Food Anal. Methods (2013) 6:781–788
DOI 10.1007/s12161-012-9486-3

Optimization of Supercritical Fluid Extraction of Phenolics from Date Seeds and Characterization of its Antioxidant Activity

Hui Liu · Zhonggao Jiao · Jiechao Liu · Chunling Zhang · Xiaowei Zheng · Shaojuan Lai · Fusheng Chen · Hongshun Yang

Received: 17 June 2012 / Accepted: 24 July 2012 / Published online: 12 August 2012

Abstract Supercritical fluid extraction (SFE) was utilized for the first time to extract phenolics in date seeds. Orthogonal array design (OA_9 (3^4)) was applied to optimize extraction variables including extraction temperature, extraction pressure, extraction time, and number of extraction. Optimum values of extraction variables were analyzed and number of extractions was found to have a significant effect on total phenolics content (TPC). Optimal SFE conditions for maximum yield of TPC were 50 °C, 350 bar, and two repeated extractions, each for 2 h. Under optimal condition, the TPC was increased to 441.57 mg gallic acid equivalents/100 g fresh weight. Several phenolic compounds were detected in date seeds including chlorogenic acid, rutin, ellagic acid, quercetin, and two unidentified compounds of phenolic acids. In addition, phenolics of date seeds showed a higher antioxidant activity than ascorbic acid at the same concentration in the range of 1.0–8.0 mg/L.

Keywords Supercritical fluid extraction · Date seeds · Optimization · Total phenolics content · Antioxidant activity

H. Liu · Z. Jiao (✉) · J. Liu · C. Zhang · X. Zheng
Zhengzhou Fruit Research Institute,
Chinese Academy of Agricultural Sciences,
Gangwan Rd,
Zhengzhou, Henan 450009, People's Republic of China
e-mail: jiaozhonggao@caas.net.cn

S. Lai · F. Chen · H. Yang (✉)
College of Food Science and Technology,
Henan University of Technology,
140 S Songshan Rd,
Zhengzhou, Henan 450052, People's Republic of China
e-mail: hongshunyang@hotmail.com

Introduction

Dates are a popular kind of fruit among many nations around the world. The fruit is composed of a fleshy pericarp and seed which constitutes between 10 and 15 % of date fruit weight (Hussein et al. 1998). Chinese production of dates reached 147,600 tons in 2010 (Food and Agriculture Organization of the United Nations 2010); among which, approximately 15,000 tons were date seeds. Currently, date seeds are mainly discarded or just used as animal feedings, which is a huge waste. The non-use of this byproduct for human food is a real economic loss since it is rich in value-added components including bioactive constituents with antimicrobial, antioxidant, and other health-promoting activities (Hamada et al. 2002; Al-Farsi et al. 2007; Al-Farsi and Lee 2008; Soong and Barlow 2004). Therefore, better utilization of such byproduct is very important to maintain dates cultivation and to increase the source of phenolics and income as well as to lessen environmental burden.

Since a large quantity of date seeds is being produced as a waste material and the seeds contain a significant amount of bioactive phenolics, an effective method to prepare phenolics from date seeds will be beneficial to better utilize this byproduct as a phenolics provider. Extraction yield of phenolics is dependent on the solvent and method of extraction (Goli et al. 2005). In addition, a good extraction should be able to obtain a fair amount of phenolics and simultaneously minimize the oxidation, degradation, and polymerization of the desired products (Zuo et al. 2002). To the current date, most extractions were based on solvent, and usually required several hours or even days of extraction and a huge volume of solvent. Apart from difficulties in the extraction process, the solvent used was usually methanol, acetone, or other hazardous chemicals (Al-Farsi and Lee 2008) which are not only toxic to the products but may result in degradation of the thermolabile components during solute/solvent separation (Liza et al. 2010).

Recently, supercritical fluid extraction (SFE) has been widely applied in food, chemical, and pharmaceutical industries for its non-toxicity and non-destruction to extracts. The combined liquid-like solvating capabilities and gas-like transport properties of supercritical fluids made it particularly suitable for the extraction of diffusion-controlled matrices including plant tissues (Liza et al. 2010; Reverchon and De Marco 2006). Moreover, the solvent strength of supercritical fluid can be controlled by modifying pressure and/or temperature; therefore, it may achieve a remarkably high selectivity. Supercritical CO_2 is the most commonly used fluid considering it is non-toxic, non-flammable, non-explosive, cost-efficient and easily approachable (Chiu et al. 2002; Liza et al. 2010). Moreover, extractions with supercritical CO_2 could achieve solvent-free products and avoid deteriorating reactions, due to low process temperatures (Vatai et al. 2009). Usually, adding a small amount of liquid cosolvent (modifier) can significantly increase the extraction yield and reduce the extraction time (Lang and Wai. 2001). Ethanol is a preferable cosolvent in SFE due to its low toxicity and being easily removed from products (Hamburger et al. 2004; Lang and Wai. 2001).

SFE has been reported as an effective method for preparing bioactive products from plant materials. However, to our best knowledge, there were no reports on phenolics extracted from date seeds by SFE so far.

The objective of this study was to optimize the extraction of phenolic yield from date seeds using supercritical CO_2 with ethanol as cosolvent. Effects of pressure, temperature, duration, and times of extraction on phenolic yield were investigated, and optimization of extraction using orthogonal assay (OA) design was performed.

Material and Methods

Samples

The "Lingbao" date fruits used in this study were procured from market in Zhengzhou, Henan, China. The dates at fully mature stage were sun-dried after harvested in September. The seeds were manually separated from the flesh and rinsed clean, air dried for 2 days in an oven at 50 °C (Al-Farsi and Lee 2008). Then, the dried date seeds were ground for 3 min. The particles were controlled between 0.425 and 0.053 mm in diameter, were sieved out for usage, packed in plastic bags, and stored in a desiccator at room temperature.

Supercritical Fluid Extraction

Extractions were done using a 7071 Spe-ed Supercritical Fluid Extraction System (Applied Separations Inc., Allentown, PA, USA), which consisted of a 50-mL extraction vessel, air pressure regulator (series 7071), air compressor (TYW-2, Nanjing, China), cryostat (SDC-6, Nanjing, China), and syringe pump unit (series 1500). Circulated malondialdehyde at 3 °C was used for cooling different zones in the SFE extraction apparatus.

Grounded date seed (10 g) was weighed accurately and placed into 50 mL extraction vessel fit with filters at the inlet and outlet to avoid haulage of the sample. Supercritical CO_2 and cosolvent (20 mL ethanol) were introduced into the extraction vessel after desired temperature was achieved. The cosolvent was maintained at 4 mL/min. Addition of cosolvent allowed the sample of date seeds to soak in the CO_2 and ethanol in order to equilibrate the mixture at desired pressure and temperature. The air pressure regulator and air compressor were loaded with CO_2 until the operating pressure was reached. The extraction was set for a static extraction. After extraction, samples were collected into a collection vessel through a micrometric valve, which was thermostated to avoid obstructions at the exit due to the solidification of CO_2. And then all the tubings in the process line were washed with ethanol to recover the extract deposited. The extraction yield was calculated as the ratio of the total mass of extract (extraction and cleaning process) to the initial mass of raw material (dry basis) (Piantino et al. 2008).

Experimental Design

Before the orthogonal experiment, several separate experiments for each independent factor were operated in order to make sure the correct levels for orthogonal experiment design were obtained. The independent variables were temperature (°C), pressure (bar), extraction time (h), and times of extraction (times). The effects of 3 or 5 levels of each factor on total phenolic content (TPC) by SFE in single experiment were investigated based on previous experiments. The single levels of these four factors were shown in Table 1.

OA with four variables and three factorial levels was applied. The coded levels were shown in Table 2 according to the results of single factor experiments. OA_9 (3^4) was chosen to reduce workload, while it could arrange the four factors, their levels, and also the interactions between these factors if they exist. In this case, the interaction between any two factors was included in the other two columns (Yang et al. 2007; Yan et al. 2009). TPC was carried out to determine the extraction efficiency. Altogether, nine runs were performed under randomized order, and the experiments on each sample were carried out in duplicate in order to evaluate the variability of measurement.

Determination of TPC

The extracts from SFE were collected and adjusted to 50 mL after cosolvent was removed by evaporation in a vacuum at 40 °C using a rotary evaporator. All of the steps were

Table 1 Factors and levels for single experiment design for effects on TPC

Factor	Level				
Extraction time (h)	1.0	1.5	2.0	2.5	3.0
Pressure (bar)	250	300	350	400	450
Temperature (°C)	40		50		60
Number of extraction (times)	1		2		3

performed with the protection from light. Folin–Ciocalteu method was used for the determination of TPC as described by Gelmez et al. (2009) with some modifications. Diluted extract (0.5 mL) was added to 2.5 mL Folin–Ciocalteu reagent and 2 mL Na_2CO_3. The mixture was incubated at 37 °C for 5 min. The absorbance at 760 nm was measured, and the concentration of TPC was expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight (FW) (Al-Farsi et al. 2007).

Table 2 Layout of OA_9 (3^4) matrix and results of analysis on the effects of four factors on TPC

Experiment No.	Coded levels				TPC (mg/100 g FW)
	A	B	C	D	
1	1(1.5)	1(300)	1(40)	1(1)	66.23d
2	1	2(350)	2(50)	2(2)	295.46ab
3	1	3(400)	3(60)	3(3)	222.59abc
4	2(2.0)	1	2	3	260.36abc
5	2	2	3	1	169.57bcd
6	2	3	1	2	307.79a
7	3(2.5)	1	3	2	264.62ab
8	3	2	1	3	193.14abcd
9	3	3	2	1	126.28cd
$k1$[a]	194.76	197.07	189.05	120.70	
$k2$[b]	245.91	219.39	227.37	289.29	
$k3$[c]	194.68	218.89	218.93	225.36	
Optimal level[d]	2	2	2	2	
R[e]	51.23	22.32	38.31	168.59	
Sequence[f]	2	4	3	1	

Values with different letters denote significant differences among the treatments at $P<0.05$.

TPC total phenolic content, *R* range

[a] TPC at level 1

[b] TPC at level 2

[c] TPC at level 3

[d] The level at which the average TPC reached the highest.

[e] The difference between the maximal and minimal average of TPC.

[f] The order of importance or effectiveness of the four factors on TPC by the *R* value

Determination of Phenolic Components by High Performance Liquid Chromatography Analysis

The phenolic components in extracts of date seeds were analyzed by high performance liquid chromatography (HPLC) method. The HPLC analysis (Waters Corp., Wilford, MA, USA) was performed with a Waters 1525 pump controller, 2996 Photodiode Array detector, and 2707 auto injector equipped with Waters Symmetry C_{18} column (4.6×250 mm). Classic Empower 2010 software was used for data processing. The phenolic compounds were eluted with a gradient elution of mobile phase, solvent A consisted of 100 % methanol (HPLC grade) and solvent B consisted of deionized water (Milli-Q, USA) adjusted to pH 2.6 with phosphate (HPLC grade). Gradient elution program was set as follows: 0 min, 15 % A, 85 % B; 15 min, 22 % A, 78 % B; 30 min, 30 % A, 70 % B; 65 min, 75 % A, 25 % B; 70 min, 100 % A; 85 min, 100 % A; and 90 min, 15 % A, 85 % B. The inject volume was 10 μL. The column temperature was set to 30 °C, flow rate of mobile phase at 0.6 mL/min, and detection scanning range from 210 to 400 nm with resolution of 1.2 nm. Standard sample was composed of 11 phenolic acids: gallic acid, protocatechuic acid, catechin, chlorogenic acid, caffeic acid, epicatechin, ferulic acid, rutin, ellagic acid, cinnamic acid, quercetin, and kaempferol. Identification of compounds was achieved by comparing their retention times as well as the UV–vis spectra of standards (Zhang et al. 2010). All the phenolic standards were obtained from Sigma-Aldrich (St, Louis, MO, USA).

Antioxidant Capacity of TPC

To determine the antioxidant activity of date seed extracts, the ability of the extracts to inhibit non site-specific hydroxyl radical-mediated peroxidation was carried out according to Hinneburg et al. (2006) with slight modifications. The reaction mixture contained 0.1 mL extract, 0.4 mL KH_2PO_4-KOH buffer (50 mmol/L, pH 7.4), 0.1 mL EDTA (100 mmol/L), 0.1 mL $FeCl_3$ (100 μmol/L), 0.1 mL H_2O_2 (0.1 mmol/L), 0.1 mL 2 mmol/L aqueous ascorbic acid, and 0.1 mL 60 mmol/L 2-Deoxy-D-ribose. Then, the mixture was incubated in a 37 °C water bath for 1 h. Thereafter, 1 mL 25 % HCl and 1 mL 1 % Thiobarbituric acid were quickly added and boiled at 100 °C for 15 min. Butanol (3 mL) was added for extraction if the mixture appeared cloudy.

The absorbance of the solution at 532 nm was measured, and the inhibition value was calculated as: Inhibition (%) $= \frac{A_c - A_s}{A_c} \times 100$. A_c and A_s meant the absorbance of a negative control and the sample, respectively. Same concentration of ascorbic acid was used as positive control.

Statistical Analysis

Statistical Product and Service Solutions (v13.0, USA) was applied for experimental design and data analysis. The importance of each of the four factors to TPC was evaluated based on to the effectiveness of each factor by calculating range (R) (the difference between the maximal and minimal means of TPC within the three levels of each factor). Analysis of variance was performed to test the significance of the effects of the four factors on TPC. Differences between means of different levels were analyzed by means of least significant difference multiple comparisons at $P<0.05$. Finally, the significance of antioxidant activity differences between extracted date seed phenolics and ascorbic acid at the same concentration was evaluated using Student's t-test.

Results and Discussion

Effects of Process Variables on Extraction of TPC

The static SFE extraction results were affected by four major factors: temperature, pressure, extraction time, and number of extraction. In order to approach a suitable range for individual factor, all parameters were tested in a wider range prior to OA design. The range of each factor level for OA test was based on the optimal conditions of separate experiment results, as shown in Fig. 1a–d.

Figure 1 a shows the effect of temperature on TPC of date seeds in SFE at 350 bar, extracted for 2 h and extracted twice. Temperature of 40 °C was widely used in the extraction of plant materials by SC–CO_2, which was just above the critical temperature for CO_2 (31.1 °C). The increase of temperature can have either a positive or a negative effect on the yield of TPC from date seeds. As shown in Fig. 1a, the yield of phenolics increased significantly as temperature increased from 40 to 50 °C. However, a further increase from 50 to 60 °C did not continue increasing the extraction yield. This agree well with the reports of Liu et al. (2011), who found an increase in the flavonoid yield by increasing the temperature from 40 to 50 °C of SFE extraction and a reversed trend when temperature reached a certain value.

In SFE, extraction conditions were defined in terms of variables directly related to the relative solvent strength, which was primarily dependent on the density (Pitzer 1955). Near the system's critical pressure, the fluid density became very sensitive to temperature. The increase of phenolic content was due to the enhancement of vapor pressure of analytes, which was greater than the reduction of CO_2 density (Liza et al. 2010). Heating might also weaken the interactions between phenol, protein, and polysaccharide in date seeds (Al-Farsi and Lee 2008). However, when temperature raised to 60 °C, density of CO_2 at constant pressure decreased with increased temperature, resulting in reduced solvent power for supercritical CO_2 (Liza et al. 2010; Wang et al. 2008). Decreased TPC could also be associated with the denaturation of phenolic compounds due to a long extraction processing at temperature above 50 °C (Ghafoor et al. 2010; Cacace and Mazza 2003).

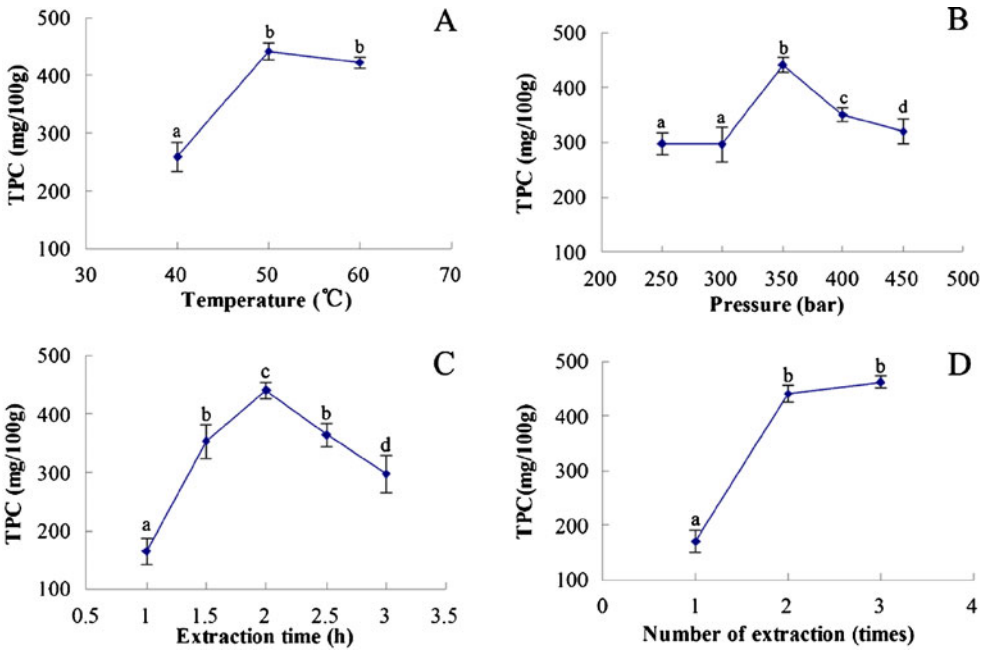

Fig. 1 Effect of temperature (**a**), pressure (**b**), extraction time (**c**) and number of extraction (**d**) on TPC by SFE. Experimental condition: **a** pressure 350 bar, extracted twice, each lasting 2 h; **b** extracted twice at 50 °C for 2 h; **c** pressure 350 bar, extracted twice at 50 °C; **d** pressure 350 bar, extracted 2 h at 50 °C. Values with different letters denote significant difference at $P<0.05$ among treatments

Figure 1b shows the effect of pressure on the extraction of TPC. A significant increase of extraction yield was observed in the range of 300–350 bar with the highest yield at 350 bar. At a constant temperature, increased fluid density was resulted from pressure increase that altered solute solubility, which was probably the major reason for the increase of total phenol extracted (Wang et al. 2008). As the density increased, the distance between the molecules decreased. Therefore, the interaction between the analytes and CO_2 increased, leading to a greater solubility of the analytes in CO_2, and a decreased phenolic yield over this range. Gomes et al. (2007) indicated the composition of extract could be controlled by adjusting pressure, and a high recovery of volatile fractions and a low recovery of non-volatile fractions were obtained at high pressure. Wang et al. (2008) demonstrated in a pressure range the yield of flavonoid from *Pueraria lobata* increased with increased pressure, consistent with our results, which might be due to the modification of volatility and polarity of extracted analytes by higher pressure. Gelmez et al. (2009) reported that pressure affected the solubility of wheat germ oil more than the solubility of phenolics with increased pressure.

Under the operational conditions previously selected of 350 bar and twice extractions at 50 °C, the effect of extraction time (h) on the TPC from date seeds was evaluated (Fig. 1c). An almost linear increase in TPC was observed with extraction up to 2 h, with the yield at 2 h being 2.5 fold of that at 1 h. This did not agree well with reports from Al-Farsi and Lee (2008) who demonstrated the yield of phenolics by solvent extraction from date seeds remained constant with increased extraction time up to 2 h. While 2 h later, yield significantly decreased with a prolonged time. Liza et al. (2010) also found the dynamic extraction time of more than 1 h did not significantly increase the yield. The increased extraction duration and constant exposure to high temperature increased the vaporization of solvent, which affected the solvent to solid ratio, thus might induce the loss of phenolics by oxidation (Al-Farsi and Lee 2008). Thereby, 2 h was chosen as optimum time for phenolic extraction from date seeds by SFE.

Heating and long extraction time might result in heat oxidation and compound degradation. Multiple-stage extraction could be an appropriate alternative to minimize these problems (Al-Farsi and Lee 2008). Effect of different extraction times on the yield of TPC of date seeds by SFE was investigated when the other three factors were fixed at 50 °C, 2 h, and 350 bar, respectively. The result (Fig. 1d) implied that TPC increased significantly from 170.75 to 441.57 mg GAE/100 g FW during the second extraction. Moreover, the yield of phenolics increased slightly after the third extraction. The result was similar to a previous report, which showed the polysaccharide yield from *Anemone raddeana* increased just a little after three extractions (Sun et al. 2009). Shi et al. (2003) and Al-Farsi and Lee (2008) also found the first two extractions for phenolic yield from grape seeds and date seeds were more efficient. Our current reports, supported by previous publications, shows that twice extractions achieved the best yield of phenolics from date seeds by SFE at fixed conditions of temperature, pressure, and extraction time. More extractions were not recommended considering the time, consumption of solvent, and supercritical fluid CO_2.

Optimization of the SFE Operating Process

The orthogonal experimental design OA_9 $(3)^4$ (Table 2) was conducted to optimize the extraction temperature (factor A), pressure (factor B), extraction time (factor C), and number of extraction (factor D) for SFE. The highest TPC (307.79 mg/100 g FW) was obtained in experiment No. 6 (50 °C, 400 bar, extraction for 1 h, and extracted twice).

To our best knowledge, no data were available in the literature regarding extraction of TPC from date seeds using SFE. In this current study, the TPC of date seeds varied from 66.23 to 307.79 mg/100 g FW. The result was similar with that of Amany et al. (2012) who reported that TPC was 90 mg GAE/100 g (methanol) to 201.5 mg GAE/100 g FW (formic acid) by a traditional solvent extraction method from "Khalas" date seeds. However, their results were much lower than those reported by Al-Farsi et al. (2007) and Habib and Ibrahim (2011) who found that TPC of date seeds in "Mabseeli", "Um-sellah", and "Shahal" cultivars were 3102, 4293, and 4430 mg GAE/100 g FW, respectively using methanol/H_2O (50:50, *v*/*v*) as extracted solvent. Habib and Ibrahim (2011) reported that the TPC of "Khalas" cultivar was 2,460 mg GAE/100 g FW using the method of Al-Farsi et al. (2007). Various extraction methods, varieties, and grinded sizes might cause the different yields.

It was not appropriate to choose the best extraction conditions only based on the outcomes in Table 2. Further orthogonal analysis was needed. Thus, the *k* and *R* values were calculated and listed in Table 2 as well. According to the ranges calculated (Table 2), the order of importance of the four factors to TPC was: D (number of extraction)>A (extraction time)>C (temperature)>B (pressure) according to the *R* values. Thus, the best combination of factors and levels for the highest TPC was $A_2B_2C_2D_2$, that was extracted twice, at 50 °C, 350 bar for 2 h. The TPC under optimal condition was detected in prior experiment as 441.57 mg GAE/100 g FW, which was much higher than the result of OA experiments in Table 2.

Numbers of extraction was the main parameter contributing to optimum extraction. Sun et al. (2009) also found that the number of extraction was the most important determinant for the yield of polysaccharides from *A. raddeana*. However, pressure was found to have least effect on TPC in our research, which was different from other reports in which pressure was the most significant variable on TPC (Gelmez et al. 2009) or total flavonoids (Liu et al. 2011). This difference may be explained by different textures of fruit materials. Date seeds were so hard that pressure may not exert significant effects on the extraction of phenolics.

We did not set up an independent column in the OA Table (Table 2) to evaluate the sum of square of error term (SSe). The reason was that, according to Zhang and Yan (2001) and Yan et al. (2009), the factor that had the smallest sum of squares can be taken as error term testing the significance of effects of the main factors. In the current report, it was found that factor B (pressure) had the smallest value of the sum of square (Table 2), thus the sum of square of factor B was used as the SSe for the test of significance. From Table 3, the effect of the number of extraction was significant on TPC ($P<0.05$), while the effects of the other factors were not significant. Through multiple comparisons by least significant difference test (data not shown), TPC of more extractions were significantly greater than single extraction, while no significant difference of TPC was observed between double extractions and triple extractions.

Phenolic Compositions Extracted from Date Seeds by SFE

HPLC results showed that the total phenolic acid compositions of date seeds in No. 6 sample from OA design runs (Fig. 2). A total of seven phenolic compounds of the date seeds could be identified in our research, as noted "S1" (gallic acid), "S2" (protocatechuic acid), "S3" (chlorogenic acid), "S4" (epicatechin), "S6" (rutin), "S7" (ellagic acid), and "S9" (quercetin). Several "unknown" compounds were also present, for example, noted as "S5" and "S8" in Fig. 2. Further studies (HPLC–MS and other techniques) were needed for elucidating the phenolic compositions from date seeds.

Among the identified phenolic acids in date seeds, chlorogenic acid, rutin, ellagic acid, and quercetin were the major phenolic acids extracted by SFE. However, Al-Farsi and Lee (2008) reported that *p*-hydroxybenzoic, protocatechuic, and *m*-coumaric acids were the major phenolic acids in date seeds. The extraction and pretreatment before HPLC might be one reason for this difference. Different solvent used could be additional reason, which had a significantly different selectivity of phenolic acids in date seeds. The extraction solvent by Al-Farsi and Lee (2008) (50 % acetone) may render the highest extraction capacity for gallic, *p*-hydroxybenzoic, caffeic, *p*-coumaric, and ferulic acids. And the hydrolysis treatments used by Al-Farsi and Lee (2008) could quantify the acids transform glycosylated and esterified phenolics into aglycon form. Furthermore, the various concentrated phenolic acid compositions of date seeds by SFE under different operating conditions might explain for the difference. Klejdus et al. (2009) found that SFE extraction temperature affected the phenolic acid composition, implicating the temperature of 100 °C was better for protocatechuic and *p*-hydroxybenzoic acids while 60 °C for chlorogenic, *p*-coumaric, and ferulic acid. All of the phenolics in the current report were extracted at low temperature (<60 °C), which resulted in more chlorogenic, *p*-coumaric, and ferulic acid extracted. On the other hand, whether the "unknown" compounds "S5" and "S8" in the current research were *p*-hydroxybenzoic or *m*-coumaric acids were not sure, requiring further experiments to characterize.

Antioxidant Properties of Phenolics Extracted from Date Seeds by SFE

Samples from the conditions of No. 6 OA design were run for evaluating the antioxidant properties of date seed phenolics extracted by SFE. The antioxidant activities of phenolics from date seeds and same concentration ascorbic acid were shown in Fig. 3. Ascorbic acid was chosen as a comparison, because deoxyribose would be degraded by hydroxyl radicals generated and ascorbic acid was a common hydroxyl radical scavenger. The result showed that date seed phenolics were more effective in eliminating hydroxyl radicals than ascorbic acid at the concentration varying from 1.0 to 8.0 mg/L. Inhibitions of 1.0, 4.0, and 8.0 mg/L date seed phenolics on hydroxyl

Table 3 ANOVA result of the four factors on TPC

Tests of between-subjects effects. TPC is the dependent variable

*$P<0.05$; significant effect of the factor on TPC

Source	Sum of squares	df	Mean square	F	Sig.
Temperature (°C)	5240.50	2	2620.25	5.38	0.16
Extraction time (h)	2431.25	2	1215.62	2.50	0.29
Number of extraction (times)	13465.71	2	21732.86	44.61	0.02*
Error	974.25	2	487.13		
Total	52111.71	8			

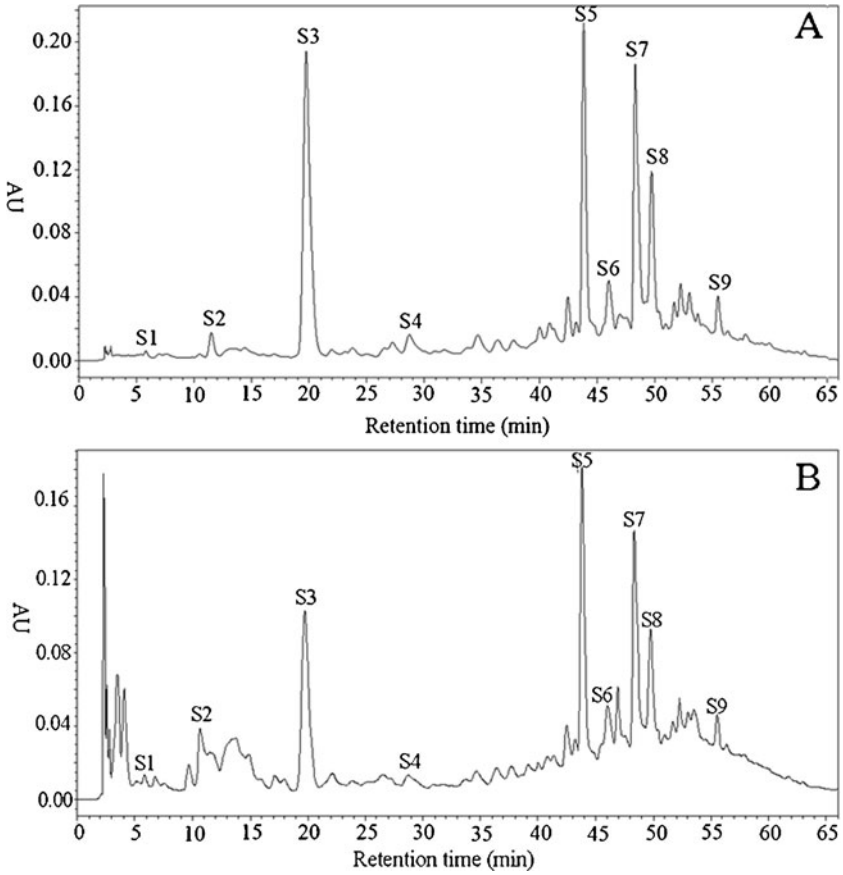

Fig. 2 HPLC analysis of date seed phenolic compositions extracted by SFE. **a** detective wavelength at 280 nm. **b** detective wavelength at 320 nm. *S1*: gallic acid, *S2* protocatechuic acid, *S3* chlorogenic acid, *S4* epicatechin, *S5* unknown, *S6* rutin, *S7* ellagic acid, *S8* unknown, *S9* quercetin. Phenolics sample was extracted twice at 40 °C, 400 bar for 2 h

radical were 66.11, 88.12, and 94.48 %, respectively, while for the same concentration of ascorbic acid, they were 50.65, 55.65, and 60.45 %, respectively. However, the difference between antioxidant activity of date seed phenolics and ascorbic acid became insignificant when the concentration increased to 10 mg/L. The high antioxidant of dates, especially date seeds due to its high phenolic contents, was supported by several reports (Al-Farsi et al. 2007; Guo et al. 2003).

Conclusions

SFE was found to be an effective technique in the recovery of biologically valuable components from date seeds. Extraction efficiency was affected by temperature, pressure, number of extraction, and extraction time, especially the number of extraction, which significantly affected TPC yield. Procedures of duplicate extractions, each for 2 h at 50 °C with 350 bar pressure, were considered the optimum for phenolic extraction from grounded date seeds by SFE. Seven phenolic acids were identified in date seeds; among which, the majority compounds were chlorogenic acid, rutin, ellagic acid, and quercetin with two other major phenolic acids unidentified. Total phenolics from date seeds were found to have a higher antioxidant activity than same concentration of ascorbic acid in the range from 1 to 8 mg/L. The results indicated that date seeds could serve as a good source of phenolics and could potentially be considered as an affordable natural antioxidant.

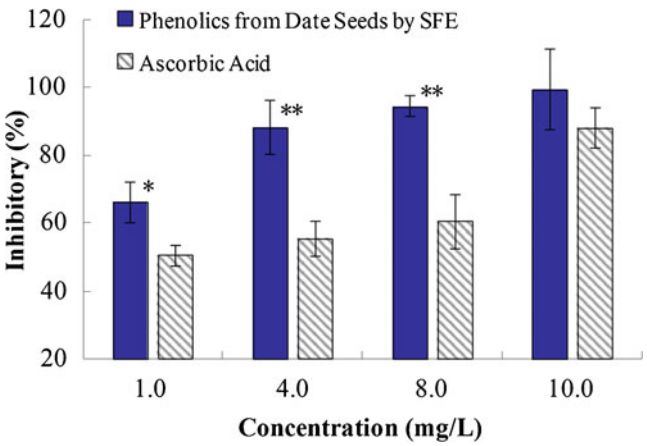

Fig. 3 Inhibition of 2-deoxy-D-ribose degradation by extracted phenolics from date seeds and ascorbic acid. *Single asterisk* indicates significant mean difference at $P<0.05$. *Double asterisks* indicate significant mean difference at $P<0.01$

Acknowledgments Projects 31071617 and 30600420 supported by National Natural Science Foundation of China contributed to this study. Projects 122300410125 supported by Foundation and Advanced Technology Research of Henan Province also contributed to this study.

References

Al-Farsi MA, Lee CY (2008) Optimization of phenolics and dietary fibre extraction from date seeds. Food Chem 108:977

Al-Farsi M, Alasalvar C, Al-Abid M, Al-Shoaily K, Al-Amry M, Al-Rawahy F (2007) Compositional and functional characteristics of dates, syrups, and their by-products. Food Chem 104:943

Amany MMB, Shaker MA, Abeer AK (2012) Antioxidant activities of date pits in a model meat system. Int Food Res J 19:223

Cacace JE, Mazza G (2003) Optimization of extraction of anthocyanins from black currants with aqueous ethanol. J Food Sci 68:240

Chiu KL, Cheng YC, Chen JH, Chang C, Yang PW (2002) Supercritical fluids extraction of *Ginkgo ginkgollaes* and flavonoids. J Supercrit Fluids 24:77

Food and Agriculture Organization of the United Nations (2010) Dates production quantity of China in 2010. http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor

Gelmez N, Kıncal NS, Yener MS (2009) Optimization of supercritical carbon dioxide extraction of antioxidants from roasted wheat germ based on yield, total phenolic and tocopherol contents, and antioxidant activities of the extracts. J Supercrit Fluids 48:217

Ghafoor K, Park J, Choi YH (2010) Optimization of supercritical fluid extraction of bioactive compounds from grape (*Vitis labrusca* B.) peel by using response surface methodology. Innov Food Sci Emerg Technol 11:485

Goli AH, Barzegar M, Sahari MA (2005) Antioxidant activity and total phenolic compounds of pistachio (*Pistachia vera*) hull extracts. Food Chem 92:521

Gomes PB, Mata VG, Rodrigues AE (2007) Production of rose geranium oil using supercritical fluid extraction. J Supercrit Fluids 41:50

Guo CJ, Yang JJ, Wei JY, Li YF, Xu J, Jiang YG (2003) Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. Nutr Res 23:1719

Habib HM, Ibrahim WH (2011) Effect of date seeds on oxidative damage and antioxidant status *in vivo*. J Sci Food Agric 91:1674

Hamada JS, Hashim IB, Sharif FA (2002) Preliminary analysis and potential uses of date pits in foods. Food Chem 76:135

Hamburger M, Baumann D, Adler S (2004) Supercritical carbon dioxide extraction of selected medicinal plants-effects of high pressure and added ethanol on yield of extracted substances. Phytochem Anal 15:46

Hinneburg I, Dorman HJD, Hiltunen R (2006) Antioxidant activities of extracts from selected culinary herbs and spices. Food Chem 97:122

Hussein AS, Alhadrami GA, Khalil YH (1998) The use of dates and date pits in broiler starter and finisher diets. Bioresour Technol 66:219

Klejdus B, Kopecký J, Benešová L, Vacek J (2009) Solid-phase/supercritical-fluid extraction for liquid chromatography of phenolic compounds in freshwater microalgae and selected cyanobacterial species. J Chromatogr A 1216:763

Lang QY, Wai CM (2001) Supercritical fluid extraction in herbal and natural product studies- a practical review. Talanta 53:771

Liu J, Lin SY, Wang ZZ, Wang CN, Wang EL, Zhang Y, Liu JB (2011) Supercritical fluid extraction of *Maydis stigma* and its nitrite-scavenging ability. Food Bioprod Process 89:333

Liza MS, Abdul Rahman R, Mandana B, Jinap S, Rahmat A, Zaidul ISM, Hamid A (2010) Supercritical carbon dioxide extraction of bioactive flavonoid from *strobilanthes crispus* (Pecah Kaca). Food Bioprod Process 88:319

Piantino CR, Aquino FWB, Follegatti-Romero LA, Cabral FA (2008) Supercrtical CO2 extraction of phenolic compounds from *Baccharis dracunculifolia*. J Supercrit Fluids 47:209

Pitzer KS (1955) The volumetric and thermodynamic properties of fluids I. Theoretical casis and virial coefficients. J Am Chem Soc 77:3427

Reverchon E, De Marco I (2006) Supercritical fluid extraction and fractionation of natural matter. J Supercrit Fluids 38:146

Shi J, Yu J, Pohorly J, Young JC, Bryan M, Wu Y (2003) Optimization of the extraction of polyphenols from grape seed meal by aqueous ethanol solution. J Food Agric Environ 1:42

Soong Y, Barlow PJ (2004) Antioxidant activity and phenolic content of selected fruit seeds. Food Chem 88:411

Sun YX, Li YJ, Li MQ, Tong HB, Yang XD, Liu JC (2009) Optimization of extraction thechnology of the *Anemone raddeana* polysaccharides (ARP) by orthogonal test design and evaluation of its anti-tumor activity. Carbohydr Polym 75:575

Vatai T, Škerget M, Knez Ž (2009) Extraction of phenolic compounds from elder berry and different grape marc varieties using organic solvents and/or supercritical carbon dioxide. J Food Eng 90:246

Wang LZ, Yang B, Du XQ, Yi C (2008) Optimisation of supercritical fluid extraction of flavonoids from *Pueraria lobata*. Food Chem 108:737

Yan LL, Zhang GF, Liu QG, Li JL (2009) Optimization of culturing the freshwater pearl mussels, *Hyriopsis cumingii* with filter feeding Chinese carps (bighead carp and silver carp) by orthogonal array design. Aquaculture 292:60

Yang H, Wang Y, Jiang M, Oh JH, Herring J, Zhou P (2007) 2-step optimization of the extraction and subsequent physical properties of channel catfish (*Ictalurus punctatus*) skin gelatin. J Food Sci 72:C188

Zhang C, Yan Y (2001) Medical Mathematic Statistics. Science Press, Beijing, p 212, in Chinese

ZhangYZ LPM, Cheng LL (2010) Developmental changes of carbohydrates, organic acids, amino acids, and phenolic compounds in 'Honeycrisp' apple flesh. Food Chem 123:1013

Zuo Y, Chen H, Deng Y (2002) Simultaneous determination of catechins, caffeine and gallic acids in green, Oolong, black and pu-erh teas using HPLC with a photodiode array detector. Talanta 57:307