Techno-Science Research Journal V13 (1) (2025) P 8-14

Content list available at ITC

Techno-Science Research Journal

Journal Homepage: http://techno-srj.itc.edu.kh/

Techno-Science Research Journal

Optimization of Extraction Condition for Oleoresin from Red Pepper Residues

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Received: 29 June 2023; Revised: 9 January 2024; Accepted: 4 June 2024; Available online: 30 April 2025

Abstract: *Oleoresin is a semi-solid mixture of volatile and nonvolatile compounds that can be extracted from plants, herbs, and spices. Red pepper is widely used as a spice in food products and herbal medicine due to the presence of various bioactive substances such as phenolics and flavonoids. The experimental design for oleoresin extraction was done to define the optimum condition that could offer the highest yield of oleoresin. This study aims to optimize the extraction condition for oleoresin from red pepper residues which are the solid waste resulting from essential oil extraction from the red pepper. The optimization for the extraction condition was designed using the Response Surface Methodology with the Central Composite approach, in which extraction temperature (30 to 70 oC) and extraction time (1 to 3 h) played a role as the investigated factors. Using ethanol as the extractant at a solid-to-solvent ratio of 3g to 40 ml of ethanol, the result showed that the extraction temperature and time significantly affected the oleoresin yield. The optimum condition for oleoresin extraction from red pepper residues was obtained at 70 oC and 3 h, providing a yield of 6.38 % (w/w). At this condition, total phenolic and flavonoid contents were found to be 3.74 mg GAE/g, and 6.81 mg QE/g, respectively. The research in this study can be useful information for designing the extraction process and valorization of essential oil by-products.*

Keywords: Optimization; Oleoresin; Total phenolic content; Total flavonoid content; Red pepper; Solvent extraction

1. INTRODUCTION[1]

Optimization is the process of minimizing the experiments under the given condition to obtain the best result. Many studies have applied response surface methodology (RSM) to identify the relation between two variables and to define the effect of variables with a statistical model for the extraction of bioactive compounds with central composite design (CCD) such as solvent extraction of phenolic compounds from Euterpe oleracea [1], and the extraction of black pepper essential oil by applying supercritical carbon dioxide extraction (SC-CO2) [2]. It is discovered that CCD is a useful experimental design for figuring out the optimum condition in the extraction that can use up from two variables to apply for different research studies including the extraction techniques for essential oil, phenolic compounds, pretreatment methods for olive oil [3], hemp [4], carao tree seeds [5], wood apple [6], and castor seed [7]. For instance, in SC-CO2 of black pepper essential oil was applied by RSM with CCD using three parameters such as the pressure of 15-30 MPa, the temperature of 40-50 oC, and dynamic extraction time of 40-80 min were found the optimum yield of 2.16 % at 30 MPa, 50 °C, and 80 min. All linear effects of the three parameters are significant and the pressure had the most impact on essential oil yield while the extraction time and temperature had a minor effect [2]. On the other hand, for the optimization of solvent extraction of bioactive compounds from wood apples with CCD of three parameters; concentration of ethanol (30-70 %), incubation temperature (37-60 °C), and solvent-to-solid ratio (20-40 ml/g) was found to be 7.14 g GAE/g of total phenolic content (TPC) at the optimal condition of 62.7 % of ethanol concentration, temperature 49.7 oC, and solvent-to-solid ratio 39.4 ml/g. This result showed that both the concentration of ethanol and temperature affected the TPC of wood apples whereas the solvent-to-solid ratio was found to be insignificant [6]. This means that extraction conditions need to be optimized to get the best yield of extract.

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Oleoresin is a semi-solid extract made up of resin, bioactive substances such as alkaloids, amides, and flavonoids, as well as essential oil. After completing an extraction, oleoresin can be made by letting the extract evaporate until all of the solvent has been dried out, providing a better distribution in finished products and taking up less storage space than the corresponding spices [8]. Oleoresin of spices can be used in food in many ways such as ground spices as it exhibits flavor and distinct aroma [9]. A recent study showed that oleoresin of black pepper functioned as a potential inhibitor of SARS-CoV-2 (COVID-19) and piperine is the potential natural compound for blocking the RNA packaging in the protein and nucleocapsid targeting of SARS-CoV-2 [10]. The piperine was found to be the major compound of oleoresin of dried black pepper which is up to 63.9 % of total bioactive compounds [11]. Moreover, yield of oleoresin of black pepper can be between 12 to 14 % of dried black pepper [12], while the total phenolic compounds of oleoresin of dried black pepper was found to be 86 µg GAE/g [13].

Oleoresin extraction has been carried out through many extraction techniques such as conventional solvent extraction [8], maceration [14], supercritical fluid carbon dioxide (SF-CO2) [9], subcritical water, and ultrasonic-assisted extractions [15]. Particularly, solvent extraction is widely used and its application is suitable for chemical industry upscaling [16]. This technique has been used to extract phenolic compounds from a variety of herbs and spices such as turmeric (221.7 mg GAE/g), lemongrass (1.2 mg GAE/g) [17], and ginger (11.84 mg GAE/g) [18]. However, application of optimization approach for the extraction of oleoresin from red pepper is still limited.

The purpose of this study is to optimize the extraction condition such as extraction temperature (30, 50, and 70 ºC) and time (1, 2, and 3 h) for the yield of oleoresin from red pepper residues. Analysis of total phenolic and flavonoid contents in the oleoresin obtained at the optimum extraction condition is also done. In this work, red pepper residue resulted after essential oil extraction was selected to be the raw material because it aimed to discover scientific data supporting the valorization of red pepper since essential oil yield from red pepper was only 0.75 to 3.6 % [18,19].

2. METHODOLOGY

2.1 Chemical reagents

Ethanol (Merck, Germany) was used as extraction solvent. For analysis of total phenolic content, gallic acid (Sigma Aldrich, Switzerland), Folin-ciocalteau (Sigma Aldrich, Switzerland), and sodium carbonate (Merck KGaA, Germany) were used. In terms of total flavonoid content, quercetin (Sigma Aldrich, Switzerland), aluminum chloride (Acros, Germany), sodium hydroxide (Merck, Germany), and sodium nitrite (Merck, Denmark) were used.

2.2 Sample preparation

Dried red pepper was bought from Kampot province. The red pepper was grounded, and its essential oil was extracted using hydro-distillation. After the essential oil extraction, the red pepper residue was collected and subjected to freeze drying at a condition of -50 ºC for 48 h. The freeze-dried sample was used for the extraction of oleoresin.

2.3 Experimental design

The extraction condition for oleoresin from red pepper residues was designed using RSM with the Central Composite Design (CCD) approach of JMP software version 16. As seen in Table 1, two variables with three levels, namely extraction temperature (X1: 30 to 70 ºC), and extraction time (X2: 1 to 3 h), were set as the experimental condition. There are 10 experiments in duplication and 4 central points. Oleoresin yield (% w/w) was set as the response. The mathematical model used for two variables of CCD is given in Eq. 1, where Y is a dependent variable (oleoresin yield); "β" _"0" is the intercept term; "β" _"1" , and "β" _"2" are linear coefficients; "β" _"12" is coefficient of interaction variables; "β" _"11" , and "β" _"22" are quadratic coefficients; and X1, X2 are extraction variables, and significant interval is 95 %.

$$\text{Oleoresin (Y, \%, w/w)} = \beta_0+\beta_1X1+\beta_1X_1X_2+\beta_1X_1^2+\beta_2^2X_2^2 \quad \text{(Eq. 1)}$$

Table 1. Experimental design for oleoresin extraction from red pepper residue

Variables	Parameters	Level		
		Low	Medium	High
Extraction temperature (ºC)	X_1	30	50	70
Extraction time (h)	X_2	1	2	3

2.4 Extraction procedure

About 3 g of dried red pepper residues was mixed with 40 ml of ethanol and placed in water bath at 150 rpm using extraction temperature ranged from 30 to 70oC, and extraction time ranged from 1 to 3 h. In order to separate the extract from the solid residues, the mixture was centrifuged at 2000 rpm for 15 min. The collected liquid was evaporated using rotary evaporator with condition of 72 mbar, 100 rpm, and 40 oC for around 20 min until dryness (see Fig. 1). The yield of oleoresin (%, w/w) was calculated by Eq. 2, where

m_0 is mass of oleoresin after evaporation (g), and m_s is mass of sample (g).

$$\text{Oleoresin (\%, w/w)} = \frac{m_0 \times 100}{m_s} \quad \text{(Eq. 2)}$$

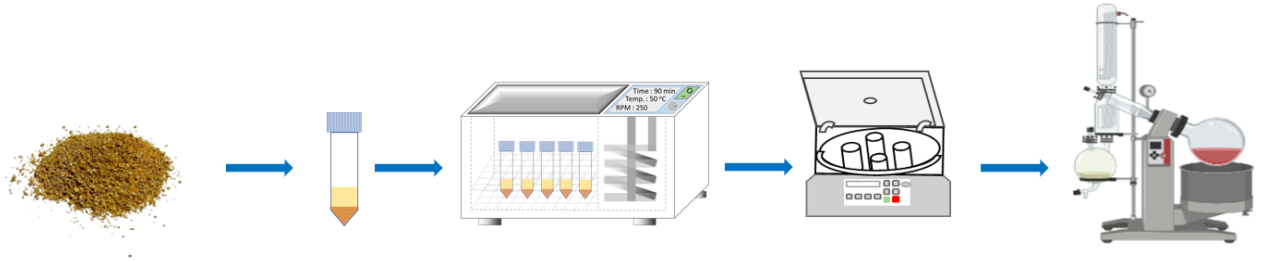

Fig. 1. Extraction procedure of oleoresin extraction from red pepper residues

2.5. Analysis of total phenolic content (TPC)

The analysis of total phenolic content (TPC) was done using Gallic acid as a standard solution and the Folin-Ciocalteu colorimetric method with some modifications from the literature [17]. The standard concentration of phenolic content in samples was determined using a concentration of gallic acid ranging from 10 to 90 µg/ml. In the analytical procedure, 2.5 ml of 10 % Folin-reagent Ciocalteu's was combined with 0.5 ml of standard (10, 30, 50, 70, and 90 g/ml) or extract solution. After keeping for 5 min, 2 ml of a 7.5 % sodium carbonate solution was added and incubated for 1 h. After that, the samples were measured in a UV-Vis spectrophotometer (UV-1280, Shimadzu, Kyoto, Japan) at a wavelength of 765 nm. Eq. 3 was used to quantify the content of total phenolic content in the sample (mg GAE/g); where C is the concentration of quercetin in extract (mg/ml); V is the volume of solution extract (ml); d is dilution factor; and m is mass of sample (g).

$$\text{TPC (mg GAE/g)} = \frac{C \times V \times d}{m} \quad \text{(Eq. 3)}$$

2.6. Analysis of total flavonoid content (TFC)

To determine the total flavonoid content (TFC) in oleoresin, an aluminum chloride colorimetric test was used [19]. Firstly, 2 ml of distilled water and 0.5 ml of extract or standard quercetin were mixed with 0.15 ml of 5 % sodium nitrite. The mixture was kept for 5 min and then added by 0.15 ml of 10 % aluminum chloride keeping for another 5 min. Following that, 1 ml of 1 M sodium hydroxide was added to the solution, and distilled water up to 5 ml of. The mixture was homogenized and measured at a wavelength of 415 nm using a UV-Vis spectrophotometer (UV-1280, Shimadzu, Kyoto, Japan). A quercetin solution containing 1 mg/ml was prepared and diluted into solutions of 100, 200, 300, 400, and 500 µg/ml concentrations. The total flavonoid content was determined by using Eq. 4, and the result was reported as mg QE/g sample; where C is the concentration of quercetin in extract (mg/ml); V is the volume of solution extract (ml); d is dilution factor; and m is mass of sample (g).

$$\text{TFC (mg QE/g)} = \frac{C \times V \times d}{m} \quad \text{(Eq. 4)}$$

2.7. Statistical analysis

In this study, oleoresin yields extracted from red pepper residues were statistically computed as an Analysis of Variance (ANOVA). The ANOVA was utilized to assess the quality of a fit model of the experimental data, the significance of the model, and the effect of extraction parameters on oleoresin yields in the extraction process. It is also noted that the significance of the model and the independent variables were determined by a p-value of less than 0.05

Table 2. Yield of oleoresin extracted from red pepper residues

No	Temperature (°C)	Time (h)	Yield (%, w/w)
1	30	1	4.71 ± 0.06
2	30	2	4.73 ± 0.05
3	30	3	5.03 ± 0.06
4	50	1	4.50 ± 0.08
5	50	2	5.05 ± 0.34
6	50	2	5.20 ± 0.66
7	50	3	5.74 ± 0.11
8	70	1	6.36 ± 0.05
9	70	2	6.56 ± 0.17
10	70	3	6.73 ± 0.04

3.2. Linear effect of the extraction parameters

The linear effect of the extraction parameters demonstrated the effect of each parameter on the oleoresin yield. Figs. 2a and b indicated the linear effect of the extraction temperature (30 to 70 oC) and extraction time (1 to 3 h), respectively. At the extraction temperature of 30 oC, oleoresin yield was found to be 4.8 % (w/w) and it is slightly increased at 50 oC. The oleoresin is gradually increased to 6.5 % (w/w) at a temperature of 70 oC. Meanwhile, the extraction time ranged from 1 to 3 h resulting in sharp raise of oleoresin yield from 5.0 to 6.0 % (w/w). Theoretically, the efficiency of mass transfer increased while raising the temperature because the solvent became more penetrable to the solid sample, thereby providing a high value of extraction yield [21]. The ANOVA result in Table 3 shows the accuracy of parameters, interactions, and experiment model. It is indicated that the model for oleoresin yield is strongly significant due to the significant p-value (<0.0001) and insignificant F-value (27.86). Moreover, since the lack of fit is insignificant, it implies that the model fits the experimental data and it is highly accurate for predicting the oleoresin yield extracted from the red pepper residue through the ethanolic extraction technique. The result in Table 3 also showed that R2 was 0.91 and adjusted R2 was 0.88, showing the high efficiency of the model for oleoresin extraction [22].

Based on statistical results in Table 3, both extraction temperature and time significantly affected on the yield of oleoresin with p-value levels of 0.0001 and 0.0018, respectively. The quadratic term of extraction temperature (X_1^2) had a p-value of 0.0010, indicating a strong effect of the parameters on the extraction yield of oleoresin. On the other hand, the p-value of the quadratic term of extraction time (X_2^2) was 0.8102, indicating less effect of extraction time compared to extraction temperature.

Table 3. Analysis of variance (ANOVA) for oleoresin yield extracted from red pepper residues

Sources	Sum of square	DF	F-value	p-value
Model	11.71	5	27.86	<0.0001a
Lack of fit	0.54	3	3.19	0.0666b
X1	8.94	1	106.38	<0.0001a
X2	1.24	1	14.76	0.0018a
X1*X2	0.001	1	0.01	0.9142b
X_1^2	1.45	1	17.27	0.0010a
X_2^2	0.005	1	0.05	0.8102b
R^2 = 0.91 Adjust R^2 = 0.88				

X1: extraction temperature (°C), X2: extraction time (h) a indicated significant parameter, b indicated insignificant parameter.

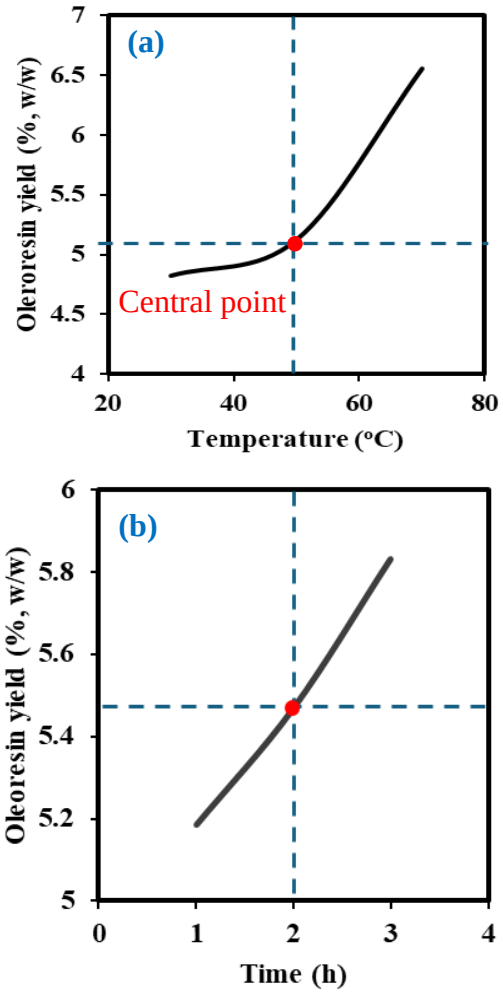

Fig. 2. Linear effect of the extraction parameters on oleoresin yield: (a) extraction temperature (oC), and (b) extraction time (h)

3.3. Interaction effect of the extraction parameters

Fig. 3 shows the interaction effect between the extraction temperature and time on oleoresin yield extracted from red pepper residues. Within the designed range of extraction temperature (30 to 70 oC) and extraction time (1 to 3 h), it is indicated that the investigated variables did not correlate with each other. Within the designed levels of the extraction temperature and time, it is seen that the effect of extraction temperature was independent with the extraction time (see Fig. 3). Based on the ANOVA result (as shown in Table 3), the p-value of the interaction of extraction temperature and time (X1X2) was 0.9142, indicating the insignificant interaction effect of both parameters. This result can confirm that the increase of oleoresin yield with the increased temperature can be due to the diffusivity of the solvent and solid matrix. Similarly, the extended time in the extraction process provided a high yield because the mass

transfer between solute and solvent required time to reach the equilibrium point [22].

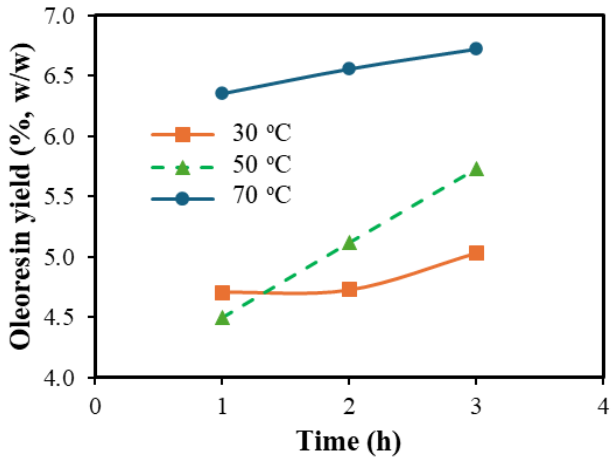

Fig. 3. Interaction effect of the extraction temperature (°C) and extraction time (h) on oleoresin yield extracted from red pepper residue

3.4. Optimization of extraction condition for oleoresin yield

Table 4 shows the regression coefficient of the intercept, linear term of each parameter, interaction between parameters, and quadratic term including their p-value, respectively. By neglecting the insignificant term as seen in Table 4, the equation for predicting oleoresin yield extracted from red pepper residue can be derived as given in Eq. 5. The plot of the experimental data and the predicted data, and the response surface of the interaction between extraction temperature and extraction time is shown in Fig. 4. The plot between the predicted and the experimental values (blue points) (see in Fig. 4a) lie close to the red line which indicated the suitable agreement of the predicted and actual data.

Table 4. Regression coefficient of extraction parameters

Variables	Regression coefficients	Standard error	p-value
Intercept			
βo	5.10	0.12	<0.0001a
Linear			
β1	0.86	0.08	<0.0001a
β2	0.32	0.08	0.0018a
Interaction			
β12	0.01	0.10	0.9142b
Quadratic			
β11	0.55	0.13	0.0010a
β22	0.03	0.13	0.8102b

β1, β2 : coefficients of extraction temperature (X1), and extraction time (X2), respectively, a indicated significant parameter, b indicated insignificant parameter

The extraction temperature of 30 oC and extraction time of 1 h provided the low yield of oleoresin (4 %, w/w) while the high yield of oleoresin extracted from red pepper residue obtained at around 7 % (w/w) at 70 °C for extraction temperature and 3 h for extraction time. Fig. 4b shows the interaction effect of each parameter on the response surface plot by combining the set levels of both parameters. At 70 oC and 3 h, it provided a high oleoresin yield (in color red) while at 30 °C and 1 h, a low yield of oleoresin was obtained (in blue color).

$$\text{Oleoresin (\%, w/w)} = 5.1 + 0.86\ X_1 + 0.32\ X_2 + 0.55\ X_1^2 \quad \text{(Eq. 5)}$$

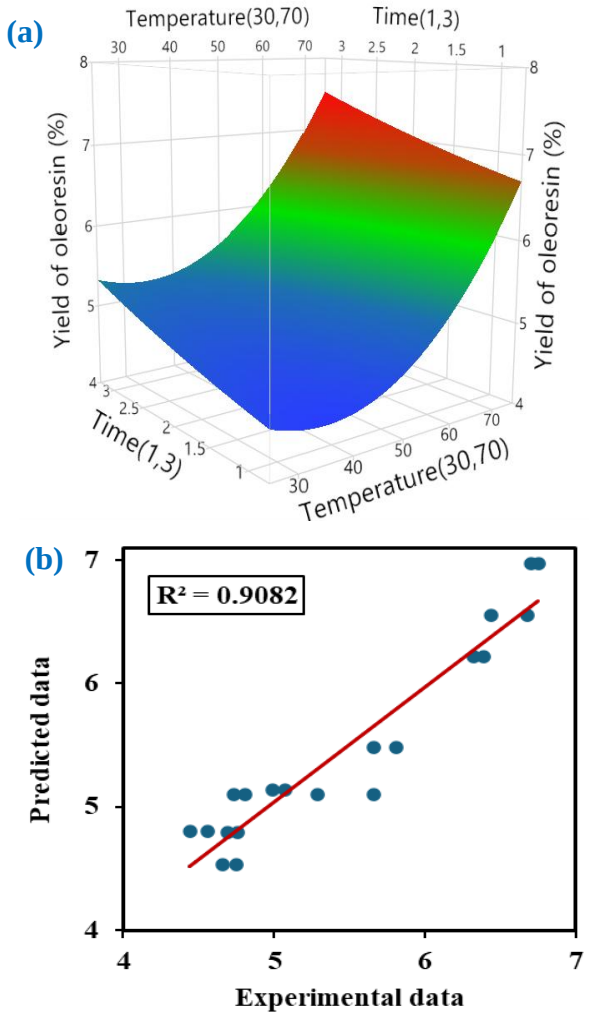

Fig. 4. Data plots showing the accuracy of regression model and optimum area: (a) the predicted and experimental data, (b) the response surface between extraction temperature and extraction time

According to Figs. 3 and 4, the optimum extraction condition for the highest yield of oleoresin yield is at the extraction temperature of 70 oC and 3 h of extraction time in which the oleoresin yield was found to be 6.38 % (w/w). At this condition, the total flavonoid and total phenolic contents of the oleoresin were 6.81 mg QE/g and 3.74 mg GAE/g, respectively. It is also noticed that, the oleoresin obtained from the dried red pepper around 10.64 % (w/w) which was significantly different. However, the residues of red pepper showed a promising result due to the recycling waste of essential oil extraction.

4. CONCLUSION

Optimization of extraction conditions for oleoresin in red pepper residues was done in this work. Each extraction variable, such as extraction temperature and extraction time, had a significant effect on the yield of oleoresin. Extraction temperature significantly affected the oleoresin yield, which was stronger than extraction time. The optimum extraction conditions for oleoresin from red pepper residues were found under an extraction time of 3 h and an extraction temperature of 70 oC, obtaining an oleoresin yield of 6.38 % (w/w), 6.81 mg QE/g, and 3.74 mg GAE/g for total flavonoid and total phenolic contents, respectively. The results of this study revealed that red pepper can be valued through the extraction of both essential oil and oleoresin. The result of this work can also be useful information for designing extraction conditions for oleoresin from red pepper.

ACKNOWLEDGMENT

The authors would like to acknowledge the Higher Education Improvement Project (HIEP Credit No. 6221-KH), Cambodian government for funding the research.

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