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Nutritional Probing and HPLC Profiling of Roasted Date Pit Powder

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Abstract: Nature has miraculously blessed many plants with such phyto-chemical profile that is valuable for mankind in various ways. Some plant materials considered waste, contain valued phyto-chemicals advantageous for humans. One such material is date pit. In present research study, date pits from three geographically different date varieties (Ajwa from Saudi Arabia, Hallawi from Iran and Aseel from Pakistan) were selected and their pits were roasted and ground into powder. The aim of this study was to determine chemical & mineral profile, dietary fibre, total phenolics, total flavonoids and total antioxidant activity of roasted date pits. Ajwa contained higher amounts of crude proteins, crude fats and crude fiber. The date pits of Ajwa varieties were also containing the higher amounts of dietary fiber especially insoluble fiber. Furthermore phenolic profiling of roasted date pits detected 10 different phenolic compounds through HPLC studies. The date pits contain higher amounts of gallic acid, chlorogenic acid and ferulic acid. However, their highest amounts were recorded in Ajwa i.e., 175.13±7.45, 82.88±3.87 and 61.46±2.81 mg/kg, respectively. The data collected from the current investigation recommend Ajwa date pits to be considered as a functional food ingredient owing to potentially excellent source of natural antioxidants.

Key words: Date pit, HPLC, dietary fibre, total phenolic content, total antioxidant capacity

INTRODUCTION

Nature endows some plant materials considered waste with excellent nutraceutical attributes due to the presence of phyto-chemicals; some of them are being used in folk and traditional medicines and have proved effective, there is global interest in the utilization of nutraceuticals on every health forum, owing to their reduced side effects (Tahraoui *et al*., 2007). Nature has not created anything ineffectual in this world. Considering this fact, utilization of waste commodities can deliver never-ending benefits to mankind otherwise these may create unlimited episodes of dilemma. Date pit is a major waste produced during the processing of date syrup, pitted dates and other date products. Date pits were considered waste in the past. Presently, these are being used, mostly as feed for animals like cattle, camel, poultry and sheep (Rahman *et al*., 2007). However, limited work has been done for their use as an ingredient for human diet (Basuny and Al-Marzooq, 2011).

Date fruit is very much popular among the Muslim community. The fruit of date plant consists of a pit enclosed by a fleshy pericarp that comprises approximately 85-90% of date fruit weight (Elleuch *et al*., 2008). The pit comprises 10 to 15% of date fruit weight (Hussein *et al*., 1998). Global date production was 6.9 million tons in 2004, from that about 863 thousand tons of date pits were produced (FAO, 2007). The region under farming of dates in Pakistan and Sindh is 84,700 and 29,300 hectares, respectively and its overall production is 426,300 and 201,100 tons, respectively (Sayed, 2007). A Continuous positive trend is being observed for world date production in every coming year, therefore exploitation of date pits containing many bioactive constituents, is very vital to benefit mankind and to enhance the income of this sector.

Date pits contain higher amount of phenolic compounds mainly phenolic acids and flavonoids. Total phenolics in date pits range from 3102-4430 mg of gallic acid equivalents/100 g fresh weight while their antioxidant activity range from 580-929 µmol of Trolox equivalents/g fresh weight. Therefore it is recommended that date pits can serve as a superior supply of natural antioxidants and may be used as a functional food or functional food ingredient (Al-Farsi *et al*., 2007). In many studies on human as well as animal subjects these phenolic compounds have shown to possess antioxidant, anticarcinogenic, antimicrobial, antimutagenic and anti-inflammatory effects (Halliwell, 1997; Butt and Sultan, 2013). They have proved to be useful against diminution of chronic disease like cardiovascular diseases (Peterson and Dwyer, 1998) and diabetes mellitus (Knekt *et al*., 2002).

Considering the above mentioned facts three geographically different date varieties (Ajwa from Saudi Arabia, Hallawi from Iran and Aseel from Pakistan) were selected to evaluate the phyto-chemical potential of their pits.

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MATERIALS AND METHODS

Plant material: The date pits of three date varieties from different ecological regions i.e., Pakistan, Iran and Saudi Arabia were used in the present study Hallawi belongs to Iran, Aseel belongs to Pakistan and Ajwa is from Saudi Arabia. The Date pits of two varieties (Hallawi and Aseel) were procured from local date processing industry while Ajwa date pits were removed from Ajwa date fruit, purchased from local market after botanical authentication.

Chemicals: 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was obtained from Applichem (GmbH, ottoweg 4, D-64291 Darmstadt Germany). All other analytical grade chemicals were procured from E. Merck (D-6100, Darmstadt F.R. Germany) except declared otherwise.

Sample preparation: Date pit powder was produced from three varieties (Aseel, Ajwa and Hallawi) by following the method of Rahman *et al.* (2007) with slight modification. Pits from fully matured date fruit (tamar stage) were cleaned. The pits were separated through straining and then they were dried at 50°C in hot air oven (Memmert UNE 200). The roasting of dried whole pits was carried out for 15 min at 220°C in Home Coffee Roasting Machine (Model-SR500) then cooled to room temperature and were ground using hammer mill (Fitz Mill model LH-DAS06) to a coarse setting size of 500 um. The roasted date-pit powders obtained from three varieties were stored in airtight conditions until used for experimentation.

The extracts of roasted date pit powder from three varieties were prepared by extracting 20 g of powders with 200 ml of 80% methanol by an orbital shaker (Thermo scientific, UK) for 8 h at room temperature. The extract was separated from the solids through filtration using Whatman No.1 filter paper. The extraction was again carried out twice with the remaining solids with the same solvent and extracts combined. A rotary evaporator (Rotavapor^{R} R-215, Switzerland) was used for concentrating the extracts at 45°C under reduced pressure. The Concentrated extracts so obtained were stored in refrigerator at -4°C until analyzed.

Nutritional analysis: The roasted date-pit powder was analyzed for the chemical analysis through recommended methods (AACC, 2000).

Dietary fiber content: The roasted date pit powder was analyzed for total dietary fiber (TDF), soluble dietary fiber (SDF), Insoluble dietary fiber (IDF) according to standard methods of AACC (2000).

Determination of mineral elements: Roasted date pit powder was subjected to mineral analysis following the instructions of AOAC (2003). Sodium and potassium were determined through Flame Photometer-410 (Sherwood 44 Scientific Ltd., Cambridge) whilst Atomic Absorption Spectrophotometer (Varian AA240, Australia) was used for measurement of calcium, zinc, iron, phosphorous, boron, copper and magnesium.

Determination of total phenolics (TP) contents: The estimation of total polyphenols was carried out by using Folin-Ciocalteau reagent in alkaline medium and results were expressed as mg of GAE/100 g (Hussain *et al.*, 2012). Total flavonoid content was estimated following the method of Hussain *et al.* (2012). Total flavonoids were expressed mg of CE/100 g.

DPPH radical scavenging assay: 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) assay was carried out as per the guidelines of Hussain *et al.* (2008) to assess the free radical scavenging action. Extract and Standard phenolic acid and flavonoid compounds concentrations in methanol (1-100 µg/mL) were mixed with 2 mL of 90 µM methanol solution of DPPH. The absorbance was read through spectrophotometer at 517 nm after incubation period of 30 min. Butylated hydroxytoluene (BHT) and butylated hydroxyl anisol (BHA) procured from Applichem (GmbH, ottoweg 4, D-64291 Darmstadt Germany) were used as standard for comparison and 90 µM DPPH solution was taken as blank. Following formula was used to determine the percent scavenging of samples:

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

where, A_{blank} represents the absorbance of the DPPH solution while A_{sample} represents the absorbance of the extract solution.

HPLC analysis of phenolics

Standard preparation: Different phenolic acids (Sigma) standard stock solutions were prepared in methanol, at concentrations of 5 mg/ml. Standard solutions were injected by auto sampler after filtering through 0.45 mm membrane filter.

Sample preparation: The dried crude extract previously prepared was dissolved in the 100 mL mobile phase. Prior to the injection into HPLC the extract was filtered through a filter paper and a 0.45 um membrane filter (Millipore) (Samee and Vorarat, 2007).

HPLC conditions: Phenolics were analyzed using a Shimadzu HPLC system (Shimadzu Corp., Kyoto, Japan) consisting of a LC-10AD pump, SCL 10A system controller and SPD-M 10A UV detector. The resolution of compounds was substantially effected by the gradient elution of solvent A [water-acetic acid (25:1 v/v)] and

solvent B (methanol). Dual pumping system was employed for varying the proportion of solvent A [water-acetic acid (25:1, v/v)] to solvent B (methanol). As a result, at a flow rate of 1.0 ml/min solvent gradients were formed. Solvent B was increased to 50% in 4 min and subsequently increased to 80% in 10 min. The system was run at a detection wavelength of 280 nm.

Statistical analysis: The data was reported as mean after analyzing each sample individually in triplicate through STATISTIX 8.1 at 5% significance level to interpret the results by one way analysis of Variance ANOVA.

RESULTS AND DISCUSSION

Proximate composition: The results for chemical analysis pertaining to the roasted date pit powder prepared from different varieties is presented in Table 1 The outcomes of proximate analysis revealed significant ($p \leq 0.05$) variations for fat, protein and NFE content among three varieties under study, while depicted non-momentous differences for moisture, fiber and ash content.

The highest ash content 1.27±0.04 g/100 g was observed in Hallawi variety trailed by Ajwa and Aseel as 1.22±0.05 and 1.19±0.06 g/100 g, respectively however, the difference was non-significant. Moreover, crude protein contents were recorded as 7.80±0.31, 6.80±0.32 and 7.23±0.34 g/100 g in Ajwa, Hallawi and Aseel, correspondingly. Similarly, crude fat and crude fibre contents were 9.50±0.42 and 18.62±0.75 g/100 g in Hallawi, 9.80±0.44 and 18.71±0.88 g/100 g in Ajwa and 9.20±0.45 and 17.90±0.85 g/100 g in Aseel pit powders. Due to similar processing conditions the moisture contents were observed to be more or less similar, however, Aseel pit powder showed less moisture content (3.12±0.11 g/100 g) compared to Hallawi (3.24±0.13 g/100 g) and Ajwa (3.33±0.13 g/100 g) roasted pit powders. Likewise, the recorded NFE values of respective samples were 61.46±2.99, 59.74±2.53 and 59.75±2.57 g/100 g, Ajwa, Hallawi and Aseel powders, respectively.

The current results for proximate analysis are found to be in line with Rahman *et al.* (2007) who reported proximate analysis of roasted date pits to contain protein 7.08%, fat 8.08%, ash 0.98%, carbohydrates 62.31%, crude fiber 21.35%. The results are further in accordance with Aldhaheri *et al.* (2004) who reported that on dry matter basis date pits contain Crude protein 6.0%, Ether extract 8.0%, Crude fibre 13.5%, Ash 1.0%, NFE 71.5%. The results are further confirmed through the findings of Ardekani *et al.* (2010) who reported that date pits contain 3.10-7.10% moisture, 2.30-6.40% protein, 5.00-13.20 fat and 0.90-1.80% ash.

The slight variations in proximate composition of instant findings might be due to various factors, basically date fruit matures after passing through three main ripening stages that are known worldwide by their Arabic names: kimri (unripe), khalal (full-size, slightly crunchy; edible), rutab (ripe, soft; edible) and tamar (ripe, reduced moisture; edible) (Ahmed *et al.*, 1995). Many researchers are of view that as date fruit progresses towards maturity ash, fibre and moisture content follow a decreasing trend (Sawaya *et al.*, 1983; Baliga *et al.*, 2011) therefore it is suggested that variations in ash, crude fiber and moisture content might be related to stage of maturity. Some researchers narrated that the ash content of date decreases from kimri to tamar stage (Hui, 2006; Shaheen and Al-Qurashi, 2007). Dates at kimri stage contain an average of 3.7% ash decreasing to 2.8% at khalal stage and 2.6% in rutab stage. The average percentage of ash in the tamar stage is 1.7% (Al-shahib and Marshall, 2003). This decrease in the khalal stage is more significant than in the tamar stage (Shaheen and Al-Qurashi, 2007) secondly, it may be due to different geographic zones (Iran, Pakistan and Saudi Arabia) having diverse climatic conditions in which these varieties are grown. Date palm is resistant to bad climatic conditions and is able to endure a wide range of temperature (from-6 to 50°C). The most appropriate area for this plant to grow is arid regions with hot and dry climates and limited rainfall, therefore arid areas of Arab countries are suitable for its growth however, Saudi Arabia is renowned for its supreme quality dates owing to the best suited climatic conditions for the growth of date palm (Hui, 2006). These vary facts further support inter varietal variations for proximate analysis observed in this study where Ajwa is leading Aseel and Hallawi in chemical composition. Nonetheless, the role of genetic differences on proximate composition could not be overseen. As far as the variation in crude fat and crude protein content is concerned it is proposed that it could be due to variety, origin and cultural practices as narrated by Aidoo *et al.* (1996).

Mineral profile of date pit powder: Mineral is an inorganic substance with a characteristic chemical composition that occurs naturally and is an essential part of the human diet. During the current study, nine different minerals were detected from roasted date pit powder. The mineral analysis of roasted date pit powder (Table 2) elucidated that Phosphorous, Boron, Calcium, Iron, Magnesium, Potassium and Zinc were present in momentous ($p \leq 0.05$) amounts whereas, non-momentous trend was observed among Sodium and copper.

The mean values for phosphorous content in roasted date pit powder from three varieties were ranged from 1.9±0.095 to 7.35±0.36 mg/100 g. The highest value 7.35±0.36 mg/100 g was detected in Ajwa followed by Hallawi (3.85±0.32 mg/100 g) and Aseel (1.9±0.095 mg/100 g). Similarly, the sodium and boron contents

Table 1: Mean values for proximate composition of roasted date pit powder of different date varieties

Varieties	Ash (%)	Moisture (%)	Crude fat (%)	Crude fiber (%)	Crude protein (%)	NFE (%)
Aseel	1.19±0.05	3.12±0.11	9.20±0.45^{b}	17.90±0.85	6.80±0.32^{b}	59.74±2.53^{b}
Ajwa	1.22±0.06	3.33±0.12	9.80±0.44^{a}	18.71±0.88	7.80±0.31^{a}	61.46±2.99^{a}
Hallawi	1.27±0.04	3.24±0.13	9.50±0.42^{ab}	18.62±0.75	7.23±0.34^{b}	59.75±2.57^{b}

Different letters show significant variations ($p \leq 0.05$), Similar letters show non-significant variations ($p \geq 0.05$), Values are expressed as means±standard deviation

were 18.1±0.75 and 1.061±0.053 mg/100 g, 16.4±0.61 and 0.935±0.047 mg/100 g and 17.2±0.85 and 0.897±0.045 mg/100 g in Ajwa, Hallawi and Aseel pit powder, respectively. Moreover, calcium and copper contents of three varieties were recorded as 57.5±2.84 and 1.12±0.06 mg/100 g in Ajwa trailed by 41.9±2.08 and 1.45±0.072 mg/100 g in Aseel and 21.6±1.08 and 1.10±0.05 mg/100 g in Hallawi pit powder, respectively. The iron and magnesium contents among the three varieties varied significantly ($p \leq 0.05$) and were found to be 23.50±1.18 and 66.01±0.30 mg/100 g, 12.85±0.64 and 59.3±2.97 mg/100 g and 6.10±0.30 and 55.02±0.25 mg/100 g in Ajwa Aseel and Hallawi roasted pit powder, respectively. Moreover, the potassium contents were found to be the highest mineral recorded among the roasted pit powders prepared from all three varieties. The potassium was found in Ajwa, Aseel and Hallawi varieties as 226.4±11.32, 172.4±8.75 and 111.9±5.59 mg/100 g, correspondingly. Additionally, the amount of zinc was noticed as 0.165±0.02, 0.149±0.01 and 0.145±0.01 mg/100 g in Ajwa, Aseel and Hallawi roasted pit powders, respectively.

The results of mineral analysis of roasted date pit powders are in harmony with the results documented earlier by Rahman *et al*. (2007), who reported that roasted date pit powder contained sodium 165 mg/kg, potassium 2540.96 mg/kg, calcium 191.76 mg/kg, iron 21.33 mg/kg, copper 5.33 mg/kg, magnesium 789.48 mg/kg, zinc 1.53 mg/kg and phosphorous 1300 mg/kg. Later, Mirghani (2012) also documented the mineral profile of date kernel powder which he later, utilized for preparing a drink. He narrated that copper, calcium, iron, manganese and magnesium in date pit powder are 0.923, 2.035, 0.911, 0.429 and 4.99 mg/100 g, respectively.

Furthermore, Basuny and Al-Marzooq (2011) determined the mineral contents of Hallawi date pit and documented the values as 160, 189.35, 2489.50, 19.23, 5.02, 1256.23, 811.3, 1.67 and 7.12 mg/kg for sodium, calcium, potassium, iron, copper, phosphorus, Magnesium, Zinc and Manganese. Later, Sadiq *et al*. (2013) analyzed Ajwa date pit for mineral contents and revealed that it contains 77.23 mg/100 g sodium, 106.2 mg/100 g potassium, 54.67 mg/100 g calcium, 9.36 mg/100 g Magnesium, 49.33 mg/100 g Iron, 42.80 mg/100 g Zinc, 8.23 mg/100 g Phosphorous.

The variations in outcomes observed in the present studies might be related to climate, soil type, varietal differences and developmental stage. The transition of date fruit from kimri to the tamar stage is associated with a reduction in the amounts of all minerals (Gasim, 1994; Shaheen and Qurashi, 2007; Ahmed *et al*., 1995). Total phosphorus increases slowly from beginning of kimri stage and then significantly decreases to the end of kimri stage. Total phosphorus decreases slightly to its minimum percentage at the tamar stage. Potassium percentage of dates slightly increases at the end of kimri stage and then gradual decrease occurs until tamar stage (El-Agamy *et al*., 2001). The percentage of iron typically decreases from kimri to tamar stage, although there is an increase in some cultivars. The percentages of calcium, magnesium, phosphorus, potassium, sodium and zinc decrease from kimri to tamar stage. The percentage of copper in various cultivars differs; it decreases in some cultivars and in other cultivars it either has no change, or increases (Al-Shahib and Marshall, 2003). However, the variation in mineral content among different varieties might also be due to genetic potential of each cultivar. The quantity of each mineral is controlled by specific group of transcription factors that affect different metabolic pathways, thus varying, the amount of minerals in this commodity. 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. Moreover, 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 macro nutrients. The winter rainfall is best for the enhancement of soil mineral pool availability, necessary for the growth of date palm as the surface deposited salts get dissolved in rain water and leach down inside the soil surface along with the rain water thus becoming available to the plant by getting in close proximity with plant roots, contrary to this summer rainfall enhances the surface deposition of salts due to water evaporation, thus rendering them unavailable to the plant. Moreover, soil chemistry of geographic zones (Iran and Pakistan) of Hallawi and Aseel face depletion in these minerals owing to the stress on the soils of these areas due to aggressive farming strategies.

Dietary fiber content: Dietary fiber is an integral part of plants and also plays vital role in human nutrition, its components include cellulose, hemicelluloses, pectins, hydrocolloids and lignins that cannot be digested by

Table 2: Mean values for mineral composition of roasted date pit powder of different date varieties (mg/100 g)

Varieties	Phosphorous	Sodium	Boron	Calcium	Copper	Iron	Magnesium	Potassium	Zinc
Aseel	1.9±0.095[c]	17.2±0.85	0.897±0.045[b]	41.9±2.08[b]	1.45±0.072[a]	12.85±0.64[b]	59.3±2.97[b]	172.4±8.75[b]	0.149±0.01[b]
Ajwa	7.35±0.36[a]	18.1±0.75	1.061±0.053[a]	57.5±2.84[a]	1.12±0.06[a]	23.50±1.18[a]	66.01±0.30[a]	226.4±11.32[a]	0.165±0.02[a]
Hallawi	3.85±0.32[b]	16.4±0.61	0.935±0.047[b]	21.6±1.08[c]	1.10±0.05[a]	6.10±0.30[c]	55.02±0.25[b]	111.9±5.59[c]	0.145±0.01[b]

Different letters show significant variations ($p \leq 0.05$), Similar letters show non-significant variations ($p \geq 0.05$), Values are expressed as means±standard deviation

Table 3: Mean values for HPLC profile of roasted date pit powder of different date varieties

	------ Varieties ------		
Components (mg/kg)	Hallawi	Ajwa	Aseel
Chromatotrophic acid	16.08±0.74[c]	54.77±2.11[a]	19.6±0.88[b]
Quercetin	11.22±0.51[b]	13.65±0.58[a]	3.29±0.14[c]
Gallic acid	129.74±5.61[b]	175.13±7.45[a]	105.58±4.26[c]
Caffeic acid	21.82±0.99[a]	18.45±0.84[b]	16.64±0.65[c]
Chlorogenic acid	31.10±1.33[c]	82.88±3.87[a]	35.56±1.45[b]
Syringic acid	6.88±0.29[c]	8.15±0.41[a]	7.52±0.32[b]
p-coumeric acid	6.15±0.28[b]	10.22±0.46[a]	1.48±0.064[c]
m-coumeric acid	6.25±0.25[b]	15.45±0.74[a]	3.11±0.15[c]
Ferulic acid	23.87±1.12[c]	61.46±2.81[a]	24.44±1.22[b]
Vitamin C	16.99±0.77[b]	29.31±1.33[a]	17.45±0.82[b]

Different letters show significant variations ($p \geq 0.05$), Similar letters show non-significant variations ($p \leq 0.05$), Values are expressed as means±standard deviation

human gastrointestinal enzymes. Generally it is categorized into soluble and insoluble dietary fiber. Soluble dietary fiber mainly includes pectic substances and hydrocolloids that are soluble in water, whereas, insoluble dietary fiber includes components like cellulose, hemicelluloses and lignin that are not soluble in aqueous phase (Bermink, 1994).

The dietary fiber analysis (Fig. 1) for roasted date pit powder showed non-significant trend for soluble, insoluble and total fiber. The soluble fiber ranged from 7.01 to 7.08 g/100 g, maximum value 7.08 g/100 g was observed in Ajwa variety whereas minimum 7.01 g/100 g was found in Hallawi variety. The insoluble fiber ranged from 71.41 to 72.18 g/100 g, maximum value 72.180 g/100 g was depicted by Ajwa variety while minimum 71.460 was observed in Aseel variety. The total fiber ranged from 78.42 to 79.260 g/100 g, maximum value 79.260 g/100 g was observed in Ajwa variety whereas minimum 78.420 g/100 g was found in Hallawi variety. Al-Farsi *et al.* (2007) evaluated three native Omani sun-dried date varieties (Mabseeli, Um-sellah and Shahal) for their dietary fiber content and reported 77.75% in Shahal, 79.84% in Mabseeli and 80.15% in Um-sellah. Al-Farsi *et al.* (2008) optimized extraction conditions of dietary fiber from date pits. They reported that date pit contain 57.87 g/100 g total dietary fiber out of which 5.17 g/100 g is soluble while 52.70 g/100 g is insoluble. Furthermore they also determined the dietary fiber (soluble, insoluble and total) of date pits remaining after extraction of phenolics with water (WDF) and 50% acetone (ADF) and it was found that after extraction of phenolics by water the date pit fiber contained 83.50 g/100 g total dietary fiber out of which 1.53 g/100 g was soluble and 81.97 g/100 g was insoluble. Moreover, it was found that after extraction of phenolics by 50% acetone the date pit fiber contained 82.17 g/100 g total dietary fiber out of which 1.10 g/100 g was soluble and 81.07 g/100 g was insoluble.

The fiber content vary as a function of progression in developmental stage and is higher at kimiri stage while low at tamer stage this is due to the fact that majority of the fiber content are hydrolyzed by different enzymes that make the date fruit soft, lowering fiber content and thus enhancing the fruit palatability. As date ripens, protopectin converts into soluble pectin through the combined action of two pectolytic enzymes, polygalacturonase and pectin methyl esterase. The quantity of pectin and fibers is usually smaller in soft dates. Before date ripening begins, the quantity of pectin compounds increases and by the time date attains maximum size, the amount of existing protopectin also reaches its maximum level; but as fruit ripening begins, the amount of soluble pectin slowly increases (Fallahi, 1996).

TPC and TFC in date pit powder: Anti-oxidant action in plants, is directly exhibited by phenolic and polyphenolic compounds that represent the main class of natural antioxidants (Awika *et al*., 2003). The TPC and TFC varied momentously ($p \leq 0.05$) among the roasted date pit powders obtained from three varieties. The mean value was 843.54 to 1204.7 mg/100 g for TPC and 371.16 to 530.05 mg/100 g TFC (Fig. 2). The maximum TPC 1204.7 mg/100 g was detected in Ajwa variety where as minimum 843.54 mg/100 g was detected in Aseel date. The TFC were recorded as 530.05, 513.66 and 371.16 mg/100 g in Ajwa, Hallawi and Aseel, respectively.

Antioxidant activity: It has been proved through recent studies that many fruits and vegetables exhibit total antioxidant activity significantly owing to the presence of many flavonoids and related polyphenols (Luo *et al*., 2002). The roasted date pit powder extract exhibited highest DPPH scaveging activity at a concentration of 10 µg/ml. The antioxidant activity shown by three varieties is presented in Fig. 3. The DPPH scavenging activity was exhibited as 73.68, 55.78 and 32.78 % in Ajwa, Hallawi and Aseel, respectively.

HPLC phenolics characterization of roasted date pit powder: High-Performance liquid chromatography (HPLC) is a fast technique that, with high precision and specificity, separates mixtures into individual ingredients. It is used as a routine procedure for food analysis.

The HPLC analysis of total phenolic contents showed substantial variations among the treatments. The HPLC analysis of Ajwa variety showed that it had 54.77±2.11 mg/kg Chromatotrophic acid, 13.65±0.58 mg/kg Quercetin, 175.13±7.45 mg/kg Gallic acid, 18.45±0.84 mg/kg Caffeic acid, 82.88±3.87 mg/kg Chlorogenic acid, 8.15±0.41 mg/kg Syringic acid, 10.22±0.46 mg/kg p-Coumeric acid, 15.45±0.74 mg/kg m-Coumeric acid, 61.46±2.81 mg/kg Ferulic acid, 29.31±1.33 mg/kg vitamin C. Similarly, Hallawi variety had 16.08±0.74 mg/g Chromatotrophic acid, 11.22±0.51 mg/kg Quercetin, 129.74±5.61 mg/kg Gallic acid, 21.82±0.99 mg/kg Caffeic acid, 31.10±1.33 mg/kg Chlorogenic acid, 6.88±0.29 mg/kg Syringic acid, 6.15±0.28 mg/kg p-Coumeric acid, 6.25±0.25 mg/kg m-Coumeric acid, 23.87±1.12 mg/kg Ferulic acid, 16.99±0.77 mg/kg vitamin C. Whereas, Aseel variety showed 19.6±0.88 mg/kg Chromatotrophic acid, 3.29±0.14 mg/kg Quercetin, 105.58±4.26 mg/kg Gallic acid, 16.64±0.65 mg/kg Caffeic acid, 35.56±1.45 mg/kg Chlorogenic acid, 7.52±0.32 mg/kg Syringic acid, 1.48±0.064 mg/kg p-Coumeric acid, 3.11±0.15 mg/kg m-Coumericacid, 24.44±1.22 mg/kg Ferulic acid, 17.45±0.82 mg/kg vitamin C.

The results of present research are in line with the previous findings of Paranthaman *et al*. (2012) who investigated the effect of roasting on the content of flavonoids in date pits through HPLC analysis. The results showed that the flavonoids content in raw date pits was found to be gallic acid (0.1 mg/gm), rutin (0.4 mg/gm) and quercetin (0.9 mg/gm). Whereas, roasted date pit contained gallic acid (0.1 mg/gm), rutin (2.2 mg/gm) and quercetin (0.52 mg/gm). They concluded that roasting of date pits is recommended to improve their chemical profile.

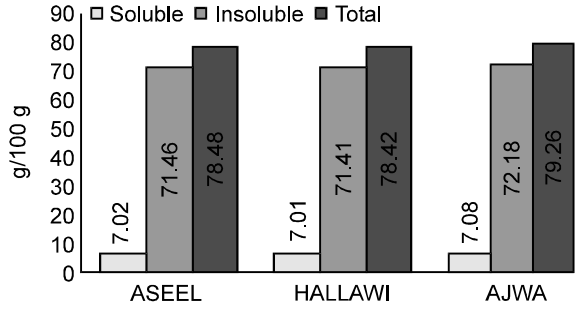

Fig. 1: Comparison of dietary fiber conent

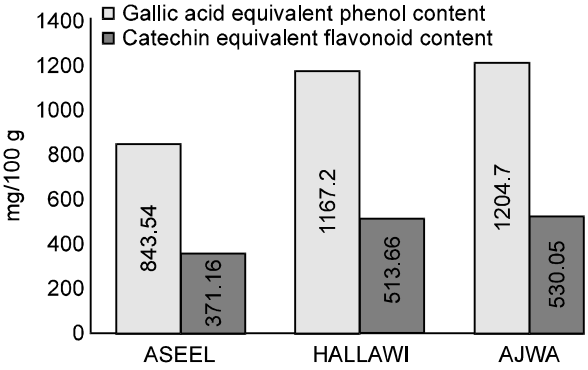

Fig. 2: Comparison of TPC and TFC

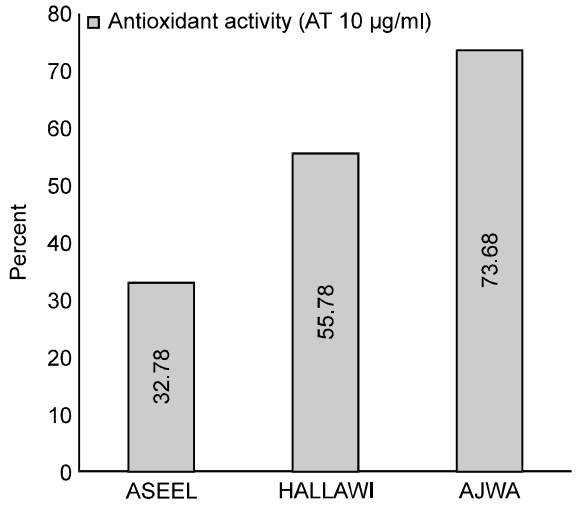

Fig. 3: Comparison of DPPH scavenging activity

Furthermore, Al-Farsi and Lee (2008) probed date pits for phenolic acid composition. They deduced that date pit contains 0.28 mg/100 g gallic acid, 8.84 mg/100 g protocatechuic acid, 9.89 mg/100 g p-hydroxybenzoic acid, 4.07 mg/100 g vanillic acid, 0.18 mg/100 g caffeic acid, 6.07 mg/100 g p-coumaric acid, 6.93 mg/100 g ferulic acid, 8.42 mg/100 g m-coumaric acid, 3.96 mg/100 g o-coumaric acid. Likewise, HPLC phenolic profile outcomes of three date varieties (Ajwa, Sukkari and Khalas) by Saleh *et al*. (2011) indicated that Sukkari contained the highest rutin concentration (8.10 mg/kg) trailed by Ajwa and Khalas as 6.50±1.70 and 3.60±0.07 mg/kg, respectively, whereas, catechin was approximately the same in Sukkari and Ajwa (7.50 and 7.30 mg/kg, respectively) while recorded 5.00±0.18 mg/kg in Khalas that has highest content of caffeic acid (7.40 mg/kg) trailed by Ajwa and Sukkari as 5.70±0.77 and 5.40±0.64 mg/kg.

Recently, supercritical fluid extraction technique was employed by Liu *et al*. (2013) to prepare date pit extracts. Furthermore HPLC analysis of date pit extract was conducted and a total of seven phenolic compounds from date pits were identified namely gallic acid, protocatechuic acid, chlorogenic acid, epicatechin, rutin, ellagic acid, quercetin.

Later, aqueous date extract of degla cultivar at besser stage was found a polyphenolic rich extract after HPLC analysis by El-Arem *et al*. (2014); they documented the presence of different polyphenolic acids like gallic, chlorogenic, protocatechuic, catechin, caffeic, syringic, 3-hydroxybenzoic, ferulic, p-coumaric, m-coumaric, o-coumaric and phenylacetic as 2.05±0.01, 2.34±0.06, 3.06±0.01, 2.48±0.02, 4.64±0.03, 3.02±0.01, 1.93±0.01, 5.50±0.02, 4.71±0.02, 2.35±0.01, 1.94±0.01 and 1.52±0.02 mg/100 g fresh weight, respectively.

The present results emphasized that roasted date pit powder holds significant antioxidant potential. Additionally, HPLC quantification revealed that Ajwa roasted date pit powder contains appreciable amount of diverse phenolic acids also justified by Saleh *et al*. (2011) who observed that water extract of Ajwa had significantly ($p \leq 0.05$) higher contents of total phenols than alcoholic extract (455.88 and 245.66 mg/100 g, respectively).

It is evident from the outcomes of present research study that Ajwa date pit powder showed highest phenolic content which is also in agreement with the findings of Eid *et al*. (2013) who previously reported high polyphenols in Ajwa date fruit, the variation in polyphenolic content in respect of other varieties might be due stage of development. As date fruit progresses towards maturity the polyphenolic content follow a decreasing trend with maximum polyphenols content at kimri stage (unripe) and minimum at tamar stage (ripe, reduced moisture; edible) this fact is endorsed to the oxidation and enzymatic browning of polyphenols that results into the production of secondary products. The high polyphenols content in Ajwa may also be due to the fact that Ajwa dates belong to a region most favorable for date cultivation in respect of temperature, precipitation and light availability. The highest maximum temperatures found in this region are the result of low humidity and long days in summer. Date palm is cultivated in arid and semi-arid regions which are characterized by long and hot summers, no (or at most low) rainfall and very low relative humidity level during the ripening period. The optimum growth temperature for date palm is 7-38°C, however, date palm can also tolerate temperature ranges from-6 to 50°C. The growth of date palm continues without any stress in red color of solar spectrum that enhances the process of photosynthesis while violet and yellow radiations in the solar spectrum tend to suppress its growth. The winter rainfall is best for the growth of date palm as the surface deposited salts get dissolved in rain water and leach down inside the soil surface along with the rain water thus becoming available to the plant by getting in close proximity with plant roots, contrary to this summer rainfall enhances the surface deposition of salts due to water evaporation, thus rendering them unavailable to the plant. These absorbed minerals play vital role of co-factors for enzymes that are in control of various metabolic pathways in the plant body for the production of different components like polyphenols and their unavailability may inhibit activity of enzyme controlling a particular pathway, involved in the synthesis of a specific phenolic compound. Saudi Arabia is gifted with all the above environmental parameters necessary for the optimum growth of date fruits. Therefore date fruits from this region are globally well known for their superior sensory and chemical attributes. The above mentioned facts are supporting Ajwa date for claiming lead position on the basis of the research outcomes of present studies.

Conclusions: From the results of the present investigation, it is concluded that date pit is an excellent source of dietary antioxidant activity, total phenolics and fiber that can serve as an excellent and economical resource of natural antioxidants. It is proposed from this research study that Ajwa date pit on account of its miraculous nature can be used as a functional food or functional food ingredient. It is further recommended that composition of phenolic acids and flavonoids should be classified and enumerated through additional research.

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