Research Article

Effects of Supercritical Carbon Dioxide Extraction (SC-CO_2) on the Content of Triterpenoids in the Extracts from *Ganoderma Lucidum*

Duc Duy Tran
Ho Chi Minh City University of Food Industry, Ho Chi Minh City, Vietnam

Huu Hanh Pham Thi
Ba Ria-Vung Tau University, Vung Tau City, Vietnam

Van Man Phan*
Ba Ria-Vung Tau College of Technology, Vung Tau City, Vietnam

* Corresponding author. E-mail: pvman.dbv@moet.edu.vn DOI: 10.14416/j.asep.2022.02.002
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Abstract
This research investigates the effect of SC-CO_2 extraction parameters on the triterpenoid recovery from *G. lucidum*. The SC-CO_2 parameters included extraction pressure, extraction temperature, and extraction time. The extraction pressure was varied between 200, 400 and 600 bar, the extraction temperature between 30, 55 and 80 °C, and the extraction time between 30, 75 and 120 min. In this study, the SC-CO_2 parameters were first optimized using a response surface methodology (RSM) for maximum triterpenoid recovery. The results showed that the optimal RSM-based SC-CO_2 conditions were 430 bar extraction pressure, 54.8 °C extraction temperature and 78.90 min extraction time, achieving the maximum triterpenoid recovery of 1.56 mg/100g. The kinetic behavior of SC-CO_2 process was subsequently characterized using a second-order kinetic model under variable extraction pressures and extraction temperatures, given a SC-CO_2 time interval. The second-order kinetic models represented well the experimental results of triterpenoid extraction by SC-CO_2 method. At these conditions, the triterpenoid extract also exhibited strong scavenging activities with IC50 values of 0.49 mg/mL for the DPPH radical scavenging activity and 0.26 mg/mL for ABTS radical scavenging activity. Thus, triterpenoids extracted from *G. lucidum* could be regarded as a potential agent for medicinal treatment. The results also suggest that the SC-CO_2 extraction can be a useful extraction method for triterpenoid extraction from *G. lucidum*.

Keywords: Antioxidant scavenging activity, *Ganoderma lucidum*, Supercritical carbon dioxide extraction, Triterpenoids

1 Introduction

Ganoderma lucidum (known as Lingzhi in China, reishi in Japan, and yeongji in South Korea) is a traditional medicinal mushroom that has been used for more than 2000 years [1], [2]. *G. lucidum* contains many bioactive and nutritional compositions, such as triterpenoids, steroids, phenolics, nucleotides, and polysaccharides [2]–[4]. Among these compounds, triterpenoids have been known as strong antioxidant compounds in *G. lucidum* [2], [3]. Triterpenoids are responsible for various ailments, such as antitumor, anti-inflammatory, antihyperlipidemic effects [5]–[7]. Numerous studies have been carried out on extraction and purification of triterpenoids from *G. lucidum*, and more than 150 triterpenoids have been isolated [8]–[11].

Extraction is an important step involved in the

discovery of triterpenoid components from *G. lucidum*. Different extraction methods have been applied to extract triterpenoid compounds from *G. lucidum* [2], [4], [10]. Currently, Soxhlet extraction (SE) and maceration extraction (ME) are the common conventional method for triterpenoid extraction from *G. lucidum* [10]. These methods have some drawbacks, such as the requirement of long extraction time, large solvent volumes, and flammable organic solvents [12]–[15]. To overcome these drawbacks, many green techniques including ultrasound assisted extraction (UAE), microwave assisted extraction (MAE), and supercritical carbon dioxide extraction (SC-CO_2) methods were used for *G. lucidum* extraction [10], [13]–[15]. Compared to the traditional extraction methods (SE and ME methods), the green techniques provide a broad range of useful properties (i.e., environmental friendly, non-toxic, shorter extraction times, high extraction efficiency, and low viscosity) [4], [10], [11]. However, MAE and UAE completely destroy the cell wall and thus the content of impurities in the extracts increase and subsequent purification is a difficult process [15], [16]. Meanwhile, some previous studies reported that SC-CO_2 can penetrate into the *G. lucidum* spores more effectively than the traditional methods [11], [14], [17]. Under SC-CO_2 extraction, higher extraction pressure and extraction temperature increase the density of supercritical CO_2, thus enhancing its ability to extract triterpenoid from *G. lucidum* fruiting body [2], [14], [15]. In addition, the selectivity of SC-CO_2 allows the attainment of high purity triterpenoids. However, triterpenoids reduce solubility in SC-CO_2 solvent [14], [15].

To enhance the recovery of polar triterpenoids and bioactive compounds, the use of a co-solvent, such as methanol, ethanol, and other polar organic solvents, has been considered [18]–[21]. Specifically, Rodrigues *et al.* experimentally extracted the triterpenoids from Acacia dealbata leaves using SC-CO_2 modified with ethanol as a co-solvent, and documented that 1% ethanol was ideal for triterpenoid extraction [20]. Pieczykolan *et al.* extracted tiliroside from *Tilia L. flowers* using ethanol as a co-solvent extraction, and concluded that the addition of small percentages (1–10%) of ethanol to carbon dioxide can improve the tiliroside yield [19]. Ethanol is a nontoxic and ecological-friendly solvent, which can be mixed with carbon dioxide solvent for extracting the triterpenoids and phenolics from plants [14], [18]–[20]. In this research, SC-CO_2 modified with ethanol as a cosolvent was used. From the previous findings, many factors, such as extraction time, extraction temperature, and extraction pressure may significantly influence the extraction efficacy of triterpenoids [18]–[20]. To our knowledge, no data on the optimization of SC-CO_2 extraction conditions on the recovery or SC-CO_2 extraction kinetics of triterpenoids from *G. lucidum* can be found in any literature.

Response surface methodology (RSM) is a valuable statistical tool, and can be used to find the optimization of extraction conditions [8], [10]. The response surface methodology reduces the numbers of experimental trials and explains the interactions between variables [8], [10], [21], [22]. In this study, RSM was employed to standardize the SC-CO_2 parameters (extraction pressure, extraction temperature, and extraction time) for maximum recovery of triterpenoids and evaluated the antioxidant activity of the triterpenoids in the extract. Furthermore, a second-order kinetic model was used to characterize the kinetics of the SC-CO_2 process under variable extraction pressure and extraction temperature, for a given sonication interval. The findings would be beneficial for enhancing the recovery of triterpenoid from *G. lucidum* by using proper SC-CO_2 processes.

2 Materials and Methods

2.1 *Materials*

The *G. lucidum* was provided by a L'ang farm store (Da Lat city, Lam Dong province, Vietnam) and dried at 85 °C for 60 min. Then, it was ground in a blender and passed through a 20-mesh sieve (0.2 mm). The final moisture content was 5.5–6.0% and thereafter *G. lucidum* powder was vacuum-packed and placed in a chiller at 4 °C for further analysis.

Ascorbic acid (99%), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ursolic acid (>90%), and ABTS (2,2′-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) were products of Sigma-Aldrich Co. (St. Louis, MO, USA). Perchloric acid 70%, glacial acetic acid (99.5%), and ethanol (99%) were obtained from Merck (Dam-stadt, Germany). Carbon dioxide (99.9%) was obtained from the Daxing Gas Co., Beijing, China. All other chemical reagents used were of analytical grade.

2.2 *Methods*

2.2.1 Extraction procedure

The supercritical carbon dioxide extraction method (SC-CO_2) was carried out using a supercritical fluid system (SFE-500F2-C5, Thar technology). Ethanol was selected to investigate the effect of controlling parameters (pressure, temperature, and time) on the extraction of triterpenoids from *G. lucidum*.

First, the effect of pressure on triterpenoid extraction was investigated. Dried *G. lucidum* powder (10 g) was placed in the supercritical fluid CO_2 extracting apparatus. Extraction was performed at 55 °C for 70 min at different extraction pressures (200–600 bar). Second, the experiments were conducted to study the effect of extraction time on the recovery of triterpenoids. The extraction was performed at 55 °C, with different extraction times (30, 55, 80, 105 and 120 min). The extraction pressure was set at the desired value of 400 bar. Finally, the influence of temperature extraction on the recovery of triterpenoids was analyzed. The temperature extraction was in a range of 30 to 80 °C, and the extraction process was performed at 400 bar for 55 min. All experiments were fixed the flow rate of CO_2 at 7 mL/min and 1 mL/min for 10% ethanol based on the preliminary survey showing a suitable extraction condition. After extraction, the solvent was evaporated in a rotatory evaporator (Buchi $R^2$10, Flawil Switzerland), and the extracts were kept refrigerated at –20 °C for further analysis.

2.2.2 Experimental design

A central composite face-centered design (CCF) was used in the implementation of a response surface methodology to optimize the extraction parameters using the MODDE software (version 5.0; Umetri, Umeå, Sweden). Three independent variables were extraction pressure (X_1), extraction temperature (X_2), and extraction time (X_3). The variation of these factors was varied at three levels (low, moderate, high), and coded as –1, 0 and +1, respectively (Table 1). The content of triterpenoids (Y) was chosen as a response, and the experimental design consisted of 17 experimental runs (Table 2). A quadratic polynomial equation model was generated to predict for optimization of the triterpenoid content [Equation (1)].

$$Y = \beta_0 + \sum_{i-1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_i X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} X^2 \quad (1)$$

Where Y is the predicted response; β_0 is a constant; X_1, X_2 and X_3 are independent variables and β_i, β_{ij} and β_{jj} are the linear coefficients (L), interaction coefficients, and quadratic coefficients (Q), respectively. The model adequacy was evaluated based on lack of fit (LOF), an F-test and the coefficient of determination (R^2) using analysis of variance (ANOVA). The Pareto analysis was used to show the contribution of each variable and their interactions on the recovery of triterpenoids.

Table 1: Coded and actual levels of three variables

Variables	Coded Levels of Variables		
	–1	**0**	**+1**
Pressure extraction (X_1, Bar)	200	400	600
Extraction temperature (X_2, °C)	30	55	80
Extraction time (X_3, min)	30	75	120

2.2.3 Kinetic model of SC-CO_2 extraction

A second-order kinetic model was applied to characterize the kinetic behavior. In this study, kinetic analysis was performed based on two scenarios: 1) different extraction pressure levels (200, 400, 600 bar), given an extraction temperature of 55 °C, and 2) different extraction temperatures (30, 55, 80 °C), given an extraction pressure of 400 bar.

For a second-order kinetic extraction model, the rate of dissolution of the triterpenoids contained in the solid to solution can be described by Equation (2).

$$\frac{dC_t}{dt} = k(C_e - C_t)^2 \quad (2)$$

Where k is the second-order extraction rate constant (100 g/g^{-1} min^{-1}). C_s is the concentration of triterpenoids at saturation in (g/100 g). C_t is the concentration of triterpenoid in the solution at any time (g/100).

By taking the initial and boundary conditions (t = 0 to $t = t$ and $C_t = 0$ to $C_t = C_t$), the integrated rate law for second order extraction was obtained as Equation (3).

$$\frac{t}{C_t} = \frac{1}{k.C_e^2} + \frac{t}{C_e} \quad (3)$$

When t approaches 0, the initial extraction rate, h, is written as in Equation (4).

Table 2: Experimental and predicted triterpenoid content under variable extraction pressures (X_1, bar), extraction temperatures (X_2,°C), and extraction times (X_3, min)

Run	Coded Variable						Experimental Value	Predicted Value
	X_1	X_2	X_3	X_1	X_2	X_3	g/100g	g/100g
1	–1	–1	–1	200	30	30	0.723 ± 0.01	0.719 ± 0.02
2	–1	+1	–1	200	80	30	1.051 ± 0.02	1.055 ± 0.02
3	0	0	–1	400	55	30	1.296 ± 0.01	1.303 ± 0.01
4	+1	0	0	600	55	75	1.412 ± 0.02	1.407 ± 0.02
5	–1	0	0	200	55	75	1.313 ± 0.02	1.294 ± 0.03
6	0	0	+1	400	55	120	1.396 ± 0.03	1.366 ± 0.01
7	–1	–1	+1	200	30	120	0.891 ± 0.01	0.878 ± 0.02
8	0	0	0	400	55	75	1.532 ± 0.02	1.556±0.03
9	+1	+1	+1	600	80	120	0.917 ± 0.01	0.926 ± 0.03
10	+1	+1	–1	600	80	30	0.938 ± 0.01	0.957 ± 0.01
11	0	0	0	400	55	75	1.556 ± 0.01	1.556 ± 0.01
12	+1	–1	–1	600	30	30	0.872 ± 0.03	0.846 ± 0.02
13	0	1	0	400	80	75	1.431 ± 0.02	1.368 ± 0.02
14	0	–1	0	400	30	75	1.298 ± 0.02	1.338 ± 0.02
15	+1	–1	+1	600	30	120	1.198 ± 0.02	1.201 ± 0.03
16	0	0	0	400	55	75	1.532 ± 0.01	1.556 ± 0.03
17	–1	+1	+1	200	80	120	0.796 ± 0.02	0.828 ± 0.01

$$h = k.C_e^2 \quad (4)$$

Where C_s and k are determined from the slope and intercept, respectively, by plotting t/C_t against t.

2.2.4 Determination of triterpenoids content

The determination of total triterpenoids was performed following the colorimetric method of Wei *et al.* with some modifications [21]. The extract (0.16 mL) was mixed with 0.4 mL of 5% vanillin/glacial acetic acid (w/v) in test tube. After that, 1.0 mL of perchloric acid solution was added successively into the mixture. The tube was incubated in a water bath at 60 °C for 30 min. Then, the sample was measured at 573 nm after adding 5.0 mL of glacial acetic acid to the cooled samples. For triterpenoid analysis, the ursolic acid was used as the standard prepare a calibration curve in the adequate range of concentrations (0.1–1.0 g/100 mL in methanol). The results were expressed in mg of ursolic acid equivalents per g of dw.

2.2.5 DPPH radicals scavenging activity

DPPH radical scavenging assay was used to determine the antioxidant activity of the extract following the Wei *et al.* method with minor modifications. 2.4 mL of DPPH (0.1M) solution were mixed with 1.6 mL of extract in methanol at different concentrations (0.125–1.15 mg/mL). The reaction mixture was vortexed thoroughly and placed in the dark for 30 min. The absorbance of the mixture was measured at 517 nm. Ascorbic acid was used as reference. Percentage of DPPH radical scavenging activity was calculated by the following Equation (5):

$$\%DPPH\ radical\ scavenging\ activity = \frac{A_0 - A_1}{A_0} \times 100\% \quad (5)$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the extractives/standard. Then percentage of inhibition was plotted against concentration, and from the graph IC_{50} was calculated.

2.2.6 ABTS radicals scavenging activity

The ABTS assay was based on the method of Wei *et al.* [21]. ABTS radical solution was prepared by adding 5 mL of 7 mM ABTS solution and 88 µL of 140 mM potassium persulfate solution. The mixture was then placed in a dark place at 10 ± 2 °C for 12–18 h before

use. For the test of ABTS radicals scavenging activity, 1 mL triterpenoid extract (0.075–0.6 mg/mL) and 2 mL of ABTS working solution was added into a test tube. The mixture was mixed and placed in the dark for 1 h at room temperature. The *G. lucidum* extract was then measured at the absorbance of 734 nm. Ascorbic acid was used as a standard with the same method. The percentage of ABTS+ scavenging activity was calculated the same as DPPH assay.

2.3 *Statistical analysis*

The experimental results were expressed as mean ± SD of three repeats. The statistical analyses were performed using Stagraphic Centrution XV (Statsoft Inc., Umeå, Sweden). The data from response surface methodology (RSM) were analyzed using Modde (version 5.0; Umetri, Umeå, Sweden) by F-test and ANOVA.

3 Results and Discussion

3.1 *The effect of extraction pressure on the content of triterpenoids*

Figure 1 illustrates the effect of extraction pressure variables on the content of triterpenoids using the SC-CO_2 method, where the extraction pressure conditions were varied between 200 bar to 600 bar. As shown in Figure 1(a), it can be seen that the content of triterpenoids obtained from the extracts significantly increased when the extraction pressure ranged from 200 to 600 bar. The highest content of triterpenoids was achieved (1.431 g/100 g) with the extraction pressure in a range of 400–600 bar. It was reported that high pressure caused cell broken resulting in the enhancement of the release of triterpenoids into the solvent. Yim *et al.* reported that the higher the pressure was used for extraction, the more solvent can enter inside the cells and more bioactive compounds can dissolve into the solvent [22]. Meanwhile, Domingues *et al.* documented that high pressure increased the rate of mass transfer of triterpenoids in solvent extraction [23]. In this study, high extraction pressure can increase the extraction yield of triterpenoids but too high pressure was not safe and expensive. Therefore, the extraction pressure of 400 bar was chosen for the extraction of triterpenoids.

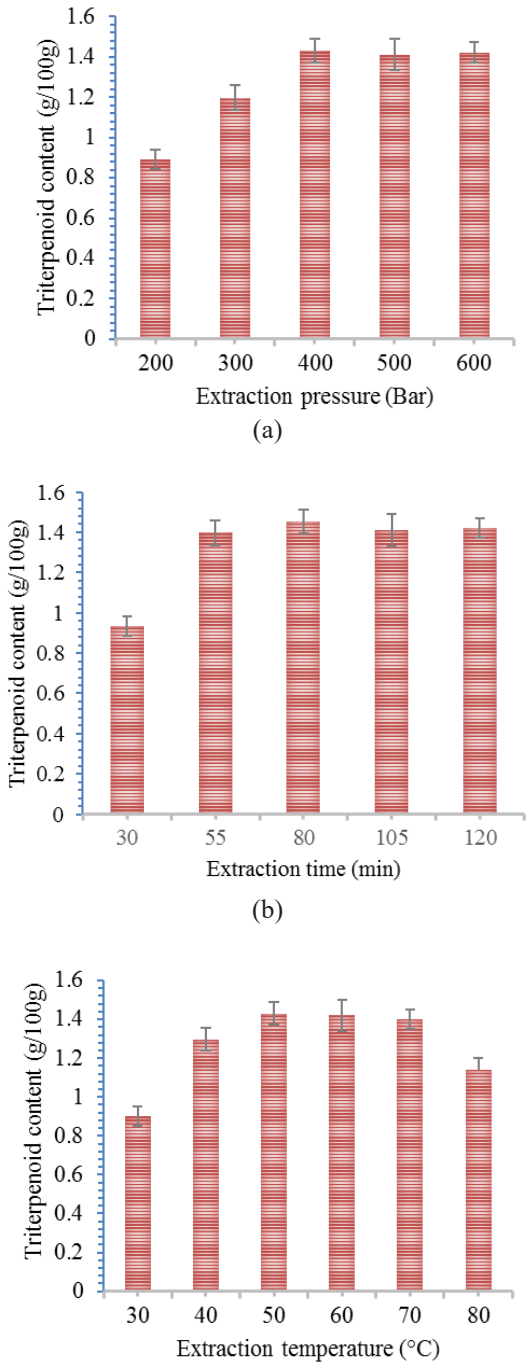


Figure 1: The effects of extraction variables on the content of triterpenoids. (a) effect of extraction pressure; (b) effect of extraction time; (c) effect of extraction temperature.

3.2 *The effect of extraction time on the content of triterpenoids*

Experiments were conducted to evaluate the effect of extraction time on the total content of triterpenoids. The extraction was performed with 400 bar extraction pressure and 50 °C at different extraction times (30, 55, 80, 105 and 120 min, respectively). Figure 1(b) shows that the content of triterpenoids increased with the increase of the extraction time. The highest level of triterpenoids (1.452 g/100 g) was obtained when the extraction time was 55 min. After 55 min extraction, the triterpenoid content remained unchanged (1.412–1.452 g/100 g). Our results were similar with Rodríguez-Pérez *et al.*, who reported that under a high pressure condition, the diffusion rate of the solvent was very high in a short extraction time, leading to the equilibrium of the content of triterpenoids between the inside and outside of the cells [14]. Thus, the extraction time of 55 min was used for the following experiments.

3.3 *The effect of extraction temperature on content of triterpenoids*

Figure 1(c) shows the effects of different extraction temperatures on the recovery of triterpenoids from the *G. lucidum* extract. In the range of 30–70 °C, the triterpenoid content increased as the temperature increased. An increase in temperature can enhance the permeability of triterpenoid molecules inside the cell into the solvent. Its finding is similar to Solana *et al.*, who reported that the increased recovery of glucosinolates and phenols from 29.2% to 38.2% when the temperature was increased from 45 °C to 75 °C, at high pressure of 300 bar [23]. As presented in Figure 1(c), the content of triterpenoids slightly decreased when the temperature exceeded 70 °C. According to Cai *et al.*, high temperature (over 60 °C) destroyed the triterpenoid molecular structure of the five rings, resulting in the low level of triterpenoids [24]. Thus, 50 °C was selected for supercritical fluid extraction of triterpenoid from *G. lucidum*.

3.4 *Regression analysis of triterpenoid extraction*

In this study, RSM design (based on CCF) was used to study the interaction among these parameters, including extraction pressure (X_1), extraction temperature (X_2) and extraction time (X_3), and optimize the triterpenoid content (Y). The results obtained from RSM experiments were summarized in Table 2. As shown in Table 2, the experimental and predicted triterpenoid values were in the range of 0.723–1.556 g/100 g.

The ANOVA results were used to check the adequacy of the regression model, and the results were shown in Table 3. The regression model was highly significant with a high F-value (95.63) and a small p-value (< 0.01). According to Maran and Manikandan, the large value of F indicates that most of the variation in the response can be explained by the regression equation [25]. Furthermore, the insignificance of the lack of fit test (p-value > 0.091) suggested that the regression model was fitted the experimental data properly [10], [21], [25], [26].

The robustness and predictive ability of the model were tested based on the coefficient of determination (R^2), the adjusted R^2, and model predictive ability (Q^2). The R^2 (0.992) and adjusted R^2 (0.982) values indicated the good agreement between the experimental and predicted values. The Q^2 was 0.993, indicating a high predictive ability model. The difference between R^2 and Q^2 was less than 0.3, which confirmed the validity of the predicted model [27].

The Pareto analysis was used to show the contribution of each variable and their interactions on the triterpenoid content at p-value < 0.05. As shown in Figure 2, it can be observed that the effect of extraction temperature (X_2) on the results was not significant,

Table 3: ANOVA results of response surface quadratic model for triterpenoid recovery

Tritepenoid Content	Degree of Freedom	Sum of Squares	Mean Square	F-value	*p*-value
Total Corrected	16	1.27663	0.079789		
Regression	9	1.26633	0.140703	95.6312	0
Residual	7	0.010299	0.001471		
Lack of Fit	5	0.009915	0.001983	10.3283	0.091
Pure Error	2	0.000384	0.000192		

R^2 = coefficient of determination = 0.992; adjusted R^2 = 0.982; Model predictive ability Q^2 = 0.933; $p < 0.05$ indicates statistical significance.

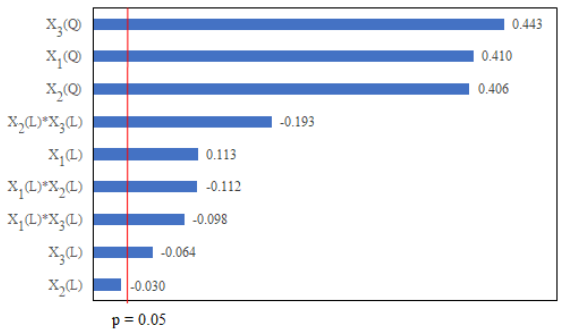


Figure 2: Pareto chart of the data analysis ($p < 0.05$).

while the pressure (X_1) and extraction time (X_3) had a considerable influence on the triterpenoid content. The quadratic regression equation was then generated as follows.

$$Y = 1.56 + 0.056X_1 + 0.032X_3 - 0.21X_1^2 - 0.20X_2^2 - 0.22X_3^2 - 0.056X_1X_2 + 0.049X_1X_3 - 0.1X_2X_3 \quad (6)$$

In Equation (6), the negative sign indicated the antagonistic effects, whereas the positive sign indicated the synergistic effects.

The 3D response surface plots of the triterpenoid recovery were obtained by plotting the response against two variables and presented in Figure 3(a)–(c). Figure 3(a) depicted the interaction between the X_1 and X_2 on the content of triterpenoids, given a 75 min SC-CO_2 time. The content of triterpenoids increased initially, and then slightly declined with an increase in X_1 (300–600 bar) and X_2 (30–80 °C), respectively. The maximum content of triterpenoids (1.56 g/100 g) was achieved with X_1 at 428 bar and X_3 at 54.9 °C, given X_2 was 75 min. The triterpenoid content significantly decreased to 1.24 g/100 g as the extraction pressure and temperature increased from 428 to 600 bar and 54.9 to 80 °C, respectively. This could be attributed to the degradation of triterpenoids by high pressure and temperature extraction. Its finding is similar to Uwineza and Waśkiewicz [28], who suggested the SC-CO_2 temperature of bioactive compounds should be fixed between 35 and 60 °C to avoid degradation, and the pressure should be around 400 bar.

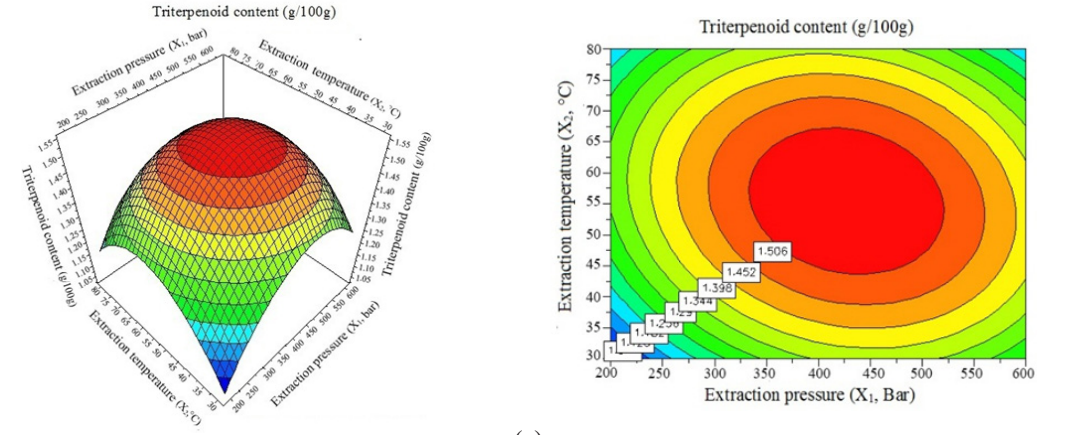
Figure 3(b) indicated the simultaneous effects of X_1 and X_3 on the triterpenoid content of the extract. The content of triterpenoids increased and achieved the maximum value (1.56 g/100 g) at X_1 (428 bar) and X_2 (75 min), given X_2 was 55 °C. The content of triterpenoids was then slightly reduced to 1.48 g/100 g as X_2 increased from 75 min to 120 min, given at 428 bar. According to Yim *et al.*, high pressure extraction and extended extraction time resulted in cell broken, thus enhancing the dissolution of triterpenoids into the solvent extraction [22]. However, Uwineza and Waśkiewicz reported that the extraction time is too long, it could affected the thermal instability of the bioactive compounds and extract a large number of impurities [28].

In Figure 3(c), X_2 and X_3 had a significant impact on the triterpenoid content. As presented in Figure 2(c), it can be seen that the content of triterpenoids increased with increasing in temperature (X_2) and time (X_3). The maximum triterpenoid value (1.56 g/100 g) was achieved with X_2 (55 °C) and X_3 (75 min) when X_1 was fixed at 400 bar. Tran *et al.* reported that high temperature and extended extraction time improved mass liquid transfer, enhancing the dissolution of triterpenoids into solvent extraction. However, high extraction temperatures (55–80 °C) and extended extraction times (75–120 min) contributed to lower levels of tritepenoids. Similar results were reported by Wei *et al.*, who documented that high temperature can accelerate the degradation of triterpenoids [21].

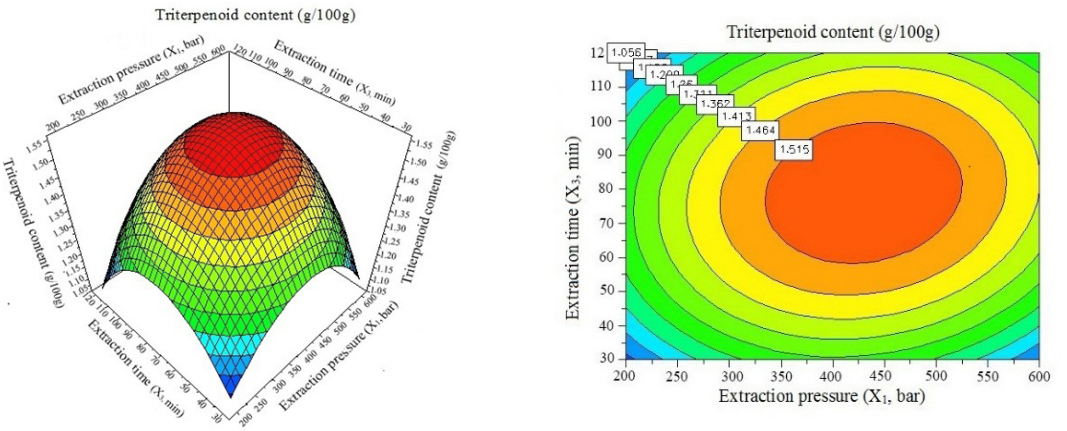
3.5 *Optimization of triterpenoid extraction*

The optimal extraction conditions for triterpenoid content from the *G. lucidum* were 430 bar, 54.8 °C, and 78.90 min. Under this condition, the predicted triterpenoid content achieved 1.56 g/100 g. To maximize the extraction conditions, these parameters were adjusted to 430 bar extraction pressure, 55 °C extraction temperature, and 79 min of extraction time. The experiments were then performed three replicates, with a recovery of 1.49 g/100g triterpenoids. Therefore, the experimental values were close to the predicted values, which indicated the response model is suitable for SC-CO_2 extraction of triterpenoids from *G. lucidum*.

Compared with traditional extraction methods, the extraction time in this study was shorter and the extraction recovery of triterpenoids was greater [13]. Zhou *et al.* used *G. lucidum* as raw materials to develop an ultrasonic extraction methodology, with an average extraction yield of triterpenoids of 1.30 g/100 g [29]. Microwave assisted extraction method (MAE)



(a)



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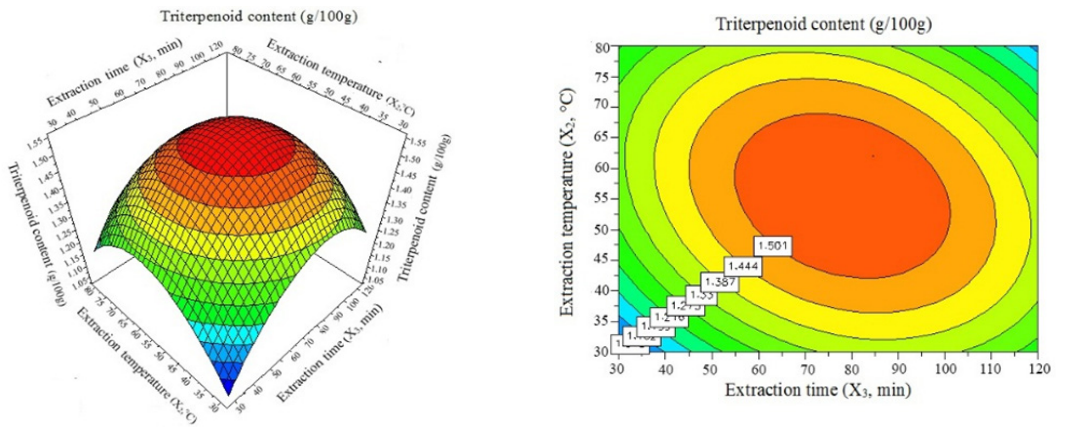
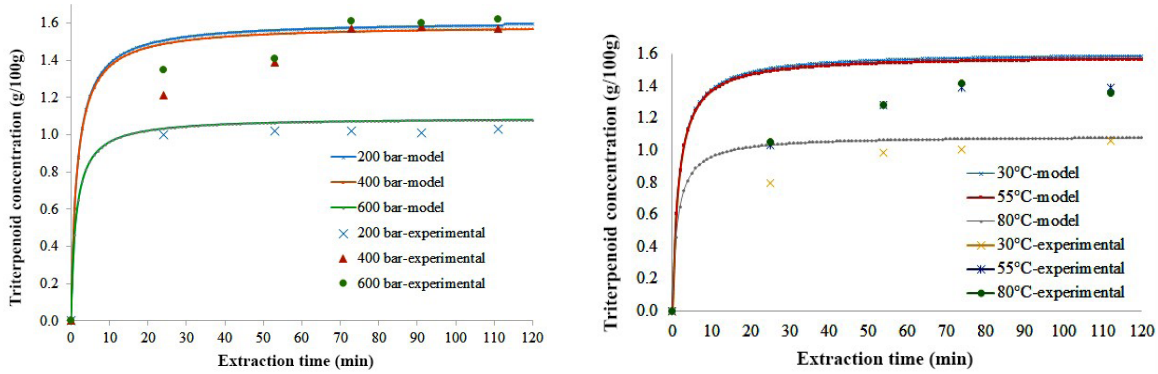


(c)

Figure 3: Response surface plots of relationships between triterpenoid recovery and extraction pressure, extraction temperature and extraction time, given: (a) Extraction time of 75 min; (b) extraction temperature of 55 °C; (c) extraction pressure of 400 bar.



(a) (b)

Figure 4: Triterpenoid concentration relative to SC-CO_2 time given: (a) extraction temperature of 55 °C; (b) extraction pressure of 400 bar.

was also used to ameliorate the triterpenoid content extracted from *G. lucidum* [10], [15]. However, MAE process can severely damage the cell wall, increasing the impurities in the extracts [15]. Thus the purification of triterpenoid was difficult to process.

3.6 *Kinetic of SC-CO_2 extraction process*

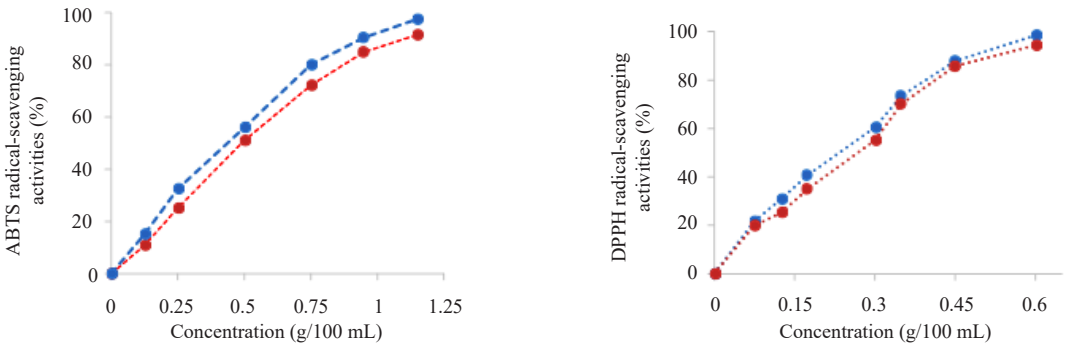
A second order kinetic model was used to describe the effects of the SC-CO_2 parameters on the extraction of triterpenoids from *G. lucidum*. The R^2 and root mean square error (RMSE) used to determine the goodness of fit of the models are shown in Table 4. As shown in Table 4, it can be seen that the high value of R^2 (0.989–0.993) and a very low value of RMSE (0.091–0.098) indicated the validity of the second-order kinetic extraction model. Similar results have been reported by Phan *et al.* for extraction gamma-oryzanol from dried rice bran soapstock. In Table 4, Cs, k and h increased with increases in extraction pressure (from 200 to 400 bar) and extraction temperature (from 30 °C to 55 °C). Meanwhile, Cs and h slightly decreased when the extraction temperature rose from 55 °C to 80 °C. Thus, high extraction temperature lead to the degradation of triterpenoid, resulting in a low extraction rate and low level of triterpenoids in the extracts.

In Figure 4(a) and (b), the kinetic results (simulation and experimental) also revealed that nearly 80% of the triterpenoids were recovered in the early extraction stage (from 0 to 65 min). The prolonger extraction time slightly increased the recovery of triterpenoids, consistent with the RSM results [Figure 3(b) and (c)]. In addition, the triterpenoid content significantly increased with increased SC-CO_2 pressure (200 bar to 400 bar) and extraction temperature (30 to 55 °C) [Figure 4(a) and (b)]. According to Domingues *et al.*, high pressure increased the rate of mass transfer of triterpenoids in solvent extraction. Meanwhile, Tran *et al.* reported that higher extraction temperatures improved the extraction rates of triterpenoids

Table 4: Kinetic parameters of triterpenoid recovery under different SC-CO_2 extraction pressures and extraction temperatures, given 120 min extraction time

Kinetic Paramters	Extraction Pressure (bar)			Extraction Temperature (°C)		
	200	400	600	30	55	80
h (g/100g)	0.166	0.392	0.422	0.177	0.402	0.397
k (100g/g.min)	0.140	0.155	0.163	0.142	0.161	0.157
Ce (g/100g.min)	1.09	1.59	1.61	1.12	1.61	1.59
R^2	0.975	0.976	0.994	0.984	0.989	0.991
RSME	0.092	0.0922	0.063	0.040	0.079	0.091

C_e = triterpenoid concentration at saturation; *k* = extraction rate constant; *h* = initial extraction rate; R^2 = coefficient of determination; RMSE = root mean square error.



(a) (b)

Figure 5: (a) ABTS and (b) DPPH radical scavenging activities of the extract (●) and ascorbic acid (●).

because under higher temperatures, the solubility and diffusivity of triterpenoids increased, and accelerating the extraction process. Therefore, it can be said that the second-order kinetic model can represent well the experimental results of triterpenoid extraction by the SC-CO_2 method, and is similar to the RSM results.

3.7 *Antioxidant activity*

The scavenging activity of $DPPH^{\bullet+}$ and $ABTS^{\bullet+}$ has been widely used to determine the free radical-scavenging activity. Based on the relationship curve between the triterpenoid concentration and the percentage of free radical activity, the value of IC_{50} was determined. Ascorbic acid was used as a reference standard. The DPPH and ABTS radical scavenging activities of the extract and ascorbic acid are shown in Figure 5(a) and (b). As presented in Figure 5(a) and (b), when the triterpenoid concentration increased, the DPPH and ABTS radical scavenging activity significantly increased.

For DPPH radical scavenging activity, the IC_{50} value of *G. lucidum* extracts was found to be 0.49 mg/mL, and non-significant difference compared with ascorbic acid (0.51 mg/mL). For ABTS radical scavenging activity, the measured IC_{50} of *G. lucidum* extract was 0.26 mg/mL and ascorbic acid was 0.27 mg/mL. This data showed that the antioxidant capacity of the extract resembled the ascorbic acid. The results were consistent with the data found by Tran *et al.*, who reported that the triterpenoid extract from *G. lucidum* exhibited strong antioxidant scavenging activities [10].

4 Conclusions

In this study, the SC-CO_2 method was developed proficiently to obtain triterpenoid from *G. lucidum*, and the RSM was applied to determine the optimal extraction conditions. The optimal conditions for triterpenoid extraction were 430 bar, 54.8 °C, and 78.90 min. Under these conditions, the highest content of triterpenoids was 1.56 mg/100 g, that was agreed with the experimented value of 1.49 mg/100 g indicating the success of RSM for optimizing triterpenoids from *G. lucidum*. The result also showed that the second order kinetic model represented well the experimental results of triterpenoid extraction by SC-CO_2 method. The antioxidant activity of the extract achieved under the optimized extraction condition using the SC-CO_2 method was then estimated and calculated for the IC_{50} values. The measured IC_{50} of the extract was 0.49 mg/mL for the DPPH radical scavenging assay, and 0.26 mg/mL for ABTS radical scavenging assay, which closely comparable to the standard ascorbic acid (DPPH value of 0.51 mg/mL and ABTS value of 0.27 mg/mL). Therefore, the *G. lucidum* extract could be considered as an agent for antioxidant, and it can be applied for functional foods and antitumor drugs in the future. Our findings suggest that SC-CO_2 is a promising method for triterpenoid extraction with antioxidant properties.

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