LWT - Food Science and Technology 44 (2011) 1172–1178

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Supercritical CO_2 cell breaking extraction of *Lycium barbarum* seed oil and determination of its chemical composition by HPLC/APCI/MS and antioxidant activity

Li Guoliang [a,b], Shi Junyou [a,b,1], Suo Yourui [a], Sun Zhiwei [a,b], Xia Lian [a,b], Zheng Jie [a,b], You Jinmao [a], Liu Yongjun [a,*]

[a] Northwest Plateau Institute of Biology, Chinese Academy of Sciences, Xining 810001, PR China
[b] Graduate School of the Chinese Academy of Sciences, Beijing 100039, PR China

ARTICLE INFO

Article history:
Received 27 September 2009
Received in revised form
8 October 2010
Accepted 13 October 2010

Keywords:
Lycium barbarum
Supercritical CO_2
Seed oil
Fatty acids
Antioxidant activity

ABSTRACT

The extraction parameters for oil extraction from *Lycium barbarum* seed including extraction pressure, temperature and time were optimized using an orthogonal test design. The optimum conditions for supercritical CO_2 extraction were as follows: extraction pressure, 30 MPa; extraction temperature, 45 °C; dynamic extraction time, 60 min; CO_2 flow, 25 kg/h. The oil yield under the conditions proposed was 19.28 g/100 g. The effect of cell wall breakage pretreatment was investigated by supercritical CO_2 rapid depressurization, and results indicated this pretreatment could result in a rapid and efficient extraction. A sensitive fluorescent reagent 2-(11H-benzo[a]carbazol-11-yl) ethyl 4-methylbenzenesulfonate (BCETS) was utilized as pre-column labeling regent to determine fatty acids (FA) from *Lycium barbarum* seed oils obtained by different extraction methods. The main FA were: C18:2, C18:1, C16, C20:6, C18:3, and C20. The oil from *L. barbarum* seed exhibited excellent antioxidant activity in 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and β-carotene bleaching test, and its antioxidant activity compared well with the references ascorbic acid and α- tocopherol.

1. Introduction

Lycium barbarum (*L. barbarum*) belongs to the plant family Solanaceae (Li, 2007). It distributes from the southeast of Europe to China and is a well-known herb in the East, which is also widely used as a popular functional food (Gao, Ali, & Khan, 2008; Li, Ma, & Liu, 2007). The use of this herbal substance was first described in the first century AD in Chinese literature. There are many ways that people consume this fruit for example: drinking juice, eating raw and/or smoothies, mixed with tea, and added to trail mix, cereals, muffins, energy bars or soups.

L. barbarum seed is the by-product in the processing, and accounts for 4% of the total mass. Traditionally, *L. barbarum* seed oil is obtained with both mechanical and chemical separation processes. Mechanical separation processes are often associated with low yields and chemical separation process such as extraction methods in the majority of cases employ solvents such as hexane, which are dangerous to handle and quite harmful to human health and environment.

Supercritical fluid CO_2 extraction (SFE) is becoming one of the promising foods processing technologies. It offers numerous potential advantages over conventional extraction processes including: non-toxic, non-explosive, environmental friendly, cost effective, reduced organic solvent volume, time saving and more selective extractions (Araújo & Sandi, 2007).

Most fatty acids show neither natural absorption in the visible or UV regions nor fluorescence; thus, the detection of them at trace levels using absorptiometry is fairly difficult. Therefore, derivatization of these analytes with labeling reagents has been widely adopted, since HPLC with UV or fluorescence detection has a higher sensitivity. In contrast with GC, use of HPLC allows fatty acids to be 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 fewer polar compounds which can be more easily analyzed by LC (Chen, Chen, & Lien, 1999; Rosenfeld, 2002; You, Zhang, & Zhang, 2001). In this study, a sensitive fluorescent reagent 2-(11H-benzo[a]carbazol-11-yl) ethyl 4-methylbenzenesulfonate (BCETS) synthesized in our laboratory was successfully applied as a pre-column labeling regent to determine fatty acids from *L. barbarum* seed oils.

* Corresponding author. Tel.: +86 531 88365576.
E-mail address: yongjunliu_1@sdu.edu.cn (L. Yongjun).
[1] This author contributed equally to this work.

0023-6438/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.lwt.2010.10.012

Up to now, research on supercritical CO_2 extraction of *L. barbarum* seed oil as well as its composition and antioxidant activity has never been reported. The aims of the present work were to (1) develop a fast, simple, and high-efficiency supercritical technique for extracting oil from *L. barbarum* seed; (2) establish an HPLC analysis procedure for fatty acids from *L. barbarum* seed oils using BCETS as a labeling regent; (3) evaluate the antioxidant activity of *L. barbarum* seed oils extracted by different methods.

2. Materials and methods

2.1. Materials

L. barbarum was collected from Qaidam Basin (elevation 2800 m), Qinghai Province, China in September 2008. The seeds were dried under a stream of nitrogen and broken into powder. The chemicals used were of analytical reagent grade that include DPPH (90% purity), β-carotene (Type I synthetic, 95%), α-tocopherol and all fatty acid standards were from Sigma Reagent Co. (USA). CO_2 (99.99% purity) was obtained from Fushan Co. (Yantai, China), 2-(11*H*-benzo[a]carbazol-11-yl) ethyl 4-methylbenzenesulfonate (BCETS) was synthesized in our laboratory. All other chemicals used in the experiments were of analytical grade.

2.2. Instrumentation

Supercritical extraction assays were conducted in a semi-bath flow extraction apparatus (Hua'an Supercritical Fluid Extraction Corp., Nantong, China). The capacity of the extractor was 2000 ml and a maximum flow rate of CO_2 was 50 kg/h. Fatty acids analysis was performed using Agilent HP 1100 Series high-performance liquid chromatography and mass spectrometry (LC-MSD Trap SL, a complete LC-MS-MS, Agilent, USA). The mass spectrometer 1100 Series LC-MSD Trap-SL (ion trap) from Bruker Daltonik (Bremen, Germany) was equipped with an atmospheric pressure chemical ionization source. Derivatives were separated on Eclipse XDB-C_8 column (4.6 × 150 mm, 5 μm). Fluorescence excitation and emission spectra were obtained on a 650-10S fluorescence spectrophotometer (Hitachi, Japan). The mobile phase was filtered through a 0.2-μm nylon membrane filter (Alltech, Deerfiled, IL, USA).

2.3. Supercritical CO_2 cell breaking extraction

Extraction assays were conducted in a semi-bath flow extraction apparatus, and the schematic flow diagram was described in detail in a previous study (Koga, Iwai, Hata, Yamamoto, & Arai, 1996). Supercritical CO_2 cell breakage treatment: in all experiments, 400 g samples of prepared *L. barbarum* seeds were placed in the extractor cylinder and after an initial air purge, liquefied CO_2 was pumped into the vessel and consequently the pressure was raised to 40 MP for 10 min. At the end of each treatment, the pressure was quickly released within 1 min (Xu, Sun, Dong, & Zhang, 2009).

The samples after cell breakage were for the consecutive SFE. The oil was collected every 15 min from the separator. Successive collected samples were weighed and analyzed. In some extractions, ethanol and acetone were added as modifiers, and the concentration was up to 10 mol % (Koga et al., 1996). The modifier was pumped into the system from the modifier bottle after the selected pressure had been reached.

2.4. Organic solvent extraction

The organic solvents used to carry out several extractions were n-hexane, petroleum ether and a mixture of chloroform, methanol and water (Bligh & Dyer, 1959). Twenty five grams of prepared *L. barbarum* seeds were macerated with 300 ml organic solvent at 45 °C for 4 h. Thereafter, the process was repeated but for 20 h at ambient temperature. The resulting extracts were combined; the solvent was removed in vacuo (40 °C); the remainder was weighed and analyzed. All extractions were carried out in duplicate.

2.5. Fatty acids analysis

2.5.1. Derivatization of fatty acids

10 mg K_2CO_3 and 200 μl DMF was added to 100 μl of a standard FA mixture in a 1 mL vial. 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 at 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 derivatized solution (10 μl) was injected directly onto the chromatograph. The typical derivatization scheme of C18 fatty acids with BCETS is shown in Fig. 1 A.

2.5.2. Separation fatty acid derivatives with HPLC

HPLC separation of BCETS derivatives was conducted on an Eclipse XDB-C8 column with a gradient elution. Eluent A was water, B was a mixed solvent of ACN/DMF (1:1.v/v), and C was acetonitrile (100%). The percentage of the mobile phase was changed after injection as follows: 45–10% (A), 50–80% (B) from 0 to 30 min; 10–3% (A), 80–87% (B) from 30 to 40 min; 3–2% (A), 87–88% (B) from 40 to 50 min; and 2–0% (A), 88–85% (B) from 50 to 70 min. The flow rate was maintained at 1.0 mL/min and the column temperature was set at 30 °C. Maximum fluorescence responses of derivatives were achieved at the excitation wavelength of 333 nm and emission wavelength of 390 nm. Under the chromatographic conditions above, all FA standard derivatives achieved a complete baseline resolution within the shortest time.

2.6. Determination of antioxidant activity

2.6.1. DPPH radical scavenging assay

The scavenging activity of seed oil towards DPPH radical was measured by the method of Amarowicz, Pegg, Rahimi-Moghaddam, Barl, and Weil (2004), with slight modification. In brief, 0.1 mL of seed oil was added with 2.4 mL of 0.0004% DPPH in ethanol. Then, the mixture was shaken vigorously and left in darkness for 60 min. Finally, the absorbance of the mixture was measured at 515 nm. Ascorbic acid (4 mg/mL) was used as a synthetic reference. Anti-radical activities of samples were calculated from the following equation:

$$\text{Inhibition percentage}\ (\%) = \left(\left[A_{\text{control}} - A_{\text{sample}}\right] / A_{\text{control}}\right) \times 100$$

Where A_{control} and A_{sample} are the absorbance values of the blank and of the tested samples.

Extracting concentration providing 50% inhibition (IC_{50}) was calculated form the plot of inhibition percentage against extract concentration.

2.6.2. β- carotene - linoleic acid assay

The β-carotene bleaching test was conducted as described by Taga, Miller, and Pratt (1984), with minor modifications. 200 μL of β-carotene (10 mg/10 ml in chloroform) was added to linoleic acid (20 mg) and Tween 80 (200 mg). The chloroform was evaporated, followed by addition of distilled water (50 ml) to prepare the β-carotene/linoleic acid emulsion. The emulsion (5 ml) was added to a tube containing 120 μL of seed oil and the absorbance was

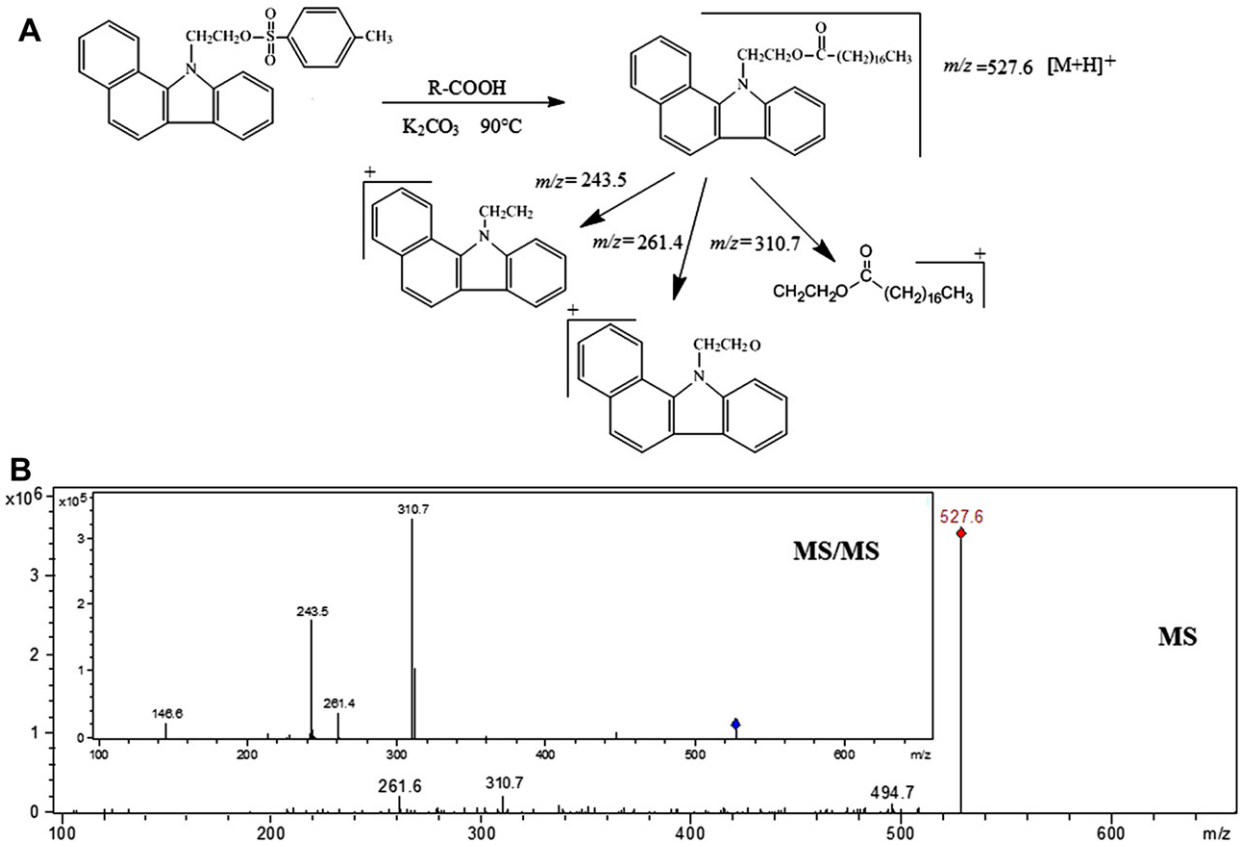

Fig. 1. Scheme of derivatization reaction and MS spectra of representative BCETS-labeled C18 derivative: (A) derivatization reaction of C18 fatty acid with BCETS and the cleavage mode of protonated molecular, (B) MS data for C18 derivative. Scanning range from 100 to 1000 amu under APCI positive-ion mode.

immediately measured at 470 nm against a blank, consisting of an emulsion without β-carotene. The tubes were placed in a water bath at 50 °C and the oxidation of the emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm over a 60 min period. Control samples contained 120 μL of water instead of seed oil. The antioxidant activity was expressed using the following equation: AA (%) = 100(DRc−DRs)/DRc.

Where AA (%) antioxidant activity; DR_C degradation rate of the control [ln(a/b)/60]; DR_S degradation rate in the presence of the sample = [ln(a/b)/60], where a absorbance at zero time; b absorbance at 60 min. Antioxidant activity in this study was expressed in mg α- tocopherol equivalents (T_{eq})/g oil.

3. Results and discussion

3.1. Optimization of supercritical CO_2 extraction conditions

L. barbarum seed powders for seed oil extraction were processed by supercritical CO_2 cell wall breakage pretreatment. In fact, the fluid pressures and temperatures, the extraction time are generally considered as the most important factors. According to preliminary experiments, CO_2 flow rate was kept constant at 25 kg/h, and the optimization of the experimental parameters were carried out step-by-step or by using a three-level orthogonal array design with an OA9 (3^3) matrix. The results were summarized in Table 1. The oil yields of Run 1 to Run 9 were ranged from 7.94 to 18.86%. According to the R values presented in Table 1, the most significant parameter on extraction yield was extraction pressure, followed by extraction temperature and extraction time. The mean values of the extraction yields for the corresponding factors at each level were calculated according to the assignments of the experiment. The mean values of three levels of each factor revealed how the extraction yield would change when the level of that factor was changed. This tend could be expressed intuitively by the Fig. 2. As it can be seen in Fig. 2, oil yields were increased with the increasing pressure. Because raising the extraction pressure, at constant temperature, leads to a higher fluid density, which increases the solubility of the analytes. Even so, considering the safety factor, the loss of machine and energy consumption, 30 MPa was chosen as the optimum

Table 1
Results of the orthogonal test design for the extraction of seed oil from *L. barbarum*.

Run	Pressure(MPa)	Time(min)	Temperature(°C)	Yields of oil (g/100 g seed)
1	20	20	40	7.94
2	25	40	40	13.43
3	30	60	40	18.86
4	25	60	45	17.11
5	30	20	45	17.66
6	20	40	45	15.52
7	30	40	50	18.56
8	20	60	50	15.59
9	25	20	50	12.02
K1	39.03[a]	37.62	40.11	
K2	42.56	47.51	50.29	
K3	54.96	51.43	46.16	
k1	13.01[b]	12.54	13.37	
k2	14.19	15.83	16.76	
k3	18.32	17.14	15.39	
R	5.31[c]	4.60	3.39	

[a] K_i^A = Σthe extraction yield of seed oil at A_i
[b] $k_i^A = K_i^A/3$
[c] $R_i^A = \max\{k_i^A\} - \min\{k_i^A\}$

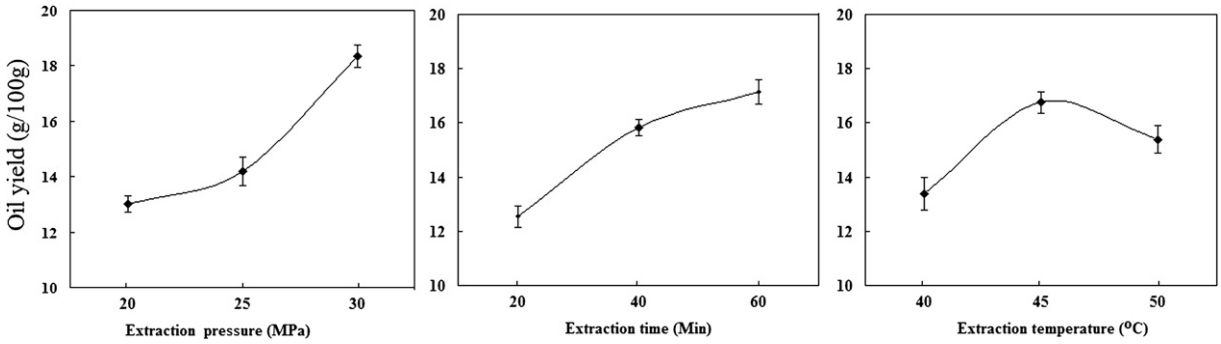

Fig. 2. Effect of extraction pressure, dynamic extraction time and temperature on the oil yields.

pressure. The extraction yields first increased, and reached a maximum extraction yield at 45 °C and then decreased at higher temperatures. This is due to that the variation of temperature affects the density and the solubility of the analytes from the matrix. By increasing the temperature, the volatilities of the analytes could be increased but the supercritical CO_2 density decreases. Thus, 45 °C was the optimum temperature. Our results are consistent with other reported studies (Cao & Ito, 2003; Papamichail, Louli, & Magoulas, 2000; Salgin, 2007; Araus, Uquiche, & del Valle, 2009). In the preliminary experiments, we found that the yield increased markedly and reached the maximum at 60 min, and the extraction yield increased slowly and tended to saturated (60–120 min).Combining with the result of orthogonal test, 60 min should be employed.

Finally, the optimum extraction conditions was obtained, they were as follows: extraction pressure, 30 MPa; extraction temperature, 45 °C; dynamic extraction time, 60 min; and CO_2 flow, 25 kg/h. Oil extractions were carried out under the optimum conditions, the average oil yield of three times was 19.28 g/100 g ($n = 3$).

3.2. Effect of supercritical CO_2 cell wall breakage pretreatment

The cell wall breakage pretreatment of *L. barbarum* seed power was investigated by supercritical CO_2 rapid depressurization. In order to investigate the effect of this pretreatment on the oil yield, supercritical CO_2 was applied to oil extraction from *L. barbarum* seed powder without pretreatment at the optimum extraction conditions above, and a lower oil yield of 15.4 g/100 g (n 3) was obtained.

Fig. 3 shows that oil yields change with the variations of extraction time (square symbol represents seed powder without cell wall breakage pretreatment; triangle symbol represents seed powder with pretreatment). As is described in Fig. 3, supercritical CO_2 cell wall breakage pretreatment can significantly speed up the extraction; the oil yield rapidly achieved the maximum value at 60 min (the curve of triangle symbol), and without this pretreatment it would take more than 150 min to reach this maximum value (the curve of square symbol). This result revealed that supercritical CO_2 cell breakage pretreatment could significantly reduce the extraction time; and an efficient extraction could be achieved in 60 min.

3.3. Fatty acids composition analysis

3.3.1. Derivative identification with MS/APCI

The ionization and fragmentation of the BCETS-fatty acid derivatives were studied by mass spectrometry (MS) with APCI in positive-ion detection mode. All FA derivatives produced intense molecular ion peaks at $[M\,H]^+$ ions, which should be attributed to the introduction of an alkalescent nitrogen atom in BCETS molecular core. The collision-induced dissociation spectra (MS/MS) of molecular ions (MS, $[M\,H]^+$ ion) produced intense and stable fragment ions at m/z 243.5 and m/z 261.4. The selected reaction monitoring, which was based on the $[M\,H]^+$ ion→ m/z 243.5 and m/z 261.4 transition, was specific for BCETS-labeled fatty acid derivatives. The cleavage mode and the MS, MS/MS spectra for C18 fatty acid derivatives were shown in Fig. 1 A and B.

3.3.2. Reproducibility, calibration, and detection limits

A standard solution of fatty acids (3.0×10^{-6} mol/L) was prepared and reproducibility of the method was examined, after which 50 pmol of each fatty acid were injected six times repeatedly and the resulting retention times and peak areas were recorded. The relative standard deviations (RSDs) were found to range from 0.04 to 0.51% and 0.15–2.68% for retention time and peak area, respectively. The calibration graph was established with the peak area (y axis) versus the fatty acid concentration (x axis: pmol, injected amount), and all fatty acids provided excellent linear responses, with correlation coefficients greater than 0.9991. For the 1.0 pmol injections, the calculated detection limits (at signal-to-noise of 3:1) of all of the derivatized fatty acids ranged from 4.08 to 6.74 nmol/L.

3.3.3. Analysis of fatty acids composition in samples

The compositions of the oil samples extracted by supercritical CO_2, supercritical CO_2 with ethanol (CO_2 ethanol), supercritical CO_2 with acetone (CO_2 acetone), Bligh and Dyer method, n-hexane and mineral ether, were analyzed by the established method above. The

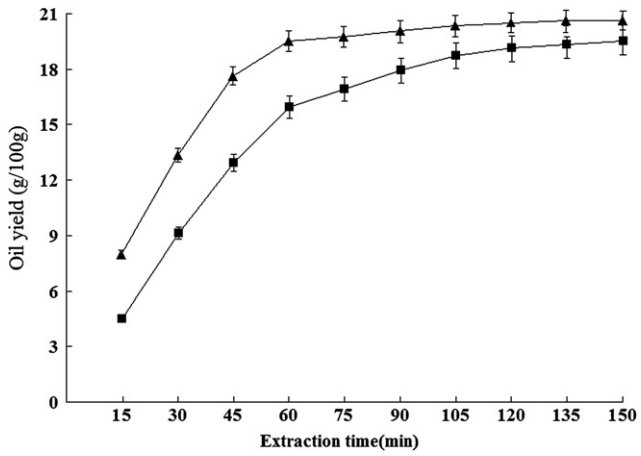

Fig. 3. Comparison of the oil yields of *L. barbarum* seed powder with cell wall breakage pretreatment and without this pretreatment (triangle symbol represents seed powder with pretreatment; square symbol represents seed powder without pretreatment).

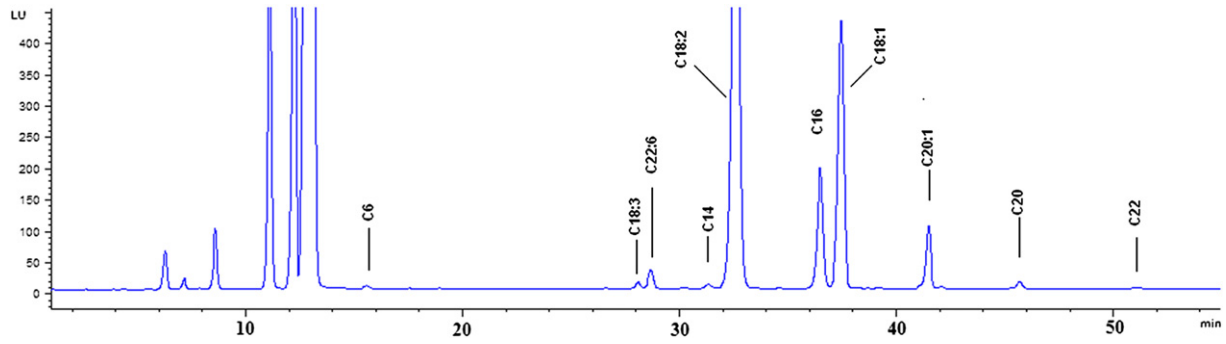

Fig. 4. Chromatograms of FA derivative in *L. barbarum* seed oil. Chromatographic conditions: Column temperature at 30 °C; excitation wavelength λex 279 nm, emission wavelength λ em 380 nm; Eclipse XDB-C_8 column (4.6 × 150 mm, 5 m); flowrate 1.0 mLmin^{-1}; Peak labels: C6 (hexanoic acid); C18:3 (8,11,14-octadecatrienoic acid); C22:6 (2,5,8,11,14,17-docosahexaenoic acid); C14 (myristic acid); C18: 2 (9,12-octadecadienoic acid); C16 (hexadecanoic acid); C18: 1 (12-octadecenoic acid); C20:1 (11-eicosenoic acid); C20(eicosoic acid); C22 (docosanoic acid).

peaks were identified by chromatographic retention time and on-line MS identification. The typical chromatogram for FA derivative in sample is presented in Fig. 4. The FA contents in oil samples are shown in Table 2. Results showed that total FA content ranged from 650.4 to 661.53 mg/mL. Unsaturated FA accounted for 84.21–87.91% and their contents were 554.67–579.32 mg/mL. C18:2 presented the highest concentrations (410.94–439.82 mg/mL), followed by C18:1 (99.63–107.29 mg/mL), C16 (45.03–47.46 mg/mL) and C18 (28.30–32.92 mg/mL). Meanwhile, C21 were present at the lowest concentration in all FA (0–0.05 mg/mL).

Results in Table 2 revealed that extraction method could affect the FA composition of the oil sample. Among these extracting methods, Bligh and Dyer method yielded the highest FA content (661.53 mg/mL), which compared with CO_2 method with significant differences (P 0.05). The next extracting methods in order of FA content were mineral ether method (659.72 mg/mL), CO_2 acetone (658.67 mg/mL), CO_2 ethanol (657.86 mg/mL), n-hexane (652.20 mg/mL) and CO_2 (650.04 mg/mL).

3.4. The antioxidant activity of seed oil

In order to evaluate the effect of extraction method on the antioxidant activity of the oil samples, DPPH free radical scavenging activity test and β-carotene bleaching assay were employed to determinate the antioxidant activity of seed oils extracted by CO_2, CO_2 ethanol, CO_2 acetone, Bligh and Dyer method, n-hexane and mineral ether.

DPPH free radical scavenging activity test can serve as a rapid and reliable test in predicting oxidative stability of fatty food such as oils, margarines and meat products (Lee, Chung, Chang, & Lee, 2007). Fig. 5A shows DPPH radical scavenging activity of *L. barbarum* seed oils obtained by different extraction methods. The DPPH radical scavenging activity ranged from 65 ± 2.08 to 91.8 ± 0.77%. Oils extracted by CO_2 ethanol possessed the highest DPPH radical scavenging activity, which was close to the reference of ascorbic acid Fig. 5A, followed by CO_2 acetone (88.2%), CO_2 (84.5%), Bligh and Dyer method (84.0%) and n-hexane (77.2%). Meanwhile, the oils extracted by mineral ether exhibited the lowest DPPH radical scavenging activity in all methods. In this assay, *L. barbarum* seed oil presented remarkable DPPH radical scavenging activity, which suggested that it could have a role in preventing free-radical-mediated chain reactions.

The β-carotene bleaching assay is one of the helpful methods in the investigation of lipophilic antioxidants, and also very suitable in evaluating the antioxidant activity of edible oils (Kulisic, Radonic, Katalinic, & Milos, 2004). Fig. 5B shows the inhibition activity of *L. barbarum* seed oils in BCB assay. Results indicated that all samples displayed great potential of quenching linoleate free radicals (generated from linoleic acid peroxidation) and shielding the carotenoid from bleaching. The antioxidant activity of oil samples were ranged from 2.8 ± 0.04 to 3.3 ± 0.05 mg T_{eq}/g oil (Fig. 5 B). The

Table 2
The content of fatty acids (FA) in the oils from *L. barbarum* seed (n 3).

FA	Different extraction methods											
	CO_2		CO_2 acetone		CO_2 ethanol		mineral ether		Bligh and Dyer		n-hexane	
	mg/mL[a]	%[b]	mg/mL[a]	% [b]	mg/mL[a]	% [b]	mg/mL[a]	% [b]	mg/mL[a]	% [b]	mg/mL[a]	% [b]
C6	0.12	0.02	1.19	0.18	1.20	0.18	0	0	0	0	0	0
C18:3	7.54	1.16	9.97	1.51	10.00[c]	1.52	8.31	1.26	6.94	1.05	7.92	1.21
C20:6	24.38	3.75	28.67	4.35	28.78[c]	4.37	28.44[c]	4.31	25.26	3.82	27.01	4.14
C14	0.61	0.09	3.82	0.58	3.88	0.59	0.89	0.14	0.00	0.00	0.80	0.12
C18:2	437.86	67.36	410.94	62.39	417.36[c]	63.44	437.16	66.27	439.82	66.49	423.86	64.99
C16	46.29	7.12	46.70	7.09	47.46	7.21	46.59	7.06	45.03	6.81	46.66	7.15
C18:1	101.68	15.64	105.09	15.95	99.63	15.14	103.60	15.70	107.29	16.22	105.38	16.16
C18	28.80	4.43	32.92[c]	5.00	31.21	4.74	28.77	4.36	28.30	4.28	32.74[c]	5.02
C20	2.77	0.43	8.07[c]	1.22	7.64c	1.16	4.24[c]	0.64	6.60[c]	1.00	5.52[c]	0.85
C21	0	0	0.06	0.01	0.05	0.01	0	0	0	0	0	0
C22	0	0	11.25	0	10.66	0	1.71	0.26	2.28	0.34	2.30	0.35
UFA	571.46	87.91	554.67[c]	84.21	555.77[c]	84.48	577.51	87.54	579.32	87.57	564.17[c]	86.50
SFA	78.58	12.09	104.00[c]	15.79	102.09[c]	15.52	82.21	12.46	82.21	12.43	88.03[c]	13.50
TFA	650.04	100	658.67	100	657.86	100	659.72[c]	100	661.53[c]	100	652.20	100

UFA: unsaturated fatty acids; SFA: saturated fatty acids; TFA: total fatty acids.
[a] absolute content absolute content (mg/mL, mg (FA)/mL(oil)).
[b] relative percent (%, ratio of the content of one FA with that of all FA).
[c] compared with CO_2 method with significant differences ($P < 0.05$).

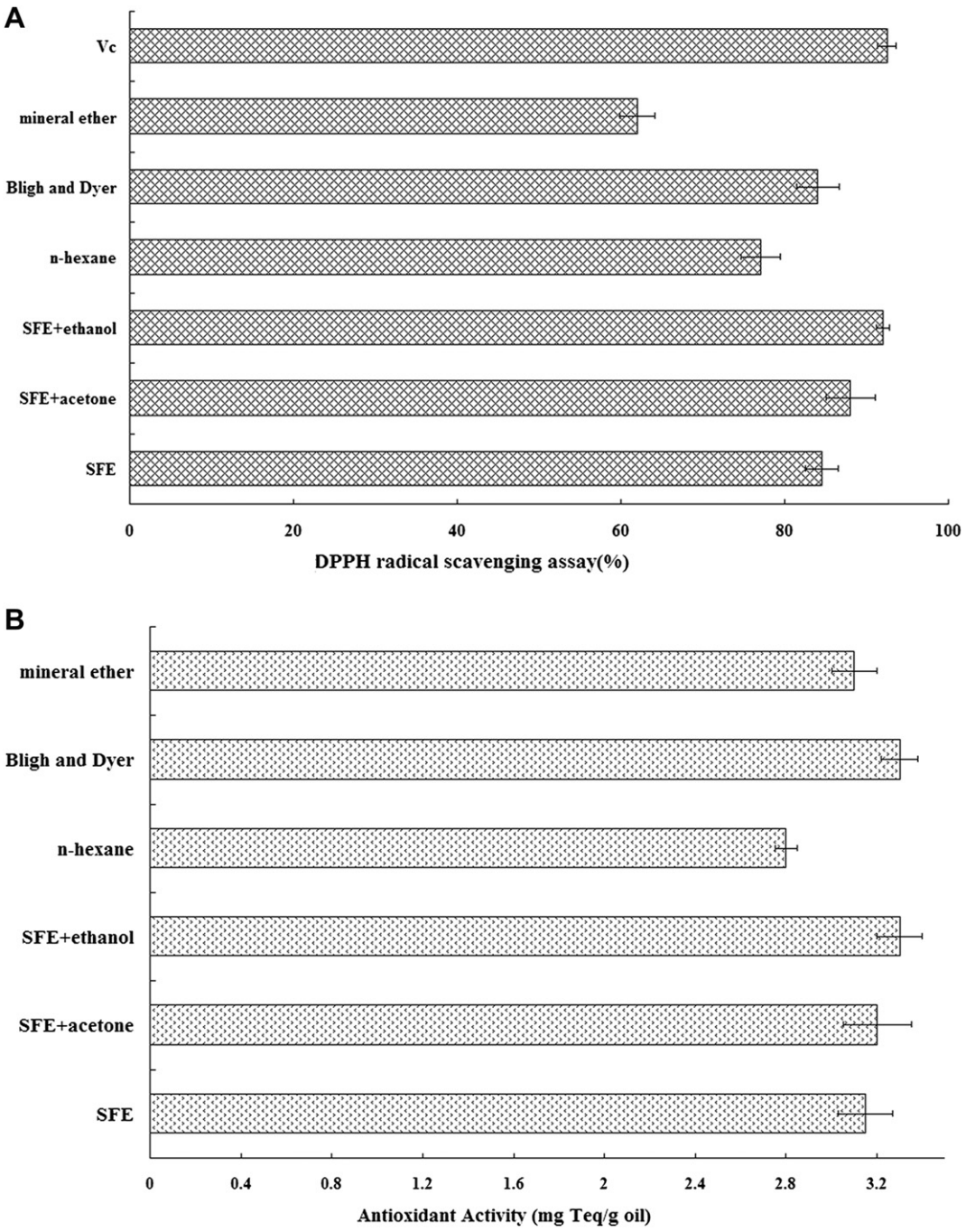

Fig. 5. The antioxidant activity of oil samples extracted by different extraction methods: DPPH radical scavenging assay (A) and β-carotene bleaching test (B).

oils extracted by CO_2 ethanol and n-hexane, respectively, represented samples with strongest and weakest antioxidant activity (P 0.05). The other samples in order of the antioxidant activity were the oils, respectively, extracted by Bligh and Dyer method (3.23 T_{eq}/g oil), CO_2 acetone (3.20 T_{eq}/g oil), CO_2 (3.15 T_{eq}/g oil) and mineral ether (3.10 T_{eq}/g oil) (Fig. 5B).

The antioxidant activities of commercial edible oils (Basso pure olive oil, Mazola soy bean oil, Mazola sunflower seed oil, Mazola corn oil, Mazola canola oil, Green love rice bran oil and Sri Murni palm olein oil) have been studied by the BCB assay, and were ranged from 0.15 to 1.85 mg Teq/g oil (Chan & Ismail, 2009). The antioxidant activity of *L. barbarum* seed oils in this study were ranged from 2.8 ± 0.04 to 3.3 ± 0.05 mg T_{eq}/g oil, which was higher than all of the commercial edible oils above.

L. barbarum seed oils exhibited excellent antioxidant activity in DPPH radical scavenging and β-carotene bleaching assay. We could conclude that the seed oils from *L. barbarum* may play potential role as a health-promoting agent with antioxidant activity in human diets, as well as providing valuable natural antioxidants for the pharmaceutical industry.

4. Conclusion

In this study, supercritical CO_2 oil extraction from *Lycium barbarum* seed oil has been optimized using an orthogonal test design. The supercritical CO_2 cell breakage pretreatment was investigated; results revealed this pretreatment could sharply reduce the extraction time and make the extraction more efficient. A developed

pre-column method for fatty acids analysis using BCETS as labeling regent was successfully applied to the fatty acids determination from oils extracted by different solvents. The results of DPPH radical scavenging assay and β-carotene bleaching test indicated *L. barbarum* seed oils possessed excellent antioxidant activity, which compared well with the references ascorbic acid and α- tocopherol.

Acknowledgements

This work was supported by the Program of "Hundreds Talent" of the Chinese Academy of Sciences (CAS).

References

Amarowicz, R., Pegg, R., Rahimi-Moghaddam, P., Barl, B., & Weil, J. (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chemistry, 84*, 551–562.

Araújo, J., & Sandi, D. (2007). Extraction of coffee diterpenes and coffee oil using supercritical carbon dioxide. *Food Chemistry, 101*, 1087–1094.

Araus, K., Uquiche, E., & del Valle, J. (2009). Matrix effects in supercritical CO2 extraction of essential oils from plant material. *Journal of Food Engineering, 92*, 438–447.

Bligh, E., & Dyer, W. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Physiology and Pharmacology, 37*, 911–917.

Cao, X., & Ito, Y. (2003). Supercritical fluid extraction of grape seed oil and subsequent separation of free fatty acids by high-speed counter-current chromatography. *Journal of Chromatography A, 1021*, 117–124.

Chan, K., & Ismail, M. (2009). Supercritical carbon dioxide fluid extraction of Hibiscus cannabinus L. seed oil: a potential solvent-free and high antioxidative edible oil. *Food Chemistry, 114*, 970–975.

Chen, S., Chen, K., & Lien, H. (1999). Determination of fatty acids in vegetable oil by reversed-phase liquid chromatography with fluorescence detection. *Journal of Chromatography A, 849*, 357–369.

Gao, Z., Ali, Z., & Khan, I. (2008). Glycerogalactolipids from the fruit of Lycium barbarum. *Phytochemistry, 69*, 2856–2861.

Koga, Y., Iwai, Y., Hata, Y., Yamamoto, M., & Arai, Y. (1996). Influence of cosolvent on solubilities of fatty acids and higher alcohols in supercritical carbon dioxide. *Fluid Phase Equilibria, 125*, 115–128.

Kulisic, T., Radonic, A., Katalinic, V., & Milos, M. (2004). Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chemistry, 85*, 633–640.

Lee, J., Chung, H., Chang, P., & Lee, J. (2007). Development of a method predicting the oxidative stability of edible oils using 2, 2-diphenyl-1-picrylhydrazyl (DPPH). *Food Chemistry, 103*, 662–669.

Li, X. (2007). Protective effect of Lycium barbarum polysaccharides on streptozotocin-induced oxidative stress in rats. *International Journal of Biological Macromolecules, 40*, 461–465.

Li, X., Ma, Y., & Liu, X. (2007). Effect of the Lycium barbarum polysaccharides on age-related oxidative stress in aged mice. *Journal of Ethnopharmacology, 111*, 504–511.

Papamichail, I., Louli, V., & Magoulas, K. (2000). Supercritical fluid extraction of celery seed oil. *The Journal of Supercritical Fluids, 18*, 213–226.

Rosenfeld, J. (2002). Application of analytical derivatizations to the quantitative and qualitative determination of fatty acids. *Analytica Chimica Acta, 465*, 93–100.

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.

Taga, M., Miller, E., & Pratt, D. (1984). Chia seeds as a source of natural lipid antioxidants. *Journal of the American Oil Chemists' Society, 61*, 928–931.

Xu, X., Sun, L., Dong, J., & Zhang, H. (2009). Breaking the cells of rape bee pollen and consecutive extraction of functional oil with supercritical carbon dioxide. *Innovative Food Science and Emerging Technologies, 10*, 42–46.

You, J., Zhang, W., & Zhang, Y. (2001). Simple derivatization method for sensitive determination of fatty acids with fluorescence detection by high-performance liquid chromatography using 9-(2-hydroxyethyl)-carbazole as derivatization reagent. *Analytica Chimica Acta, 436*, 163–172.