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Assessment of Biochemical changes in Date palm (*Phoenix dactylifera* L.) cultivars during fruit growth and ripening

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Abstract

The study on biochemical changes in date palm (*Phoenix dactylifera* L.) cultivars was conducted at ICAR-Central Institute for Arid Horticulture, Bikaner during the year 2014. The observations on the growth of fruits were recorded from 15th April till harvesting at 15 days intervals. The assessment of biochemical changes in date palm cultivars Halawy, Khalas, Khadrawy and Shamran at different stages of growth and maturity differed significantly among the cultivars. The qualitative characters such as sugar contents (total, reducing sugars and non-reducing sugars) and ascorbic acid content increased from initial stage to *doka* stage of harvesting whereas, acidity, protein, tannin content and anti-oxidant activity increased at initial stage but exhibited a decreasing trend at *doka* stage of fruit maturity. Per cent acidity was found minimum in cv. Halawy and Khalas (0.150 %). Ascorbic acid content was highest in cv. Khadrawy (11.22 mg/100g pulp) followed by Khalas (10.61 mg /100 g pulp) at harvesting stage. Sugar contents accumulated gradually during fruit development in all cultivars were also varied among four cultivars. Cultivar Halawy found highest in sugar content (36.30% total sugars, 5.96% non-reducing sugars and 30.34% reducing sugars). Protein content varied from 1.49 % in cv. Khalas to 1.73 % in cv. Khadrawy. Tannin content varied from 0.72% in cv. Halawy to 1.00% in cv. Khalas. Anti-oxidant activity was recorded maximum in cv. Halawy (3.50 mmolTE/g) followed by. Khalas (3.22 mmolTE/g). The results revealed that cultivar Halawy is better in respect of quality parameters followed by cv. Khalas under hot arid conditions.

Key words: *Date palm, cultivars, anti-oxidant activity, sugars, protein, tannin.*

Introduction

Date palm (*Phoenix dactylifera* L.; Family Arecaceae) also known as *khajoor, kharek* is a basic dietary component of people living in semi arid and arid regions in the world. Date palm is the most successful and extremely important subsistence crop in many of the hot arid regions of the world (Zaid *et al*,2007, Chandra *et al*,1990) . In India, maximum area of date cultivation is about 17,072 ha with production of 1, 37,476 million tonne in Kachchh region of Gujarat. In Rajasthan, its cultivation has great potential in Jaisalmer, Barmer, Bikaner, Jodhpur and some parts of Churu, Sri Ganga nagar, Hanumangarh, Nagaur districts, which falls under arid climate and provided with irrigation facilities. It is a drought hardy fruit plant species and tolerates salinity. Date fruit is used for fresh consumption as well as for processed products such as wine, starch vinegar, *arak*, jam, chutney, beverages, juice and toffees. It is highly nutritions fruit and contains high calorific value (3150 calories/kg of fresh fruits), 60-65% sugar, fair amount of fibre (2.5%), protein (2.0%), fat (2.0%), vitamins (vitamin A = 220 I.U., vitamin B_1 = 0.35 mg, vitamin B_2 = 0.38 mg), minerals up to (2.0%) i.e. iron, potassium, calcium, copper, magnesium, chlorine, sulphur, phosphorus, etc. (Gopalan *et al.*, 1985). Caramel colour produced from dates which can be used in food and pharmaceuticals industries (Zaid *et al.*, 2007). The date fruit goes through five distinct growth and ripening stages (Shobana *et al.*, 1981)). These five stages are usually referred to in terms derived from Iraqi Arabic as *"Hababouk"*, *"Kimri"*, *"Khalal"*, "Rutab" and "Tamer". *"Hababouk"* stage which starts soon after fertilization and is characterized by loss of two unfertilized carpels. The *Kimri* or *gandora* represent the pea stage and immature green colour, *doka* or *Khalal* – mature full coloured and Rutab *or dang* -soft brown, Tamer *or pind*- hard raisin like (Chandra *et al.*, 1992). In our country, maximum dates are harvested at hard ripen *doka or Khalal* stage because of early rains in July month. However in Gulf countries, fruit maturity goes up to *pind* or Tamer stage. The growth and development of the fruit follows a unique pattern in the date palm cultivars (Al-Qureshi, 2010; Iqbal *et al.*, 2012). Although, work has been reported by different workers on physical and biochemical attributes in different date palm cultivars (Chandra and Pareek, 1992; Osman, 2008 and El-Agamy *et al.*, 2001). Keeping in view, the present study was undertaken to assess the biochemical changes in date palm cultivars during fruit growth under hot arid conditions.

Material and Methods

The present study was carried out at ICAR-Central Institute for Arid Horticulture Bikaner, Rajasthan during the year 2014 having four replications. The four commercial date palm cultivars *viz.*, Halawy, Khalas, Khadrawy and Shamran were taken for fruit growth and

biochemical study. The fruit samples were collected at 15 days interval (15th April, 30th April, 15th May, 30th May, 15th June, 30th June, 15th July and at harvesting) in the month of April to July 2014 for assessment of biochemical changes during fruit growth period of date palm fruits. Percent acidity and ascorbic acid and sugars were observed in fruit samples as per standard methods described by Ranganna, (2001).

Total sugars were estimated by Anthrone reagent method described by Thayumanavan and Sadasivam (1984). The absorbance was recorded at 630nm against a reagent blank in the UV-VIS spectrophotometer (Decibel, Delhi, India). The concentrations of total soluble sugars were calculated according to the glucose standard curve.

The tannin content was analyzed using Folin-Denis method described by Schander, (1970) on dry weight basis. The absorbance was recorded at 700 nm against a reagent blank in the UV-VIS spectrophotometer (Decibel, Delhi, India). The standard graph was made by using 0-100µg of tannic acid and the concentration of tannin was expressed as percentage of fresh weight.

The soluble proteins were estimated by Lawry's method (1951). The absorbance was recorded at 660nm against a reagent blank in the UV-VIS spectrophotometer. The concentrations of soluble proteins were calculated according to the standard bovine serum albumin curve.

Total antioxidant activity was analysed by CUPRAC (cupric reducing antioxidant capacity) assay recently developed by Apak *et al.*(2004) which measured the cupper (II) ion reducing ability of polyphenols, vitamin C and vitamin E. The 2 g of fruit material was extracted using 20 ml mixture of ethanol and distilled water (80:20, v/v) by centrifuging at 10000 x g for 10 min. The supernatant was used for analysis. The absorbance was recorded at 450 nm in the UV-VIS spectrophotometer (Decibel, Delhi, India). The units were expressed in µmol Trolox/ 100g.

The experimental values were analysed statistically by using Completely Randomized Design (CRD). Means were compared using soft ware. Stat package CCSHAU, Hisar at 5% level of significance.

Results and Discussion

The biochemical attributes viz., total soluble solids, acidity percentage, ascorbic acid, tannin, protein content, sugars and anti-oxidant activity of different date cultivars were assessed at 15days intervals from *kimri* to *doka* stage of fruit growth and maturity. Fruits were harvested at *doka* stage in all cultivars. The significant differences in biochemical changes were observed among the cultivars in all the stages of fruit growth and development.

Total Soluble Solids (TSS ° Brix)

The results clearly showed that an increasing trend in TSS of fruits at all fruit development stages was recorded in date palm cultivars studied (table -1). TSS of fruits in date palm variety Halawy was 41.28 °Brix at harvesting stage which was 13.85 °Brix at initial stage of fruit development that gradually increased on 15th May to harvesting stage. It is due to accumulation of carbohydrates and decrease in tannins in fruits. The highest TSS was recorded in Halawy (41.28 °Brix) followed by Khalas (39.17°Brix), Shamran (39.13°Brix) while it was lowest in cv/ Khadrawy (37.39 °Brix %). A significant difference in TSS of fruits was recorded at fruit growth, development and harvesting stages. The findings is similar to earlier workers reported by Haider *et al* (2014) ,Bacha *et al* (1987).

Acidity (%)

The acidity content followed a decreasing trend as the fruit develops in all cultivars (Table-2).The significant difference was noticed in acidity content in fruits among cultivars. It is indicated that acidity of fruits in date palm on fresh weight basis in cv. Halawy 0.150% was at harvesting stage which was 0.160% at initial stage of fruit development that gradually increased on 30th April to 30th May and decreased from 15th June to Harvesting stage. The same pattern of increase and decrease in acidity was noticed in all cultivars during fruit growth. The highest acidity percent in fruits was recorded in Shamran (0.168%) followed by Khadrawy (0.160%) and it was at par in cultivars Halawy and Khalas (0.150%). The acidity percentage in cv. Halawy and Khalas was found less. It may be due to less astringency and more sweetness at fully matured fruits in these cultivars in comparison to other cultivars. Similar findings have been reported by Abbas *et al.*, (2002) and Ebtehal and Al-Tamin (2014). Moreover, it may possibly be due to accumulation and metabolism of organic acids in the fruit during their growth and development.

Ascorbic acid (mg/100 g fruit pulp)

The data presented in table 3 revealed that ascorbic acid content of fruits in date palm on fresh weight basis increased from initiation of fruit development to harvesting stage.). Difference among the cultivars in ascorbic acid content was significant at all the fruit development stage. In cv. Halawy ascorbic acid content was 8.04 mg/100 g at harvesting stage which was 1.04 mg/100 g at initial stage of fruit development that gradually increased on 30th April to 15th July and it was highest at Harvesting stage. The same pattern of increase in ascorbic acid was noticed in other varieties at different stages.

The highest ascorbic acid content was recorded in cultivar Khadrawy (11.22 mg/100 g) followed by Khalas (10.61 mg/100 g), Shamran (10.37 mg/100 g) and lowest in Halawy (8.04 mg/100 g. Ascorbic acid content increased from initial stage of fruit growth and was highest at *doka* stage of ripening. Sawaya *et al.*, (1983) reported that vitamin C content was higher at *Khalal* stage. Marbet et al. (2008) reported vitamin C content 24 to 46 mg/100 g whereas, Ebtehal and Al-Tamin (2014) reported 10.52 mg/100 vitamin C in Saudi Sukhari and Egyptian Sewi date palm samples. Similar results have been reported by Chandra and Choudhary (1990).

Total sugars (%)

The total sugar content of fruits in date palm cultivar Halawy was 36.30% at *doka* stage of harvesting which was 4.25% at initial stage of fruit development that gradually increased on 15th May to Harvesting. On 15th May the magnitude of sugar was 6.32%, which increased to 12.60% by 30th May. It nearly doubled (24.48%) by 15th June and then slightly increased to 28.25% by 30th June and reached to the level of 34.40% by 15th July. Total sugar content in cultivar Khalas 34.40% at harvesting stage which was 4.05% at initial *kimri* stage of fruit development which subsequently increased from 15th May to 15th July and highest at harvesting stage. Increase in total sugar was observed from *Kimri* to *doka* stage of harvest. The highest total sugars content was recorded in Halawy (36.30%) followed by Khalas (34.40%), Shamran (34.06%) while lowest in Khadrawy (32.49%). A significant difference in total sugars content in different date palm cultivars was found at fruit development stages(table-4). Total sugars are a varietal character and its quantity depends on cultivar. Total sugars increased from initial stage of fruit development which was highest at *doka* stage of harvesting. Elahmer *et al*., (2007) reported total sugars were found highest in Ammi (49.49%) followed by Tabouni (38.54%) and lowest in Brinsi (35.87%). Muhhamad *et al* (2011) reported the total sugar content was much higher in date palm grown in Dera Ismail Khan (71.05%). Similar resuhs were observed by Gasim *et al*., (1994); Awad *et al*., (2011); Taain *et al*., (2013); Ebtehal and Al-Tamin (2014).

Reducing sugars (%)

It is clearly indicated that highest reducing sugar content of fruits in date palm cultivar Halawy was 30.34% at harvesting stage which was 3.91% at initial stage of fruit development that gradually increased on 15th May to Harvesting (table -5).

Reducing sugars content was highest in Halawy (30.34%) followed by Khalas (29.05%) and Shamran (29.03%) while it was lowest in Khadrawy (27.52%). Difference among the cultivars was significant in relation to reducing sugar content at all the fruit development stages. Carbohydrate metabolism is the most activated pathway at every single stage of the fruit development and ripening in date palm with in this also, the genes directly associated with sugar metabolism are much more activated at the later stage of fruiting. This pattern accounts for gradually increasing reducing sugar content in date palm. The high level of total sugars in date fruits are due to the up gradation of sucrose phosphate synthase and inverts at the later stage of fruit growth.

Protein content (%):

The soluble protein content decreased from *kimri* to *doka* stage of maturity. Data clearly showed that protein content of fruits on fresh weight basis in cv. Halawy was 1.67% at harvesting stage which was 1.5% at initial stage of fruit development that gradually increased on 30th April to 30th May and decreased from 15th June to Harvesting stage (table 6). The significant difference among cultivars in respect of protein content was recorded. The highest protein content was recorded in Khadrawy (1.73%) followed by Halawy (1.67%) and Shamran (1.56%) while it was lowest in cv. Khalas (1.49%).The finding is similar to the results reported by Haider *et al* (2013).

Tannin content (%)

There is a significant difference in tannin content of date palm fruits at growth and development stages. (Table 7). The tannin content was estimated in fruit samples from 15th May onwards in date palm cultivars. Tannins content of fruits in date palm on dry weight basis in cv. Halawy was 0.72% at harvesting which was 1.4% at initial stage of fruit development that gradually increased on 30th May to 15th June and decreased from 30th June to Harvesting stage. Increase in tannin content was noted at initial stage of fruit growth which showed the decreasing trend of tannin content at later stage of fruit development during July month. It is because of breakdown of tannins and protein compounds in to simple soluble sugars due to the role of enzymes in fruit development process.

The highest tannin content was recorded in cultivar Khalas (1.00%) followed by Khadrawy (0.95%), and Shamran (0.82%) while it was lowest in cv. Halawy (0.72). This is in general agreement with findings of Mougthith *et al* (1976).

Anti–oxidant activity (mmolTE/g)

There is a significant difference in anti-oxidant activity among date palm cultivars at different fruit growth and development stages (table-8). The maximum anti-oxidant activity was recorded in Halawy (3.505 mmolTE/g) followed by Khalas (3.225 mmolTE/g) and Shamran cultivar (3.158 mmolTE/100 g). Lowest anti-oxidant was observed in Khadrawy cv. (3.083 mmolTE/g) at harvesting stage. The results showed the increasing trend in anti-oxidant activity, tannins contents from *kimri* to *doka* stage in all cultivars. The tannin content and total phenols are the major contributors to the antioxidant activity. An increase in anti-oxidant activity may due to high tannin and phenolic compounds during fruit development stages. However, gradual decreasing in anti-oxidant activity possibly is due to decreasing trend in tannin and other phenolic compounds at colour turning and maturity in fruits.

The finding is in accordance to results reported by Al-Farsi *et al*, (2005) in date palm cultivars.

In the present study, the changes in metabolite composition were assessed from kimri to doka stage of date fruits. It was observed that majority of the metabolites increases with the advancement of growth stage. Those such changes occur in date fruits has been demonstrated by Abbas and Fandi (2002), Ebtehal and Al-Tamin (2014), etc. In the present study too, it was reported that metabolites such as total sugars and reducing sugars increased with the stage of development. This is on account of fact that fruit acts as sink and accumulates the carbohydrates produced in the leaves. This accumulation then leads to changes in TSS, which is also apparent in our study. Thus, our study shows that fruits are highly

active sinks which accumulates the carbohydrates in the form of reducing and non reducing sugars which are then converted into other complex forms with the growth and development of the fruit. Among the varieties studied, cv. Halawy was found to be best since it has more number of berries, high TSS, sugar content, anti-oxidant activity and minimum acidity.

Table 1. TSS (°Brix) of different Date palm cultivars during fruit growth and maturity stages.

Treatments	April	May		June		July	Harvesting
	30	15	30	15	30	15	Final
Halawy	13.85	15.26	16.68	19.72	30.41	39.00	41.28
Khalas	11.45	13.84	14.06	17.33	24.06	36.04	39.17
Khadrawy	11.23	12.51	13.67	15.90	23.43	33.75	37.39
Shamran	12.45	13.17	13.75	16.09	19.86	34.03	39.13
CD at 5%	1.512	1.054	0.908	1.184	2.232	1.807	1.489

Table 2. Acidity (%) in fruits of different Date palm cultivars during fruit growth and maturity.

Treatments	April		May		June		July	Harvest
	15	30	15	30	15	30	15	Final
Halawy	0.160	0.268	0.330	0.343	0.320	0.270	0.265	0.150
Khalas	0.170	0.280	0.340	0.375	0.320	0.285	0.270	0.150
Khadrawy	0.180	0.188	0.353	0.563	0.360	0.293	0.285	0.160
Shamran	0.170	0.180	0.595	0.615	0.428	0.320	0.310	0.168
CD (P=0.05)	0.013	0.048	0.061	0.154	0.068	0.018	0.017	0.013

Table 3. Ascorbic acid (mg/100 g) in fruits of different Date palm cultivars

Treatments	April		May		June		July	Harvest
	15	30	15	30	15	30	15	Final
Halawy	1.04	1.28	1.70	2.14	3.65	6.34	7.19	8.04
Khalas	0.59	1.07	1.58	1.64	5.85	9.27	10.24	10.61
Khadrawy	1.22	1.37	1.82	2.29	4.51	8.78	10.73	11.22
Shamran	0.49	1.04	1.34	2.19	6.09	7.07	10.00	10.37
CD (P=0.05)	0.09	0.16	0.20	0.16	0.65	0.96	0.92	1.60

Table 4. Total sugars (%) in fruits of different Date palm cultivars

Treatments	April	May		June		July	Harvest
	30	15	30	15	30	15	Final
Halawy	4.25	6.32	12.60	24.48	28.25	34.40	36.30
Khalas	4.05	5.44	10.71	21.81	23.72	32.49	34.40
Khadrawy	4.17	5.88	12.29	23.72	24.48	28.91	32.49
Shamran	3.41	5.36	9.91	19.49	21.81	31.54	34.06
CD (P=0.05)	0.48	0.59	0.23	0.39	0.42	0.24	0.23

Table 5. Reducing Sugars (%) in fruits of different Date palm cultivars

Treatments	April	May		June		July	Harvest
	30	15	30	15	30	15	Final
Halawy	3.91	4.99	9.47	21.00	23.72	29.57	30.34
Khalas	3.76	4.26	7.55	18.10	18.88	27.55	29.05
Khadrawy	3.69	4.32	9.57	19.83	19.94	24.11	27.52
Shamran	3.09	4.17	7.14	16.50	17.82	27.37	29.03
CD (P=0.05)	0.07	0.04	0.06	0.06	0.14	0.06	0.08

Table 6. Protein content (%) in fruits of different Date palm cultivars

Treatments	April		May		June		July	Harvest
	15	30	15	30	15	30	15	Final
Halawy	1.50	2.64	4.76	5.21	3.94	2.88	2.23	1.67
Khalas	1.25	2.33	4.61	5.03	3.84	2.78	2.08	1.49

Khadrawy	1.56	2.74	4.86	5.32	4.20	3.11	2.30	1.73
Shamran	1.31	2.41	4.67	5.13	3.89	2.79	2.12	1.56
CD (P=0.05)	0.07	0.14	0.10	0.06	0.18	0.09	0.13	0.05

Table 7. Tannin content (%) in fruits of different Date palm cultivars

Treatments	May		June		July	Harvest
	15	30	15	30	15	Final
Halawy	1.42	1.64	2.23	2.08	1.07	0.72
Khalas	1.77	1.99	2.70	2.45	1.41	1.00
Khadrawy	1.61	1.74	2.40	2.22	1.29	0.95
Shamran	1.47	1.64	2.00	2.17	1.20	0.82
CD (P=0.05)	0.06	0.05	0.07	0.05	0.05	0.04

Table 8. Anti-oxidant activity (mmolTE/g) in fruits of different Date palm cultivars

Treatments	April		May		June		July	Harvest
	15	30	15	30	15	30	15	Final
Halawy	1.395	3.043	5.535	6.015	4.443	4.173	3.683	3.505
Khalas	1.263	2.970	5.310	5.793	4.195	3.703	3.505	3.225
Khadrawy	1.203	2.365	5.203	5.670	3.798	3.593	3.230	3.083
Shamran	1.230	2.515	5.170	5.643	3.890	3.663	3.393	3.158
CD (P=0.05)	0.089	0.479	0.081	0.071	0.073	0.074	0.099	0.153

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