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Physicochemical Composition of Date Fruit (*Phoenix dactyifera* L.) From Offshoots and Cultured Cells at Different Stages

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Abstract: The objective of this study was to investigate, the changes in physicochemical characteristics of date fruit (*Phoenix dactyliferaL.*) cultivars at Kimri, Khalal, Rutab and Tamar state by two different propagation methods. Weight and length of whole fruit and seeds were significantly higher *in vitro* cultivars (16.74±0.38, 4343±0.15) as compare to offshoot cultivars (1.53±0.02, 2.93±0.15), could be the result of older productivity. Ash (1.77±0.44), protein (3.23±0.08) and Carbohydrate (8.48±0.49) were recorded maximum at Tamar stage. Significant amount of fructose and glucose were calculated higher at Tamar stage from *in vitro* cultivars. Higher percentage of Potassium (419.64±12.12) and Iron (2.3±0.04) among micro nutrients were detected in date fruits, which is considered a front contributor in Saudi Arabian diet.

Key words: *In vitro*, offshoot, *Phoenix dactylifers* L. Kimri, Rutab, Khalal, Tamar

INTRODUCTION

Date fruits (*Phoenix dactyifera* L.) are an important commercial crop in Middle East. It is estimated 6.7 millions tons production world wide (www.FAO.org.) and 734000 tons year^{-1} in kingdom of Saudi Arabia. Climate condition in the various date producing region are not always same cause's low production of the date cultivars. Some fruit ripen early whilst others are not until the last of the season. Different time ripen fruit can be vary in the orgenleptic and physico chemical changes (Dowson, 1982). Limited production through off shoots as compared to the demand, researchers propagated *in vitro* technique, which has been revolutionized as the growing industry with genetically superior products (Al-Ghamdi, 1989; Martin, 1980; Tisserat, 1981; Mahdia and Tisserat, 1985). In spite of socio-economic changes in the kingdom, date fruits are considered a good source of energy, due to their high percentage of sugars and significant amount of fiber (Kulkarni *et al*., 2008; Swaya *et al*., 1982; Husain *et al*., 1993). Date fruit are also, a good source of vitamin and macro nutrient such as potassium, magnesium, iron and calcium (Hafid *et al*., 2007; Gamil *et al*., 1980; Al-Shahib and Marshal, 2003; Elleuch *et al*., 2008). All edible varieties of date have four distinct stages of ripening as Kimri (immature), Khalal (turning to color), Rutab (mature ripe) and Tamar (ripe) states.

Although, the potential *in vitro* propagation of date is well documented (Mahdia and Tisserat, 1985; Booji *et al*., 1993). A little information has been published of date fruit about the changes in chemical composition and comparative study between off shoot and *in vitro* date fruits at different maturation stages. Therefore, it was subjected to judge the physico chemical changes in culture and offshoot fruit at stages of ripening for the benefit of national interest.

MATERIALS AND METHODS

Experimental design and method: The Experiment of cultured and the offshoots propagated khlass date palm was conducted in the experimental station at research center in Riyadh, Saudi Arabia.

Each plant were subjected to the normal culture practice at all location. During flowering season the plants was pollinated with pollen from the same date palm to eliminate effects of metaxinia. Date fruit were collected at each stage of development i.e., Kemri (immature), Khalal (turning of color), Rutab (mature-ripenning) and Tamar (ripe). The samples collected at random from three palms and picked at random from strands located at different parts of three randomly chosen bunches. On the day of harvesting, the fruits were transferred to the laboratory in King Saud University and stored at 4°C for experimental purpose.

Physical characteristics: Physical properties were calculated in 15 fruits of each replicates as follows:

- Fruits and seeds diamentions (weight, length and width) were measured in centimeters using vernier caliper. The ratio of length/width of fruit and seed were calculated

- Fruit and seed weight in grams were determined using an electronic balance. The percentage and flesh seed ratio were calculated
- Colour and fruit shape were determined visually

Chemical analysis: Date fruits were cut, deseeded and the pulp portion was homogenized in blender for analysis. Moisture, protein, fat, crude fiber, pH, carbohydrate was determined according to AOAC (2000). Brix was determined using ABBE mark 11-digital refractrometer (Cambridge Instrument Inc., Buffalo, NY, USA). Mineral analysis carried as, 5 g portion of each sample was ignited and ashed at about 750°C in a muffle furnace for overnight.

The total ash was weight and calculated as percentage. The ash was then dissolved in 5 mL of fuming nitric acid and transferred to 100 mL volumetric flask using double distilled demonized water. The solution analyzed by ICP-Integra (GBC Co., Australia). Glucose, fructose and sucrose were determined by HPLC (Scimadzu LC-10AD, Shimadzu Corporation, Japan). All sugars were separated using LC-NH_2 column (Bellefonte, PA, USA).

The mobile phase was acetonitrile 80% in distill water, setting flow rate 1.5 mL min^{-1}. Data were recorded and analyzed using chromatopack-CR7-A and compare with standard of glucose, fructose and sucrose (Sigma, chemicals, ST, Louis, MO, USA). Data Analysis statistically (ANNOVA) using analysis of variance (Steel and Torrie, 1980) and difference among the means were determined for significance at $p<0.05$.

RESULTS AND DISCUSSION

Morphological comparisons for offshoots and *in vitro* plants and significant difference were observed at all stages of whole fruit in weight, length and width, respectively (Table 1). The weight of individual fruit ranged from (8.65±1.12) in offshoot at Tamar stage to (16.74±0.38 g) *in vitro* at Khalal stage. The length and diameter of whole date fruits varies from 2.50±0.03-4.43±0.15 and 1.86±0.20-2.50±0.10 cm among the samples. Seed weight, length and width were calibrated in range as (0.79±0.09-1.53±0.02), (2.16±0.05-2.93±0.15) and (0.73±0.05-1.06±0.05), respectively. The pattern followed in whole date fruits and seed as well. The weight and size in seeds were found higher *in vitro* as compare to offshoot particularly at Kimri stage, which could be due to the older productivity. The overall difference in physical properties certainly a cause of environmental effects, which is also supported by the finding of Booji *et al.* (1993), Ferry *et al.* (1988). Proximate composition at each stage of date fruit as Kimir, Khalal, Rutab and Tamar are presented (Table 2). The moisture content was calculated higher in Kimir (84.27±0.21), which was lost 85.87% at Tamar stage (11.90±0.26). Unripe contain excess water follows the same pattern, reported by Swaya (1986) and Imad *et al.* (1995). Significant differences in crude fiber was observed in all samples between tissue culture and offshoots plant fruits (Table 3). Crude fiber were higher in offshoot range (1.41±0.06-2.08±0.06) as compare to tissue culture fruits (1.16±0.00-1.56±0.56). Significantly changes in chemical properties were observed such as, fat, which was

Table 1: Physical characteristic at different stages of ripening *in vitro* culture and Off shoot date fruit (*Phoenix dactylifera* L.) mg element/100 g wet weight

Stages	Sample	Wt whole date (g)	Lth whole date (cm)	Wt whole date (cm)	Wt pit (g)	Lth pit (cm)	Wt pit (cm)
Kimri	O	11.25±1.43^{b}	3.96±0.20^{b}	2.43±0.11^{a}	1.09±0.02^{b}	2.34±0.05^{b}	0.80±0.00^{b}
	T	15.10±1.44^{a}	4.43±0.15^{a}	2.46±0.05^{a}	1.53±0.20^{a}	2.93±0.15^{a}	1.06±0.05^{a}
Khalal	O	13.10±0.88^{b}	3.90±0.1^{b}	2.30±0.00^{b}	0.94±0.09^{b}	2.40±0.10^{a}	0.86±0.05^{a}
	T	16.74±0.38^{a}	4.20±0.10^{a}	2.50±-0.10^{a}	1.21±0.19^{a}	2.60±0.01^{a}	0.90±0.00^{a}
Rutab	O	10.16±0.67^{b}	3.40±0.17^{b}	2.26±0.05^{b}	0.79±0.02^{b}	2.16±0.05^{b}	0.66±0.05^{b}
	T	11.11±0.35^{a}	3.96±0.11^{a}	2.50±0.10^{a}	0.96±0.04^{a}	2.34±0.11^{a}	0.83±0.05^{a}
Tamar	O	8.65±1.12^{b}	2.50±0.30^{b}	1.86±0.20^{b}	0.93±0.08^{a}	2.20±0.10^{a}	0.86±0.05^{a}
	T	11.06±0.04^{a}	3.53±0.32^{a}	2.33±0.11^{a}	0.86±0.05^{a}	2.36±0.05^{a}	0.73±0.05^{b}

*Means of replicated raw followed by different letters are significantly different ($p = 0.05$) ±SD; Wt: Weight; Lth: Length; Wth: Width; O: Offshoot; T: *in vitro*

Table 2: Proximate composition at different stages of ripening *in vitro* culture and off shoot date fruit (*Phoenix dactylifera* L.) (g/100 g wet weight)

Stages	Sample	Moisture	Ash	Fat	Protein	Cruse fiber*	Carbohydrate
Kimri	O	84.57±0.21^{a}	0.53±0.01^{a}	0.09±0.01^{a}	0.73±0.02^{b}	1.41±0.06^{a}	12.65±0.21^{b}
	T	83.74±0.44^{b}	0.49±0.04^{a}	0.05±0.01^{b}	0.79±0.02^{a}	1.16±0.07^{b}	13.75±0.41^{a}
Khalal	O	70.66±0.45^{a}	0.72±0.04^{a}	0.06±0.01^{a}	0.99±0.03^{b}	1.72±0.02^{a}	25.83±0.49^{b}
	T	66.46±0.41^{a}	0.46±0.03^{b}	0.06±0.01^{a}	1.09±0.02^{a}	1.33±0.09^{b}	30.40±0.23^{a}
Rutab	O	25.87±2.254^{a}	1.49±0.11^{a}	0.06±0.01^{a}	2.40±0.07^{a}	2.30±0.12^{a}	67.83±2.22^{a}
	T	26.59±2.62^{a}	1.40±0.21^{a}	0.07±0.02^{a}	2.51±0.09^{a}	1.84±0.22^{b}	67.57±2.44^{a}
Tamar	O	17.04±0.67^{a}	1.61±0.07^{b}	0.04±0.01^{a}	2.98±0.07^{b}	2.08±0.06^{a}	76.23±0.75^{b}
	T	11.90±0.26^{a}	1.77±0.04^{a}	0.05±0.02^{a}	3.23±0.08^{a}	1.56±0.15^{b}	81.48±0.49^{a}

Means of replicated raw followed by different letters are significantly different ($p = 0.05$) ±SD; O: Offshoot; T: *in vitro*

Table 3: Sugar composition at different stages of ripening *in vitro* culture and off shoot date fruit (*Phoenix dactylifera* L.) (g/100 g wet weight)

Stages	Samples	Fructose	Glucose	Sucrose
Kimri	O	2.56±0.12^{a}	5.69±0.53^{a}	2.52±0.30^{a}
	T	2.50±0.02^{a}	5.56±0.47^{a}	2.32±0.09^{a}
Khalal	O	4.78±0.26^{b}	7.38±0.29^{a}	8.44±0.30^{a}
	T	6.37±0.67^{a}	7.55±0.99^{a}	8.39±1.69^{a}
Rutab	O	24.30±0.23^{b}	25.10±0.39^{a}	-
	T	25.7±0.69^{a}	25.48±0.42^{a}	-
Tamar	O	27.82±0.29^{b}	28.53±0.10^{a}	-
	T	33.24±0.22^{a}	27.84±0.20^{b}	-

Table 4: Ash percent and minerals at different stages of ripenning *in vitro* culture and offshoot date fruit (*Phoenix dactylifera* L.) (mg/100 g wet weight)

Stages	Samples	Ca	Cu	Fe	K	Na	Mg	Zn
Kimri	O	21.85±3.34^{a}	0.25±0.06^{a}	0.29±0.05^{a}	132.98±23.41^{a}	1.59±0.27^{a}	14.45±1.68^{a}	0.56±0.25^{a}
	T	15.78±3.30^{a}	0.11±0.01^{b}	0.16±0.01^{b}	110.43±11.00^{a}	1.73±0.01^{a}	11.15±0.53^{b}	0.55±0.30^{a}
Khalal	O	27.65±2.87^{a}	0.47±0.08^{a}	0.44±0.04^{a}	178.80±5.05^{a}	6.68±1.93^{a}	21.17±0.03^{a}	0.78±0.22^{a}
	T	23.11±2.46^{b}	0.20±0.04^{b}	0.35±0.01^{b}	128.76±15.58^{b}	7.47±2.00^{a}	24.70±2.52^{a}	0.92±0.34^{a}
Rutab	O	42.69±2.31^{a}	0.55±0.20^{a}	1.57±0.25^{a}	377.06±0.07^{a}	1.76±0.03^{a}	14.45±1.68^{a}	0.56±0.25^{a}
	T	33.12±2.91^{b}	0.30±0.01^{a}	1.54±0.47^{a}	282.19±7.91^{b}	1.73±0.01^{a}	11.15±0.53^{b}	0.55±0.30^{a}
Tamar	O	50.81±2.87^{a}	0.40±0.05^{a}	2.15±0.03^{a}	419.62±12.42^{a}	8.35±1.06^{a}	21.15±0.01^{a}	0.78±0.22^{a}
	T	45.38±3.30^{a}	0.32±0.08^{a}	2.13±0.04^{a}	336.76±12.14^{b}	7.47±2.00^{a}	24.70±2.52^{a}	0.92±0.34^{a}

Means of replicated raw followed by different letters are significantly different (p = 0.05) ±SD. Offshoot (O); *in vitro* (T)

extracted more at kimri stage in off shoot fruits, while the percentage of Ash (1.77±0.04), protein (3.23±0.08) and carbohydrate (81.48±0.49) analyzed in cultured fruit were higher as compare of *in vitro* plants at mature stage. This difference could be due to evaporation of moisture and excess of nutrients provided at the time of culture. The rapid build up fructose and glucose at Rutab and Tamar stage is an excellent source of carbohydrate, which contributes approx. 100 g person^{-1} in Saudi Arabian diet (El-Sharawy *et al*., 1989). Intake of similar amount of fructose as compare the glucose play a role to lower the hyperglycemia is maximum at ripe-Tamar stage (33.24±0.2) as compare to offshoot (22.82±0.29) in cultured fruit (Table 4). The concentration of reducing sugar ratio 2:1 at early stage of the development decreases slowly during maturation in both the cultivars and reaches its ration 1:1 at Tamar stage. This ratio is the same as reported early (Swya *et al*., 1982; Melgarejo *et al*., 2000). The result of minerals at all stages of *in vitro* and offshoots are shown in Table 4. Potassium was present in the largest amount at all stage in offshoot cultivars but maximum values were found at Tamar stage (419.62±12.42) as compare to tissue culture (336.76±12.14), followed by calcium (45.38-50.81), magnesium (21.15-24.70), sodium (7.47-8.35), respectively. Among micro nutrients Iron (2.3-2.15) was calculated higher as compared to zinc (0.78-.92) and copper (0.32-0.40). The overall composition of minerals is markedly varied among various stages at Kimri, Khalal, Rutab and Tamar state, respectively. The amount of potassium, calcium and magnesium were calculated higher at Tamar stage in offshoot fruit cultivars as compare to *in vitro* cultivars. The other elements in descending order (mg/100g) were sodium, iron, zinc and copper. Their respective values of nutrients at maturation stages in both the cultivars were comparable as reported (Imad *et al*., 1995). These variations could be due to agro climatic changes. Higher level of potassium and iron an essential minerals as in many fruits like pomegranate and grape (Salah and Ahmad, 2002) are a front contributor in Arabian diet for controlling salt balance.

CONCLUSION

This study provide an important data as a reference to compare the physicochemical changes at four different stages (Kimri, Rutab, Khalal and Tamar) of dates fruits harvested on tree by *in vitro* culture and traditional propagation methods. More study on physico chemical characteristics at different stages is needed, especially in different agro system in relation with *in vitro* and off shoot cultivars.

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