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ORIGINAL PAPER

Optimization of Supercritical Carbon Dioxide Extraction of Gardenia Fruit Oil and the Analysis of Functional Components

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Abstract Supercritical carbon dioxide (SC-CO_2) extraction of whole fruit oil from *Gardenia jasminoides* Ellis was performed. The effect of extraction pressure, temperature and CO_2 flow rate on the oil yield was investigated by response surface methodology (RSM). The results showed that experimental data had a good fit to the proposed model ($R^2 = 0.938$). Extraction pressure, CO_2 flow rate, the quadratics of pressure, and the interaction between pressure and flow rate showed significant effects on the oil yield ($p < 0.05$). The optimum parameters that maximized the yield of gardenia fruit oil (GFO) were: extraction pressure of 36.8 MPa, temperature of 65 °C, and CO_2 flow rate of 15 kg/h. The main fatty acid of GFO was linoleic acid (about 44%), followed by palmitic acid (about 26.4%) and oleic acid (about 24.6%). α-Tocopherol was dominant in the total tocopherols of GFO, and showed the main antioxidant activity. The fatty acid composition and tocopherols content of GFO were not remarkably affected by the extraction by SC-CO_2 and *n*-hexane.

Keywords Supercritical carbon dioxide extraction · Gardenia fruit oil · Response surface methodology · Fatty acids · Tocopherol · Antioxidant activity

Introduction

Gardenia (*Gardenia jasminoides* Ellis, Rubiaceae) fruits as traditional Chinese herbal medicine have been used for their anti-inflammatory, hepatinica, diuretic, and cholagogue properties [1]. The oval shaped fruits are reddish yellow, and the pigment is widely used as a natural colorant in the food industry [2]. The other main constituents of gardenia fruits are iridoid glycosides which are focused on because of their pharmacological activities [3]. However, information regarding the extraction and main components of gardenia fruit oil (GFO) is limited.

Generally speaking, lipids from biological materials were always extracted by conventional organic solvent. However, the method usually involves to some extent hazardous solvent residues and is laborious and time-consuming. As an alternative processing method for lipids extraction, supercritical carbon dioxide (SC-CO_2) has gained wider attention due to its advantages of high solvent power, non-toxicity, and nonflammability. Moreover, supercritical carbon dioxide has a relatively low viscosity and high molecular diffusivity, and can penetrate into the sample matrix more effectively than liquid solvents. Meanwhile, vegetable oils can be extracted at moderate temperatures on account of its low critical temperature (31.1 °C). In addition, SC-CO_2 displays other benefits, such as the extracts being free of solvent residues, selective extraction, and low separation cost [4]. Owing to these advantages, SC-CO_2 has been widely used in the extraction of vegetable oils including apricot kernel oil [5], sea buckthorn oil [6], and pomegranate seed oil [7].

Many factors can affect the efficiency of SC-CO_2 extracted oil plant materials, among which extraction pressure, extraction temperature and CO_2 flow rate are three relatively important factors. The change of extraction pressure and/or extraction temperature can alter the CO_2 density, thereby manipulating the solvation power of CO_2. Carbon dioxide flow rate can affect mass transfer rates of extracts and thus change the extraction efficiency. Therefore, it is important to study the effects of these factors on

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oil yield in SC-CO_2 in order to improve the extraction efficiency [8].

Response surface methodology (RSM) has the advantage over traditional methods for optimizing processes due to its efficiency and that lower amounts of data are required [9]. Nowadays, RSM has been successfully used to model and optimize SC-CO_2 extraction for various materials [5–7]. However, the SC-CO_2 extraction of GFO optimized by RSM has not yet been reported.

Antioxidants are important to the oxidative stabilization of oils. Tocopherols, phospholipids, phytosterols and some phenolic compounds were found to be natural antioxidants in crude oils [10]. Recently, sensitive on-line HPLC methods for analyzing free radical scavenging activity have been developed [11, 12]. The determination of antioxidant activity was based on a decrease in absorbance at 517 or 734 nm after post-column reaction of HPLC separated antioxidants with the 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\cdot}$) or 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$). The methods can effectively screen free radical scavengers, especially phenolic compounds, from natural products. These methods and their applications have been systematically reviewed by Niederländer et al. [13]. However, these methods in the analysis of vegetable oils are still scarce and may have great potential in the analysis of antioxidants in oils.

The purpose of this study is to extract the gardenia fruits oil by using SC-CO_2. Extraction variables such as extraction pressure, temperature, and CO_2 flow rate were optimized using response surface methodology (RSM) with a central composite design (CCD), in order to obtain the extraction parameters with the highest oil yield from the whole gardenia fruits. The fatty acid composition and tocopherols content of GFO were also analyzed. The main antioxidants and antioxidant activities of GFO were measured by using on-line HPLC methods and a spectrophotometry assay.

Experimental Procedures

Materials

The dried Gardenia (*Gardenia jasminoides* Ellis) fruits were kindly supplied by Jiangxi Tianshun Industry Development Co., Ltd (Fuzhou, China). The gardenia fruits were packed and kept in the shade until used. Whole gardenia fruits were ground and fractionated by a series of sieves (0.3, 0.6 and 0.9 mm). The distribution of the particle sizes was as follows: <0.3 mm (28.8%), 0.3–0.6 mm (51.6%), 0.6–0.9 mm (17.5%) and >0.9 mm (2.11%). And the water content of the material was 6.27 ± 0.02%.

Chemicals and Reagents

2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), α-tocopherol, β-tocopherol, γ-tocopherol and mixture standards of fatty acid methyl esters (FAME) were purchased from Sigma (St. Louis, MO, USA). Carbon dioxide (99.5%) was supplied by the Pute Gas Co. (Beijing, China). All the solvents (HPLC grade) were acquired from E. Merck (Darmstadt, Germany) and other chemicals (analytical grade) from Beijing Chemical Co. (Beijing, China), unless otherwise stated.

Supercritical CO_2 Extraction (SC-CO_2)

Extractions were conducted in a 1-L stainless steel treatment vessel with a Huali (Huali Pump Co., Ltd, Hangzhou, China) supercritical fluid extractor. The schematic diagram of SC-CO_2 extraction is shown in Fig. 1. The major components of the apparatus included a high-pressure extraction vessel and two separator vessels. Samples (260 g) of whole gardenia fruits powder were placed in the extractor. After an initial air purge, liquefied carbon dioxide was supplied from an inner storage vessel and pumped into the extraction vessel by a high-pressure pump to a given pressure. The pressure was controlled by a back pressure regulator with an accuracy of ±0.5 MPa. The temperature inside the vessel was raised and maintained at the desired level (with an accuracy of ±0.5 °C) by an electrical water bath heating jacket. The flow rate of CO_2 was regulated by adjusting the length of the pumping stroke. After 2 hours of extraction, the oil was collected in the first separator (45 °C, 6.5 MPa). Water and volatile components were recovered in the second separator (35 °C, 5.5 MPa). The oil samples obtained from the first separator were further analyzed. The amount of extracted oil was measured

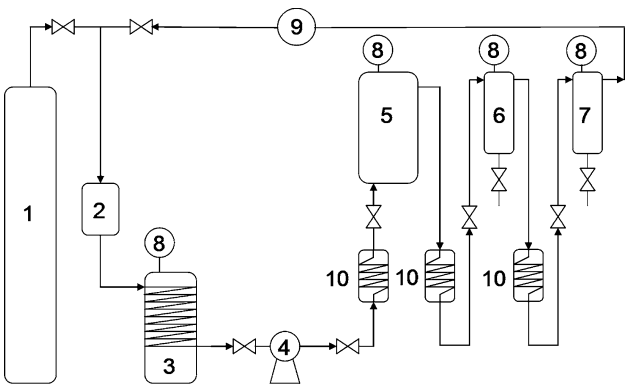


Fig. 1 Schematic diagram of the SC-CO_2 extraction apparatus: *1* CO_2 feed tank, *2* filter, *3* cold bath, *4* pump, *5* extractor, *6* separator I, *7* separator II, *8* pressure gauge, *9* flow meter, *10* preheater

gravimetrically after collection, and then the oil yield (%) was expressed as a percentage of the mass of extracted oil to the mass of gardenia fruit powder loaded into the extraction vessel.

Soxhlet Extraction (SE)

The powder of whole gardenia fruits (40 g) was placed in a Soxhlet apparatus and then continuously extracted for 9 h using *n*-hexane (68 °C). After extraction, the solvent was evaporated by a rotary vacuum evaporator (30 °C) and the extracts were treated with nitrogen gas to remove residual solvent. The oils from SE were weighed and the yield of oil was measured in triplicate. Fatty acid composition and tocopherols content of the oil were analyzed and compared with ones from SC-CO_2.

Fatty Acid Content of Gardenia Fruit Oil

The fatty acid composition was analyzed as fatty acid methyl esters (FAME), according to the method described by Xu et al. [6] with minor modifications. The analysis of FAME was performed on an Agilent 6890N GC-FID equipped with a fused silica capillary column (30 m × 0.25 mm i.d., 0.32 μm film thickness, J&W Scientific, US) coated with polyethylglycol (PEG). The sample (1 μL) was injected with a split ratio of 30:1 and the inlet temperature and detector temperature were set at 250 °C. The oven temperature was initially set at 40 °C for 0.5 min, then increased to 195 °C at a rate of 25 °C/min, 3 °C/min to 205 °C, then 8 °C/min to 230 °C and kept for 4 min, and 5 °C/min to final temperature 240 °C and kept for 5 min. Nitrogen was used as the carrier gas with a flow rate of 2.0 mL/min. Identification of FAME was based on comparing their retention times with those of authentic compounds analyzed under exactly the same conditions. The fatty acid composition in the sample was analyzed in triplicate. The composition of the fatty acids was calculated from their peak areas.

Tocopherol Analysis

The tocopherols were investigated by using an HPLC method. Gardenia fruit oil (GFO) was dissolved in *n*-hexane at a concentration of 0.2 g/mL, and 10 μL was injected into an NP-HPLC (Normal phase-HPLC, Agilent 1100 series, US) equipped with a diode array detector (DAD). Samples were separated on an Agilent Zorbax NH_2 column (250 mm × 4.6 mm i.d., 5 μm particle size) at 30 °C. The mobile phase was a mixture of *n*-hexane and isopropanol (97:3, v/v) with a flow rate of 0.6 mL/min, and the peaks were detected at 292 nm. The tocopherols were identified taking into account the retention times and the UV spectra of the corresponding standards, and quantified using the calibration curves prepared from the corresponding standards. The tocopherols analyses were carried out in triplicate.

Assay of DPPH Radical Scavenging Activity

The stable DPPH free radical scavenging activity of GFO was determined by the method of Valavanidis et al. [14] with minor modifications. The DPPH chloroform solution (10^{-4} M, 3 mL) was added to 1 mL of chloroform solution of GFO (0.04 g/mL), and 1 mL of chloroform was used as the blank. The mixture was shaken vigorously for 20 s and allowed to stand for 30 min in the dark at ambient temperature. The decrease in absorbance of the mixture was measured at 517 nm against the blank using a UV-Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The DPPH radical scavenging activity of each GFO was expressed as α-tocopherol equivalent antioxidant capacity using a calibration curve with α-tocopherol standard (mg α-tocopherol/g oil). The DPPH radical scavenging activity in the sample was analyzed in triplicate.

On-line HPLC Methods for Analyzing Antioxidant Activities

On-line HPLC coupled with DPPH and ABTS assays were performed using the methods developed by Koleva et al. [11, 12] with some modifications. The DPPH free radical solution was prepared by diluting with *n*-hexane to an absorbance of 0.70 ± 0.02 at 517 nm. The ABTS free radical solution was prepared as follows: a stock solution containing 3.5 mM potassium persulfate and 2 mM ABTS was prepared and kept at room temperature in darkness for 16 h in order to stabilize the radical. The stock solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 734 nm. The extracts (10 μL) were injected into an Agilent Normal-Phase HPLC system. Samples were separated on an Agilent Zorbax NH_2 column (250 mm × 4.6 mm i.d., 5 μm particle size) at 30 °C. The mobile phase was a mixture of *n*-hexane and isopropanol (97:3, v/v) with a flow rate of 0.6 mL/min. The chromatographic peaks were detected at 292 nm, and spectral data from all peaks were accumulated in the range of 200–500 nm. The HPLC analytes separated from the column then arrived at a T-junction, where the DPPH or ABTS reagent was added. The radical reagent flow rate was 0.7 mL/min delivered by a Waters Reagent Pump (Waters Corporation, USA). The negative peaks were measured by DAD at 517 or 734 nm for DPPH or ABTS, after the eluates mixed with radical reagent in a reaction coil (15 m × 0.25 mm i.d. PEEK tubing) maintained at 30 °C with a Waters Temperature Control Module (Waters Corporation, USA). Methanol was used as the control by replacing the radical reagent by the

above procedure. Data were analyzed using Agilent Chemstation Software.

Experimental Design

Response surface methodology (RSM) was applied to evaluate the effects of extraction pressure, temperature, and CO_2 flow rate on the oil yield. The experimental design was based on the central composite design (CCD) using a 2^3 factorial and star design with three central points as shown in Table 1. All experiments, which included eight factorial points, six axial points and three center points, were randomly performed. Experiments at the center point were conducted for evaluation of the experimental error. A second-order polynomial equation was applied to express the oil yield (Y) as a function of the independent variables,

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i \neq j=1}^{3} \beta_{ij} X_i X_j \quad (1)$$

where Y represents the dependent variables, β_0 is the intercept term, β_i, β_{ii} and β_{ij} are the linear, quadratic and interactive coefficients, respectively. X_i and X_j are the levels of the independent variables. The model was built based on the variables with confidence levels of 95%. The coefficients of the response surface equation were estimated by using Design-Expert 7.1.6 (Stat-Ease Inc., Minneapolis, MN, USA). Analysis of variance (ANOVA) was performed to evaluate the adequacy of the generated mathematical model.

Results and Discussion

Fitting the Model

To minimize the experimental runs and time for optimizing the oil extraction variables, an experimental design was adopted on the basis of response surface methodology. The levels of these independent variables were determined based on preliminary experiments. The actual and predicted yields of oil obtained from all seventeen simplified experimental runs are listed in Table 1. The experimental data were analyzed to calculate the coefficients of the second-order polynomial equation which are showed in Table 2. The analysis of variance (ANOVA) for the response surface model is also given in Table 2. The regression equation for the oil yield was highly significant ($p < 0.01$), which shows that the fitting degree of regression equation was better on the border of the independent variables. Moreover, the resultant second-order polynomial model adequately represented the experimental data ($R^2 = 0.938$). However, lack of fit of regression equation was significant ($p < 0.05$), which shows that the fitting degree of regression equation was weaker within the independent variables.

Analysis of variance was used to evaluate the significance of the coefficients of the models (Table 2). For any of the terms in the model, a large regression coefficient and a small p-value would indicate a more significant effect on the respective response variables [9]. Thus, as shown in Table 2, the variable with the largest effect on the oil yield

Table 1 Experimental and predicted data for the oil yield obtained from the central composite experimental design

Experiment number[a]	Pressure (MPa)	Temperature (°C)	Flow rate (kg/h)	Yield (%)	
				Experimental	Predicted
1	40	65	15	9.77	10.37
2	20	45	5	5.04	3.95
3	30	71.8	10	8.35	8.63
4	20	65	15	9.27	7.96
5	13.2	55	10	1.62	2.89
6	30	55	10	8.38	8.41
7	30	55	18.4	8.69	9.36
8	40	45	15	8.42	7.83
9	30	55	10	8.35	8.41
10	40	65	5	8.96	8.70
11	30	38.2	10	8.42	8.83
12	46.8	55	10	9.54	8.97
13	20	45	15	8.35	8.12
14	20	65	5	1.08	1.18
15	40	45	5	7.96	8.78
16	30	55	10	8.62	8.41
17	30	55	1.6	4.42	4.44

[a] Experiments were performed in random order

Table 2 Results of the analysis of variance to the response surface quadratic model

	Regression coefficients	Estimated coefficients	Standard error	Degree of freedom	Sum of squares	Mean squares	F-value	p-value[a]
Intercept	β_0	8.41	0.579	1				
	β_1	1.808	0.272	1	44.64	44.64	44.22	0.0003
Linear	β_2	−0.059	0.272	1	0.048	0.048	0.047	0.8340
	β_3	1.461	0.272	1	29.15	29.15	28.88	0.0010
	β_{11}	−0.878	0.299	1	8.70	8.70	8.62	0.0218
Quadratic	β_{22}	0.113	0.299	1	0.14	0.14	0.14	0.7161
	β_{33}	−0.534	0.299	1	3.21	3.21	3.18	0.1177
	β_{12}	0.674	0.355	1	3.63	3.63	3.60	0.0997
Interaction	β_{13}	−1.279	0.355	1	13.08	13.08	12.96	0.0087
	β_{23}	0.654	0.355	1	3.42	3.42	3.39	0.1083
Total model				9	106.06	11.78	11.68	0.0019
Lack of fit				5	7.02	1.40	64.12	0.0154
Pure error				2	0.044	0.022		
Residual				7	7.07	1.01		
R^2	0.938	Adjusted R^2	0.857					

[a] $p < 0.01$ highly significant; $0.01 \leq p < 0.05$ significant; $p \geq 0.05$ not significant

was the linear term of pressure ($p < 0.001$), followed by the linear term of flow rate ($p < 0.01$), the interaction term between pressure and flow rate ($p < 0.01$) and the quadratic term of pressure ($p < 0.05$).

Analysis of Response Surfaces

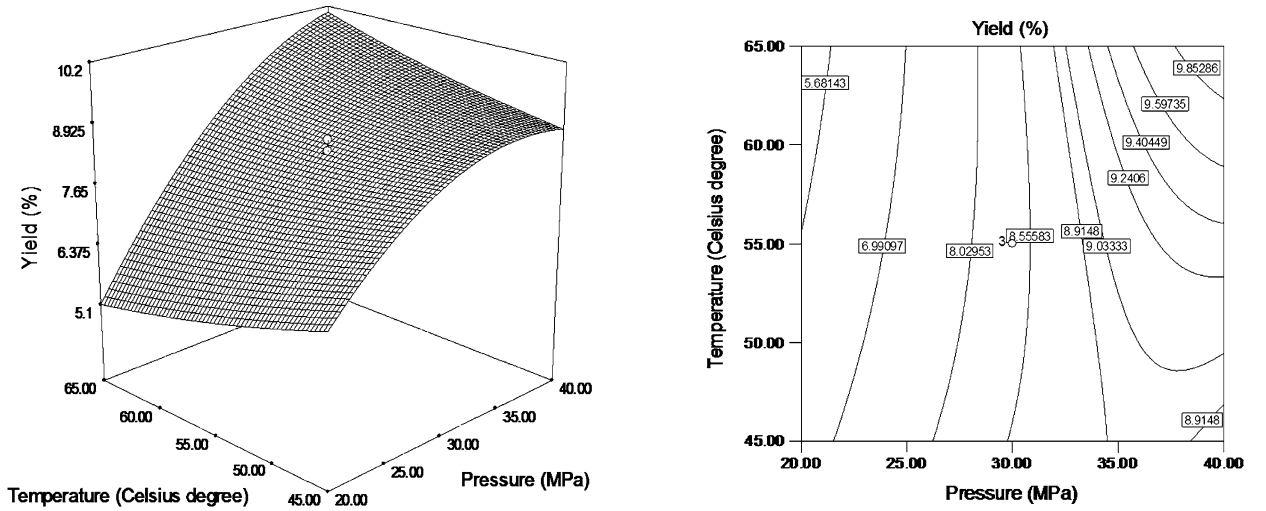
In order to illustrate the effect of the independent variables on the oil yield, surface responses and contour plots of the model were constructed by varying two variables within the experimental range and holding the third variable at the central point. Figure 2a shows the effect of pressure and temperature on the oil yield at the fixed CO_2 flow rate of 10 kg/h. Low pressure had a positive linear effect on oil yield, and the result can be explained by the fact that the pressure increases CO_2 density resulting in an increase in the solvating power [15]. However, there was a negative quadratic effect at high pressure levels (Table 2). The probable reason was that the highly compressed CO_2 with the rise of pressure makes the interactions of repulsive solute–solvent increase [6, 16].

Temperature had a negative linear effect on the oil yield at low-pressure levels, while it showed a positive effect at high-pressure levels. The oil yield decreased with increasing temperature when the pressure was lower than 30 MPa. However, at higher pressures (>30 MPa), the yield increased with the elevation of temperature. It was likely due to the fact that the density of CO_2 decreased with the rise of temperature at low pressures. Nevertheless, at higher pressures, increasing temperature induces a vapor pressure increase of solutes, leading to an increase in the solubility of the oil in CO_2 [8]. Similar crossover pressure phenomena were also reported for the extraction of other oils by SC-CO_2 [5, 6].

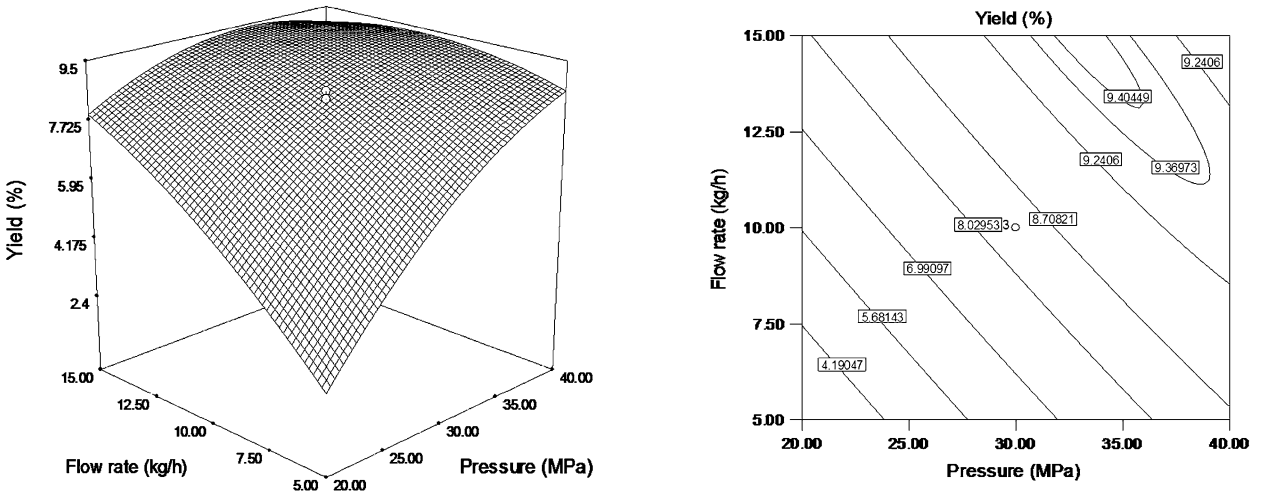
Figure 2b depicts the effect of extraction pressure and CO_2 flow rate on the oil yield at 55 °C. The CO_2 flow rate had a positive linear effect on oil yield, while the interaction between CO_2 flow rate and pressure had a negative effect (Table 2). At low pressure levels, the oil yield increased rapidly with the rise of CO_2 flow rate at a fixed pressure, probably due to the decrease in the mass transfer resistance [5]. However, at higher pressures, the oil yield increased and then decreased as the CO_2 flow rate increased at a given extraction pressure.

Figure 2c exhibits the interaction between extraction temperature and CO_2 flow rate. It revealed that the oil yield decreased with the rise of temperature at low CO_2 flow rate levels. However, the effect of temperature on the oil yield began to reverse when the CO_2 flow rate was over about 12 kg/h.

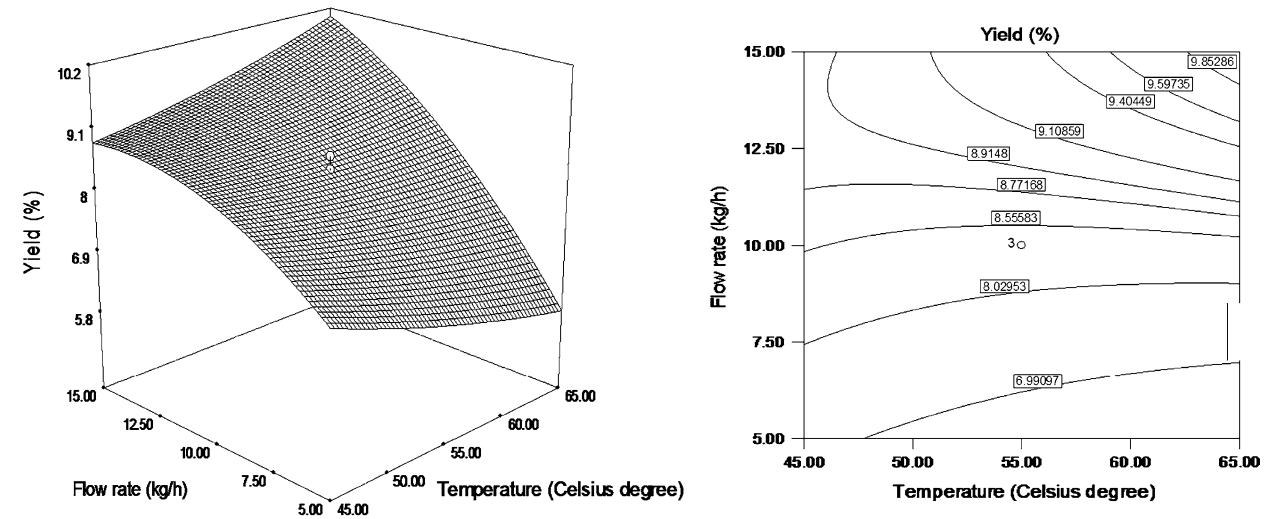
Considering all effects of different variables, the extraction pressure had the most significant effect (linear effect: $p < 0.001$; quadratic effect: $p < 0.05$) on the oil yield. The effects of the other variables on the oil yield became less remarkable in the following order: CO_2 flow rate and extraction temperature. Moreover, it has been elucidated that the physical characteristics of material, such as moisture content and particle size, can affect the oil yield of SC-CO_2 extraction [15], but these factors were not considered in the present study. Therefore, further



(a) Fixed variable: 10kg/h



(b) Fixed variable: 55°C



(c) Fixed variable: 30MPa

Fig. 2 Response surfaces and contour plots of the oil yield (%) from gardenia fruit as affected by extraction pressure, extraction temperature and CO_2 flow rate. The scales of the *y*-axes are predicated values

investigation is still necessary to evaluate the effect of these variables on the oil yield.

Optimization of Extraction Parameters

The optimal parameters obtained using the proposed model were as follows: extraction pressure of 36.8 MPa, extraction temperature of 65 °C, and CO_2 flow rate of 15 kg/h. Under these optimal condition, the oil yield was predicted from the model to be 10.46%, which is slightly lower than that extracted with *n*-hexane (11.58 ± 0.21%). The difference has already been reported, and a probable reason was that *n*-hexane was much less selective than SC-CO_2 in the extracted oil and it included some undesirable compounds [17].

Fatty Acid Composition and Tocopherols Content of Gardenia Fruit Oil

Fatty acid composition and tocopherols content of gardenia fruit oil extracted by SC-CO_2 at the central point level and by the conventional Soxhlet method using *n*-hexane are listed in Table 3. The fatty acid composition and tocopherols content had minor differences between the oils extracted by SC-CO_2 and *n*-hexane. This phenomenon was generally consistent with those formerly reported [6, 18].

In the oil extracted by SC-CO_2, polyunsaturated fatty acids (PUFA) including linoleic acid (about 44%) and linolenic acid (about 1.3%) were the main fatty acids in the gardenia fruit oil. Saturated fatty acids (SFA) were the second most abundant at approximately 29%. Palmitic acid was the most abundant in SFA. In addition, monounsaturated fatty acids (MUFA) accounted for more than 25% of the total fatty acids, mainly oleic acid. In regard to tocopherols, α-tocopherol was dominated in GFO, followed by γ-tocopherol and β-tocopherol. The total tocopherols content was 52.20 ± 0.79 mg/100 g oil, which was similar to linseed oil and linola oil [19]. Therefore, gardenia fruit oil is a rich source of unsaturated fatty acids (>70%), essential fatty acids (linoleic acid and linolenic acid) and tocopherols, which may be supplied as a good nutritional supplement for the human diet [18].

Antioxidant Activity of Gardenia Fruit Oil

On-line HPLC coupled with DPPH or ABTS assays, as effectively screening and evaluating antioxidants methods, were used to identify antioxidant constituents in gardenia fruit oil. As shown in Fig. 3, compounds **1** and **2** have significant free radical scavenging activities. They were identified as α-tocopherol and γ-tocopherol on the basis of their UV spectrum and retention times of standard compounds. Furthermore, β-tocopherol showed weak antioxidant

Table 3 Fatty acid composition and tocopherols content of gardenia fruit oil extracted by supercritical carbon dioxide and *n*-hexane

	n-Hexane	SC-CO_2
Fatty acids (%)		
Palmitic acid, $C_{16:0}$	26.43 ± 1.77[a]	25.15 ± 0.06[a]
Palmitoleic acid, $C_{16:1}$	0.37 ± 0.08[a]	0.49 ± 0.02[a]
Heptadecanoic acid, $C_{17:0}$	0.18 ± 0.01[a]	0.17 ± 0.01[a]
Heptadecenoic acid, $C_{17:1}$	0.11 ± 0.00[a]	0.11 ± 0.00[a]
Stearic acid, $C_{18:0}$	2.66 ± 0.09[a]	2.57 ± 0.01[a]
Oleic acid, $C_{18:1n-9}$	24.56 ± 0.60[a]	24.88 ± 0.11[a]
Linoleic acid, $C_{18:2n-6}$	44.00 ± 0.93[a]	44.61 ± 0.29[a]
Linolenic acid, $C_{18:3n-3}$	1.30 ± 0.03[a]	1.28 ± 0.02[a]
Eicosanoic acid, $C_{20:0}$	0.33 ± 0.03[a]	0.41 ± 0.05[a]
Eicosenoic acid, $C_{20:1}$	0.06 ± 0.02[a]	0.33 ± 0.03[b]
MUFA	25.06 ± 0.62[a]	25.71 ± 0.10[a]
PUFA	45.27 ± 0.96[a]	45.70 ± 0.30[a]
SFA	29.58 ± 1.65[a]	28.20 ± 0.11[a]
Tocopherols (mg/100 g oil)		
α-Tocopherol	40.11 ± 0.68[a]	36.27 ± 0.45[b]
β-Tocopherol	0.64 ± 0.02[a]	0.56 ± 0.04[a]
γ-Tocopherol	11.45 ± 0.10[a]	11.11 ± 0.82[a]
Total	52.20 ± 0.79[a]	47.94 ± 1.31[a]

Results of the fatty acids extracted are expressed as a % of the total content (relative content). All data presented are means ± standard deviation ($n = 3$). Means in the same row with different superscript letters are significantly different at $p < 0.05$ level. Supercritical carbon dioxide extraction was carried out at 30 MPa and 55 °C with a CO_2 flow rate of 10 kg/h

MUFA monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *SFA* saturated fatty acids

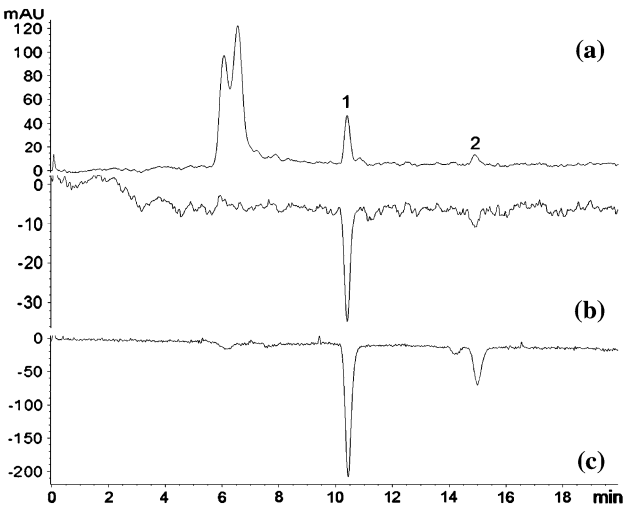


Fig. 3 HPLC chromatograms of gardenia fruit oil detected at 292 nm (**a**), and negative peaks detected by HPLC coupled with DPPH (**b**, 517 nm) and ABTS (**c**, 734 nm). Peak **1**, α-tocopherol; peak **2**, γ-tocopherol

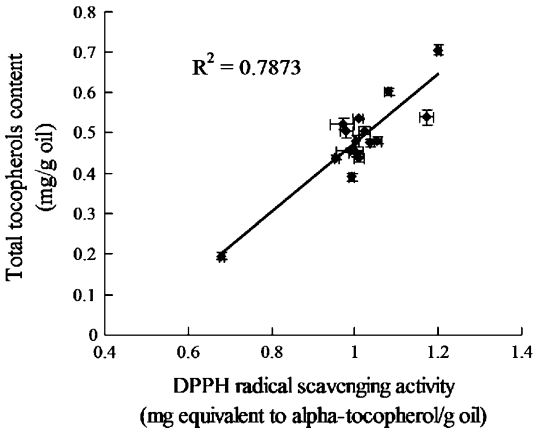


Fig. 4 The correlation between the DPPH scavenging activity and total tocopherols content of the gardenia fruit oils extracted by SC-CO_2. Each value is represented as the mean ± standard deviation of triplicate measurements

activity on the on-line HPLC with ABTS assay (retention time about 14 min), due to its low level in GFO. Therefore, tocopherols mainly α-tocopherol were the major antioxidants in GFO.

For a validation of the on-line HPLC method, the spectrophotometry assay to measure the DPPH radical scavenging activity of GFO was performed. The correlation analysis of the DPPH radical scavenging activity versus the total tocopherols content was carried out using the Microsoft Excel program, shown in Fig. 4. A linear correlation was found with a good correlation coefficient ($R^2 = 0.7873$). Therefore, it can be concluded that the tocopherols showed major antioxidant activity in GFO. In addition, Fig. 4 shows that about 0.5 mg/g oil of the total tocopherol content has an equivalent antioxidant capacity to a calibration oil sample with 1 mg/g oil α-tocopherol concentration. Hence, the oil probably contains other components with higher antioxidant capacity or the synergic effects of different tocopherols are extremely strong.

Compared with the spectrophotometry assay, the on-line HPLC method exhibited better sensitivity to evaluate antioxidant activity in GFO. Additionally, some phenolic compounds and phospholipids in oils exhibited potent antioxidant activities reported by some previous studies [10], however, they can not be found in GFO.

Conclusion

The current study showed that the second-order polynomial model could be used to optimize extraction of gardenia fruit oil (GFO) for maximizing the oil yield within the experimental ranges. Extraction pressure, CO_2 flow rate, the quadratics of pressure, and the interaction between pressure and flow rate showed significant effects on oil yield. The optimum parameters for oil yield were an extraction pressure of 36.8 MPa, an extraction temperature of 65 °C, and a CO_2 flow rate of 15 kg/h. Unsaturated fatty acids, especially polyunsaturated fatty acids, were the dominant fatty acids in GFO. α-Tocopherol is the major tocopherol and antioxidant constituent in GFO. Response surface methodology (RSM) is an effective technique for analyzing and optimizing the extraction processes by supercritical carbon dioxide. On-line HPLC methods coupled with a free radicals scavenging reaction show a potential advantage for the rapid detection of antioxidants in oils.

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