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Nutritional composition and biochemical characteristics of five date palm fruit (*Phoenix dactylifera* L.) varieties at the Khalal stage grown in Kuwait.

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Abstract

Palm dates are among the most highly cultivated and consumed fruits in Kuwait and other Arabian Gulf countries. However, data on the nutritional content of Kuwaiti date fruits remain scarce. The purpose of the current study was to determine the nutritional composition and biochemical characteristics of five date palm fruit varieties at the Khalal stage grown in Kuwait. Samples of five different cultivars (Berhi (samples from 2 different locations), Ikhlas, Saamaran, and Khanizi) grown in Kuwait were analyzed for energy, protein, carbohydrates, sugars, antioxidants, and micro-minerals. Varieties studied had high energy values ranging between 351.63 kcal/100g and 368.35 kcal/100g. They were rich in potassium (4450-7128mg/100g), with significant amounts of calcium (287.8-469.1mg/100g), magnesium (130.3 -294.5 mg/100g), sodium (70.7-123.3 mg/100g), iron (2.45-3.2 mg/100g) and manganese (0.7-1.55 mg/100g). Gallic (19.97-5.47 mg/100g), chlorogenic (0-0.712mg/100 g), and ascorbic (0.624-0.875 µg/1mg) acids were the main antioxidants. The data indicate that the Kuwaiti date fruit is a rich source of essential nutrients making it an important local product that requires further investigations for its nutritional properties and industrial utilization prospects.

Keywords: Date fruits; nutrition content; antioxidant, khalal stage, Kuwait

1. Introduction

Date palm (*Phoenix dactylifera L.)* is a monocotyledon from the *Palmaceae* family. The date palm fruit is mainly cultivated in the Middle Eastern countries mostly Egypt, Iran, Saudi Arabia, Algeria, Iraq, Pakistan, Oman, United Arab Emirates, Tunisia and Libya (Eid *et al*., 2014). The fruit maturation can be divided into five stages: hababouk, kimri, khalal (or besser), rutab, and tamar, respectively, with different characteristics at each stage (Abdul-Afiq *et al*., 2013).

The date fruit contains naturally occurring compounds that exhibit antibacterial and antioxidant activities (Samad *et al*., 2016), mainly due to the presence of significant amounts of polyphenolics, anthocyanin and other bioactive compounds. Samad *et al*., (2016) reported that the methanolic extract of Ajwa demonstrated an antibacterial activity against four different types of bacteria, indicating that dates can be utilized as a natural antibacterial compound.

Date palm fruit has received special attention from the nutritional and therapeutic perspective. They are known as rich sources of sugars and thus considered a high energy food source (Al-Shahib *et al*., 2003). Furthermore, dates are a rich source of dietary fiber.

Date palm has been a major agricultural crop in Kuwait for over 90 years. Approximately 601,563 trees are planted in 4,181 registered farms located in three main regions of Kuwait: Abdhally, Wafra and Sulaibia. Cultivation area has increased progressively from 870 ha in 1998 to 5,099 ha in 2011 with an increase in annual fruit production from 6,662 mt in 1998 to 33,562 mt (FAO, 2013). However, data on nutritive as well as bioactive compounds of the date palm varieties grown in Kuwait are scarce.

The purpose of the current study, therefore, was to determine the nutritional composition and biochemical characteristics of five date palm fruit varieties at the Khalal stage grown in Kuwait.

2. Material and Methods

2.1. Sample

Fresh date fruits grown in Kuwait were donated by farms located in Wafra, Sabhan, Sabah area and Abdali, which are the main date plantation areas in Kuwait. Five samples were obtained from the varieties Berhi (samples from 2 different locations), Ikhlas, Saamaran, and Khanizi. Samples were freeze-dried and stored under -40^{O}C until analysis.

2.2. Ash content

Ash was determined using the standard laboratory method for ash determination by ashing in a furnace at 550°C for 6 hr.

2.3. Energy content

Samples were ground into powder and analyzed in Parr Bomb 6400 Calorimeter (Parr Instrument, IL. USA) in which the energy values were determined automatically.

2.4. Macronutrient analysis

2.4.1. Total protein

Nitrogen content was determined using 0.1 g of powdered sample from each cultivar in Elemental Analyzer (Leco Truespec, St. Joseph, MI. USA). Total protein was calculated from nitrogen content using the equation %N x 6.25 (Habib and Ibrahim, 2011).

2.4.2. Total carbohydrates and sugars

A 1.5 g ground sample of each cultivar was mixed with 7.5 ml of HPLC grade water. Reaction mixtures were sonicated for 30 min at 40°C and centrifuged at 8000 rpm for 15 min at 10°C. The supernatant was filtered through 0.45 µM membrane filter, and a 20 µl sample of filtrate was injected into Agilent 1260 Infinity II HPLC (Agilent, Santa Clara, CA, USA), equipped with a RI detector, and connected with Thermo Scientific™ HyperREZ™ XP Carbohydrate column Pb++ (7.7 id x 300 mm, particle size: 8 microns). The mobile phase was DI water at a flow rate of 0.600 ml/min at 80°C.

2.5. Micronutrient Analysis

A one g sample of each cultivar was cold-digested with HNO_3 overnight, followed by further digestion with HNO_3, HCl, and 30% H_2O_2 at 95°C. Prepared samples were then analyzed with Inductively Coupled Plasma – Optical Emission Spectrometer (ICP-OES) Optima 7300 (Perkin Elmer, Waltham, MA, US).

2.5.1. Ascorbic Acid

A 0.5 g of powdered sample was mixed with 30 ml of 70:30 v/v methanol-water extraction solvent. The mixture was sonicated for 20 min at 27±3°C, diluted up to mark, filtered through a 0.45 µm membrane, and injected into Nexera X2 UFLC HPLC (Shimadzu, Japan). The column was Zorbax SB-C18 (4.6 x 250 mm, 5 microns), with flow rate of 1 ml/min, UV detector at 254nm, mobile phase A – 25mM NaH_2PO_4 pH 2.5 and Mobile phase B – Methanol.

2.5.2. Phenolics

A five-gram sample of each cultivar was mixed with 25 g of methanol. The reaction mixture was stirred for 24 hours. The supernatant was filtered using Whatman No. 1 filter paper and stored at 4°C until analysis. A sample of 20 microliters were injected into Nexera X2 UFLC HPLC system (Shimadzu, Japan) equipped with a UV at 254 nm detector and connected with Waters Nova-pak C18 column (4.6 x 150 mm, 5 microns). The mobile phase was 0.4% phosphoric acid: acetonitrile: methanol (80:10:10), flow rate: 0.7 ml/min, ambient temperature, and isocratic elution.

2.6. Statistical analysis

Statistical analysis was performed using IBM SPSS (version 25.0). Multivariate Analysis of variance (MANOVA) and Post Hoc LSD tests ($p \leq 0.05$) were performed to evaluate the influence of cultivar on nutritional composition of the date samples and to determine significant differences between means.

3. Results

3.1 Energy, protein, and ash

Table 1 shows the data for calories, protein, and ash in the date samples. Energy values ranged from 351.63 to 368.35 kcal/100 g. Berhi from Wafra farm had the highest energy (368.3 kcal/100 g), followed by Khanizi from Wafra (367.9 kcal/100 g), Berhi from Sabah Area (365.2 kcal/100 g), Ikhlas from Wafra (364.29 kcal/100 g), and Saamran from Sabhan (351.6 kcal/100 g). The difference between the first two varieties was not statistically significant while Saamaran from Sabhan was significantly ($p \leq 0.05$) different from the rest.

The highest protein content was in cultivars from Wafra farms: Berhi (4.77% ± 0.01), Khanizi (4.23% ± 0.01), and Ikhlas (4.11% ± 0.04). Berhi from Sabah Area has 3.56% ± 0.05 protein, with the least being in Saamaran from Sabhan farms (2.82% ± 0.21). All differences were significant ($p \leq 0.05$) across all samples. This result could possibly indicate that cultivation location, and/or related factors such as soil, weather, and farming techniques may have a significant effect on protein content, possibly even more than cultivar.

Saamran contained the least value of ash (2.08% ± 0.12), with the highest being that of Ikhlas from Wafra farms (6.84% ± 0.06). All the differences were statistically significant ($p \leq 0.05$) between cultivars.

Table 1. Energy, Protein, and Ash contents of five varieties of dates at Khalal stage

Cultivar (Area)	Kcal/100 g[1]	% Protein	% Ash
Berhi (Sabah Area)	365.2 ±1.86^{b,c}	3.56 ± 0.05^{b}	4.56 ± 0.17^{b}
Berhi (Wafra)	368.4±0.85^{d}	4.77 ± 0.01^{d}	4.88 ± 0.54^{b}
Ikhlas (Wafra)	364.3±0.17^{b}	4.11 ± 0.04^{c}	6.84 ± 0.06^{c}
Khanizi (Wafra)	367.9±0.17^{c,d}	4.23 ± 0.01^{c}	4.28 ± 0.16^{b}
Saamaran (Sabhan)	351.6±0.17^{a}	2.82 ±0.21^{a}	2.08 ± 0.12^{a}

[1]Weight of edible portion of dried Khalal.
Values are shown as mean ± standard deviation of two replicates.
Means within a column with different letters differ significantly ($p \leq 0.05$).

3.2. Sugars

Table 2 shows the data for the different sugars in the date samples analyzed. All cultivars were high in fructose and glucose, in equal proportions, and had non-detectable sucrose. The variations in sugar content across cultivars in this study was high and significant ($p \leq 0.05$). The highest cultivar in glucose, fructose, and total sugars was Berhi from Wafra (34.4 g/100 g, 33.6 g/100 g, 68.0 g/100 g, respectively), followed by Khanizi from Wafra (29.6 g/100 g, 30.35 g/100 g, 60.1 g/100 g, respectively), Saamaran from Sabhan (25.2 g/100 g, 25.7 g/100 g, 50.9 g/100 g, respectively), Berhi from Sabah area (22.5 g/100 g, 20.9 g/100 g; 43.4 g/100 g, respectively). The glucose/fructose ratios across all samples were close to 1, indicating almost equal amounts of glucose and fructose were present.

Table 2. Sugar contents of five varieties of dates at Khalal stage

Cultivar (Area)	Sucrose (g/100 g)[1]	Glucose (g/100 g)[1]	Fructose (g/100 g)[1]	Glu/Fru	Total Sugars (g/100 g)[1]
Berhi (Sabah Area)	0000^{a}	22.5 ± 0.3^{b}	20.9 ± 0.2^{b}	1.07	43.4 ± 0.5^{b}
Berhi (Wafra)	0000^{a}	34.4 ± 0.5^{e}	33.6 ± 0.4^{e}	1.02	68.0 ± 0.9^{e}
Ikhlas (Wafra)	0000^{a}	19.7 ± 0.05^{a}	18.9 ± 0.09^{a}	1.04	38.6 ± 0.06^{a}
Khanizi (Wafra)	0.22 ± 0.4^{a}	29.5 ± 0.6^{d}	30.4 ± 0.3^{d}	0.97	60.1± 1.3^{d}
Saamaran (Sabhan)	0000^{a}	25.2 ± 0.1^{c}	25.7 ± 0.1^{c}	0.98	50.9± 0.2^{c}

[1]Weight of edible portion of dried Khalal.
Values are shown as mean ± standard deviation of four replicates.
Means within a column with different letters differ significantly ($p \leq 0.05$).

3.3. Minerals

Table 3 shows the data for mineral content of the dates varieties studied. The highest mineral in all cultivars was potassium, followed by calcium, magnesium, and sodium, with trace amounts of iron and manganese. All other minerals tested were below detection limits. Minerals commonly found in other cultivars, such as copper, zinc, phosphorous and selenium, were not detectable in the samples of this study. This can be attributed to either the nature of the cultivars, the maturation stage, the nature of Kuwaiti soil and farming conditions, or the detection limits of the methods employed.

Potassium was found in high amounts, ranging from 7128 ± 103 mg/100g (Khanizi, Wafra) to 2786 ± 76.4 mg/100g (Saamaran, Sabhan). The second most abundant mineral was calcium, ranging from 469.10 ± 4.38 mg/100 g (Khanizi, Wafra) to 287.8 ± 7.92 mg/100g (Berhi, Wafra).

Table 3. Mineral content of five varieties of dates at Khalal stage

Cultivar (Area)	K (mg/100 g)[1]	Ca (mg/100 g)[1]	Mg (mg/100 g)[1]	Na (mg/100 g)[1]	Fe (mg/100 g)[1]	Mn (mg/100 g)[1]	% of fruit weight
Berhi (Sabah Area)	4485±3.54 ^{b}	331.0±1.98^{c}	229.2± 1.06^{b}	103.2±0.28^{b}	2.85±0.071^{a,b}	1.2±0.00^{c}	5.2
Berhi (Wafra)	4450±48.1 ^{b}	287.8±7.92^{a}	217.2±3.47^{b}	70.7± 2.62^{a}	2.80±0.141^{a,b}	1.55±0.071^{d}	5
Ikhlas (Wafra)	5506±77.1^{c}	292.55±3.46^{a}	130.3± 2.33^{a}	123.3±1.56^{c}	2.45±0.071^{a}	0.90±0.00^{b}	6
Khanizi (Wafra)	7128±103.2^{d}	469.10± 4.38^{b}	294.5±4.67^{c}	100.9±2.26^{b}	3.2±0.28^{b}	1.20±0.00^{c}	8
Saamaran (Sabhan)	2786±76.4 ^{a}	299.0±0.00^{a}	137.2±5.8^{a}	76.4±0.92^{a}	3.1±0.14^{b}	0.70±0.00^{a}	3.3

[1]Weight of edible portion of dried Khalal.
Values are shown as mean±standard deviation of two replicates.
Means within a column with different letters differ significantly ($p \leq 0.05$).

Magnesium values decreased from 294.5±4.67 mg/100g (Khanizi, Wafra) to 229.2±1.06 mg/100g (Berhi, Sabah Area) to 217.2±3.47 mg/100g (Berhi Wafra) to 137.2±5.8 mg/100g Saamaran Sabhan and finally to 130.3±2.33 mg/100g (Ikhlas, Wafra). Ikhlas, Wafra, had the highest sodium content (123.3±1.56 mg/100g), followed by Berhi from Sabah Area (103.2±0.28 mg/100g), Khanizi from Wafra (100.9±2.26 mg/100g), Saamran from Sabhan (76.4± 0.92 mg/100g), and finally Berhi from Wafra (70.7±2.62 mg/100g).

Macro-mineral differences between the samples were significant. Iron ranged from 3.2 ±0.283 mg/100g (Khanizi, Wafra) to 2.45±0.071 mg/100g (Ikhlas, Wafra), but the differences between cultivars was mostly insignificant. Manganese ranged between 1.55±0.07 mg/100g (Berhi, Wafra) and 0.70±0.00 mg/100g (Saamaran, Sabhan), with cultivars varying significantly, except for Khanizi (Wafra) and Berhi (Sabah Area). Khanizi (Wafra) was the richest cultivar in minerals, except in sodium and manganese. The difference between it and other Wafra-based cultivars was significant, possibly indicating that cultivar had a larger effect on mineral composition than soil content and farming conditions.

3.4. Antioxidants

3.4.1 Phenolics

Table 4 shows the data for phenolic content of the date samples. Among the phenolic acids, gallic acid was the highest in the samples, followed by chlorogenic acid, while caffeic acid and coumaric acid were below detection limits. The highest amount of gallic acid was 19.97±3.52 mg/100g (Khanizi, Wafra), followed by 11.9±3.2 mg/100g (Berhi, Sabah Area), 9.59±0.83

mg/100g (Ikhlas, Wafra), 8.98±0.62 mg/100g (Berhi, Wafra) and 5.47±0.151 mg/100g (Saamaran, Sabhan). Only Khanizi was significantly different from the rest.

Table 4. Values of phenolic content of the five varieties of dates at Khalal stage

Cultivar (Area)	Gallic Acid (mg/100 g)	Chlorogenic acid (mg/100 g)	Caffeic acid (mg/100 g)	Coumaric Acid (mg/100 g)
Berhi (Sabah Area)	11.9±3.20^{b}	0.71±0.006^{b}	0.0±0.0	0.0±0.0
Berhi (Wafra)	8.98±0.62^{a,b}	0.12±0.01^{c}	0.0 ± 0.0	0.0 ± 0.0
Ikhlas (Wafra)	9.59± 0.83^{a,b}	0.064± 0.01^{b}	0.0 ± 0.0	0.0 ± 0.0
Khanizi (Wafra)	19.97± 3.52^{c}	0.17± 0.002^{d}	0.0 ± 0.0	0.0 ± 0.0
Saamaran (Sabhan)	5.47± 0.15^{a}	0.0± 0.0^{a}	0.0 ± 0.0	0.0 ± 0.0

Weight of edible portion of dried Khalal.
Values are shown as mean ± standard deviation of three replicates.
Means within a column with different letters differ significantly ($p \leq 0.05$).

Berhi (Sabah Area) was significantly the highest in chlorogenic acid (0.71 ± 0.006 mg/100g), followed by Khanizi (Wafra) (0.17 ± 0.00263 mg/100g), Berhi (Wafra) (0.12± 0.01 mg/100g), Ikhlas (Wafra) (0.064± 0.01 mg/100g), and the lowest was Saamaran, which was below detection limits. Caffeic acid and coumaric acid were also below detection limits.

3.4.2. Ascorbic acid

Table 5 shows the values for ascorbic acid. Ascorbic acid ranged from 0.88±0.03 μg/mg (Khanizi, Wafra) to 0.62±0.008 μg/mg (Berhi, Sabah Area). Ikhlas (Wafra) had 0.75± 0.02 μg/mg, Saamaran (Sabhan) had 0.72 ± 0.1 μg/mg, and Berhi (Wafra) had 0.71± 0.03 μg/mg. All difference between cultivars were significant. ($p \leq 0.05$).

Table 5. Ascorbic acid content of five date varieties at Khalal stage

Cultivar (Area)	μg/mg	mg/100 g[1]
Berhi (Sabah Area)	0.62 ± 0.008^{a}	6.2E-6 ± 8.1E-8^{a}
Berhi (Wafra)	0.71 ± 0.03^{b}	7.1E-6 ± 2.6 E-7^{b}
Ikhlas (Wafra)	0.75 ±0.02^{b}	7.4E-6 ± 2.3 E-7^{b}
Khanizi (Wafra)	0.88 ± 0.03^{c}	8.7E-6 ± 2.7 E-7^{c}
Saamaran (Sabhan)	0.72 ± 0.1^{a}	7.1E-6 ± 9.7 E-7^{a}

[1]Weight of edible portion of dried Khalal.
Values are shown as mean ± standard deviation of four replicates. Means within a column with different letters differ significantly ($p \leq 0.05$).

4. Discussion

4.1. Energy, Protein, and Ash

Date fruits are a rich source of energy, with values varying according to cultivar (Ghnimi *et al*. 2017). A review by Nasir *et al*. (2015) indicated that the average calorie from fresh dates is 314 kcal/100g and 213 kcal/100g for dried dates. The averages in the current study are slightly higher than the reported averages. The energy value of the Berhi cultivar from Sabah area was significantly lower than that from Wafra farm, suggesting that cultivar may not be the only determinant of nutritional composition. Differences in farming location and factors related to that may have significant impact.

Dates have limited amounts of ash and protein (Assirey, 2015). Proteins make 1-3% of the date fruit and have a good amino acid profile (Sidhu, 2012). However, protein, amino acid, and ash concentrations decrease across developmental stages (Al-Hooti *et al*. 1997; Sidhu, 2012). The current study indicated that dates were not high in protein. The dates with the highest protein - Berhi, Khanizi, and Ikhlas - were from Wafra farms, a possible indication that location, soil, weather, farming techniques etc., may have a significant impact on protein content, possibly even more than cultivar.

The ash concentrations of dates in this study were similar to previous studies on Kuwaiti dates at Tamr stage, where the ash content constituted 5% of the total weight of all varieties. However, these averages are higher than the reported average of 1.67g/100g for dates (Nasir *et al*. 2015).

4.2.Sugars

All cultivars were high in fructose and glucose, in equal proportions, and had non-detectable amounts of sucrose, similar to previous reports that major sugars in dates are glucose and fructose in equal proportions with negligible sucrose (Sidhu, 2012; Vayalil, 2012). The variation in sugar content across cultivars in the current study is high and significant ($p \leq 0.05$). A study on Berhi grown in Iran was found to have fructose and glucose as the most abundant sugars, with a Glu/Fru ratio of 1.03, and with negligible sucrose (Mortazavi *et al*., 2010). The Glu/Fru ratios across all samples in the current study are close to 1, indicating the presence of equal amounts of glucose and fructose.

4.3. Minerals

Palm dates are among the richest fruits in minerals, with the most abundant being potassium, in addition to micro-minerals (Vayalil, 2012; Ghnimi, *et al*., 2017; Al-Alawi *et al*., 2017). The total amount of minerals for most samples in the current study were significantly higher than the averages reported. Minerals constituted a relatively high percentage of the total fruit weight, with an average of 6.5%. This is higher than previously reported data which do not exceed 0.916% (Nasir *et al*. 2015). The highest mineral in all the cultivars studied was potassium, followed by calcium, magnesium, and sodium, with trace amounts of iron and manganese. Calcium and Magnesium values were higher than the averages previously reported (Mohamed *et al*., 2014). The values in these studies, however, were reported for dates at the tamr stage.

Minerals commonly found in other cultivars, such as copper, zinc, phosphorous and selenium, were not detectable. This may be due to either the nature of the cultivars, the maturation stage, the nature of Kuwaiti soil and farming conditions, or the detection limits of the methods employed.

Minerals are reported to decrease across maturation stages (Al-Alawi *et al*., 2017). In the current study, values for calcium, sodium, potassium and magnesium for Behri and Khalas cultivars were all higher than those found in U.A.E. Berhi and Khalas in the Tamr stage (Habib and Ibrahim, 2011).

4.4. Antioxidants

Among the phenolic acids, gallic acid was the highest in the varieties studied, followed by chlorogenic acid. Caffeic acid and coumaric acid were below detection limits. Gallic acid was previously reported to be the most abundant phenolic compound in Omani and Saudi dates at the Tamr stage (Al-Harthi *et al*., 2015; Hamad *et al*., 2014; Hamad *et al*., 2015).

The highest and lowest amount of gallic acid in the current samples were similar to that of gallic acid in Saudi and Omani date cultivars (Hamad *et al*., 2015; Al-Harthi *et al*., 2015). This means that Kuwaiti dates are an important source of gallic acid, which is a powerful antioxidant. Gallic acid is well absorbed by the body and has anticarcinogenic effects.

Berhi (Sabah Area) was significantly the highest in chlorogenic acid and the lowest is Saamaran, which was below detection limits. This range is also similar to the results for chlorogenic acid concentrations reported by Hamad *et al*., (2015).

Ascorbic acid is an antioxidant that contributes to date fruits' health-enhancing effects (Tang *et al*., 2013), although its concentration is relatively low. Ascorbic acid in the cultivars of the current study ranged from 0.624±0.00806 µg/mg (Berhi, Sabah Area) to 0.875±0.0266 µg/mg (Khanizi, Wafra). A report by Al-Gboori and Krepl (2010) on Iraqi dates at the Tamr stage showed dates to be insignificant source for ascorbic acid at this stage. Other studies had shown that antioxidant activity sharply decreases across maturation stages (Allaith, 2007).

5. Conclusion

The aim of the present study was to determine the nutritional composition and biochemical characteristics of five date palm fruit varieties at the Khalal stage grown in Kuwait. The date fruits studied in the current research had higher than average energy values, most of which came from sugars. The most abundant sugars in the date cultivars examined were glucose and fructose. The cultivars were high in potassium. Calcium was also present in considerable amounts, followed by magnesium and sodium, with trace amounts of iron and manganese. The cultivars also contained antioxidants such as gallic acid, chlorogenic acid, and ascorbic acid. Ash and protein were low in amount compared to other components.

This study may serve as the starting point for future research on the nutritional properties of Kuwaiti date fruits, and their potential effects on health. The nutritional compositions of the different cultivars at various stages need to be studied further, in addition to the effect of various confounding factors such as farming conditions and locations on the nutritional components.

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