OPTIMIZATION OF TRITERPENES EXTRACTION FROM *Ganoderma lucidum* BY SUPERCRITICAL CARBON DIOXIDE

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ABSTRACT

Ganoderma lucidum is one of the popular mushrooms in Asia. Nowadays, *Ganoderma lucidum* is used all over the world as a tonic or functional food. The bioactive properties of *Ganoderma lucidum* are mainly from polysaccharides, peptidoglycan, and triterpenes. Water is normally used to obtain *Ganoderma lucidum* liquid. However, this traditional method only collects the polarized active substance as a polysaccharide. In recent years, the extraction by the supercritical solvent to improve the resulted extraction and assure the biological actives quality has been mentioned in some research. In this study, we used supercritical CO_2 for *Ganoderma lucidum* extraction. The results showed that the best condition to extract with supercritical CO_2 was by ethanol 14% (w/w) with a flow rate of 14 g/min for 120 mins, at 59 ^oC temperature and 153 bar pressure. At this condition, the triterpene yield extraction and the total soluble dry matter were 88.9% and 39.2%, respectively. As compared to *Ganoderma lucidum* prepared by enzyme assistance, the supercritical CO_2 method gave a higher extraction yield. However, the extraction of the total soluble dry matter was not higher than by applying the enzyme method.

Keywords: Extraction, *Ganoderma lucidum*, optimize, triterpenes, supercritical CO_2.

1. INTRODUCTION

Ganoderma lucidum (*G. lucidum)* is mainly planted in Asia. Chinese, Japanese, and others use *G. lucidum* as a plant-sourced medicine for health improvement and long life [1]. *G. lucidum* is classified into 6 kinds based on the colors: green, red, yellow, white, black, and purple; the main ingredients include ash (1.8%), carbohydrates (26-28%), lipids (3-5%), fiber (59%) and protein (7-8%) [2-4]. Besides, *G. lucidum* also contains many biological actives such as terpenoids, steroids, phenolics, nucleotides, and their derivatives. The biological actives of *G. lucidum* are mainly from polysaccharides, peptidoglycan, and triterpenes [5-7]. The polysaccharides of *G. lucidum* have anti-inflammatory effects, hypoglycemia, anti-ulcer, anti-tumor formation and improve immunity [1]. More than 50 triterpenes have been found in *Ganoderma lucidum*, most of the triterpenes are ganoderic acid and lucidenic acid [8]. They possess lots of functional biological activities to health such as antioxidants and decreasing fat quantity in our body. These substances contribute to the bitterness of *G. lucidum* [9].

Currently, the supercritical method by the solvent is used popularly for production to increase the selectivity of the concerned ingredients, improving retrieving, and preserving the compound activity. A fluid in critical status has the diffusive ability as a gas and the dissolving ability as a liquid [10]. CO_2 is the best choice in the natural compounds extraction industry [11, 12] due to its advantage of the critical level condition is not high (P = 73 atm, T = 30,9 ^oC).

The critical level condition of CO_2 is not high (P = 73 atm, T = 30,9 ^oC) thus it has less energy consumption for operation, rapid mass transfer capacity and high selectivity of the extracted constituents. On the other hand, it is an inert substance hence limiting the side effects, non-toxic to our body, and non-equipment corrosive. To increase the extraction efficiency for a constituent group with different properties, the auxiliary solvent can be added which plays a role as a co-solvent in the extraction method by supercritical fluid. Therein, methanol, ethanol, and propanol are fluid which is frequently used as co-solvent [13, 14].

Normally, water is the solvent used to extract the substances in *G. lucidum*. However, this traditional method can only extract polar active ingredients such as polysaccharides. Weakly polar or non-polar active compounds cannot be extracted by water [15,16]. Recently, the supercritical fluid extraction method has also become a popular method in the world to recover natural compounds such as essential oils and similar compounds. This is a promising method to replace traditional solvent extraction methods. The purpose of this study is to determine the optimal conditions in the process of extracting *G. lucidum* using supercritical CO_2 with ethanol as a co-solvent.

2. MATERIALS AND METHODS

2.1. Material

In this study, red *G. lucidum* originated from Da Lat. They were dried to 10% moisture and finely grounded (particle diameter is about 0.2 mm) before experimenting.

The supercritical extraction equipment (model SFE500, Thar Technologies Inc., USA) was at the key laboratory, Department of Machine and Equipment, Faculty of Chemical Engineering, Ho Chi Minh City University of Technology.

Analysis equipment HPLC (model 1200 series, Agilent Technologies Inc., USA) was at the Biomass laboratory, Ho Chi Minh City University of Technology.

2.2. Examination of the elements affecting supercritical CO_2 ($ScCO_2$) extraction

The purpose of the experiment was to determine the effective elements on the recovery efficiency of triterpenes, polysaccharides, and total dissolved dry matter. The examination elements consist of an ethanol ratio of 5-20% (w/w), flowing rate of 8-20 g/min, temperature of 40-80 ^oC, pressure at 100-225 bar, and duration of 30-240 mins.

5 g of *G. lucidum* was extracted by supercritical CO_2 and obtained the solution. The control sample was prepared by weighting 5 g *Ganoderma lucidum*, adding ethanol 60% with a ratio of 1:35 (w/v), and extracted at 70 ^oC for 30 mins. Then it was filtered by air vacuum. 35 mL distilled water was added to the residue to extract polysaccharide at 90 ^oC for 30 mins. *G. lucidum* solution was determined for triterpenes, polysaccharides, and total dissolved dry matter.

2.3. Optimizing parameters by the experimental method

Two factors which are most affected by the extraction were detected by the Plackett-Burman matrix experiment with 3 levels -1, 0, +1 (Table 1). The optimal value of two major factors was determined at 5 levels -α, -1, 0, +1, +α (Table 2). The mathematical formula was presented by the quadratic equation (1) :

$$Y = b_o + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2 \quad (1)$$

In which: b_0 is the freedom coefficient, b_1, b_2 are first-order coefficients; b_{11}, b_{22} are second-order coefficients; b_{12} is the interaction coefficient; Y is the yield of polysaccharides or triterpenes; X_1, X_2 are coded independent variables.

The experiment matrix is determined and processed by Mode 5.0 software. The optimum values of these factors are the optimum results of extraction.

Table 1. Plackett-Burman design

Independent variable	Factor	Survey range	Independent variable levels		
			-1	0	1
X_1	The ratio of ethanol (% w/w)	11 – 17	11	14	17
X_2	Flow rate (g/min)	12 – 16	12	14	16
X_3	Temperature (^oC)	50 – 70	50	60	70
X_4	Pressure (bar)	125 - 175	125	150	175
X_5	Time (min)	90 - 150	90	120	150

Table 2. Central composite design

Independent variable	Factor	Survey range	Independent variable levels				
			-α	-1	0	+1	+α
X_1	Temperature (^oC)	46 - 74	46	50	60	70	74
X_2	Pressure (bar)	115 - 185	115	125	150	175	185

2.4. Comparison of the treatment of supercritical CO_2 and treatment of enzyme

G. lucidum was treated by $ScCO_2$ or enzyme at the optimum condition. The extracted fluid is analyzed by HPLC to compare ursolic acid and ganoderic acid content (two triterpene ingredients in *G. lucidum* and compare the degree of constituent selectivity of two mentioned methods.

The sample treated by enzymes was prepared as follows: 5g of *G. lucidum* was added with distilled water at a ratio of 1:35. pH was adjusted to 5.0 with 0.1N HCl. Hemicellulase enzyme (trade name is Viscozyme L) was added at a concentration of 0.15% (v/w). The sample was incubated in a thermostatic bath at 50 ^oC for 51 mins to allow enzyme actives. Then the sample was vacuum filtered, and the extract was collected.

2.5. Determination of polysaccharides content

The polysaccharides content was determined by the phenol-sulfuric acid method with reference standard D-glucose. Each specimen is withdrawn 1 mL of the extraction to put into the tube with a cover. Afterwards, 1 mL phenol 5% and 5 mL H_2SO_4 98% were added to each tube. The mixture was shaken vigorously and boiled for 2 mins, then was kept at room temperature for 30 mins. The absorbance was measured at the wavelength of 625 nm. The total contents of polysaccharides were identified based on the standard curve of glucose $y = 121.6x – 1.2882$ (y: Glucose content (ppm), x: Absorbance). The results were presented by grams of glucose in 100 grams of material.

2.6. Determination of triterpenes content

The content of triterpenes was determined based on the reaction between triterpenes and vanillin in the acid environment which produces violet complex and ursolic acid standard. 1 mL triterpenes solution was continuously wholly evaporated by placing it in a hot tub at 100 ^oC. Then 0.4 mL vanillin-acetic acid 5% and 1 mL perchloric acid were added and the solution was incubated in the water tub at 60 ^oC for 15 mins. It was frozen rapidly, then 5 mL of acetic acid was added and placed at room temperature for 15 mins. The total triterpenes

content was determined based on ursolic acid standard y = 0.1381x + 0.0048 (y: Ursolic acid content (mg), x: Absorbance). The results were expressed in grams of ursolic acid counted from 100 grams of initial material [17].

The revoked contents of triterpene and soluble dry matter were calculated by the following equation (2):

$$H\% = \frac{m_1}{m_0}.100 \quad (2)$$

There m_1 is the triterpenes content or the soluble dry matter content in the extract, m_0 is the triterpenes or the soluble dry matter content in dry material.

2.7. Determination of total soluble dry matter content

The mass of soluble dry matter was determined by drying to a constant value [18]. After filtration, 50 mL of the extracted solution was placed into a petri dish and dried at 105 °C for 30 minutes. Afterward, the petri dish was weighed, and the mass of soluble dry matter was calculated according to equation (3):

$$W = \frac{B-A}{50} \times V \quad (3)$$

There is W (g) is total soluble dry matter content, A (g) is the weight of the petri dish without the sample, B (g) is the weight of the petri dish after drying, and V (mL) is the volume of the extract.

2.8. Identifying the types of triterpenes by HPLC

Triterpenes were analyzed by HPLC performed by following Wang's method. In short, The HPLC system equipped with a UV-Vis detector, Eclipse XDB-C18 (5 µm, 4.6 x 150 mm) with the following procedure: flow rate was 1 mL/min; the mobile phase consisted of a mixture of 30% (A) acetonitrile and 70% (B) 0.03% aqueous phosphoric acid (v/v). The detection wavelength was set at 270 nm [19].

2.9. Statistical analysis

In this research, each experiment was conducted in triplicate. The results were calculated as average in 100g ingredients.

These experiments were analyzed by ANOVA (α= 5%) statistical system on Statgraphics Centurion XVI software. Optimization experiments were designed by Mode 5.0 software.

3. RESULTS AND DISCUSSION

3.1. The affection of co-fluid ratio to the extraction yield

The content variation following to co-fluid is presented in Figure 1. In the case of triterpene content extraction, the $ScCO_2$ sample was lower than the sample extracted by ethanol at 60% if the co-fluid was lower than 10% (w/w). When co-fluid was increased the triterpene content trended to increase rapidly and was higher as compared to the control sample. However, the speed was unchangeable when the co-fluid rate was higher than 14% ethanol (w/w). According to Hsu et al., the exploit of supercritical fluid which did not use the co-fluid could not obtain polarized matter such as triterpene [20]. Co-fluid not only increased the dissolvent of the extraction but also the flexibility of supercritical fluid, which made it obtain more extraction content. This indicates that ethanol increased the polarization of supercritical matter and helped extract more polarized triterpene. Corresponding to the ratio of 14% ethanol, the triterpene content reached 0.741g, higher 37.1% than the control sample.

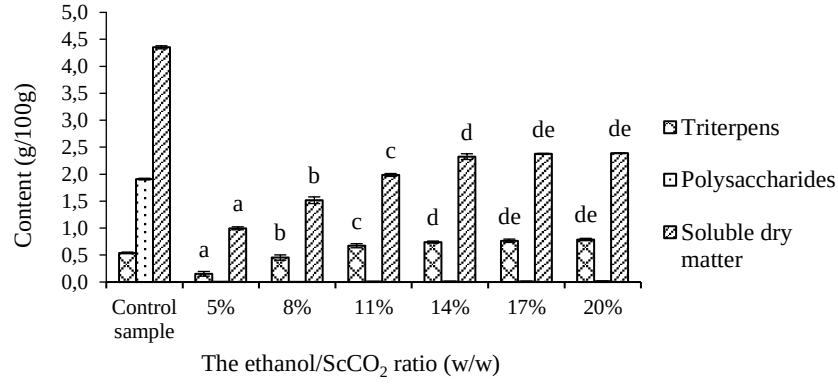

Figure 1. The affection of ethanol concentration to the extraction

In theory, the obtained soluble dry matter content has a similar rule to triterpene, which means the soluble dry matter content is raised when the co-fluid reaches 14%, and then there is no difference if ethanol continues to rise. However, the soluble dry matter content was always lower than the control sample treated with ethanol 60%. It is suggested that *G. lucidum* has abundant dissolved matter which has rich polarization while SO-CO_2 was impossible to use to extract. With the ethanol concentration of 14%, the soluble dry matter content obtained was lower than 46.6%.

On the other hand, the polysaccharide content obtained was extremely low. The highest matter was 0.013 g corresponding to 0.7% as compared to the control sample which was extracted by ethanol of 60%. This could be explained by SO-CO_2 being a liquid that is very poor in polarization. It was more suitable for the complex, which is polarized or non-polarized, meanwhile, the polysaccharide was a highly polarized matter.

3.2. The affection of flow rate to the extraction yield

Figure 2 expressed the affection of the flow rate SO-CO_2 to the extraction results. Triterpene content reached the highest content of 0.794 g equivalent to the flow rate of 14 g/min and higher than the control sample of 46.9% (0.541 g). When the flow rate increased, the triterpene content was not different. Similarly, the obtained soluble dry matter content reached 2.513 g at 14 g/min which was higher than that of ethanol 60% extraction 42.3% (4.352 g). Dunford et al. (2003) reported that the flow rate increases lead to the ratio of fluid/material also rising thus the triterpene content is obtained more [21]. However, matter contents increased to a definite level when the flow rate changed. Besides, polysaccharides changed when the flow rate rose nonetheless it was not remarkable.

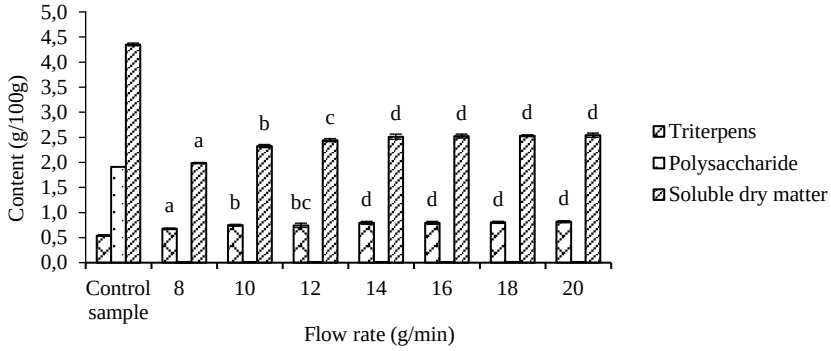

Figure 2. Affection of flow rate SO-CO_2 to triterpenes content

In the research of Michielin et al. (2005) when the flow rate changes from 1.85×10^{-5} to 4.73×10^{-5} kg CO_2/s the duration of the obtaining period is shortened from 720 mins to 288 mins. Similarly, the treatment duration was down to half if the flow rate was increased from 1 g/min to 2 g/min in peach oil extraction [22].

In general, the flow rate increases which shorten the treatment duration. In this research, the flow rate of 14g/min was the most suitable to perform the subsequent experiment. With this flow rate, the revoked content of triterpene and soluble dry matter was 49.5% and 27.7%, respectively.

3.3. Affection of treatment temperature to the extraction yield

In general, the obtained triterpene content with the $ScCO_2$ method at different temperatures was higher than the standard sample (figure 3). Triterpene content increased rapidly when it gradually reached 60 ^oC and then decelerated. With the treatment of 60 ^oC, the obtained triterpene content was 0.902 g which was higher 66.8% than the control sample which was extracted by ethanol at 60%. The obtained soluble dry matter content had a similar rule to triterpene. However, it was still 32,1% lower than the sample which was extracted by ethanol 60% at 60 ^oC. Hsu et al. stated that temperature increases lead to escalating the diffusion ability of supercritical fluid [20]. Michielin et al. also found that when the temperature increases from 45 ^oC to 60 ^oC the yield rises by 0.05% [23]. Vasapoloo et al. described a similar affection when lycopene from tomatoes was extracted from 45 ^oC to 60 ^oC leading to a proportional increase in the resulting efficiency [24].

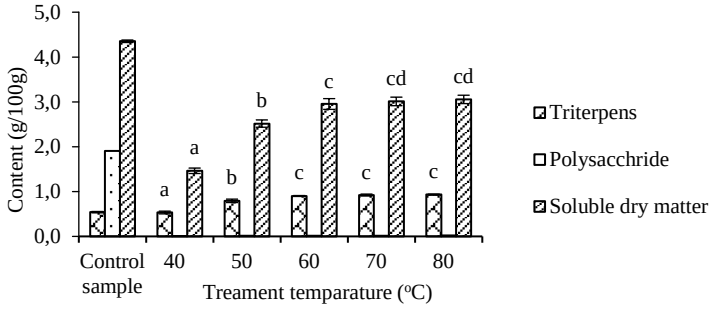

Figure 3. The affection of treatment temperature with $ScCO_2$ to triterpene and total soluble dry matter contents

Therefore, in this research, the temperature of 60 ^oC is suitable for extracting *G. lucidum* by $ScCO_2$. The obtained extraction efficiency of triterpene and soluble dry matter content was 56.3% and 32.5%, respectively.

3.4. Affection of treatment pressure to the extraction yield

The results of the $ScCO_2$ are presented in Figure 4. The triterpenes and soluble dry matter contents reached the maximum value of 0.982 g and 3.127 g at the pressure of 150 bar, the triterpene content was 81.7% higher than the control sample. Meanwhile, the soluble dry matter content was lower by 28.2%. Despite increasing even higher in pressure, these two obtained contents were not changed. Hsu et al., stated that the pressure affects the density of $ScCO_2$, the higher the density of the fluid, the greater the solubility of the substances [20]. Fluid density was increased when the pressure was enhanced. Although the increase of soluble dry matter is insignificant when the pressure of treatment changes, increasing the pressure is important to enhance the triterpene content. Besides, high pressure also affects to break up of

the spores to contribute to releasing matter [25]. Michielin et al. also found that higher processing pressure results in higher concentrations of Equisetum extraction [23].

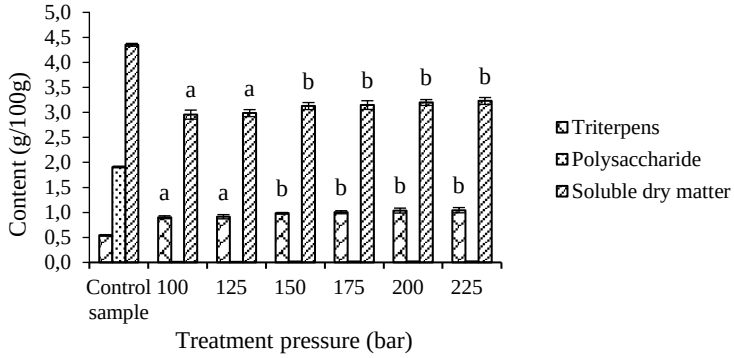

Figure 4. The affection of treatment pressure with $ScCO_2$ to triterpene and total soluble dry matter contents

Whereas the extraction yield of triterpene and soluble dry matter content by $ScCO_2$ with pressure at 150 bar was 62.3% and 34.5%, respectively. This pressure level was chosen to perform the next experiments.

3.5. Affection of treatment duration to the extraction yield

As the experiment results presented in Figures 5 and 6, the triterpene extraction content by $ScCO_2$ was always higher than the sample when the treatment duration was extended. However, the difference between the two methods was decreased when the duration was longer. The main reason was due to the longer time of extraction the lesser remnants content amount hence the extracting speed slowed down. The triterpene content at 120 mins of treatment was 1.414 g, higher by 41.4% compared to the control sample. In contrast, the soluble dry matter content was lower than the control sample which was extracted by ethanol 60%. The difference between these two methods was initially increased gradually and afterward unchangeable. Since the matter in *G. lucidum* initially still was abundant the extraction rate with ethanol 60% increased sharply when the content of matter was nearly exhausted causing unchangeable speed. The soluble dry matter content extracted by $ScCO_2$ after 60 mins was 3.443 g, lower by 43.2% than the control sample. Michielin et al. (2005) also found a similar effect of treatment duration on the extraction content of Equisetum [23]. Specifically, triterpene content was gradually increased when the treatment duration rose from 0 to 100 mins. Maximum triterpene content when the duration reached 270 mins nevertheless prolonged treatment duration with $ScCO_2$ the content tended to decrease.

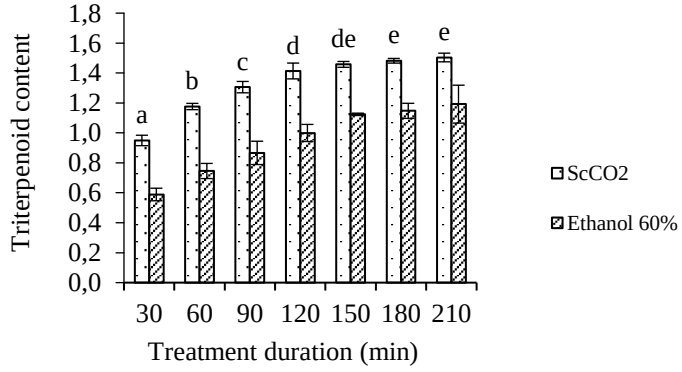

Figure 5. The affection of treatment duration of $ScCO_2$ to triterpene content

Therefore, the duration of 120 mins was chosen to perform the following critical experiment. With this treatment duration, the yield of triterpene and soluble dry matter reached 88.2% and 38.6%, respectively.

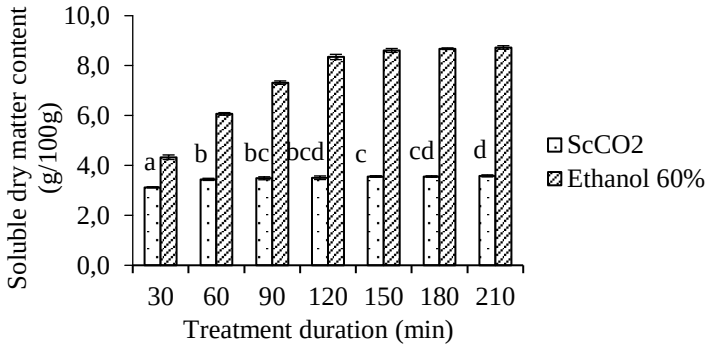

Figure 6. The affection of treatment duration of $ScCO_2$ to dry matter content

3.6. Optimizing for extraction of *Ganoderma lucidum*

First, within 5 factors there were 2 factors have the most influent extraction efficiency were chosen. The results in Table 3 showed the temperature and pressure of $ScCO_2$ had a higher influence than other factors ($p < 0.05$). For instance, the obtained triterpene content under the conditions of temperature and pressure were 0.078 and 0.091, respectively. Therefore, temperature and pressure were chosen for matrix design to discover the optimal values. The optimal results of the CCD model are shown in Table 4.

After determining the regression coefficient, these coefficients were examined to control for the relevance of the equation. A polynomial model describing the correlation between the extraction yield of *G. lucidum* and two variables was obtained as follows:

$$Y_1 = -0.184X_1^2 - 0.296X_2^2 - 0.033X_1 + 0.058X_2 + 1.424$$

$$Y_2 = -0.423X_1^2 - 0.325X_2^2 - 0.061X_1 + 0.096X_2 + 3.508$$

Table 3. The affection of $ScCO_2$ parameters to the extraction

Factors	Affection rate	
	Triterpen	Soluble dry matter
Ethanol ratio	-0.071[a]	-0.010[b]
Flow rate	-0.001[b]	0.003[b]
Temperature	0.078[a]	0.028[a]
Pressure	0.091[a]	0.032[a]
Duration	0.021[a]	0.004[a]

[a] Significant at $\alpha = 0.95$; [b] Insignificant at $\alpha = 0.95$

The ANOVA analysis results for examining the compatibility of the triterpene regression equation are presented in Table 5. The regression model has high compatibility because $P < 0.05$. The R^2 (0.987), and R^2_{adj} (0.977) values indicate the suitability between the experimental value and the predicted value. The Q^2 value (0.920) indicates the high prediction accuracy of the model. The ANOVA results for examining the compatibility of the soluble solids content regression equation give similar results.

Table 4. Experimental triterpene and total soluble dry matter content under variable extraction temperatures (X_1, °C), and extraction pressures (X_2, bar).

Run	Real value		Code value		Triterpenoid content (g/100g) (Y_1)	Total soluble dry matter content (g/100g) (Y_2)
	Temperature (°C)	Pressure (bar)	Temperature (X_1)	Pressures (X_2)		
1	50	125	-1	-1	0.823	2.584
2	70	125	1	-1	0.872	2.698
3	50	175	-1	1	1.051	2.929
4	70	175	1	1	0.938	2.782
5	46	150	$-\sqrt{2}$	0	1.151	2.833
6	74	150	$\sqrt{2}$	0	1.008	2.512
7	60	115	0	$-\sqrt{2}$	0.796	2.751
8	60	185	0	$\sqrt{2}$	0.917	2.988
9	60	150	0	0	1.413	3.508
10	60	150	0	0	1.424	3.578
11	60	150	0	0	1.432	3.435
12	60	150	0	0	1.396	3.539
13	60	150	0	0	1.456	3.479

Table 5. ANOVA results of response surface quadratic model for triterpenoid recovery

Triterpene content	Degree of freedom	Sum of squares	Mean square	F-value	p-value
Total Corrected	12	0.810917	0.067576		
Regression	5	0.800258	0.160052	105.107	0
Residual	7	0.010659	0.001523		
Lack of Fit	3	0.008666	0.002889	5.79851	0.061
Pure Error	4	0.001993	0.000498		
N = 13	Q2 =	0.92	Cond. no. =	2.8971	
DF = 7	R2 =	0.987	Y-miss =	0	
	R2 Adj. =	0.977	RSD =	0.039	

Table 6. Coefficients of the regression equation

Triterpene content	Coeff. SC	P
Constant	1.42421	1.09E-11
X1	-0.03328	0.046634
X2	0.058146	0.003965
X1*X1	-0.18412	4.99E-06
X2*X2	-0.29566	1.97E-07
X1*X2	-0.0405	0.076566

Soluble dry matter content	Coeff. SC	P
Constant	3.5078	1.45E-12
X1	-0.06087	0.048123
X2	0.09553	0.007151
X1*X1	-0.42341	1.12E-06
X2*X2	-0.32488	6.74E-06
X1*X2	-0.06525	0.112849

In the triterpenes polynomial model, the regression coefficient of triterpene of X_1^2 (0.184) is smaller X_2^2 (0.296) showing that the treatment temperature had less influence than the treatment pressure (table 6). On the other hand, these factors do not interact with each other due to the coefficient of X_1X_2 was insignificant with α= 0,95 (p > 0.05). In contrast, treatment temperature has a greater influence on soluble dry matter content than pressure. The triterpenes and total soluble dry matter contents reached the maximum value of 1.429 g and 3.517 g respectively at the treatment temperature and pressure corresponding to 59°C and 153 bar (Figure 7, 8). The examined experiment results were not noticeably different as compared to the prediction (Table 7).

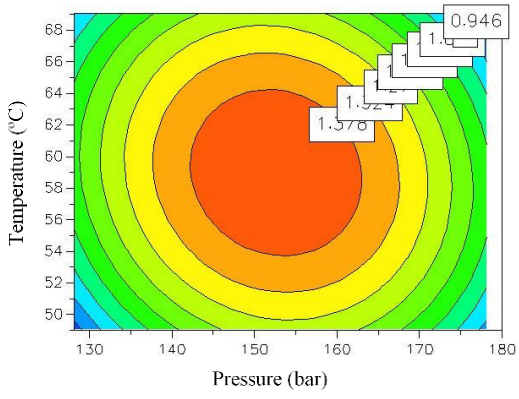

Figure 7. The response surface of triterpene content

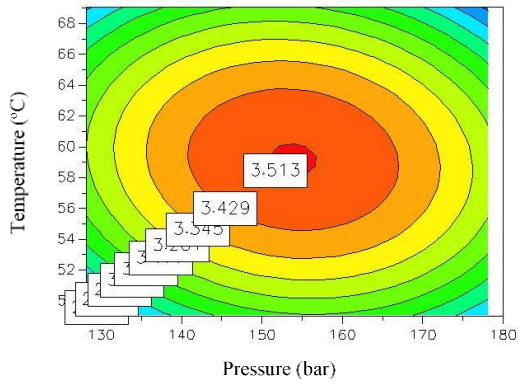

Figure 8. The response surface of total soluble dry matter content

Table 7. Experimental and predicted triterpenoid content and total soluble dry matter content under optimization conditions of $ScCO_2$ extraction

Temperature (°C)	Pressure (bar)	Triterpenoid content (g/100g)		Total soluble dry matter content (g/100g)	
		Predicted value	Experimental value	Predicted value	Experimental value
59	153	1.429	1.424 ± 0.022	3.517	3.556±0.033

In general, the optimal conditions for the two goals were at 59 °C treatment and 153 bar pressure. In these conditions, triterpenes content achieved 1.424 g covalent to 88.9% efficiency, the soluble dry matter content was 3.556 g covalent to 39.2% efficiency. Previously, Yan et al. (2010) optimized triterpenes extraction from *G. lucidum* by $ScCO_2$ without using co-solvent, the result of obtained triterpenes contents at optimal conditions only reached 17.6%. Therefore, it is possible to conclude that using co-fluid in $ScCO_2$ gives a better result than purified CO_2 [26].

3.7. The comparison of $ScCO_2$ and enzyme-assisted extraction methods

G. lucidum was extracted at optimization conditions by $ScCO_2$ and enzyme-assisted methods. Following the analyzed HPLC result of two methods (Figure 9). Only ursolic acid was detected by enzyme extraction. In the case of $ScCO_2$, ursolic acid, and garnoderic A acid were found. However, the obtained ursolic acid from the enzyme treatment method was more than another method (Table 8). The ratio of 2 triterpenes of the $ScCO_2$ sample was 8.3% higher than the sample treated by an enzyme which was 6.1%. It implies that the purity of the triterpene sample treated by $ScCO_2$ was higher than by the enzyme method. However, polysaccharides and total soluble dry matter content extracted by $ScCO_2$ were lower (Table 9). This was explained

by Sihvonen et al. (1999), $ScCO_2$ has the character of dissolving the nonpolar ingredient extracted as triterpenes. Hence the impurities also decreased noticeably.

Table 8. The contents of some ingredients are extracted by two methods.

Treatment method	Triterpene (g/100g)	Polysaccharide (g/100g)	Total soluble dry matter (g/100g)
$ScCO_2$	1.429 ± 0.046	0.022 ± 0.001	3.553 ± 0.034
Viscozyme L	1.316 ± 0.029	3.185 ± 0.018	8.654 ± 0.049

Table 9. Result of HPLC analysis with two-method extraction

Treatment method	Triterpenes	Time (s)	Peak area (mAU*s)	Peak rating (%)
$ScCO_2$	Ursolic acid	2.308	17.427	1.826
	Garnoderic acid	9.820	61.374	6.432
Viscozyme L	Ursolic acid	2.391	135.435	6.062

4. CONCLUSION

$ScCO_2$ extraction used with ethanol fluid has effective results, selectively extracting non-polarized or less-polarized matters such as triterpenes. Consequently, the extracted products have higher purity, and shorter extraction duration as compared to the traditional methods that utilize solvent. The optimum condition for $ScCO_2$ was 14% ethanol, a flow rate of 14g/min, a treatment duration of 120 mins at 59 ^oC, and 153 bar pressure. At this condition, the extraction efficiency of triterpene reached 88.9%, and the recovery efficiency of total soluble dry matter was 39.2%. However, this method was less effective in extracting polarized matter such as polysaccharides. Hence, it is necessary to continue extracting polysaccharides by traditional methods after being extracted by $ScCO_2$. Accordingly, polysaccharide recovery efficiency could be improved.

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TÓM TẮT

TỐI ƯU HÓA QUÁ TRÌNH TRÍCH LY TRITERPENS TỪ NẤM LINH CHI (*Ganoderma lucidum*) BẰNG PHƯƠNG PHÁP CO_2 SIÊU TỚI HẠN

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Nấm Linh Chi (*Ganoderma lucidum*) là một loài nấm thường được tìm thấy ở các nước Á Đông. Hiện nay, Linh Chi được dùng khắp nơi trên thế giới như một loại thuốc bổ hay một loại thực phẩm chức năng. Hoạt tính sinh học của nấm Linh Chi chủ yếu do các polysaccharide, peptidoglycan và triterpene mang lại. Thông thường, nước là dung môi được sử dụng để thu dịch Linh Chi. Tuy nhiên, phương pháp truyền thống này chỉ thu được các hoạt chất phân cực chẳng hạn như các polysacharide. Trong những năm gần đây, kỹ thuật trích ly bằng dung môi siêu tới hạn được đề cập trong nhiều nghiên cứu, nhằm tăng hiệu quả thu hồi chất chiết đồng thời bảo toàn chất lượng các hợp chất có hoạt tính sinh học. Trong nghiên cứu này chúng tôi sử dụng CO_2 siêu tới hạn để trích ly các hoạt chất từ nấm Linh Chi. Kết quả cho thấy, điều kiện tối ưu để trích ly với CO_2 siêu tới hạn là nồng độ đồng dung môi ethanol 14% (w/w), lưu lượng dòng 14g/phút, thời gian xử lý 120 phút, nhiệt độ xử lý 59 ^oC và áp suất xử lý 153 bar. Ở điều kiện này, hiệu suất thu hồi triterpen và chất khô hòa tan lần lượt là 88.9% và 39.2%. So với phương pháp xử lý nấm Linh Chi dưới sự hỗ trợ của enzyme, phương pháp CO_2 siêu tới hạn cho hiệu suất thu hồi triterpen và độ tinh sạch của sản phẩm cao hơn nhưng hiệu suất trích ly chất khô hòa tan thì không bằng phương pháp enzyme.

Từ khóa: CO_2 siêu tới hạn, nấm linh chi, tối ưu hóa, trích ly, triterpenes.