

Food Science

REGULAR ARTICLE

Biochemical attributes of dates at three maturation stages

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Abstract

The date fruit is a primary component of the human diet in many countries with arid and semiarid climates. The present study reflects the relationship of different biochemical attributes with progressive date fruit developmental stages. The study involved eight date palm (*Phoenix dactylifera* L.) cultivars from Pakistan examined at three different edible stages of dates (khalal, rutab and tamar). The antiradical efficiency (2.14–0.36), antioxidant enzymes (catalase and peroxidase), total phenolic contents (468.99–108 mg GAE/100g, FW) and the soluble protein contents (5.73–2.75 g/100g) were higher in higher at khalal and thereafter, but declined at fully ripened (tamar) stage. Moreover, glucose (16.92–31.66%) and fructose (15.25–30.58%) have lower quantity at khalal and higher quantity at tamar stage, whereas non-reducing (sucrose) sugars were present only at khalal and rutab stage fruits. Makran and Chohara cultivars revealed best overall values in examined compounds. Our results revealed that variation in different biochemical attributes is mainly depended on the difference in fruit maturity stage and cultivar. Cultivars exhibiting high values of beneficial biochemical attributes may be considered for the expansion of date palm cultivation.

Key words: Antioxidant activity, Date palm, Enzymes, Phenolic contents, Protein contents, Sugars profile

Introduction

The fruit of date palm (*Phoenix dactylifera* L.) is a basic dietary component of people living in the arid and semiarid regions in the world. It has a significant share of the economic and social role in livelihoods for the people of particular regions (El-Hadrami and Al-Khayri, 2012). Pakistan is the sixth largest producer of dates after Egypt, Saudi Arabia, Iran, Algeria and Iraq, contributing 10.3% of the total world production. World date production is about 7.2 million mt, whereas the contribution of Pakistan is 700,000 mt (FAO, 2011). About 325 date cultivars are reported in Pakistan (Jamil et al., 2010). Date fruit is a single-seeded berry with a fleshy pericarp and fibrous endocarp (Amira et al., 2011).

Date fruit attains stepwise maturation stages

that are internationally denominated by Arabic terms such as kimri (19 weeks after pollination: unripe, astringent, green and firm), khalal (29 weeks after pollination: partially-ripe, colored yellow or red depending on cultivar), rutab (30 weeks after pollination: fully-ripe, light-brown and soft) and tamar (31 weeks after pollination: dark-brown and soft, semidry or dry, highly sweet and storable) as explained by Kader and Hussein (2009). The saccharinity, texture, aroma and taste of date fruits are closely associated with the ripening stages (Al-Shahib and Marshal, 2003).

Date flesh is a readily-accessible source of energy because of its high sugar content (70-85% FW) with a significant share of reducing sugars (glucose and fructose) and very small amount of non-reducing sugars (sucrose) (Al-Farsi and Lee, 2008; Rastegar et al., 2012). Dates possess important radical scavenging activity due to the presence of non-enzymatic antioxidants (phenolic, flavonoid and ascorbic acid) and enzymatic antioxidants (catalase, peroxidase and superoxide dismutase), compounds (Biglari et al., 2008; Awad et al., 2011a) known to have health benefits (Vayalil, 2011). Antioxidants are the complex

Received 28 August 2013; Revised 11 October 2013; Accepted 22 October 2013; Published Online 15 October 2014

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moieties which can quench and neutralize the free radicals and can prevent oxidation of additional molecules and promote health benefits (Mansouri et al., 2005).

Chemically date fruit is composed of total sugars, dietary fibers, proteins, vitamins, fat, mineral contents and a very small starch content (Baliga et al., 2011; Vayalil, 2011), each of which may vary, depending on cultivar type, fruit maturation stage, soil type and agronomic practices (Al-Farsi et al., 2007b; Amira et al., 2011). Moreover, some related studies have also been reported on date fruits from Saudi Arabia and the USA (Al-Turki et al., 2010), Oman (Al-Farsi et al., 2005; 2007a), Algeria (Mansouri et al., 2005), Iran (Biglari et al., 2008) and Bahrain (Allaith, 2008). However, limited studies have been done on antioxidant activity, total phenolic contents and sugar profiling of date fruits during their development. Therefore, the objective of the present study was to examine the overall composition of phytochemicals varying with stage of maturity and their interaction. The compounds examined include antioxidant activity (DPPH), total phenolic contents, antioxidant enzymes, sugars profile and soluble protein contents.

Materials and Methods

Date Fruit samples

The fruits of eight date palm cultivars (Akhrot, Hillawi-I, Qantar, Makran, Chohara, Kokna, Danda and Shamran-I) were harvested at three maturation stages: khalal, rutab and tamar at the Date Palm Research Station, Jhang, Pakistan during the 2012 harvest season. The cultivars selected for this study are of important market value and highly appreciated among consumers. The fruits were graded for consistency of size and color and kept at -80°C until analysis.

Extraction of date fruits

A sample of date (flesh) at each maturity stage (0.5 g) was ground in a mortar and pestle with 2 ml methanol (95% v/v) at room temperature, 25°C ±4, for antioxidant activity and phenolic contents, following the method of Ainsworth and Gillespie (2007); while extraction of date flesh (0.05 g) using potassium phosphate buffer (pH 7) for assessing enzyme and protein contents were carried out at three developmental stages as described by Naqvi et al. (2011). The extracts were filtered and centrifuged at 13,000xg, at 4°C for 5 min. The residues were discarded and the supernatant separated and stored at 4°C until use.

DPPH radical scavenging assay

The antioxidant activity of the date fruit (flesh) extracts was evaluated for scavenging of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as described by Amira et al. (2012). The 50 µl aliquot of various concentrations (25, 50, 75, 100 µg/ml) of the date fruit extracts was added to 5 ml of 0.004% DPPH in methanol. After incubation (30 min) period at room temperature, the absorbance was taken against a blank at 517 nm. Butylated hydroxytoluene (BHT) was used as a positive control. Three replicates were recorded for each sample. The disappearance of DPPH was read using microplate (BioTek, USA). Inhibition of free radical by DPPH in percent (%) was calculated according to the following equation:

$$I\% = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100;$$

where, A_{blank} (absorbance of only control reaction mixture), and A_{sample} (absorbance of the examined sample). IC_{50} values, which represented the concentration of date fruit extracts that caused 50% neutralization of DPPH radicals, were calculated from the plot of inhibition percentage against concentration.

Estimation of total phenolic contents

The total phenolic content (TPC) of date fruits was calculated by using Folin-Ciocalteu (FC) reagent following the method as described by Ainsworth and Gillespie (2007). In 100 ml of each sample, 200 µL of FC-reagent was added and vortexed thoroughly. The 800 µL of 700 mM Na_2CO_3 was added to each sample and incubated at room temperature for 2 h. The sample (200 µL) was transferred to a clear 96-well plate and absorbance of each well was measured at 765 nm. The amount of TPC was calculated using a calibration curve for gallic acid. The results were expressed as gallic acid equivalent (GAE) per dry matter.

Enzymatic antioxidant activity

The activity of catalase (CAT) and peroxidase (POD) were measured using the method of Liu et al. (2009) with some modifications as described by Naqvi et al. (2011). CAT uses hydrogen peroxide as a substrate and generates water and oxygen, while POD generates water and an activated donor molecule. The CAT reaction solution (3 ml) contained 50 mM phosphate buffer (pH 7), 5.9 mM H_2O_2 and 0.1 ml enzyme extract. The change in absorbance of the reaction solution was read at 240 nm. The POD reaction solution contained 50 mM phosphate buffer (pH 5), 20 mM guaiacol, 40 mM H_2O_2 and 0.1 mL enzyme extract. The change in absorbance of the reaction solution was taken at 470 nm.

Sugar profile (HPLC)

Sugar profile was calculated using high-performance liquid chromatography (HPLC) as reported by Amira et al. (2011). The edible part of date (1 g) was added to 2 ml of distilled water with constant stirring for 10 min to support dissolving the sugars in water. The fruit extract was centrifuged at 13000xg for 10 min and the supernatant was parted in sterilized eppendorf tubes.

HPLC conditions

The separation was carried out at room temperature on a Razex RCM-Monosaccharides Ca²⁺, Phenomenex. The mobile phase was 100% (v/v) double distilled water. The HPLC was linked to a refractive index detector (RID) RID-10 AL (Shimadzu, Japan). The injection capacity and flow speed was 20 µL and 0.6 ml/min, respectively. Identified sugars were quantified on the basis of peak areas of external standards consisting of glucose (2%), fructose (2%) and sucrose (2%) solutions. Each sample was carried out from integrated peak areas of the sample against the corresponding standard graph. Results were expressed as (g/100g).

Soluble protein contents

The soluble protein contents were quantified by the Bradford method (Bradford, 1976); 50µl of the sample was taken in microcentrifuge tube and 2 ml of Bradford reagent was added. Blank contain Bradford reagent. Absorbance was taken at 595 nm. Protein content was determined by a standard curve prepared with different concentrations of bovine serum albumin (BSA).

Statistical analysis

The experimental data was analyzed statistically using one-way analysis of variance (ANOVA) and each treatment contained three replications. Means were compared using Duncan's

Multiple Range (DMR) test at 5% probability with the help of MINITAB (15.0) and SPSS (20.0).

Results and Discussion

Antiradical efficiency of date fruits

The antiradical efficiency (AE) of date fruits showed significant ($p<0.05$) differences among eight examined cultivars as well as within stages of maturity as shown in Figure 1. The AE of date fruits gradually starts decreasing from immature stage (khalal) through rutab and finally at fully-ripened stage (tamar). The highest AE was recorded at khalal stage and lowest value at tamar stage. At both khalal and rutab stages all date cultivars showed highest AE, whereas Danda showed highest antiradical efficiency (2.14 and 1.34 AE) and Chohara cv. showed lowest (1.18 and 1.02 AE) at khalal and rutab stage, respectively. However, tamar stage showed the lowest values, whereas Kokna cv. showed (0.86 AE) and Chohara (0.36 AE). This gradual reduction in AE was due to reduction in tannins, ascorbic acid and β-carotene contents during maturation of dates (Allaith, 2008). The high level of AE particularly at khalal stage is due to a higher level of total phenolic contents at the same stage. This is due to a positive correlation between AE and TPC (Al-Turki et al., 2010; Awad et al., 2011a). The antioxidant activity of Tunisian and American date fruit cultivars as examined by DPPH assay was similar to our study (Vinson et al., 2005; Saafi et al., 2009). Biglari et al. (2008) reported Iranian dates possessed the highest AA (22.83–54.61 µmole TE/100g FW) which reflects the highest activity compared to our results. The differences in AE among cultivars are genotype-dependent and influenced by fruit maturation stage and storage time. The soil conditions and dose of fertilizers were also reported to be responsible for affecting the AE of date fruits (Mansouri et al., 2005).

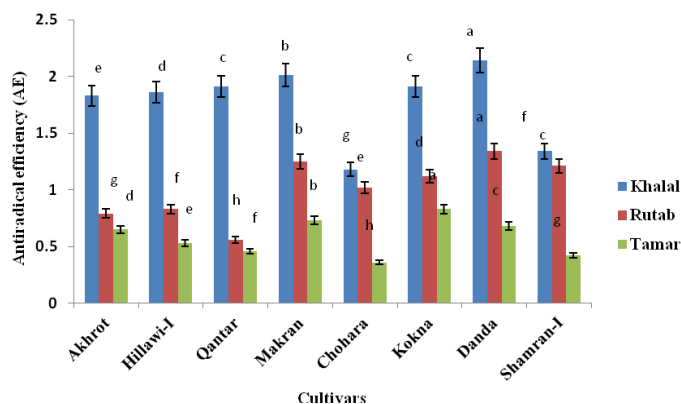


Figure 1. Antioxidant activity of date fruits at three different developmental stages.

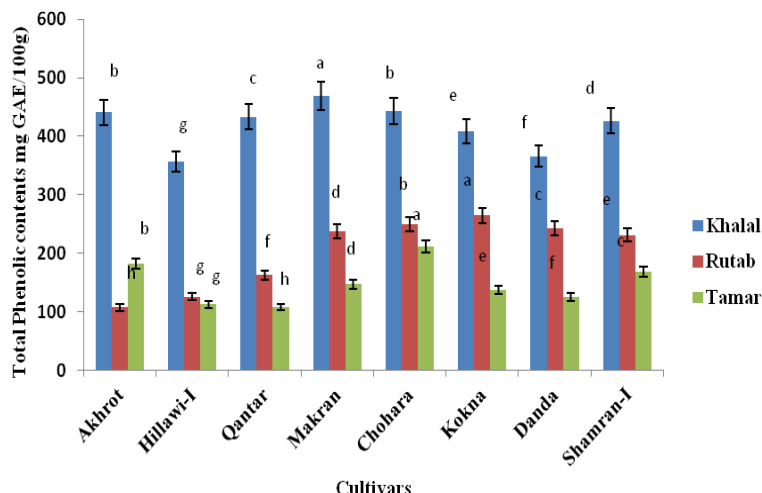


Figure 2. Total phenolic contents (TPC) of date fruits at three different developmental stages.

Total phenolic contents

There was a significant ($p < 0.05$) difference between the mean values of TPC among examined cultivars as well as within fruit developmental stages as shown in Figure 2. The mean values of TPC decreased significantly from khalal to tamar stage. At khalal stage Makran and Hillawi-I cvs. exhibited (468.99 and 356.99 mg GAE/100g, respectively) a higher level of TPC among all selected date palm cultivars; although, at rutab and tamar stages, respectively, Chohara cv. exhibited the highest values (249.58 and 211.64 mg GAE/100g) of TPC. TPC was higher at early (khalal) stage and gradually decreased at final stage (tamar). The declining trend may be due to a gradual decrease of tannins or oxidation of TPC by polyphenol oxidase that distinguish the developmental stages as well as the decreasing trend of radical scavenging assay (antioxidant activity) (Amiot et al., 1995; Myhara et al., 1999; Amira et al., 2012). These decreasing values of TPC in date fruits were also reported in previous published data (Amoros et al., 2009; Awad et al., 2011a). Our findings of TPC are the same as Omani (Al-Farsi et al., 2005; 2007b), Saudi Arabia and American (Al-Turki et al., 2010) dates. The differences in the TPC values may be due to variety, ripeness, cultivation region, environmental factors and storage time and conditions (Biglari et al., 2009). The storage duration affects TPC due to ethylene action which stimulates the activity of phenylalanine ammonia lyase that actively

biosynthesize the phenolic compounds (Hwang et al., 1994; Ritenour et al., 1995).

Specific activity of antioxidant enzymes

The specific activity of CAT was higher at khalal (2.18–0.87 IU/mg of protein) then gradually decreased at rutab (1.97–0.33 IU/mg of protein) and tamar (1.35–0.02 IU/mg of protein) stage fruits as shown in Figure 3. A similar decreasing trend in specific activity of POD was observed in all selected cultivars. The specific activity of POD range was (2.99–0.90 IU/mg of protein), (2.23–0.38 IU/mg of protein) and (0.95–0.39 IU/mg of protein) at khalal, rutab and tamar, respectively, as presented in Figure 5 The higher specific activity of CAT was observed in Akhrot cv. at khalal and rutab stages, followed by Shamran-I which also has higher activity at tamar stage among all studied cultivars. Similarly, the specific activity of POD was higher in Danda cv. at khalal stage, Akhrot cv. at rutab stage and Shamran-I cv. at tamar stage fruit. In general, the specific activity of both enzymes (CAT and POD) was higher at khalal and thereafter, a gradual decline was found at rutab and tamar stages. This decreasing trend was similar to the trend of antioxidant enzymes found in Tunisian dates during different developmental stages (Awad et al., 2011a). Catalase has the ability to convert H_2O_2 into water and oxygen forms, whereas, POD enzyme was found profoundly responsive to temperature and its activity decreased if exposed to heat for a longer period (Lee et al., 2003).

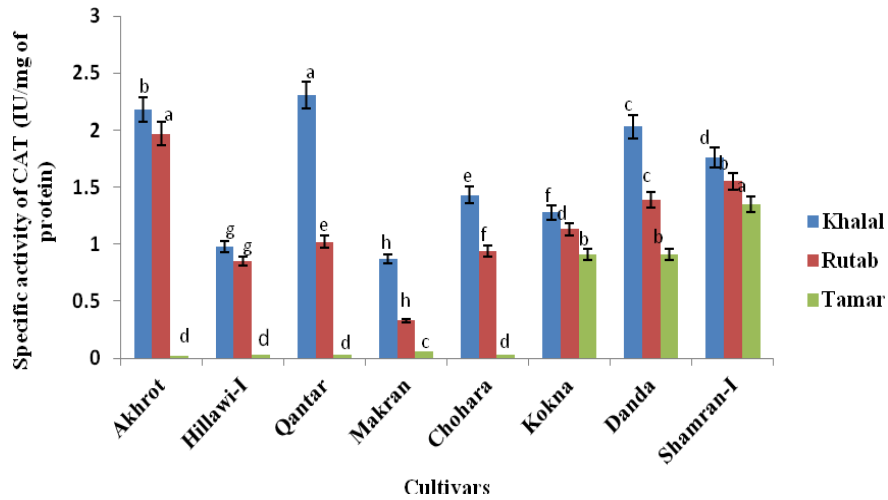


Figure 3. Specific activity of catalase (CAT) of date fruits at three different developmental stages.

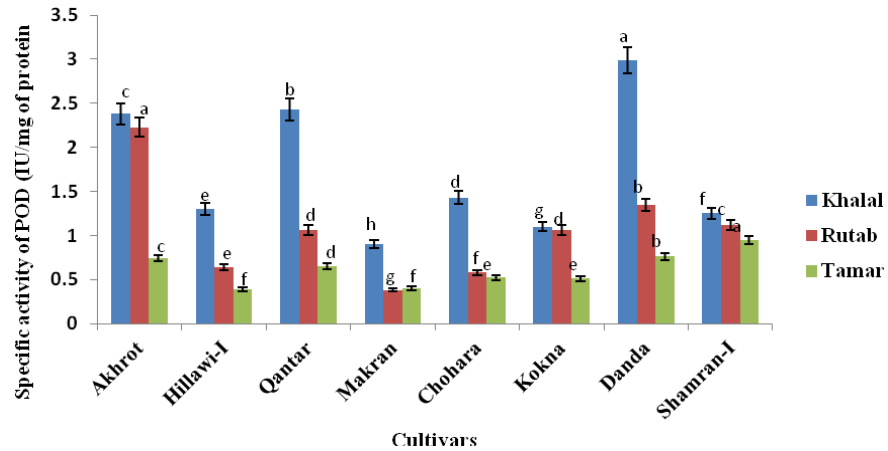


Figure 4. Specific activity of peroxidase (POD) of date fruits at three different developmental stages.

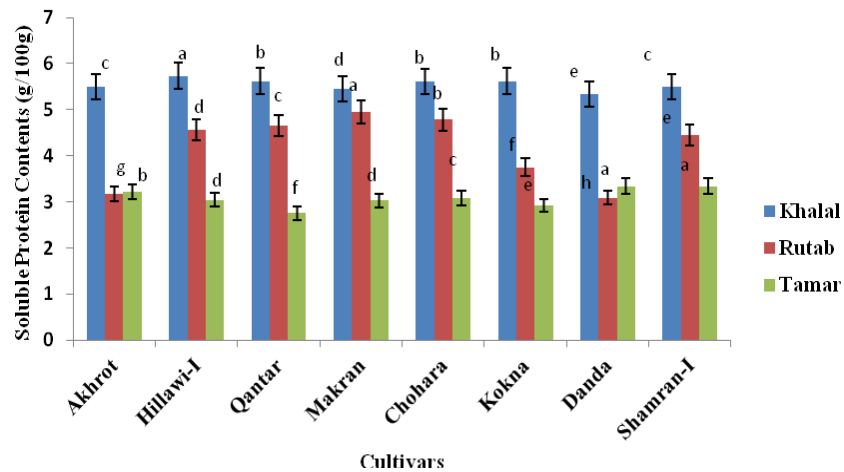


Figure 5. Soluble protein contents of date fruits at three different developmental stages.

Table 1. Sugars profile of eight date palm cultivars at three different developmental stages as quantified by HPLC method.

Cultivars	Ripening stage	Sucrose (g/100g)	Glucose (g/100g)	Fructose (g/100g)	Reducing Sugars (g/100g)	G/F
Akhrot	Khalal	15.14±1.02d	16.99±1.2f	15.25±0.2g	32.24±0.6g	1.11
	Rutab	3.76±0.98d	23.9±1.1e	22.48±0.5d	46.38±0.2e	1.06
	Tamar	nd	30.54±0.7e	28.47±1d	59.01±0.6d	1.07
Hillawi-I	Khalal	15.76±1.1b	21.73±1.1a	19.06±0.8c	40.78±1.2b	1.14
	Rutab	4.7±0.7b	25.91±1.1c	23.7±0.7c	49.18±0.8c	1.09
	Tamar	nd	31.45±0.9c	30.31±0.7b	61.76±0.2b	1.03
Qantar	Khalal	10.74±0.99f	16.92±0.8g	16.3±0.3f	33.22±0.5f	1.03
	Rutab	3.2±1e	20.55±0.4h	19.45±1g	40±0.7 h	1.05
	Tamar	nd	27.31±0.7h	25.71±1.3e	53.05±1f	1.06
Makran	Khalal	15.05±0.5e	21.24±0.5c	19.3±1.01b	40.54±0.3c	1.1
	Rutab	4.8±0.9b	26.92±1b	24.53±0.3b	51.45±0.01b	1.09
	Tamar	nd	30.91±0.3b	29.04±0.6c	59.95±0.5c	1.06
Chohara	Khalal	17.95±1.06a	21.71±0.2b	21.42±0.9a	43.13±0.7a	1.01
	Rutab	5.21±0.5a	28.59±1.2a	26.13±0.7a	54.72±0.8a	1.09
	Tamar	nd	31.66±1.1a	30.58±1.2a	62.24±0.2a	1.03
Kokna	Khalal	8.69±0.4h	20.06±1.03d	18.93±0.5d	38.93±1.2d	1.05
	Rutab	1.1±0.3g	25.18±1d	23.67±1c	48.85±1d	1.06
	Tamar	nd	26.61±0.2d	24.62±1.3g	51.23±0.1g	1.08
Danda	Khalal	10.40±1.13g	16.48±1.02h	15.33±0.03g	31.73±0.4h	1.01
	Rutab	2.4±0.87f	22.21±0.6g	20.96±0.2e	43.17±0.3f	1.08
	Tamar	nd	27.81±0.9g	25.43±0.4f	53.24±0.4e	1.14
Shamran-I	Khalal	15.34±1.1c	19.26±0.8e	17.48±1e	36.74±0.02e	1.1
	Rutab	4.32±0.4c	22.37±0.3f	20.61±1f	42.98±1.1g	1.08
	Tamar	nd	25.7±0.2f	22.48±0.7h	48.18±0.9h	1

nd: not detected, G/F: glucose fructose ratio.

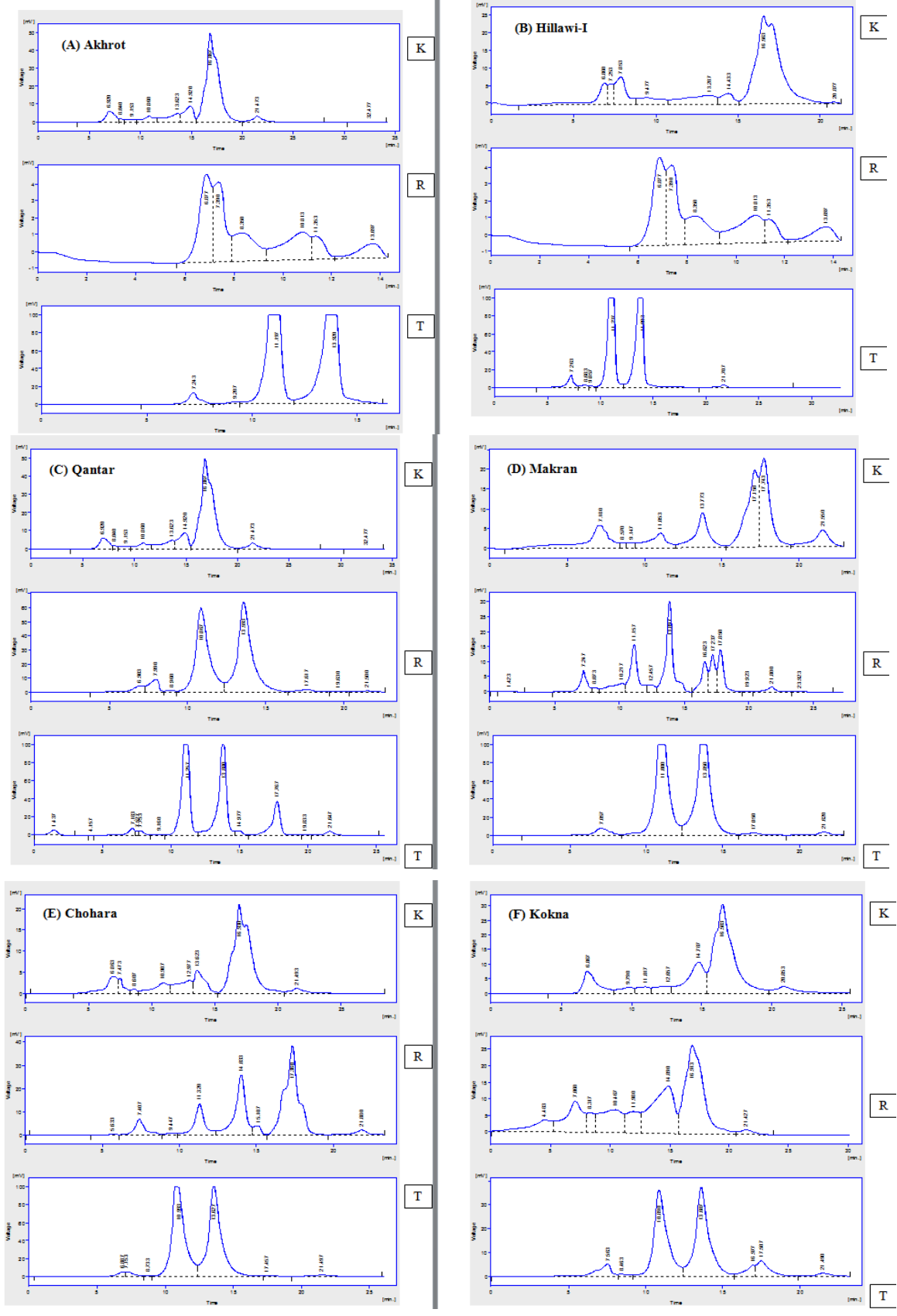
Sugar profiles by HPLC

Table 1 contains the arrangement and aggregate of sugars and Figure 6 shows the HPLC graphical representation of sugars of date fruits at different maturity levels. Analysis of variance revealed that total sugars of interest including sucrose, glucose and fructose, the profiles had significant ($p<0.05$) variation within all examined cultivar in relation to maturity. Chohara cv. showed highest values (17.95 g/100 g) while, the lowest values (8.69 g/100 g) of sucrose contents were quantified in Kokna cv. at khalal stage, whereas, drastic decrease in sucrose contents was observed at rutab stage and was not detected at tamar stage. However, gradual increases in glucose (16.92–31.66 g/100 g) and fructose (16.3–30.58 g/100 g) were observed overall in all cultivars during the fruit maturation process. The type and quantity of sugars vary according to the cultivar and their specific fruit developmental stage. In the presented results, sucrose contents were higher at khalal stage and after conversion a very small amount was present at rutab stage, while the accumulation of reducing sugars (glucose and fructose) started from khalal and was higher with different concentrations at tamar stage depending on the cultivars (Sawaya et al., 2006; Amoros et al., 2009). The reduction in sucrose and further increase in glucose and fructose contents is due to increasing activity of the invertase enzyme, which converts sucrose into reducing sugars (Amira et al., 2011). Generally, sucrose undergoes a complete hydrolysis, especially at tamar stage. However, our results are in agreement with previous reports on

Tunisian dates (Amira et al., 2011) and Iranian dates (Rastegar et al., 2012). The glucose and fructose (G/F) ratio are less, equal to those previously reported by Sahari et al. (2007) and Elleuch et al. (2008).

Quantification of protein contents

The soluble protein contents expressed as (g/100 g) significantly decreased ($p<0.05$) from khalal to tamar stage of the fruit maturity, as shown in Figure 5. The khalal stage showed highest values; while rutab and tamar stage showed comparatively less amount of protein content at date fruit maturity. Hillawi-I cv. showed highest value (5.73, 4.56 and 3.04 g/100 g), followed by Qantar (5.62, 4.62 and 2.75 g/100 g); while Danda cv. showed lowest values (5.34, 3.09 and 3.34 g/100 g) at khalal, rutab and tamar, respectively. The soluble protein contents in our study decreased from khalal to the tamar stage fruits which is in agreement with a previously published report (Awad et al., 2011b). The decreasing values of protein contents during maturation of fruits are because these proteins, pigments and phospholipids may undergo degradation by free radicals as the trend of radical scavenging system declines (Prochazkova et al., 2001) as well as the rising activities of hydrolytic and proteolytic enzymes like proteases (Rastegar et al., 2012). Our results showed similar values to those earlier reported by Rastegar et al. (2012). The existing differences may be due to different ecological conditions and cultivar origin.



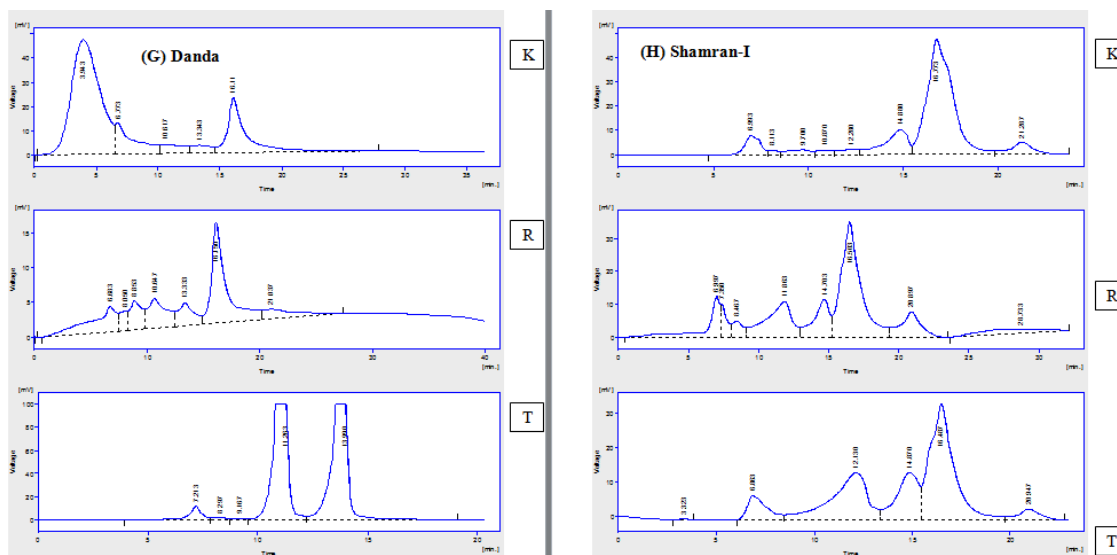


Figure 6. HPLC data for eight date palm cultivars at different fruit maturation stages khalal (K), rutab (R) and tamar (T). (a) cv. Akhrot, (b) cv. Hillawi-I, (c) cv. Qantar, (d) cv. Makran, (e) cv. Chohara, (f) cv. Kokna, (g) cv. Danda and (h) cv. Shamran-I.

Conclusions

It was concluded that local date palm germplasm has radical scavenging capacity especially at khalal stage of the ripening process. Moreover, local dates are a rich source of sugar and protein content, as compared to other commonly consumed date cultivars. These cultivars can be taken into consideration by growers for their wider cultivation.

Acknowledgement

We would like to share our deep acknowledgments to Central High Tech Lab, University of Agriculture, Faisalabad and Citrus Sanitation Lab, Institute of Horticultural Sciences, University of Agriculture, Faisalabad for providing lab facilities and help in conduction this research.

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