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Analytical Methods

Extraction of tocopherol-enriched oils from Kalahari melon and roselle seeds by supercritical fluid extraction (SFE-CO_2)

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ABSTRACT

Tocopherol-enriched oil was extracted by supercritical fluid extraction of carbon dioxide (SFE-CO_2) from Kalahari melon and roselle seeds. The SFE-CO_2 process was optimised using response surface methodology (RSM) with central composite design (CCD). Three SFE-CO_2 parameters namely extracting pressure, extracting temperature, and flow rate of carbon dioxide were examined. The optimal SFE-CO_2 conditions were determined and the quadratic response surfaces were drawn from the mathematical models. The optimal SFE-CO_2 conditions for the extraction tocopherol-enriched oil from Kalahari melon seeds were: extracting pressure 290 bar, extracting temperature 58 °C, and flow rate of carbon dioxide 20 ml/min. The optimum conditions for roselle seeds were extracting pressure 200 bar, extracting temperature 80 °C, and flow rate of carbon dioxide 20 ml/min. These optimum conditions yielded tocopherol concentration of 274.74 and 89.75 mg/100 g oil from Kalahari seed and roselle seed, respectively. No significant ($P > 0.05$) differences were obtained between the experimental and predicted values.

1. Introduction

Interest in the application of supercritical fluid extraction (SFE-CO_2) has grown continuously (Balachandran et al., 2008; Gomes, Mata, & Rodrigues, 2007; Gouveia et al., 2007; Lu et al., 2007; Salgin, 2007; Vaughn Katherine, Clausen Edgar, King Jerry, Howard Luke, & Danielle Julie, 2008) because SFE-CO_2 showed several advantages over classical extraction processes with organic solvents. Under the supercritical fluid conditions, supercritical fluid extraction (SFE) technology is suitable to decrease volatility and thermal degradation during compounds extraction. In comparison with conventional liquid extraction, supercritical fluids have a higher diffusivity and lower density, viscosity, and surface tension (Li, Zhang, & Zheng, in press). The most commonly used SF, supercritical CO_2, is nontoxic, nonflammable, and noncorrosive, inert to most materials, cheap, and readily available in bulk quantity with satisfied purity (Ge, Ni, Chen, & Cai, 2002a).

Extraction of vitamin E from natural sources has received increasing interest due to the high antioxidant activity associated with this family of compounds. Besides its well-known antioxidant activity, studies have demonstrated that synthetic vitamin E, is less effective than natural vitamin E (Hadolin, Skerget, Knez, & Bauman, 2001). Vitamin E has been extracted from several natural sources using SFE-CO_2. Hadolin et al. (2001) studied the extraction of vitamin E-rich oil from a plant (*Silybum marianum*) that naturally grows in the Mediterranean area. Another important source of vitamin E is wheat germ. Two early studies conducted with this raw material were published by Saito and Yamauchi (1990), Saito, Yamauchi, Inomata, and Kottkamp (1989). More recent studies have confirmed that SFE-CO_2 can achieve extraction yields for tocopherols from wheat germ similar to traditional hexane extraction (Munoz, Gomez, & Martinez de la Ossa, 1999). Also, the production of wheat germ oil with high contents of tocopherols using SFE-CO_2 has been studied by Munoz et al. (1999) and Panfili, Cinquanta, Fratianni, and Cubadda (2003). Ge et al. (2002a) and Ge et al. (2002b) have optimised the extraction of vitamin E from wheat germ under the following extracting conditions: 275 bar, 40 °C and CO_2 flow rate equal to 2 ml/min for 90 min. The amount of total vitamin E extracted under these conditions was higher than those obtained using traditional extraction methods (with *n*-hexane or chloroform/methanol mixtures). Recently, Wei Liu and co-workers (2009) reported the use of the supercritical carbon dioxide technique to extract oil from *Opuntia dillenii* Haw. The seed oil demonstrated marked antioxidant activity in DPPH radical scavenging assay and β-carotene bleaching test. Consequently, supercritical fluid extraction of carbon dioxide (SFE-CO_2) is an ideal extraction technology for natural products (Saito, 1995).

Kalahari melon seed oil is particularly interesting to the cosmetic industry where it is used by a number of prominent

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European cosmetics companies for moisturising, regenerating and restructuring skin-care formulations. The oil of Kalahari melon seeds is also used as cooking oil in some countries in West Africa and the Middle East (Kamel, Deman, & Blackman, 1982). It would be feasible to commercialise the production. Our previous study showed that Kalahari melon seed oil has a potential to be used as a new source of seed oil with high content of tocopherols (Nyam, Tan, Lai, Long, & Che Man, 2009).

Roselle seeds are the waste that is left behind during processing of roselle for juices or other roselle related products. Disposing of wastes is highly undesirable both economically and environmentally. Roselle seeds contain about 14.9% protein, 21.2% crude fibres, 14.6% fats and oils, 35.6% carbohydrate by weight. Other components of functional lipids, which are also likely to be present in roselle seed oil, include phytosterols and tocopherols (Nyam et al., 2009). These compounds have various physiological and therapeutic functions such as antioxidant activity, prevention of cardiovascular diseases and effective chemopreventive agent's types of cancer. Emmy Hainida, Amin, Normah, Mohd.-Esa, and N (2008) have reported the nutritional and amino acid contents of roselle seeds grown from Malaysia.

The aim of this study was to optimise the recovering tocopherols from Kalahari melon (*Citrullus lanatus*) and roselle seeds (*Hibiscus sabdarriffa* Linn) by SFE-CO_2 extraction. In order to obtain basic technological information for the SFE-CO_2, the performance of extraction operation through the approach of response surface was used. The extraction parameters studied were extracting pressure (200–400 bar), extracting temperature (40–80 °C) and flow rate of carbon dioxide (10–20 ml/min).

2. Materials and methods

2.1. Materials

Dried Kalahari melon (*C. lanatus*) seed was obtained from the northern part of Namibia. Dried roselle (*H. sabdarriffa* Linn) seed was obtained from Malaysian Agricultural Research and Development Institute (Selangor, Malaysia). The seeds were ground in a blender (MX-291-N, National, Selangor, Malaysia). The seeds were ground in large quantity and kept in a cold room prior to suspension preparation. The ground Kalahari melon seeds used in the extraction experiments were between 0.6–1.0 mm whilst ground roselle seeds were less than 0.6 mm.

2.2. Reagents and standards

Tocopherols (α, β, γ and δ), absolute ethanol and hexane HPLC were purchased from Merck (Darmstadt, Germany); The CO_2 with 99.995% purity was obtained from Malaysian Oxygen Berhad (Selangor, Malaysia).

2.3. Methods

2.3.1. Experimental designs

Response surface methodology (RSM) with central composite design (CCD) was employed to investigate the effect of SFE-CO_2 extraction on tocopherol concentration from Kalahari melon seeds and roselle seeds. Three independent parameters namely, extracting pressure, extracting temperature and supercritical fluid flow rate at three different levels each, were employed. The parameters chosen and their levels were based on preliminary experiments carried out in our laboratory. The experimental plan was designed and the results obtained were analysed using Design Expert version 6.0 (Stat-Ease Inc., Minneapolis, MN) software to build and evaluate models and to plot the three-dimensional response surface curves. For this study, for each oil seed, a total of 20 experiments were carried out (Tables 1 and 2). The experimental design consisted of eight (2^3) factorial points, six extra points (star points) to form a central composite design and six replicates for the centre point. Optimisation was performed using a rotatable CCD with an alpha value of ±1.00 for four factors. The experiments were run in random order to minimise the effects of unexpected variability in the observed responses due to extraneous factors.

2.3.2. Extraction procedures

A laboratory-scale SFE system (Thar Model 500, Thar Technologies Inc., Pittsburgh, PA, USA) was used in this study. The 500-ml extraction cell was of 316 stainless-steel, with a stainless-steel frit. Seeds were mixed with glass wool in the ratio of 25:1 (w/w), packed in a sample cartridge. The filled cartridge was inserted into the thermal-controlled extraction cell. Liquefied CO_2 was introduced into the sample cartridge through a piston pump with a cooling jacket. Both the pressure and temperature of the cartridge were automatically reached and maintained by a control unit according to settings. After the desired pressure and temperature were reached, the cell was placed in the oven cavity and connected to the manifold and the restrictors. The system was held for 30 min under the desired conditions, and then carbon dioxide was allowed to flow continuously through the extractor for 3 h. The flow rate of CO_2 was regulated by both the pressure-releasing valve and a thermal-controlled restrictor and monitored by a flow metre. Extracts were finally separated from the CO_2 phase and collected in collector at ambient temperature and atmospheric pressure. Extracts obtained by SFE at the different conditions were tested for tocopherol concentration (Table 1 and 2).

2.3.3. Determination of tocopherol concentration of the extract

Tocopherols contents were determined according to the modified method of Kamal-Eldin, Stefanie, Jan, and Anna-Maija (2000). Tocopherols were analysed by an HPLC system (Shimadzu, Kyoto, Japan). The chromatographic system included a fluorescence detector and a 250 × 4 mm i.d. LiChrospher Si 60–5, 5 μm, column (Merck, Darmstadt, Germany). Separation of all tocopherols was based on isocratic elution when the solvent flow rate was maintained at 1 ml/min. The solvent system selected for tocopherol elution was *n*-hexane/isopropanol (99:1, v/v) and was detected fluorimetrically (λ_{ex} = 290 nm, λ_{em} = 330 nm). Prior to HPLC analysis, the oils were diluted with hexane, filtered (0.45 μm nylon syringe filter) and a 20 μl sample was injected.

3. Results and discussion

In the present work, multiple regression analyses were carried out using response surface analysis to fit the mathematical models to the experimental data aiming at an optimal region for the response variables studied (extracting pressure, extracting temperature and flow rate of carbon dioxide) to define the relationship between three independent variables and the criteria of response variables as presented in Tables 1 and 2.

3.1. Regression modelling

Multiple regressions give a mathematical relationship between responses and independent variables. Experimental results were analysed using response surface methodology. The second-order polynomial fitted (Ahn & Kwak, 1999; Meyer, 1971) was:

$$Y = B_o + \Sigma B_i X_i + \Sigma B_{ii} X_i^2 + \Sigma B_{ij} X_i X_j$$

where, Y represents the experimental response, B_o, B_i, B_{ii} and B_{ij} are constant and the regression coefficients of the model, and X_i and X_j are independent variables in coded values. The whole model includes linear, quadratic and cross-product terms.

Table 1

Experimental data and the observed response values with different combinations of pressure (X_1), temperature (X_2) and supercritical fluid flow rate (X_3) for the extraction of tocopherol from Kalahari melon seed oil by supercritical fluid extraction.

Run	Coded parameter			Actual parameter values			Tocopherol concentration (mg/100 g)
	X_1	X_2	X_3	X_1	X_2	X_3	
1	0	0	0	300	60	15	241.11
2	0	−1	0	300	40	15	231.05
3	−1	1	1	200	80	20	231.77
4	−1	1	−1	200	80	10	243.83
5	0	1	0	300	80	15	247.32
6	1	1	1	400	80	20	213.63
7	−1	0	0	200	60	15	195.76
8	0	0	0	300	60	15	238.32
9	1	−1	−1	400	40	10	227.35
10	0	0	−1	300	60	10	258.96
11	1	−1	1	400	40	20	261.26
12	1	0	0	400	60	15	190.45
13	−1	−1	−1	200	40	10	209.56
14	0	0	0	300	60	15	232.05
15	0	0	0	300	60	15	236.87
16	−1	−1	1	200	40	20	243.12
17	0	0	0	300	60	15	255.29
18	1	1	−1	400	80	10	233.73
19	0	0	0	300	60	15	240.64
20	0	0	1	300	60	20	266.87

Table 2

Experimental data and the observed response values with different combinations of pressure (X_1), temperature (X_2) and supercritical fluid flow rate (X_3) for the extraction of tocopherol from roselle seed oil by supercritical fluid extraction.

Run	Coded parameter			Actual parameter values			Tocopherol concentration (mg/100 g)
	X_1	X_2	X_3	X_1	X_2	X_3	
1	0	0	0	300	60	15	30.40
2	0	−1	0	300	40	15	41.80
3	−1	1	1	200	80	20	94.88
4	−1	1	−1	200	80	10	49.23
5	0	1	0	300	80	15	66.85
6	1	1	1	400	80	20	65.14
7	−1	0	0	200	60	15	40.32
8	0	0	0	300	60	15	40.02
9	1	−1	−1	400	40	10	39.38
10	0	0	−1	300	60	10	39.94
11	1	−1	1	400	40	20	30.65
12	1	0	0	400	60	15	44.48
13	−1	−1	−1	200	40	10	39.25
14	0	0	0	300	60	15	34.28
15	0	0	0	300	60	15	35.62
16	−1	−1	1	200	40	20	50.56
17	0	0	0	300	60	15	35.07
18	1	1	−1	400	80	10	53.04
19	0	0	0	300	60	15	39.16
20	0	0	1	300	60	20	41.41

$$Y = B_o + B_1X_1 + B_2X_2 + B_3X_3 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 + B_{11}X_1^2 + B_{22}X_2^2 + B_{33}X_3^2$$

Table 3 shows the test statistics for the model (F-test and probability) of Kalahari melon and roselle seeds. It can be seen that there was a high statistically significant multiple regression relationship between the independent variables and the response variable. The probability (P) values of all regression models were less than 0.0001. The complete quadratic model showed an excellent fit and appeared to reasonably represent the data. Within the experimental design, the responses were sufficiently explained by the regression equation, allowing it to establish response surfaces, and it was feasible using these regression models to predict the effects of various SFE-CO_2 parameters on tocopherol extraction from Kalahari melon and roselle seeds by SFE-CO_2. The R^2 values for these response variables were higher than 0.90 (Kalahari melon and roselle seeds, 0.902 and 0.921, respectively). The response surface models could explain more than 90% of the variation in the response variables studied, thus indicating that the variability of responses was explained well by the models. The lack of fit, which measures the fitness of models, resulted in no significant F-value ($P > 0.05$) in terms of the response variables studied, indicating that the models were sufficiently accurate for predicting those response variations.

The coefficients of the regression equation and P values for Kalahari melon and roselle seeds are shown in Table 4, respectively. In view of these mathematical models, the significance of the investigated factors and their interactions were examined. For the Kalahari melon seed, the second-order terms of extracting pressure and flow rate of carbon dioxide (X_1^2, X_3^2) and the cross-product term between extracting temperature and flow rate of carbon dioxide (X_2X_3) had highly significant ($P < 0.01$) effects on recovery of tocopherols, whilst the cross-product term between extracting pressure and extracting temperature (X_1X_2) had significant ($P < 0.05$)

Table 3
Analysis of variance for response surface quadratic model for tocopherol concentration from Kalahari melon seed by supercritical fluid extraction.

Seed	Source	Sum of squares	Degree of freedom	Mean square	F-value	$P > F$[a]
Kalahari melon	Oil recovery[b]					
	Model	7029.87	7	1004.27	15.78	<0.0001
	Residual	763.69	12	63.64		
	Lack of fit	455.50	7	65.07	1.06	0.4937
	Pure error	308.19	5	61.64		
	Total	7793.56	19			
	Coefficient of variation = 3.40%, R^2 = 0.9020					
Roselle	Oil recovery[b]					
	Model	4045.85	6	674.31	25.25	<0.0001
	Residual	347.12	13	26.70		
	Lack of fit	286.00	8	35.75	2.92	0.1262
	Pure error	61.12	5	12.22		
	Total	4392.96	19			
	Coefficient of variation = 11.34%, R^2 = 0.9210					

[a] Defined by Eq. (1).
[b] $P < 0.05$ indicates statistical significance.

Table 4
Regression coefficients and P-values for tocopherol concentration from Kalahari melon and roselle seeds by supercritical fluid extraction after backward elimination.

Seed	Variables[a]	Regression coefficients	P-values[b]
Kalahari melon	Intercept	238.60	<0.0001
	X_1	0.24	0.9264
	X_2	−0.21	0.9363
	X_3	4.32	0.1124
	X_1^2	−38.55	<0.0001
	X_3^2	31.25	<0.0001
	X_1X_2	−8.02	0.0148
	X_2X_3	−12.45	0.0008
Roselle seed	Intercept	38.07	<0.0001
	X_1	−4.15	0.0245
	X_2	12.75	<0.0001
	X_3	6.18	0.0023
	X_2^2	15.01	<0.0001
	X_1X_3	−6.70	0.0028
	X_2X_3	6.90	0.0023

[a] Pressure (X_1), temperature (X_2) and supercritical fluid flow rate (X_3).
[b] $P < 0.05$ indicates statistical significance.

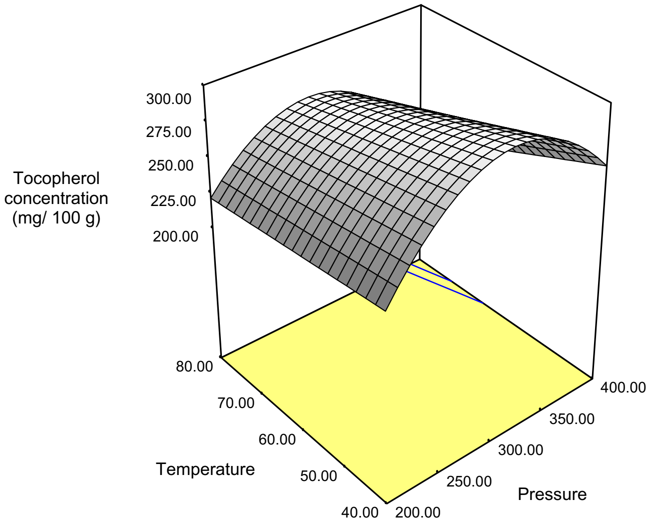

Fig. 1a. Response surface plot of the interaction between pressure (X_1) and temperature (X_2) at a high level of flow rate of carbon dioxide (X_3) on tocopherol concentration of Kalahari melon seed oil.

effects. All first-order terms of various processing parameters (X_1, X_2, X_3), second-order terms of extracting temperature (X_2^2) and cross-product term between extracting pressure and flow rate of carbon dioxide (X_1X_3) had no significant ($P > 0.05$) effects on recovery of tocopherol in the SFE-CO_2.

For the roselle seed, all first-order terms of extracting pressure, extracting temperature and flow rate of carbon dioxide (X_1, X_2, and X_3, respectively) of various processing parameters, the second-order term of extracting temperature (X_2^2), the cross-product terms between extracting pressure and flow rate of carbon dioxide (X_1X_3) and the cross-product terms between extracting temperature and flow rate of carbon dioxide (X_2X_3) had highly significant ($P < 0.01$) effects on recovery of tocopherols. The second-order terms of extracting pressure and flow rate of carbon dioxide (X_2^2, X_3^2) and cross-product term between extracting pressure and extracting temperature (X_1X_2) had no significant ($P > 0.05$) effects on recovery of tocopherol in the SFE-CO_2.

3.2. Optimisation procedure

Graphical optimisation procedures were carried out for predicting the exact optimum level of independent variables leading to the highest recovery of tocopherols during the SFE-CO_2 process. First, an optimal treatment was found *via* response surface plotting of the data. The 3D surface plots were drawn to illustrate that the interactive effects of the independent variables corresponded to the response variable. Figs. 1a and b and 2a and b visualise the surface plot for the yield of tocopherols as a function of extracting pressure, extracting temperature and flow rate of carbon dioxide for Kalahari melon and roselle seeds, respectively.

For the Kalahari melon seed, the flow rate of carbon dioxide and extracting pressure were fixed as 20 ml/min and 290 bar in Fig. 1a and Fig. 1b, respectively. Based on the response surface plot (Fig. 1a), which was constructed for a high level of flow rate of carbon dioxide, it was observed that tocopherol concentration increased at central level of pressure and decreased at a lower level and higher level of pressure under same flow rate of carbon dioxide. Fig. 1b shows that the tocopherol concentration increased with an increase in temperature and flow rate of carbon dioxide under isobaric conditions.

For the roselle seed, the extracting temperature and extracting pressure were fixed as 80 °C and 200 bar in Fig. 2a and Fig. 2b, respectively. Based on the response surface plot (Fig. 2a), which was constructed for a high level of extracting temperature, it was observed that tocopherol concentration increased at a high level of flow rate of carbon dioxide at isothermal conditions. Fig. 2b

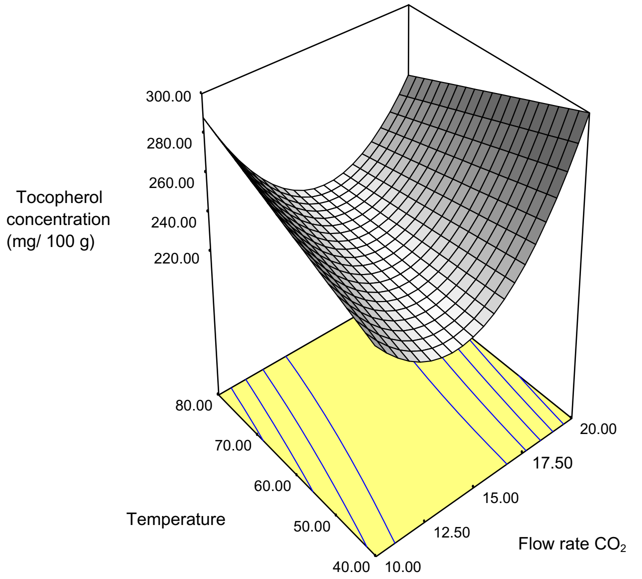

Fig. 1b. Response surface plot of the interaction between temperature (X_2) and supercritical fluid flow rate (X_3) at central level of pressure (X_1) on tocopherol concentration of Kalahari melon seed oil.

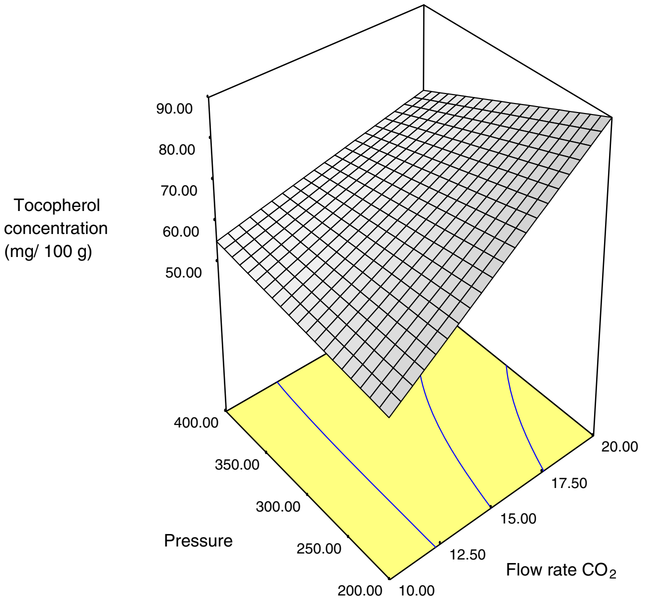

Fig. 2a. Response surface plot of the interaction between pressure (X_1) and supercritical fluid flow rate (X_3) at a high level of temperature (X_2) on tocopherol concentration of roselle seed oil.

shows that the tocopherol concentration increased with an increase in temperature and flow rate of carbon dioxide at isobaric conditions.

3.3. Fitting the response surface model

The response surface was generated based on the second-order equation after backward elimination.For Kalahari melon seed:

$$Y = 238.60 + 0.24X_1 - 0.21X_2 + 4.32X_3 - 38.55X_1^2 - 31.25X_3^2 - 8.02X_1X_2 - 12.45X_2X_3 \quad (1)$$

The second-order term of extracting pressure is the most active and important processing parameter in the course of SFE-CO_2. When the extracting pressure exceeded 300 bar, the yield of tocopherol increased slowly and then decreased. Use of high extracting pressure will increase the equipment and processing cost, therefore, the optimal extracting pressure was selected around 290 bar.

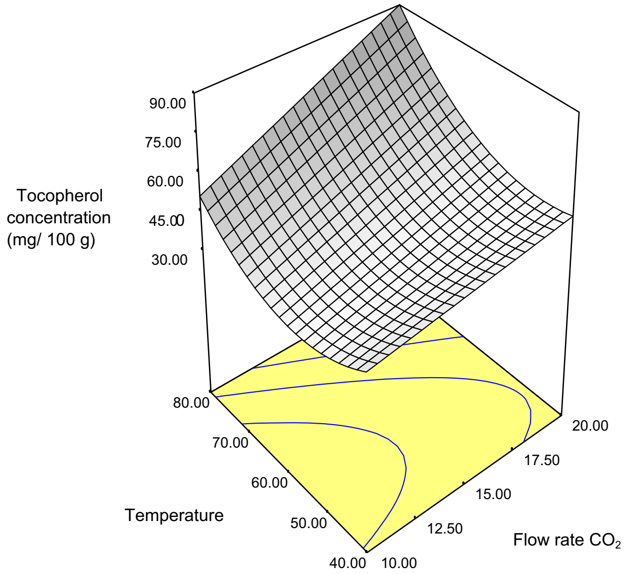

Fig. 2b. Response surface plot of the interaction between temperature (X_2) and supercritical fluid flow rate (X_3) at low level of pressure (X_1) on tocopherol concentration of roselle seed oil.

Furthermore, considering the decimal-place limitation of the SFE apparatus' parameters, the optimal processing conditions of tocopherol extraction from Kalahari melon seeds by SFE-CO_2 were finally determined as the extracting pressure of 290 bar, extracting temperature of 58 °C and flow rate of carbon dioxide of 20 ml/min.For roselle seed:

$$Y = 38.07 - 4.15X_1 + 12.75X_2 + 6.18X_3 + 15.01X_2^2 - 6.70X_1X_3 + 6.90X_2X_3 \quad (2)$$

The first-order and second-order terms of extracting temperature are the most active and important processing parameter in the course of SFE-CO_2. When the extracting temperature increased, the yield of tocopherol increased slowly.

3.4. Verification of optimised models

The adequacy of the model equations for predicting the optimum response values was tested using the recommended optimum conditions. This set of independent variables was determined to be optimum by an RSM optimisation approach, which was also used to experimentally predict the value of responses using the models.

For Kalahari melon seeds, the optimum response values was tested in the experiment using the extraction pressure of 290 bar, extracting temperature of 58 °C and flow rate of carbon dioxide of 20 ml/min. This set of conditions was determined to be optimum by the RSM optimisation procedure and balance of various aspects. A mean value of 269.56 mg/100 g ($N = 3$), obtained from actual experiments, demonstrated the validation of the RSM model. A chi-square test was performed to verify the adequacies of the models established. Chi-square test showed that there were no significant ($P > 0.05$) differences between the observed and predicted values for SFE-CO_2 of Kalahari melon seed oil. The good correlation between these results confirmed that the response model was adequate for reflecting the expected optimisation. The results of analysis indicated that the two groups of experimental values were in good agreement with the predicted ones, and also suggested that the model of Eq. (1) is satisfactory and accurate.

For roselle seed, an optimum point (89.75 mg/100 g) was produced with an extraction pressure of 200 bar, extracting temperature of 80 °C and flow rate of carbon dioxide of 20 ml/ min. A value of 83.55 ($n = 3$) was obtained. The good correlation between these two results verified the validity of the response model and the existence of an optimal point. Chi-square test showed that there were no significant differences ($P > 0.05$) between the predicted and observed values for supercritical extraction. Therefore, both models were adequate to predict the tocopherol concentration from Kalahari melon and roselle seeds by supercritical fluid extraction with high accuracy.

3.5. Comparison of SFE-CO_2 with Soxhlet extraction on the tocopherol concentration in Kalahari melon and roselle seeds

A comparison of SFE-CO_2 and Soxhlet extraction was made. The tocopherol concentration in the Kalahari melon seed as estimated by Soxhlet extraction was 165.89 mg/100 g, whilst the roselle seed was 65.07 mg/100 g. The results show that the content of tocopherol in the extract obtained by extraction with SFE-CO_2 was higher than those extracted in the Soxhlet equipment which used petroleum ether as solvent, whilst the extracting time of SFE-CO_2 were far lower than Soxhlet extraction.

4. Conclusion

The central composite design (CCD) was found to be a valuable tool to estimate the effect of the extracting pressure, extracting temperature, flow rate of carbon dioxide and their interactions for optimising the extraction of tocopherols from Kalahari melon and roselle seeds. Analysis of variance shows that the regression model for tocopherol-enriched oil from Kalahari melon and roselle seeds extracted by SFE-CO_2 were statistically good with a significance level of $P < 0.0001$ and the models had no significant ($P > 0.05$) lack of fit. Thus, well-fitting models for the extraction of tocopherol-enriched oils were successfully established. The optimal conditions for the extraction tocopherol-enriched oil from Kalahari melon seeds were: extracting pressure 290 bar, extracting temperature 58 °C and flow rate of carbon dioxide 20 ml/min yielded tocopherol concentration of 274.74 mg/100 g oil. Whilst the optimal conditions for roselle seed were extracting pressure 200 bar, extracting temperature 80 °C, and flow rate of carbon dioxide 20 ml/min. These optimum conditions yielded a tocopherol concentration of 89.75 mg/100 g oil from roselle seed. The response fitted models were verified using the recommended optimum conditions. The experimental values were shown to be in agreement with those predicted, thus indicating adequacy of the fitted models.

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References

Ahn, J., & Kwak, H. S. (1999). Optimizing cholesterol removal in cream using b-cyclodextrin and response surface methodology. *Journal of Food Science, 64*(4), 629–632.

Balachandran, C., Mayamol, P. N., Thomas, S., Sukumar, D., Sundaresan, A., & Arumughan, C. (2008). An ecofriendly approach to process rice bran for high quality rice bran oil using supercritical carbon dioxide for nutraceutical applications. *Bioresource Technology, 99*, 2905–2912.

Emmy Hainida, K. I., Amin, I., Normah, H., & Mohd-Esa, N. (2008). Nutritional and amino acid contents of differently treated roselle (*Hibiscus sabdariffa* L.) seeds. *Food Chemistry, 111*, 906–911.

Ge, Y., Ni, Y., Chen, Y., & Cai, T. (2002a). Optimization of the supercritical fluid extraction of natural vitamin E from wheat germ using response surface methodology. *Journal of Food Science, 67*, 239–243.

Ge, Y., Yang, H., Hui, B., Ni, Y., Wang, S., & Cai, T. (2002b). Extraction of natural vitamin E from wheat germ by supercritical carbon dioxide. *Journal of Agricultural and Food Chemistry, 50*, 685–689.

Gomes, P. B., Mata, V. G., & Rodrigues, A. E. (2007). Production of rose geranium oil using supercritical fluid extraction. *The Journal of Supercritical Fluids, 41*, 50–60.

Gouveia, L., Nobre, B. P., Marcelo, F. M., Mrejen, S., Cardoso, M. T., Palavra, A. F., et al. (2007). Functional food oil coloured by pigments extracted from microalgae with supercritical CO_2. *Food Chemistry, 101*(2), 717–723.

Hadolin, M., Skerget, M., Knez, Z., & Bauman, D. (2001). High pressure extraction of vitamin E-rich oil from *Silybum marianum*. *Food Chemistry, 74*, 355–364.

Kamal-Eldin, A., Stefanie, G., Jan, P., & Anna-Maija, L. (2000). Normal-phase high-performance liquid chromatography of tocopherols and tocotrienols. Comparison of different chromatographic columns. *Journal of Chromatography A, 881*, 217–227.

Kamel, B. S., Deman, J. M., & Blackman, B. (1982). Nutritional fatty acid and oil characteristics of different agricultural seed. *Food Technology, 17*, 263–267.

Li, J. L., Zhang, M, & Zheng, T. (in press). The in vitro antioxidant activity of lotus germ oil from supercritical fluid carbon dioxide extraction. *Food Chemistry*.

Lu, T. J., Gaspar, F., Marriott, R., Mellor, S., Watkinson, C., Al-Duri, B., et al. (2007). Extraction of borage seed oil by compressed CO_2: Effect of extraction parameters and modeling. *The Journal of Supercritical Fluids, 41*, 68–73.

Meyer, R. H. (1971). *Response surface methodology* (1st ed.). Boston, Mass: Allyn and Bacon.

Munoz, M. A. B., Gomez, A. M., & Martinez de la Ossa, E. (1999). Optimisation of the extraction process of tocopherol from wheat germ by liquid and supercritical carbon dioxide. *Grasas y Aceites, 50*, 275–279.

Nyam, K. L., Tan, C. P., Lai, O. M., Long, K., & Che Man, Y. B. (2009). Some physicochemical properties and bioactive compounds of seed oils. *LWT-Food Science and Technology, 42*, 1396–1403.

Panfili, G., Cinquanta, L., Fratianni, A., & Cubadda, R. (2003). Extraction of wheat germ oil by supercritical CO_2: Oil and defatted cake characterization. *Journal of American Oil Chemists' Society, 80*, 157–161.

Saito, M., Yamauchi, Y., Inomata, K., & Kottkamp, W. (1989). Enrichment of tocopherols in wheat-germ by directly coupled supercritical fluid extraction with semipreparative supercritical chromatography. *Journal of Chromatographic Science, 27*, 79–85.

Saito, M., & Yamauchi, Y. (1990). Isolation of tocopherols from wheat-germ oil by recycle semipreparative supercritical fluid chromatography. *Journal of Chromatography A, 505*, 257–271.

Saito, S. (1995). Research activities on supercritical fluid science and technology in Japan – A review. *The Journal of Supercritical Fluids, 8*(3), 177–204.

Salgin, U. (2007). Extraction of jojoba seed oil using supercritical CO_2 + ethanol mixture in green and high-tech separation process. *The Journal of Supercritical Fluids, 39*, 330–337.

Vaughn Katherine, L. S., Clausen Edgar, C., King Jerry, W., Howard Luke, R., & Danielle Julie, C. (2008). Extraction conditions affecting supercritical fluid extraction (SFE) of lycopene from watermelon. *Bioresource Technology, 99*, 7835–7841.

Liu, Wei, Fu, Yu-Jie, Zu, Yuan-Gang, Tong, Mei-Hong, Wu, Nan, Liu, Xiao-Lei, et al. (2009). Supercritical carbon dioxide extraction of seed oil from *Opuntia dillenii* Haw. and its antioxidant activity. *Food Chemistry, 114*, 334–339.