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ORIGINAL PAPER

Potential of date seeds as a functional food components

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Abstract

The aim of this study was to determine proximate compositions, dietary fiber contents, technological properties and bile acid binding capacity of the date seeds of three cultivars (Safawi, Suhgai, and Mebruum) and to suggest their potential utilization as dietary fibre sources and bile acid binders. Total dietary fiber content of date seeds was in the range of 85.6–87.4% where the insoluble and soluble dietary fiber contents were in the range of 82.1–84.4% and 2.8–3.5%, respectively. Water-holding capacity ranged between 5.96 and 6.87 g/g DM for the all samples. Safawi seed contain the highest amount of total phenolic compounds (TPC) and the highest antioxidant activity. The bile acid bound by Safawi (94.11%) was significantly higher ($p < 0.05$) than the others. The higher bile acid binding capacity attributed to the hydrophobic interactions between dietary fiber fractions and bile acids. The results indicate that date seeds are more bound bile acids by providing more dietary fiber in the diet. The physicochemical and antioxidant properties of date seeds may also promote a valuable source to use as a novel food ingredient and supplemental product for health.

Keywords Date seed · Dietary fiber · Antioxidant activity · Bile acid binding capacity · Technological properties

Introduction

Recently there have been attempts to use agri-food industries by-products for economic and environmental advantages. Many by-products are commonly investigated as to be converted into valuable ingredients for commercial products [1]. These by-products have been found to contain important substances, such as, dietary fiber, phenolics, antioxidants, and other bioactive compounds known to possess many beneficial effects on human health [2, 3].

Date seeds are by-products of date (*Phoenix dactylifera* L.) serve as good sources of dietary fiber and potential antioxidant compared with most fresh, dried fruits and their by-products [2, 4]. Date seeds represent about 10–15% of the date fruits weight and have phytochemical composition as a good nutritional value. However, date seeds generally utilized as livestock feed and have very little and inadequate consumption as food [2, 3]. They contain high amount of dietary fiber as well as bioactive compounds that provide economic, nutritive and protective benefits to many industries such as cosmetic, nutraceutical and especially food [5, 6]. Dietary fibers are the part of the plants that are resistant to intestinal digestion and include polysaccharides, oligosaccharides, lignin and other non-carbonhydrate components [7]. They are classified as soluble and insoluble depending on their solubility in water. Soluble dietary fiber (SDF) has been associated to increase stomach and intestinal transit times, absorbs cholesterol or bile acids and forms gels [8]. SDF reduces cholesterol by binding the cholesterol and bile acids in the gut and promoting their excretion. Insoluble dietary fiber (IDF) has only ability to bind water not being soluble in water that has been related to promoting regular bowel movements and softening and bulking of stool in the gastrointestinal tract [9]. Dietary fibers, especially lignin had the highest impact on bile acid binding while passing in small intestine without digestion [10, 11]. Bile acids are responsible from cholesterol utilization in the human digestion tract. Binding of bile acids by dietary fiber from its circulation would promote cholesterol conversion into additional bile acids. Consequently, bile acids are synthesized from cholesterol by the liver, lowering blood cholesterol levels [12, 15].

The epidemiological studies show that consumption of dietary fiber and natural antioxidant have protective effects against cardiovascular diseases, gastrointestinal diseases,

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colon cancer and obesity [13, 14]. Increased intake of dietary fiber per day for human in diets has been found to alter gut transit time, alert the colonic microflora, increase faeces weight, lower insulin responses, decrease appetite effects, absorb toxins and bind bile acids [15]. The determination of bile acids adsorption capacity by dietary fiber is known regarding the mechanisms for the hypocholesteremic effect and an increase in the faecal loss of bile acids [11, 16, 17]. Interestingly, the date seeds have antihyperlipidemic activity like reducing plasma triglycerides, total and LDL cholesterol [18].

The objectives of this study were to determine the potential of date seeds as functional food components. The proximate compositions, antioxidant activities and in vitro bile acid binding capacity of three varieties of Madinah date seeds (Safawi, Sughai, Mabrum) were determined. The outcomes of the current study will improve the knowledge on the important functional properties of date seeds.

Materials and methods

Proximate compositions

Date seeds were obtained from date palm three cultivars (Safawi, Sughai, Mabrum) grown in Madinah, Saudi Arabia. These were purchased from a local market in Turkey. They were removed from date flesh and washed with distilled water to get rid of any adhering date flesh. The seeds of each variety were oven dried at 50 °C for 1 day and then finely ground in a hammer mill to pass through a 250 µm sieve. The dry matter, protein, lipid and ash content of date seeds were determined by using standard AOAC methods [19]. Mineral constituents (Ca, Na, K, P, Mg and Fe) were analyzed separately, using Inductively Coupled Plasma—Mass Spectrometer (Agilent 7500ce, Japan) [20]. Soluble and insoluble dietary fiber was determined according to Prosky et al. by using the Megazyme total dietary fiber analysis kit (Megazyme International Ireland Ltd, Wicklow, Ireland) [21]. Lignin content (Klason lignin) was determined gravimetrically according to Theander et al. [22].

Technological properties

Water-holding capacity (WHC) and oil holding capacity (OHC) were determined according to Bchir et al. [7]. Firstly, 1 g sample was added to 15 ml of distilled water in a 50 ml centrifuge tube and soaked overnight at room temperature. Then the mixture was centrifuged at 10,000×g for 30 min. The residue was weighted after centrifugation. WHC was expressed as gram of water per gram of sample, while OHC was determined using sunflower oil instead of distilled water, expressed as gram of oil per gram of sample. Swelling capacity (SWC) was measured with some modifications. 1 g of sample was weighed in a graduated cylinder and 10 ml of distilled water added and the mixture was left at room temperature for 24 h [1]. The volume of the sediment was recorded and SWC was expressed as ml sediment per gram of sample dry weight. Each assay was performed in triplicate.

Extraction, total phenolic contents and antioxidant activities

Total phenolic contents

0.5 g of powdered date seeds was mixed with 10 ml of 80% methanol at room temperature for 2 h and then centrifuged. Supernatant was taken and diluted 50 fold with distilled water and used for the total phenolic content and antioxidant activity analyses.

The total phenolic content of date seeds was determined according to the method of Al-Farsi et al. [13]. Briefly, 200 µl of the extract was added to 1500 µl of a 1/10 dilution of Folin–Ciocalteu reagent in water, and then 1500 µl of sodium carbonate solution (60 g/l) was added. After 90 min at room temperature and the absorbance was measured at 725 nm. The calibration curve was prepared using Gallic acid. The total phenolic compounds were expressed as Gallic acid equivalent in mg/100 g dry weight (DW).

Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power determination was carried out according to Thaipong et al. [23]. The FRAP reagent was prepared by mixing 100 ml of acetate buffer (0.3 M) at pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution 10 mM prepared in HCl (40 mM) and 5 ml of ferric chloride solution ($FeCl_3$) (20 mM). 150 µl of the extract was added to 2.85 ml of the freshly prepared FRAP solution for 30 min in the dark and then the absorbance was measured at 593 nm. The calibration curve was prepared using TROLOX (6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid) and result was expressed as TROLOX equivalent (TE) in mM/g of dry weight (DW).

DPPH radical scavenging activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was assessed with slight modifications [23]. The stock solution was prepared by dissolving 25 mg DPPH with 100 ml of methanol. Date seed extracts (150 µl) were added to 2850 µl of the DPPH solution and the mixture was waited for 3 h in the dark. Then the absorbance was measured at 517 nm. The standard curve was between 0 and 1000 mM Trolox. Absorbance response (y) of Trolox

($y = -0.4794x + 0.7959$, R2 = 0.9964) concentrations was linear. Results are expressed in µM TE/g DW.

Bile acid binding

The method given by Sayar et al. [11] was applied for the determination of bile acid binding capacity. At the end of the in vitro digestion process the mixtures were centrifuged and the supernatants were used for bile acid binding capacity, using commercial kit from Trinity Biotech plc, Bray Co. Wicklow, Ireland. Cholestyramine was used as a positive control that bind bile acids. The bile acid- binding capacity was calculated as mmol bound bile acids/100 g dry sample.

Statistical analysis

Results were expressed as mean ± standard deviation (SD) (n = 3) on an extract. Statistical significance (t-test: two-sample equal variance, using two-tailed distribution) was determined using Microsoft Excel statistical software (Microsoft Corporation, Microsoft Office Excel 2010, Redmond, WA). Differences at $p < 0.05$ were considered to be significant.

Results and discussion

Proximate compositions

Date seed compositions are shown in Table 1. Protein content ranged between 5.42 and 5.60%. Shugi had the highest protein content whereas Safawi had the lowest. Fat content ranged between 6.99 and 8.14% with Mebruum containing the lowest and Safawi had the highest fat content. The lowest contents of ash (0.81%) were determined in Safawi seeds, while Mebruum contained the highest (0.97%).

Table 1 Crude protein, fat, ash content and mineral compositions of three varieties of date seed

Composition (% DW)	Safawi	Mebruum	Shugi
Protein	5.42 ± 0.19	5.56 ± 0.17	5.60 ± 0.22
Fat	8.14 ± 0.24	6.99 ± 0.08	7.19 ± 0.16
Ash	0.81 ± 0.04	0.97 ± 0.02	0.95 ± 0.01
Mineral compositions (mg/100 g DW)			
Na (Sodium)	30.10 ± 0.25	29.66 ± 0.34	23.32 ± 0.07
Mg (Magnesium)	83.92 ± 0.41	84.63 ± 0.29	92.25 ± 0.32
P (Phosphor)	79.14 ± 0.16	89.68 ± 0.08	92.58 ± 0.14
K (Potassium)	235.35 ± 0.93	236.63 ± 0.88	294.22 ± 0.95
Ca (Calcium)	21.89 ± 0.17	32.00 ± 0.09	21.32 ± 0.19
Fe (Iron)	1.56 ± 0.04	2.57 ± 0.07	1.92 ± 0.03

All values shown are means of three determinations ± standard deviation (n = 3)

DW dry weight

The analysis of eighteen minerals content was done for date seeds. However, only the major of these are given in Table 1. Potassium was the highest mineral content in all date seeds ranged between 294.22 mg/100 g DW contained in Shugi and 235.35 mg/100 g DW in Safawi, followed by phosphorus (92.58–79.14 mg/100 g DW), magnesium (92.25–83.92 mg/100 g DW), calcium (32–21.32 mg/100 g DW) and sodium (30.10–23.32 mg/100 g DW). The iron was the lowest; it changed between 1.56 mg/100 g DW in Safawi and 2.57 mg/100 g DW in Mebruum seeds. Phosphorus and magnesium were found at highest amount in Shugi, whereas at lowest amount in Safawi. Calcium and sodium were determined at lowest in Shugi. These results are in agreement with those found with slight difference [3, 13, 24]. The difference in proximate compositions could be explained by cultivars, climate conditions, mineral contents of soil and harvested time. Our results and other studies in the literature clearly show that date seeds have significant protein, fat and mineral contents compared to date flesh [3, 25, 26]. Thus, date seeds may provide important health benefits.

Dietary fiber and lignin content of the three date seed varieties are given in Table 2. Total dietary fiber (TDF) contents in Safawi, Mebruum and Shugi were 85.63, 87.35, and 87.21% respectively. Safawi seeds contain significantly lowest amounts of TDF and IDF. Soluble dietary fiber varied between 2.84 and 3.54% for date seeds studied. The Shugi had the highest Klason lignin content (24.81%), where the Mebruum had the lowest. TDF contents in date seeds were reported for different varieties from different countries ranging between 57.87 and 80.2 g/100 g [5, 13, 27]. The results obtained from the current study were higher than previous ones given in the literature. In addition, these TDF were higher than other fruits and their by-products such as grape fruit, orange, mango, apple pomace and date pomace (28–81.1 g/100 g DM) [6, 22]. TDF of date seeds in this study include high proportions of IDF, similar to the results found [5, 7, 28]. The KL content of date seeds in this study are lower than the KL content of date fiber reported by [6] but it was relatively higher than cereals, fruit, vegetable and their by-products [29, 30]. Thus, these date seeds varieties

Table 2 Total dietary fiber (TDF), insoluble fiber (IDF), soluble fiber (SDF) and Klason lignin (KL) of three varieties of date seed

Variety	TDF	IDF	SDF	KL
Safawi	85.63 ± 0.12^{a}	82.09 ± 0.17^{a}	3.54 ± 0.07^{a}	22.24 ± 0.05^{a}
Mebruum	87.35 ± 0.23^{b}	84.38 ± 0.31^{b}	2.97 ± 0.16^{b}	21.00 ± 0.12^{b}
Shugi	87.21 ± 0.19^{b}	84.37 ± 0.23^{b}	2.84 ± 0.15^{c}	24.81 ± 0.08^{c}

All values shown are means of three determinations ± standard deviation (n = 3). The different letters in the same column were significant different ($p < 0.05$). Based on % of date seed DW could be a valuable resource of dietary fiber compared with the other varieties and most fresh, dried fruits.

In most cases, the IDF concentration was higher than the SDF concentration. Date seeds showed the greatest difference between IDF and SDF content. This large amount of IDF present in date seeds may consist mainly of celluloses, hemicelluloses and lignin. A high fraction of IDF in the date seed could be utilized as low calorie functional ingredient. Because IDF are be ability of binding water to help reducing the serum cholesterol level and functioning of the human gastrointestinal tract [1].

In addition, a high IDF content could have health-promoting effects related to increases in satiety and faecal excretion, activate intestinal function and help in digestion and stimulate the immune response; and potential reduction in the risk for colorectal cancer [1, 16]. It has been also reported that a positive relationship between BA-binding and IDF content and is related to lignin content or hemicellulose content [10]. KL, also presented high content in date seeds, have potentially important role in the mechanism of bile acid binding and are responsible the cholesterol lowering effect [10, 29].

Technological properties

Water holding capacity (WHC) is related to the porous matrix structure of the polysaccharide chains, where water is trapped and retained through hydrogen bonding [31]. As shown in Table 3, WHC ranged between 5.96 and 6.87 g/g DM; the highest value was found in Safawi and the lowest value was found in Shugi ($p < 0.05$). WHC of date seeds was not found in the literature search; therefore, compared with date flesh. Date flesh of the different cultivars showed higher WHC (15.5 g/g DW) than our results [28]. Whereas our values were close to those reported by Borchani et al. [32] who founded WHC of date flesh from eleven varieties ranged between 3.97 and 6.20 g/g DW.

Swelling capacity (SWC) is other important hydration property directly related to the cellulose components present in the fibre [21]. Safawi was ($p < 0.05$) the highest SWC (7.01 g/ml), followed by Mebruum (5.94 g/ml) and Shugi (5.89 g/ml). The SWC is related to the chemical structure of the component polysaccharides [22].

Oil-holding capacity (OHC) is also a technological property related to the chemical structure of plant polysaccharides and depends on their chemical and physical structure [1]. The oil holding capacities (OHC) were ranged between 2.08 g oil/g DW for Shugi and 2.25 g oil/g DW for Safawi, with no significant differences between them ($p > 0.05$). A similar oil-holding capacity of date flesh fiber was observed 2.3 g oil/g DM [32]. According to Martínez et al. [1] and Bchir et al. [7], the differences of WHC, SWC and OHC depend significantly on dietary fiber compositions, physical structure and particle size.

Hydration property shows the ability of dietary fiber to retain water when applied to external forces. This property could supply colon regulations such as increasing stool weight and the rate of blood cholesterol and intestinal adsorption of glucose. This by-product could also be considered utilization for food industry due to avoid the syneresis, improve the textural properties, reduce calories and provide stability during storage time.

Table 3 Technological properties of three varieties of date seed

Variety	WHC	SWC	OHC
Safawi	6.87 ± 0.12^a	7.01 ± 0.16^a	2.25 ± 0.08^a
Mebruum	6.05 ± 0.23^b	5.94 ± 0.19^b	2.11 ± 0.03^b
Shugi	5.96 ± 0.09^b	5.89 ± 0.21^b	2.08 ± 0.05^b

All values shown are means of three determinations ± standard deviation (n = 3). The different letters in the same column were significant different ($p < 0.05$)

WHC water holding capacity (g/g), *SWC* swelling capacity (g/ml), *OHC* oil holding capacity (g/g)

Total phenolic contents and antioxidant activities

Results of total phenolic contents (TPC) and antioxidant activity using two in vitro assays, FRAP and DPPH scavenging (expressed as TEAC), are presented in Table 4. Usually, these methods measure the ability of antioxidants to scavenge specific radicals, to inhibit lipid peroxidation or to chelate metal ions [1].

The highest amount on TPC (49.66 mg GAE/g DW), FRAP (1.38 mM TE/g DW) and DPPH (6.17 mM TE/g DW) were found in Safawi seed, whereas the lowest contents of TPC (29.26 mg GAE/g DW), FRAP (1.03 mM TE/g DW) and DPPH (4.84 mM TE/g DW) were determined in Mebruum seed. These results were in agreement with previous reports who found that TPC of different varieties of date range between 26.97 and 53.42 mg GAE/g of seed [12, 32, 33]. The principle of FRAP assay is the reduction of ferric to

Table 4 Total phenolic content (TPC) and antioxidant activity of three varieties of date seed

Variety	TPC[1]	DPPH[2]	FRAP[2]
Safawi	49.66 ± 0.01^a	6.17 ± 0.02^a	1.38 ± 0.01^a
Mebruum	29.26 ± 0.05^b	4.84 ± 0.03^b	1.03 ± 0.01^b
Shugi	31.17 ± 0.05^b	4.86 ± 0.04^b	1.09 ± 0.01^b

Means ± SD followed by the different letter within a column are significantly different ($p < 0.05$)

[1] Data are expressed as milligrams of gallic acid equivalents (GA) per g ± SD (n = 3) on DW

[2] Data are expressed as micromoles of Trolox equivalents (TE) per g ± SD (n = 3) on DW

Table 5 In vitro bile acid binding

Sample	Bile acid bound (mmol/100 g DM)	Binding relative to cholestyramine (%)
Safawi	7.67 ± 0.14^a	94.11
Mebruum	7.02 ± 0.45^b	86.13
Shugi	6.96 ± 0.29^b	85.39
Cholestyramine	8.15	100

Means ± SD followed by the different letter within a column are significantly different ($p < 0.05$)

ferrous iron using reagent. However, antioxidant activity of three date seed varieties were reported lower amounts, range between 10.96 mM TE/100 g DW and 22.86 mM TE/100 g DW [18]. It seems that this variation was related to different extraction system, variety and geographic origin. There are no studies regarding antioxidant activities of date seeds as measured using the DPPH method, expressed as TEAC. Because, different methods were used for the studies [4, 13], thus the results are not directly comparable.

These results indicate that dates by-products could be considered as rich sources of total phenolics and antioxidants, in comparison with fresh fruits [1, 22, 29] and dried fruits [13]. These extractable high value-added compounds could be utilized in cosmetics, pharmaceuticals and food industries. Further studies are necessary to assess the potential of their components as effective natural remedies.

Bile acid binding

Several studies indicated the potential bile acid-binding capacity of fruits, vegetables, cereals and ready to eat cereals [9, 11, 34, 35]. However, there is no information in the literature on the bile acid binding properties of date seeds. It was determined to bind bile acids using the in vitro method, in addition to be associate the potential relationships between bile acid binding capacity and functional properties. The BA binding of three varieties of date seeds, cellulose and cholestyramine on dry weight-basis are shown in Table 5. Cholestyramine, the positive control, bound 8.15 µmol BA/100 mg DW, which is equal to 86.33% of the total added BA. Relative bile acid binding were calculated by assigning the bile acid binding capacity of cholestyramine as 100%. Therefore, the relative bile acid binding of Safawi (94.11%) were significantly higher ($p < 0.05$) than Mebruum (86.13%) and Shugi (85.39%).

These BA binding values were higher and compared with those reported for fruits range between blueberries (7%), and apples (1%) [9]; green vegetables between spinach (8.6%) and cauliflower (2.4%) [35, 36]; pulse between black gram bean (35%) and lupin (19%) [12, 37]; cereal by products between wheat bran (20%) and corn bran (2.9%) [11], which could be due to differences in the in vitro bile acid binding assay, the raw materials, or proximately and phytochemical compositions. Therefore, results indicate that Klason lignin has been considered to play an important role in the mechanism of bile acid binding because it is neither digested nor absorbed in the human small intestine. The high bile acid-binding effects was based on direct binding forces, such as hydrophobicity, as known Klason lignin and correlated at increasing contents of IDF [15]. In addition, bile acid binding was not solely attributed to lignin by recent studies, but also to their phytonutrients (antioxidant compounds) present and may contribute possible synergic effects [9, 11, 12]. Binding of bile acids and increasing their fecal excretion has been hypothesized as a possible mechanism by which dietary fiber lowers cholesterol This is believed to be the mechanism by which food fractions lower cholesterol and prevent risk of cancer [9].

The physicochemical and antioxidant properties of date seeds may promote their potential utilization in functional food and supplemental products and benefits for health.

Conclusion

The dietary fibre content, antioxidant potential, functional properties and bile acid binding capacity of these varieties of date seeds from Madinah have been demonstrated for the first time. Date seed IDF: SDF ratios were found to be higher than most fruits, vegetables, cereals and their by-products. They are also good sources of Klason lignin (range of 21–24.81%). Therefore, it provides important benefits such as intestinal regulation and reduction of cholesterol. In vitro bile acid binding capacity of these date seeds relative to cholestyramine were found between 85.39 and 94.11%. This result confirms that date seed has great potential as cholesterol-lowering supplement. The higher bile acid binding capacity might be attributed to the hydrophobic interactions between dietary fibre fractions and bile acids. The antioxidant activities of date seeds studied in both DPPH and FRAP assays, increased with high total phenolic contents. These by-products show considerable levels of antioxidant capacity and may be used not only for dietary fibre enrichment and reduction of energy in food formulations, but also as ingredient in nutraceuticals, pharmaceuticals and medicine.

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