IJFANS
International Journal of Food And Nutritional Sciences

Volume 3, Issue 4, Jul-Sep 2014, www.ijfans.com e-ISSN: 2320-7876

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

IMPACT FACTOR ~ 1.021

Official Journal of IIFANS

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

e-ISSN 2320 –7876 *www.ijfans.com*
Vol.3, Iss.4, Jul-Sep 2014

Research Paper *Open Access*

FLESH AND FIBER CHARACTERIZATION AT THREE DIFFERENT EDIBLE STAGES OF DATE FRUIT DEVELOPMENT

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Received on: 8th June 2014 **Accepted on: 10th July 2014**

ABSTRACT

In the present study the effect of different biochemical attributes on date fruits at their three different edible stages were studied in Aseel, Dhakki (Pakistan) and Deglet Nour (Algeria) cultivars. The results depicted that total phenolic contents, antioxidant activity (DPPH), antioxidant enzymes (CAT, POD) and protein decreased gradually from khalal to tamar stage during date fruit development in all selected cultivars. Despite, the amount of glucose (23.89-32.31%) and fructose (20.34-30.45%) increased significantly during ripening process among examined cultivars. The characterization of fibers of date fruits at three edible stages by Fourier transform infrared spectroscopy (FTIR) showed lignin (1514 cm^{-1}), amide (1649 cm^{-1}), cellulose-I and cellulose-II (1635 and 1420 cm^{-1}), respectively, whereas, Scanning Electron Microscopy (SEM) revealed crystalline surface morphology of date fruit fibers at last three edible stages. Furthermore, our results revealed that variation in chemical composition and a significant variability in all the characterization techniques were recorded of date fruit fibers during ripening process.

Keywords: Date palm, Fibers, Antioxidant, Total phenolic contents, scanning electron microscopy**.**

INTRODUCTION

The date (*Phoenix dactylifera* L.) fruit is an important fruit for the population living in Pakistan. It has always engaged in an economic, social and environmental role for the people of this area. The date fruit is delivering nutritious diet to millions of individuals worldwide from thousands of years. Out of worldwide reported 5000 date palm cultivars 325 cultivars are present in Pakistan (Jamil *et al*., 2010). Date production is a world agri-business producing about 7.5 million tonnes (MT) of fruit and contribution of Pakistan is 72 thousand tonnes (FAO, 2011).

Date fruit passes through several ripening steps, viz. hababouk, kimri, khalal, rutab and tamar to attain maturity after pollination (Fadel *et al*., 2006), whereas, harvested and consumed at last three stages depending on market demand and environmental conditions. These stages are collectively termed by variation in color, texture, aroma and flavor. Fresh dates are considered nutritionally better-quality and appealing than dried dates (Vinson *et al*., 2005). Date flesh is the quick accessible supply of energy because of their highly rapid sugar (monosaccharide) contents such as glucose, fructose and sucrose (Vayalil, 2011); whereas, sucrose is also known as invert sugar because it is completely converted into glucose and fructose especially at rutab and tamar stages (Rastegar *et al*., 2012). Chemically, date fruit contains balance composition of macro and micro nutrients like; dietary fiber, protein, vitamins, fat, minerals and very little starch reliant on the cultivars (Vayalil, 2011).

The date fruits possessed a panel of antioxidant compounds and antioxidant enzymes (Awad *et al*., 2011). Still, some related studies have been reported on date fruits from, Tunisia (Amira *et al*., 2012), Oman (Al-Farsi *et al*., 2007), Algeria (Mansouri *et al*., 2005) and Iran (Biglari *et al*., 2008). The date fruit is considered very rich source of total phenolic contents among other consumable fruits and it can also be amplified by drying them under the sun light (Al-Farsi *et al*., 2005). To the best of our limited knowledge, elite biochemical studies have not been studied by our cultivars in Pakistan.

Therefore, the objective of present study was to conduct a comprehensive study on the nutritive importance of dates by analyzing various biochemical analyses during the ripening process and characterization of date dietary

fibers for their surface morphology, crystalinity, stability and composition.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

The chemicals used in these experiments were of analytical grade and were procured from USA (Sigma-Aldrich, Fluka) and Germany (Riedel-de-Haen, Merck).

PLANT MATERIAL

For this experiment the fruit samples of three date palm cultivars (Deglet Nour, Aseel and Dhakki) were collected from Date palm Research Station, Jhang, Pakistan during 2012 harvest season at different stages (khalal, rutab and tamar). Immediately after harvesting, date fruits were sorted for uniformity in size, defects and color, and stored at -80 ^{0}C until further analysis.

EXTRACTION OF DATE FLESH

The edible part (flesh) of date palm fruits (0.5 g) at three maturity stages was grinded in mortar and pestle with 2 mL methanol water (95% v/v) at room temperature 25 ^{0}C ±4 following the method of Ainsworth and Gillespie, (2007) for examining total phenolic contents and antioxidant activity; while extraction in potassium phosphate buffer (pH: 7.0) was carried out as described by Naqvi *et al*. (2011) for soluble protein contents and antioxidant enzymes (CAT and POD). The extracts were filtered and centrifuged (Hettich, Germany) at 13,000 x*g*, at 4^{0}C for 5 mint and the supernatant were separated in sterilized eppendorf tubes and used for further analysis.

CHEMICAL COMPOSITION

TOTAL PHENOLIC CONTENTS (TPC)

TPC was determined by using Folin-Ciocalteu reagent method as described by Ainsworth and Gillespie, (2007). In each sample (100 mL), the FC-reagent (200 µL) was added and vortex carefully. Then 800 µL of 700 m*M* Na_2CO_3 was added into each sample and incubated at room temperature for 2 h. The date fruit extract (200 µL) was transferred to a clean 96-well plate and absorbance of each well was measured at 765 nm. Amount of TPC was calculated using a calibration curve for Gallic acid. The results were expressed as gallic acid equivalent (GAE).

2, 2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH)

The antioxidant activity of the date fruits (flesh) extracts was assessed by measuring their scavenging abilities to 2, 2-diphenyl-1-picrylhydrazyl stable free radicals. Antioxidant activity was determined by 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 fruits extracts were added to 5 mL of a 0.004% methanol solution of DPPH. After a 30 minutes incubation period at room temperature, the absorbance was read against a blank at 517 nm. Butylated hydroxytoluene (BHT) was used as a positive control. For each sample, three replicates were recorded using microplate reader (BioTek, USA). Inhibition of free radical by DPPH in percent (%) was calculated in the following way:

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

Where A_{blank} is the absorbance of the control reaction mixture excluding the test compounds, and A_{sample} is the absorbance of the test compounds. 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.

IDENTIFICATION AND QUANTIFICATION OF SUGARS BY HPLC

Sugar profile was quantified using high-performance liquid chromatography (HPLC) as reported by Amira *et al*. (2011). Date flesh (1 g) was taken in 2 mL of distilled water with continuous stirring for 10 min to aid dissolving the sugars in water. The extract was centrifuged at 13000 x*g* for 10 min and the supernatant was separated.

The separation was carried out at room temperature on a Razex RCM-Monosaccharides Ca^{+2}, Phenomenex. The mobile phase was 100% (v/v) double distilled water. The HPLC was connected to a refractive index detector (R_ϵID) RID-10 AL (Shimadzu, Japan). The injection volume and flow rate 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 (1%), fructose (1%) and sucrose (1%) solutions. Each sample was carried out from integrated peak areas of the sample against the corresponding standard graph. Results were expressed as percentage of dry weight.

SOLUBLE PROTEIN CONTENTS

The soluble protein contents of the samples were determined by Bradford method (Bradford, 1976), bovine serum albumin **(**BSA) was used as standard and absorbance was taken at 595 nm.

ANTIOXIDANT ENZYME ANALYSIS OF DATE FLESH

The specific activity of catalase and peroxidase were measured using the method as described by Naqvi *et al*. (2011). The CAT reaction solution (3mL) contained 50 m*M* phosphate buffer (pH: 7), 5.9 m*M* H_2O_2 and 0.1 mL enzyme extract and the absorbance was read at 517 nm. The POD reaction solution contained 50 m*M* phosphate buffer (pH: 5), 20 m*M* guaiacol, 40 m*M* H_2O_2 and 0.1 mL enzyme extract and the absorbance was read at 470 nm. The results were presented in international unit (IU) per milligram of protein.

EXTRACTION OF DIETARY FIBERS

Hot plate magnetic stirrer was used for extraction of dietary fibers from milled date flesh. Hot sterilized water separated the fibers from date flesh due to

continuous stirring of the dates. After five rinsing's with double distilled water, allowed the fibers free from sugars using the method of Shafiei *et al*. (2010). The dietary fibers were stored at normal refrigerated temperature (4 ^{o}C) to avoid degradation of date dietary fibers.

SCANNING ELECTRON MICROSCOPY (SEM) OF DATE DIETARY FIBERS

The gross microstructure and surface morphology of the date dietary fibers was determined using SEM (Perkin Elmer, Diamond series, USA). Sputter coater was used for the date fruit fibers sample preparation.

FOURIER TRANSFORMATION INFRA-RED (FT-IR) SPECTRAL ANALYSIS OF DATE DIETARY FIBERS

Infrared of date dietary fibers were recorded with FT-IR (Bruker FT-IR, 2000, U.S.A) spectrometer with encompass software in the range of 3500/500 cm^{-1}, using Zinc Selinite (Zn-Se) method.

STATISTICAL ANALYSIS

The experiment was laid out Completely Randomized Design (CRD). Data was analyzed statistically by one way analysis of variance (ANOVA) and means were compared for significant differences using Duncan's Multiple Range (DMR) at (p=0.05) using IBM SPSS 20.0 (SPSS Inc, Chicago, IL, U.S.).

RESULTS AND DISCUSSION

The date fruit attained four internationally accepted stages of development after pollination i.e. kimri, khalal, rutab and tamar. The dates are mainly harvested, marked and consumed at last three ripening stages. Though, the fruits of three date palm cultivars Aseel, Dhakki (Pakistan) and Deglet Nour (Algeria) were harvested at khalal, rutab and tamar stage to determine total phenolic contents, antioxidant activity, total sugars (sucrose, glucose and fructose) and soluble protein contents.

TOTAL PHENOLIC CONTENTS (TPC)

Analysis of variance revealed ($p<0.05$) that significant variation was found in the mean values of TPC at three developmental stages as shown in table (1). Dhakki, Deglet Nour and Aseel cultivars possessed (498.99 more than 219 more than 37.05 mg GAE/100 g), (441.07 more than 93.19 more than 58.56 mg GAE/100 g) and (393.54 more than 243.1 more than 173.41 mg GAE/100 g), respectively showed declining trend in total phenolic contents during maturation process, ranging khalal to tamar stage. These results showed similar values and trend with previous reporting of Al-Turki *et al*. (2010). Amira *et al*. (2012), reported TPC at different ripening stages which are in agreement with these findings. The tamar stage values of Aseel and Deglet Nour showed lower values than reported by Al-Farsi *et al*., 2007. These three examined cultivars exhibited higher level of TPC compared to Algerian dates (Mansouri *et al*., 2005). The gradual reduction in the TPC of date fruits during maturation process is due to the reduction in tannins during ripening process (Awad *et al*., 2011). The differences in results may be due to origin, location, cultivars, soil type, exposure to sunlight, applied irrigated water etc.

ANTIOXIDANT ACTIVITY (DPPH)

Antioxidant activity as quantified by DPPH assay depicted that antiradical efficiency ($AE=1/IC_{50}$) of three cultivars showed significant ($p < 0.05$) declining trend from khalal upto tamar stage, respectively as shown in table 1. Our cvs. exhibited antioxidant values ranging (2.07-1.56 AE), (1.83-1.37 AE) and 1.17-0.91AE) during development process at khalal, rutab and tamar stage, respectively. Amira *et al*. (2012) analyzed the antioxidant activity using DPPH assay in Tunisian dates during development and reported that the trend and AE values were found similar to this study. The AE of American and Algerian dates showed values 2.17 µmol TE and 0.08-0.22 AE, respectively and observed similar from ours (Vinson *et al*., 2005; Mansouri *et al*., 2005). These variations in results may reflect difference in cultivars, cultural operations, amount of fertilizer and different analytical approaches for the quantification of antioxidant activity.

SUGARS PROFILING (HPLC)

The percentage of most important sugars presented in table 2. Analysis of variance showed significant difference ($p < 0.05$) between important sugars (sucrose, glucose and fructose) of all selected cultivars. The amount of sucrose was only present at khalal stage some minor amount was also present at rutab stage as well; while the values of glucose and fructose showed increasing trend from khalal to tamar stage, respectively, which clearly indicate the rising activity of invertase enzyme and conversion of sucrose into monosaccharide components (El-Sharnouby *et al*., 2009). These selected cvs. showed (18.78<3.1%) of sucrose, (20.61>32.31%) of glucose and (17.76>30.45%) of fructose contents during ripening process of fruits. These results are comparable with previously published reports of different date cultivars (Amira *et al*., 2011; Vayalil, 2011). Rastegar *et al*, (2012) analyzed the sugar profile of three Iranian date palm cultivars ranged 52.6 to 63% at tamar stage which was similar to our finding. During ripening process the sucrose undergoes thorough hydrolysis and converted into reducing sugars at tamar stage (Rastegar *et al*., 2012). The differences in results may be due to the ecological and genetic influences that may disturb the quantitative and qualitative configuration of the sugar profile by altering the action of the enzymes involved in modification and synthesis practices.

SOLUBLE PROTEIN CONTENTS QUANTIFICATION

The analysis of variance ($p < 0.05$) revealed significant variation in final values of protein contents (g/100g) in all three examined cultivars. The cv. Deglet

Nour has overall highest values (5.45, 4.84 and 3.35 g/100g), then Aseel (5.74, 4.18 and 3.09 g/100g) and Dhakki (5.62, 4.01 and 3.23 g/100g) of protein contents during three different maturation stages as shown in table (2). The cv. Aseel has higher protein contents at khalal stage but cv. Deglet Nour showed higher contents at rutab and tamar stage. This study reflected that soluble protein contents were higher at early stage (khalal) and decrease to reach lower concentration at tamar stage. It is due to that when free radical scavenging system declines during senescence of tissues; it starts degradation of proteins, particularly accruing difference in protein due to activation of protease enzyme in the ripening process (Prochazkova *et al*., 2001; Rastegar *et al*., 2012). Our cultivars showed higher values of protein contents as compared to some previous reported data (2.10-3.03%) in other date cvs. (Elleuch *et al*., 2008). (Rastegar *et al*., 2012) also depicted the enzyme and biochemical variability in Iranian dates during developmental stages and described that the protein decreased significantly upto the full ripe stage. The cvs. Khalas and Barhee of UAE contained 2.5% and 3.6% protein contents respectively (Ismail *et al*., 2006). The results revealed that differences may possibly due to diverse geo-ecological conditions.

ANTIOXIDANT ENZYMATIC ACTIVITY

The specific activity of antioxidant enzymes (catalase and peroxidase) was variable at three developmental stages and directly proportional to the antioxidant activity. The specific activity of CAT (1.12, 0.99 and 0.91 IU/mg of protein), (0.99, 0.86 and 0.035 IU/mg protein) and (1.67, 1.39 and 0.024 IU/mg of protein) for Deglet Nour, Dhakki and Aseel, respectively at three maturation stages as shown in table 1. Similarly, the specific activity of POD of Deglet Nour (0.78, 0.73, 0.67 IU/mg of protein), Dhakki (1.06, 0.96, 0.59 IU/mg of protein) and Aseel (1.69, 1.29, 0.6 IU/mg of protein) during maturation stages as shown in table 1. The results are comparable to (Awad *et al*., 2011), reported that activity of CAT and POD was high at early (kimri) stage and gradual decrease was found as fruit leans forward to the maturity. This increase or decrease in specific enzyme activity during maturation stage may be due to the particular enzyme requirement of ripening stage, exposure to temperature, moisture loss, cultivar genetics, etc. during the process.

Table 1: TPC, AE, CAT and POD contents of Deglet Nour, Dhakki and Aseel at three different edible stages of fruit development

Cultivars	Ripening stage	TPC (mg GAE/100g)	AE	CAT (IU/mg of proteins)	POD (IU/mg of proteins)
Deglet Nour	Khalal	441.08±1.85b	2.07±0.43a	1.12±0.08b	0.78±0.13c
	Rutab	93.19±1.18c	1.83±0.14a	0.99±0.07b	0.73±0.06c
	Tamar	58.56±1.08b	0.91±0.54c	0.91±0.09a	0.67±0.03a
Aseel	Khalal	393.54±1.74c	1.56±0.37b	1.67±0.01a	1.67±0.09a
	Rutab	243.11±1.79a	1.46±0.79b	1.39±0.04a	1.29±0.02a
	Tamar	173.42 ±0.64a	1.17±0.32a	0.24±0.22c	0.60±0.20b
Dhakki	Khalal	498.93±1.01a	2.07±1.10a	0.99±0.03c	1.06±0.12b
	Rutab	219.00±1.28b	1.37±0.28c	0.86±0.03c	0.96±0.03b
	Tamar	37.05± 0.38c	1.15±0.14b	0.35±0.15b	0.59±0.12c

Table 2: Sugars profiling (HPLC) and soluble protein contents (Bradford method) of Deglet Nour, Dhakki and Aseel at three different edible stages of fruit development

Cultivars	R.S.	Sucrose (%)	Glucose (%)	Fructose (%)	RS (%)	G/F	Proteins (µg/g)
Deglet Nour	Khalal	18.78±1.02a	20.62±1.1b	20.34±0.2a	40.96	1.01	5.45±0.13c
	Rutab	5.35b±0.98a	25.42±1.2b	24.93±0.5b	50.35	1.01	4.84±0.38a
	Tamar	ND	31.37±0.7b	30.45±1a	61.42	1.03	3.35±0.3a
Aseel	Khalal	13.72±1.1c	23.89±1.09a	20.21±0.3b	44.09	1.18	5.74±0.14a
	Rutab	3.1±0.7c	27.48± 1.1a	25.05±1.01a	52.33	1.09	4.18±0.08b
	Tamar	ND	32.31± 0.9a	28.03±0.4b	60.34	1.15	3.09±0.05c
Dhakki	Khalal	14.69±0.9b	18.39± 0.8c	17.76± 1.1c	36.15	1.03	5.62±0.18b
	Rutab	3.8b±1.0b	24.63±0.4c	21.62± 0.9c	46.24	1.13	4.01±0.16c
	Tamar	ND	28.94± 0.6c	27.53±0.7c	56.47	1.05	3.23±0.13b

R.S. represent ripening stage, ND represents not detected; RS represents reducing sugars; TS represents total sugars; G/F represents glucose, fructose ratio.

FOURIER TRANSFORM INFRA-RED (FT-IR) SPECTROSCOPY

The FT-IR interferogram of date dietary fibers of three date palm cultivars at three maturity phases are shown in fig (1) and data is presented in table (3). The region 810-1000 cm^{-1} revealed (C-H) out of-plane bending vibrations (Kumar *et al*., 2005; Sundaraganesan *et al*., 2009). Mostly, region of 1430–1650 cm^{-1} form a C=C stretching vibrations in aromatic compounds (Mahadevan *et al*., 2011). The region of carbonyl variations (1500 to 1800 cm^{-1}) has feeble band at 1653cm^{-1} (amide I), IR band at 1649 cm^{-1} (amide I, in-plane bending of H_2O) and near 1559 cm^{-1} (Amide II) as shown in table (3). The frequency band near 1635 cm^{-1} revealed (OH of H_2O absorbed from cellulose) indicating cellulose I (1635 cm^{-1}) and Cellulose II (1425 cm^{-1}), (Carrilo *et al*., 2004), whereas, band at 1514 cm^{-1} showed the aromatic rings indicating the presence of lignins (Shafiei *et al*., 2010). The vibration band at 1200-1445 cm^{-1} mainly indicate the plane ring denaturation including CH and OH bending modes (Novac *et al*., 2012), while near 1420 cm^{-1} revealed cellulose II and band near 853cm^{-1} and 880cm^{-1} indicated α-glucans, though is very sensitive to anomeric structure nearby glycosidic bonds repectively, α- and β- configuration of the polysaccharides (Mohacek-Grosev *et al*., 2001). The vibrations near 1072 are typically indication for β-glucan (Sandulla *et al*., 1999).

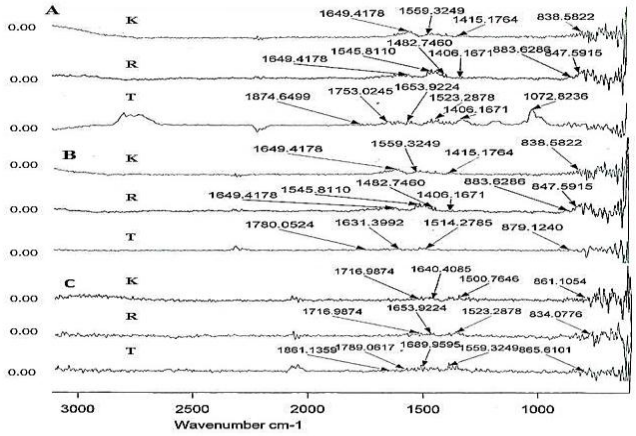

Fig (1). FTIR images of (A) Aseel, (B) Dhakki and (C) Deglet Nour at (K) khalal, (R) rutab and (T) tamar stages of date fruit fibers

CHARACTERIZATION OF DATES DIETARY FIBERS BY SEM

The SEM images of different date dietary fibers of three date palm cultivars at three developmental stages are shown in fig (2). Surface morphology of the date fruit fibers shows the complex interaction within stage of maturity and between different cultivars. Roughness of the apparent date dietary fibers at microscopic level can be principally endorsed to alteration, heterozygosity of a suspension including cell wall fragments and in a slight extent, to high viscidness (extensive relaxation interval) in the ultimate stages of solidification. Spongy structure of the date dietary fibers reflected as an essential element for preparation of new products to take over surgery, in frameworks for micropropagation, and as wound healings (Kil'deeva *et al*., 2006). Several of these products are expected to be based on the polyurethane and presences of apertures are indispensable in this situation. We consider that porosity is not the important parameter for their application in the case of polysaccharide based films.

Table 3: Frequencies of the FT-IR bands for date dietary fibers of three cultivars at three different edible stages of fruit development

Frequency (cm^{-1})	Assignment	Comments
809-1000	(C-H)	out of-plane bending vibration
853, 880	α-glucan	
1200-1445	CH and OH	Bending modes
1415	CH	
1430-1650	(C=C)	Stretching vibrations
1689	(C=O)	Stretching
1653	Amide-I	
1649	Amide-I	In-plane bending of H_2O
1640	Amide-I (C=O)	Amide (1) associated with carbonyl
1635	Cellulose-I	OH of H_2O absorbed from cellulose
1559	Amide-II	
1514	Lignin	
1500	(C=C) υ	
1420	Cellulose-II	
1072	β-glucan	

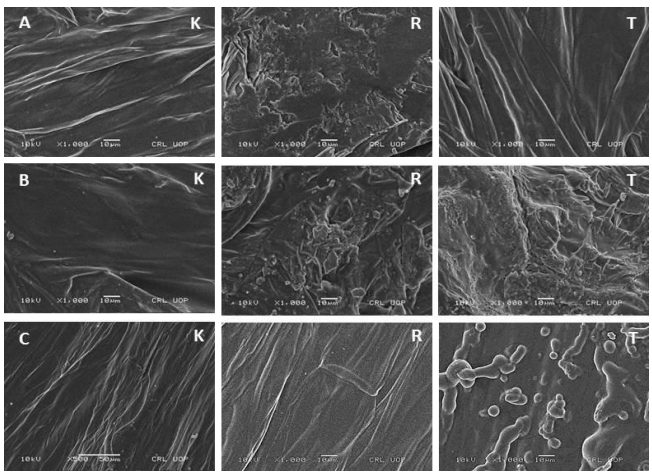

Fig (2). SEM images of (A) Aseel, (B) Dhakki and (C) Deglet Nour at (K) khalal, (R) rutab and (T) tamar stages of date fruit fibers

CONCLUSION

The assessment of biochemical composition of three date fruit cultivars showed that Pakistani indigenous cultivars are rich in sugars mainly glucose and fructose; while lower in TPC, antioxidant activity, antioxidant enzymes and protein contents specially at tamar stage. These findings showed that our date cultivars have the potential to compete with the world's most capable promoted variety (Deglet Nour). Our results revealed that variation in chemical composition and a significant variability in all the characterization techniques were recorded of date fruit fibers during ripening process hence, the farmer and consumer could take these cultivars under consideration for further cultivation.

ACKNOWLEDGEMENT

I would like to express my sincere acknowledgements to **International Centre for Development and Decent Work (ICDD) program**, **Germany-Pakistan, Punjab Agriculture Research Board (PARB), National Institute of Food Science and Technology (NIFSAT), Central High Tech Lab, University of Agriculture, Faisalabad** and Centralized Resource Lab, Peshawar for facilitating me in terms of funds and laboratory space for this research.

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