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Chemical composition and antioxidant activity of date (*Phoenix dactylifera* L.) varieties at various maturity stages

Yasmeen BANO¹ , Allah RAKHA¹*, Muhammad Issa KHAN¹, Muhammad ASGHER²

Abstract

The current study was conducted to determine the effect of four maturity stages on the chemical composition and antioxidant activity of date (*Phoenix dactylifera* L.) varieties. Four varieties of date palm viz., Zahidi, Aseel, Halawi, and Dhaki were used at four ripening stages (Kimri, Khalal, Rutab, and Tamer). The protein and fat contents of all the selected varieties of dates decreased from Kimri to the tamer stage of development. In all the date varieties the glucose, fructose, and total sugar contents were increased up to the full ripe stage. On the other hand, the total phenolic content, antioxidant activity (DPPH and FRAP), and total flavonoid content decreased gradually in all the selected date varieties from Kimri to Tamer stage of development. The highest amount of TPC, TFC, DPPH, and FRAP was observed in the Dhaki variety. During the fruit maturation process, minerals and dietary fiber contents also decreased from Kimri to Tamer stage of development in all varieties. The dietary fiber including both soluble and insoluble fiber was observed highest in Dhaki variety at the Kimri stage.

Keywords: date palm fruit; ripening stages; minerals; sugars; bioactive compounds; dietary fiber.

Practical Application: Phytochemicals (dietary fiber and bioactive compounds) of plant source food protect the body from several physiological threats. Date fruit has both nutritious and medicinal importance, it contains carbohydrates, dietary fiber, bioactive compounds, and macro minerals like calcium and potassium. Date fruit at early maturity stages based on phytochemicals can be used to develop date fruit enriched products to combat cardiovascular diseases. But there were no comparative studies on the phytochemicals of different varieties of date fruit at various maturity stages.

1 Introduction

The date palm (Phoenix dactylifera L.) belongs to the Arecaceae family and is cultivated in the hot arid regions of the world. The date palm tree is also known as the "Tree of life". It is cultivated in 34 countries around the world and 100 million date palms are cultivated in these countries. The major cultivators of the date palm are Tunisia, Oman, Pakistan, Libya, Iraq, Egypt, Saudi Arabia, Iran, and UAE (Aljasass et al., 2016; Younas et al., 2020; Oladzad et al., 2021; Shehzad et al., 2021; Ahmad et al., 2021). Several varieties of the date palm are produced in the southern regions of Pakistan and the prominent among these are Dhaki, Aseel, and Zahidi (Soomro et al., 2022). The date fruit is responsible for providing nutrition to millions of people around the globe (Jamil et al., 2010). Date fruit contributes to the diet of people living in Pakistan and is an important source of nutrition. Pakistan is ranked as the world's 6th largest producer of date fruit. Sindh province produced the majority of dates in Pakistan (Food and Agriculture Organization, 2021). Date fruit is the third commercial fruit after mango and citrus in Pakistan and the production of date fruit is 7,25,000 metric tons by cultivating 97,300 hectares area according to the Pakistan Horticulture Development and Export Board (Fatima et al., 2016).

In Pakistan, date palm is grown in the areas of Makran, Kalat, Kech, Turbat, Baluchistan, Punjgur, Khairpur, and Suthar District in Sindh while in the areas of Southern Punjab Multan, Muzaffargarh, Jhang, Vehari, and Dera Ghazi Khan are predominantly involved in the cultivation of date palm (Osman et al., 2014). In PakistanBaluchistan and Sindh contribute to 90% of date fruit production, and these two regions are then followed by southern Punjab in the production of date fruits (Nadeem et al., 2011). Date fruit passes through a series of several ripening steps for obtaining maturity after pollination. The names of various maturity stages are written and pronounced in Arabic as Kimri, Khalal, Rutab, and Tamer stages respectively (Othmani et al., 2020). The fruit in the Kimri stage is green in color. The Khalal stage is different from other sages in such a way that the color of the fruit changes from green to yellow or green to red, and this transition of color depends on the variety of the date palm and moisture content decrease and the taste of the date fruit becomes sweeter as compared to the earlier stage of maturation. After the completion of the Khalal stage of maturation of date fruit, the fruit is now mature physically and it is hard in texture also the size and weight gain of date fruit are maximum at the end of the Khalal stage of maturation (Rahmani et al., 2014). In the Rutab stage, the texture becomes soft along with the ripening of the fruit. The date fruit attains a firm texture and dark brown or black color in the last stage of maturation as known as the "Tamer" stage of maturation (Baliga et al., 2011).

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The stages of the date fruit at which it is consumed are characterized by the aroma, flavor, color, and texture of the fruit. Fresh ripened dates due to their high monosaccharide sugar content are the source of quickly accessible energy. The monosaccharide sugars present in the fresh dates are glucose, and fructose along with sucrose which is a disaccharide (Vayalil, 2012). Date palm is a food that is characterized as energy-dense and contains almost all the micronutrients (Al-Farsi & Lee, 2008). It contains a balanced quantity of both micro and macronutrients (Vayalil, 2012). The date palm plant is cultivated for various benefits including energy boosters as a date-based energy drinks, functional date bars, sweet fruit, hunger pacifiers, and many medical properties such as gastroprotective, hepatoprotective, antimutagenic, and immunostimulant (Baliga et al., 2011; Mostafa, 2022; Safdar et al., 2022). Date fruit is also a rich source of bioactive compounds such as phenolics and flavonoids all of which are beneficial against oxidative stress (Allaith, 2008). It also contains antioxidant components along with antioxidant enzymes (Awad et al., 2011). Date fruit possesses different nutraceutical properties. It also provides various health benefits to the consumers which are neuroprotection, hepatoprotection, nephroprotection antihyperlipidemic, anticarcinogenic, and anti-inflammatory properties (Chandrasekaran & Bahkali, 2013; Dayem et al., 2016; Khalid et al., 2017; Mostafa et al., 2021). Keeping in view the health benefits and good nutritional composition of date fruits, the present study was designed to investigate the chemical composition and antioxidant activity of four different varieties of date fruits at different maturity stages.

2 Materials and methods

2.1 Raw materials procurement

Four locally grown date fruit varieties namely, Zahidi, Aseel, Halawi, and Dhaki were collected at four distinct developmental stages e.g., Kimri, Khalal, Rutab, and Tamer. Merck, Darmstadt, and Sigma-Aldrich standards and reagents (analytical and HPLC grade) were procured from the local market for the analysis.

2.2 Sample preparation

The samples were graded, washed, and blanched before pitting. Subsequently, dates were chopped and dried by using the dehydrator. Samples were grounded by using an electric grinder, packed, and stored at -20 °C for further analysis.

2.3 Protein and fat contents

Date fruit samples were analyzed for protein and fat contents using their specific experimental methods explained (Association of Official Analytical Chemists, 2016).

2.4 Total Dietary Fiber (TDF)

The Samples were analyzed for TDF according to the method of American Association of Cereal Chemists (2011).

2.5 Mineral's profile

Minerals were analyzed by the following protocol (Association of Official Analytical Chemists, 2016). Mineral elements from samples were quantified via Atomic Absorption Spectrophotometer (AAS) and Flame Photometer. Minerals including K, Ca and Na were estimated using Flame Photometer-410; Sherwood Scientific Ltd., Cambridge, UK while Zn, Mg, and Fe through AAS; Hitachi Polarized Zeeman AAS, Z-8200, Japan.

2.6 Sugar contents

The sugar contents of the samples were analyzed by following the protocol of (Chaira et al., 2009) with minor modifications. HPLC grade water (25 mL) was used to reflux the date powder (1g), then using an orbital shaker the mixture was homogenized for 5 min at 280 rpm, and then the mixture was allowed to rest for 2 h. Afterwards, for 10 min the mixture was centrifuged at 5000 rpm. To filter the supernatant 0.45 μ m membrane filter was used. In date samples, the reducing and non-reducing sugars were quantified by using HPLC. Lichrospher* 100 NH2 Purospher*STAR NH2 5 μ m column was used for the separation which was conducted at 20 °C. Acetonitrile and ultrapure water (80/20 v/v) were used as the medium in the mobile phase.

2.7 Bioactive compounds and antioxidant activity of date fruit

Preparation of date flesh extract

The 100 mL of 75% ethanol was used for 3 h in an Orbital Shaker (KS-260 Edmund Buhler Gmg H-Ks 15, Germany) for the extraction of 10 g of date powder. Centrifugation of the samples was done for 15 min at 5000 rpm. Whatman filter paper was used for the filtration of the sample. The filtrate was separated, and the solvent was evaporated by using a rotatory evaporator (Eyelia, Japan) (Haimoud et al., 2016).

Total Phenolic Content (TPC)

TPC were determined in date samples using Folin-Ciocalteau (FCA) gallic acid and reagent as a set standard (Lemine et al., 2014). Accordingly, 0.2 mL aliquots of each sample were evaluated. In each test tube, Folin-Ciocalteu phenol reagent (0.2 M/L) 0.5 mL was added and for 5 min these test tubes were kept at room temperature. In these test tubes, 0.4 mL of 7.5% sodium carbonate (Na₂CO₃) was added and then mixed thoroughly. At 25 °C these samples were incubated for 60 min. By using UV-Vis Spectrophotometer the absorbance was taken at 750 nm. Results were expressed as milligram gallic acid equivalents (GAE)/100 g DM. The gallic acid standard was used at different concentrations to prepare the standard curve.

Total Flavonoids Content (TFC)

The procedure explained by Hasnaoui et al. (2012) was used to measure the total flavonoid content. Distilled water and extract were added to a 10 mL volumetric flask. After adding the extract to the flask, 0.3 mL of 5% sodium nitrite was poured into the flask and then 1 M sodium hydroxide was added after 6 to 12 min. The sodium hydroxide was diluted to volume with distilled water. By using UV-Vis Spectrophotometer the absorbance of the solution was measured immediately at 510 nm. Calibration of the measurements was done by using the standard curve of prepared quercetin solution, and the total flavonoid content was expressed as milligrams of quercetin equivalents (mg QE)/100 g of dry weight.

DPPH

The protocol of (Al-Najada & Mohamed, 2014) with minor modifications was used to estimate the ability of the date fruit to donate a hydrogen atom or an electron. Date extract (0.5 mL) was incorporated into 2.5 mL methanolic DPPH solution which was freshly prepared. An equal amount of methanol was used in comparison to the control sample. The optical density (OD) was measured at 517 nm using a UV-Visible Spectrophotometer after incubating the samples in the darkroom for 30 min. The DPPH activity was expressed as µg/mL.

FRAP

Al-Najada & Mohamed (2014) described the protocol which was used to perform the FRAP analysis with minor modifications. FRAP analysis is based on the principle of reduction of a ferric 2,4,6-tripyridyl-s-triazine complex (Fe3+- TPTZ) to ferrous, which is the colored form (Fe2+-TPTZ), and this process takes place in the presence of antioxidants. Accordingly, the preparation of the FRAP reagent was carried out by mixing 25 mL acetate buffer (pH 3.6, 0.3 M) with 2.5 mL 2,4,6-tris-(2-pyridyl)-S-triazine (10 mM) and 2.5 mL of ferric chloride (20 mM). In 20 μ L of extract, 280 μ L of FRAP solution was added and for 30 min it was placed in a dark place for incubation at room temperature. The absorbance was observed at 593 nm using UV-Visible Spectrophotometer. The calibration curve was obtained using Trolox, and µmol TE/100 gm DW, was used to express the results.

2.8 Statistical modeling

For each parameter, data were analyzed statistically to determine the level of significance. Statistical Package (Statistix 8.1) was used. The 2-factor factorial CRD was employed to determine the level of significance. Whereas posthoc Tukey's HSD test was applied to test means differences (Montgomery, 2008).

3 Results and discussion

The present study was designed to collect data about the compositional and functional properties of the four distinct date varieties of Pakistan at four stages of maturity. The parameters studied include protein, fat, mineral profile, sugar profile, dietary fiber, phenolic contents, flavonoid contents, and antioxidant activity was also examined for all four varieties at Kimri, Khalal, Rutub, and Tamer stage.

3.1 Protein and fat

The results of the protein and fat contents are presented in Figure 1. The protein contents were found to be in decreasing trend in the date powder from Kimri to the Tamer stage in all date varieties. The results revealed that there is a significant difference in the protein contents of the four varieties at four different maturity stages. The percentage of protein was high at earlier stages and then decreased slowly up to the Tamer stage. The highest protein content $5.28 \pm 0.01\%$ was found in the Dhaki variety at the Kimri stage followed by $5.16 \pm 0.01\%$ of the Zahidi variety at the Kimri stage. The lowest protein content of $4.51 \pm 0.01\%$ was observed in Halawi at the Tamer stage. These findings close are in line with (Rastegar et al., 2012). Minor variations, on the other hand, may be due to type of variety, harvesting time, as well as environmental and experimental conditions. Furthermore, the concentration of protein decreased from the Kimri to the Tamer stage of date fruit development. The protein in date fruit may vary due to differences in genotype along with the areas where the dates are grown and the developmental stage (Ibourki et al., 2021). Fat content was decreased from Kimri to Tamer stage in all studied date varieties. The maximum amount $(0.89 \pm 0.01\%)$ of fat was observed in Dhaki at the Kimri stage, while the minimum amount (0.61 \pm 0.01%) was observed in the Halawi variety at the Tamer stage. These findings strongly resemble those (Amira et al., 2011), who also observed that fat content decreased with the increase in the ripening of the date

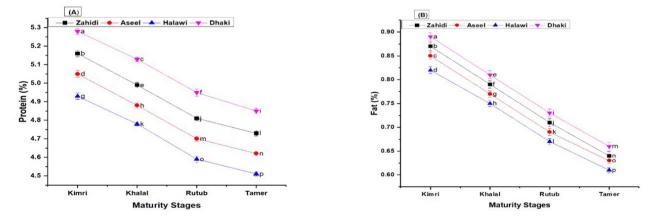


Figure 1. Protein and fat analysis of four date varieties at four maturity stages. Graphs (A) and (B) are representing protein and fat percentages on a dry weight basis, respectively. Different letters represent a significant difference in protein and fat contents among the varieties in the maturity stage.

fruit. It was reported that fat contents in different date varieties are very low and date powder is not considered a good source of fat. Variations in fat content may be attributed to genotypic influences, environmental factors, and the stage of ripening at which date fruits are harvested (Benmeziane-Derradji, 2019).

3.2 Dietary fiber

The results of the dietary fiber analysis including soluble and insoluble fiber are presented (Figure 2). It was found that DF contents decrease gradually as the ripening stages increases. A significant variation exists among all varieties at four different stages of maturity. The highest amount of TDF was observed in the Dhaki (9.83 \pm 0.10%) at the Kimri stage followed by the Zahidi (9.41 \pm 0.16%), Aseel (8.67 \pm 0.137%), and Halawi (8.15 \pm 0.16%) which declines with the maturation of date fruits and lowest being found in Halawi (5.48 \pm 0.14%) at Tamer stage. A similar decrease from Kimri to Tamer stage was noted for the soluble and insoluble fibers. The highest percentage of soluble fiber (3.59 \pm 0.19%) and insoluble fiber (6.24 \pm 0.129%) were also measured in Dhaki at the Kimri stage.

The Zahidi contained $3.43 \pm 0.13\%$ and $5.98 \pm 0.18\%$ soluble and insoluble fiber respectively. The amount of these also declines up to the Tamer stage of development. The lowest amount of soluble and insoluble fiber was found in Halawi at the Tamer stages. The results of this research regarding TDF are in line with (Kamal-Eldin et al., 2020), they found TDF in various date varieties at the Tamer stage in the range of 5.19 ± 0.23 to $8.29 \pm 0.23\%$. The difference in the percentages of dietary fiber including soluble and insoluble fiber might be attributed to the fiber degrading enzymes mainly pectinases and cellulases that hydrolyze fiber to the smaller molecules (Ghnimi et al., 2017). Fiber content is desirable in the food sector because DF offers major therapeutic advantages such as anti-diabetic, anti-obesity, cholesterol absorption (limiting access to the body), and gut-health promotion via bulking effects and the production of short-chain fatty acids (Ötles & Ozgoz, 2014). Dietary fiber is considered a very important parameter concerning functional properties especially cholesterol-lowering properties. The leading cause of mortality around the globe is CVDs. Cardiovascular diseases mainly arise due to the deposition of fat in blood vessels especially due to a higher level of low-density lipoproteins (LDLs). Dietary

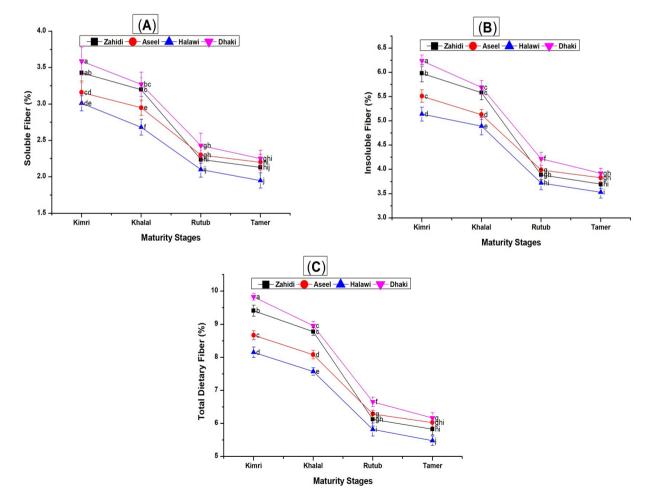


Figure 2. Dietary fiber analysis of different date varieties at four maturity stages. Graphs (A), (B), and (C) are representing soluble fiber, insoluble fiber, and TDF percentages on a dry weight basis, respectively. Different letters represent a significant difference in fiber contents among the varieties at a maturity stage.

fiber is also useful in food products development such as the formation of gel, emulsification effects, etc. (Hussain et al., 2020).

3.3 Mineral analysis

The mineral elements are expressed as mg/100 g dry weight of the date varieties at four ripening stages are presented (Figure 3).

Analysis of the mineral contents reflects that potassium was detected as one of the most abundant minerals in the different date varieties at different maturity stages. The maximum amount of potassium ($2659 \pm 16.40 \text{ mg}/100 \text{ g}$) was found in Dhaki dates at the Kimri stage followed by $2549 \pm 19.49 \text{ mg}/100 \text{ g}$ in Zahidi at the same stage. In all varieties, a decreasing trend was observed in the potassium contents from Kimri to Tamer stage. A similar

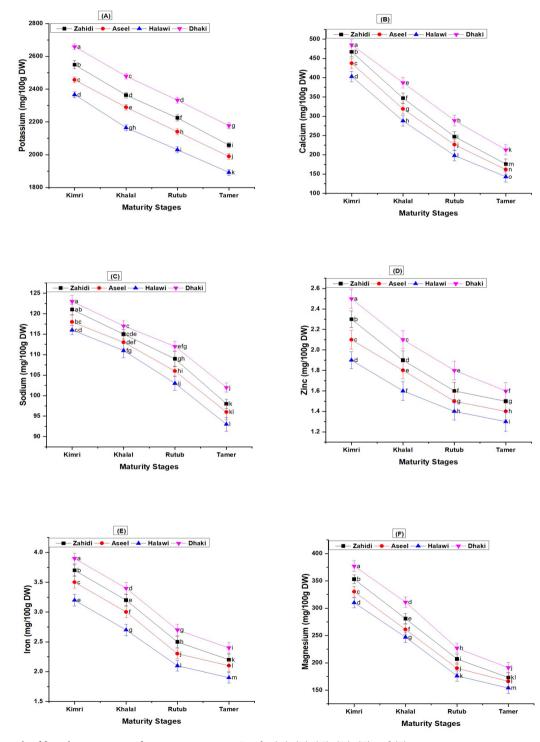


Figure 3. Minerals of four date varieties at four maturity stages. Graphs (A), (B), (C), (D), (E) and (F) are representing potassium, calcium, sodium, zinc, iron, and magnesium percentages respectively. Different letters represent a significant difference among the varieties at a maturity stage.

decreasing trend was observed in the case of calcium contents. At the Kimri stage, all varieties possess a maximum amount of calcium in the range of 403 ± 13.39 to 485 ± 13.41 mg/100 g. The Dhaki variety contains the highest (485 ± 13.41 mg/100 g) of calcium followed by 467 ± 12.97 mg/100 g in the Zahidi variety at the Kimri stage. However, the lowest concentration (143 ± 13.41 mg/100 g) was found in Halawi at the Tamer stage. The data recorded for the sodium contents revealed that sodium contents decrease with the maturity stages. The highest amount was observed at the Kimri stage while the lowest was observed at the Tamer stage.

The Dhaki variety was found with the highest amount of sodium $(123 \pm 1.47 \text{ mg}/100 \text{ g})$ at the Kimri stage followed by Zahidi $(121 \pm 1.31 \text{ mg}/100 \text{ g})$ at the same stage. The lowest amount was in the Halawi variety $(93 \pm 1.67 \text{ mg}/100 \text{ g})$ at the Tamer stage. The concentration of Zn recorded for the different varieties of the dates at different maturity stages presented in Figure 3 showed that Zn was in the range of 1.3 ± 0.09 to $2.5 \pm 0.09 \text{ mg}/100 \text{ g}$. All varieties exhibit the same pattern of decreasing concentration from the Kimri to Tamer stage. The highest amount was found in Dhaki $(2.5 \pm 0.09 \text{ mg}/100 \text{ g})$ at the Kimri stage followed by Zahidi $(2.3 \pm 0.08 \text{ mg}/100 \text{ g})$. It was found that there was a significant difference among all the varieties at different maturity stages. The lowest amount was in Halawi $(1.3 \pm 0.09 \text{ mg}/100 \text{ g})$ at the Tamer stage.

The different date varieties were also statistically analyzed for the Fe determination at four different maturity stages. The Fe contents decrease in the dates as the maturity stages increase from Kimri to the Tamer stage. The concentration of iron was in the range of 1.9 \pm 0.08 to 3.9 \pm 0.09 mg/100 g. The maximum Fe contents were observed in the Dhaki $(3.9 \pm 0.09 \text{ mg}/100 \text{ g})$ at the Kimri stage. The lowest amount of Fe was observed in Halawi (1.9 \pm 0.08 mg/100 g) at the Tamer stage. The data recorded for the Mg contents of the four date varieties at four different stages has been shown (Figure 3). The data revealed a decreasing trend in all varieties from Kimri to Tamer stage $(154 \pm 9.93 \text{ to } 377 \pm 9.63 \text{ mg}/100 \text{ g})$. The maximum amount of Mg was recorded in the Dhaki variety $(377 \pm 9.63 \text{ mg}/100 \text{ g})$ at the Kimri stage followed by Zahidi $(353 \pm 8.08 \text{ mg}/100 \text{ g})$. The results regarding the minerals profile are in close confirmation with those of Rastegar et al. (2012), potassium was maximum among all the minerals in the selected date varieties followed by calcium and magnesium. Mineral contents in the date varieties varied from each other at different maturity stages depending upon fertilizer application, climatic conditions, and type of soil. The results are also in line with those of Chaira et al. (2009) and Juhaimi et al. (2014), and they found the minerals contents in the date varieties varied and potassium was the highest among all the minerals. Several factors, including variety, geographic origin, cultivation, maturity, fertilization, and soil type may affect the differences found in mineral contents in date fruits (Benmeziane-Derradji, 2019).

3.4 Sugar profile

The data of sugars in date varieties at four different maturity stages are presented (Figure 4). The statistical analysis showed a significant difference (p < 0.05) between the values of reducing

sugars and sucrose for all the varieties used in the study at different stages of maturity. The percentage and the type of sugar varied according to the maturation stage and variety. The amount of reducing sugars varied from $31.04\% \pm 0.47$ to $55.1\% \pm 0.57$, $36.22\% \pm 0.53$ to $61.2\% \pm 0.64$, $33.86\% \pm 0.50$ to $58.84\% \pm 0.60$ and $32.69\% \pm 0.48$ to $57.76\% \pm 0.56$ in Dhaki, Halawi, Aseel, and Zahidi respectively throughout the fruit growth. The Halawi showed significantly higher contents of sugars as compared to other varieties. In Halawi the greater increase in reducing sugars was observed at Khalal and Rutab stage which further increased at the Tamer stage. The lowest reducing sugars were found at the Kimri stage $(31.04\% \pm 0.47)$ in the Dhaki variety. Among reducing sugars, both glucose $(31.21\% \pm 0.39)$ and fructose $(29.99\% \pm 0.24)$ were found in the maximum amount in Halawi at the Tamer stage. The glucose was in a higher amount as compared to fructose. The data regarding the sucrose analysis showed a significant difference (p < 0.05) among all varieties at all stages of ripening. Unlike glucose and fructose, the sucrose content decreases at the Tamer stage. The maximum amount of sucrose was observed in Halawi at the Khalal stage which decreases up to $2.77\% \pm 0.01$ at the Tamer stage. The lowest amount of sucrose was in Dhaki (1.23%) \pm 0.01) at the Tamer stage.

Although there are several forms of dates on the market, such as fresh dates, date paste, and date syrup, each of which is used for a different purpose, date powder would be extremely useful in improving shelf life, ease of handling, and blend-ability with a variety of foods prepared at home and in industry. The higher concentration of glucose and fructose might be attributed to the activity of the invertase enzyme that converts sucrose to glucose and fructose. That's why the sucrose contents decrease with maturity (Haider et al., 2013). The change in sugar concentration can be attributed to environmental and genetic variables that may influence the qualitative and quantitative composition of the sugar fraction by affecting the activity of enzymes involved in the synthesis and breakdown processes along with maturation. At the Tamar stage, sucrose was hydrolyzed into reducing sugar. The quantity of reducing sugars also varies by variety and is directly connected to date texture. The results of this study are in close collaboration with those of (Rastegar et al., 2012), who found a similar trend of reducing sugars and sucrose in the three selected date varieties i.e Shahani, Piarom, and Deiry.

3.5 Bioactive compounds and antioxidant activity

TPC

The TPC of the four different date varieties at four different maturity stages is given (Figure 5). It is obvious from statistical analysis that TPC differs significantly among different varieties. The TPC was found in greater quantity at the Kimri stage which goes on to decline up to the Tamer stage. The highest TPC was recorded in the Dhaki variety ($457.68 \pm 3.73 \text{ mg GAE}/100 \text{ g}$) followed by Zahidi ($442.3 \pm 3.25 \text{ mg GAE}/100 \text{ g}$) at the Kimri stage. The TPC ranges from Kimri to Tamer stage as $457.68 \pm 3.73 \text{ mg GAE}/100 \text{ g}$ to $389.20 \pm 4.41 \text{ mg GAE}/100 \text{ g}$ in Dhaki, $434.39 \pm 4.654 \text{ mg GAE}/100 \text{ g}$ to $374.95 \pm 3.09 \text{ mg GAE}/100 \text{ g}$ in Aseel, $442.30 \pm 3.25 \text{ mg GAE}/100 \text{ g}$ to $379.34 \pm 4.08 \text{ mg GAE}/100 \text{ g}$ in Zahidi and $424.09 \pm 3.54 \text{ mg GAE}/100 \text{ g}$ to $364.75 \pm 2.79 \text{ mg GAE}/100 \text{ g}$

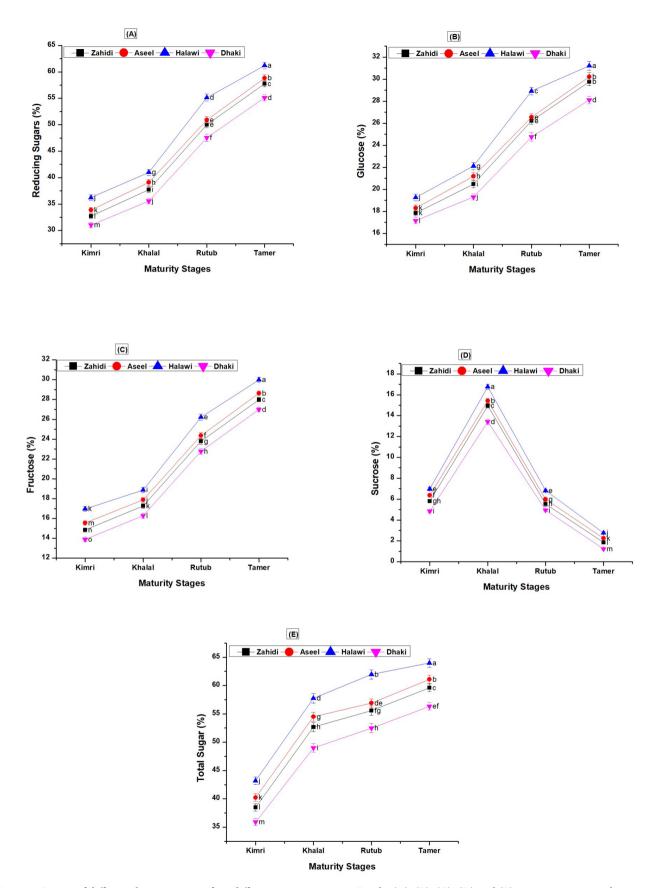


Figure 4. Sugars of different date varieties at four different maturity stages. Graphs (A), (B), (C), (D) and (E) are representing reducing sugars, glucose, fructose, sucrose, and total sugars percentages respectively. Different letters represent a significant difference among the varieties at a maturity stage.

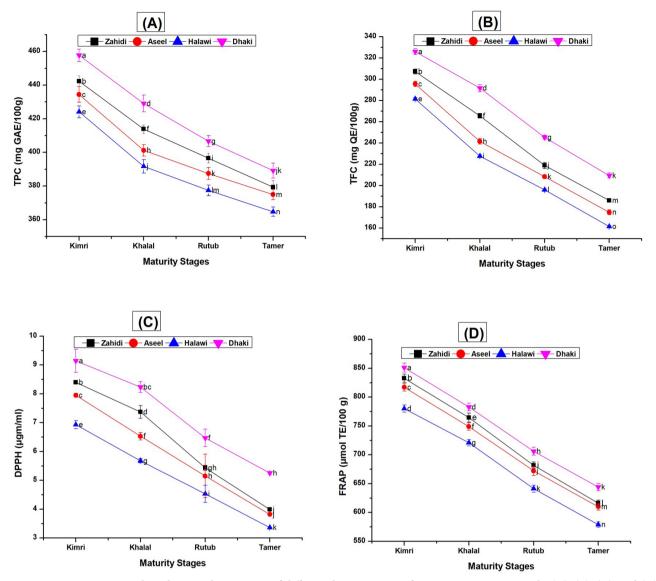


Figure 5. Bioactive compounds and antioxidant activity of different date varieties at four maturity stages. Graphs (A), (B), (C), and (D) are representing TPC, TFC, DPPH, and FRAP respectively. Different letters represent a significant difference among the varieties at a maturity stage.

in Halawi variety. The lowest TPC was recorded as $364.75 \pm 2.79 \text{ mg GAE}/100 \text{ g}$ in Halawi at the Tamer stage among all the varieties. Phenolic contents of the date represent the nutraceutical potential of the food and its antioxidant properties. The difference in all varieties might be due to several factors such as type of soil, maturity stage, variety, and environmental conditions. The TPC concentration decreases because these are oxidized by polyphenol oxidase enzyme as the fruit passes the maturity stages. The results of the present study are close to the findings of Haider et al. (2018), who found that TPC contents decrease with the progressive maturation stages.

TFC

The total flavonoid contents of the four different date varieties at four different maturity stages are presented (Figure 5). It is clear from statistical data analysis that TFC differs significantly from each other of the different varieties. The TFC was found in decreasing order from the Kimri stage to the Tamer stage. The highest TFC were recorded in the Dhaki variety $(326.10 \pm 2.66 \text{ mg QE}/100 \text{ gm})$ followed by Zahidi $(307.25 \pm 2.41 \text{ mg QE}/100 \text{ gm})$ at the Kimri stage. The TPC ranges from Kimri to Tamer stage as 326.10 \pm 2.662 mg QE/100 gm to 209.35 ± 2.237 mg QE/100 gm in Dhaki, 307.25 ± 2.41 mg QE/100 gm to 185.85 ± 1.42 mg QE/100 gm in Zahidi, 295.62 ± 2.50 mg QE/100 gm to 174.78 ± 2.31 mg QE/100 gm in Aseel and 281.42 ± 1.33 mg QE/100 gm to 161.44 ± 0.715 mg QE/100 gm in Halawi variety. The lowest TFC was recorded in Halawi at the Tamer stage among all the varieties. While in TPC, the TFC concentration decreases because of the type of soil, maturity stage, variety, and environmental conditions. Geographical origin, growing and environmental conditions (mainly light and temperature) throughout fruit development, and harvest time, all play a role in these variations. The results of the present study are close to the findings of Amira et al. (2012),

who found that TFC decreases with the progressive maturation stages in different date varieties.

DPPH

The DPPH of date varieties at four different maturity stages are shown in Figure 5. The DPPH activity was found in the range of $3.36 \pm 0.01 \,\mu\text{g/mL}$ to $9.14 \pm 0.40 \,\mu\text{g/mL}$ in all four varieties. The maximum DPPH activity was observed in Dhaki at the Kimri stage. The minimum radical scavenging activity was found in the Halawi $(3.36 \pm 0.16 \,\mu\text{g/mL})$ at the Tamer stage. The DPPH activity decreases as ripening proceeds in all the varieties. Date fruits contain a higher number of phytochemicals so play an important role in scavenging the free radicals after the oxidative stress through the formation of antioxidants. The analysis of antioxidant activity by using the DPPH radical scavenging method was carried out to evaluate the different date fruit varieties at four different maturity stages. The DPPH activity decreases as ripening proceeds in all the varieties. This might be due to the activity of specific antioxidant enzymes, which decreases from Kimri to Tamer, the ripening stage. This might be due to changes in agricultural practices and ecological factors across regions, such as temperature, water stress, or mineral nutrient supply. The antioxidant activity of harvested fruits is also influenced by soil types and fertilizer parameters, which impact the water and nutrient availability to the plant. The results are confirmation of those of (Amira et al., 2012), who found the antioxidant potential of Tunisian date cultivars and increase focus on the impact on health-promoting antioxidant compounds in those cultivars during the three maturation stages.

FRAP

The antioxidant activity of date fruit based on FRAP assay is represented (Figure 5). The analysis of FRAP is used for the assessment of the antioxidant activity of the dietary phenol. The basic principle of FRAP assay is the determination of the ability of the antioxidant to reduce ferric ions. The results regarding the FRAP analysis show that all four varieties exhibited a good reducing power which varied significantly (P < 0.05) from 851.06 ± 7.78 to $579.07\pm4.64~\mu mol$ TE/100 g. The maximum activity of FRAP was recorded in the Dhaki variety (851.06 \pm 7.78 µmol TE/100 g) at the Kimri stage followed by Zahidi $(832.68 \pm 6.91 \mu mol TE/100 g)$. The lowest activity was found in Halawi (579.07 \pm 4.64 µmol TE/100 g) at the Tamer stage. The FRAP activity decreases from Kimri to Tamer stages in all studied date varieties. This might be due to the activity of specific antioxidant enzymes which decreases as ripening proceeds from the Kimri to Tamer stage. This might be due to changes in agricultural practices and ecological factors across regions, such as temperature, water stress, or mineral nutrient supply. The antioxidant activity of harvested fruits is also influenced by soil types and fertilizer parameters, which impact the water and nutrient availability to the plant. These results showed that the date fruit had a similar trend of antioxidant activity obtained by the FRAP assay compared to those of dates, grown in Morocco (Bouhlali et al., 2017). These findings are similar to the findings of Souli et al. (2018), who also found that different varieties of dates have varied FRAP activity depending upon the different

factors. Humans maintain their health by eating healthy fruits and vegetables having antioxidants. The antioxidants vary according to variety and ripening stage of fruits (Shoukat et al., 2022).

4 Conclusion

Date fruit has both nutritious and medicinal importance, it contains carbohydrates, dietary fiber, bioactive compounds, and significant amounts of potassium, calcium, and magnesium. Dates are high in phytochemicals, which were maximum in dhaki at the Kimri stage followed by khalal stage. Dates are also a good source of natural sugar at the Tamer stage that can be used as a sugar replacer in various value-added products development. Date fruit at various maturity stages has various amounts of bioactive compounds, minerals and dietary fiber, its composition at earlier maturity stages can make it suitable to develop date fruit enriched products to combat cardiovascular diseases. The utilization of date fruit to act as a functional food to meet the specific dietary requirements of the human body requires further extensive research. It is also required to validate the potential of the date fruit phytochemicals concerning maturity stages, in avoiding the health problems in the problem afflicted countries.

Conflict of interest

Authors declare no potential conflicts of interest.

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