

Characteristic Analysis, Antioxidant Components and Antioxidant Activity of Date Fruits, Date Seeds and Palm Shell

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Abstract

The present study describes the chemical composition and antioxidant components of date fruits, date seeds and palm shell. Tocopherol, phenolic compounds, benzoic acid, cinnamic acid, quercetin and kaempferol of date fruits, date seeds and palm shell were determined by HPLC. Date fruits contained high amounts of total phenols 2515.6 mg/ 100 g and tocopherol 22.722 µg/ 100 g, while palm shell contained high amounts of total tannins 1728.03 mg/ 100 g. Date seeds contained high amounts of kaempferol 14.878 mg/ 100 g and protocatechuic acid 6.672 mg/ 100 g. The antioxidant activity of date fruits, date seeds and palm shell were evaluated and exhibited 91.87, 81.85 and 63.77%, respectively. Our findings demonstrate that the date fruits, date seeds and palm shell can be considered as good source of natural antioxidants.

Keywords: Date fruits; Date seeds; Palm shell; Antioxidants; Total phenols; Total tannins; Tocopherol; Kaempferol; HPLC

Introduction

Dates are produced in 35 countries worldwide and cultivated in about 2.9 million acres of land. The world production estimate of date in 2004 was 6772,068 metric tons, Kingdom of Saudi Arabia and Egypt have 13.5% and 17% of world production, respectively (FAO, 2004). The date *Phoenix dactylifera* L. is one of the most cultivated palms around the world. It is commonly found in the Afro-Asiatic dry-band, which stretches from North Africa to the Middle East [1]. This plant is considered as an important crop in arid and semiarid regions of the world. It has always played an important part in the economic and social lives of the people of these regions. The fruit of the date is well known as a staple food and composed of a fleshy pericarp and seed [2]. Date seeds may have extractible high value-added components. However, very little use is made of these components: they are discarded or used in animal feed. Little research has been undertaken on date seeds; this has focused particularly on their chemical composition [3-6]. Date seeds are known to be waste product of many industries based on the technological transformation of date fruits [7-9].

Antioxidants have become one among the most important topics in human nutrition because of high concentrations of free lipid radicals, both in food and *in vivo* after food ingestion.

Are natural antioxidants better and safer than synthetic antioxidants? Antioxidants are necessary in the Western diet as it is rich in polyenoic fatty acids, which are easily oxidized with formation of free radicals that are harmful if present in higher amounts. Consumers prefer natural antioxidants to synthetic antioxidants, mainly for emotional reasons. The common Western daily diet contains about 1 g natural antioxidants even if no natural antioxidants have been added for lipid stabilization. Their aim sources are cereals,

fruits, vegetables, and beverages. Only a part of the natural antioxidants is absorbed and used as free-radical scavengers *in vivo*. Natural antioxidants should be added to food in larger amounts than synthetic antioxidants as they are less active, but the actual activity depends very much on particular conditions and food composition. Nevertheless, the addition of additional antioxidants is still negligible in comparison with the dietary supply of native antioxidants. The safety limits of natural antioxidants are mostly not known, but they are hardly safer than synthetic antioxidants. The best protection would be to replace high-polyenoic oils in the diet with high-oleic oils, and to use alternative methods of food protection against autoxidation.

Natural antioxidants are obtained from the biological system. Human diet contains an array of different compounds that possess antioxidant activities. The most prominent representatives of dietary antioxidants are vitamin C, tocopherols, carotenoids, flavonoids, antioxidant polysaccharides and amino acids and its compounds. The need of antioxidants in food industry is components of nutraceutical food. Intake of antioxidant rich diet protects against deleterious degenerative diseases. In food system, naturally occurring antioxidant mechanism are often lost during processing or storage. This necessitates the addition of exogenous food additives either natural or synthetically produced antioxidants. Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), tert-butyl hydroquinone (TBHQ) are the frequently used synthetic antioxidant in food. However, antioxidants are effective at very low concentration; higher doses may generate toxic effects. The cytotoxicity of BHA and BHT have been found in human promyelocytic leukemia cell lines (HL-60) and squamous carcinoma cell lines [10]. This food safety issue with these synthetic antioxidants limits their use as additive.

Nowadays, the consumption of fruit and vegetables is regarded as important and good for health. Indeed, recent epidemiological studies have indicated that a high intake of fruit and vegetables is associated with reduced risk for a number of chronic diseases [11]. The recent explosion of interest in the bioactivity of the flavonoids of higher

plants is due, at least in part, to the potential health benefits of these polyphenolic compounds as important dietary constituents [12]. Thus, it is important to have a clear idea of the major phenolic families of which fruit and vegetables are comprised and the levels contained therein [13]. Vitamin E is an important natural antioxidant in foods, especially those rich in polyunsaturated fatty acids [14]. Due to its role as a scavenger of free radicals, vitamin E is also believed to protect our bodies against degenerative malfunctions, mainly cancer and cardiovascular diseases [15]. The date fruit possesses antioxidants and antimutagenic properties *in vitro* [16]. The antioxidant properties of fruits vary depending on their content of phenolic components and vitamins C and E, carotenoids, flavonoides [17]. One of the principal causes of food quality deterioration is lipid peroxidation [18]. Lipid peroxidation results in formation of reactive oxygen species and free radicals; which are purportedly associated with carcinogenesis, mutagenesis, inflammation, DNA changes, aging and cardiovascular disease [19,20]. Though vegetable oils, the ideal cooking media of the day, are beneficial and popular due to their cholesterol lowering effects, but they are more susceptible to oxidation in comparison to animal fats, which predominantly contain saturated fatty acids and hence do not react readily with other chemicals especially oxygen [21]. The aim of the present study is to: 1- explore the difference between date, date seeds and palm shell chemical composition, total phenols, total tannins, tocopherols and phenolic compounds contents. 2- Identify antioxidant components by HPLC. 3-Determine the antioxidant activity of date, date seeds and palm shell.

Materials and Methods

Materials

Date (Rozez) and palm shell were obtained from A-Ahas, date seeds was obtained from a local market in Dammam, Saudi Arabian.

Methods

Determination of crude protein, moisture, ash, oil, crude fiber and hydrolysable carbohydrate: The recommended methods of the Association of Official Analytical Chemists AOAC (1999) were adopted to determine the levels of crude proteins, moisture, ash, oil and crude fibers. Nitrogen content was determined using the Kjeldahl method and multiplied by a factor 6.25 to determine the crude protein content. Total carbohydrate content was estimated by difference of mean values, i.e., 100 (sum of percentages of moisture, ash, proteins, lipids and crude fiber) [22].

Determination of total phenols and total tannins: Total phenolic content was measured by vanillin-hydrochloric acid [23]. Tannins were determined using vanillin hydrochloric acid (V-HCl) method [24].

Determination of date fruits, date seeds and palm shell antioxidant by HPLC: Date, date seeds and palm shell phenolic compounds, benzoic acid, cinnamic acid, quercetin and kaempferol were determined by HPLC (HP 1050) according to the method [25]. Tocopherol was extracted and analyzed by HPLC according to the method [26].

Extraction of antioxidant: A weighed portion (10 g) of dried date fruits, date seeds and palm shell were extracted with 50 ml of water, ethanol, methanol and ethyl acetate for 24 h. The antioxidants from ethanol, methanol and ethyl acetate extracts were filtered and dried to dryness at room temperature. While, the extract of water antioxidant

was filtered and evaporated at 40°C to dryness in a rotary evaporator (RE 300/MS).

DPPH radical-scavenging activity: The DPPH free radical-scavenging activity of date fruits, date seeds and palm shell were measured using the method [27]. A 0.1 mM solution of DPPH in methanol was prepared. An aliquot of 0.2 mL of sample was added to 2.8 mL of this solution and kept in the dark for 30 minutes. The absorbance was immediately measured at 517 nm. The ability to scavenge the DPPH radical was calculated with the following equation:

$$\text{Inhibition percentage} = (I\%) = [(A_0 - A_1) / A_0]$$

Where A₀ is the absorbance of the control, A₁ is the absorbance in the presence of sample. All samples were analyzed in three replicates.

Statistical analysis of data: Mean values of data were obtained from triplicate determination. Values expressed are mean ± SD. Significance of differences between control and treated samples were evaluated using Duncan's multiple range tests at 5% level.

Results and Discussion

A sample of date fruits, date seeds and palm shell were subjected to chemical analysis where ash, crude proteins, crude oils, crude fibers and hydrolysable carbohydrates were determined.

Constituents	Date fruits	Date seeds	Palm shell
Ash	2.11 ± 0.011	1.30 ± 0.008	13.39 ± 0.074
Crude Proteins	4.70 ± 0.031	3.12 ± 0.017	2.66 ± 0.021
Crude Oils	1.14 ± 0.006	6.79 ± 0.036	1.06 ± 0.006
sCrude Fiber	4.64 ± 0.058	14.54 ± 0.081	51.93 ± 0.289
Hydrolyzable	88.55 ± 0.492	74.25 ± 0.437	30.96 ± 0.172
Carbohydrate			

Table 1: Chemical composition of date fruits, date seeds and palm shell on a dry weight basis. Values are mean ± SD, n=3.

Table 1 shows the approximate chemical composition of date fruits and date seeds of palm shells. Date was characterized by the high levels of crude proteins and hydrolysable carbohydrates, being about 1.5 and 1.7 times as great as that in date seeds of palm shell, respectively. On the other hand, date seeds contained high levels of crude oil, being 5.95 and 6.4 times as high as that in date fruits of palm shell, respectively. Palm shell was distinguished by having remarkable higher levels of ash and crude fibers being 6.34, 10.3 and 11.19, 3.57% times as great as that in date fruits and date seeds, respectively [28]. They found that protein in date seeds ranged from 2.3 – 6.4%, fat 5.0 – 13.2%, ash 0.9 – 1.8% and dietary fiber 22.5 – 80.2% [29] used date pits in the feeding of ruminant animals and found that crude proteins, crude fats, crude fibers and ash were in the range of 5 – 7%, 4 – 10%, 12 – 27% and 1 – 2%, respectively. Date (*Phoenix dactylifera*) fruits (DPF) contained carbohydrates (total sugars 44 – 88%), fats (0.2 – 0.5%), proteins (2.3 – 5.6%), dietary fibers (6.4 – 11.5%), minerals (0.1 to 916 mg/ 100 g date) and vitamins C, B1, B2, A, riboflavin and niacin [30-32]. Antioxidant activity is closely related to the phenolic content of plants (3). Date flesh contained 11% moisture. Also, 0.56% crude lipid, 2.16% total protein and 1.64% ash of dry matter. The total carbohydrates content was found in the range of 83.46% [33].

Samples	Aqueous extract	Ethanol extract	Methanolic extract	Acetyl acetate extract
Date fruits	16.68 ± 0.088	1.23 ± 0.006	47.43 ± 0.21	0.16 ± 0.001
Date seeds	2.39 ± 0.011	2.37 ± 0.008	12.44 ± 0.062	3.81 ± 0.012
Palm shell	3.49 ± 0.017	0.36 ± 0.001	1.87 ± 0.009	0.25 ± 0.001

Table 2: Antioxidant extracts from date fruits, date seeds and palm shells. Values are mean ± SD, n=3.

Table 2 shows that date fruits methanolic and aqueous extracts contained high amount of antioxidants and their levels were 47.43% and 16.68%, respectively. On the other hand, palm shell ethanolic and acetyl acetate extracts contained lower amount (0.36, 0.25%) of antioxidants, respectively. Palm shell aqueous extract (3.49%) gave a best result compared with ethanolic, methanolic and acetyl acetate extracts (0.36, 1.87, 0.25%), respectively. Meanwhile, methanolic date and date seeds extracts (47.43, 12.44%) gave a high amount of antioxidants compared with palm shell. This results agree with [34]. They reported that non-polar compounds were dominated in date seed, which were extracted highly in ethanol and acetone, compared to water.

Sample	Total phenols (mg/ 100 g)	Total tannins (mg/ 100 g)	Tocopherol (µg/ 100 g)
Date fruit	2515.6 ± 13.24	306.47 ± 1.61	53.92
Date seeds	730.43 ± 4.06	1565.06 ± 8.69	0.93
Palm shell	437.28 ± 2.43	1728.03 ± 10.16	31

Table 3: Total phenols, tannins and tocopherol contents in date fruits, date seeds and palm shell. Values are mean ± SD, n=3.

The total phenols and tannins of date, date seeds and palm shell were determined and the results are shown in Table 3. Data was characterized by having higher quantities of total phenols, being about 3.44 and 5.75 as high as that in date seeds of palm shell, respectively. On the other hand, palm shell contained greater amounts of tannins, being 1.10 and 5.63 times as high as that in date seeds and date, respectively. The results demonstrated that date had higher amounts of total phenols and lower amounts of total tannins. Also, palm shell contains higher amounts of total tannins and lower amounts of total phenols. These results are in accordance with those reported by [35] they mentioned that the different date fruit varieties contained phenolic compounds in the range of 2.49 ± to 8.36 ± 0.060 mg gallic acid equivalents (GAE) per 100 g fresh fruit. However, [28] reported the total phenolic content (TPC) values ranged between 172 and 246 mg gallic acid equivalents/ 100 g fresh weight of Omani date, that were closer to the TPC value of Kharak date. It has been suggested that date fruits may contain a higher level of TPC among other fresh and dried fruits [35-37]. The TPC values of two date varieties studied by Wu et al. [38] were 572 – 661 mg gallic acid equivalents/ 100 g fresh weight which were higher than those reported by [28,35]. Total polyphenols in Ajaw date fruit water extract (455.88 mg/100 g) was the highest compared to sukkari date fruit 377.66 mg/100 g and khalas date fruit 238.54 mg/100 g extracts (P ≤ 0.05). Also, total polyphenols in alcohol

extract of the three date varieties followed the same trend as in water extract. However, polyphenols in the water extract was higher significantly compared to the alcohol extract (P ≤ 0.05) [37].

Tocopherol contents of date fruits, date seeds and palm shell are shown in Table 4. It was found that date fruits contained the higher amounts of tocopherol (53.92 µg/100 g), followed by palm shell (31.00 µg/ 100 g) and date seeds (0.93 µg/ 100g). In this respect [38] reported that palm oil as a source of α-tocopherol, α-tocotrienol, γ-tocotrienol and δ-tocotrienol.

Chemical Constituents	Date fruit	Date seeds	Palm shell
Ferulic acid	9.096	7.676	–
Syringic acid	22.722	20.478	10.5
Cinnamic acid	4.802	3.513	2.2
Protocatechuic acid	5.239	6.672	–
Vaniline	0.153	0.068	38.6
Coumarin	2.504	0.663	3.7
Caffic	0.84	0.512	0.57
p- Coumaric acid	2.027	1.004	0.39
Resorcinol	1.499	0.919	12.86
Salicylic acid	13.689	7.835	2.66
Benzoic acid	0.255	0.128	_____
Apigenin	0.23	_____	_____
Quercetin	_____	_____	3.69
Kaempferol	6.688	14.878	2.42

Table 4: Analysis of the HPLC for date fruit, date seeds and palm shell phenolic compounds, benzoic acid, cinnamic acid, quercetin and kaempferol.

Table 4 shows analysis of the HPLC for date, date seeds and palm shell. The results indicated that date amounted to high level from ferulic acid (9.096 mg/ 100 g), syringic acid (22.722 mg/ 100 g), cinnamic acid (4.802 mg/ 100 g), caffic (0.840), p-coumaric acid (2.027 mg/ 100 g), salicylic acid (13.689 mg/ 100 g), benzoic acid (0.255 mg/ 100 g) and apigenin (0.230 mg/ 100 g) compare with date seeds (7.676, 20.478, 3.513, 0.512, 1.004, 7.835, 0.128, zero mg/ 100 g) and palm shell (zero, 10.500, 2.200, 0.570, 0.390, 2.660, zero, zero mg/ 100 g), respectively. On the other hand, date seeds content a high amount of protocatechuic acid (6.672 mg/ 100 g) and kaempferol (14.878 mg/ 100 g) compared with date (5.239, 6.688 mg/ 100 g) and palm shell (zero, 2.420 mg/ 100 g), respectively. Meanwhile, palm shell was content high amount of vaniline (38.600 mg/ 100 g), commarine (3.700 mg/ 100 g), resorcinol (12.860 mg/ 100 g) compare with date (0.153, 1.499, 1.499 mg/ 100 g) and date seeds (0.068, 0.663, 0.919 mg/ 100 g), respectively. The present findings are in agreement with those obtained by [39]. They said that 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,

ρ -coumaric acid, ferulic acid, m-coumaric and o-coumaric acid). Among the identified phenolic acids in date seeds, ρ -hydroxybenzoic (9.89 mg/ 100g), protocatechuic (8.84 mg/ 100 g), and m-coumaric (8.42 mg/ 100 g) were the major phenolic acids.

Sample	Antioxidant activity (mg/ ml)
Date fruit	91.87 \pm 4.83
Date seeds	81.85 \pm 4.56
Palm shell	63.77 \pm 3.35

Table 5: Antioxidant activity of date fruits, date seeds and palm shell. Values are mean \pm SD, n =3.

Date fruit, date seed and palm shell demonstrated marked antioxidant activity in the DPPH radical- scavenging assay (Table 5). The results indicated that palm shell exhibited the lowest inhibition ratio (63.77 \pm 3.35), while, date fruit have a good activity and exhibited the highest inhibition ratio (91.87 \pm 4.83). It could be resulted to the high content of total phenols and tocopherol. The antioxidant activity of seeds obtained from different date fruits changed between 78.03 (mg/ml) (Monaif) and 79.94 (mg/ml) (Barhi cv). In addition, the total phenol contents of seeds were found between 1.98 mg GAE/100 g (Barhi) and 4.65 mg GAE/100 g (Soughi cv) [40].

Conclusion

Our study concluded that the date palm fruits, date seeds and palm shall consider a good source of natural polyphenolic compounds and have high antioxidant potentials. These fruits date seeds and palm shall can be used as natural antioxidants agents for various food and food products. Tese findings may enhance our knowledge for the value of using the date palm fruits in our daily diet as a functional food. We can recommend it as valuable and healthy food and providing a wide range of essential nutrients and potential health benefits. Also, Date seeds and palm shell could potentially be used as ingredients in the production of some functional foods for human consumption through enhancing the nutritional value of several food products because they have high level of fibers.

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