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Assessment of Biochemical and Nutraceutical Components in Date Palm (*Phoenix dactylifera* L.) Cultivars

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ABSTRACT

Present study was conducted to know the variability of antioxidant activity and its components among different cultivars at two different fruit development stages i.e. gandora and doka stage. The total antioxidant activity, total phenols, total flavonoids, total sugars and calcium content was increased from gandora stage to doka stage, while a decreasing trend was observed for acidity, proteins and tannin contents. The highest antioxidant activity (1.88 µmolTrolox/100g) at doka stage was recorded in the cultivar Zahidi, which is at par with the cultivar Halawy (1.84 µmolTrolox/100g) and the lowest antioxidant activity was recorded in the cultivar Chip Chap (1.24 µmolTrolox/100g). The maximum total phenols (158.98 mg GAE/100g) and flavonoid (86.0 mg QE/100g) contents were noticed in the cultivar Zahidi and minimum (84.31 mg GAE/100g and 50 mg QE/100g) was in Chip Chap, respectively. The cultivar Zahidi recorded the highest total sugars (43.14 %) and calcium (4.65%) at doka stage. The maximum soluble protein (6.31 %) and tannin (1.92 %) was noticed cultivar Chip Chap and Dayari, respectively at gandora stage. Results revealed that the total antioxidant activity mainly depends on the amount of total phenol content present in the fruits at both gandora (R^2=0.567) and doka (R^2=0.592) stage. The variation in the nutraceutical attributes primarily influenced by the type of cultivars and fruit development stages, meanwhile it also reveals that the doka stage of harvesting is highly suitable for consumption due to its higher level of total antioxidant activity, total phenols and total flavonoid content, regardless of cultivar.

Key words: *Date palm, Antioxidant activity, Phenolics, Gandora, Doka.*

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the oldest domesticated fruit crops and is considered as one of the world's first cultivated fruit trees. It is one of the most important fruit crops which can grow in hot arid regions of the world and only crop able to withstand high temperature and low humid conditions at bearing stage. Date fruit is a high-energy food owing to high sugar content, a good source of iron, calcium, potassium, and iodine, as well as low in fats.

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Fresh dates are highly nutritious than dry dates[23] because of its rich sugar content in the form of fructose, glucose, and sucrose[7] and as a good source of dietary fibre[16] and essential minerals and vitamins[5]. Al-Farsi *et al.,*[1] and Mansouri *et al.,*[13] reported that dates contain a variety of polyphenols, including phenolic acids, hydroxycinnamates, flavonoid glycosides, and proanthocyanidins which make it an interesting fruit from a pharmaceutical and medicinal point of view[9]. Date fruit not only provide antioxidant, antimutagenic, and immunomodulatory benefits to health but also has diverse medicinal values, including antihyperlipidemic, anticancer, gastro protective, hepatoprotective, and nephroprotective properties[21]. In this respect, phytochemicals from fruits have been shown to possess significant antioxidant capacities that may be associated with lower incidence and lower mortality rates of degenerative diseases in human. The antioxidant properties of fruits vary depending on their content of phenolic components and vitamins C and E, carotenoids, flavonoids. They have good antioxidants potential and can be used to produce novel natural antioxidants as well as flavouring components for various food products.

Now-a-days the phenolic compounds in fruits have drawn a lot of attention due to their potential in preventing cancer and heart diseases[11]. There is a lot of scope for antioxidants which are of natural origin over synthetic antioxidants due to their carcinogenicity. The fruits and vegetables are the most important sources for natural antioxidants. Hence present study was conducted to know the variability of antioxidant activity and other bioactive compounds in different cultivars at two different fruit development stages *i.e. gandora* and *doka*.

MATERIAL AND METHODS

The fruits of ten commercial date cultivars *viz*., Zahidi, Braim, Chip Chap, Khalas, Medjool, Shamran, Dayari, Halawy, Khadrawy and Khunezi were harvested randomly at two different stages *i.e., gandora* (Kimri) and *doka* stage (Khalal) in the month of June and July 2014, from date palm orchard of ICAR-Central Institute for Arid Horticulture, Bikaner, Rajasthan. The fruits were washed under running tap water to remove field heat and dust from the surface. The fruits from each variety were separated and homogenised. Required quantity of homogenised fruit samples were taken separately for the estimation of different biochemical components using quartering method. In Indian conditions maximum dates are harvested at doka stage for fresh consumption and processing due to occurrence of early rains.

Determination of total antioxidants activity:

Total antioxidant activity was analysed by CUPRAC (cupric reducing antioxidant capacity) assay recently developed by Apak *et al.,*[3] which measured the copper (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.

Determination of total phenol content (TPC)

The content of total phenolics was estimated as per the method suggested by Singleton *et al*.,[20] using Folin-Ciocalteu reagent. Phenolic compounds were extracted from 2g of fresh fruit sample with 20 ml mixture of 99% ethanol and distilled water (80:20, v/v) by centrifugation at 10000 x g for 10 min. The supernatant was collected and the absorbance was recorded at 725 nm in a double beam UV-VIS spectrophotometer (Decibel, Delhi, India). Gallic acid was used to prepare the standard curve and result was expressed as mg per gallic acid equivalent (GAE)/100 g of fresh weight.

Determination of total flavonoid content (TFC)

The flavonoids concentration was determined colorimetrically according to the method reported by Dewanto *et al.*,[6]. The yellow colour produced by the reaction between flavonoids present fruit samples and aluminium chloride reagent was quantified at 510 nm using quercetin as standard. The concentration of total flavonoids was expressed as mg quercetin equivalent (QE)/100 g fresh weight.

Tannins

The tannin content was analyzed using Folin-Denis method described by Schanderl[19] 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.

Total soluble sugars

The total soluble sugars were estimated by using anthrone method described by Thayumanavan and Sadasivam[22]. The absorbance was recorded at 630 nm 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.

Soluble proteins

The soluble proteins were estimated by Lawry's method[12]. The absorbance was recorded at 660 nm against a reagent blank in the UV–Vis spectrophotometer. The concentrations of soluble proteins were calculated according to the standard bovine serum albumin curve.

Calcium (%): The total calcium content of fruits was estimated by using flame photometer on dry weight basis.

Statistical analysis

The experimental values were analyzed statistically by using completely randomized design (CRD) with three replications of each cultivar. Means were compared using WASP (WEB Agri Stat Package, ICAR Research Complex, Goa) test at 1% level of significance.

RESULTS AND DISCUSSION

Phenolic compounds and flavonoids are the major group of compounds that contribute to the antioxidant activity of vegetables, fruits, cereals and other plant based materials. The composition of biochemical and nutraceutical compounds v*iz.*, total antioxidants, total phenols, total flavonoids, soluble proteins, tannins and calcium of date palm cultivars are represented in Table 1 and 2.

Significant differences were observed in total antioxidants activity of date fruits at two developmental stages among different cultivars (Table 1). The total antioxidants were increased from *gandora* stage to *doka* stage. At *gandora* stage maximum antioxidant activity was observed in cultivar Dayari (1.55 µmolTrolox/100g) followed by Khadrawy (1.51 µmolTrolox/100g) and minimum was in cultivar Chip Chap (1.05 µmolTrolox/100g) and Khalas (1.03 µmolTrolox/100g). Where as in *doka*, highest amount of total antioxidant activity was observed in the cultivar Zahidi (1.88 µmolTrolox/100g) which was found on par with the cultivar Halawy (1.84µmolTrolox/100g) and least amount of antioxidant activity was noticed in the cultivar Chip Chap (1.24µmolTrolox/100g).

The total phenol content was increased from *gandora* to *doka* stage fruits (Table 1). The cultivar Dayari (115.79 mg GAE/100g) recorded the highest phenol content at *gandora* stage which was on par with cultivar Khadrawy (114.59 mg GAE/100g) and the least was observed in cultivar Chip Chap (61.97 mg GAE/100g). However, at *doka* stage the highest amount of total phenols was observed in the cultivar Zahidi (158.98 mg GAE/100g) and followed by cultivar Halawy (139.60 mg GAE/100g) and Khadrawy (139.25 mg GAE/100g) and lower amount was observed in cultivar Chip Chap (84.31 mg GAE/100g) contain its low amount.

The significant differences were observed in total flavonoid content at two different stages of fruit development among

the cultivars (Table 1). The more amount of flavonoid content was observed in cultivar Dayari (57.0 mg QE/100g) followed by Khadrawy (56.0 mg QE/100g) and low content was observed in cultivar Chip Chap (34.0 mg QE/100g) at *gandora* stage. In *doka* stage the cultivar Zahidi (86.0 mg QE/100g) recorded maximum content and minimum amount of total flavonoids were noticed in the cultivar Chip Chip (50.0 mg QE/100g).

The results clearly showed the increasing trend in total antioxidant activity, total phenols and flavonoid content from *gandora* to *doka* stage in the all cultivars. The total phenols are the major contributors to the total antioxidant activity at both *gandora* (R^2=0.567) and *doka* (R^2=0.592) stage. These results agree to previous studies of AL-Faris *et al.*,[1], Biglari *et al.*,[4], Mertia *et al.*,[14] and Faquir Mohammad *et al.*,[8]. It is often described that the values of total phenolic content found increasing trend from *gandora* stage to *doka* stage of fruit development in relation to maturity. It is clear that the cultivar which is having higher phenol content will show higher antioxidant activity.

The acidity content followed a decreasing trend as the fruit develops in all the cultivars (Table 1). The significant differences were noticed in acidity content at two different stages of fruit development among the cultivars. The higher acidity was noticed in cultivar Dayari (0.41 %) at *gandora* stage which was on par with cultivar Khadrawy (0.38 %) and less acidity was noticed in cultivar Braim (0.16 %). At *doka*, cultivar Zahidi (0.16 %) have more acidity followed by Khadrawy (0.14 %) and low content was noticed in cultivar Medjool and Khuneizi (0.10 %). The acidity content decreases due to increase in the total soluble sugars and other biochemical compounds. These results fall in line with Moughith *et al.*,[15].

There was a significant difference in total soluble sugar content at two different stages among the cultivars (Table 2). At *gandora*, maximum sugar content was noticed in cultivar Halawy (18.93 %) followed by cultivars Khunezi (11.28 %) and minimum content was noticed in cultivar Dayari (6.35 %). The total soluble sugars increased 2-3 times from *gandora* stage to *doka* stage depending upon the cultivars. The cultivar Zahidi recorded the highest total soluble sugar (43.14 %) at doka stage which is on par with Khadrawy (42.92 %) and the lowest was recorded in cultivar Medjool (31.06 %) at *doka* stage. Similar results were obtained by Moughith *et al.*,[15].

The soluble protein content decreased from *gandora* stage to *doka* stage of dates (Table 2). The significant differences were observed at two different stages of dates among the different cultivars. In *gandora* stage the maximum protein content was recorded in cultivar Chip Chap (6.31 %) which was on par with Dayari (6.25 %) followed by cultivar Medjool (6.04 %) and the minimum was noticed in cultivar Braim (2.80 %). The cultivar Dayari (4.74 %) contains the maximum amount of proteins followed by cultivar Shamran (3.0 %) and the lowest was noticed in the cultivar Braim (1.87 %) in *doka* stage.

The highest protein content was observed in *gandora* stage which followed a decreasing trend towards maturity[18]. The rising activities of enzymes towards maturity may be one of reason for decrease protein content[19].

There was a significant difference in tannin content of date fruit at two developmental stages among different cultivars (Table 2). The cultivar Dayari recorded the highest tannin content at *gandora* stage (1.92 %) followed by Zahidi (1.66 %) which was on par with cultivar Medjool (1.62 %) and the lowest was recorded in cultivar Khadrawy (0.32%). The cultivar Medjool (0.84 %) recorded high amount of tannin content in *doka* stage followed by Khunezi (0.76 %) and low content was present in cultivar Halawy (0.02 %) and Chip Chap (0.04 %) at *doka* stage. Thus tannin content reduced from *gondora* to *doka* stage in all the cultivars. The total soluble sugar content increased as the fruit matured. It is because of the breakdown of tannins and protein compounds into simple soluble sugars due to the role of

enzymes in fruit developmental process. This is in general agreement with Moughith *et al.*,[15].

Significant differences were observed in calcium content at two different fruit developmental stages among the cultivars (Table 2). The calcium content in date fruits showed an increasing trend from *gandora* stage to *doka* stage. The high content of calcium was observed in variety Zahidi (3.89 %) which was on par with cultivars Khalas (3.79 %), Chip Chap (3.78%) & Braim (3.73 %) and low content was in cultivar Halawy (2.69 %) at *gandora* stage. At *doka* stage, cultivar Zahidi (4.65%) recorded more calcium content which is on par with the cultivar Dayari (4.54%) and the lowest was noticed in the cultivar Halawy (4.0 %). The calcium content also varied significantly among the cultivars at two different fruit developmental stages, which is found to be in close agreement with Al-Shahib and Marshall[2].

Table 1: Biochemical profile of ten date cultivars at two different developmental stages

Cultivars	Total Antioxidants (µmolTrolox/100g)		Total Phenols (mg GAE/100g)		Total Flavonoids (mg QE/100g)		Acidity (%)	
	Gandora	*Doka*	*Gandora*	*Doka*	*Gandora*	*Doka*	*Gandora*	*Doka*
Zahidi	1.29	1.88	89.35	158.98	42.00	86.00	0.35	0.16
Braim	1.12	1.64	72.26	93.03	40.00	73.00	0.16	0.11
Chip chap	1.05	1.24	61.97	84.31	34.00	50.00	0.35	0.11
Khalas	1.03	1.41	67.75	102.85	39.00	66.00	0.17	0.13
Medjool	1.43	1.65	75.01	128.08	47.00	63.00	0.19	0.11
Shamran	1.45	1.51	70.20	135.02	43.00	71.33	0.17	0.10
Dayari	1.55	1.76	115.79	131.56	57.00	74.67	0.41	0.13
Khadrawy	1.51	1.77	114.59	139.25	56.00	77.33	0.38	0.14
Halawy	1.41	1.84	94.25	139.60	51.00	75.33	0.34	0.12
Khuneizi	1.12	1.62	73.65	98.40	41.00	63.33	0.33	0.10
LSD(P<0.01)	0.05	0.07	4.77	4.11	7.31	3.98	0.03	0.01

Table 2: Effect of fruit development on Total Soluble Sugars, Proteins, Tannins and Calcium content in date palm cultivars

Cultivars	Total Soluble Sugars (%)		Proteins (%)		Tannins (%)		Calcium (%)	
	Gandora	*Doka*	*Gandora*	*Doka*	*Gandora*	*Doka*	*Gandora*	*Doka*
Zahidi	11.07	43.14	4.63	2.57	1.66	0.56	3.89	4.65
Braim	7.18	35.37	2.80	1.87	0.68	0.08	3.73	4.22
Chip Chap	7.56	41.56	6.31	3.00	0.86	0.04	3.78	4.22
Khalas	9.02	37.09	4.53	2.16	0.52	0.42	3.79	4.58
Medjool	8.12	31.06	6.04	2.77	1.62	0.84	2.82	4.17
Shamran	7.48	41.12	5.32	2.00	1.43	0.15	3.41	4.36
Dayari	6.35	36.62	6.25	4.74	1.92	0.57	2.94	4.54
Khadrawy	10.95	42.92	4.35	2.25	0.32	0.20	2.91	4.30
Halawy	18.93	41.50	5.39	2.63	1.00	0.02	2.69	4.00
Khuneizi	11.28	39.62	2.90	2.08	1.10	0.76	3.33	4.06
LSD(P<0.01)	**0.86**	**1.48**	**0.11**	**0.08**	**0.08**	**0.03**	**0.26**	**0.14**

CONCLUSION

The results concluded that there is a huge variation in biochemical and nutraceutical compounds among the cultivars and fruit developmental stages. It is mainly influenced by the type of cultivars and stage of fruit development. The cultivars Zahidi, Halawy, Khadrawy and Dayari are superior for consumption at *doka* stage due to high content of total antioxidant activity and other nutraceutical compounds which may be used as a variety with high nutraceutical value.

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