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Phytochemical characterization of the date palm (*Phoenix dactylifera* L.) linked to chemical characteristics of the soil of backyard orchards

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Abstract

The fruits of *Phoenix dactylifera* L. contain bioactive compounds such as phenols, flavonoids, carotenoids, vitamins and minerals. However, its composition and content exhibit great variability depending on genetic and environmental factors, especially the physico-chemical properties of the soils where date palms develop. To elucidate the above, samples of dates from three backyard orchards and soil samples from each orchard were collected to determine the content of bioactive compounds and chemical characteristics of the substrate and to determine the correlations between these. For total contents of polyphenols and flavonoids, antioxidant activity and soluble solids, the following methods were used - folin-Ciocalteu reagent based total polyphenol estimation; aluminium chloride based total flavonoid estimation; 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical inhibition and a hand-held refractometer for soluble solid content determination. Each orchard was considered as a separate treatment and a completely randomized design was used, with the Tuckey's test (α=0.05) applied to compare means. Pearson's linear correlation analysis was applied to establish the link between bioactive compounds of dates and chemical characteristics of the soils. The hypotheses were that the bioactive compounds present in dates obtained in different backyard orchards could vary depending on the chemical characteristics of their soils. The results reflected significant differences only for the soluble solids content of the dates. Statistically significant correlations were observed between two soil characteristics; organic matter (OM) and electrical conductivity (EC); and the soluble solids content of dates. It was concluded that higher organic matter in the soils is associated with higher soluble solids content in the fruit, while higher electric conductivity in the soils is linked to lower soluble solids content in the dates.

Keywords

antioxidant activity; bioactive compounds; date tree; environmental factors; organic matter; soil characteristics; soluble solids

Introduction

In addition to basic nutrition, functional foods or nutraceuticals provide considerable benefits to human health. It is widely recognized that the consumption of fruits and vegetables reduces the risk of heart disease, certain types of cancer and degenerative diseases, due to their high content of fibre, antioxidants, vitamins and minerals, among other components (1, 2). The capacity of some plants to synthesize and accumulate various chemical compounds, such as sugars, vitamins and secondary metabolites, is of great importance. These compounds serve as cheaper and more environmentally friendly sources for bio-stimulation and growth promotion than other synthetic substances of a similar nature (3).

Like many fruits, dates contain a wide variety of bioactive compounds such as phenols, flavonoids, carotenoids, vitamins and minerals (calcium (Ca), magnesium (Mg), phosphorus (P), iron (Fe), copper (Cu) and zinc (Zn)) and they also contain large amounts of dietary fiber. Dates are rich in antioxidant compounds, have high concentrations of potassium (K) (due to their high sugar content), are a source of rapid and high levels of energy and their sodium (Na) content is relatively low (2, 4-6). These compounds impart various nutritional characteristics to dates, including antioxidant, anti-inflammatory, antimicrobial and cardio-protective properties that benefit human health (7, 8).

The production of secondary metabolites, both in quality and quantity, by plant species is affected by geographical, seasonal and environmental variations (9). There is also evidence that secondary metabolites- non-nutritional bioactive substances - are generated through metabolic pathway when the plants suffer stress conditions like, a) biotic, when suffering from attack by fungi, insects, or viruses; and b) abiotic, due to the impact of environmental factors such as ultraviolet radiation, salinity, light intensity, temperature changes, drought, among others. Likewise, it is known that these metabolites are generated due to environmental stress and through signalling processes and pathways to achieve their structural and functional stability (10).

In response to the above, the composition and contents of bioactive compounds in dates can reflect great variability depending on genetic and environmental factors, especially the physical and chemical properties of the soils in which date palms grow (11). In the same sense, the edaphic parameters such as pH, OM, as well as nitrogen (N), P and K contents, significantly affect the differential accumulation of phenolic compounds and carotenoids in fruits. If the nutritional demand of crops is partially satisfied, the generation of bioactive compounds will be visibly affected (12). Therefore, the use of humic acids in nutrient solutions, alongside the reduced use of synthetic fertilizers, contributes to sustainable production without affecting the yield and quality of the fruits, in terms of their content of bioactive compounds (13).

About the role of edaphic factors on the content of secondary metabolites, the existence of correlations between a) atranorin with pH, OM and sand content, b) fumarprotocetraric acid with the contents of OM and sand and c) usnic acid with pH and OM. With reference to dates, the available literature reports variable effects of soil chemical characteristics on phytochemical composition (14). However, studies focusing on backyard gardens are scarce (15). Therefore, the objective of this study was to determine the phytochemical profile of dates, as well as the physico -chemical characteristics of the soils from three backyard orchards and establish correlations between these characteristics.

Materials and Methods

Vegetal material

The date samples were collected from three backyard orchards located in Villa de Bilbao, municipality of Viesca (25° 25' 33.439" N; 102° 53' 50.539" W), Coahuila de Zaragoza, Mexico. The pulp and seeds of the fruits were separated manually, without mixing the fruits from each orchard. Each orchard was considered a treatment and for the pulp obtained, material was randomly selected to create three repetitions. These samples were stored in plastic bags, properly labelled and chilled at 4°C before being sent to the laboratory of the Desarrollo de Productos Alimenticios at the Facultad de Estudios Profesionales, Zona Huasteca, Universidad Autónoma de San Luis Potosí, Mexico, for the corresponding analysis.

Sample preparation

For each treatment and repetition, 1 g of sample was weighed and diluted in 5 mL of absolute ethanol and allowed to rest for 24 hr. After 24 hr, the supernatant was extracted to carry out the respective analysis. The next analysis was carried out in triplicate.

Determination of total polyphenol content (TPC)

This determination was carried out using a colorimetric oxidation-reduction reaction (16). In a test tube, 50 µL of the sample was mixed with 3 mL of distilled water, followed by the addition of 250 µL of Folin-Ciocalteu reagent (grade analytic, Merck) and stirred in vortex mixer for 10 sec. After 3 min of reaction, 750 µL of sodium carbonate [Na_2CO_3, (20%; w/v)] was added and stirred for 10 sec, then 950 µL of distilled water was added, vortexed again and allowed to react for 2 hr in the dark, wrapped in aluminium foil. The absorbance of the solution was determined at a wavelength of 760 nm using the Genesys 10UV UV spectrophotometer (Thermo-electron®), with gallic acid dissolved in absolute ethanol as the standard. The results are expressed in milligrams of gallic acid equivalents per 100 g (mg GAE . 100 g^{-1}) of fresh fruit pulp.

Determination of total flavonoid content (TFC)

Total flavonoids were quantified based on the formation of a complex between Al (III) ions and the carbonyl and hydroxyl groups of the flavonoid (16). In test tubes, 250 µL of the sample, 1.25 mL of distilled water and 75 µL of sodium nitrite [$NaNO_2$, 5% (w/v)] were placed, vortexed and allowed to rest for 5 min. Subsequently, 150 µL of aluminium chloride [AlCl3.H2O, 10% (w/v)] were added, vortexed again and allowed to rest for 6 min. Then, 500 µL of 1 M NaOH and 275 µL of distilled water were added and vortexed once more. The colorimetric estimation was performed using Genesys 10UV UV spectrophotometer (Thermo-electron®) at 510 nm. Quercetin standard dissolved in absolute ethanol was used as standard. The results are expressed in milligrams of quercetin per 100 g (mg quercetin equivalents• 100 g^{-1}) of fresh fruit pulp.

Determination of antioxidant activity (AA)

The antioxidant capacity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH*) radical method, which involves quantifying the elimination of free radicals by antioxidant compounds with DPPH* (17). The radical is stable and has a violet color that progressively decolorizes in the presence of radical-scavenging substances. A solution of 12 mg of DPPH* in 50 mL of methanol was prepared, adjusting the absorbance of the solution to 1.1 ± 0.002 at a wavelength of 515 nm. To achieve this adjustment, 5 mL of stock solution was diluted with 33 mL of ethanol, which was used as the blank.

To quantify the antioxidant capacity, 150 µL of the sample was mixed with 2850 µL of DPPH* solution, shaken briefly and immediately read in the Genesys 10UV UV spectrophotometer (Thermo-electron®) at 515 nm. The percentage of inhibition was calculated using the equation:

$$\text{DPPH* radical inhibition percentage} = \frac{\text{A initial - A final}}{\text{A initial}} \times 100 \quad \text{(Eqn. 1)}$$

Where, A initial: Absorbance of DPPH solution without extract and A final: Absorbance of the extract with DPPH. A greater inhibition percentage indicates greater antioxidant activity.

Determination of soluble solids content (SS)

For the quantification of soluble solids (SS) in the dates, 15 fruits were used per treatment, with repetition (18). A manual Master-T refractometer (Atago®), with a scale range of 0-80% °Brix, was used. The calibration of this device was carried out with distilled water, adjusted to 0% °Brix. With the help of a manual juicer, the juice from the dates was extracted, two drops of the juice were placed on the lens of the device and the scale values were recorded.

Determination of the chemical characteristics of soils

The determination of these properties in the soil samples was carried out in the Soil Laboratory of the Universidad Autónoma Agraria Antonio - Unidad Laguna. The organic matter content was determined by measuring the organic carbon content using the Walkey and Black method, which is based on the oxidation of soil organic carbon using a solution of potassium dichromate and in the presence of concentrated sulfuric acid (19). N was determined (20). K and micronutrient content were assessed using a Perkin model 2380® atomic absorption spectrometer, while P was evaluated using the Olsen method (21). pH values were obtained using a pH meter (Thermo Orion model 420®) and electrical conductivity was determined with a conductivity meter (Hanna model H1 2314®) using the saturation paste extract.

Statistical Analysis

Each orchard was considered a treatment and a completely randomized experimental design was used. The data obtained for TPC, TFC, antioxidant activity and soluble solids were subjected to analysis of variance (ANOVA) and comparison of means performed using the Tukey's 0.05 test. The SAS statistical package version 9.0 was used (22). Pearson's correlation analysis was also applied to establish the relationship between the phytochemical characteristics of dates and the chemical properties of the soils from the backyard orchards under study.

Results and Discussion

ANOVA and the comparison of means of Tukey's test ($p \leq 0.05$) for the variables evaluated in date fruits from the three orchards under study are presented in Table 1. As shown in this table, only the content of soluble solids recorded statistical differences ($p \leq 0.05$) due to the effect of sampling locations. Dates are fruits that provide great significant energy values due to their abundant sugar content (2).

Table 1. Average values and statistical significance of the variables evaluated in dates from three orchards in the Villa de Bilbao municipality of Viesca, Coahuila de Zaragoza, Mexico

Orchard	TPC^{ns} (mg GAE. 100 g^{-1} sample)	TFC^{ns} (mg Cat. 100 g^{-1} sample)	AA^{ns} (% Inhibition DPPH)	SS* (°Brix)
Enrique	202.67	760.65	44.60	41.5 ab
Melitón	226.14	716.24	41.03	52.4 a
Apolinar	232.25	877.86	56.3	48.6 ab

TPC = Total polyphenol content; TFC = Total flavonoid content; AA = Antioxidant activity; SS = Soluble solids; ns = Not significant; * = Statistical difference at 5%. The means of the columns with the same letter are not significantly different.

Total polyphenol content (TPC)

Dates are very rich in polyphenolic compounds, both in quality and quantity. These fruits also exhibit antiviral, antibacterial and antifungal properties, which is why they are used as a remedy against certain diseases and prevent chronic inflammation. Additionally, they inhibit pathogenic and parasitic organisms (23).

The TPC in the dates obtained from the three backyard orchards showed only numerical differences (Table 1), with values that ranged between 202.67 and 232.25 mg GAE.100 g^{-1} sample. These values significantly exceeded the TPC reported for dates from the varieties: Rushudiah, Osailah, Sbakah, Rothanh Qassim and Nabtat Ali obtained in a market in the province of Riyadh, Saudi Arabia, where the contents ranged from 50.64 and 98.61 mg GAE.100 g^{-1} of sample (6).

On the other hand, the TPC recorded in the present study was moderately higher than the TPC range of 116.74 to 189.60 mg GAE.100 g^{-1} of sample determined in dates from three varieties, Mech Degla, Degla-Beida and Deglet-Nour, from southeastern Algeria harvested at full maturity (24). It also widely exceeded the range of 3.82 and 5.30 mg GAE.100 g^{-1} sample, determined in dates of the Medjool cultivar, female recipients with pollen from four cultivars (Deglet Noor, Medjool, Khadrawy and Zahidi) (25). On the other hand, the TPC range recorded in the present study, turned out to be very different from the ranges reported by previous study, whose values ranged from 91.86 to 366.35 and from 119.13 to 410.39 mg GAE.100 g^{-1} dry sample, in fruits from four date palm varieties (Khalt, Khal, Jdar Lahmer and Rasse Tamar) during the years 2021 and 2022 (26).

The variations in TPC in dates described above can be attributed to 1) the different varieties used; 2) various factors such as environmental conditions (climate, temperature, humidity, number of daylight hours); 3) agronomic management of the crop (till practices, fertilizers); 4) processing methods; and 5) the storage conditions of the fruits (6). Dates are an abundant source of phenolic compounds, which together with selenium favour their high antioxidant activity (AA) (23, 25).

Total flavonoid content (TFC)

Flavonoid compounds are found in abundant amounts in ripe dates (21). Previous study reported a content of 289.2 mg Cat. 100 g^{-1} sample in fresh dates of the Khalas variety (27). On the other hand, in six date palm cultivars (Ahmar dli, Ahmar denga, Bou seker, Tenterguel, Lemdina and Tijib), at two edible stages of development [ripening (Blah) and fully ripe (Tamr)], the average total flavonoid content was 119.6 and 67.3 mg quercetin equivalents.100 g^{-1} of dry matter, respectively. Both contents were significantly exceeded by the TFC determined in the present study, which ranged from 760.65 to 877.86 mg Cat.100 g^{-1} sample (28). Similarly, the range of TFC determined in the present study widely exceeded the ranges reported by other researchers, with values ranging from 46.59 to 118.8 and 59.30 to 100.85 mg quercetin equivalents.100 g^{-1} dry matter, during the years 2021 and 2022, in four varieties: Khalt, Khal, Jdar Lahmer and Rasse Tmar (26).

The TFC in fruits is mainly attributed to several factors. According to different authors, the TFC of fruits is strongly influenced by genetic factors and can vary significantly between different varieties of the same species (29). The different stages of maturation mainly influence the TFC and environmental factors such as UV radiation, temperature and water stress can also influence the biosynthesis and accumulation of flavonoids in fruits (30, 31).

Antioxidant activity (AA)

Date palms are adapted to grow in hostile environments, which prevail in arid and semi-arid regions, such as those that predominate in a large part of the municipality of Viesca, Coahuila, from where the samples for the present study were collected. This adaptation may explain the great AA exhibited by its fruits (32). Additionally, it is known that the antioxidant properties of dates depend on TPC and other antioxidant compounds such as vitamin C and α-tocopherol (24).

Dates have great AA, surpassing other fruits consumed by humans, such as strawberries *Fragaria* × *ananassa* (Duchesne ex Weston) Duchesne ex Rozier, kiwis *Actinidia chinensis* var. deliciosa (A.Chev.) A.Chev., guavas (*Psidium guajava* L.), white pomegranates (*Punica granatum* L.) and purple blackberries (*Morus nigra* L.) (32). The difference in the AA recorded in the dates from the backyard orchards of the Villa de Bilbao was only numerical (Table 1). The AA values ranged from 41.5 to 52.4% of inhibition of the DPPH* radical which were significantly higher than the AA range of 6.61 to 10.72% inhibition of the DPPH* radical determined in samples of six varieties of dates - Rushudiah, Osailah, Sbakah, Rothanh Qassim and Nabtat Ali obtained from a market in the provincial Riyadh, Saudi Arabia (6).

Additionally, the AA values determined in dates from three palm varieties: Mech-Degla, Deglet-Nour and Degla-Beida, which ranged from 89.73 to 93.41% inhibition of the DPPH* radical, were twice as high as the AA values recorded in the present study (24).

Different authors highlight that polyphenols and other non-phenolic components can favour the AA of the date fruit (6, 32). The variations in these characteristics across different dates may be due to their ripening, genetic factors, variety, temperature, geographical location, as well as agronomic management conditions. On the other hand, its AA can be reduced due to factors such as processing of dates, sun drying and storage, probably due to enzymatic oxidation or the decomposition of natural antioxidants, including the conversion of soluble tannins into insoluble tannins (23, 32).

Soluble solids (SS)

The SS content recorded in dates from Mr. Melitón's orchard, 52.4% °Brix, exceeded the lowest sugar content of 50% °Brix, reported in the fruits of the Deglet Nour variety. However, it was significantly lower than the SS contents (76, 74, 70, 70 and 60% °Brix) recorded in dates from the varieties Deglet, Timjouhert, Takerbouch, Lahmira and Adam Boullah, respectively, from Algeria (24). It was also surpassed by the range of 64.45 to 67.50% °Brix determined in dates of the Medjool cultivar, which were pollinated with pollen from four cultivars (Deglet Noor, Medjool, Khadrawy and Zahidi) (8). The differences in SS content could largely be attributed to the application of synthetic fertilizers and the management practices of the crop in those locations, as synthetic fertilizers were not applied at the backyard level in this study.

General considerations

In general, the differences recorded in the total contents of polyphenols, flavonoids, soluble solids and antioxidant activity in the present study with respect to the results published by other authors may largely be due to the climatic and soil conditions, as well as the agricultural practices, which prevail in the different regions where the dates are produced. The above claim is supported by the production of secondary metabolites (flavonoids, polyphenols, among others) both in quality and quantity, because plant species is affected by geographical, seasonal and environmental variations (33). Similarly, there is evidence that the generation of these bioactive compounds occurs through their metabolic pathway, when plant species suffer biotic or abiotic stress conditions (34).

Correlation coefficients

Table 2 presents the Pearson correlation coefficients (R^2) determined between the quality and phytochemical variables of the dates and the characteristics of the soils from the backyard orchards where the date samples were collected for the present study. The table shows that significant correlations were exclusively recorded for the SS content, with respect to OM and EC. Regarding OM, the positive correlation (0.699*) is considered moderate, while the negative correlation with EC (-0.806**) is very strong (35). Similarly, correlations have been determined between soil characteristics and secondary metabolites, for example: a) atranorin with pH, OM and sand content; b)

Table 2. Pearson correlation coefficients for phytochemical variables, date quality and soil characteristics, from three backyard orchards in the Villa de Bilbao, municipality of Viesca, in the state of Coahuila de Zaragoza, Mexico

Quality and phytochemical variables	Soil characteristics			
	pH	OM (%)	EC (mS.cm^{-1})	CEC (meq.100 g^{-1}soil)
SS (°Brix)	-0.515	0.699*	-0.806**	0.247
pH_D	-0.510	-0.427	-0.276	-0.618
TPC (mg GAE.100 g^{-1} PF)	-0.531	0.187	-0.581	-0.156
TFC (mg Cat.100 g^{-1} PF)	-0.404	-0.374	-0.201	-0.517
AA (% Inhibition DPPH)	-0.365	-0.310	-0.195	-0.445

SS = Soluble solids; pH_D = pH of dates; TPC = Total polyphenol content; TFC = Total flavonoid content; AA = Antioxidant activity; OM = Organic matter; EC = Electrical conductivity; CEC = Cation Exchange Capacity; *, ** = Significant correlation at 0.05 and 0.01 (two-sided), respectively.

fumarprotocetraric acid with OM and sand contents; and c) usnic acid with pH and OM (14, 36).

Conclusion

The results showed significant differences only in the soluble solids content of the dates. Significant statistical correlations were recorded between the soluble solids content of the dates and two soil characteristics: organic matter and electrical conductivity. It was determined that higher organic matter content in the soils was associated with higher soluble solids content in the dates, while higher electrical conductivity in the soils was linked to lower soluble solids content in the fruits.

Authors' Contributions

AMR and JLRC were responsible for the conceptualization of the study, conducting research and drafting the manuscript. JHJ and JLRC contributed to the validation and formal analysis of the findings. OAG and JLRC took charge of designing the study and performing the statistical analysis. The design and coordination aspects were managed by AMR, ARM and SYMG. AMR played a crucial role in acquiring funding for the project. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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