Research Article

Received: 26 November 2009 Revised: 29 March 2010 Accepted: 29 April 2010 Published online in Wiley Interscience: 14 June 2010

(www.interscience.wiley.com) DOI 10.1002/jsfa.4031

Supercritical CO_2 oil extraction from Chinese star anise seed and simultaneous compositional analysis using HPLC by fluorescence detection and online atmospheric CI-MS identification

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Abstract

BACKGROUND: Supercritical CO_2 was utilised to extract Chinese star anise seed oil (CSASO), and a three-level Box–Behnken factorial design from response surface methodology was applied to optimise the extraction conditions, including pressure, temperature and amount of modifier (ethanol). The compositional analysis of fatty acids in CSASO was performed by HPLC with fluorescence detection using 2-(11*H*-benzo[a]carbazol-11-yl)-ethyl-4-methylbenzenesulfonate (BCETS) as labelling reagent. Identification was carried out by online atmospheric chemical ionisation–mass spectrometry.

RESULTS: The optimum extraction conditions were as follows: extraction pressure, 27.72 MPa, extraction temperature, 46.22 °C, and amount of modifier, 8.58 vol.%. The experimental result showed that the maximum extraction yield was 25.31 ± 0.22% (w/w) under the conditions proposed. The compositional analysis indicated that CSASO mainly contained C18 : 2, C18 : 1, C18 : 3, C20 : 4, C16, C18 and C20 fatty acids.

CONCLUSION: In this study, a fast, simple and high-efficiency supercritical technique for extracting oil from Chinese star anise seed was developed. Simultaneous determination of fatty acids in CSASO using BCETS as the labelling reagent with HPLC fluorescence detection and online mass spectroscopy identification has been successfully achieved.

Keywords: Chinese star anise seed oil; supercritical CO_2 fluid extraction; response surface methodology; fatty acids; HPLC/APCI/MS

INTRODUCTION

Chinese star anise (*Illicium verum* Hook. f.), the seed pod of an evergreen tree grown in eastern Asia, has a pungent, licorice-like flavour. It is used as a spice in cooking and for treatment of dyspeptic complaints and catarrhs of the respiratory tract in traditional Chinese medicine.[1,2] However, in the last year, intoxications after consumption of Chinese star anise have been reported due to adulterations of *I. verum* with the morphologically similar Japanese star anise (*I. anisatum* L.) containing anisatin and other related toxic sesquiterpene lactones.[2,3] Most previous studies were focused on the essential oils in the fruit peel of Chinese star anise,[1,4,5] but there is little study on the Chinese star anise seed oil (CSASO).

The most valuable part in Chinese star anise is the essential oil, which have a wide range of commercial applications in the production of perfumes, cosmetics, soaps, food and beverage flavourings.[6] The seeds are a by-product of the process, and account for 20.4% of the total fruit. It has an estimated annual production potential of one million metric tons in China. Usually they are treated as waste disposal.

Supercritical CO_2 fluid extraction (SFE) offers numerous potential advantages over conventional extraction processes, including the facts that it is non-toxic, non-explosive, environmentally friendly, cost-effective, has a lower consumption of organic solvent, is time-saving and has high selectivity.[7] SFE has been widely used for seed oil extractions.[8–11] However, to the best of our knowledge, the effect of SFE parameters on the CSASO yield and the optimum operation conditions for CSASO 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

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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. It has also been extensively applied to optimise processing parameters in the production of food, drugs and other commodities.[12–15]

Quantitative determination of the fatty acids composition is helpful to control the quality of CSASO, which has not been reported yet. Most fatty acids show neither natural absorption in the visible or ultra-violet (UV) regions nor fluorescence; thus, their detection at trace levels using absorptiometry is fairly difficult. Therefore, derivatisation of these analytes with labelling reagents has been widely adopted, since high-performance liquid chromatography (HPLC) with UV or fluorescence detection has a higher sensitivity.[16–18] In this study, 2-(11*H*-benzo[a]carbazol-11-yl) ethyl 4-methylbenzenesulfonate (BCETS), which is a new fluorescent labelling regent, is utilised to analyse the fatty acids composition in CSASO.

The aims of the present work were to (1) optimise the SFE conditions including extraction pressure, extraction temperature, the added amount of modifier, and study on the interaction effect of the parameters using RSM; (2) to develop a sensitive method using BCETS as labelling reagent for the simultaneous determination of saturated and unsaturated fatty acids; and (3) to determine and quantify the total fatty acids (TFA) and free fatty acids (FFA) in CSASO.

MATERIALS AND METHODS

Materials

The dried Chinese star anise seeds were purchased in Guangxi (China), and were ground into powder with a cyclone mill and passed through a 60 mesh sieve. All fatty acids were purchased from Sigma Reagent Co. (St Louis, MO, USA). Water was purified on a Milli-Q system (Millipore, Bedford, MA, USA). 2-(11*H*-benzo[a]carbazol-11-yl) ethyl 4-methylbenzenesulfonate (BCETS) was synthesised in our laboratory (the synthesis of BCETS is not shown). All other chemicals and solvents used were of analytical grade.

Cell wall breakage pretreatment and supercritical CO_2 extraction of CSASO

Cell breakage pretreatment and extraction measurements were carried out by a semi-bath flow extraction apparatus (Hua'an Supercritical Fluid Extraction Corp., Nantong, China).The schematic flow diagram was described in detail in a previous study.[19]

The cell wall breakage pretreatment was carried out by the method described by Xu *et al.*[20] with minor modifications. The prepared samples (400 g) were placed into a steel cylinder equipped with mesh filters on both ends to prevent the particles being flushed out. The loaded cylinder was then introduced into the extraction vessel and liquefied CO_2 was pumped into the vessel by a high pressure pump. Extraction pressure, extraction temperature and CO_2 flow rate were controlled by adjusting the valves on the front panel. The pressure and temperature were controlled to an accuracy of 45 ± 0.5 MPa and 40 ± 0.5 °C, respectively, and kept for 10 min. At the end of each treatment, the pressure was quickly released to atmospheric pressure within 1 min.

The next step was consecutive oil extraction, which was conducted at the specified extraction conditions (see Table 1).

Table 1. Experimental and predicted data for the oil yield obtained from the Box–Behnken design ($n = 3$)

Run number	Independent variables: X_1, pressure (MPa)	X_2, temperature (°C)	X_3, modifier (vol.%)	Oil recovery (%, w/w): Experimental	Predicted
1	22.5(0)	40.0 (0)	7.5 (0)	22.11	22.02
2	22.5(0)	40.0 (0)	7.5 (0)	22.60	22.02
3	15.0 (−1)	30.0 (−1)	7.5 (0)	10.80	10.21
4	30.0 (+1)	40.0 (0)	0.0 (−1)	21.90	21.00
5	15.0 (−1)	40.0 (0)	15.0 (+1)	14.70	15.60
6	22.5 (0)	30.0 (−1)	0.0 (−1)	12.10	12.45
7	22.5 (0)	40.0 (0)	7.5 (0)	21.78	22.02
8	30.0 (+1)	40.0 (0)	15.0 (+1)	23.70	23.45
9	15.0 (−1)	50.0 (+1)	7.50 (0)	10.40	9.85
10	22.5 (0)	40.0 (0)	7.50 (0)	21.40	22.02
11	22.5 (0)	40.0 (0)	7.50 (0)	22.20	22.02
12	30.0 (+1)	50.0 (+1)	7.50 (0)	23.72	24.32
13	15.0 (−1)	40.0 (0)	0.00 (−1)	6.80	7.05
14	22.5 (0)	50.0 (+1)	15.0 (+1)	21.50	21.15
15	22.5 (0)	50.0 (+1)	0.00 (−1)	12.60	12.90
16	30.0 (+1)	30.0 (−1)	7.50 (0)	17.00	17.55
17	22.5(0)	30.0 (−1)	15.0 (+1)	15.53	15.20

With some extraction procedures, ethanol, which was used as modifier, was pumped into the system from the modifier bottle after the selected pressure and temperature had been achieved. Each extraction lasted for 90 min, since longer extraction times did not significantly increase the yield of oil. 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

The single-factor experimental design (extracting pressure, extracting temperature, extracting time and the added amount of modifier) were carried out before RSM experiments (data not shown). Three factors (extracting pressure, extracting temperature, and the added amount of modifier) were chosen for further optimisation by employing a three-level, three-variable Box–Behnken factorial design (BBD) from RSM.[21] 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 1). Each experiment was performed in triplicate and the average oil yield (%, w/w) was taken as the response, Y. Based on the experimental data, regression analysis was performed and was fitted into an empirical second-order polynomial model:

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

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.

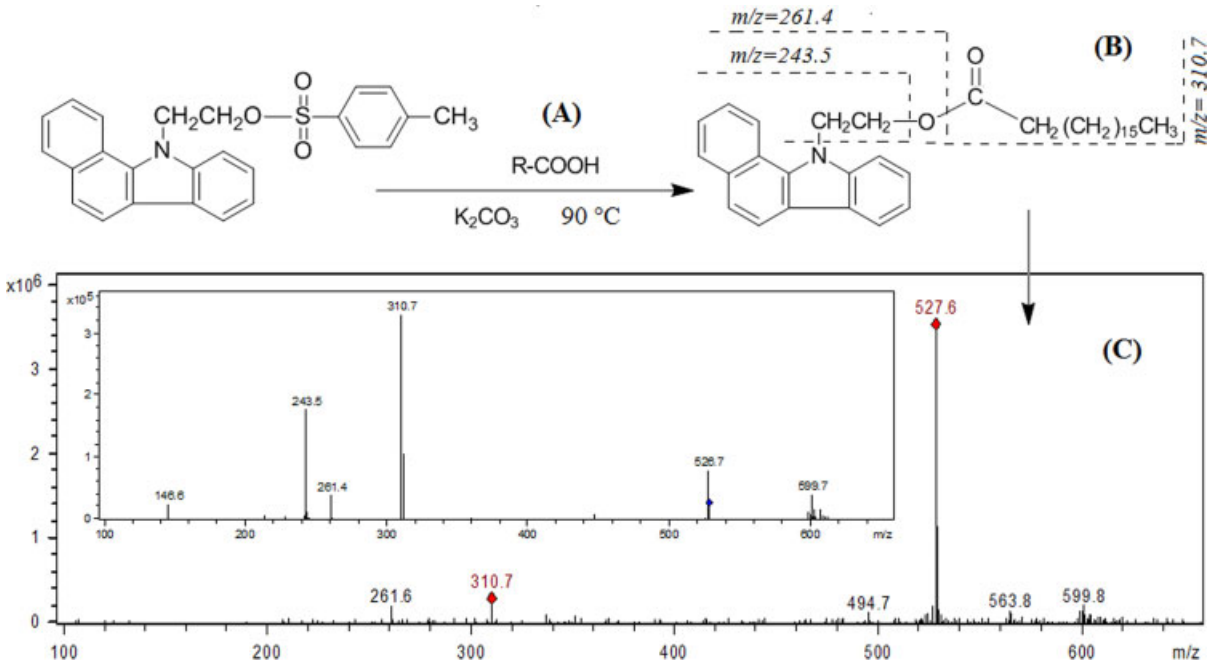

Figure 1. Derivatisation and identification of fatty acids. (A) Derivatisation scheme of fatty acids with BCETS; (B) the MS/MS cleavage mode of a BCETS–fatty acid derivative; (C) typical LC/MS profile of the C18 acid derivative (BCETS-C18) from full scanning range from 100 to 600 amu with APCI in positive-ion detection mode and typical APCI-MS/MS profile of C18 acid derivative (BCETS-C18) from full scanning range from 100 to 600 amu with APCI in positive-ion detection mode.

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; Waldbronn, Germany). All the HPLC system devices were from the HP 1100 series (Waldbronn, Germany) and consisted of a vacuum degasser (model G1322A), a quaternary pump (model G1311A), an autosampler (model G1329A), a thermostatic column compartment (model G1316A), a fluorescence detector (FLD; model G1321A), and a diode array detector (DAD; model G1315A). The mass spectrometer, from Bruker Daltonik (Bremen, Germany), was equipped with an APCI ion-source. The mobile phase was filtered through a 0.2 µm nylon membrane filter (Alltech, Deerfield, IL, USA).

Saponification of seed oil

To a 10 mL test tube, 0.1 g seed oil and 2.0 mL potassium hydroxide/methanol solution (2 mol L^{-1}) were added. After being sealed, the test tube was immersed in a water bath at 90 °C for 2 h. After cooling, the contents were transferred to a centrifugal test tube, to which 2 mL water was added, and the pH was adjusted to 7.0 with 2 mol L^{-1} hydrochloric acid solution. This solution was extracted with chloroform three times (3 mL × 3). The combined chloroform was filtered and evaporated under a stream of nitrogen. The residue was re-dissolved in 50 mL N, N-dimethylformamide (DMF), filtered through a 0.2 mm nylon membrane filter, and stored at −10 °C until HPLC analysis.

Pre-column derivatisation of fatty acids

To a 1 mL vial, 50 µL BCETS, 10 mg K_2CO_3, 100 µL fatty acid mixture and 200 µL DMF was 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. The derivatisation procedure is shown in Fig. 1A.

Separation fatty acid derivatives with HPLC

HPLC separation of BCETS–fatty acid derivatives was carried out on a reversed-phase Eclipse XDB-C_8 column (150 mm × 4.6 mm, 5 µm; Agilent, Agilent Technologies, USA) with a gradient elution. Eluent A was water, B was a mixed solvent of acetonitrile (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 nm and λ_{em} 380 nm, respectively. The gradient elution program was as follows: initial = 45% A, 50% B; 30 min = 10% A, 80% B; 40 min = 3% A, 87% B; 50 min = 2% A, 88% B; 70 min = 0% A, 85% B.

Quantitative analysis

Quantitative conversion of fatty acids from CSASO to their BCETS derivatives was ensured by using an excess of BCETS. All fatty acids were quantified using the external standard method. The calibration curves for each fatty-acid derivative were obtained by linear regression plotting peak area versus concentration (see Table 2).

RESULTS AND DISCUSSION

Model fitting and statistical analysis

The conditions for supercritical CO_2 oil extraction from Chinese star anise seed were optimised using different parameters by the combinations of the Box–Behnken design (3^3 factorial). Table 1 shows the experimental and predicted oil yields. The regression coefficients of the intercept, linear, quadratic and interaction terms of the model were calculated using the least square technique,

Table 2. Linear regression equations, correlation coefficients, detection limits, and repeatability

Fatty acid	Regression equation	Correlation coefficient	Detection limit (fmol)	Retention time (RSD%, $n = 6$)	Peak area (RSD%, $n = 6$)
C5	$Y = 1.70X + 2.10$	0.9994	20.71	0.21	0.47
C6	$Y = 1.18X - 1.07$	0.9997	23.67	0.18	0.70
C7	$Y = 1.21X + 5.32$	0.9994	17.07	0.13	0.59
C8	$Y = 1.05X + 7.02$	0.9999	32.06	0.10	0.58
C9	$Y = 0.84X + 5.16$	0.9997	20.25	0.09	0.49
C10	$Y = 0.90X + 4.23$	0.9995	19.76	0.07	0.40
C11	$Y = 0.89X - 1.93$	0.9994	20.25	0.08	0.50
C12	$Y = 0.93X + 0.38$	0.9998	18.92	0.06	0.47
C20:5	$Y = 1.19X + 2.90$	0.9996	16.32	0.06	0.38
C13	$Y = 0.60X + 3.47$	0.9994	34.19	0.06	0.22
C18:3	$Y = 1.23X + 3.60$	0.9996	15.65	0.06	0.25
C22:6	$Y = 1.09X + 4.20$	0.9998	16.19	0.06	0.30
C14	$Y = 0.74X + 4.49$	0.9994	20.79	0.04	0.27
C20:4	$Y = 1.08X + 2.65$	0.9999	15.18	0.07	0.33
C18:2	$Y = 1.42X - 5.38$	0.9996	14.26	0.04	0.27
C15	$Y = 0.87X - 1.81$	0.9994	12.06	0.04	0.19
C16	$Y = 1.26X - 3.36$	0.9994	10.79	0.04	0.11
C18:1	$Y = 1.94X - 3.47$	0.9994	12.32	0.03	0.14
C17	$Y = 0.86X - 3.63$	0.9998	12.06	0.03	0.19
C18	$Y = 0.92X - 2.24$	0.9999	12.06	0.02	0.18
C20:1	$Y = 1.14X - 2.76$	0.9995	15.34	0.04	0.20
C19	$Y = 0.82X - 4.19$	0.9996	10.79	0.02	0.19
C20	$Y = 0.74X + 0.01$	0.9997	14.65	0.05	0.40
C22:1	$Y = 0.76X - 1.56$	0.9998	18.74	0.09	0.52
C21	$Y = 0.97X - 2.09$	0.9999	15.78	0.07	0.88
C22	$Y = 0.67X - 2.45$	0.9997	16.34	0.10	1.25
C24:1	$Y = 0.54X + 0.87$	0.9996	20.48	0.18	1.14
C23	$Y = 1.03X - 5.89$	0.9994	28.05	0.12	1.38
C24	$Y = 1.01X - 2.48$	0.9999	24.75	0.19	1.92
C25	$Y = 1.08X - 11.64$	0.9996	25.62	0.24	2.30
C26	$Y = 0.91X - 4.33$	0.9997	26.79	0.23	2.96

Y is the peak area, and X is the amount injected (pmol).

and are presented in Table 3. 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:

$$Y = 22.02 + 5.45X_1 + 1.62X_2 + 2.75X_3 - 2.59{X_1}^2 - 3.94{X_2}^2 - 2.65{X_3}^2 + 1.78X_1X_2 - 1.53X_1X_3 + 1.38X_2X_3.$$

The analysis of variance (ANOVA) for the experimental results of BBD is shown in Table 3. Obviously, all of the linear parameters, interaction parameters 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 (0.9912) revealed that the experimental data was in good agreement with the predicted values of the oil yield. The value of adj-R^2 (0.9800) suggested that the total variation of 98% for the yield of seed oil was attributed to the independent variables and only about 2% of the total variation could not be explained by the model.

The lack of fit was an indication of the failure for a model, if there is a significant lack of fit which could be indicated by a low probability value, the response predictor is discarded.[22] The F value for the lack of fit was insignificant ($P > 0.05$), meaning that this model was sufficiently accurate for predicting the relevant responses. The coefficient of variation (CV%) of less than 4.42% indicated that the model was reproducible.[23]

Response surface analysis and optimisation of SFE conditions

The three-dimensional (3D) response surface and 2D contour plots can provide 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 amount 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 increasing extraction pressure (Fig. 2A), most likely due to the increase of solvent density resulting in the improvement of oil solubility.[24] However, the extraction temperature showed the different results compared to extraction pressure. Oil yields increased with increasing extraction temperature and reached a maximum value, followed by a decline with its further increase (Fig. 2A). That was probably due to the fact that the density of CO_2 decreased at further high temperature. The temperature showed a negative quadratic effect, while the complex interaction between temperature and pressure had a positive effect on the oil yield (see Table 3).

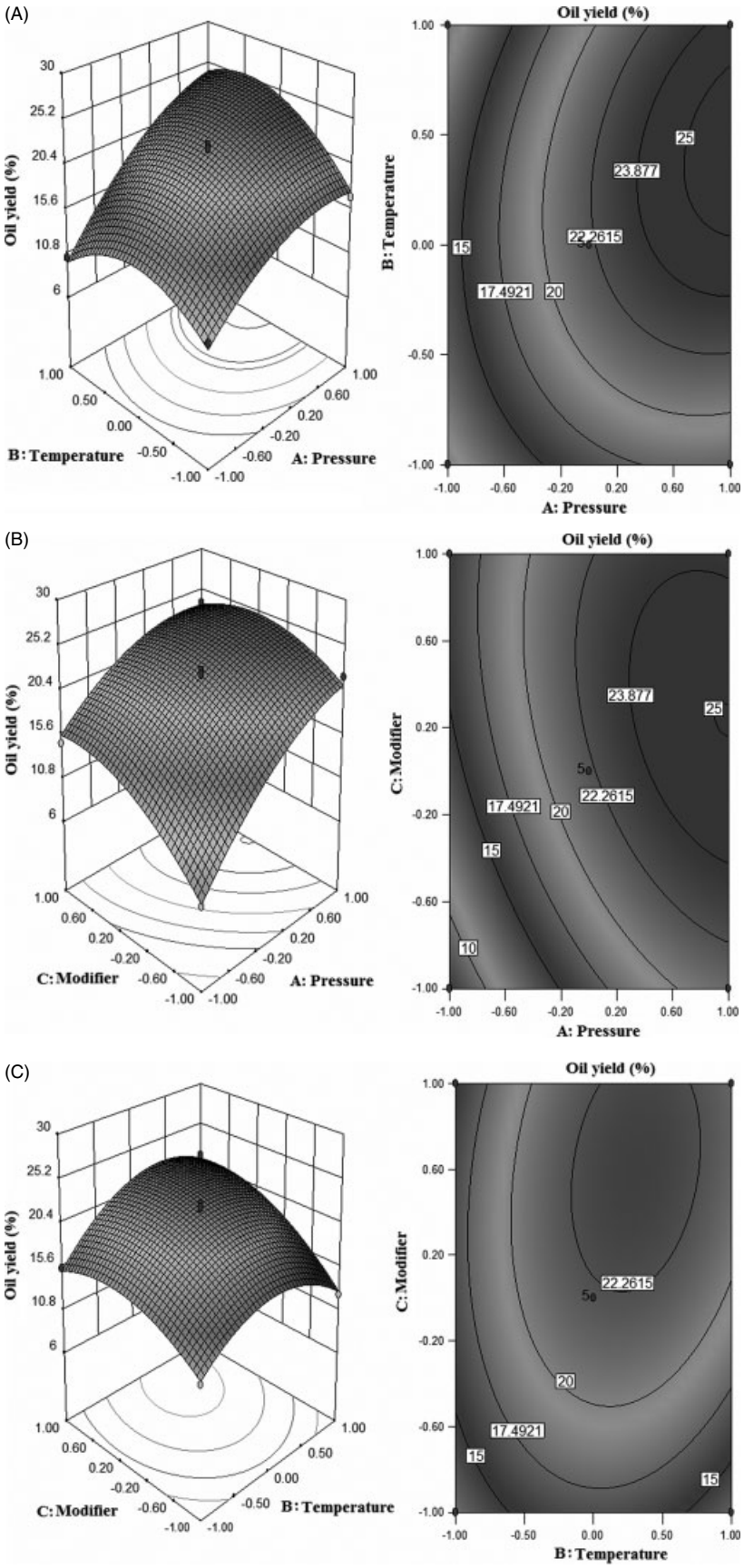

Figure 2. The 3D response surface and 2D contour plots of the oil recovery affected by extraction pressure, extraction temperature, and the amount of modifier.

Table 3. Estimated regression coefficients for the quadratic polynomial model and ANOVA for the experimental results

Regression coefficient	Estimated coefficients	Standard error	Degrees of freedom	Sum of squares	*F* value	Prob > *F*
β_0	22.02	0.35	1	485.48	88.02	<0.0001
Linear						
β_1	5.45	0.28	1	237.84	388.08	<0.0001
β_2	1.60	0.28	1	20.54	33.52	0.0007
β_3	2.75	0.28	1	60.50	98.72	<0.0001
Interaction						
β_{12}	1.78	0.39	1	12.67	20.68	0.0026
β_{13}	−1.53	0.39	1	9.30	15.18	0.0059
β_{23}	1.38	0.39	1	7.56	12.34	0.0098
Quadratic						
β_{11}	−2.59	0.38	1	28.33	46.23	0.0003
β_{22}	−3.94	0.38	1	65.50	106.87	<0.0001
β_{33}	−2.65	0.38	1	29.55	48.21	0.0002
Lack of fit	–	–	3	3.47	5.65	0.064
Pure error	–	–	4	0.82	–	–
R^2	0.9912	–	Adjusted R^2	0.9800	–	–

A small amount of a liquid modifier can enhance significantly the extraction efficiency of polar compounds, and counterbalanced the hydrogen bonds and ionic forces between the membrane-associated lipids and proteins allowing the lipids to be available for extraction by the supercritical CO_2.[25,26] In this study, ethanol was used as modifier.[27,28] With a fixed extraction temperature of 40 °C, the interaction relationship between the extraction pressure and amount of modifier is shown in Fig. 2B. At a given amount of modifier, the oil yield increased rapidly with increasing extraction pressure. With a given pressure, oil yields increased with increasing amount of modifier and reached a maximum value, and with further increasing amount of modifier, no obvious increase in oil yield was observed. As can be seen from Table 3, the complex interaction between extraction pressure and modifier had a negative effect on the oil yield.

With a fixed extraction pressure of 22.5 MPa, the interaction relationship between the extraction temperature and amount of modifier is shown in Fig. 2C. At a given amount of modifier, the oil yield increased rapidly with increasing temperature, and reached the highest value, followed by a decline with its further increase. The temperature and modifier exhibited a negative quadratic effect, while the complex interaction between them had a positive effect on the oil yield (see Table 3).

Calculating the regression equation gave the following results: $X_1 = 0.70$, $X_2 = 0.62$, $X_3 = 0.144$. The optimal values of the variables given by the software were as follows: extraction pressure, 27.72 MPa; extraction temperature, 46.22 °C; and the added amount of modifier, 8.58 vol.%. Under these conditions proposed, the model gave the predicted values of *Y* being 25%.

To compare the predicted result with the practical value, experimental validation was performed using the optimised conditions and the mean oil yield was 25.31 ± 0.22% (w/w). The value was close to the theoretical predicted values, indicating that the experimental design model may better reflect the extraction parameters of CSASO.

Effect of supercritical CO_2 cell wall breakage pretreatment

Figure 3 shows the variations of oil yield with the variations of extraction time (square symbol represents seed powder with cell wall breakage pretreatment; the triangle symbol represents seed powder without pretreatment). In order to evaluate the effect of cell wall breakage pretreatment on the oil yield, supercritical CO_2 was applied for oil extraction from Chinese star anise seed powder without pretreatment at the optimum extraction conditions above, and a low oil yield of 20.04 ± 0.61% (w/w) was obtained. As is described by Fig. 3, cell wall breakage pretreatment can significantly speed up the extraction; the oil yield rapidly achieved the maximum value at 90 min (the curve of square symbol), and without this pretreatment it took more than 150 min to reach this maximum value (the curve of triangle symbol). It is possible that the breakage of the seed cell wall could facilitate the removal of the cell contents and increase the available surface area between supercritical CO_2 and the cells.

Fatty acids composition analysis

Derivative identification with APCI-MS

The ionisation and fragmentation of the isolated the BCETS–fatty acid derivatives were studied by mass spectrometry (MS) with an APCI ion source in positive-ion detection mode. As expected, the BCETS–fatty acid derivative produced an intense molecular ion peak at *m/z* $[M+H]^+$. The collision-induced dissociation spectra of *m/z* $[M+H]^+$ produced the specific fragment ions at *m/z* 310.7 $[M'+CH_2CH_2]^+$ and *m/z* 261.4 and *m/z* 243.5 (where M′ is the molecular mass of the fatty acids). The specific fragment ion *m/z* 261.4 was from the molecular core structure. The selected reaction monitoring, based on the *m/z* $[M+H]^+ \rightarrow$ *m/z* $[M'+CH_2CH_2]^+$ and *m/z* 261.4 transition, was specific for fatty acid derivatives. The cleavage mode and MS/MS analysis for the C18 derivative are shown in Fig. 1B and C. MS data of BCETS–fatty acid derivatives, which are detected in samples, are presented in Table 4.

Reproducibility, calibration and detection limits

Reproducibility, calibration and detection limits are presented in Table 2. Method repeatability was investigated by the analysis of a standard containing 50 pmol FA derivatives. The relative standard deviations (RSD) of retention times and peak areas were from 0.04% to 0.25%, and 0.11% to 2.96%, respectively. The calibration

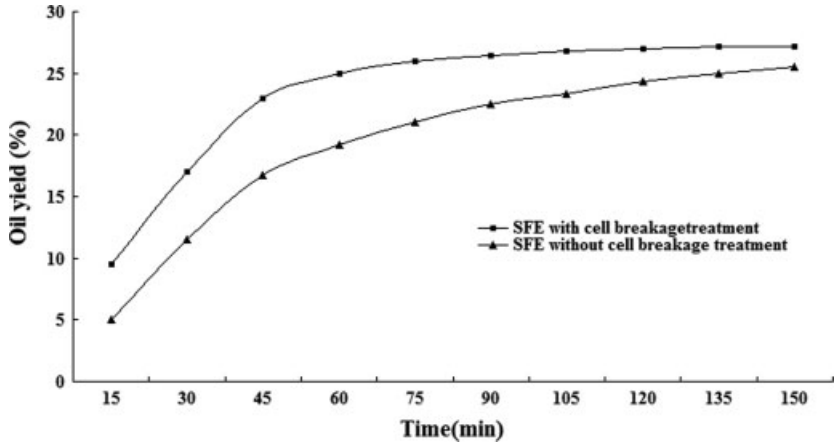

Figure 3. Comparison of the oil yields of Chinese star anise seed powder with cell wall breakage pretreatment and without (the squares represent seed powder with pretreatment; and the triangles represent seed powder without pretreatment).

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

Fatty acid	Molecular weight	$[M+H]^+$	Specific MS/MS data	TFA[1] % (μg mL^{-1})[a]	TFA[2] % (μg mL^{-1})[a]	FFA[3] % (μg mL^{-1})[a]
C9	401	402.3	261.4, 185.4	0.16 (15.13)	0.12 (12.03)	0.31 (1.46)
C10	415	416.3	261.4, 199.5	0.09 (8.66)	0.08 (8.06)	0.05 (0.22)
C18 : 3	521	521.9	261.4, 304.9, 503.9	0.46 (44.15)	0.46 (45.78)	0.34 (1.61)
C20 : 4	547	547.9	261.4, 330.9, 529.9	0.19 (17.80)	0.17 (16.52)	0.00 (0.00)
C18 : 2	523	523.9	261.4, 306.7, 516.0	47.15 (4518.20)	45.10 (4473.24)	46.94 (223.45)
C16	499	500.2	261.4, 283.5	20.38 (1953.09)	21.08 (2091.09)	24.99 (118.95)
C18 : 1	525	525.8	261.4, 309.0, 507.7	26.33 (2522.81)	25.77 (2555.61)	22.67 (107.90)
C18	527	528.3	261.4, 311.3	4.81 (461.00)	6.65 (659.55)	4.30 (20.48)
C20	555	556.3	261.4, 339.4	0.44 (42.30)	0.57 (56.67)	0.42 (1.99)
Total SFA	–	–	–	26 (2480.17)	29 (2827.39)	31 (143.10)
Total UFA	–	–	–	74 (7102.97)	71 (7091.15)	69 (332.96)

[1] Total fatty acids in CSASO extracted under the optimum conditions without modifier.
[2] Total fatty acids in CSASO extracted under the optimum conditions.
[3] Free fatty acids in CSASO extracted under the optimum conditions.
[a] a: mass percent (%, ratio of the mass of a fatty acid with that of all fatty acids), absolute content (μg fatty acid mL^{-1} oil).

graph was established with the peak area (*y* axis) versus the fatty acid concentration (*x* axis: pmol,), and all of the fatty acids provided excellent linear responses, with correlation coefficients >0.9994. For the 1.0 pmol injections, the calculated detection limits (at a signal-to-noise of 3 : 1) of all of the derivatised fatty acids ranged from 10.79 to 34.19 fmol.

Analysis of fatty acids in CSASO

The total fatty acids (TFA) and free fatty acids (FFA) from CSASO extracted under the optimum conditions were determined and quantified by HPLC/APCI/MS. The representative chromatograms of fatty acid standards and total fatty acids from CSASO are presented in Fig. 4 (chromatograms of other samples not shown). The result in Table 4 indicated that the seed oil extracted mainly contained C16, C18, C18 : 1, C18 : 2, C18 : 3 and C20 : 4 fatty acid. Unsaturated fatty acids (UFA) accounted for 71% at a concentration order of C18 : 2 > C18 : 1 > C18 : 3 > C20 : 4. In order to investigate the effect of modifier on the content of fatty acids, the composition of fatty acids in CSASO obtained under the optimum conditions without modifier was determined and the result is also presented in Table 4. As is shown in Table 4, SFE without modifier yielded 9583 μg mL^{-1} of TFA; when ethanol was added as modifier, 9918 μg mL^{-1} was obtained. The result indicated that modifier in SFE has great impact on the content of fatty acids. FFA was also determined and the composition was basically consistent with the total fatty acids (Table 4), but the C20 : 4 fatty acid was not detected in FFA.

In this study, the fatty acid content in the sample was determined by pre-column derivatisation (using BCETS as the labelling reagent) using HPLC/APCI/MS by fluorescence detection. This method proved to be sensitive and quantitative for analysing the fatty acids.

CONCLUSION

In this work, the SFE parameters for CSASO were optimised using BBD from RSM, and under the optimum conditions, the experimental yield (25.31 ± 0.22%, w/w) was well matched with the predicted yield (25.00%, w/w). The effect of cell wall breakage pretreatment by supercritical CO_2 rapid depressurisation was significant, which could greatly reduce the extraction time and increase the oil yield. Simultaneous determination of fatty acids in CSASO using BCETS as labelling reagent with HPLC fluorescence detection and online MS identification has been successfully achieved. The established method can be applied to the extraction

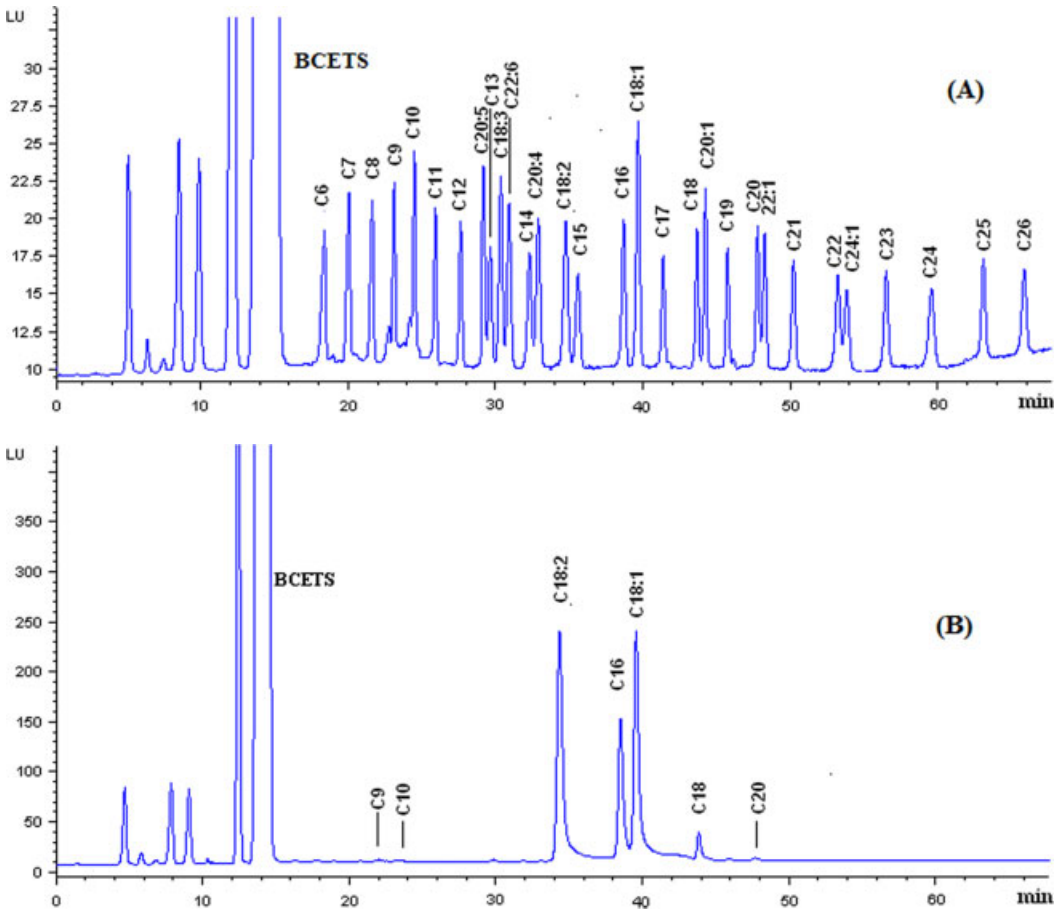

Figure 4. Chromatogram of fatty acids standards derivative (A) and fatty acids derivative in extracted oil (B). Chromatographic conditions: column temperature at 30 °C; excitation wavelength, λ_{ex}, 279 nm, emission wavelength, λ_{em}, 380 nm; Eclipse XDB-C_8 column (4.6 × 150 mm, 5 µm); flow rate, 1.0 mL min^{-1}. Peak labels: 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).

and determination of fatty acids from various food, drugs, plants and biochemistry samples.

ACKNOWLEDGEMENT

This work was supported by the National Science Foundation of China (No. 20075016) and supported by the 100 Talents Programme of The Chinese Academy of Sciences (No. 328).

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