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RESEARCH ARTICLE

Extraction of β-sitosterol from *Swietenia mahagoni* seeds by using supercritical carbon dioxide (SC-CO_2) extraction

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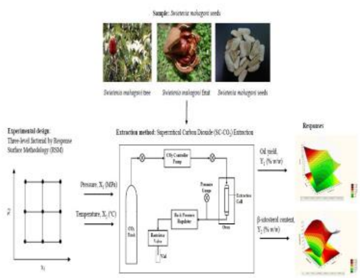
Graphical abstract



Abstract

This work investigates the effect of supercritical carbon dioxide (SC-CO_2) extraction conditions (pressure and temperature) on the oil yield and β-sitosterol content extracted from *Swietenia mahagoni* seeds by using response surface methodology (RSM). The experimental data obtained were fitted to a second-order polynomial model and the obtained oil yields were 1.49-14.45%, while β-sitosterol content obtained were 3.12-9.20 mg/g. The best conditions within the ranges studied were 30 MPa and 40°C to extract β-sitosterol in the highest amount. The present findings show that *S. mahagoni* seeds extract has a high concentration of β-sitosterol.

Keywords: *Swietenia mahagoni* seeds, β-sitosterol, supercritical CO_2 extraction, response surface methodology

INTRODUCTION

Swietenia mahagoni is also known as 'tunjuk langit' in Malaysia (Fig. 1) is used traditionally to treat various diseases such as diabetes and high blood pressure (Goh *et al.*, 2010). *Swietenia mahagoni* tree is 30 meters or taller (Eid *et al.*, 2013) and the wood, usually being used for making furniture (Falah *et al.*, 2008). Meanwhile, the bark can be used for natural colorant (Haque *et al.*, 2013). The fruit of *Swietenia mahagoni* is woody and consisting of capsules containing winged seeds (Blundell *et al.*, 2003). Whereas, the seed of *Swietenia mahagoni* can be obtained by removing the wing. In Malaysia, the raw seeds were used for treating hypertension and diabetes (Balijepalli *et al.*, 2014). In addition, *Swietenia mahagoni* seeds have been reported to have various biological activities such as anti-inflammatory activity, anticancer and antitumor activity (Goh *et al.*, 2011) and also antidiabetic activity (Maiti *et al.*, 2009). Moreover, the seeds contain a number of bioactive compounds as has been noted by Hashim *et al.*, (2013) and presented in Table 1.

To date, no study was found on the quantification of β-sitosterol from *Swietenia mahagoni* seeds using high performance liquid chromatography (HPLC). Recently, attention on the importance of natural compounds from plants and herbs has been reassessing. As a matter of fact, bioactive compounds from plant sources are chemically sensitive and present in low concentration, hence supercritical carbon dioxide (SC-CO_2) extraction is an appropriate extraction method to use. SC-CO_2 is a separation process of matters by using supercritical carbon dioxide as a solvent. In this case, thermolabile and non-polar compounds can be extracted by using SC-CO_2 extraction due to the low operating temperature of 30°C without any degradation. It cannot be used to extract polar compounds since SC-CO_2 extraction is more appropriate to extract non-polar nature compounds (Vilegas *et al.*, 1997). Previously, β-sitosterol has been extracted from various plants using SC-CO_2 since β-sitosterol is a non-polar compound. Therefore, no co-solvent is needed in the extraction of β-sitosterol by using SC-CO_2 extraction.

(a) (b) (c) (d)

Fig. 1 *Swietenia mahagoni* also known as 'tunjuk langit' in Malaysia (a) tree, (b) fruit, (c) winged seeds and (d) seeds.

Moreover, carbon dioxide (CO_2) is the most frequently solvent used because it is environmental friendly (fairly non-toxic), low cost and can be easily removed from the extract (Liza *et al.*, 2010). The elimination of CO_2 is easily achieved since CO_2 is in a gas state at room temperature. In addition, CO_2 in the supercritical state is in a moderate critical temperature (31.3°C) and pressure (7.38 MPa). Supercritical state is when gas and liquid are indistinguishable where at this state it is compressible but possessing a density of a liquid. In a word, supercritical CO_2 makes a good solvent because of the gas-like state that attributed the low viscosity and high diffusion coefficient and the liquid-like state that gave the solvating power (Aionicesei *et al.,* 2008).

Table 1 Primary compounds found in *S. mahagoni* seeds determined by gas chromatography-mass spectrometry (Hashim *et al.,* (2013).

Compounds	Molecular formula
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
n-Hexadecanoic acid	$C_{16}H_{32}O_2$
9-Octadecenoic acid (Z)- methyl ester	$C_{19}H_{36}O_2$
9,12-Octadecadienoic acid (Z,Z)-Linoleic acid	$C_{18}H_{32}O_2$
Gamma-tocopherol	$C_{28}H_{48}O_2$
Fucosterol	$C_{29}H_{48}O$
β- sitosterol	$C_{29}H_{50}O$

Furthermore, the extraction of β-sitosterol from various plants using SC-CO_2 extraction have been reported in the extraction of saw palmetto berries (Catchpole *et al.,* 2002), *Vitex agnus castus* fruit (Cossuta *et al.,* 2008) and sea buckthorn seeds (Sajfrtová *et al.,* 2010). Sajfrtová *et al.,* (2010) has reported that low temperature in the extraction of β-sitosterol as low as 50°C didn't cause the degradation of β-sitosterol since the degradation was occurred at temperature exceeding the temperature mentioned. Also, the yield of β-sitosterol increased slightly as pressure increased and the highest yield found from *Vitex agnus castus* fruit was 1.1 mg/g at a pressure of 45 MPa and a temperature of 40°C (Cossuta *et al.,* 2008). In this context, the extraction of β-sitosterol can be manipulated or controlled by pressure and temperature. Pressure and temperature are the most relevant parameters in supercritical carbon dioxide (SC-CO_2) extraction. In general, quantitative recovery of analytes influence by the increase in pressure lead to the increase in solvent power. Solvent power is described as the solvent density in any given conditions. Significantly, high pressure and moderate temperature favor the extraction of β-sitosterol from plants using SC-CO_2.

Therefore, the aim of this work is to determine the effect of pressure and temperature of supercritical carbon dioxide (SC-CO_2) extraction on the oil yield and β-sitosterol content from *Swietenia mahagoni* seeds by using response surface methodology (RSM).

EXPERIMENTAL

Materials

Swietenia mahagoni seeds were bought in the local market of Johor, Malaysia. Commercial grade liquid carbon dioxide (purity 99.99%) used in SC-CO_2 extraction was purchased from Kras, Instrument and Services, Johor, Malaysia. Methanol grade HPLC and β-sitosterol standard were purchased from Sigma-Aldrich, Germany

Sample preparation

The seeds were rinsed with tap water to remove any foreign particles and dirt prior to drying. Then, the cleaned seeds were cut into small pieces and dried by using oven at temperature of 50°C for a week to remove moistures. The seeds were ground by using a blender (Waring® Commercial blender) and sieved to approximately 0.50 mm of particle size.

Supercritical carbon dioxide (SC-CO_2) extraction

Supercritical fluid extraction (SFE) machine in Center of Lipids Engineering and Applied Research (CLEAR), Universiti Teknologi Malaysia is consisted of CO_2 gas cylinder, CO_2 controller pump (Lab Alliance), co-solvent pump (Lab Alliance), oven (Memmert, Germany), 10 ml stainless steel extraction vessel, pressure gauge (Swagelockk, Germany), automatic back pressure regulator (Jasco BP 2080- Plus) and restrictor valve. A schematic diagram of CLEAR SFE apparatus is illustrated in Fig. 2.

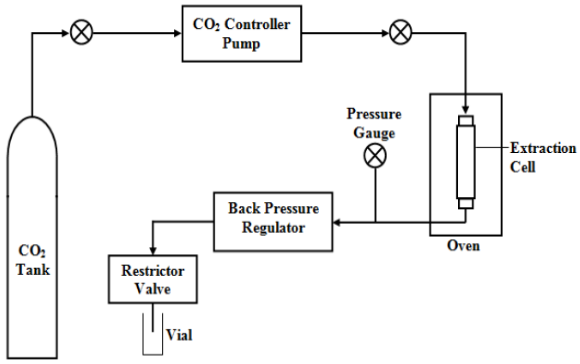


Fig. 2 Schematic diagram of CLEAR supercritical fluid extraction (SFE) machine

The parameters and constant parameters used in extraction process are presented in Table 2. Five gram of sample was placed in 10 ml stainless steel extraction vessel and sealed tightly in the oven. All the parameters (temperature, pressure and flowrate of CO_2) was fixed, and the extraction process was started after all the parameters were attained. The extract was collected by depressurizing the system. The oil yields were collected after 120 minute extraction time.

Table 2 The process parameters for SC-CO_2 extraction.

Parameter	Range/value
Temperature (°C)	40-60
Pressure (MPa)	20-30
Flowrate of CO_2 (ml/min)	2.00
Particle size (mm)	0.50
Mass of sample (g)	5.00
Extraction time (min)	120

The oil yield was calculated as percentage of oil yield using Eq. (1) as follow:

$$Oil\ Yield\ (\%) = (M_0 / M_1) \times 100 \qquad (1)$$

where M_0 is the mass of oil extract in gram and M_1 is the mass of sample in gram.

Design of experimental for response surface methodology (RSM)

Response surface methodology (RSM) is a technique used to describe the behavior of a set of data. The main purpose is to optimize the variables so that the best system performance could be obtained. Three-level factorial design was employed to optimize the oil yield and β-sitosterol content from *Swietenia mahagoni* seed. The number of experiments is calculated by expression of Eq. (2) (Bezerra *et al.,* 2008) below :

$$N = 3^k \qquad (2)$$

where N is the number of experiment and k is the number of factor.

Three-level factorial is suitable for second-order polynomial model of two factors. In supercritical fluid extraction, three level factorial usually been used to optimize the number of factors for obtaining the highest yield of extract (Sharif *et al.,* 2014). The coded and un-coded values are shown in Table 3. Moreover, the analysis of variance (ANOVA) and the regression analysis were all obtained by using Statistica software version 7.0 (STatSoft, EUA). ANOVA analysis was used to analyze the significance of the results at 95% of confidence level.

Table 3 The extraction process variables in coded and un-coded levels.

Coded factors level	Un-coded factors level	
	Pressure, X_1 (MPa)	Temperature, X_2 (°C)
Low (-1)	20	40
Middle (0)	25	50
High (+1)	30	60

High performance liquid chromatography (HPLC) analysis

Identification of β-sitosterol was conducted by using a Waters HPLC system (Milford, MA, USA) consisting of a pump and system controller (Model Waters e2695) with photo-diode array detector (Model 2998). The method of identification for β-sitosterol was referred to the previous method (Sánchez-Machado *et al.,* 2004) with a slight modification. C18 reserved phase Kinetex Biphenyl column (5 µm, 4.6 × 150 mm) with a flow rate of 1.0 ml/min was used for compound separation. The mobile phase was consisted of methanol (60%)/ acetonitrile (40%), in an isocratic program. The injection volume of sample was 20 µL and all samples were filtered with 0.45 µm nylon filters prior to injection. The detection was monitored at 210 nm and data were integrated by Empower 3 software (Waters) (Milford, MA, USA).

RESULTS AND DISCUSSION

β-sitosterol content

The β-sitosterol content of *Swietenia mahagoni* seeds extract with different conditions in SC-CO_2 extraction were identified and quantified. The highest β-sitosterol content was 9.2 mg/g obtained at 30 MPa and 40°C, meanwhile the lowest one (3.12 mg/g) was obtained at 20 MPa and 50°C. Previous researches on the β-sitosterol content of other plants using SC-CO_2 extraction were compared with the result in this study as shown in Table 4. Notably, the temperature of 40°C shows better extraction of β-sitosterol from plants since low temperature can avoid the degradation of compound. The temperature in SC-CO_2 extraction influenced the yield of β-sitosterol because of the solvent density changed. The solvent density increases with decreasing temperature, hence the solubility of β-sitosterol increases by increasing the solvating power. Moreover, high pressure also increased the solvent density.

This finding is accordance with previous researches in the extraction of β-sitosterol by using SC-CO_2 extraction (Catchpole *et al.,* 2002, Simandi *et al.,* 2002, Andras *et al.,* 2005, Cossuta *et al.,* 2008). Catchpole *et al.,* (2002) reported the extraction of β-sitosterol from saw palmetto berries using SC-CO_2 at pressures of 25 and 28 MPa and temperature of 40°C. The maximum β-sitosterol content was achieved at 28 MPa and 40°C. It can be stated that high pressure and low temperature favor to be applied in the extraction of β-sitosterol from plants. Fig. 3 and 4 shows the HPLC chromatograms of the standard (β-sitosterol) at a concentration of 80 ppm and β-sitosterol compound detected in *Swietenia mahagoni* oil extract, respectively.

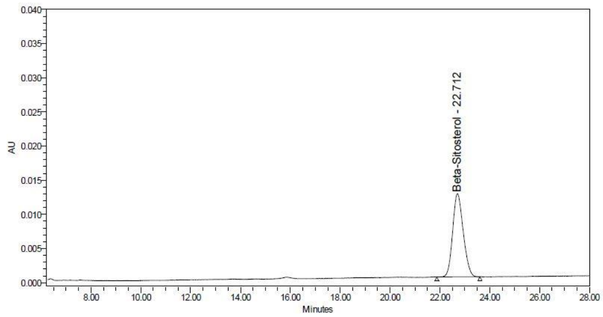


Fig. 3 HPLC chromatogram of the standard (β-sitosterol) at concentration of 80 ppm

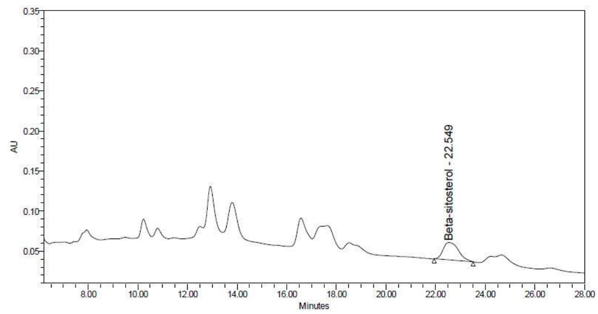


Fig. 4 HPLC chromatogram of β-sitosterol compound detected in *S. mahagoni* oil extracted at 30 MPa and 40°C

Optimization of supercritical carbon dioxide (SC-CO_2) extraction

Optimization in experimental design for supercritical fluid extraction referred to as a separation performance to achieve high extraction efficiency by improving different operating conditions of various processes (Sharif *et al.,* 2014). Experimental design for *Swietenia mahagoni* seed was based on three level factorial with 13 set of experiments with four repetition at middle point, as shown on Table 5.

Table 4 Extraction of β-sitosterol by SC-CO_2 extraction.

Raw material	Extraction conditions		β-sitosterol content (mg/g)	Reference
	Pressure (MPa)	Temperature (°C)		
Saw palmetto berries	28	40	2.3	[13]
Vitex agnus castus fruit	45	40	1.1	[14]
Sea Buckthorn seeds	15	40	5.0	[15]
Swietenia mahagoni seeds	30	40	9.2	This study

Table 5 Experimental matrix and values of the observed responses

Run	Pressure, X_1 (MPa)	Temperature, X_2 (°C)	Coded level		Extraction yield (%)		β-sitosterol concentration (%)	
					Actual	Predicted	Actual	Predicted
1	20	40	-1	-1	6.56	7.28	0.35	0.35
2	20	50	-1	0	3.68	3.31	0.31	0.27
3	20	60	-1	+1	1.49	1.13	0.59	0.64
4	25	40	0	-1	6.64	5.78	0.70	0.81
5	25	50	0	0	4.79	4.93	0.56	0.61
6	25	60	0	+1	4.56	5.87	0.87	0.86
7	30	40	+1	-1	7.02	7.16	0.92	0.82
8	30	50	+1	0	8.61	9.43	0.37	0.50
9	30	60	+1	+1	14.45	13.50	0.67	0.63
10	25	50	0	0	4.95	4.93	0.56	0.61
11	25	50	0	0	6.03	4.93	0.69	0.61
12	25	50	0	0	5.06	4.93	0.64	0.61
13	25	50	0	0	4.28	4.93	0.67	0.61

Fitting the response surface model

The selection of a model for the experimental data was selected based on correlation coefficient (R^2) and *Fisher F*-test (Rastogi *et al.*, 1999). In addition, R^2 can be expressed as a proportion of variance in a set of data explained by a statistical model. When R^2 value approaching or approximately 1, the model can be said well fitted to the actual data (Sin *et al.*, 2006). Typically, R^2 value more than 0.75 is considered accurate in developing statistical model or equation (Henika., 1982). Fig. 5 and 6 are illustration of the experimental data (observed) and predicted values of oil yield and β-sitosterol content, respectively. The R^2 values for oil yield and β-sitosterol concentration at 95% confident level were 0.94 and 0.85, respectively.

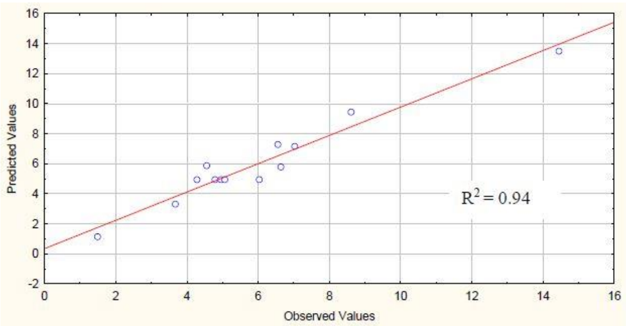


Fig. 5 Experimental data (observed) versus predicted values for *S. mahagoni* seeds oil yield

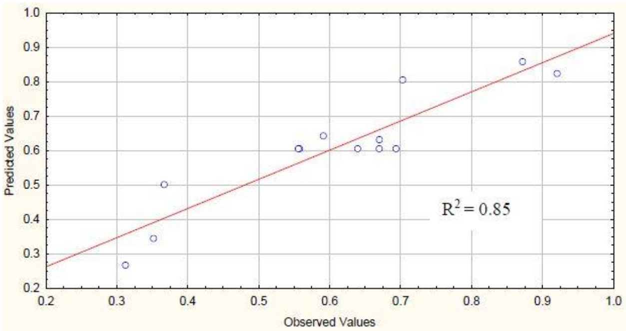


Fig. 6 Experimental data (observed) versus predicted values for β-sitosterol

Furthermore, the F-calculated values from ANOVA for oil yield and β-sitosterol content were also considered in selecting an adequate model for the process. Table 6 and 7 show the analysis of variance for oil yield and β-sitosterol, respectively, fitted in the second-order polynomial model. The calculated F-value defined as the ratio of the mean square of model or regression to the mean square of residual. The larger the F-value, the greater significance of the model or equation in the set of data (Vogel and Todaro., 1997).

Table 6 Analysis of variance (ANOVA) for the response surface second-order polynomial model for the yield of *S. mahagoni* seed obtained by SC-CO_2 extraction

Source	Sum of squares	Degree of freedom	Mean square	$F_{calculated}$
Due to Regression	107.66	5	21.53	23.08
Residual	6.53	7	0.93	
Total	114.20	12		

Table 7 Analysis of variance (ANOVA) for the response surface second-order polynomial model for β-sitosterol obtained by SC-CO_2 extraction

Source	Sum of squares	Degree of freedom	Mean square	$F_{calculated}$
Due to Regression	0.35	5	0.069	7.81
Residual	0.062	7	0.009	
Total	0.41	12		

Based on the analysis of variance for both oil yield and β-sitosterol content fitted in the second-order polynomial model, the calculated F-values are 23.08 and 7.81, respectively. To determine the significant of the calculated F-values obtained, the tabulated F-values from the table of the critical value of F with 0.05 of significance level were compared. Thereby, the calculated F-value obtained are greater than tabulated $F_{(5, 7, 0.05)}$ obtained which is 3.97. It indicates the significance between independent variables with the responses at 95% confidence level. Hence, second-order polynomial model was chosen to depict the relationship between the oil yield and β-sitosterol content with the independent variables (temperature and pressure) The second-order polynomial model equations for oil yield, Y_1, and β-sitosterol content, Y_2 (dependent variables), with pressure, X_1, and temperature, X_2, (independent variables) are shown in Eq. (3) and (4), respectively:

$$Y_1 = 125.7830 - 5.3882 X_1 + 0.0575 X_1^2 - 2.4506 X_2 + 0.0089 X_2^2 + 0.0625 X_1X_2 \quad (3)$$

$$Y_2 = - 3.0321 + 0.5881 X_1 - 0.0088 X_1^2 - 0.1628 X_2 + 0.0023 X_2^2 + 0.0025 X_1X_2 \quad (4)$$

The multiple regression coefficients (individual linear, quadratic and interaction terms) of the oil yield and β-sitosterol content were determined and summarized in Figure 7 and 8, respectively, together with the Pareto charts. Regression coefficients indicate the ability of any term(s) toward the response variable(s) (Mironeasa *et al.*, 2016). All the terms in the polynomial were analyzed by the degree of significance (*p*-value) of each term. Thus, the term that is considered significant (p <0.05) has an influenced on the process (Cvjetko., 2012).

Based on Fig. 7, the oil yield regression coefficients were significant except for temperature in quadratic (X_2^2) and linear terms (X_2) with p >0.05. Therefore, the temperature has no influence on the oil extraction. The pressure in a linear term (X_1) showed a negative effect on the response (oil yield) with p <0.05. While the pressure in the quadratic term (X_1^2) and interaction of pressure and temperature term (X_1X_2) gave a positive effect on oil yield with p >0.05 and p >0.01, respectively. Hence, pressure is a dominant factor on the oil yield. The solvent density increases with increasing pressure hence the interaction of inter-molecules and solutes increase (Pereira and Meireles., 2009).

Variable(s)	Regression coefficients estimation (Oil yield, Y_1)
Intercept	125.7830[a]
X_1	- 5.3882[b]
X_1^2	0.0575[b]
X_2	- 2.4506
X_2^2	0.0089
X_1X_2	0.0625[a]

[a] p < 0.01, [b] p < 0.05.

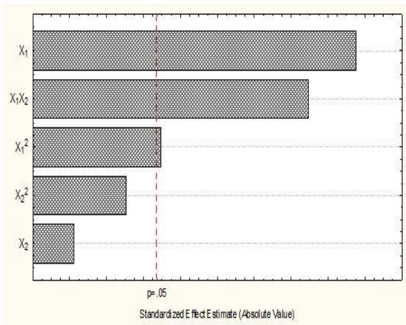


Fig. 7 Multiple regression coefficients and Pareto chart of the oil yield

Pareto chart in statistical analysis is used to demonstrate the effect of the factor to the response (Nei *et al.*, 2009). When the bars that represent each independent variables exceeding the line at p =0.05 indicate that the independent variables are significant at 95% confidence level (Rodriguez-Nogales *et al.*, 2005). Based on the Pareto chart, the most influence independent variable is pressure in a linear term (X_1), meanwhile to least influence is pressure in a quadratic term (X_1^2). The temperature in a linear term (X_2) is not significance to the response.

Subsequently, the regression coefficients for β-sitosterol content in Fig. 8 also shows that all the terms were significant except for temperature in a linear term (X_2) with p >0.05 and temperature in a quadratic term (X_2^2) with p >0.01 . Thus, the temperature in linear and quadratic terms do not affect the β-sitosterol concentration. The pressure in quadratic (X_1^2) and interaction of pressure and temperature (X_1X_2) terms shows a positive effect on β-sitosterol concentration with *p* >0.01 and *p* >0.05, respectively. Inversely, the pressure in a linear term (X_1) shows the negative effect on β-sitosterol content with *p* >0.05. Hence, pressure in the recovery of β-sitosterol is crucial.

Variable(s)	Regression coefficients estimation (β-sitosterol concentration, Y_2)
Intercept	- 3.0321[a]
X_1	0.5881[b]
X_1^2	- 0.0088[a]
X_2	- 0.1629
X_2^2	0.0023[a]
X_1X_2	- 0.0025[b]

[a] $p < 0.01$, [b] $p < 0.05$.

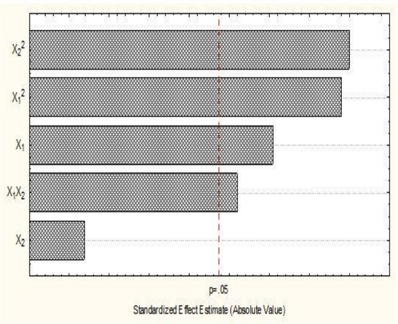


Fig. 8 Multiple regression coefficients and Pareto chart of the β-sitosterol content

Pressure is significant to the recovery of β-sitosterol. Theoretically, by increasing the pressure, the density of solvent also increase (Pereira and Meireles., 2009, Liza *et al.,* 2010). This will also enhance the solvating power and increase the solute solubility (Pereira and Meireles., 2009) resulting in a higher recovery of β-sitosterol. Based on the Pareto chart, the most influence independent variable is the pressure in a quadratic term (X_1^2). Whereas, the temperature in a linear term (X_2) and temperature in a quadratic term (X_2^2) are not significance to the response.

Analysis of response surface

Fig. 9 show the surface plot for the response of the oil yield. When the temperature decreases from 60-40°C, the oil yield slightly increases, while as pressure increases from 20-30 MPa, the oil yield increases. It concluded that pressure is a dominant factor for the extraction of oil yield from *Swietenia mahagoni* seeds, whereas temperature has a minimal effect on the oil yield. According to Qiuhui *et al.,* (2007), the extraction of *Chlorella pyrenoidosa* resulted in the increase of oil yield as pressure increase from 25 to 40 MPa due to the change in solubility of oil in $SC\text{-}CO_2$. The increase in solubility of oil in the solvent will increase the extraction rate because of the solvating power. De-Castro *et al.,* (1994) stated solvating power is the interaction of intermolecular solvent and solute.

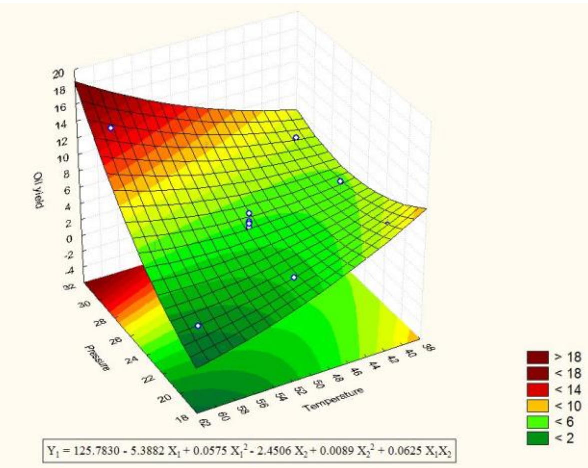


Fig. 9 Surface plot of oil yield from *S. mahagoni* as a function of pressure and temperature

In addition, Mustapa *et al.,* (2009) reported that the increase in intermolecular interactions of solvent and solute resulted in the increase of solvent density, thereby the extraction rate increases. Similar finding was reported by de Azevedo *et al.,* (2008), the extraction of green coffee oil ranging pressures from 15.2 to 35.2 MPa found that the extraction rate correlates with the increase in solvent density. The authors added that pressure also attributed to the increase in solvating power and the intermolecular physical interactions between solvent and solute.

Moreover, similar trends were reported in the extraction of *Vitex agnus castus* (Cossuta *et al.,* 2008) and virgin coconut oil (Nik Norulaini *et al.,* 2009) where the extraction rate increases as pressure increases due to the solvent power. In the extraction of *Vitex agnus castus* fruit at the pressure of 10 to 45 MPa increased the extraction rate. The authors also related the solubility parameter in the study with the solvent power of $SC\text{-}CO_2$ that increased significantly as the pressure increased from 10 to 27.5 MPa. Meanwhile, the effect of pressure in the extraction of virgin coconut oil found that yield obtained also depended on the pressure, where a 100% oil yield was obtained at the highest pressure.

The study of the extraction of bottle gourd seed oil by Said *et al.,* (2014) reported that the direct relationship of pressure and $SC\text{-}CO_2$ gave the dominant effect of pressure toward the mass transfer rate as well as the extraction rate. Viganó *et al.,* (2016) stated that the recovery of extraction yield is related to the solvent power where the increase in pressure at constant temperature resulted in the increase of extraction yield due to the increase of CO_2 density as well as solvent power.

Subsequently, the minimal effect of temperature in the extraction of *Swietenia mahagoni* seeds as the drop of temperature from 60-40°C, increases the extraction yield. This phenomena can be related to the study of Lee *et al.,* (1991), where the solvent solubility increased at the lower temperature due to the changes in density. Jerry *et al.,* (2001) also reported that the maximum oil yield was extracted at lower temperature in the extraction of *Vernonia galamensis* seeds. This is due to the increase in density of extraction fluid ($SC\text{-}CO_2$) when the temperature decreases from 100-40°C.

Azizi *et al.,* (2007) reported the similar result in the extraction of *Parkia Speciosa* seeds using $SC\text{-}CO_2$. The oil yield decreased as the temperature increased due to the retrograde vaporization behavior. This behavior referred to the increase in the solvent solubility at lower temperature up to cross over pressure zone as the density increases. Meanwhile, in the extraction of *Vitex agnus castus* fruit by Cossuta *et al.,* (2008) found that as temperature increases, the solubility parameter also decreases as well as the extraction yield. Solubility parameter in the author's study refer to the relative solvency behavior of $SC\text{-}CO_2$. This finding can be related to the study in the extraction of passion fruit bagasse by Viganó *et al.,* (2016), where the reduction in oil yield as temperature increase because of the density of CO_2 decrease.

Fig. 10 shows the response surface plot of β-sitosterol content as a function of pressure and temperature. The effect of pressure on the extraction of β-sitosterol shows a positive quadratic effect. As pressure increases from 20 to 25 MPa, the β-sitosterol content in extract increases as the solubility of β-sitosterol in the solvent but decreases as it reaches 30 MPa, which shows the interaction of repulsive solute-solvent increases (Liu *et al.,* 2009). This may be due to the compressed solvent at high pressure in the extractor.

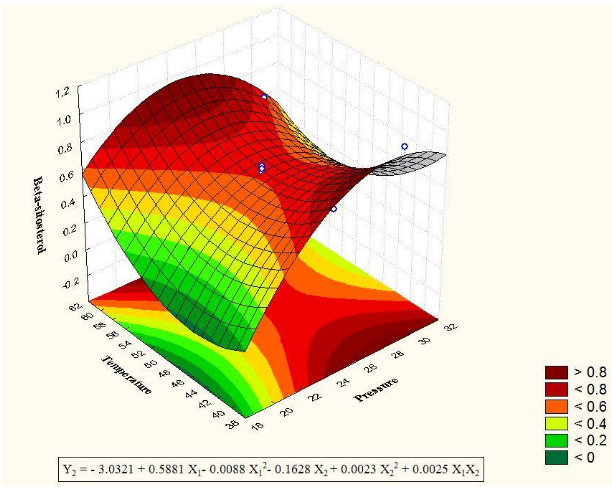


Fig. 10 Surface plot of β-sitosterol content from *S. mahagoni* as a function of pressure and temperature

Similar finding reported by Hartati *et al.*, (2014) in the extraction of *Swietenia mahagoni* seeds. The negative quadratic effect at high pressure is resulted from the highly compressed CO_2 that facilitates solute-solvent repulsion. The authors suggested that high pressure is not always recommended because it can potentially induce the complex extraction. Catchpole *et al.*, (2002) performed the extraction of β-sitosterol from saw palmetto berries at the pressure of 25 and 28 MPa and at the temperature of 40°C. The highest concentration of β-sitosterol found in the extract was at 28 MPa and 40°C.

According to Cossuta *et al.*, (2008), the increase of the pressure slightly increased the yield of the β-sitosterol. Pressure had the strongest effect on the concentration of phytostreol in roselle seed (Nyam *et al.*, 2010). At high pressure, the CO_2 density increases hence the solvent power to dissolve the analyte also increases (Machmudah *et al.*, 2007). On the other hand, at higher temperature, the concentration of β-sitosterol decrease in both studies. Similarly, the concentration of β-sitosterol decreased with an increase in temperature of 40-80°C in Kalahari melon seed oil (Nyam *et al.*, 2010).

The evaluation of the effect of temperature toward the extraction of β-sitosterol is much more complex due to the dual effects. In the Fig. 10, it shows the negative quadratic effect of temperature. Dual effects of temperature are when the temperature at 40°C to 50°C, the decrease in β-sitosterol content due to the reduce in the solubility of β-sitosterol in the solvent but as temperature further increasing to 60°C, the β-sitosterol content in the extract increases. This is because of the mass transfer of β-sitosterol in the solvent as the solubility of mentioned analyte increases.

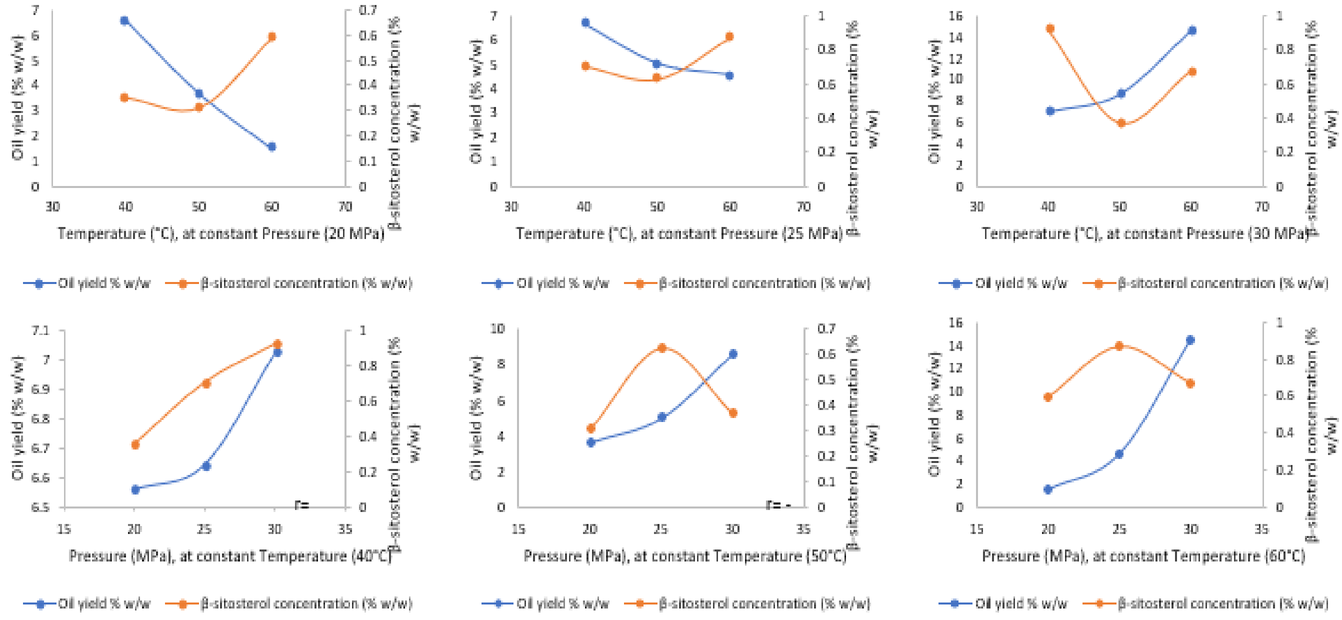


Fig. 11 The correlation of oil yield and β-sitosterol content in the extracts

Correlation of oil yield and β-sitosterol

Moreover, several studies had suggested the act of oil as co-solvent in the extraction of compound (Vasapollo *et al.*, 2004, Sun *et al.*, 2006, Krichnavaruk *et al.*, 2008, Viganó *et al.*, 2016). Thus, the correlation of oil yield and β-sitosterol content of extracts was examined as shown in Fig. 11. At the constant pressure of 25 and 30 MPa, it shows no correlation between extraction of oil yield and β-sitosterol content since r value is nearer to zero. On the contrary, at the constant pressure of 20 MPa it shows a negative correlation. A negative correlation means that the oil yield decreases, while the β-sitosterol content increases. This result is the opposite as the other studies mentioned. This may be due to the low pressure of extraction led to the decrease in the density of the fluid due to the distance between the molecules, that resulted in reduce in the solubility of β -sitosterol content.

Meanwhile, at the constant temperature of 50°C and 60°C it shows no correlation between extraction of the oil yield and the β-sitosterol content, however at the constant temperature of 40°C, the r value is 0.88 that shows a positive correlation. A positive correlation means that the oil yield increases, and the β-sitosterol content also increases. This shows that the domination of solute vapor pressure at low temperature (Kawahito *et al.*, 2008). Hence, it is proven that the influence of extracted oil as co-solvent in SC-CO_2 was influenced by pressure and temperature in the extraction of the compound.

CONCLUSION

Supercritical carbon dioxide (SC-CO_2) extraction applied to extract β-sitosterol from *Swietenia mahagoni* seeds shows that pressure and temperature influenced the extraction of β-sitosterol. The maximum yield of the extract was 14.45% obtained at 30 MPa and 60°C, and the maximum of β-sitosterol content was 0.9204% obtained at 30 MPa and 40°C. This work is the first to report the quantification of β-sitosterol from *Swietenia mahagoni* seeds and had succedded in obtaining the optimized parameters for obtaining highest valued of β-sitosterol from *Swietenia mahagoni* seeds.

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