Original article

Optimisation of red pepper seed oil extraction using supercritical CO_2 and analysis of the composition by reversed-phase HPLC-FLD-MS∕MS

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Summary   Oil extractions from red pepper seed were performed by supercritical CO_2. Three-level Box–Behnken factorial design (BBD) from response surface methodology (RSM) was applied to optimise the main extraction conditions including pressure, temperature and concentration of modifier (ethanol). The optimum conditions were as follows: extraction pressure, 27.17 MPa; extraction temperature, 47.67 °C; and the added concentration of modifier, 8.11 vol.%. Under the optimum conditions, the oil yield of 18.4% was obtained, which was well matched with the predicted yield. A simple, stable and sensitive method for the simultaneous determination of saturated and unsaturated free fatty acids in extracted oils using 2-(11H-benzo[a]carbazol-11-yl)-ethyl-4-methylbenzenesulfonate (BCETS) as labelling reagent with fluorescence detection has been developed. All of free fatty acids (FFA) were found to give a linear response with correlation coefficients of > 0.9991. The detection limits at a signal-to-noise ratio (S∕N) of three are in the range of 19.06–41.19 fmol. This method should have powerful potential for the trace analysis of short- and long-chain FFA from edible oils, foodstuff and other complex samples.

Introduction

The fruits of varieties of *Capsicum* (family Solanaceae) vary widely in size, shape, flavour, colour and pungency. *Capsicum annuum* L. has been used worldwide as red peppers powder, vegetables, in folk medicines and also as a source of food additives. Red pepper seed oil (RPSO) is widely used as condiment oil in China and many other nations. Red pepper seeds are obtained as by-products in the preparation of red pepper powder. Traditionally, RPSO is obtained with both mechanical and chemical extraction processes. Mechanical extraction processes are often associated with low yields and chemical extraction process such as extraction methods in the majority of cases employ solvents such as hexane, which are dangerous to handle, and unacceptable as they are quite harmful to human health and environment.

Supercritical CO_2 fluid extraction (SFE) has become one of the promising foods processing technologies. Compared to mechanical and chemical separation processes, it offers numerous potential advantages over conventional extraction processes including nontoxic,

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nonexplosive, environmental friendly, cost-effective, reduced organic solvent volume, time-saving and more selective extractions. Supercritical CO_2 has been utilised for the extraction of oleoresin and capsaicinoid from the peel of red peppers (Illés *et al.*, 1999; Jaren-Galan *et al.*,1999; Sato *et al.*, 1999; Gnayfeed *et al.*, 2001; Duarte *et al.*, 2004; Uquiche *et al.*, 2004). However, to the best of our knowledge, the effect of SFE parameters on the RPSO yield and the optimum operation conditions for RPSO remain poorly investigated. For a possible industrial application, the optimisation and assessment of the extraction process with mathematical modelling seem to be essential. In classical methods, process parameters are optimised by conducting experiments concentrating on one factor at a time. This method is also troublesome and time-consuming as well as ignoring the interaction effect of parameters. Compared to the classical methods, response surface methodology (RSM) is more efficient, requires fewer data and provides interaction effects on the response besides factor effects.

Quality edible oil is not only healthy but can also add flavour to dishes. The quality of edible oil is determined by various factors, and free fatty acids (FFA) are one of the most frequently determined quality indices during

production, storage and marketing (price dictated by FFA content). FFA are usually one undesirable content in edible oils, it results in lower flavour quality and stability of the oil, and too high levels of FFA will results in rancidity of the oil. Thus, quantitative determination of FFA is equally important to the quality control, trading and storage of the edible oils.

Determination of FFA in edible oils and fats has been investigated (Cocito & Delfini, 1994; Ai, 1997; Man & Moh, 1998; Mariotti & Mascini, 2001; Kondyli *et al.*, 2002; Poveda & Cabezas, 2006; Saad *et al.*, 2007), but these methods cannot determine each free fatty acid accurately and are frequently interfered by sample components. The methods usually used for the determination of FFA are based on liquid–liquid extraction using methyl tert-butyl ether, derivatization to the respective methyl-trimethylsilyl-esters and analysis using gas chromatography (GC) or GC/MS analysis. In contrast with GC, use of HPLC allows the FFA to be directly converted to a large number of different derivatives. Derivatization can overcome some problems, such as tailing peaks and low detector sensitivity, by the formation of less polar compounds, which can be more easily analysed by LC. Therefore, derivatization of these analytes with labelling reagents has been widely adopted; because HPLC with UV, especially fluorescence detection, has higher sensitivity. In this study, 2-(11*H*-benzo[a]carbazol-11-yl)-ethyl 4-methylbenzene-sulfonate (BCETS), which is a new fluorescent labelling regent synthesised in our research group, was developed for the simultaneous determination of thirty saturated and unsaturated FFA in RPSO.

The composition of total fatty acids in RPSO has been reported in previous researches (Domokos *et al.*,1992; Al-Khalifa, 1996; El-Adawy & Taha, 2001a,b). However, FFA composition of RPSO remains poorly investigated.

The aims of the present work are (i) to develop a fast, simple and highly efficient supercritical technique for the extraction of oils from red pepper seed; (ii) to develop a simple, stable and sensitive method for simultaneous determination of saturated and unsaturated FFA in RPSO using BCETS as a labelling regent.

Materials and methods

Materials

The matured fruits of *C. annuum* L. were grown in the planting base of Taian, Shandong province (China), red pepper seeds were dried under a stream of nitrogen and ground into a powder of particles with 0.5 mm diameters for supercritical and organic solvent extraction. All FFA were purchased from Sigma Reagent Co. (St. Louis, MO, USA). Water was purified on a Milli-Q system (Millipore, Bedford, MA, USA). BCETS was synthesised according to our previous study (Shi *et al.*,

2005). All other chemicals and solvents used were of analytical grade.

Supercritical CO$_2$ oil extraction

SFE was carried out using the Model HA121-50-01 extraction system (Hua'an Supercritical Fluid Extraction Corp., Nantong, China) equipped with a 1000 cm^3 extraction vessel in which 400 g of seed powder was loaded for each experimental run. The schematic diagram of supercritical fluid extraction was described in detail in a previous study (Koga *et al.*, 1996). The flow rate of CO$_2$ was adjusted by the pump speed and monitored by a mass flow meter. Pressures in the extractor and separators were regulated by micrometering valves. The operating temperatures in the extractor and separators were maintained by water baths, which could keep the temperature change within ± 0.5 °C, and they were monitored continuously by temperature transducers. The operating pressure in the extractor was measured by pressure transducers. In ethanol-modified supercritical CO$_2$ experiments, ethanol was added gradually by the modifier pump to reach the desired concentration. At the end of the extraction, supercritical CO$_2$ was depressurised by a flow control valve to atmospheric pressure, and the oil was collected in a collection vial. The oil yield was calculated by the weight increased.

Experimental design and statistical analysis

Three factors (extracting pressure, extracting temperature and the concentration of modifier) were chosen based on preliminary experiments for further optimisation by employing a three-level, three-variable BBD from RSM. The coded and uncoded independent variables used in the RSM design and their respective levels were listed in Table 1. A total of 17 experiments were designed (Table 2). Each experiment was performed in triplicate, and the average oil yield (%) was taken as the response, Y.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$$

Table 1 Codes and levels of independent variables of pressure (P), temperature (T) and the concentration of modifier (M) in RSM design

Symbols	Independent variable	Coded levels		
		−1	0	+1
X_1	P (MPa)	15	22.5	30
X_2	T (°C)	35	45	55
X_3	M (vol.%)	0	7.5	15

RSM, response surface methodology.

Table 2 Experimental and predicted data for the oil yields obtained from Box–Behnken design ($n = 3$)

Run	X_1	X_2	X_3	Oil recovery (%)
1	15	45	15	11.6
2	22.5	45	7.5	17.4
3	15	45	0	5.5
4	22.5	45	7.5	17.1
5	30	45	0	15.2
6	22.5	35	0	11.9
7	22.5	45	7.5	17.4
8	22.5	45	7.5	17.5
9	30	35	7.5	16.1
10	30	45	15	17.4
11	22.5	45	7.5	17.0
12	30	55	7.5	17.8
13	15	35	7.5	8.6
14	22.5	55	0	14.4
15	22.5	35	15	16.3
16	22.5	55	15	17.5
17	15	55	7.5	11.3

Where Y represents the response variable; β_0 is a constant term; β_1, β_2 and β_3, are linear coefficients; β_{11}, β_{22} and β_{33} are quadratic coefficients; β_{12}, β_{13} and β_{23} are interaction coefficients.

A software DESIGN-EXPERT 7.1.3 Trial (State-Ease, Inc., Minneapolis MN, USA) was used to obtain the coefficients of the quadratic polynomial model. The quality of the fitted model was expressed by the determined coefficient (R^2), and its statistical significance was checked by an *F*-test.

Organic solvent extraction

The organic solvents used to carry out several extractions were n-hexane and mineral ether. Twenty-five grams of prepared samples was macerated with 300 mL organic solvent at 45 °C for 4 h. Thereafter, the process was repeated twice. The resulting extracts were combined; the solvent was removed by a rotary evaporator (40 °C); the remainder was weighed and analysed.

Fatty acids analysis

Instrumentation
Experiments were performed with an LC-MSD-Trap-SL liquid chromatograph mass spectrometer (1100 Series LC-MSD Trap, a complete LC–MS–MS instrument). All the HPLC system devices were from the HP 1100 series and consisted of a vacuum degasser (model G1322A), a quaternary pump (model G1311A), an autosampler (model G1329A), a thermostatted column compartment (model G1316A) and a fluorescence

detector (FLD; model G1321A). The mass spectrometer, from Bruker Daltonik (Bremen, Germany), was equipped with an APCI ion-source. Fluorescence excitation and emission spectra were acquired with a 650-10 S fluorescence spectrophotometer (Hitachi). The mobile phase was filtered through a 0.2-μm nylon membrane filter (Alltech, MA, USA).

Precolumn derivatization of fatty acids
The schematic diagram of derivatization procedure is shown in Fig. 1. In brief, to a 1-mL vial 50 μL BCETS, 10 mg K_2CO_3, 100 μL fatty acid mixture and 200 μL DMF were successively added. The vial was sealed and allowed to react in a water bath at 90 °C with shaking in 5 -min intervals for 30 min. After the reaction was completed, the mixture was cooled to room temperature. A 200-μL volume of the acetonitrile solution (CH_3CN/H_2O 1:1, v/v) was added to dilute the derivatization solution. The diluted solution (10 μL) was injected onto the chromatograph.

Separation of fatty acid derivatives with HPLC
HPLC separation of FA derivatives was carried out on a reversed-phase Eclipse XDB-C_8 column (150 mm × 4.6 mm, 5 μm, Agilent) with a gradient elution. To achieve optimal separation, DMF were dissolved in the mobile phase B, which could raise solubility of fatty acid derivatives to obtain fast separation with sharp peaks. Eluent A was water, B was a mixed solvent of ACN and DMF (1:1.v/v), and C was acetonitrile (100%). The flow rate was constant at 1.0 mL min^{-1}, and the column temperature was set at 30 °C. The injection volume was 10 μL. The fluorescence excitation and emission wavelengths were set at λ_{ex} 279 and λ_{em} 380 nm, respectively. The gradient elution program is presented in Table 3.
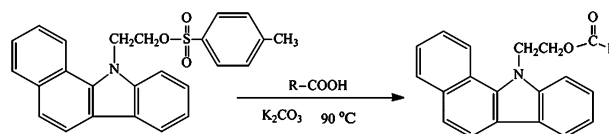
Figure 1 Scheme of derivatization reaction of 2-(11*H*-benzo[a]-carbazol-11-yl)-ethyl-4-methylbenzenesulfonate with fatty acids.

Table 3 Chromatographic gradient conditions

Time	Eluent A	Eluent B	Eluent C
0	45	50	5
30	10	80	10
40	3	87	10
50	3	87	10
70	0	85	15

Quantitative analysis

Quantitative conversion of fatty acids in seed oil to their BCETS derivatives was guaranteed by using an excess of BCETS. The calibration curves for each BCETS-fatty acid derivative were obtained by linear regression plotting peak area vs. concentration.

Results and discussion

Effect of extraction time and the flow rate of supercritical fluid on the oil yield

The extraction time and the flow rate of supercritical fluid for SFE were determined before the RSM optimisation. The extraction time was investigated at a extraction pressure of 22.5 MPa, a temperature of 45 °C, a modifier concentration of 7.5 vol.% and a supercritical fluid flow rate of 20 kg h^{-1}. The extraction times were 20, 40, 60, 80, 100, 120, 140, 160 and 180 min, respectively. The increase in the extraction time increased oil yield within 20–120 min. After 120 min, the oil yield did not increase, thus 120 min was chosen as the optimum for the subsequent tests. The effect of supercritical fluid flow rate was studied at a extraction pressure of 22.5 MPa, a temperature of 45 °C, a modifier concentration of 7.5 vol.% and a extraction time of 120 min. The supercritical fluid flow rates were 5, 10 15, 20, 25, 30 and 35 kg h^{-1}. The maximum oil yield was obtained at a supercritical fluid flow of 20 kg h^{-1}. The higher flow rate cannot lead to the increase in the oil yield. Hence, the supercritical fluid flow rate of 20 kg h^{-1} was chosen for the subsequent tests.

Model fitting and statistical analysis

The conditions for supercritical CO_2 oil extraction from red pepper seed were optimised using different parameters by the combinations of the Box–Behnken design (3^3 factorial). Table 1 shows the experimental data. The independent and dependent variables were analysed to obtain a regression equation that could predict the response within the given range. The predicted second-order polynomial model was the following:

$$Y = 17.29 + 3.69X_1 + 1.01X_2 + 1.98X_3$$
$$- 3.2X_1^2 - 0.64X_2^2 - 1.64X_3^2 - 0.25X_1X_2$$
$$- 0.98X_1X_3 - 0.34X_2X_3$$

The regression coefficients of the intercept, linear, quadratic and interaction terms of the model were calculated using the least square technique and are presented in Table S1. The analysis of variance (ANOVA) for the experimental results of the Box–Behnken Design is also shown in Table S1. Obviously, the linear param-

eters and quadratic parameters were found to be significant at the level of $P < 0.05$ or $P < 0.01$. In this experiment, the value of R^2 (0.997) revealed that the experimental data were in good agreement with the predicted values of the yield of seed oil. The value of adj-R^2 (0.994) suggested that the total variation of 99.4% for the yield of seed oil was attributed to the independent variables, and only about 0.6% of the total variation could not be explained by the model. *F*-value for the lack of fit was insignificant ($P > 0.05$), meaning that this model was sufficiently accurate for predicting the relevant responses.

Optimisation of supercritical CO_2 fluid extraction conditions

The 3D response surface and 2D contour in Fig. 2 provided a method to visualise the relationship between responses and experimental levels of each variable and the type of interactions between two test variables. Figure 2a shows the effect of the extraction pressure and temperature on the oil yield at a fixed modifier concentration of 7.50 vol.%. With a definite extraction temperature, pressure had a positive linear effect on the oil yield, the oil yield increased significantly with the extraction pressure (Fig. 2a), most likely because of the increase in solvent density resulting in the improvement in oil solubility (Abbasi *et al.*,2008). However, the extraction temperature showed the different results compared to extraction pressure. Oil yields increased with the extraction temperature and reached a maximum value, followed by a decline with its further increase (Fig. 2a). That was probably because of the fact that the density of supercritical fluid decreased at further high temperature. The positive regression coefficient for B_2 and the negative for β_{12} (see Table S1) indicated extraction temperature showed a positive linear effect on the oil yield, the complex interaction between temperature and pressure had a negative effect. With a fixed extraction pressure of 22.5 MPa, the interaction relationship between the extraction temperature and concentration of modifier is shown in Fig. 2b. The temperature and modifier exhibited negative quadratic and interaction effect on the oil yield (see Table S1). At a given concentration of modifier, the oil yield increased rapidly with the temperature and reached the highest value, followed by a decline with its further increase. A small amount of a liquid modifier enhanced significantly the extraction efficiency of less polar compounds and dissociated them from their bindings with the membranes (Koga *et al.*, 1996; Sánchez-Vicente *et al.*, 2009). In this study, ethanol was used as modifier. With a fixed extraction temperature of 45 °C, the interaction relationship between the extraction pressure and concentration of modifier is shown in Fig. 2c. At a given concentration of modifier, the oil yield increased rapidly
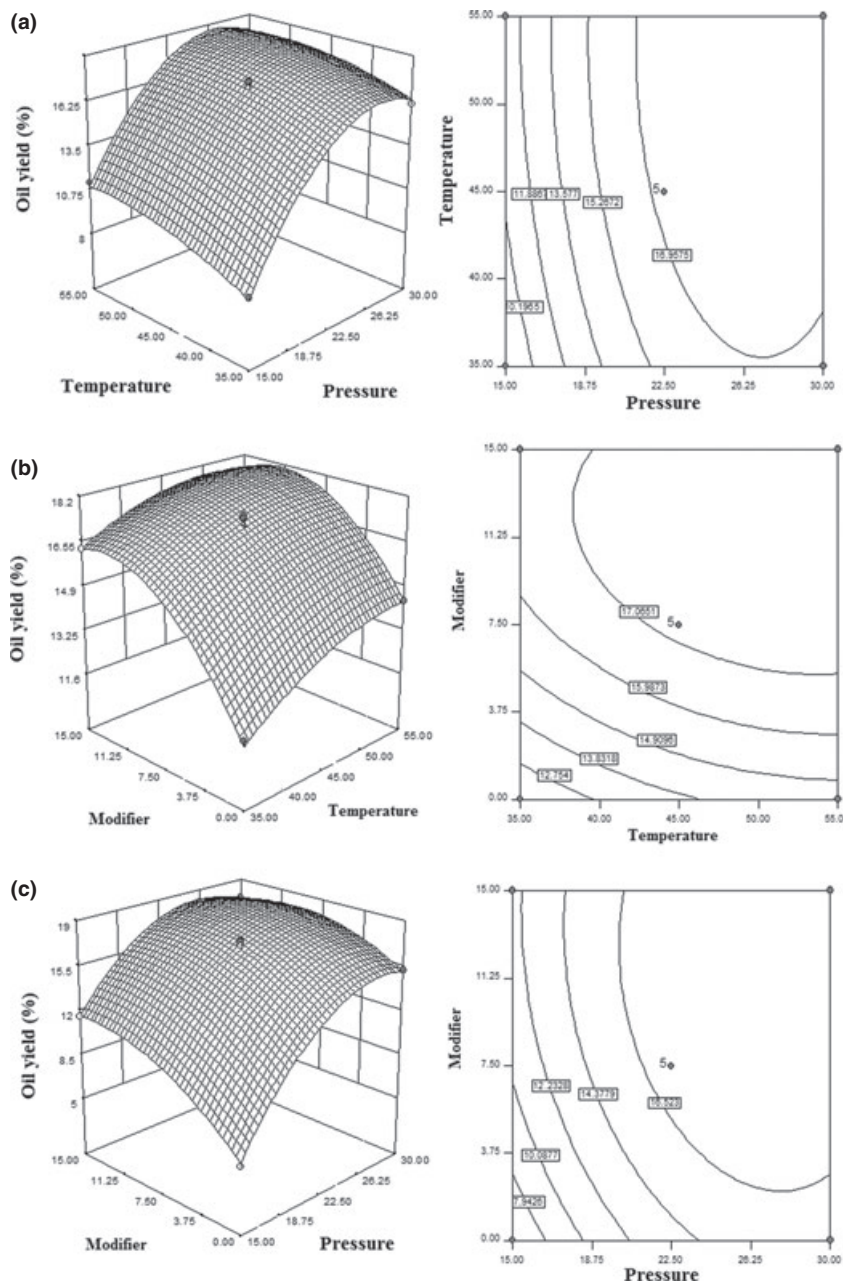
(a)

(b)

(c)

Figure 2 The 3D response surface and 2D contour plots of the oil recoveries affected by extraction pressure, extraction temperature and the concentration of modifier: (a) Effect of pressure and temperature on the oil yield at a modifier concentration of 7.50 vol.%; (b) Effect of modifier concentration and temperature on the oil yield at a extraction pressure 22.5 MPa; (c) Effect of pressure and modifier concentration on the oil yield at a extraction temperature 45 °C.

with the extraction pressure. That was probably because of that the increase in the pressure leaded to the increase in CO_2 and ethanol mixtures density, which induced the improvement in solvent power. With a given pressure, oil yields increased with the concentration of modifier and reached a maximum value. With further increasing concentration of modifier, the oil yields decreased slightly. That might be because at the beginning the added ethanol could significantly improve the solvent power, when the added ethanol reached a certain concentration, the further increase in ethanol would

affect the density of supercritical fluid and lead to the decrease in solvent power (Gülü-üstündag & Temelli, 2005). The complex interaction between extraction pressure and the concentration of the modifier had a negative effect on the oil yield (Table S1).

To further validate optimal values, first partial derivative of regression equation was taken and made to be zero. Calculating the equation gave the following results: $X_1 = 0.62$, $X_2 = 0.27$, $X_3 = 0.08$. The optimal values of the variables given by the software were as follows: extraction pressure, 27.17 MPa; extraction temperature,

47.67 °C; and the added concentration of modifier, 8.11 vol.%. Under the conditions proposed, the model gave the predicted values of Y being 18.6%.

To compare the predicted result with the practical value, experimental validation was performed using the optimised conditions and the mean oil yield was 18.4% ($n = 3$). The positive correlation between these results confirmed that the response model was adequate to reflect the expected optimisation.

Organic solvent extractions were carried out for comparison. The complete organic extractions were achieved within 12 h. The oil yields obtained by n-hexane and mineral ether were 19.2% and 19.8%, respectively. Compared to organic solvent extractions, supercritical fluid extraction in this study exhibited the obvious advantages containing satisfactory extraction efficiency, time-saving, cost-effective, reduced organic solvent volume, etc.

Free fatty acids analysis

MS identification
With the chromatographic conditions described earlier, excellent separation of thirty compounds was achieved with high peak's symmetry (see Fig. S1). The ionisation and fragmentation of the separated BCETS-fatty acid derivatives were studied by APCI/MS in positive-ion detection mode. The MS/MS analysis and corresponding cleavage mode for a representative BCETS-C_{18} derivative are shown in Fig. S2. MS and MS/MS data for the FFA derivatives in RPSO are shown in Table 4. As expected, the BCETS-C_{18} fatty acid derivative produced an intense molecular ion peak at m/z [M + H]$^+$. With MS/MS analysis of fatty acid derivatives, the collision-induced dissociation spectra of m/z [M + H]$^+$ produced the specific fragment ions at m/z 310.7, m/z 243.5 and m/z 261.4 (see Fig. S2). The specific fragment ions at m/z 310.7 was corresponding the protonated fatty acid moiety, which was specific for C_{18} fatty acid derivatives. The identification of other fatty acid derivatives was similar as the earlier statement. With APCI in positive-ion detection mode, intense ion current signals for fatty acid derivatives should be attributed to the introduction of the weakly basic nitrogen atoms in the corresponding BCETS molecular core structure, resulting in high ionisation efficiency.

Reproducibility, calibration and detection limits
The reproducibility of the method was ascertained by carrying out five assays on the same sample over 2 days; each solution was injected twice. The relative standard deviations (RSDs) of retention times and peak areas are 0.04–0.26% and 0.12–2.97% (Table S2), respectively. The linearity was established over a 2500-fold concentration range for FFA with analysis of serial dilutions of the standard derivatized mixture ranging from 0.045 to 112.5 μM. All of FFA found to give a linear response with correlation coefficients of > 0.9991. The calculated detection limits with an injection of 1.0 pmol of each derivatized FFA, for a signal-to-noise ratio of 3:1, ranged from 19.06 to 41.19 fmol (Table S2).

Analysis of free fatty acids in samples
The representative chromatogram of FFA in the RPSO extracted under optimum conditions is shown in Fig. 3.

Table 4 MS data of BCETS-fatty acid derivatives and content of fatty acids from RPSO ($n = 3$)

Fatty acids	Molecular weight	[M+H]$^+$	Specific MS/MS data	SFE without ethanol % (μg mL^{-1})[b]	SFE with ethanol[a] % (μg mL^{-1})[b]	Mineral ether % (μg mL^{-1})[b]	n-hexane % (μg mL^{-1})[b]
C14	471	472.3	243.5, 255.6	0.84 (7.93)	0.83 (9.80)	0.83 (7.96)	0.83 (7.41)
C18:3	521	521.9	243.5, 304.9, 503.9	7.24 (68.09)	7.33 (86.53)	7.23 (69.24)	7.15 (63.55)
C18:2	523	523.9	243.5, 306.7, 516.0	46.49 (437.05)	48.20 (568.79)	48.12 (460.95)	45.88 (407.91)
C16	499	500.2	243.5, 283.5	17.90 (168.26)	17.23 (203.31)	16.71 (160.07)	17.66 (157.04)
C18:1	525	525.8	243.5, 309.0, 507.7	14.38 (135.18)	14.99 (176.91)	14.20 (136.08)	14.19 (126.17)
C17	513	514.4	243.5, 297.1	4.34 (40.81)	3.65 (43.03)	4.32 (41.40)	4.28 (38.09)
C18	527	528.3	243.5, 310.7	2.24 (21.07)	2.62 (30.90)	2.32 (22.21)	2.55 (22.69)
C20	555	556.3	243.5, 339.4	3.16 (29.73)	2.14 (25.30)	3.18 (30.42)	3.60 (32.01)
C22	583	584.1	243.5, 367.5	3.38 (31.74)	2.94 (34.67)	3.07 (29.46)	3.84 (34.18)
SFFA				31.88 (299.53)	29.47 (347.02)	30.45 (291.52)	32.77 (291.42)
UFFA				68.12 (640.32)	70.53 (832.23)	69.55 (666.26)	67.23 (597.63)
TFFA				100 (939.85)	100 (1179.24)	100 (957.78)	100 (889.05)

SFFA, saturated free fatty acids; UFFA, unsaturated free fatty acids; TFFA, total free fatty acids; BCETS, 2-(11H-benzo[a]carbazol-11-yl)-ethyl-4-methylbenzenesulfonate.
[a]SFE + ethanol: supercritical CO_2 extraction under optimum conditions; RPSO, red pepper seed oil.
[b]Mass per cent (%), ratio of the mass of a FFA with that of all FFA, absolute content [μg (FFA)/mL (oil)].
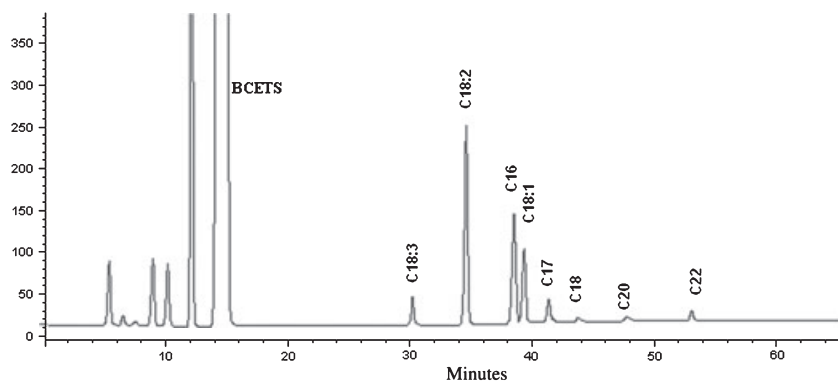
Figure 3 Chromatogram of fatty acid derivatives in red pepper seed oil. Chromatographic conditions: Column temperature at 30 °C; excitation wavelength λex 279 nm, emission wavelength λ em 380 nm; Eclipse XDB-C$_8$ column (4.6 × 150 mm, 5 μm); flow-rate = 1.0 mL min^{-1}. Peak labels: C18:3 (8,11,14-octadecatrienoic acid); C18: 2 (9,12-octadecadienoic acid); C16 (hexadecanoic acid); C18: 1 (12-octadecenoic acid); C17 (heptadecanoic acid); C18 (stearic acid); C20(eicosoic acid); C22 (docosanoic acid).

Chromatographic peaks were doubly identified by retention times and MS identification. Results showed RPSO was rich in unsaturated free fatty acids (UFFA) (see Table 4), and the high to low concentrations were as follows: linoleic acid (C18:2), oleic acid (C18:1), linolenic acid (C18:3). The main saturated free fatty acids (SFFA) were hexadecanoic acid (C16), heptadecanoic acid (C17), docosanoic acid (C22), octadecanoic acid (C18) and eicosoic acid (C20).

The RPSO extracted by SFE under the optimum conditions (SFE with ethanol), SFE without ethanol and organic solvents extractions (n-hexane and mineral ether) was analysed quantitatively for comparison, and the results are presented in Table 4. Results indicated oil samples extracted by different methods showed different FFA content ranging from 889.05 to 1179.24 μg mL^{-1} (see Table 4). Figure 4 intuitively presents the FFA content in RPSO samples. As is shown in Fig. 4, FFA content of samples at a high to low order is as follows: SFE with ethanol > mineral ether > SFE without ethanol > n-hexane. Under the same extracting conditions, SFE without ethanol yielded 939.85 μg mL^{-1} of FFA, when ethanol was added; 1179.24 μg mL^{-1} of

FFA was obtained (see Table 4). This result indicated ethanol as modifier can affect the FFA content in RPSO.

Conclusion

In this study, SFE parameters for RPSO were optimised using BBD from RSM, the optimum conditions were as follows: extraction pressure, 27.17 MPa; extraction temperature, 47.67 °C; and the added concentration of modifier, 8.11 vol. %. Under the optimum conditions, the experimental yield (18.4%) was well matched with the predicted yield (18.6%). Simultaneous determination of thirty FFA in RPSO using BCETS as labelling reagent with HPLC fluorescence detection and on-line MS identification has been successfully achieved. This method exhibited powerful potential for the trace analysis of short- and long-chain FFA from edible oils, foodstuff and other complex samples. In RPSO, the most abundant FFA was C18:2. Other FFA in order of abundance were C16, C18:1, C18:3, C17, C22, C18 and C20. FFA content in RPSO extracted by different extraction methods at a high to low order is as follows: SFE with ethanol > mineral ether > SFE without ethanol > n-hexane.

Figure 4 The content of total free fatty acids, saturated free fatty acids and unsaturated free fatty acids in red pepper seed oils extracted by supercritical CO$_2$ fluid extraction (SFE) without ethanol, SFE with ethanol, mineral ether and n-hexane, respectively.

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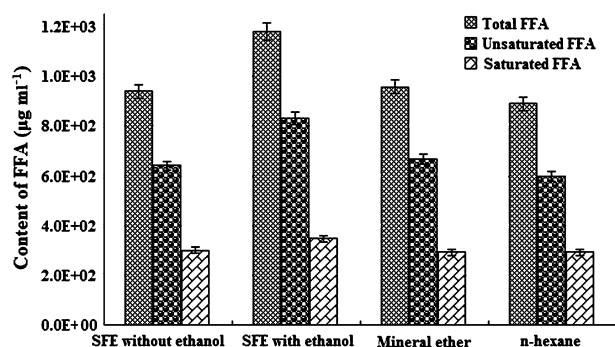
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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Chromatogram of fatty acids standards derivative in red pepper seed oil. Chromatographic conditions: Column temperature at 30 °C; excitation wavelength λex 279 nm, emission wavelength λ em 380 nm; Eclipse XDB-C_8 column (4.6 × 150 mm, 5 μm); flowrate = 1.0 mL min^{-1}. (a) Peak labels: C6 (hexanoic acid); C7 (heptoic acid); C8 (octanoic acid); C9 (nonanoic acid); C10 (decoic acid); C11 (undecanoic acid); C12 (dodecanoic acid); C20:5 (5,8,11,14,17-eicosapentaenoic acid); C13 (tridecanoic acid); C18:3 (8,11,14-octadecatrienoic acid); C22:6 (2,5,8,11,14,17-docosahexaenoic acid); C14 (myristic acid); C20: 4 (6,9,12,15-arachidonic acid); C18: 2 (9,12-octadecadienoic acid); C15 (pentadecanoic acid); C16 (hexadecanoic acid); C18: 1 (12-octadecenoic acid); C17 (heptadecanoic acid); C18 (stearic acid); C20:1 (11-eicosenoic acid); C19 (nonadecanoic acid); C20(eicosoic acid); C22:1 (12-docosenoic acid); C21 (heneicosanoic acid); C22 (docosanoic acid); C24:1 (20-tetracosenoic acid); C23 (tricosanoic acid); C24 (tetracosanoic acid); C25 (pentacosanoic acid); C26 (hexacosanoic acid).

Figure S2. MS spectra and cleavage mode of representative C18 fatty acid derivative: (a) molecular ion MS and (b) MS/MS, scanning range from 100 to 600 amu with APCI in positive-ion detection mode.

Table S1. Estimated regression coefficients for the quadratic polynomial model and ANOVA for the experimental results.

Table S2. Linear regression equations, correlation coefficients, detection limits and repeatability.

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