modern extraction techniques are being developed for the extraction of bioactive components from *Camellia sinensis*. Sokmen applied sequential supercritical fluid extraction to isolated catechins from green tea; the highest yield of catechin was 2.9% under the condition of pressure 25 MPa, temperature 60 °C, extraction time 3 h, and ethanol modifier flow rate at 0.5 mL/min [9]. Vahid Ghasemzadeh-Mohammadi proposed microwave-assisted extraction and ultrasonic extraction for the optimal isolation of tea catechins. The microwave-assisted extraction was more efficient in which the best yield of catechin was 6.25% under the conditions of ultrasonic time 7.8 min, microwave power 180 W, and extraction temperature 65 °C [10]. Pressurized liquid extraction was successfully deployed for the extraction of catechin. Four different species of green tea had been experimented with and the optimum condition was found for the extraction of catechin, which had pressure values around 150 bars with a constant temperature of around 75 °C for 60 min [11].

While not a very new extraction technology, SWE offers numerous advantages. It was found to require less solvent volume as well as the time and cost of samples per experiment [12]. Furthermore, the water, which is used as the solvent, has several benefits like a minor impact on the environment and worker's health as well as easy carrying and storage. In the experiments, the use of heat can drastically change the physical and chemical properties of water. When the temperature is increased, not only do the viscosity and surface tension of water decrease, but the dielectric constant also becomes lower [13]. In particular, the dielectric constant of water drops from 53 to 36.5 when the temperature has risen from 110 °C to 190 °C. This parameter, indicating the polarity of water, has shown to be comparable to organic solvents such as ethanol at ambient temperature [14]. Recently, subcritical water has shown excellent potential as a substitute solvent for the extraction of natural material. They are vanillin and coumarin from vanilla beans and tonka beans [15], caffeine from black tea leaves [16], and asiatic acid and asiaticoside from *Centella asiatica* [17].

The regular one-factor-at-a-time method in which one factor adjusts at a time while all others are retained steady has several shortcomings, including its more laborious and time-consuming protocol. Response surface methodology (RSM), a statistical experimental design, can be the alternative method to maximize the yield of extraction. RSM consists of mathematical and statistical techniques used to explore a suitable functional interaction between a response of interest and some process variable [18]. When applied with the subcritical water extraction method, optimal extraction procedures can be created to serve the purpose of research as well as for industrial applications [19–21].

At present, there were few investigations on the optimization of extraction of EGCG from green tea using SWE technology combined with RSM. Therefore, we reported the potential of the SWE for the extraction of EGCG from green tea. High-Performance Liquid Chromatography (HPLC) was employed to analyze the EGCG levels. The stability of EGCG is investigated as a function that depends on temperature and extraction time. Based on the response surface methodology (RSM) experimental design, this study determines the optimum conditions for the EGCG extraction process. Moreover, the extraction process was also modeled and evaluated at a larger scale in order to apply to industrial production.

2. Materials and Methods

2.1. Plant Materials

Green tea (*Camellia sinensis*) leaves were obtained as a dry sample from Thai Nguyen province, Vietnam. They were grounded and sieved to a particle size of less than 2.5 mm. The sample was

stored in the dry environment at ambient temperature. Moisture content (7.75%) was determined before extraction.

2.2. Subcritical Water Extraction (SWE) Procedure

The SWE was performed using an Accelerated Solvent Extractor (ASE) 350 system from Dionex Corporation (Sunnyvale, CA, USA). Two grams of sample were mixed with 4 g of diatomaceous earth and placed in a 100 mL stainless extraction cell. A stainless-steel frit and a cellulose filter (Dionex) were placed at the bottom of the extraction cell to prevent the contamination of the powder from infiltrating into the collection bottle. The extraction cells were arranged in a cell tray and the samples were extracted under certain conditions. After extraction, the obtained extracts were transferred into collection bottles and stored at 4 °C in the refrigerator for further analyses.

2.3. Conventional Water Extraction

Green tea leaves extracts were heated (2 g of grounded green tea leaves, mixed with 40 mL of water (ratio 20 mL:1 g)) at 60 °C for 2 h. The extraction mixture was continuously stirred with a magnetic stirrer. After that, the extraction mixture was cooled and filtered through a filter. The extraction solution was centrifuged at a speed of 4000 rpm for 10 min, and the supernatant was accumulated; the solvent was vaporized under vacuum and stored at 4 °C in the refrigerator for following evaluation [22].

2.4. HPLC Analysis

Here, an HPLC method was applied for catechins (EGCG and GCG) analysis. The HPLC was performed using the Shimadzu SPD-20A system (Shimadzu Co., Ltd., Kyoto, Japan) with C18 column (250 mm × 4.6 mm, 5 μm). A mixture of methanol-0.1% phosphoric acid solution was used as the mobile phase. The elution mode was a binary, high-pressure gradient system, and the elution gradients were: 0–38 min, 25% methanol; 38–40 min, 100% methanol. Other running conditions included the detection wavelength (272 nm), the flow rate (1 mL/min), the injection volume (10 μL), and the column temperature (25 °C).

2.5. Degradation Assays

The stability of catechins (EGCG and GCG) during extraction by SWE system was investigated. Independent experiments applied a series of extraction times (1, 3, 5, 7, and 9 min) and temperatures (60 °C, 80 °C, 100 °C, 120 °C, 140 °C, and 160 °C) with purified water were tested. Therefore, one experimental configuration resulted in 30 conditions for the extraction of EGCG.

2.6. Single-Factor Analysis

Prior to the development of the RSM study, the set of tests was carried out to select the experimental ranges for the independent variables. Three factors which respond to yield include: extraction time (min), extraction temperature (°C), and sample/solvent ratio (g/mL). When optimizing experimental factors, one factor was modified, while other factors were maintained at a specified value.

2.7. RSM Procedure

From the results of the single factor analysis, the three level-three factor Box–Behnken design was used in this study, requiring 17 experiments and five center points shown in Table 1. Multiple regressions explained the behavior of the system to fit a second-order polynomial model as follows:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=1}^{3} \beta_{ij} X_i X_j \quad (1)$$

Y is the response function, β_0 is an intercept, and β_i, β_{ii}, and β_{ij} are the coefficients of the linear, quadratic, and interactive terms, respectively. Accordingly, X_i and X_j represent the coded independent variables.

Design-Expert 7.1.6 (Trial Version, State-Ease Inc., Minneapolis, MN, USA) program package was run to design the experiment and handled the data. Analysis of variance (ANOVA) was conducted to check the fitness of the model and the statistical significance of the regression coefficients. Last, optimal conditions were counted from the final model and verified by an actual experiment attempt.

Table 1. Independent process variables, range, and levels used for Box–Behnken design.

Independent Variable	Factors	Coded Levels		
	X	−1	0	+1
Extraction temperature (°C)	X_1	80	100	120
Extraction time (min)	X_2	3	5	7
Solvent/sample ratio (mL/g)	X_3	40	50	60

2.8. Modeling of the Extraction Process

Extracts of active ingredients from natural products are involved in mass transfer from solid to liquid. The kinetics of the active ingredients are based on two concurrent processes: the rapid part is the washing stage, where the active ingredients in the cell and surface are quickly extracted by direct washing with the solvents; and the slow one is diffusion stage, where remaining active ingredients in the cell are transferred by diffusion from the solid particles to the solvent [23]. The pace of the extraction process depends on these slow steps, and the rate is minimal when transported through the solid matrix.

Based on previous studies [24,25], the steady-state kinetic model leads to a first-order rate equation as shown in Equation (2):

$$\ln\left(\frac{C_\infty}{C_\infty - C}\right) = kt + b \tag{2}$$

where C_∞ is the concentration (mg/10 g) in infinite time ($t = \infty$), C is the concentration of the extracted ingredients in the solution (mg/10 g) at time t, k is the overall rate constant, and b is an intercept.

When replacing the maximum yield (Y_m) in the experiment with C, Equation (2) becomes:

$$\ln\left(\frac{Y_m}{Y_m - Y}\right) = kt + b \tag{3}$$

We will use Equation (3) to be suitable with the experimental data, and to obtain values for Y_m and k.

3. Results and Discussion

3.1. Degradation Experimental Design

The effects of extraction time (min) and temperature (°C) on the yield of catechins from green tea leaves were represented in Figure 1. The concentration of catechins increased to the maximum, then decreased with the rise in temperature. More specifically, the maximum content of EGCG was 4.898% under extraction temperature/time conditions of 100 °C/5 min. After that, the efficiency of EGCG extraction was significantly reduced with the temperature ≥140 °C, such as 160 °C (yield = 1.648% at 9 min). This phenomenon was also true for GCG. However, the highest temperature point was 140 °C (yield = 2.492% at 9 min) and the yield decreased when extraction temperature/time reached 160 °C/5 min (yield = 1.798%). These results were in agreement with Sharma's study which found that degradation of flavonoids could occur at 150 °C or higher temperature [26].

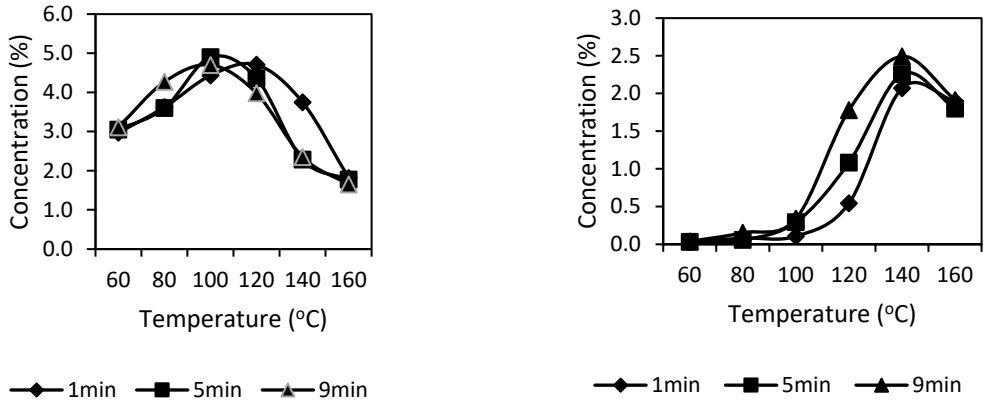


Figure 1. Effects of extraction temperature and duration of the SWE method on the yield of (**a**) EGCG and (**b**) GCG.

A number of factors such as the solvent type, time of extract, temperature, and the ratio between solvent and sample can affect the flavanol extraction [14]. In this study, the obtained EGCG amount was also found to depend on the extraction duration. As shown in Figure 1a, the extracted EGCG concentration decreased drastically for longer extraction times. This can be seen in a set of experiments at extraction temperature 140 °C; EGCG content reached 3.745% when the extraction time was 1 min, which is higher than the results of two experiments at the time points 5 min and 9 min. This loss of EGCG contents probably occurred due to oxidation, dimerization, and polymerization reaction [27].

In addition to possible degradation, epimerization could be observed simultaneously through the extraction process. Epimers of tea catechins partially become their isomers in particular conditions, such as high temperatures or prolonged storage. The epimerization process probably took place in the high-temperature extract, in which EGCG was converted into GCG [28,29]. The data in Figure 1 indicated that the epimerization would occur over a long time of extraction, which reduced the proportions of EGCG and gradually increased GCG, simultaneously. When the extraction temperature was fixed at 120 °C, the EGCG concentration was dropped from 4.708% to 3.980% while GCG content was rising from 0.543% to 1.778%. EGCG was epimerized more quickly at higher extraction temperatures, so the pattern was repeated; the EGCG decreased while GCG increased. At the 5-min extraction time tests, EGCG was highest at 100 °C (yield = 4.898%), then was reduced to 2.285% at 140 °C. Meanwhile, at the same rising of temperature condition, GCG concentration was improved from 0.292% to 2.527%. However, at temperatures of >140 °C, epimerization rarely happened [30], and the remaining flavanols were destroyed by thermal degradation.

According to these results, the loss of EGCG in the extraction process is influenced by parameters such as temperature, extraction time, and epimerization. These factors will be carefully considered while optimizing the condition for EGCG extraction of green tea leaves.

3.2. *Single Factor Analysis*

As can be seen in Figure 2a, the influence of temperature (°C) on extraction yield (%) was studied. The temperature was adjusted from 60 °C to 160 °C, while other extraction variables were set as follows: a ratio of sample to water of 1:80 and extraction time of 5 min. The yield of EGCG increased with the temperature up to 100 °C and began to drop off, and the maximum extraction value was 4.898% at 100 °C. This result pointed out that the temperature improved the extraction of EGCG to a positive level, followed by its possible loss, due to the degradation at a higher temperature as shown in Figure 1. The outcome of this experiment can be explained by the fact that the temperature changes the properties of water like solubility, viscosity, and surface tension considerably. For example, the viscosity of water decreases threefold as the temperature rises from 25 °C to 100 °C [31]. All of

the changes will stimulate the interaction of water with the compounds and, therefore, boost the yield of extraction [32]. Although the positive influence of higher temperatures on the extraction yield is substantial, this cannot be increased endlessly. When the temperature reaches some point, the degradation and epimerization of tea catechins could take place in thermal procedures and reduce the extraction efficiency.

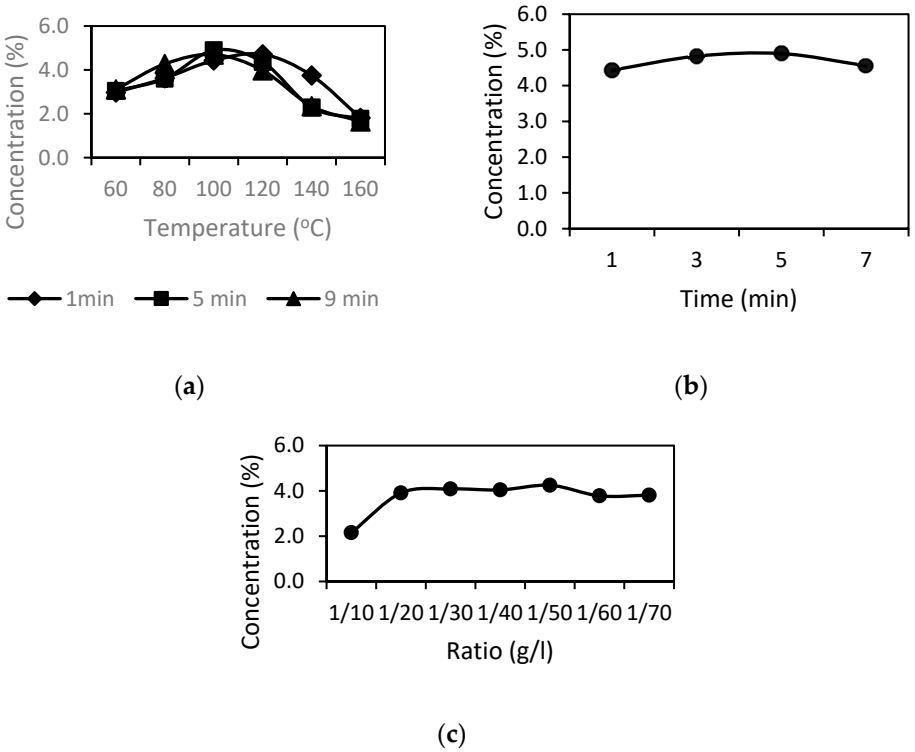


Figure 2. Effects of extraction factors on yield of EGCG extraction: (**a**) temperature; (**b**) time; (**c**) sample/solvent ratio.

Similarly, Figure 2b revealed the time extraction (min) effect on the yield of EGCG (%). In this series of experiments, the duration was varied from 1 min to 7 min when other extraction factors were kept at fixed values: a ratio of sample to water of 1:80 and temperature of 100 °C. When the extraction time increases from 1 to 7 min, the yield of the extracted EGCG continuously increases from 4.435 to 4.898% at 5 min, while it is reduced to 4.557% at 7 min because of the degeneration of EGCG caused by prolonged vulnerability at high temperature. In this matter, the yield of extraction increases when the extraction time is lengthened in a specific time range. However, after the equilibrium is reached, the extraction will not be affected by the change of time [33]. Apart from that, because of the unstable structure, EGCG tends to degrade when extending the extraction time [14]. Moreover, additional extraction time not only delays the process, but it also leads to additional energy and, as a result, leads to extra operational costs. Therefore, the extraction time was chosen as 5 min for the following experiments.

In the third experiment, the yield of EGCG extraction (%) by the various ratio of material to water (g/mL) from 1:10 to 1:70 was presented in Figure 2c. The extraction temperature and extraction time were fixed at 100 °C and 5 min, respectively. The extraction yields of EGCG built up from 2.153% to 4.249%, with a step up in the ratio from 1:10 to 1:50. These results match up well to those of Pan et al., [34], who described a boost in the extraction of polyphenolic compounds as the sample to solvent ratio increased. The increase in the ratio of sample to solvent can enlarge the total extraction surface area so the solutes will have higher propensities to move from the matrix to the solvent. However, such an increase in the EGCG content was not detected when increasing the ratio from 1:50 to 1:70 in the present study. Instead, it went to be stable. This might be because of the exhaustion of the

content of the extractable compound, where the additional solvent cannot obtain any further EGCG from the sample.

According to the single-parameter study, we adopted an extraction temperature of 80–120 °C, extraction time of 3–7 min, and the ratio of raw material to water 1:40–1:60 for RSM experiments.

3.3. Optimization of Extraction Using RSM

BBD model was used to enhance the three variables of extraction: extraction time, extraction temperature, and the ratio of sample to water. Table 2 showed the results of 17 experiments under particular conditions influencing the performance of EGCG extract. From the table, EGCG extraction varied from 3.437% to 4.651%. Data from the BBD model were carried out for the regression analysis in which the second-order polynomial model was presented to reveal the relationship between extraction efficiency and three aspects.

Table 2. Experimental results for the response variables, temperature, ratio, and time.

Std	Run	Factor 1A: Temperature	Factor 2B: Ratio	Factor 3C: Time	Response 1: Yield
2	1	120	40	5	4.432
8	2	120	50	7	3.755
14	3	100	50	5	4.438
3	4	80	60	5	3.714
12	5	100	60	7	4.009
1	6	80	40	5	3.814
16	7	100	50	5	4.319
11	8	100	40	7	4.651
6	9	120	50	3	4.423
10	10	100	60	3	4.336
15	11	100	50	5	4.182
13	12	100	50	5	4.651
5	13	80	50	3	3.437
4	14	120	60	5	3.889
17	15	100	50	5	4.564
7	16	80	50	7	3.866
9	17	100	40	3	4.058

The analysis of ANOVA results in Table 3 indicated that the terms of the model were significant with p-values < 0.05. The lack of fit testing was utilized to find out the adequacy of the fit. With p-value > 0.05, the model was able to fit sufficiently with the experimental data. The R^2 of 0.9138 reasonably settled with the adjusted R^2 of 0.8029 (both > 0.8), which showed that the model had a solid correlation between experimental data and the data predicted by the model. The high reliability of the experiments was likewise reflected in the low CV value of 5.29. Table 2 also showed that the linear coefficients (X1) and quadratic coefficients (X1X3; X2X3; $X1^2$) were statistically significant with p-values < 0.05. Therefore, X1, $X1^2$, X1X3, and X2X3 were variables that affected EGCG extraction efficiency.

The effects of independent variables and their synergy with the yield of the EGCG extract was characterized by 3D response surface and 2D contour line in Figure 3. Visually, extraction efficiency increased proportionally with condition specifications. However, as these conditions exceeded the optimal point (120 °C, 6 min, 1:40 g/mL), the EGCG content obtained stops increasing, and eventually began to decrease. The condition collected from the software was then employed to perform actual extraction for verification. After a batch of tripled experiments, the efficiency of EGCG extraction was 4.665% ± 0.196%, which was closed to the prediction of the model (4.617%). Given the results, the predictability of the model for the extraction of EGCG from *Camellia sinensis* was confirmed in the experimental condition.

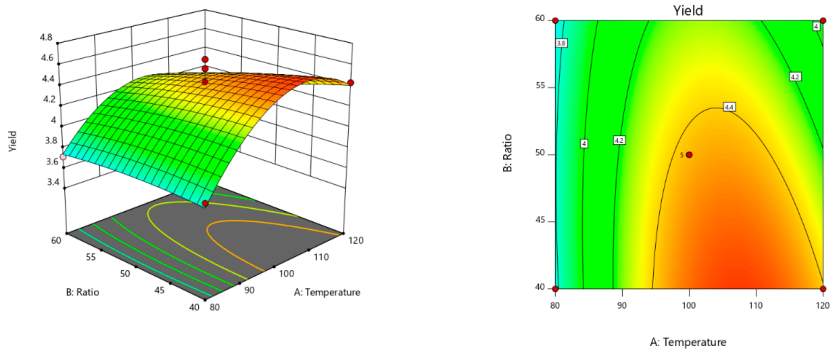
Table 3. ANOVA for quadratic model results. (**A**) Response 1: Yield; (**B**) Fit Statistics.

Source	Sum of Squares	df	Mean Square	*F*-Value	*p*-Value	
Model	1.93	9	0.2146	8.24	0.0055	significant
A- Temperature	0.3520	1	0.3520	13.52	0.0079	
B- Ratio	0.1243	1	0.1243	4.77	0.0652	
C- Time	0.0001	1	0.0001	0.0035	0.9545	
AB	0.0469	1	0.0469	1.80	0.2216	
AC	0.3009	1	0.3009	11.56	0.0115	
BC	0.2116	1	0.2216	8.13	0.0247	
A^2	0.7773	1	0.7773	29.85	0.0009	
B^2	0.0056	1	0.0056	0.2143	0.657	
C^2	0.0721	1	0.0721	2.77	0.1399	
Residual	0.1823	7	0.0260			
Lack of Fit	0.0416	3	0.0139	0.3940	0.7649	Not significant
Pure Error	0.1407	4	0.0352			
Cor Total	2.11	16				

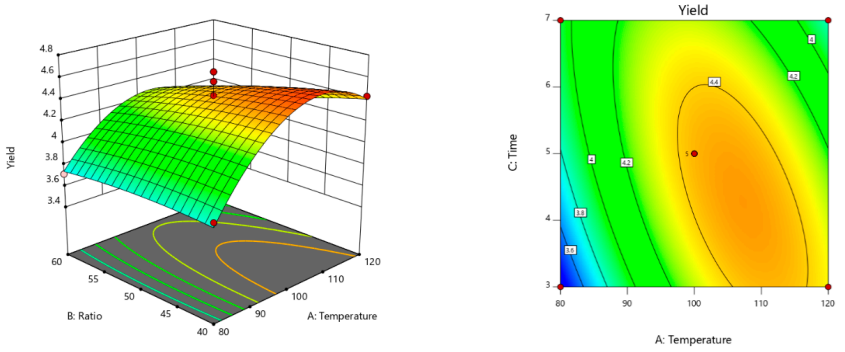
(**A**)

Std. Dev.	0.1614	R^2	0.9138
Mean	4.15	Adjusted R^2	0.8029
C.V. %	3.89	Predicted R^2	0.5814
		Adeq Precision	10.0086

(**B**)

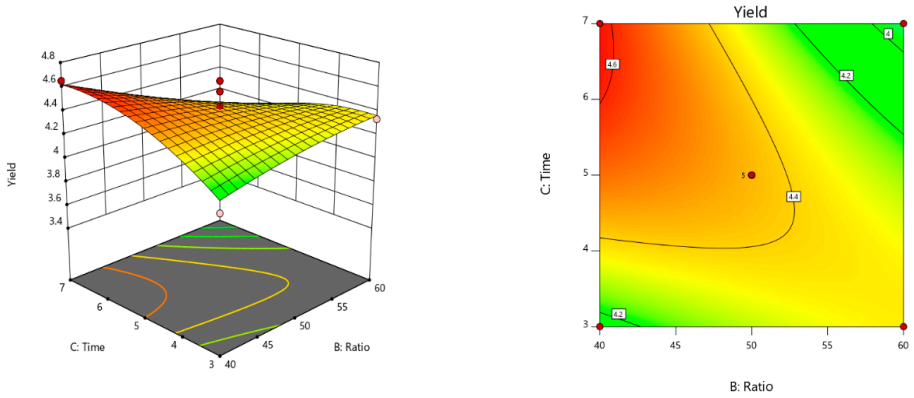


(**a**)



(**b**)

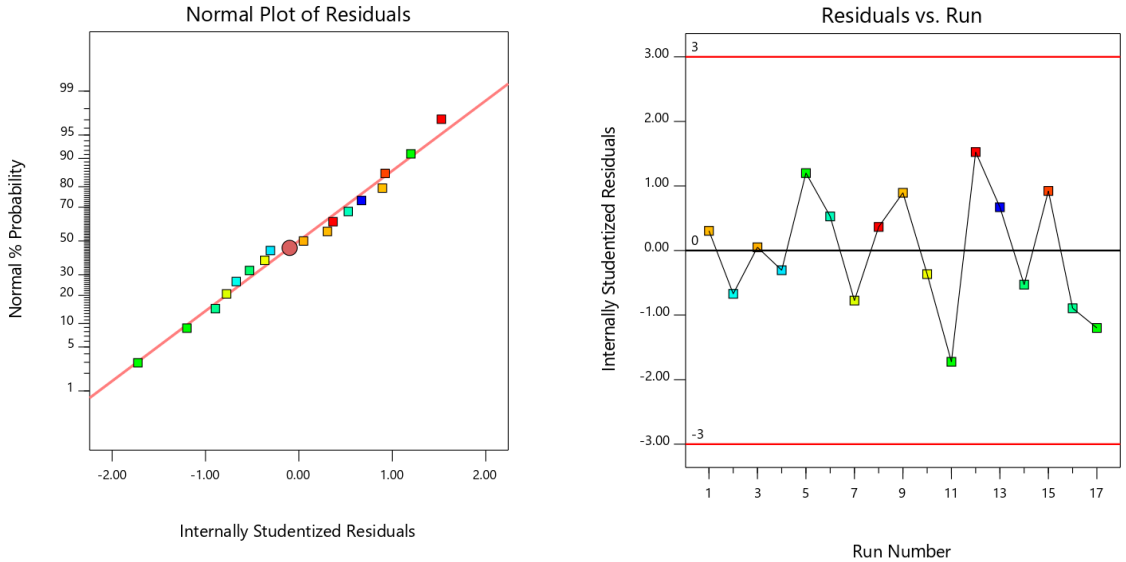
Figure 3. *Cont.*



(**c**)

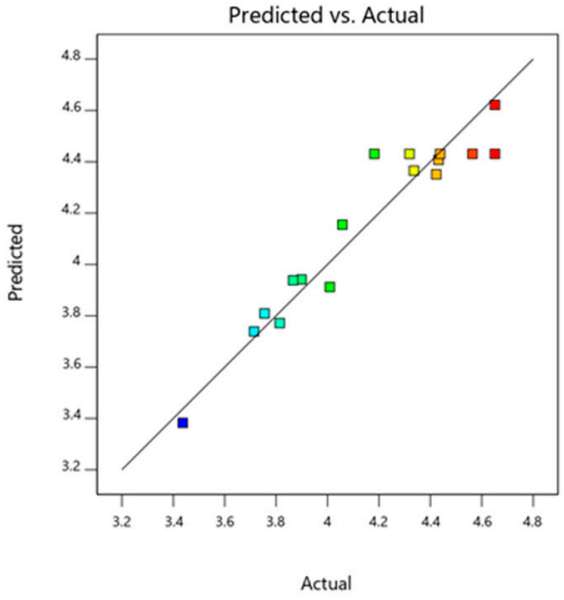
Figure 3. Response surface and contour plots for factors influencing yields of EGCG extraction include (**a**) temperature and ratio; (**b**) temperature and time; and (**c**) ratio and time.

To assess the data fitting, a probability distribution histogram model could be used for the residuals to evaluate the contrast between the predicted and empirical values. Figure 4a showed the residuals in a straight line to confirm the normal distribution model and the analytical hypothesis. To check if the order of observations affects the results, the residuals versus experimental runs were performed for analysis of experimental data. From Figure 4b, all data points were within the allowed limits. Figure 4c showed the relationship between the actual and predicted values of EGCG yield, which demonstrated that the model was sufficient due to the small residuals and the matter the residuals are closely related to the diagonal line.



(**a**) (**b**)

Figure 4. *Cont.*



(**c**)

Figure 4. (**a**) The normal probability plot of residuals, (**b**) plot of internally studentized residuals versus experimental runs, and (**c**) plot of predicted and actual values.

The comparison of EGCG yield and extraction time of this study with other reported extraction methods are shown in Table 4. Kwang Jin Lee extracted EGCG from green tea using the dipping method—specifically, using water as solvent for 60 min. The resulting concentration of EGCG was found to be 0.90% [35]. Sena Saklar Ayyildiz reported that ground green tea was treated with hot water and with ultrasonic processors for 52.49 min, by which 4.81% EGCG was obtained [36]. Using microwave-assisted water extraction proposed by Ezzohra Nkhili, EGCG content in the extracts was 5.84% under the condition of temperature 100 °C and 20 min extraction duration [37]. From Table 4, EGCG yield by SWE is not much different from other reported research values, while SWE is also the most expeditious and cheapest method. These facts indicate that SWE is not only "green" chemistry but is also a valuable and competent method.

Table 4. Comparison of time and EGCG yield with reported extraction methods.

Extraction Method	Extraction Time (min)	EGCG Yield (%)	Reference
Leaching extraction	60	0.09	Lee, K.J, et al., 2008
Ultrasonic extraction	52.49	4.81	Ayyildiz, S.S, et al., 2018
Microwave-assisted extraction	20	5.84	Nkhili, E, et al., 2009
Subcritical water extracation	6	4.67	This study

In our experiments, SWE extraction (120 °C, 6 min, 1:40 g/mL) extracted higher amounts of EGCG from green tea (4.665%) than hot water extraction (4.090%). Overall, the contents of EGCG obtained using SWE extraction were higher than those obtained using conventional water extraction. Additionally, the time used for SWE extraction was much shorter (6 min) than that used for conventional solvent extraction (120 min). It is confirmed that increased extraction time leads to the degradation of bioactive molecules of tea due to fractional epimerization of EGCG into GCG [38].

Conventional solvent extractions with organic solvents and Shoxlet have been commonly operated for the isolation of bioactive compounds from plant material, especially in green tea [39]. In most cases, the conventional system for tea extraction is an old, time- and solvent-consuming, and strenuous process with limited yield efficiency. All these characteristics are main reasons to explain why their application in the industry or studies including the analysis of numerous samples is not a simple

matter. In addition to these properties, environmental acceptability of green extraction techniques is gaining attention over traditional extraction techniques for isolation of natural products, which reduces chances of environmental hazards. SWE is one of the newer methods for tea extraction in noxious organic solvent-free attempts [40]. In some cases, SWE can reduce extraction times by up to 50% when compared with conventional extraction methods [41–43]. Furthermore, subcritical water is an applicable solvent for a variety of extraction, and a sustainable alternate for toxic organic solvents [44].

3.4. Modeling Extraction Process

As presented in Figure 5 and Table 5, the EGCG yield enhanced considerably with the extraction time increasing from 1 to 3 min, while the yield was not quite altered after 3 min. This is probably for reaching a dynamic equilibrium between internal and external diffusion in 3 min to 5 min. The R^2 value (R^2 = 0.8358) suggests that the model seems to be valid in illustrating experimental data (Figure 6), indicating that the model might be reasonable for analyzing the extraction processes under the condition that the product was not decomposed.

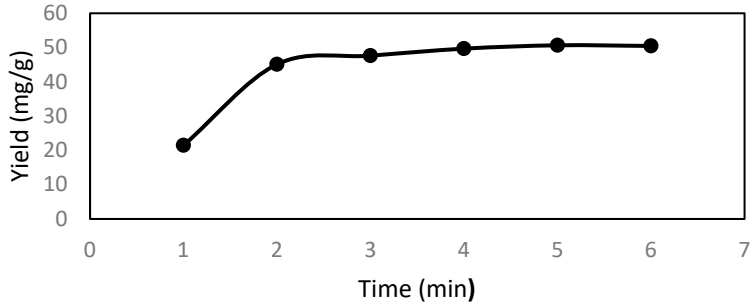


Figure 5. Extraction yield (mg/g) per time.

Table 5. k and R^2 obtained from model.

Extraction Method	Subcritical Water Extraction with Dionex ASE 350
k (1/min)	0.0479
R^2	0.8358

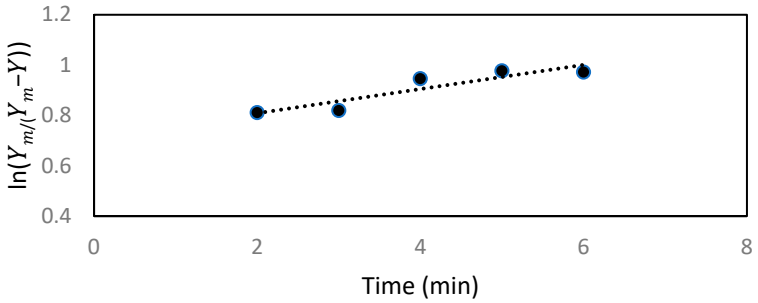


Figure 6. $\ln\left(\frac{Ym}{Ym-Y}\right)$–T data from EGCG extraction at different condition.

4. Conclusions

The application of SWE is a reasonable replacement for the conventional extraction methods, due to the facts that quicker extraction time needs to be utilized for cost-effective extraction and the commonly available water is used as an extraction solvent in this technology, and because of the chance to directly use acquired extracts as semi-products or products for food or pharmaceutical industry without an additional process of separation or purification. Therefore, in this study, SWE was used for the recovery of EGCG from *Camellia sinensis*. Moreover, the response surface methodology was used and verified to be suitable for the optimization of the SWE condition extraction. RSM results validate

that optimal values of extraction time, extraction temperature, and solvent/solid ratio are, respectively, 6 min, 120 °C, and 40 mL/g. Additionally, EGCG yield reached 4.665% under the above-optimized extraction conditions. The concentration of EGCG in the extracts was determined by HPLC. Moreover, our results confirmed that mathematical modeling of the extraction of EGCG from green tea is possible, yielding a useful tool for process control, even though there remains the problem of the complex interaction of the extraction conditions. This model also the presents potential for analysis of extraction processes on other active ingredients from natural products.

Overall, SWE is not only competent and environmentally friendly, but is also a highly selective and fast method. A main disadvantage of SWE is the high functional pressure, which needs expensive equipment. However, in the case of bioactive compounds such as antioxidants like EGCG, cost should not play a restricting role. Because natural antioxidants could be wanted, food components and expenses are given back by other compensations such as the high purity of extracts and the effectiveness of the technique. Therefore, SWE is a candidate to take the place of organic solvents used in the extraction of weak polar and even non-polar natural compounds. Further studies to set up a cost-efficiency pilot version of commercial equipment and scale-up of the extraction process are ongoing.

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Abbreviations

Accelerated solvent extractor	ASE
Analysis of variance	ANOVA
Box–Behnken design	BBD
Epicatechin	EC
Epicatechin gallate	ECG
Epigallocatechin	EGC
Epigallocatechin gallate	EGCG
Gallocatechin gallate	GCG
High-performance liquid chromatography	HPLC
Subcritical water extraction	SWE
Response surface methodology	RSM

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