INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 15, No. 3, p. 1-14, 2019

RESEARCH PAPER OPEN ACCESS

Characterization of compositional and functional characteristics of date seeds and oil (*Phoenix dactylifera* L.) from three varieties

Muhammad Qasim Raza[1*], Muhammad Umair Arshad[2], Muhammad Sajid Arshad[2], Faqir Muhammad Anjum[2,3]

[1]*Institute of Home and Food Sciences, Government College University, Faisalabad 38040, Pakistan*

[2]*Institute of Home and Food Sciences, Faculty of Life Sciences, Government College University, Faisalabad, Pakistan*

[3]*University of The Gambia (UTG), Banjul, Gambia*

Key words: Date seeds and oil, proximate analysis, mineral contents, antioxidant indices, HPLC quantification.

http://dx.doi.org/10.12692/ijb/15.3.1-14 Article published on September 14, 2019

Abstract

Current study on characterization of date seeds (*Phoenix dactelifera* L.) particularly and their oil especially from three different varieties with respect to their composition in addition to the functional constituents is of its very first kind in Pakistan. Core objective of the current investigation was to find the most appropriate date seed variety with proven health claims, apposite antioxidant potential and suitability of its oil that could be further utilized as an active constituent for the preparation of functional foods and nutraceuticals. Seeds of three date palm cultivars i.e. Karblain, Halawi and Ajwa were comparatively examined for their proximate composition, mineral contents, characterization of date seed oil and antioxidant indices by their standard methods. High Performance Liquid Chromatography (HPLC) analysis was carried out for quantification of phenolic acids and flavonoids. Results showed that carbohydrates were the principal constituents trailed by moisture, while crude fat, crude protein and ash contents were also detected in small proportions. Potassium was the predominant mineral while TPC and TFC values showed significant variations ($p \leq 0.05$) in all three date seed varieties. On characterization date seed oils revealed that being dominant in unsaturated fatty acids of plant origin with good antioxidant potential made it superior in quality as compared with the other conventional edible oils. Outcomes of this current investigation proposed that date seeds are found very rich in nutritional and functional moieties including minerals and natural antioxidants with a noticeable quantity of good quality edible oil that may further be exploited for human consumption.

* **Corresponding Author:** Muhammad Qasim Raza, ✉ qasimraza1648@gmail.com

Introduction

Very imperative linkages have been recognized among the nutrition and human health, which in turn are rerouting the demand towards plant based natural products owing to the treatment of diverse diseases (Jayasekera *et al.*, 2011). Communities with high population ratios as compared to their low income are being diverted towards the diet-based regimen because of the high cost of medication in addition to their health promoting benefits. Natural products like dates and date seed oil (DSO) are vital in this background due to their abundance and availability, squat cost and associated human health benefits. Different studies revealed that plant constitutes are very helpful and beneficial in restricting different diseases (Arshad *et al.*, 2014).

Date (*Phoenix dactylifera* L.) is believed to be the oldest food produce being consumed by the mankind for more than 6000 years (Kwaasi, 2003), included in the family *Palmae* and is under cultivation across the globe because of its popularity among the masses and its wide acceptability to an extensive range of ecological regions including arid to semiarid areas (Al-Farsi and Lee, 2008a). Dates are mostly consumed in fresh form, while the surplus quantity is preserved in its dried form especially in the arid region in addition to a number of products that are also being processed worldwide (Omezzine *et al.*, 1997).

Date fruit is comprised of two major parts namely, fruit pericarp that is used either as such fresh or dried, or in its any of the processed forms while, date seed commonly called the pit is the most neglected waste part of the date fruit having very limited usage (Besbes *et al.*, 2004a). Date seeds are reported having a weight of 9-15 % of fruit weight and is believed that it is comprised of date seed oil (DSO) which is almost 8-10% on an average basis (Hussein *et al.*, 1998; Al-Farsi *et al.*, 2007). In 2011, the area under cultivation of dates was reported as 2.9 million acres in almost 35 countries worldwide, with a produce of 7200000 tons, means with an annual waste material of approximately 720,000 tons of date seeds without any productivity and utilization (FAO, 2011). World's economic survey shows that Egypt is the largest producer with a 17% of the world's total production and ranked number one in the list, followed by Iran with a 14% of world's total date production (FAO, 2004). Pakistan is ranked 6th in the list, with an annual production of almost 650,000 tons of dates. It means that over 60,000 tons (date seeds) were produced in the form of over 300 varieties with an estimated area of around 90.1 thousand hectares including 29,300 hectares under cultivation (GOP, 2008). With its dominance in Sindh province with a production of 201,100 tons as compared to an area of 84,700 hectares and a production of 426,300 tons in Pakistan across the country (Sayed, 2007).

General consideration about the date pits/seeds is a waste or a by-product/discarding material without any productivity or usage in the processing line either after the domestic consumption or otherwise discarded as such after various processing operations in industries, using dates for the manufacturing of date jams, date bars, date paste or date syrups and in many other different processed products. A number of researchers have conducted their work on the chemical composition and antioxidant potential of date seeds in addition to the characterization of date seed oil (DSO) after its extraction from date seeds (Nehdi *et al.*, 2010; Sadiq *et al.,* 2013; Suresh *et al.*, 2013). In the present research work date seed oil from three different varieties were evaluated for their suitability as a potential alternative source of conventional fats on the basis of characterization, rate of recovery and antioxidant activity of date seed oils. Up-till now very limited data in terms of research work is available for the efficient utilization of date seeds and its active ingredients in human diets (Basuny and Al-Marzooq, 2011).

Flavonoids in general and flavanols and flavonols specifically have proven health benefits against curtailing various diseases and disorders including cardiac complications, obesity, cancer and hyperglycemia (Hassimotto *et al.*, 2005). In this context active biochemical constituents of flavonoids

and non-flavonoid contents consisting rutin, catechin and caffeic acid were analyzed from the date seed oil of three different verities through HPLC. Date seeds being good source of physico-chemical and functional moieties with a considerable amount of 8-10% of date seed oil mainly comprised of MUFAs and PUFAs considered healthful (Siscovick *et al.*, 2017) were further explored for the individual active ingredients of flavonoid and non-flavonoids origin in order to quantify their potential health benefits. Prime objective of this study was to evaluate the proximate and nutritional profile of the seeds from three prominent date varieties and to characterize their fatty acid profiles, antioxidantal activity and estimation of individual phenolic constituents of their lipid fractions for the value addition of the neglected/waste by-products of dates.

Materials and methods

Procurement of raw materials

Three date seed varieties i.e. Karblain, Halawi and Ajwa were procured from two different locations, Karblain and Halawi were purchased from date palm research center Jhang, Punjab, (Pakistan) and Ajwa being native to Saudi Arabia was procured locally from Faisalabad, Punjab, (Pakistan).

The seeds (pits) of three varieties (Karblain, Halawi and Ajwa) were separated from the date fruits after obtaining the proper identification certificate. Similarly, different reagents as per specifications and standards were obtained from Sigma Aldrich (Seelze, Germany), Megazyme International (Ireland), E. Merck (Darmstadt, Germany), Lab-Scan (Dublin, Ireland) and Randox laboratories (UK).

Preparation of date seed powder

Date seed powder of three varieties Karblain, Halawi and Ajwa was prepared (Rahman *et al.,* 2007) with minor changes.

The seeds were cleaned and separated in the shade, then were desiccated in hot air oven at 50°C (Memmert UNE 200, Germany). Separated date seeds were then ground using hammer mill (Fitz Mill model LH-DAS06) to a possible minimum size of 500µm. Resultant powder obtained from each variety was kept hermetically sealed in food grade containers until further use in research work.

Chemical analysis of raw materials

Each sample in triplicates was analyzed for the proximate analysis by means of standard methods (AACC, 2000). Moisture contents (MC) were estimated by the standard method No. 44-15A while crude fat (CF) was analyzed by following the method No. 30-10. Similarly, crude protein (CP) was measured by method No. 46-13 calculated as (N) and C.P was determined as N x 5.70. Further, Cf was estimated by the standard procedure No. 32-10 (AACC, 2000), Total ash was quantified in accordance with method No. 08-01 while NFE was determined according to the following equation: -

NFE% = 100 – (CP% + CF% + Cf% + Ash%).

Estimation of mineral contents

Samples of date seed powder from three date varieties were further subjected to mineral estimation (AOAC, 2006). Flame Photometer was employed for the determination of sodium and potassium, while Atomic Absorption Spectrophotometer (Varian AA240, Australia) was used for the estimation of (P), (B), (Mg), (Ca), iron, zinc, magnesium and copper.

Dietary fiber (DF) estimation

DF content of the date seed powder was estimated by their standard method (AOAC, 2006).

Antioxidant activity of date seed powders

Preparation of extracts

Extraction was prepared by taking a known 20g of date seed powder from all three varieties in 200ml of 80% methanol (Al-Farsi and Lee, 2008a).

DPPH radical scavenging assay

2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) assay was estimated using the given expression (Rababah *et al.*, 2011).

$$Scavenging(\%) = \left(\frac{A_{blank} - A_{sample}}{A_{blank}}\right) \times 100$$

TPC and TFC quantification

Total polyphenols contents (TPC) and total flavonoids contents (TFC) in the samples of date seed powder were estimated by following Folin-Ciocalteu protocol (Suman Chandra *et al.*, 2014).

HPLC analysis of phenolic acids and flavonoids

HPLC study was conducted with standards of the system named (Shimadzu CBM-20A) from Shimadzu Corporation, Kyoto, Japan as per standard conditions.

The hydrolysis of previously prepared extracts was conducted (Nuutila *et al.*, 2012) followed by the preparation of calibration curves of the respective standards of different phenolic acids and flavonoids.

Extraction of date seed oil (DSO)

Extraction of DSO was done from date seed powders (Mortadha *et al.*, 2015) in accordance with (AOAC, 1990).

$$\text{Yield of oil extraction} = \frac{(\text{weight of oil extracted})}{(\text{weight of seed sample})} \times 100$$

Characterization of date seed oil (DSO)

DSO was analyzed for following parameters of SG, RI, smoke point, FFA, IV, FA profile and PV, according to their respective methods (AOAC, 1990; AOCS, 1998).

Fatty acid analysis

Fatty acid methyl esters were prepared using transesterification with methanolic potassium hydroxide through chromatographically in triplicate (Juhaimi *et al.*, 2012).

Free radical scavenging ability of date seed oil

Free radical scavenging activity of extracts was estimated by DPPH method (Juhaimi *et al.*, 2012) with trivial changes.

Antioxidant activity of date seed oil

Antioxidant activity of all three date seed oils were carried out by following a combination of two tests in a model system based on coupled oxidation of β-carotene and linoleic acid assay and FRAP (ferric reducing antioxidant power) of extracts (Singleton *et al.*, 1999; sun *et al.*, 2010).

Determination of rutin, catechin and caffeic acid

Catechin, rutin and caffeic acid (nonflavonoid) contents were estimated (Al-Farsi *et al.*, 2005; Lunn, 2000; Scalbert, 2000) respectively.

Statistical analysis

For proximate analysis and characterization studies all data was obtained in triplicate and expressed as mean ± SE. Two-way ANOVA was applied to the all data obtained with a value of ($p \leq 0.05$) was expressed as highly significant (Steel *et al.*, 1997).

Results and discussion

Proximate composition

Quality of the raw material is assessed by quantifying their proximate composition which plays a pivotal role in further utilization of the raw material and evaluation of the suitability and desired quality of the final product. Means of different prominent parameters of date seed powders of Karblain, Ajwa and Halawi varieties are mentioned in Table 1.

Table 1. Proximate analysis of date seeds from three varieties (g/100g).

Variety	Moisture	Crude Fat	Crude Protein	Crude Fiber	Ash	NFE
Karblain	3.13±0.03c	9.14±0.02a	6.81±0.01c	17.98±0.02c	1.19±0.02b	61.75±0.08a
Ajwa	3.40±0.02a	9.57±0.59a	7.78±0.04b	18.83±0.02a	1.24±0.01a	59.18±0.58c
Halawi	3.26±0.02b	9.24±0.02a	7.24±0.01a	18.20±0.03b	1.28±0.03a	60.77±0.05b

Means showing similar letter(s) in a row/column are non-significant ($P>0.05$, $n = 3$) Mean ± SE.

Chemical analysis of all three date pits showed that the highest Ash content was found as 1.28±0.03g/100g in Halawi date seed powder (DSP) trailed by Ajwa and Karblain date seed powders as 1.24±0.01g/100g and 1.19±0.02g/100g respectively. Furthermore, crude protein content was estimated as 7.78±0.04g/100g, 7.24±0.01g/100g and 6.81±0.01g/100g in Ajwa, Halawi and Karblain

respectively. Likewise, CF & Cf contents showed the values of 9.24±0.02 &18.20±0.03g/100g in Halawi seed powder, 9.57±0.59 & 18.83±0.02g/100g in Ajwa seed powder and 9.14±0.02 &17.98±0.02g/100g in Karblain seed powder. Due to similar processing conditions the moisture content was noticed almost similar in all three date seed powders but Karblain variety showed comparatively lesser moisture contents (3.13±0.03g/100g) as compared to Hallawi (3.26±0.02g/100g) and Ajwa (3.40±0.02g/100g) seed powders. Similarly, the resulted nitrogen free extract values were in the following order, Ajwa (59.18±0.58) < Hallawi (60.77±0.05) < Karbalain (61.75±0.08g/100g) seed powders respectively.

Table 2. Mineral contents of three date seed varieties (mg/100g).

Minerals	Karblain	Ajwa	Halawi
Phosphorous	1.88±0.02c	7.34±0.03a	3.88±0.01b
Sodium	17.30±0.20b	18.57±0.15a	15.70±0.17c
Boron	0.87±0.03c	1.03±0.00a	0.99±0.00b
Calcium	41.50±0.26b	57.50±0.26a	20.63±0.12c
Copper	1.44±0.03a	1.14±0.02b	1.03±0.02c
Iron	12.77±0.03b	23.56±0.02a	6.22±0.02c
Magnesium	59.33±0.25b	66.04±0.02a	54.03±0.03c
Potassium	170.63±0.25b	226.30±0.26a	112.30±0.26c
Zinc	0.19±0.02a	0.16±0.00b	0.14±0.00c

Means showing similar letter(s) in a row/column are non-significant (P>0.05, n = 3) Mean ± SE.

Outcomes of current study for chemical profiling of date seed powders are in accordance with the previous outcomes of Rahman *et al.* (2007), who narrated values as 7.08, 8.08, 0.98, 62.31 & 21.35% for CP, CF, Ash, carbohydrates and Cf, respectively. Current findings are also justifiable with that of Ardekani *et al.* (2010), who concluded MC, CP, Cf, AC and DF as 3.10-7.10, 2.30-6.40, 5.00-13.20, 0.90-1.80 and 22.50-80.20% correspondingly.

Table 3. Phenolics(mg/kg), TPC, TFC(mg/100g) and dietary fiber(g/100g) in date seeds of three varieties.

Phenolics	Karblain	Ajwa	Halwai
Chromatotrophic acid	18.67±0.25b	55.09±0.02a	16.06±0.03c
Quercetin	3.35±0.03c	14.55±0.03a	10.56±0.01b
Gallic acid	103.14±0.03c	179.16±0.03a	130.08±0.01b
Caffeic acid	15.46±0.03c	18.45±0.01b	22.29±0.01a
Chlorogenic acid	37.24±0.03b	80.89±0.02a	33.04±0.02c
Syringic acid	7.38±0.01b	9.08±0.02a	6.88±0.02c
p-coumeric acid	1.54±0.01c	11.22±0.01a	7.19±0.03b
m-coumeric acid	3.34±0.02c	14.33±0.01a	5.99±0.02b
Ferulic acid	26.43±1.16b	61.46±0.01a	24.96±0.03c
Vitamin C	16.98±0.01c	30.05±0.02a	17.33±0.01b
TPC(mg/100g)	840.07±0.83c	1,207.63±0.25a	1,157.63±0.21b
TFC(mg/100g)	375.47±0.03c	533.05±0.02a	519.38±0.03b
%DPPH scavenging	21.96±0.02c	79.75±0.02a	57.24±0.03b
Soluble Dietary Fiber	8.87±0.03b	9.05±0.02a	7.10±0.03c
Insoluble Dietary Fiber	67.70±0.20c	72.60±0.02a	72.27±0.03b
Total Dietary Fiber	76.57±0.19c	81.65±0.02a	79.36±0.03b

Means showing similar letter(s) in a row/column are non-significant (P>0.05, n = 3) Mean ± SE.

The instant results are also in line with the findings of Suresh *et al.* (2013). The trivial differences in the present results of proximate composition are due to the effects of a number of factors including ripening stages (Baliga *et al.*, 2011), different geographic zones (Hui, 2006) and varietal differences or cultural practices (Aidoo *et al.*, 1996).

Mineral profile

In the current investigation a total of nine minerals were quantified from date seed powders of three date varieties. The mineral analysis of date seed powders expound that predominant minerals were observed in the following order potassium > magnesium > calcium > sodium > iron > phosphorous were present in highest proportions followed by some lesser amounts of copper, boron and zinc as well. The results for mineral analysis are presented in Table 2. The values for phosphorous contents were ranging from 1.88±0.02 to 7.34±0.03mg/100g.

The highest value 7.34±0.03mg/100g was observed in Ajwa trailed by Halawi (3.88±0.01mg/100g) and Karblain (1.88±0.02mg/100g). Likewise, Na and B contents were 18.57±0.15 and 1.03±0.00mg/100g, 15.70±0.17 and 0.99±0.00mg/100g and 17.30±0.20 and 0.87±0.03mg/100g in Ajwa, Halawi and Karblain seeds individually.

Table 4. Characterization of date seed oils from three varieties.

Parameters	Karblain	Ajwa	Halawi
Peroxide value(meq/kg)	1.06±0.02b	1.03±0.02b	1.10±0.01a
Acid value(meq/kg)	1.83±0.02a	1.02±0.01c	1.35±0.02b
p-An value(meq/kg)	2.18±0.01c	3.04±0.02a	2.39±0.00b
Refractive index (n)	1.51±0.02a	1.43±0.03b	1.56±0.03a
Saponification value	228.33±2.52a	201.67±1.15c	214.33±2.89b
Iodine value(mg/g)	47.33±1.15a	40.33±0.58b	47.67±1.53a

Means showing similar letter(s) in a row/column are non-significant (P>0.05, n = 3) Mean ± SE.

Furthermore, Ca & Cu contents of all DSPs were measured as 57.50±0.26 and 1.14±0.02mg/100g in Ajwa trailed by 41.50±0.2.6 and 1.44±0.03mg/100g in Karblain and 20.63±0.12 and 1.03±0.02mg/100g in Halawi seed powder in the same way. Maximum contents amongst all minerals detected were of Potassium.

Potassium was estimated in Ajwa, Karblain and Halawi varieties as 226.30±0.26, 170.63±0.25 and 112.30±0.26mg/100g respectively. Moreover, zinc was detected as 0.16±0.00, 0.19±0.02 and 0.14±0.00mg/100g in Ajwa, Karblain and Halawi seed powder respectively.

Current outcomes are in strong agreement with the findings of Mirghani, (2012b), who narrated that Cu, Ca, Fe, Mn & Mg in DSPs were found as 0.923, 2.035, 0.911, 0.429 and 4.99mg/100g respectively. The observed variations in the outcomes of instant findings may be due to the differences of climatic conditions, soil type, varietal differences and developmental stage. However, the variation in mineral content among different varieties might also be due to genetic potential of each cultivar, as quantity of each mineral is controlled by specific group of transcription factors, which affect different metabolic pathways thus varying amount of minerals in this commodity were observed.

The present study results showed that Ajwa was leading other varieties in mineral composition that may be endorsed to the unique and superior genetic potential of this variety compared to other cultivars.

Ajwa belongs to Saudi Arabia that has best-suited climatic conditions for date cultivation. Likewise, the soil type of this region has added role in the rich mineral profile owing to the presence of vital micro and macronutrients.

Antioxidant indices

Values of total phenolic contents and total flavonoid contents are presented in Table 3. Significant variations ($p \leq 0.05$) were observed among the date seed powders obtained from three cultivars.

The maximum TPC content was detected as 1207.63±0.25mg/100g in Ajwa date seed trailed by Halawi 1157.63±0.21mg/100g and Karblain 840.07±0.83mg/100g respectively, whereas, TFC value was recorded as 533.05±0.02 in Ajwa followed by 519.38±0.03 in Halawi and 375.47±0.03mg/100g in Karblain individually.

Table 5. Fatty acid composition of date seed oils from three varieties(g/100g).

Fatty acids	Karblain	Ajwa	Halawi
Caprylic acid	0.38±0.003a	0.38±0.001a	0.36±0.003b
Pelargonic acid	0.78±0.006a	0.00±0.000c	0.21±0.006b
Capric acid	0.55±0.003a	0.48±0.006c	0.50±0.003b
Undecylic acid	0.46±0.007a	0.18±0.002b	0.17±0.005b
Lauric acid	25.68±0.182b	30.80±0.132a	30.50±0.268a
Myristic acid	13.24±0.120b	6.93±0.006c	16.75±0.081a
Palmitic acid	11.95±0.040b	13.08±0.059a	13.06±0.043a
Linoleic acid	6.94±0.027a	4.44±0.024b	4.40±0.044b
Oleic acid	37.58±0.290a	31.77±0.099b	31.48±0.130b
Stearic acid	2.32±0.032a	1.87±0.023b	1.80±0.015b

Means showing similar letter(s) in a row/column are non-significant ($P>0.05$, n = 3) Mean ± SE.

It is evident from the outcomes of a number of studies that many fruits have shown significant ($p \leq 0.05$) antioxidant activity due to the presence of different functional moieties (Luo *et al.*, 2002). Therefore, date seed powder from three varieties (Ajwa, Halawi and Karblain) was probed for its free radical scavenging activity and it was deduced that at 10µg/ml the date seed powder exhibited maximum free radical scavenging activity as 79.75±0.02, 57.24±0.03 and 21.96±0.02% for Ajwa, Halawi and Karblain, respectively.

Current outcomes are in strong agreement with the outcomes of (Al-Farsi and Lee, 2008b; Ardekani *et al.*, 2010), who analyzed 14 Iranian date seed varieties for antioxidant activity and total phenolic contents, TFC were estimated as 381-3541mg/100g.

HPLC phenolics characterization

Phenolic contents in date seed powders were analyzed through HPLC analysis and it showed significant ($p \leq 0.05$) variations among the treatments. The HPLC analyses of phenolics are presented in Table 3. HPLC quantification of phenolics revealed that Ajwa variety showed 55.09±0.02mg/kg Chromatotrophic acid, 14.55±0.03mg/kg Quercetin, 179.16±0.03mg/kg Gallic acid, 18.45±0.01mg/kg Caffeic acid, 80.89±0.02mg/kg Chlorogenic acid, 9.08±0.02mg/kg Syringic acid, 11.22±0.01mg/kg *p*-Coumeric acid, 14.33±0.01mg/kg *m*-Coumeric acid, 61.46±0.01mg/kg Ferulic acid and 30.05±0.02mg/kg vitamin C. Similarly, Halawi variety exhibited 16.06±0.03mg/kg Chromatotrophic acid, 10.56±0.01mg/kg Quercetin, 130.08±0.01mg/kg Gallic acid, 22.29±0.01mg/kg Caffeic acid, 33.04±0.02mg/kg Chlorogenic acid, 6.88±0.02mg/kg Syringic acid, 7.19±0.03mg/kg *p*-Coumeric acid, 5.99±0.02mg/kg *m*-Coumeric acid, 24.96±0.03mg/kg Ferulic acid and 17.33±0.01mg/kg vitamin C. Whereas, Karblain variety exhibited 18.67±0.25mg/kg Chromatotrophic acid, 3.35±0.03mg/kg Quercetin, 103.14±0.03mg/kg Gallic acid, 15.46±0.03mg/kg Caffeic acid, 37.24±0.03mg/kg Chlorogenic acid, 7.38±0.01mg/kg Syringic acid, 1.54±0.01mg/kg *p*-Coumeric acid, 3.34±0.02mg/kg *m*-Coumeric acid, 26.43±1.16mg/kg

Ferulic acid and 16.98±0.01mg/kg Vitamin C. Current results are in strong agreement with the outcomes of Paranthaman *et al.* (2012), who studied the effects of high temperature like roasting by means of HPLC analysis on the TPC and TFC contents in the date seed powders of different varieties.

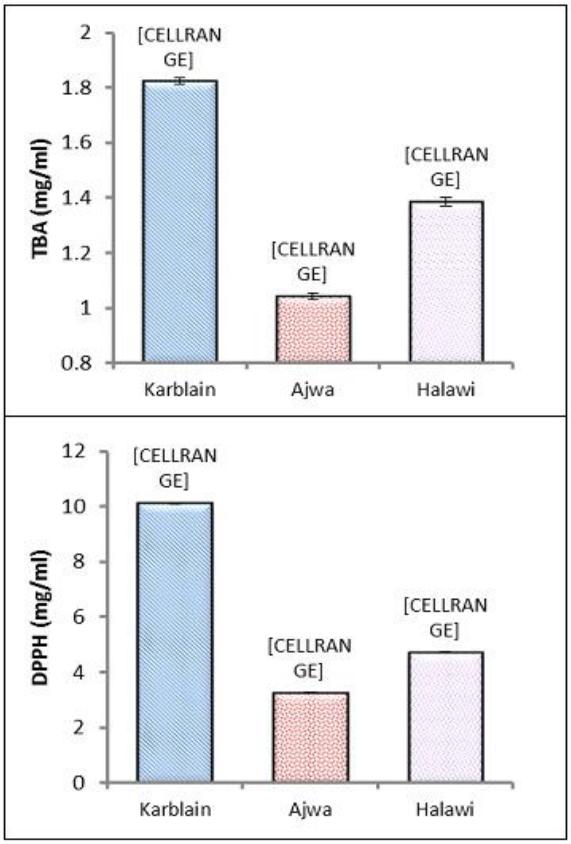

Fig. 1. Antioxidant activity of date seed oils from three varieties.

Estimation of dietary fiber (Df)

Mean values of soluble, insoluble and total dietary fiber from date seed powders of three cultivars with significant differences ($p \leq 0.05$) are presented in Table 3. Values for soluble Df were observed from 7.10±0.03 to 9.05±0.02g/100g, maximum value 9.05±0.02g/100g was detected in Ajwa date seed powder while, Halawi exhibited a value of 7.10±0.03g/100g. Values of insoluble Df were found ranging from 67.70±0.20g/100g in Karblain date seed powder to 72.60±0.02g/100g in Ajwa date seed powder. Values for total Df were ranging from 76.57± 0.19 to 81.65±0.02g/100g, maximum value 81.65±0.02g/100g was found in Ajwa date seed powder where minimum 76.57± 0.19g/100g was found in Karblain variety.

The research outcomes of Al-Farsi *et al.* (2007) are in line with the instant findings of the present research work, who evaluated three different date seed varieties for Df content and it was found ranging from 77.75% in Shahal, to 79.84% in Mabseeli and to 80.15% in Um-sellah. Dietary fiber is an essential component of plants that plays a vital role in human nutrition; its components include cellulose, hemicelluloses, pectin, hydrocolloids and lignins that cannot be digested by human gastro intestinal enzymes. Generally, it is categorized into soluble and insoluble Df. Soluble Df mainly includes pectic substances and hydrocolloids that are soluble in water, whereas insoluble as the name indicates are water insoluble (Bermink, 1994). The dietary fiber content vary as a function of progression in developmental stage and is higher at kimiri stage while lower at tamer stage that is due to the fact that majority of the fiber contents are hydrolyzed by different enzymes that make the date fruit soft, lowering fiber content and thus enhancing the fruit palatability.

Date seed oil characterization

Table 4 illustrates the outcomes of date seed oils with respect to physicochemical characterization. The AV of DSO ranges from 1.0±0.01 to 1.83±0.02meq/kg for Ajwa and Karblain and 1.35±0.02meq/kg for Halawi seed oil. It is evident that instant findings are in line with the results of (Besbes *et al.*, 2004; Boukouada and Yousfi, 2009) for the date seed oils of Tunisian and Algerian verities correspondingly. Their findings predicted that Bousthammi date seed oil which was comparable with the FFA contents of olive oil as evaluated by Borchani *et al.* (2010) had lower amount of FFA than Majhoul variety which indicated that Bousthammi DSO could be used for human consumption. Saponification value (SV) gives information about the fat and average molecular weight in addition to the nature of the FA. In our study date seed oil showed a range of SV between 201.67±1.15meq/kg and 228.33±2.52meq/kg for Ajwa and Karblain seed oils respectively. The extraordinary SV of DSO indicated that high numbers of carbon atoms are present in the fatty acids, indicating that

DSO could even after hydrogenation be utilized as an alternative of conventional oils in the manufacturing of shampoo and soap industry (Akintayo and Bayer, 2002; Falade *et al.*, 2008). Higher the POV of HSO (1.10±0.01meq O_2/kg) means that it is at greater risk of autoxidation than a lower POV of KSO (1.06±0.02meq O2/kg).

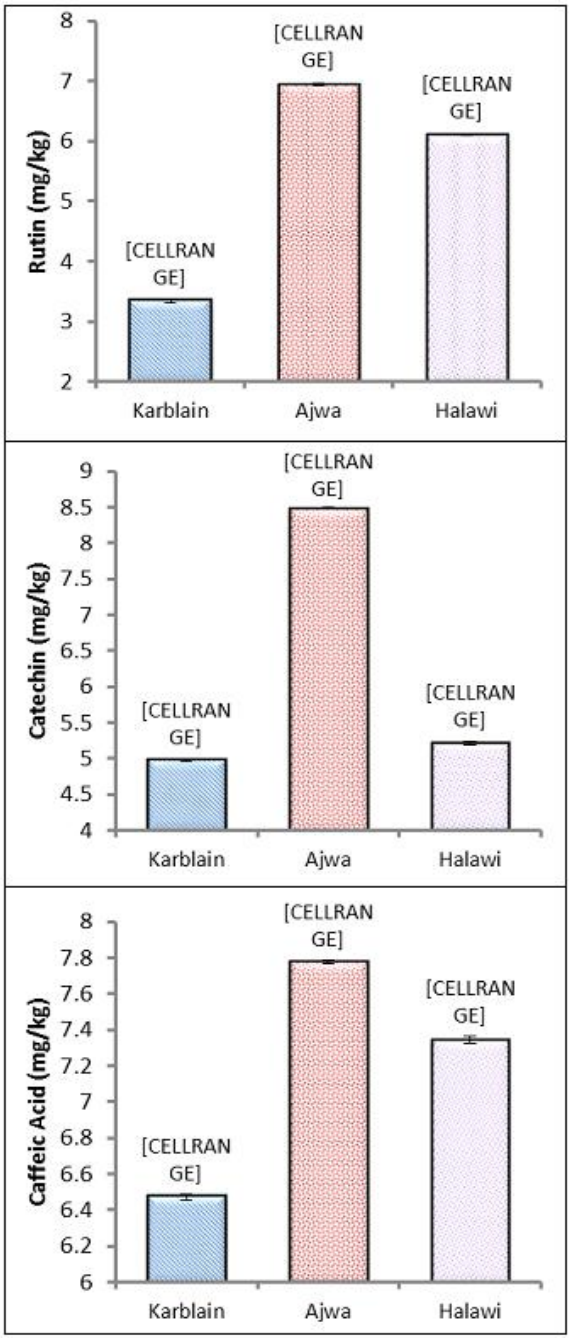

Fig. 2. Individual flavonoids and non-flavonoid contents in date seed oils from three varieties.

This disparity depends on a number of factors like degree of unsaturation, storage conditions, contact to the sunlight and other catalyzing agents like metals and other oxidation compounds (Choe and Min, 2006).

The PV for Ajwa seed oil determined in our study was 1.03±0.02meq O_2/kg that is in accordance with the findings of Gotoh and Wada, (2006) suggested that a lower PV than 30meq peroxide/kg could be recommended as innocuous for the consumptions of humans as GRAS. Degree of unsaturation is generally termed as Iodine value and a variation from 40.33±0.58mg/g and 47.67±1.53mg/g for ASO and HSO was estimated correspondingly. In the current study the IV for Karblain was in the range of 47.33±1.15mg/g. This reflects that lower amounts of unsaturated fatty acids were present in ASO than HSO and KSO. Findings of our research work are slightly in-disagreement with the results of Besbes *et al.* (2004) which is slightly lower than our values while the outcomes of Boukouada and Yousfi, (2009) are also in disagreement being higher than ours.

Fatty acid composition of DSO

The outcomes of fatty acid composition estimation in date seed oils are presented in Table 5. A total of ten fatty acids were estimated and oleic was the predominant (31.48±0.130 to 37.58±0.290g/100g) followed by lauric (25.68±0.182 to 30.80±0.132g/100g), myristic (6.93±0.006 to 16.75±0.081/100g), palmitic (11.95±0.040 to 13.08±0.059g/100g), linoleic (4.40±0.044 to 6.94±0.027g/100g) and stearic (1.80±0.015 to 2.32±0.032g/100g) that accounts for more than 98% of the oil. Some fatty acids were detected in lower amounts including tricosylic, arachidic, behinic, lignoceric and gadoleic acid while some others were of insignificant amounts including margaric, palmitoleic, myristoleic and linoleinic. 53.96% unsaturation for Ajwa oil and 57.32% for Karblain oil was estimated that is in line with the outcomes of (Besbes *et al.*, 2004; Juhaimi *et al.*, 2012).

Mean of SFAs in the present research work of lauric, myristic, palmitic, caprylic, capric and stearic acids for three cultivars were 28.993, 12.306, 12.696, 0.3733, 0.511 and 1.9966% correspondingly. Hence

the remaining ones are associated with USFAs of undecylic, linoleic and oleic in Karblain, Ajwa and Halawi, correspondingly. Types of fatty acids in the oil from three verities were similar except for the Ajwa seed oil that was deficient in pelargonic acid. No significant relationship was observed for amount of every fatty acid in all three verities but differences in amounts of different fatty acids were observed.

Antioxidant activity of DSO

Subsequently there is non-existence of a single test that could elucidate the activity of antioxidants in natural extracts especially oils, so therefore it was the dire need of time to use the alternative methods to be vitalized against the determination of antioxidant activity that could in turn give information about the effectiveness of a targeted samples towards the free radicals as defined by (Caturla *et al.*, 2003; Tsao *et al.*, 2005). In current study for the above said purpose two methods namely TBARS and DPPH in combination were used. It is evident from Fig. 1 that the pre-requisite quantity required for reducing 50% of lipid peroxidation was significantely ($p<0.05$) less in Ajwa and Halawi (1.04±0.01, 1.39±0.02mg/ml) than in Karblain (1.82±0.01mg/ml). Moreover, to inhibit 50% of lipid peroxidation the values were also been found significant ($p<0.05$) for the aqueous extracts of Ajwa and Halawi that showed 50% of its concentration in alcoholic solvent.

Outcomes of current research work are in line with the results of Al-Farsi *et al.* (2005), who narrated that most of the antioxidant types in dates are hydrophilic. Falvanols are attributed for antioxidant activity and shielding from oxidative dilapidation, having the ability to react on both sides of water and lipid layers of cell membrane (Vayalil, 2002; Verstraeten *et al.*, 2003). In another study conducted by Vayalill, (2002) found that by applying TBA test at early fresh (Rutab) stage of maturity which is supposed to be very rich in antioxidant activity than tamer stage, to find out the antioxidant activity it requires 1.9mg/ml of aqueous extract in the reduction of 50% lipid peroxidation which is similar to those values found in the current study (Al-Farsi *et al.*, 2005).

Flavonoids and Phenolic compounds

Flavonoids in general and specifically flavanols and flavonols have proven health benefits against curtailing various diseases and disorders including cardiac complications, obesity, cancer and hyperglycemia (Hassimotto *et al.*, 2005). In this context the active biochemical constituents of flavonoids and non-flavonoid contents consisting rutin, catechin and caffeic acid were analyzed from the date seed oil of three different verities through HPLC. Fig. 2 demonstrates that the estimated rutin content in the DSO of all three verities was in the following significant order Ajwa (6.94±0.02mg/kg) > Halawi (6.11±0.01mg/kg) > Karblain (3.34±0.02mg/kg). Whereas, catechin content was found in the following significant order: ASO (8.49±0.01mg/kg) > HSO (5.22±0.02mg/kg) > KSO (4.97±0.02mg/kg), furthermore, non-significant differences were observed for caffeic acid contents amongst ASO and HSO (7.78±0.01mg/kg and 7.35±0.02mg/kg) whereas it was bit lower in Karblain as 6.47±0.02mg/kg.

Our findings are in line with the results of Scalbert and Williamson, (2000), who concluded that the range of caffeic acid for different date seed cultivars was almost similar as in case of current study but not in agreement with the outcomes of Al-Farsi, (2005) being too much lower than his results of 76 mg/kg. These deviations might be due to the protocols followed and the selected varietal differences. These findings suggested that natural antioxidantal potential of DSO could be vitalized in the formulations of functional foods and nutraceuticals.

Conclusion

Recent scientific findings have confirmed the presence of a variety of bioactive constituents in date seeds and their oil that is dually verified by the current outcomes of the present study. Results from physicochemical characterization and functional attributes revealed that Ajwa date seeds and its oil ranked higher than seeds and oil from other two varieties. It is recommended that ASO is of prime quality with proven health claims and improved

nutrition that could further be used in the preparation of novel functional food products as an alternative source of commercial hydrogenated vegetable fats believed as unhealthy.

Abbreviations

Total phenolic contents (TPC), Total flavonoid contents (TFC), Date seed oil (DSO), Ajwa seed oil (ASO), Halawi seed oil (HSO), Karblain seed oil (KSO), Specific gravity (SG), Refractive index (RI) Date seed powder (DSP).

Acknowledgments

The authors are grateful to the Supervisory and the supporting staff at Institute of Home and Food Sciences for their support to carry out the present research. The authors are also highly obliged to the Library Department, Government College University Faisalabad (GCUF) and IT Department, HEC for access to journals, books and valuable database.

Conflicts of Interest

No conflict of interest in this case.

References

AACC. 2000**.** Approved Methods of American Association of Cereal Chemists. American Association of Cereal Chemists, Incorporation, St. Paul, Minnesota, USA.

Aidoo KE, Tester RF, Morrison JE, MacFarlane D. 1996. The composition and microbial quality of pre packed dates purchased in greater Glasgow. International Journal of Food Science and Technology **31(5),** 433-38. http://dx.doi.org/10.1046/j.1365-2621.1996.00360.x

Akintayo ET, Bayer E. 2002. Characterisation and some possible uses of *Plukenetia conophora* and *Adenopus breviflorus* seeds and seed oils. Bioresource Technology **85(1),** 95-97. http://dx.doi.org/10.1016/S0960-8524(02)00073-1

Al-Farsi M, Alasalvar C, Al-Abid M, Al-Shoaily K, Al-Amry M, Al-Rawahy F. 2007. Compositional and functional characteristics of dates, syrups and their by-products. Food Chemistry **104(3),** 943-47. http://dx.doi.org/10.1016/j.foodchem.2006.12.051

Al-Farsi M, Alasalvar C, Morris A, Baron M, Shahidi F. 2005. Comparison of Antioxidant Activity, Antho-cyanins, Carotenoids, and Phenolics of Three Native Fresh and Sun-Dried Date (*Phoenix dactylifera* L.) Varieties Grown in Oman. Journal of Agricultural and Food Chemistry **53,** 7592-99. http://dx.doi.org/10.1021/jf050579q

Al-Farsi MA, Lee CY. 2008a. Nutritional and Functional Properties of Dates: A Review. Critical Reviews in Food Science and Nutrition **48(10),** 877-87. http://dx.doi.org/10.1080/10408390701724264

Al-Farsi MA, Lee CY. 2008b. Optimization of phenolics and dietary fibre extraction from date seeds. Food Chemistry **108,** 977-85. http://dx.doi.org/10.1016/j.foodchem.2007.12.009

AOAC. 1990. Association of Official Analytical Chemists, Official Methods of Analysis. (15th ed.). Association of Official Analytical Chemists, Washington DC, USA.

AOAC. 2006. Association of Official Analytical Chemists, Official Methods of Analysis (18th ed.). Association of Official Analytical Chemists, Gaithersburg, Maryland, USA.

AOCS. 1998. Official Methods and recommended Practices of AOCS. (5th ed). American Oil Chemists Society Campaign, Illinions, USA.

Ardekani MRS, Khanavi M, Ajimahmoodi M, Jahangiri M, Hadjiakhoond A. 2010. Comparison of Antioxidant Activity and Total Phenol Contents of some Date Seed Varities from Iran. Iranian Journal of Pharmaceutical Research **9(2),** 141-46.

Arshad HR, Salah MA, Habeeb A, Babiker AY, Srikar S, Amjad AK. 2014. Therapeutic effects of date fruits (*Phoenix dactylifera* L.) in the prevention of diseases via modulation of anti-inflammatory, anti-oxidant and anti-tumour activity. International Journal of Clinical and Experimental Mediicine **7(3),** 483-91.

Balaiga MS, Balaiga BRV, Kandathil SM, Bhat HP, Vayalil PK. 2011. A review of the chemistry and pharmacology of the date fruits (*Phoenix dactylifera* L.). Food Research International **44(7),** 1812-22. http://dx.doi.org/10.1016/j.foodres.2010.07.004

Basuny AMM, Al-Marzooq MA. 2011. Production of mayonnaise from date pit oil. Food and Nutrition Science **2,** 938-43. http://dx.doi.org/10.4236/fns.2011.29128

Bermink MR. 1994. Fiber analysis. Introduction to the chemical Analysis of Foods. In: (Nielson SS ed). Jones and Bartlett Publishers, Incorporation Boston, USA.

Besbes S, Blecker C, Deroanne C, Bahloul N, Lognay G, Drira NE, Attia H. 2004a. Date seed oil: phenolic, tocopherol and sterol profiles. Journal of Food Lipids **11(4)**, 251-265. http://dx.doi.org/10.1111/j.1745-4522.2004.01141.x

Besbes S, Blecker C, Deroanne C, Lognay G, Drira NE Attia H. 2004. Quality characteristics and oxidative stability of date seed oil during storage. Food Science and Technology International **10(5),** 333-38. http://dx.doi.org/10.1177%2F1082013204047777

Borchani C, Besbes S, Blecker C, Attia H. 2010. Chemical characteristics and oxidative stability of sesame seed, sesame paste, and olive oils. Journal of Agriculture Science and Technology **12,** 585-96.

Boukouada M, Yousfi M. 2009. Phytochemical study of date seeds lipids of three fruits (*Phoenix dactylifera* L) produced in Ouargla region. Annales de la Faculté des Sciences et Sciences de l'Ingénieur **1(3)**, 66-74.

Caturla N, Vera-Samper E, Villalain J, Mateo CR, Micol V. 2003. The Relationship between the Antioxidant and the Antibacterial Properties of Galloylated *Catechins* and the Structure of Phospholipid Model Membranes. Journal of Free Radical Biology and Medicine **34(6),** 648-62. https://doi.org/10.1016/S0891-5849(02)01366-7

Choe E, Min DB. 2006. Mechanisms and factors for edible oil oxidation. Comprehensive Reviews in Food Science and Food Safety **5(4),** 169-86. http://dx.doi.org/10.1111/j.1541-4337.2006.00009.x

Falade QS, Adekunle AS, Aderogba MA, Atanda SO, Harwood C, Adewusi SR. 2008. Physicochemical properties, total phenol and tocopherol of some Acacia seed oils. Journal of Science of Food and Agriculture **88(2),** 263-68. http://dx.doi.org/10.1002/jsfa.3082

FAO. 2004. Date Palm Production Chapters 1 and 2. http://www.FAO.org]on2007/05/24

FAO. 2011. Statistical Databases. Food and Agricultural Organization of the United Nation. http://www.FAOstat.FAO.org

GOP. 2008. Fruit, Vegetable and Condiments. Statistics of Pakistan. Ministry of Food, Agriculture and Livestock (Economic Wing), Islamabad, Pakistan.

Gotoh N, Wada S. 2006. The importance of peroxide value in assessing food quality and food safety. Journal of the American Oil Chemists' Society **83(5),** 473-74.

Hassimotto NM, Genovese MI, Lajolo FM. 2005. Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. Journal of Agriculture and Food chemistry **20**, 2928-35. https://doi.org/10.1021/jf047894h

Hui YH. 2006. Fruit and Fruit Processing, Black well Publishing. Ames, Lowa, USA. 391-411.

Hussein AS, Alhadrami GA, Khalil YH. 1998. The use of dates and date pits in broiler starter and finisher diets. Journal of Bioresource Technology **66(3),** 219-23. https://doi.org/10.1016/S0960-8524(98)00054-6

Jayasekera S, Molan AL, Garg M, Moughan PJ. 2011. Variation in antioxidant potential and total polyphenol content of fresh and fully fermented Sri Lankan tea. Food Chemistry **125(2),** 536-41. https://doi.org/10.1016/j.foodchem.2010.09.045

Juhaimi FA, Ghafoor K, Ozcan MM. 2012. Physical and chemical properties, antioxidant activity, total phenol and mineral profile of seeds of seven different date fruit (*Phoenix dactylifera* L.) varieties. International Journal of Food Science and Nutrition **63(1),** 84-89. https://doi.org/10.3109/09637486.2011.598851

Kwaasi AAA. 2003. Date palm and sandstorm borne allergens. Journal of Clinical and Experimental Allergy **33(4),** 419-26. https://doi.org/10.1046/j.1365-2222.2003.01635.x

Lunn G. 2000. HPLC Methods for Pharmaceutical Analysis, Interscience Publication, New York, USA.

Luo XD, Basile MJ, Kennelly EJ. 2002. Polyphenolic Antioxidants from the fruits of *Chrysophyllum cainito* L. (Star Apple). Journal of Agriculture and Food Chemistry **50(6),** 1379-82. https://doi.org/10.1021/jf011178n

Mirghani MES. 2012b. Processing of Date Palm Kernel (DPK) for Production of Nutritious Drink. Advances in Natural and Applied Sciences **6(5),** 575-82.

Mortadha AA, Tahseen AAH, Imad AAH. 2015. Extraction of date palm seed oil (*Phoenix dactylifera* L.) by soxhlet apparatus. International Journal of Advances in Engineering and Technology **8,** 261-71.

Nehdi I, Omri S, Khalil MI, Al-Resayes SI. 2010. Characteristics and chemical composition of date palm (*Phoenix canariensis* L.) Seeds and seed oil. Industrial Crops and Products **32(3),** 360-65. https://doi.org/10.1016/j.indcrop.2010.05.016

Nuutila AM, Kammiovirta K, Oksman KM. 2012. Comparison of methods for the hydrolysis of flavonoids and phenolic acids from onion and spinach for HPLC analysis. Food Chemistry **76(4),** 519-525. https://doi.org/10.1016/S0308-8146(01)00305-3

Omezzine A, Chebaane H, Zaibet L. 1997. Reallocations of agricultural water in Oman. A potential advantage in higher valued crops. International Symposium on water and Arab Gulf Development, Center of Arab Gulf Studies, University of Exeter, UK.

Paranthaman R, Kumar P, Kumaravel S. 2012. HPLC and HPTLC Determination of Caffeine in Raw and Rosted Date Seeds (*Phoenix dactylifera* L.). Journal of Chromatography **01,** 4172. http://dx.doi.org/10.4172/scientificreports.249

Rababah TM, Ereifej KI, Esoh RB, Al-udatt MH, Alrababah MA, Yang W. 2011. Antioxidant activities, total phenolics and HPLC analyses of the phenolic compounds of extracts from common Mediterranean plants. Natural Product Research **25(6),** 596-605. https://doi.org/10.1080/14786419.2010.488232

Rahman MS, Kasapis S, Al-Kharusi NSZ, Al-Marhubi IM, Khan AJ. 2007. Composition characterization and thermal transition of date pits powders. Journal of Food Engineering **80(1),** 1-10. https://doi.org/10.1016/j.jfoodeng.2006.04.030

Sadiq IS, Izuagie T, Shuaibu M, Dogoyaro AI, Garba A, Abubakar S. 2013. The nutritional evaluation and medicinal value of date palm (*Phoenix dactylifera* L.). International Journal of Modern

Chemistry **4(3),** 147-54.

Sayed AR. 2007. Agriculture Statistics of Pakistan. Government of Pakistan, Ministry of Food, Agriculture and Livestock, Islamabad, Pakistan. 33.

Scalbert A, Williamson G. 2000. Dietary Intake and Bioavailability of Polyphenols. Journal of Nutrition **130(8),** 2073-85.
https://doi.org/10.1093/jn/130.8.2073S

Singleton VL, Orthofer R, Lamuela-Raventos RM. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology **299,** 152-78.
https://doi.org/10.1016/S0076-6879(99)99017-1

Siscovick DS, Barringer TA, Fretts AM, Wu JH, Lichtenstein AH, Costello RB, Kris-Etherton PM, Jacobson TA, Engler MB, Alger HM, Appel LJ. 2017. Omega-3 polyunsaturated fatty acid (fish oil) supplementation and the prevention of clinical cardiovascular disease: a science advisory from the American Heart Association. Circulation **135(15),** 867-84.

Steel RGD, Torrie JH, Dickey DA. 1997. Principles and Procedures of Statistics: A Biometrical Approach, 3rd ed, p 352-358. McGraw Hill Book Company, Incorporation, New York, USA.

Suman Chandra S, Khan S, Avula B, Lata H, Yang MH, Mahmoud A, ElSohly, Khan IA. 2014. Assessment of Total Phenolic and Flavonoid Content, Antioxidant Properties, and Yield of Aeroponically and Conventionally Grown Leafy Vegetables and Fruit Crops: A Comparative Study. Evidence Based Complementary and Alternative Medicine **25,** 3875-83.
http://dx.doi.org/10.1155/2014/253875

Sun C, Fu J, Chen J, Jiang L, Pan Y. 2010. On-line HPLC method for screening of antioxidants against superoxide anion radical from complex mixtures. Journal of Separation Science **33(8),** 1018-23.
https://doi.org/10.1002/jssc.200900588

Suresh S, Guizani N, Al-Ruzeiki M, Al-Hadhrami A, Al-Dohani H, Al-Kindi I, Rehman MS. 2013. Thermal characteristics, chemical composition and polyphenol contents of date pits powder. Journal of Food Engineering **119(3),** 668-79.
https://doi.org/10.1016/j.jfoodeng.2013.06.026

Tsao R, Yang R, Xie S, Scokovie E, Khanizadeh S. 2005. Which Polyphenolic Compounds Contribute to the Total Antioxidant Activities of Apple? Journal of Agricultural and Food Chemistry **53(12),** 4989-95.
https://doi.org/10.1021/jf048289h

Vayalil PK. 2002. Antioxidant and Antimutagenic Properties of Aqueous Extract of Date Fruit (*Phoenix dactylifera* L. *Arecaceae*). Journal of Agricultural and Food Chemistry **50(3),** 610-17.
http://dx.doi.org/10.1021/jf010716t

Verstraeten SV, Keen CL, Schmitz HH, Fraga CEG, Oteiza PI. 2003. Flavan-3-Ols and Procyanidins Protect Liposomes against Lipid Oxidation and Disruption of the Bilayer Structure. Journal of Free Radical Biology and Medicine **34(1),** 84-92.
http://dx.doi.org/10.1016/S0891-5849(02)01185-1