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ScienceDirect
Food Chemistry 108 (2008) 977–985
Food Chemistry
www.elsevier.com/locate/foodchem

Optimization of phenolics and dietary fibre extraction from date seeds

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Received 20 July 2007; received in revised form 1 December 2007; accepted 4 December 2007

Abstract

This work was conducted to optimise extraction conditions of phenolics and dietary fibre from date seeds. The effects of solvent to sample ratio, temperature, extraction time, number of extractions and solvent type on phenolic extraction efficiency were studied. At two-stage extraction, each stage 1 h duration at 45 °C with a solvent to sample ratio of 60:1, is considered optimum. Acetone (50%), and butanone were the most efficient solvents for extraction and purification, increasing the yield and phenolic contents of seed concentrate to 18.10 and 36.26 g/100 g, respectively. The total dietary fibre of seeds (57.87 g/100 g) increased after water and acetone extractions to 83.50 and 82.17 g/100 g, respectively. Nine phenolic acids (free and liberated) were detected in seeds with *p*-hydroxybenzoic (9.89 mg/100 g), protocatechuic (8.84 mg/100 g), and *m*-coumaric (8.42 mg/100 g) acids found to be among the highest. After extraction and purification, total phenolic acid content increased significantly from 48.64 to 193.83 mg/100 g. Protocatechuic, caffeic and ferulic acids were the major phenolic acids found in the concentrates. Based on this study, we believe date seed concentrates could potentially be an inexpensive source of natural dietary fibre and antioxidants and possibly used as a functional food ingredient.

Keywords: Solvent; Purification; Flavonoids; Antioxidants; Phenolic acids

1. Introduction

Dates of date palm tree (*Phoenix dactylifera* L.) are popular among the population of the Middle Eastern countries. The fruit is composed of a fleshy pericarp and seed which constitutes between 10% and 15% of date fruit weight (Hussein, Alhadrami, & Khalil, 1998). The date seeds considered a waste product of many date processing plants producing pitted dates, date syrup and date confectionery. At present, seeds are used mainly for animal feeds in the cattle, sheep, camel and poultry industries. With world production of dates reaching 6.9 million tonnes in 2004, from this approximately 863 thousand tonnes of date seeds are produced (FAO., 2007). Thus, utilization of such waste is very important to date cultivation and to increase the income to this sector.

Chemical and nutritional constituents of date seeds were reported by Besbes, Blecker, Deroanne, Drira, and Attia (2004), Aldhaheri, Alhadrami, Aboalnaga, Wasfi, and Elridi (2004), Hamada, Hashim, and Sharif (2002); as well as Sawaya, Khalil, and Safi (1984). Most current literature was limited to proximate and mineral compositions. Beyond compositional analysis, there is the work of Al-Farsi et al. (2007) who researched the functional properties of date seeds. Their reported composition was 3.1–7.1% moisture, 2.3–6.4% protein, 5.0–13.2 fat, 0.9–1.8% ash and 22.5–80.2% dietary fibre. Also, seeds contain high levels of phenolics (3102–4430 mg gallic acid equivalents/100 g), antioxidants (580–929 μm trolox equivalents/g) and dietary fibre (78–80 g/100 g; Al-Farsi et al., 2007).

Antioxidant compounds have been identified in grape seeds (Kallithraka, Garcia-Viguera, Bridle, & Bakker, 1995; Shi et al., 2003) blackcurrant seeds (Lu & Foo, 2003), mango seeds (Puravankara, Boghra, & Sharma, 2000), the seeds of citrus (Alessandra, Marie-Elisabeth, Hubert, & Claudette, 1998), sesame (Shahidi, Amarovicz, Abou-Gharbia, & Shehata, 1997), flaxseeds (Oomah, Kenaschuk, & Mazza, 1995). Soong and Barlow (2004) have reported that the total phenolics content of seeds of several

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doi:10.1016/j.foodchem.2007.12.009

fruits, such as mango, avocado, and jackfruit, were higher than their edible flesh. Therefore, these seeds could be considered a valuable source of phenolics. However, studies relating to the antioxidant activity of date seeds are limited.

Phenolic compounds of fruit seeds mainly phenolic acids and flavonoids, have been shown to possess such benefits as antioxidant (Peterson & Dwyer, 1998; Von Gadow, Joubert, & Harsmann, 1997), anti-carcinogenic (Bailey & Williams, 1993; Block, 1992), antimicrobial (Takechi, Tanaka, Nonaka, & Nishioka, 1985), anti-mutagenic (Liverio, Puglisi, Morazzoni, & Bombardelli, 1994), and anti-inflammatory activities (Landolfi, Mower, & Steiner, 1984), as well as reduction of cardiovascular diseases (Diplock et al., 1998; Halliwell, 1997). Thus, it is considered important to increase the antioxidant intake in the human diet and one way of achieving this is by enriching food with phenolics. As some synthetic antioxidants may exhibit toxicity, require high manufacturing costs and have lower efficiency than natural antioxidants (Madhavi, Despande, & Salunke, 1996; Soong & Barlow, 2004), there is a need to identify natural and possibly to devise more economical ways to obtain effective antioxidants with potential to be incorporated into foods. At present, the natural antioxidants commercially produced include tocopherols, ascorbic acid and plant extracts such as rosemary, sage and green tea (Djarmati, Jankov, Schirtlich, Djulinac, & Djordjevic, 1991; Ramarathnam, Osawa, Ochi, & Kawakishi, 1995; Tena, Valcarcel, Hidalgo, & Ubera, 1997; Yoshida, Kiso, & Goto, 1999).

The good nutritional value of date seeds is also based on their dietary fibre content, which makes them suitable for the preparation of fibre-based foods and dietary supplements. Dietary fibre has important therapeutic implications for certain conditions such as diabetes, hyperlipidemia, and obesity and may exhibit a protective effect against hypertension, coronary heart disease (CHD), cholesterol, colorectal and prostate cancers, and intestinal disorders (Johnson & Southgate, 1994; Kritchevsky, 1988; Tariq et al., 2000).

The extraction and purification of antioxidants from natural sources is desired, since these bioactive substances are often used in functional foods, food additives, nutraceuticals, pharmaceuticals and cosmetic industries (Shahidi & Naczk, 2004). Extraction yield is dependent on the solvent and method of extraction (Goli, Barzegar, & Sahari, 2005), which must enable complete extraction of the phenolics as well as to minimize the oxidation, degradation, or polymerization of the desired products (Zuo, Chen, & Deng, 2002). Water, ethanol, methanol and acetone and their aqueous mixtures are commonly used (Murga, Ruiz, Beltran, & Cabezas, 2000; Shahidi & Naczk, 2004; Sun & Ho, 2005; Zuo et al., 2002) The objective in extracting phenolics from their plant sources is to liberate these compounds from the vacuolar structures where they are found, either through rupturing plant tissue or through a process of diffusion. Solvent choice, solvent to sample ratio, contact time and temperature may significantly influence the extraction efficacy (Cacace & Mazza, 2003). The role of each factor in the extraction process is not always obvious; the chemical characteristics of the solvent and the diverse structure and composition of the natural products ensure that each material-solvent system shows different behaviour, which cannot be predicted. It is also necessary to purify the extract obtained, removing inert and undesirable components, to improve the antioxidant activity of the extract and reduce its odour, taste, and colour as effectively as possible.

Since a large quantity of date seeds in being produced as a waste material and the seeds contain a significant amount of bioactive phenolics and dietary fibre, the aim of this study was to optimise the extraction of phenolics and dietary fibre from date seeds under conditions compatible with food requirements. An optimum extraction method will produce extract that can be further purified and concentrated by solvent fractionation and evaporation process.

2. Material and methods

2.1. Chemicals

All chemicals were obtained from Sigma–Aldrich–Fluka Co. Ltd. (St. Louis, MO, USA) unless otherwise specified.

2.2. Seed extraction

The date seeds of Mabseeli used in this study were procured from the Batinah region, Oman. The seeds were oven dried for 2 days at 50 °C and then finely ground (1.5 mm) in a hammer mill (W4SS, Buffalo, NY, USA). Fig. 1 shows the extraction process of date seeds to produce phenolic and fibre enriched concentrates. The dried samples were subjected to extraction at a constant stirring rate of 120 rpm using a magnetic stirrer plate (320VWR, Thermolyne Corporation, Dubuque, IW, USA). Water and 50% aqueous acetone solvents were used for optimisation of seed extraction (Khokhar & Magnusdottir, 2002; Tabart et al., 2007). Solvent to sample ratio (10:1, 20:1, 40:1, 60:1 and 80:1), extraction temperature (25, 35, 45, 55 and 65 °C), extraction time (0.5, 1, 2, 3 and 4 h) and number of extraction (1, 2, 3 and 4 times) were applied one at a time to identify the optimum conditions. Solids were separated by centrifugation and filtration by Whatman No. 4 filter paper. Extracts were evaporated to dryness at 60 °C under vacuum using rotary evaporator (Heidolph Instruments, Laborota 4003-Control, Schwabach, Germany).

After selecting the optimum condition for extraction, seven solvents (water, ethanol, methanol, acetone, 50% ethanol, 50% methanol and 50% acetone) were used to identify the proper solvent for extraction. Butanol and butanone were used to purify phenolics from the extract by triple solvent fractionation process using funnel separation. These solvents were chosen because they show a high affinity for molecules containing hydroxyl groups, as well as taking into account economic considerations (Peschel et al., 2006). Total phenolics, flavonoids and antioxidants analyses were carried out to determine the extraction and purification

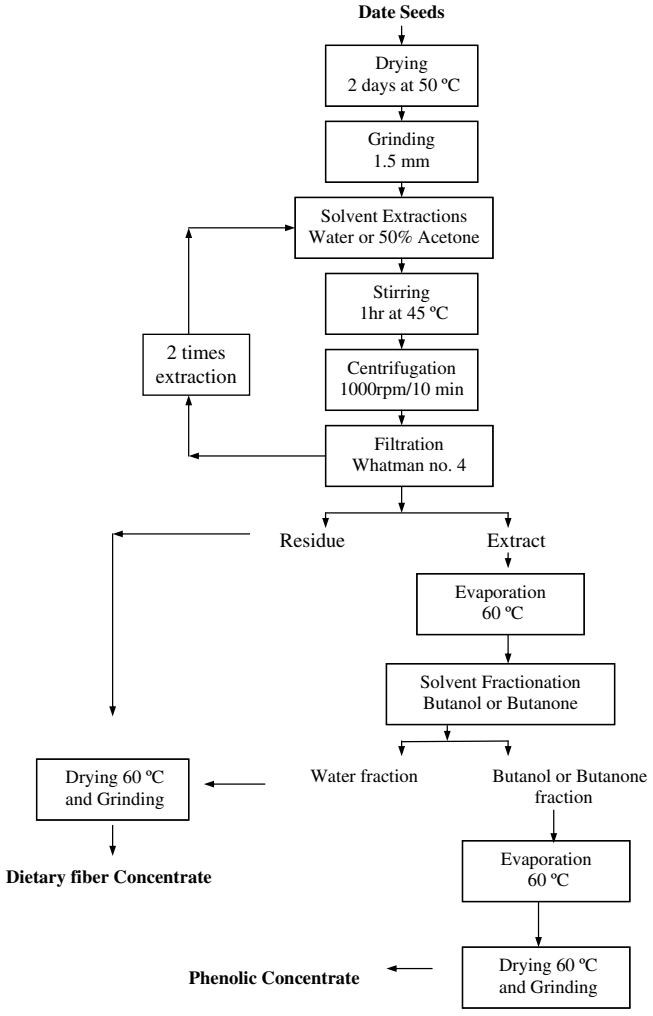

Fig. 1. Flow diagram of phenolic and dietary fiber enrichment from date seeds.

efficiency. Phenolics and dietary fibre concentrates produced from this process were oven dried at 60 °C and ground into a powder.

2.3. Dietary fibre

Dietary fibre content was determined by enzymatic-gravimetric method (AOAC, 1995). Triplicate samples were gelatinized with heat stable α-amylase, then enzymatically digested with protease and amyloglucosidase to remove the protein and starch present. Total dietary fibre was calculated as the sum of soluble dietary fibre and insoluble dietary fibre after correcting for ash and undigested protein. Dietary fibre was expressed as grams per 100 g of sample on a wet weight basis.

2.4. Total phenolic

The total phenolic content of seed extracts was determined according to the Folin–Ciocalteu method (Yoo, Lee, Park, Lee, & Hwang, 2004) with some modifications. Briefly, 1 ml of seed extract was mixed with 1 ml of Folin–Ciocalteu's phenol reagent and allowed to react for 5 min. Then, 10 ml of 7% sodium carbonate solution (w/v) were added, and the final volume was made up to 25 ml with deionised water. After 1 h of reaction at room temperature, the absorbance at 750 nm was read using a Thermo Spectrophotometer (Electron Corporation, Cambridge, England). Measurements were calibrated to a standard curve of prepared ferulic acid solution, and the total phenolic concentration was expressed as grams of ferulic acid equivalents per 100 g of sample on a wet weight basis.

2.5. Total flavonoid

The total flavonoid content of seed extracts was determined according to Zhishen, Mengcheng, and Jianming (1999). One millilitre of seed extract was placed in a 10 ml volumetric flask containing 5 ml of deionised water. Then 0.3 ml of 5% sodium nitrite was added; after 5 min, 0.3 ml of 10% aluminum chloride was added. After 6 min, 2 ml of 1 M sodium hydroxide were added and diluted to volume with deionised water with mixing. Immediately the solution absorbance was measured at 510 nm. Measurements were calibrated to a standard curve of prepared catechin solution, and the total flavonoid content was expressed as grams of catechin equivalents per 100 g of sample on a wet weight basis.

2.6. Total antioxidants

The total antioxidants activity method developed by Kim, Lee, Lee, and Lee (2002) was used. The peroxyl radical 2,2-azobis (2-amidinopropane) dihydrochloride (AAPH) 1 mM with 2.5 mM of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) were mixed in 100 mM potassium phosphate buffer (pH 7.4). After the mixture was heated in a water bath at 70 °C for 30 min, the blue-green ABTS radical solution was adjusted with phosphate buffer solution to an absorbance of 0.650 at 734 nm. Twenty microlitres of the sample solution added to 980 μl of the ABTS radical solution were incubated in a water bath at 37 °C for 10 min. The decrease of absorbance at 734 nm was measured at 10 min. Ascorbic acid was used as a standard of 10–50 mg/100 ml in 50% methanol. Total antioxidants were expressed as grams of ascorbic acid equivalents per 100 g of sample on a wet weight basis.

2.7. Phenolic acids extraction and quantification

Phenolic acids in date seeds were determined according to the high-performance liquid chromatographic (HPLC) method of Alasalvar, Al-Farsi, and Shahidi (2005) with modification. A 0.5 g sample was accurately weighed into 30 ml brown glass tube and stirred in 20 ml of a mixture of methanol and 10% acetic acid (85:15, v/v) for 5 min, then ultrasonicated for 30 min. After that, 5 ml of distilled water and 5 ml of 5 M NaOH were added into the test tube, bubbled with nitrogen, sealed, and stirred using a

magnetic stirrer at room temperature for 4 h. The solution was then adjusted to pH 2, then the free and liberated phenolic acids (total) were extracted three times with 20 ml of a mixture of cold diethyl ether (DE) and ethyl acetate (EA) 1:1 (v/v), with manual shaking and centrifuging. DE/EA layers were combined, evaporated to dryness, and dissolved in 2 ml of methanol. The HPLC run was carried out after filtering the sample through a 0.45 μm PTFE filter (National Scientific Company, Rockwood, TN, USA).

Twenty microlitres of the sample and standard extracts were injected into a Prodigy ODS-2 column (250 mm × 3.2 mm, Phenomenex, Cheshire, UK). The equipment consisted of a G1311A Quat pump, G1315A diode array detector and G1322A degasser (Hewlett Packard Agilent 1100 Series, Waldbronn, Germany). Gradient elution (filtered through a 0.2 μm Nylaflo filter before use) was used with a mobile phase consisting of 50 mM phosphoric acid, pH 2.5 (solution A) and acetonitrile (solution B) as follows: 0–5 min, isocratic elution 95% A and 5% B; 5–55 min, linear gradient 80% A and 20% B; 55–60 min, linear gradient 95% A and 5% B. Flow rate of the mobile phase was 0.9 ml/min. The wavelengths of the diode array detector were set at 254, 270, 280 and 329 nm for monitoring of phenolic acids. Fourteen phenolic acid standards (gallic, protocatechuic, *p*-hydroxybenzoic, chlorogenic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, ellagic, sinapic, *m*-coumaric, *o*-coumaric and salicylic) were used for identification. Retention time, peak spectra and internal standards were used to tentatively identify phenolic acids. All phenolic acids were quantified using calibration curves for standard concentrations ranging from 1 to 100 μg/ml of methanol, and expressed as milligrams of phenolic acid per 100 g of sample on a wet weight basis.

2.8. Statistical analysis

Results were expressed as mean of triplicate determinations ± standard deviation on a wet weight basis. Statistical significance (*t*-test: two-sample equal variance, using two-tailed distribution) was determined using the Microsoft Excel Statistical Data Analysis. Differences at $p < 0.05$ were considered to be significant.

3. Results and discussion

3.1. Solvent to solid ratio

Fig. 1 shows flow diagram of phenolics and dietary fibre enriched concentrate from date seeds. Stirring was used as a mixing technique in this process as it shows the second highest efficiency to extract phenolics compared with four other techniques, sonication, shaker, reflux and accelerated solvent extraction, the later gave the highest efficiency (Luthria & Mukhopadhyay, 2006). The total phenolic content extracted from date seeds by water and 50% acetone using five different solvents to solid ratio (v/w) (10:1, 20:1, 40:1, 60:1 and 80:1) is shown in Fig. 2. Solvent to solid ratio has a positive effect in extraction by increasing total phenolics significantly ($p < 0.05$), almost linearly with solvent ratio. Phenolics extracted with water increased from 2.5 to 5.5 g/100 g with increasing solvent ratio to the 60:1 level, whereas phenolic extracted with 50% acetone increased from 5.0 to 10.1 g/100 g when solvent to solid ratio increase to 80:1. This is consistent with mass transfer principles; the driving force during mass transfer is the concentration gradient between the solid and the liquid, which is greater when a higher solvent to solid ratio is used. Similar results about the effect of solvent to solid ratio on the extraction of phenolic compounds were reported for grape pomace by Pinelo, Rubilar, Jerez, Sineiro, and Nunez (2005), dried sage by Durling et al. (2007) and milled berries by Cacace and Mazza (2003). As the extraction of phenolics are limited among the last three ratios (40:1, 60:1 and 80:1), and to minimize solvent and energy costs associated with evaporation of the solvent, the 60:1 ratio was selected as the optimal solvent to solid ratio.

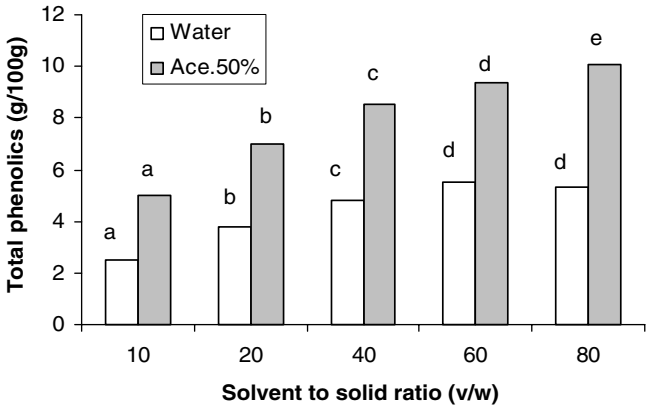

Fig. 2. Influence of solvent to solid ratio (v/w) on extraction efficiency of total phenolics from date seeds. Values are mean of three determinations, values marked by the same letter, are not significantly different ($p > 0.05$).

3.2. Extraction temperature

Fig. 3 shows the influence of temperature on extraction efficiency of total phenolics from date seeds. The solvents, water and 50% acetone showed a different pattern in phenolic extraction with extraction temperature. Water showed a steady increase in the phenolics content with temperature increases, from 5.3 to 8.2 g/100 g at 65 °C while 50% acetone increased the efficiency to 45 °C and then started to decrease. Increasing temperature favoured extraction by enhancing both the solubility of solute and the diffusion coefficient. Heating also might soften the plant tissue and weaken the phenol–protein and phenol–polysaccharide interactions in the seeds (Shi et al., 2003), thus more phenolics would distribute to the solvent. However, if 50% acetone is used for extraction, the temperature cannot go very high because acetone is volatile with a boiling point of 56.2 °C. Thus, the evaporation of acetone from aqueous-acetone solution will change the acetone–water ratio.

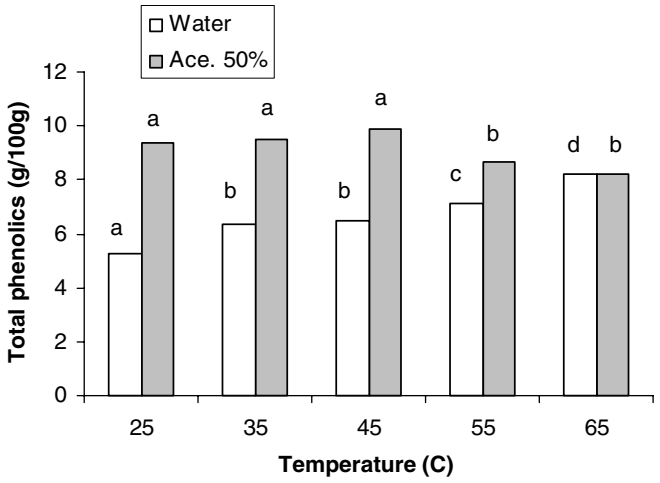

Fig. 3. Influence of extraction temperature on extraction efficiency of total phenolics from date seeds. Values are mean of three determinations, values marked by the same letter, are not significantly different ($p > 0.05$).

Heat has been found to enhance the recovery of phenolic compounds, as described by Durling et al. (2007). They found 40 °C the optimum for sage extraction. Pinelo et al. (2005) reported that 50 °C maximized the phenolic extraction from grape pomace. Shi et al. (2003) found 65 °C the best for extracting phenolics from grape seeds. Despite the positive effects of higher temperatures on the phenolics extraction, this cannot be increased indefinitely; phenolic stability and the denaturation of membranes can happen at temperatures >50 °C (Cacace & Mazza, 2003; Schwartzberg & Chao, 1982). Therefore, 45 °C was selected as optimum temperature to extract phenolics from date seeds.

3.3. Extraction time

The total phenolic compounds extracted from date seeds at different extraction times are presented in Fig. 4. There were no significant differences in phenolics extracted by water at 0.5, 1, 2, 3 and 4 h. As the extraction time was increased the total phenolics recovery remained constant. Acetone (50%) followed the same pattern as water with slight increase at 2 and 4 h extraction times. The increased extraction time and sustained exposure to high temperature throughout a large scale potentially increases the loss of solvent by vaporization, which affects the solvent to solid ratio, and increases the loss of phenolics by oxidation. Therefore, the 1 h extraction time was selected as optimum time for phenolic extraction from date seeds. Durling et al. (2007) found 3 h as the optimum extraction time for extracting phenolics from dried sage, whereas Nepote, Grosso, and Guzmán (2005) found 10 min the optimum time to extract phenolics from peanut skin.

3.4. Number of extraction

The total phenolic compounds extracted with water and 50% acetone from the date seeds in four stages are shown in Fig. 5. Heating and long extraction times may result in oxidation and decomposition of the desired compounds. Thus, multiple-stage extraction could be a good practice to minimize these problems. Phenolics extracted with water increased from 5.3 to 6.3 g/100 g after three extraction stages, with using 50% acetone, phenolics increased from 9.4 to 10.2 g/100 g after three stages. Both water and acetone efficiency decreased after four stages. As a consequence, using more than two stages is not recommended because the time and solvent required to achieve 100% extraction would not be justified by such a low difference in the yield of extraction. Shi et al. (2003) found two-stage extraction more efficient to extract phenolics from grape seeds, whereas Nepote et al. (2005) found three-stage extraction the optimum to extract phenolics from peanut skin.

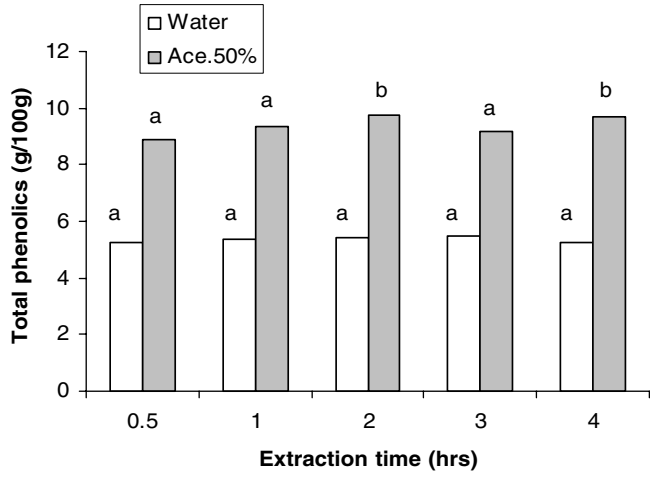

Fig. 4. Influence of extraction time on extraction efficiency of total phenolics from date seeds. Values are mean of three determinations, values marked by the same letter, are not significantly different ($p > 0.05$).

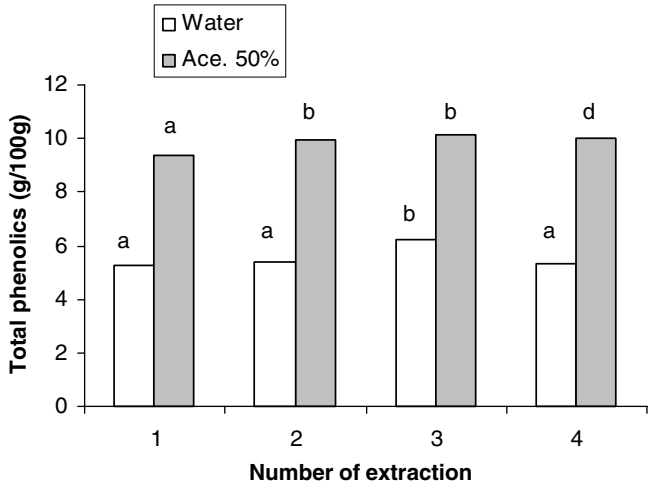

Fig. 5. Influence of number of extraction on extraction efficiency of total phenolics from date seeds. Values are mean of three determinations, values marked by the same letter, are not significantly different ($p > 0.05$).

3.5. Solvents

The selected optimum conditions for extracting phenolics from date seeds (60:1 as solvent to solid ratio, 45 °C temperature, 1 h extraction time and two-stage extraction), were used to extract phenolics with seven different solvents as shown in Fig. 6. Flavonoids and phenolics determinations were used to determine solvent extraction efficiency. Flavonoids and phenolics contents have similar extraction patterns, the lowest achieved using 100% acetone (0.74 g/100 for flavonoids and 1.5 g/100 g for phenolics) and the highest with 50% acetone (5.4 g/100 g for flavonoids and 10.3 g/100 g for phenolics). Ethanol, methanol and their aqueous 50% solutions, followed 50% acetone in the ability of extraction of flavonoids and phenolics. Similar results were reported by Tabart et al. (2007); they found 50% acetone was better than methanol, acetate or glycine buffer in extracting phenolics from black currant leaves and buds. Also, Zhao et al. (2006) reported higher phenolic extraction with 80% acetone compared to 80% ethanol, 80% methanol and water, the latter was the lowest. Acetone/water mixtures are good solvents for polar antioxidants (Lu & Foo, 2000) and more useful for extracting phenolics from protein matrices, since they appear to dissolve the phenolic–protein complexes (Kallithraka et al., 1995). Also, according to the classification of residual solvents by risk assessment, acetone is placed in class 3 solvents, which are the lowest toxic solvents to humans, compared to methanol which is in class 2, solvent to be limited (Wypych, 2001).

Water extraction in this study show low ability to extract flavonoids (2.6 g/100 g) and phenolics (5.5 g/100 g) due to their low solubility in water. Proteins and polysaccharides could be extracted at a higher temperature when water was used alone for extraction. These molecules caused filtration difficulty, compared to using aqueous acetone. Also, the cumulative cost of the concentration operation would increase since water is more difficult to remove than acetone. Therefore, acetone/water mixture could be the better extracting solvent in terms of yield and cost. However, Khokhar and Magnusdottir (2002) found water to be a better solvent for extracting Zea polyphenols than were 80% methanol or 70% ethanol. Also, Nasis-Moragher, Svanoe, & Seroy, 1999) reported a patent described the process of using hot water at high temperature and high pressure to extract procyanidins from grape seeds.

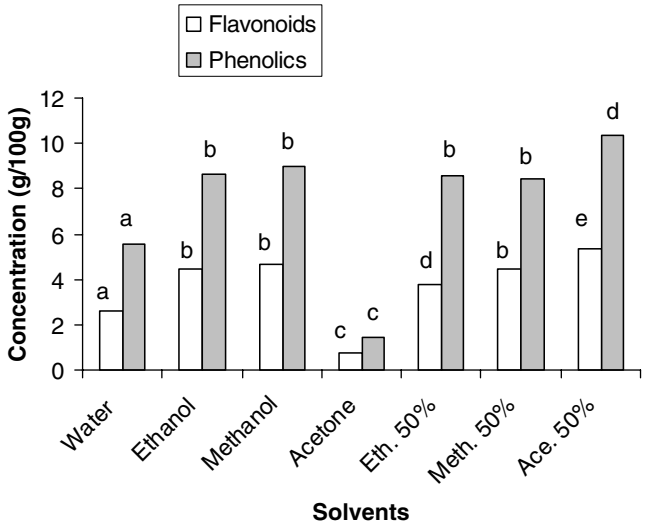

Fig. 6. Influence of solvent on extraction efficiency of phenolics and flavonoids from date seeds. Values are mean of three determinations, values marked by the same letter, are not significantly different ($p > 0.05$).

3.6. Extraction yield

Table 1 shows the dietary fibre and phenolics concentrate yields produced by extraction (water or 50% acetone) and fractionation (butanol or butanone). This amounted to four different processes; water butanol (WBL), water butanone (WBE), acetone butanol (ABL) and acetone butanone (ABE). Butanol and butanone were chosen to purify the extract after extraction because they show a high affinity to dissolve molecules containing hydroxyl groups, they have low level of toxicity (class 3) and also taking into account economic considerations, such as low evaporation temperature (Peschel et al., 2006; Wypych, 2001). There were no significant differences between dietary fibre yields produced by those four different processes, except with ABE, which has less fibre. Water extraction left more fibre (91.20–93.49 g/100 g) compared to acetone extraction (81.90–88.79 g/100 g), which extracted part of fibre along with phenolic extraction, due to acetone high extraction

Table 1
Dietary fibre and phenolic concentrates produced from date seeds

	Yield (g/100g of seed)		(g/100 g of phenolic concentrate)		
	Fibre concentrate	Phenolic concentrate	Total phenolics	Total flavonoids	Total antioxidants
WBL	93.49 ± 4.87^{a}	6.51 ± 0.27^{a}	26.79 ± 0.67^{a}	8.13 ± 0.70^{a}	1.22 ± 0.05^{a}
WBE	91.20 ± 5.29^{a}	8.80 ± 0.23^{b}	36.26 ± 0.18^{b}	15.75 ± 0.81^{b}	1.88 ± 0.07^{b}
ABL	88.79 ± 6.21^{a}	11.21 ± 0.37^{c}	14.48 ± 0.27^{c}	6.39 ± 0.30^{c}	1.01 ± 0.08^{c}
ABE	81.90 ± 4.07^{b}	18.10 ± 0.45^{d}	23.86 ± 0.58^{d}	15.93 ± 0.98^{b}	1.79 ± 0.12^{b}

WBL: Water extract of seed, followed by fractionation using butanol. WBE: Water extract of seed, followed by fractionation using butanone. ABL: Acetone 50% extract of seed, followed by fractionation using butanol. ABE: Acetone 50% extract of seed, followed by fractionation using butanone. Values are mean ± SD of three determinations on wet weight basis. Means ± SD followed by the same letter, within a column, are not significantly different ($p > 0.05$).

efficiency. In contrast, phenolic concentrate yield increased with acetone extraction (11.21–18.10 g/100 g) compared to water (6.51–8.80 g/100 g). ABE and WBE had the highest yield of phenolic concentrates, 18.10 and 8.80 g/100 g, respectively, compared to ABL (11.21 g/100 g) and WBL (6.51 g/100 g). This indicated the ability of butanone to separate higher phenolic yield compared to butanol.

Table 1 also present the total phenolics, flavonoids and antioxidants determinations of phenolics concentrate produced from date seeds. There was no general correlation between phenolics, flavonoids and antioxidants of the concentrates produced from date seeds. Although WBE and ABE had significantly higher phenolics, flavonoids and antioxidants compared to WBL and ABL. Butanone had a better ability to separate phenolics from water extract (36.26 g/100 g) than from acetone extract (23.86 g/100 g). This could be due to the high solubility of butanone in water compare to 50% acetone, which leads to better separation of phenolics. Thus butanone had achieved better yield and purity of phenolic concentrates than butanol for both the water and 50% acetone extractions.

3.7. Dietary fibre

The dietary fibre determination (soluble, insoluble an total) of date seeds and the fibre remaining after extraction of phenolics with water (WDF) and 50% acetone (ADF) are shown in Table 2. The total dietary fibre contents in date seeds was 57.87 g/100 g, whereas insoluble fibre was the major fraction (52.70 g/100 g). Al-Farsi et al. (2007) reported a higher content of total dietary fibre in three seed varieties ranging between 77.8 and 80.2 g/100 g. Also, Hamada et al. (2002) reported 64.5 to 68.8 g/100 g for total dietary fibre of three date seed varieties. These differences could be related to the stage of maturation, and varietals differences. The total dietary fibre of WDF and ADF extractions were increased significantly to 83.50 and 82.17 g/100 g, respectively, as well as their insoluble fibre to 81.97 and 81.07 g/100 g, respectively. This is clearly due to extracting phenolics as well as other components, such as protein, fat and mono- and di-saccharides with water and 50% acetone, which lead to increase the insoluble dietary fibre, (cellulose and hemicellulose). Most soluble fibre (pectins, inulin and gums) was extracted with phenolic extraction which lead to their reduction in the WDF and ADF extractions, 1.53 and 1.10 g/100 g, respectively. Thus, phenolic extraction from date seeds produced a by-product rich in dietary fibre (82–84%).

Table 2
Dietary fibre composition of date seeds, water and acetone extraction fibre

	Soluble (g/100g)	Insoluble (g/100 g)	Total (g/100 g)
Seed	5.17 ± 0.22^{a}	52.70 ± 0.35^{a}	57.87 ± 0.45^{a}
WDF	1.53 ± 0.13^{b}	81.97 ± 0.50^{b}	83.50 ± 0.17^{b}
ADF	1.10 ± 0.05^{c}	81.07 ± 0.81^{b}	82.17 ± 0.76^{b}

WDF: Dietary fibre remaining after extracting phenolics from seed by water. ADF: Dietary fibre remaining after extracting phenolics from seed by acetone. Values are mean ± SD of three determinations on wet weight basis. Means ± SD followed by the same letter, within a column, are not significantly different ($p > 0.05$).

3.8. Phenolic acid composition

Table 3 shows the total phenolic acids (free and liberated) composition of date seeds, WBE concentrate (extracted with water and purified using butanone) and ABE concentrate (extracted with 50% acetone and purified using butanone). Hydroxybenzoic and hydroxycinnamic acid derivatives in fruits are commonly present in bound forms, as esters or glycosides (Shahidi & Naczk, 1995). In order to quantify these acids strong hydrolysis treatments were needed to hydrolyze the bond between phenolic acid and the substance to which they were conjugated (glucose, quinic, tartaric, tannins, lignins). The hydrolysis transforms glycosylated and esterified phenolics into their aglycon form, which can then be detected as free phenolic acids (Shahidi and Naczk, 1995). A total of nine phenolic acids were detected in date seeds, of which four consisted of hydroxylated derivatives of benzoic acid (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid and vanillic acid) and five were cinnamic acid derivatives (caffeic acid, *p*-coumaric acid, ferulic acid, *m*-coumaric and *o*-coumaric acid). Among the identified phenolic acids in date seeds, *p*-hydroxybenzoic (9.89 mg/100 g), protocatechuic (8.84 mg/100 g), and *m*-coumaric (8.42 mg/100 g) were the major phenolic acids. In contrast, protocatechuic, caffeic and ferulic acids were the major phenolic acids in WBE and ABE concentrates. Total phenolic acids of date seeds (48.64 mg/100 g) were significantly ($p < 0.05$) increased in WBE concentrate (160.22 mg/100 g) and ABE concentrate (193.83 mg/100 g). The extraction and purifying process released and increased phenolic acids, mainly protocatechuic, caffeic and ferulic, substantially increasing their total.

For the extraction capacity and selectivity of solvent, 50% acetone significantly ($p < 0.05$) showed the highest

Table 3
Phenolic acid composition of date seeds and its concentrates

Phenolic acids	Seed (mg/100 g)	WBE (mg/100 g)	ABE (mg/100 g)
Gallic	0.28 ± 0.01^{a}	1.08 ± 0.02^{b}	13.96 ± 0.41^{c}
Protocatechuic	8.84 ± 0.18^{a}	71.71 ± 2.14^{b}	70.06 ± 3.53^{b}
p-hydroxybenzoic	9.89 ± 0.12^{a}	9.05 ± 0.32^{b}	12.98 ± 0.53^{c}
Vanillic	4.07 ± 0.21^{a}	12.92 ± 0.45^{b}	7.20 ± 0.21^{c}
Caffeic	0.18 ± 0.01^{a}	40.81 ± 1.82^{b}	58.23 ± 1.31^{c}
p-coumaric	6.07 ± 0.15^{a}	5.82 ± 0.21^{a}	12.49 ± 0.21^{b}
Ferulic	6.93 ± 0.11^{a}	12.94 ± 0.22^{b}	18.43 ± 0.32^{c}
m-coumaric	8.42 ± 0.35^{a}	5.89 ± 0.12^{b}	0.48 ± 0.02^{c}
o-coumaric	3.96 ± 0.15	nd	nd
Total	48.64 ± 2.89^{a}	160.22 ± 9.22^{b}	193.83 ± 11.29^{c}

WBE: Phenolic concentrate produced from water extraction of seed followed by butanone fractionation. ABE: Phenolic concentrate of 50% acetone extraction of seed followed by butanone fractionation. Values are mean ± SD of three determinations on wet weight basis. nd: not detected. Means ± SD followed by the same letter, within a row, are not significantly different ($p > 0.05$).

extraction capacity for gallic, *p*-hydroxybenzoic, caffeic, *p*-coumaric and ferulic, compared to water. Zhao et al. (2006) reported a similar result with extraction phenolics from barley. They found 80% acetone rendered the highest extraction for catechin, ferulic, caffeic, vanillic and *p*-coumaric, than 80% methanol, 80% ethanol and water. Furthermore, there was no significant difference ($p > 0.05$) in protocatechuic acid content between water and 50% acetone extraction from date seeds. It also noted that *o*-coumaric acid was not detected in water and 50% acetone, this could be due to oxidation during extraction process, which included evaporation and drying. The result from Table 3 shows that different solvent mixtures had significantly different extraction capacities and selectivity of phenolic acids in date seeds.

4. Conclusions

The optimum conditions for lab scale were to run extraction at 45 °C, solvent to solid ratio of 60:1, two-stage extraction each stage for 1 h is considered as the best procedure for extraction phenolics from ground date seeds. Extracting solvent significantly affected total phenolics and antioxidant contents extracted from date seeds. Acetone (50%) was the most efficient solvent for phenolic extraction; whereas, butanone was the most efficient solvent for purifying phenolic extract. The dietary fibre and phenolic concentrates obtained from this process could be used as natural additives for fortified food products to enhance their functional properties. These results are the first steps for large scale implementation process. Although, acetone is regarded as a low toxicity solvent, safety concerns associated with the use of organic solvents for extractions include solvent residues in the product, handling, and recovery of waste solvents; therefore, safer and more cost efficient techniques to separate phenolics from date seeds are required.

Acknowledgements

We would like to acknowledge the financial support from the Council for International Exchange of Scholars (Fulbright), USA. The authors also acknowledge Nancy Smith for her valuable contribution.

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