



EVALUATION OF SUGARS, PHENOLICS, CAROTENOIDS AND ANTIOXIDANT ACTIVITIES IN 22 SAUDI ARABIAN DATE CULTIVARS

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ABSTRACT

Saudi Arabia consumes a significant quantity of date fruits through traditional diets. However, information about the glucose, fructose and sucrose contents in most Saudi Arabia date cultivars at different maturity stages, especially the stage at which date consumed has not been completely reported previously. Therefore, fresh fruits of 22 Saudi Arabian date cultivars were evaluated for their sugars, phenolics, carotenoids and antioxidant activities. Most of tested date cultivars contained high amounts of glucose and fructose whereas sucrose was either not detected or present in low concentrations. Glucose and fructose comprised between 26.6%-43.1% and 25.9% -39.5% of fresh fruits, respectively. Glucose constituted more than 40% of sugars existed Mabroom, Shbib, Anbra, Um-elkashab, Um-elhamam, Barhi, Nabot Soltan, and Khalas fruits. The glucose/fructose ratios of all tested cultivars were presented in the range 1.02-1.27. Higher concentrations of sucrose were detected in the Qurawia (49.4%) and Nabtat Manea (13.3%). Total sugars content varied greatly among tested cultivars (64.4% and 80.8%) and the highest concentrations were recorded in Khalas (80.8%), Mabroom (80.3%) and Shbib (80.1%). Total phenolic content was found in the range of 215 to 514 mg gallic acid/100g, and fruits of Nabtat Manea and Metwah contain the highest concentrations (514 and 479 mg gallic acid/100g, respectively). Total carotenoids content of tested date cultivars ranged from 2.0 to 5.46 mg/kg. The antioxidant activities varied between tested cultivars, it ranged between 1.93-14.73 $\mu\text{mol Trolox/g}$ (DPPH method) and 8.28-20.19 $\mu\text{mol Trolox/g}$ (FRAP method). The highest value of antioxidant activity was recorded for Nabtat Manea, while Khalas and Anbra cultivars had the lowest values.

KEYWORDS: *Date fruit; Saudi Arabian; Sugar types; Phenolics; Carotenoids; Antioxidant activity, Cultivars*



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Received on: 26-12-2016

Revised and Accepted on: 07-03-2017

DOI: <http://dx.doi.org/10.22376/ijpbs.2017.8.2.b342-347>

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is an important and one of the oldest trees cultivated by man¹. For over 5000 years, date palm has provided food, ornament, and material for shelter, fibre, and fuel in a harsh environment where relatively few other plants are able to thrive. Thus, date palm cultivation is always blended with the entire social, cultural, religious and economical development of the people in the hot and arid regions of the world extending from Middle East to Northern Africa². According to a recent economics survey in the Kingdom of Saudi Arabia, there were 172,297 hectares date palms produce annually almost 1,122,820 tons of date fruits, which represents about 15% of the total world production³. Annual consumption of dates in Saudi Arabia was reached to about 25 kg per capita⁴. Sugars contribute the most prevalent single component. In the ancient date production countries the date has been used more as a sugar source than as a fruit due to their high carbohydrate content (44–88%)⁵⁻⁶. Borchani et al.⁷ analyzed the main chemical components of date fruits from 11 Tunisian cultivars and found that they were rich in sugar (799.3–880.2 g kg⁻¹ dry matter). Whereas, Amoros et al.⁸ found that the total sugar concentration in Caqui 24 and Caqui 22 date fruits ranged from 424 to 542 g kg⁻¹. They added that fructose and glucose are the major sugars in most date cultivars and are found almost in equal amounts. Ismail et al.⁹ reported a higher fructose concentration at the tamer stage in five different UAE date cultivars (Khalas, Barhee, Fard, Boumaan and Ruzeiz). Ali et al.¹⁰ found that the total sugar concentration in three Omani date cultivars (Khalas, Khsab and Fardh) ranged from 685.3 to 753.7 g kg⁻¹, the highest level being observed in Khalas cultivar. They observed an overall glucose/fructose ratio of 1:3 in the three test cultivars. However, sucrose was found to be the dominant sugar in many Saudi Arabia date cultivars such as Sukary and Nabtat Ali. Al-Humaid et al.¹¹ found that the concentration of fructose, glucose and sucrose were 9.17%, 12.45% and 35.94% for Sukary cultivar, 15.92%, 19.0% and 28.48% for Nabtat Ali cultivar as well as 23.39%, 26.59% and 9.27% for Rashodia cultivar, respectively. Levels of sugar types in different date cultivars affect on fruit ripening and consumer health, however, information about the glucose, fructose and sucrose contents in most Saudi Arabia date cultivars at different maturity stages, especially the stage at which date consumed has not been completely reported previously. Date fruit is renowned for the presence of many classes of bioactive components such as carotenoids, polyphenols especially phenolic acids isoflavons, lignans, flavonoids, tannins, and sterol¹². The quantity and composition of the phytochemicals present in date fruit vary widely depending on the variety, stage of maturation, storage, postharvest processing, extent of hydration, and the geographical origin of the dates^{8,13,14}. Date provides a significant source of daily dietary health affecting compounds, antioxidant, in regions that consume significant quantities through traditional diets. The interest in antioxidants has been increasing because of their high capacity in scavenging free radicals related to coronary heart disease, cardiovascular disease, cancer, aging and

neurodegenerative diseases^{15,16}. The total phenolics and antioxidant activity of Omani dates was 172–246 mg gallic acid per 100 g and 146–162 μ mol Trolox equivalents per g fresh weight, respectively¹⁷. Date fruits were found to be a good source of antioxidants components and could potentially be considered as a functional food or functional food ingredient¹⁸. Al-Humaid et al.¹¹ found that mixing camel milk with dates (2:1, v:w) increased milk antioxidant between 49-81% and enhanced the body defense against free radicals generated by the lead acetate poisoning rats¹¹. The antioxidant properties of date fruits found to be a good source of antioxidant components such as phenolics, flavonoids, carotenoids and vitamins C^{4,19,20}. Date provides a significant source of daily dietary sugars and healthy beneficial compounds, in Saudi Arabia that consume significant quantities through traditional diets. However, studies pertaining to the detailed identification, characterization, and quantification of nutritional and health affecting compounds in different Saudi Arabian date cultivars are still insufficient, detailed studies in these directions are very important. Therefore, this study was undertaken to evaluate the composition of sugars, carotenoids and phenolics as well as antioxidant activities of date fruits from 22 Saudi Arabian cultivars.

MATERIALS AND METHODS

Preparation of fruits for analysis

Mature date trees of 22 cultivars genetically different from each other, represent the most economical value for the date cultivars in Saudi Arabia, were selected for this study. Date fruits of the selected cultivars were collected and transferred immediately to the laboratory, Plant Production and Protection Department, College of Agric. & Vet. Med., Qassim Univ., Saudi Arabia. Fruit samples similar in shape, color, and degree of development were divided into groups, wiped free of dirt, weighed, stored until performing the required analysis, and then were subjected to determine nutritional, antioxidant components and antioxidant activity.

Determination of sugar constituents by HPLC

Fresh fruits (about 1g) was homogenized in 40 mL of ethanol: water (80% v/v) extraction solution. The mixture was sonicated for 15 min at 60 °C and cooled at room temperature. The solution then completed to 50 mL total volume with the extraction solution and filtered through 0.42 μ m filter. The chromatographic analyses was done in a Shimadzu high-performance liquid chromatography equipped (LC-20) with a double pump, a 7725 Rheodyne manual injector (Cotati, CA, USA) with a 20 μ L loop, a RID-6A Shimadzu refractive index detector and a C-R6A chromatopac integrator. Chromatographic separation was achieved with a Tracer carbohydrates column (5 μ m particle size; 250 mm \times 4.6 mm i.d.), and an NH₂ precolumn (13 mm \times 3 mm i.d.), both from Tracer (Teknokroma, Barcelona, Spain). Chromatographic separation was undertake with an isocratic elution mobile phase of acetonitrile–water (75:25, v/v) and degassed before use²¹. The flow-rate of this eluent is 1.8 ml/min and the volume of the sample injected was 20 μ l (filling the loop completely). Column temperature maintained at 25 °C. Peaks will identified

by comparing retention times with sugar standards. The respective peak areas were used for the quantitative analysis. Calibration curves for each sugar was prepared at seven levels, from 0.5 to 10 mg/ml for fructose, glucose and sucrose.

Determination of total phenolics

Total phenolics was determined in 70% acetone extract by using the Folin-Ciocalteu reagent and gallic acid as a standard²², and expressed as gallic acid equivalents (mg gallic acid/100 g FW).

Determination of carotenoids

Total carotenoids was extracted and determined according to the method of Talcott and Howard.²³ The sample was extracted using 25 mL acetone/ethanol (1:1, v/v) with 200 mg/L butyl hydroxyl toluene and centrifuged at 1500 g for 15 min. The supernatant adjusted to 100 mL with the extraction solvent, and absorbance at 470 nm measured using a UV-1601 Shimadzu spectrophotometer. Total carotenoids calculated according to Gross²⁴.

Determination of antioxidant activity

2,2-Diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity.

The DPPH assay was performed according to the method of Brand-Williams et al.²⁵ with some modifications. The stock solution is prepared by dissolving 24 mg 1,1-diphenyl-2-picrylhydrazyl (DPPH) in 100 ml methanol and then stored at -20 °C until needed. The working solution was obtained by mixing 10 ml stock solution with 45 ml methanol to obtain an absorbance of 1.1±0.02 units at 515 nm using the spectrophotometer. Extracts of fresh fruits with 70% acetone (750 µl) were allowed to react with 1500 µl of the DPPH solution for 5 min in the dark. Then the absorbance is recorded at 515 nm. The standard curve was done using 25 and 800 µmol Trolox. The results were expressed in µmol Trolox g⁻¹ FW.

Ferric reducing antioxidant power assay (FRAP)

The FRAP was done according to Benzie and Strain²⁶ with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g C₂H₃O₂Na·3H₂O and 16 ml C₂H₄O₂), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mM HCl, and 20 mM FeCl₃·6H₂O. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml FeCl₃·6H₂O solution and then warmed to 37 °C before use. Extracts of fresh fruits with 70% acetone (150µl) were allowed to react with 2850 µl of the FRAP solution for 30 min in the dark. The absorbance of the colored

product was recorded at 593 nm. The standard curve was done using 25 and 800 µmol Trolox and the results were expressed in µmol Trolox g⁻¹ FW.

Statistical analysis

Conventional statistical methods were used to calculate means and standard deviations. Analysis of variance and comparison of treatment means (LSD, 5% level) were performed using Stat graphics Plus Version 5.1.

RESULTS AND DISCUSSION

Sugars in date fruit

Data presented in Table 1 indicated that glucose and fructose had been detected to be present in date fruits in abundant amounts. Except Qurawia, fruits of all tested cultivars contained high amounts of glucose and fructose, whereas sucrose either not detected or present in low concentrations. In these cultivars, glucose and fructose comprised between 26.6%-43.9% and 25.9%-39.5% of the fresh fruits, respectively. Glucose constituted more than 40% of sugars existed fruits of Mabroom, Shbibbi, Anbra, Um-elkashab, Um-elhamam, Barhi, Nabot Soltan, and Khalas cultivars. The highest fructose concentrations were observed in Khalas (39.5%), Dakani (38.4%), Nabot Soltan (37.9%), Barhi (37.7%), Um-elhamam (37.1%) and Shbibbi (37.1%). In comparison, higher concentrations of sucrose were detected in the fruits of Qurawia (49.4%) and Nabtat Manea (13.3%) cultivars. Total sugars varied greatly between tested cultivars (Table 1) and ranged between 65.7% and 80.8% of fresh fruits. Khalas (80.8%), Mabroom (80.3%) and Shbibbi (80.1%) cultivars had the highest sugar concentrations. Whereas, the total sugars content of Nabtat Manea, Romana and Metwah cultivars did not reach 70% of date fruit. As shown in Table 1, glucose/fructose ratios of examined date cultivars were presented in the range 1.02-1.26. Um-elkashab (1.26), exhibited the highest ratios, followed by Romana (1.24), Mabroom (1.21), Shbibbi (1.21) and Qurawia (1.20). While, the lowest ratio (1.02) represented Khalas, Dakani, Nabtat Manea and Safawi cultivars. The observed variations in sugar contents between examined Saudi Arabian date cultivars were previously mentioned for Tunisian and Moroccan cultivars by Saafi et al.²⁷; Hasnaoui et al.²⁸ Presence of great amounts of sugars (65.7%-80.8%) in tested cultivars, mainly in glucose and fructose forms are in general agreement of those observed by Ali et al.¹⁰. They found that the total sugars in three Omani date cultivars (Khalas, Khsab and Fardh) ranged from 68.53 to 75.37%, the highest level being observed in Khalas cultivar.

Table 1
Sugar contents (g/100g FW) in different date fruit cultivars

Cultivars	Glucose	Fructose	Sucrose	Total sugars	Glucose/fructose ratio
Khalas	40.4±1.8	39.5±2.2	0.7±0.1	80.8±4.3	1.02
Fankha	37.9±1.8	36.7±1.5	1.0±0.1	76.5±3.9	1.03
Sagal	37.7±1.2	36.7±1.9	Nd	74.5±3.4	1.03
Dakani	39.1±1.7	38.4±1.9	0.7±0.1	78.2±4.1	1.02
Korasi	35.6±1.4	34.0±1.4	3.6±0.2	73.3±3.6	1.05
Barhi	40.7±2.6	37.7±1.9	Nd	78.4±4.3	1.08
Um-elhamam	41.2±2.7	37.1±1.9	Nd	78.2±4.6	1.11
Rizizi	37.7±1.1	34.9±1.7	2.1±0.1	74.6±3.7	1.08
Menifi	39.6±2.2	35.7±2.1	0.3±0.0	75.5±4.6	1.11
Nabtat Manea	26.6±1.9	25.9±1.1	13.3±0.9	65.7±4.0	1.02
Nabot Soltan	40.7±2.8	37.9±1.9	Nd	78.6±4.3	1.07
Lebana	35.0±1.9	31.6±1.9	6.8±0.3	73.4±3.6	1.11
Anbra	41.8±2.3	36.5±2.1	0.6±0.1	78.9±4.8	1.15
Romana	36.9±2.1	29.8±1.6	2.0±0.0	68.7±3.9	1.24
Eidia	39.9±2.4	34.3±1.9	2.3±0.1	76.5±4.6	1.16
Safawi	35.1±2.1	34.5±1.6	0.7±0.1	70.3±4.0	1.02
Metwah	34.6±1.9	32.5±1.2	Nd	67.1±3.5	1.06
Mabroom	43.9±2.9	36.4±1.8	Nd	80.3±4.9	1.21
Shbibbi	43.1±2.4	37.1±1.7	Nd	80.1±4.6	1.21
Sefri	39.3±2.7	33.5±1.9	Nd	72.8±3.9	1.17
Um –elkashab	41.7±2.8	33.2±1.7	Nd	74.9±4.4	1.26
Qurawia	12.5±0.9	10.4±0.8	49.4±2.8	72.3±4.1	1.20
Mean	37.3	33.8	-	75.0	1.11
LSD _{5%}	2.6	2.7	-	6.9	0.16

Moreover, Al- Farsi & Lee¹²; Vayalil²⁹ mentioned that in the most date cultivars, the major sugars present was glucose and fructose in almost equal amounts, which do not require enzyme systems to digest and can be readily absorbed into the circulation. Meanwhile, the ratio glucose/fructose may be of interest because fructose is about twice as sweet as glucose. This ratio of 22 examined cultivars ranged between 1.02-1.26, and are in agree with the previous studies^{9,30}. These results showed that the sucrose was the major sugar in fruits of Qurawia cultivar. In line with these results, Al-Humaid et al.¹¹ found that sucrose was considered the dominant sugar in many Saudi date cultivars such as Sokari (35.94%) and Nabtat Ali (28.48%), which is most likely due to low invertase activity compared to other date cultivars.

Total phenolic contents

Great differences in the total phenolics were observed between date cultivars (Table 2). Among 22 tested

cultivars, phenolics in fresh dates ranged between 235-514 mg gallic acid/100g. The highest values of phenolics were observed for Nabtat Manea (514 mg gallic acid/100g), followed by Metwah (479 mg gallic acid/100g), Korasi (450 mg gallic acid/100g) and Safawi (425 mg gallic acid/100g). The least phenolic contents exhibited Qurawia (235 mg gallic acid/100g), Eidia (241 mg gallic acid/100g) and Anbra (244 mg gallic acid/100g). These results showed that the levels of phenolics in date fruits grown in Saudi Arabia are in agreement with those of Oman dates^{17,18}, Bahrain dates⁴ and Tunisia dates³¹. Its worthy to mention that these data indicated that date fruit was considered as a good source of phenolics and some of date cultivars had much higher total phenolic content than many fruits and vegetables³². Extremely high levels of phenolics in dates may be due to greater exposure to extreme temperatures and sunlight compared to other fruits³³.

Table 2
Phenolics and carotenoids contents and antioxidant activity of date cultivars.

Cultivars	Phenolics (mg gallic acid/100g FW)	Carotenoids (mg/kg FW)	Antioxidant activities		(µmol Trolox/g FW)
			DPPH Assay	FRAP Assay	
Khalas	290±12	4.04±0.17	1.93±0.12		8.89±0.27
Fankha	336±15	4.92±0.11	3.33±0.21		10.59±0.35
Sagal	346±10	4.24±0.12	4.00±0.14		11.03±0.33
Dakani	398±16	4.76±0.15	5.59±0.22		15.06±0.31
Korasi	450±17	5.64±0.17	9.75±0.36		16.36±0.19
Barhi	339±19	3.80±0.16	3.97±0.13		11.90±0.25
Um-elhamam	373±12	3.00±0.11	4.20±0.22		10.12±0.22
Rizizi	355±19	3.44±0.14	5.89±0.29		11.76±0.21
Menifi	309±15	3.22±0.16	4.31±0.17		8.28±0.12
NabtatManea	514±21	3.28±0.19	14.73±0.34		20.19±0.33
Nabot Soltan	315±16	3.52±0.18	3.86±0.18		11.26±0.22
Lebana	268±14	2.00±0.13	4.25±0.21		10.92±0.18
Anbra	244±15	2.72±0.14	2.82±0.14		9.55±0.17
Romana	323±12	3.24±0.15	4.31±0.19		12.70±0.14
Eidia	241±12	3.04±0.19	3.71±0.16		10.86±0.22
Safawi	425±16	3.40±0.19	7.69±0.23		17.77±0.34
Metwah	479±15	4.92±0.18	8.79±0.13		18.87±0.33
Mabroom	321±18	5.56±0.22	4.45±0.16		15.27±0.23
Shbib	305±11	4.00±0.19	4.38±0.12		15.10±0.22
Sefri	323±12	2.64±0.17	5.51±0.19		13.23±0.22
Um -elkashab	281±18	5.30±0.22	5.31±0.17		12.52±0.23
Qurawia	235±19	3.08±0.19	4.52±0.16		11.65±0.15
Mean	340	3.81	5.33		12.86
LSD _{5%}	16	0.23	0.18		0.43

Total carotenoid contents

As shown in Table 2, total carotenoids content of 22 date cultivars was in the range of 2.0 to 5.64 mg/kg fresh weight. The highest amounts of carotenoids were obtained in Korasi (5.64 mg/kg), Mabroom (5.56 mg/kg) and Um-elkashab (5.3 mg/kg). In contrast, the carotenoids content in the date fruits of Lebana, Anbra and Sefri cultivars did not reach 3 mg/kg. Results of this study are in agreement with the values given in the reference. Analysis of total carotenoids found in date fruit had revealed that dates was rich in carotenoids, it ranged between 2.2 mg/kg and 30 mg/kg depending on maturity, storage, postharvest processing, extent of hydration, experimental conditions used for the analysis, and the geographical origin of the dates and date cultivar^{18,20,34}.

Antioxidant activities

Antioxidant activities of 22 date cultivars were assayed by DPPH and FRAP methods, the results are presented in Table 2. Great differences in antioxidant activity were observed between tested cultivars, and these differences followed phenolic differences. Antioxidant activities ranged between 1.93-14.73 µmol Trolox/g FW as assayed by DPPH method as well as between 8.28-20.19 µmol Trolox/g FW as assayed by FRAP method. In general, assaying antioxidant activity of 22 date cultivars with DPPH nearly had the similar trend of those of FRAP (Table 2). The highest antioxidant activities were recorded for Nabtat Manea (14.73 and 20.19 µmol Trolox/g, respectively), followed by Korasi (9.75 and 16.36 µmol Trolox/g, respectively), Metwah (8.79 and 18.87 µmol Trolox/g, respectively) and Safawi (7.69 and 17.77 µmol Trolox/g, respectively). In contrast, among the tested cultivars, the lowest antioxidant activities were possessed fruits of Khalas (1.93 and 8.89 µmol Trolox/g, respectively) and Anbra (2.82 and 9.55 µmol Trolox/g, respectively). The interest of antioxidants has been increasing because of their high capacity in scavenging free radicals related to coronary heart

disease, cardiovascular disease, cancer, aging and neurodegenerative diseases¹⁵. The obtained results indicated that date fruit provides a significant source of daily dietary health affecting compounds, antioxidant, in regions that consume significant quantities through traditional diets. Guo et al.³⁵ reported that dates had the second highest antioxidant value of 28 fruits commonly consumed in China. Moreover, Al-Farsi et al.¹⁸ found that date fruit was considered a good source of antioxidants components and could potentially be used as a functional food or functional food ingredient. In a previous study, it was found that mixing camel milk with dates (2:1, v:w) increased milk antioxidant between 49%-81% and enhanced the body defense against free radicals generated by the lead acetate poisoning rats.¹¹ Our results indicated that antioxidant activity nearly had the similar trend of phenolic contents. A linear relationship between antioxidant activity and total phenol content of date fruit was reported by Biglari et al.³⁶ The high antioxidant properties of date fruits may be resulted from its high contents from antioxidant components such as phenolics, flavonoids, carotenoids and vitamins C^{4,19-20}.

CONCLUSION

The above results confirmed that most Saudi date cultivars contained high amounts of sugars mainly in glucose and fructose forms. Beside sugars, the fruit contained a relatively high amount of antioxidant components such as phenolics and carotenoids possessing antioxidant activity through scavenging free radicals as demonstrated by DPPH and FRAP methods. Date cultivars varied quantitatively and qualitatively in their contents of sugars, phenolics, carotenoids and antioxidant activities. In general, date fruits of Saudi Arabian cultivars are considered as a good source of daily dietary sugars and antioxidants for Saudi Arabian people, who consumed significant quantities through traditional diets.

ACKNOWLEDGMENT

The authors thank Saleh Kamel, Chair for Date Palm Research, Qassim University, Saudi Arabia for his financial support.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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We sincerely thank the above reviewers for peer reviewing the manuscript